Nutrient Requirements For Preterm Infant Formulas[1,2,3]

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ABSTRACT   Achieving appropriate growth and nutrient accretion of preterm and low birth weight (LBW) infants is often difficult during hospitalization because of metabolic and gastrointestinal immaturity and other complicating medical conditions. Advances in the care of preterm-LBW infants, including improved nutrition, have reduced mortality rates for these infants from 9.6 to 6.2% from 1983 to 1997. The Food and Drug Administration (FDA) has responsibility for ensuring the safety and nutritional quality of infant formulas based on current scientific knowledge. Consequently, under FDA contract, an ad hoc Expert Panel was convened by the Life Sciences Research Office of the American Society for Nutritional Sciences to make recommendations for the nutrient content of formulas for preterm-LBW infants based on current scientific knowledge and expert opinion. Recommendations were developed from different criteria than that used for recommendations for term infant formula. To ensure nutrient adequacy, the Panel considered intrauterine accretion rate, organ development, factorial estimates of requirements, nutrient interactions and supplemental feeding studies. Consideration was also given to long-term developmental outcome. Some recommendations were based on current use in domestic preterm formula. Included were recommendations for nutrients not required in formula for term infants such as lactose and arginine. Recommendations, examples, and sample calculations were based on a 1000 g preterm infant consuming 120 kcal/kg and 150 mL/d of an 810 kcal/L formula. A summary of recommendations for energy and 45 nutrient components of enteral formulas for preterm-LBW infants are presented. Recommendations for five nutrient:nutrient ratios are also presented. In addition, critical areas for future research on the nutritional requirements specific for preterm-LBW infants are identified.   J. Nutr. 132: 1395S-1577S, 2002.

PREFACE

The Life Sciences Research Office (LSRO) of the American Society for Nutritional Sciences (ASNS) provides scientific assessments of topics in the biomedical sciences. Reports are based on comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in relevant areas of biology and medicine. This LSRO/ASNS report was developed for and supported in part by the Center for Food Safety and Applied Nutrition, Food and Drug Administration (FDA) under Task Orders #11 & 13 of Contract No. 223-92-2185. During the course of this project, administrative responsibility for LSRO transitioned from the Federation of American Societies for Experimental Biology in 1998 through the ASNS to separate incorporation as LSRO, Inc in 2001. The ASNS acknowledges the cooperation of LSRO, Inc. in preparation of this report.

An Expert Panel provided scientific oversight and direction for all aspects of the project. The LSRO independently appointed members of the Panel based on their qualifications, experience, and judgment, with due considerations for balance and breadth in the appropriate professional disciplines. Notices in the *Federal Register* of November 15, 1996 and January 15, 1998, invited submission of data, information, and views bearing on the topic under study. LSRO held two Open Meetings, March 26, 1997 and March 27, 1998, and accepted written submissions. The Expert Panel convened six times (four full meetings and two conference calls) to assess the available data. Drs. William Heird, C. Lawrence Kien, Michael Georgieff, Ephraim Levin, and J. Cecil Smith made significant contributions to the writing and construct of sections of the report. Special appreciation is expressed to Dr. William Hay for his contributions during final review. Because the Committee on Nutrition of the American Academy of Pediatrics, the Food and Nutrition Board of the Institute of Medicine, and Health Canada provide professional advice on issues related to the topics of this report, these organizations received notices of progress of this study and opportunity for review. The LSRO staff, special consultants, and members of the Expert Panel considering all available information drafted the report, incorporated reviewers' comments and provided additional documentation and viewpoints for incorporation into the final report. The final report was reviewed and approved by the Expert Panel and the LSRO Board of Directors. On completion of these review procedures, the report was approved and transmitted to the FDA by the Executive Officer, ASNS, and the Executive Director, LSRO.

The listing of members of the Expert Panel and others who assisted in preparation of this report does not imply endorsement of all statements in the report. Although this is a report of the LSRO/ASNS, it does not necessarily reflect the opinion of the

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membership of the ASNS. The ASNS accepts full responsibility for the study conclusions and accuracy of the report.

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1. INTRODUCTION

This report is the second prepared for the U. S. Food and Drug Administration (FDA) under FDA contract 223-92-2185 by the Life Sciences Research Office (LSRO) to review the medical and scientific literature regarding the nutrient needs of infants and the composition of infant formulas. The first report, *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a), focused on formulas for term infants. The present report concerns the nutrient requirements for certain premature and low birth weight (LBW) infants and the composition of formulas intended for feeding these infants.

Formulas for preterm infants were developed with a nutrient profile different from that of human milk and term formulas, in part because of early observations that preterm infants grew better when formula, human milk, or banked human milk was supplemented with protein and minerals (Atkinson et al., 1981; Atkinson et al., 1983; Lucas et al., 1984). Achieving appropriate growth and nutrient accretion is often difficult because of the special needs of the preterm infant as a result of metabolic and gastrointestinal immaturity, compromised immune function, and other complicating medical conditions (Georgieff, 1999; Wright et al., 1993). Advances in the care of preterm infants, including improvements in delivery of appropriate nutrition, have reduced mortality rates for infants born weighing less than 2500 g from 9.6% to 6.2% between 1983 and 1997 (U.S.Department of Health and Human Services. Centers for Disease Control and Prevention. National Center for Health Statistics, 2000). Larger changes were seen within certain weight ranges. For example, the mortality rate of infants born weighing between 1000 and 1499 g decreased from 16.2% to 6.2% during this period. As newer knowledge related to special nutritional requirements of preterm infants becomes available, existing formulas may be modified and new products developed.

Birth weight and length of gestation are strong predictors of an infant's future health and survival. Of 3,959,419 births in the United States in 1999, 11.8% were preterm (less than 37 completed weeks of gestation) and 7.6% were LBW (less than 2500 g) (Ventura et al., 2001). In 1998, 65% of all infant deaths occurred among LBW infants, whereas the mortality rate was less than 1% for infants born 2500 g and above (Mathews et al., 2000). From 1990 to 1999, the percentage of preterm births rose 11% and the rate of LBW births rose 9% (Ventura et al., 2001).

The FDA, under the provisions of the federal Food, Drug, and Cosmetic Act (FDCA) as amended, is responsible for ensuring the safety and nutritional quality of infant formulas. Regulations for infant formulas are codified in Title 21 Part 107 of the Code of Federal Regulations (21 CFR 107). Formulas for infants of LBW are regulated as exempt infant formulas under the Infant Formula Act of 1980 and its 1986 amendment. Exempt infant formulas are typically prescribed by a physician and are distributed directly to institutions such as hospitals rather than through retail channels. Such formulas are also generally represented and labeled solely to provide dietary management for specific diseases or conditions that are clinically serious or life threatening, and they are usually required for prolonged periods (21 CFR 107.50). Exempt infant formulas may have nutrients or nutrient levels that are different from those specified in 21 CFR 107.100 after FDA review of data submitted by the manufacturer pertaining to the medical, nutritional, scientific, or technological rationale, including any appropriate animal or human clinical studies.

Regulations establishing quality factors for infant formulas are to be consistent, to the extent possible, with current scientific knowledge. Consequently, the FDA requires evaluation of the scientific literature and expert opinion constituting current scientific knowledge of nutrient requirements for formulas for preterm or LBW infants.

SCOPE OF WORK

The LSRO performed an independent assessment of the nutrient requirements for formulas for preterm-LBW infants to answer questions posed by the FDA in task orders defining the scope of work. These questions are as follows:

What scientific basis is there to support requirements for energy and macronutrients (protein, fat, and carbohydrate) in infant formulas intended for use by preterm infants as distinct from the requirements for energy and macronutrients in formulas for term infants? The American Academy of Pediatrics (AAP), the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN), and the Canadian Paediatric Society (CPS) have proposed some nutrient requirements for preterm infants distinct from those for term infants. Has scientific knowledge advanced to the point that distinct composition standards for energy and macronutrients in formulas for these preterm infants are warranted?

Nutrient requirements of hospitalized preterm infants who are fed enteral formulas are sometimes described according to stages such as a first or transition stage (between birth and 10 days of age), a stable growing stage (from about 10 days until discharge from the hospital, 6–8 weeks after birth), and a postdischarge stage (from discharge home to approximately 1 year of age). Is there scientific evidence for more than one set of energy and macronutrient requirements to support growth and development of the hospitalized preterm infant at the different stages of development? If so, how should the stages be defined? Are the energy and macronutrient requirements for infant formulas for term infants sufficient for healthy postdischarge preterm infants? Is there scientific evidence to support specific deviations from current nutrient standards for healthy postdischarge preterm infants and if so, what would they be and at what stage (age/weight) should these special formulas be given?

Does available evidence establish essentiality of addition of subcomponents of the macronutrients [specifically, taurine, carnitine, and long-chain polyunsaturated fatty acids (LCPUFAs)] to formulas for preterm infants, and if so, does the evidence establish what the amount and ratios of these compounds should be in the formula? For example, the Canadian Guidelines for the Composition and Clinical Testing for Formulas for Preterm Infants finds that term infant formulas containing adequate and balanced 18:2n-6 and 18:3n-3 fatty acids do not require addition of the 20- and 22-carbon n-6 and n-3 fatty acids. Is there available evidence to suggest that this is also true for preterm infant formulas? If so, is there an optimum level and ratio of 18:2n-6 and 18:3n-3 fatty acids in formulas for preterm infants?

Does the available evidence address the issue of safety of various sources of these LCPUFAs for use in preterm infant formulas? If so, is there a safe source of LCPUFAs?

Does available evidence establish the essentiality of addition of nucleotides to formulas for preterm infants and, if so, does the evidence establish what the amounts should be in the formulas?

What scientific basis is there to specify requirements for micronutrients in infant formulas intended for use by LBW premature infants? The possibility that the micronutrient requirements of LBW infants may be different from those of term infants was recognized by their classification as "exempt" infant formulas by the Infant Formula Act. The AAP, ESPGAN, and CPS have proposed nutrient requirements for LBW infants distinct from those for term infants. Has scientific knowledge advanced to the point that distinct micronutrient composition standards for formulas for these preterm infants are warranted?

Micronutrient requirements of preterm infants fed enteral formulas are sometimes described according to a first or transition stage (between birth and 10 days of age), a stable growing stage (from about 10 days until discharge from the hospital, often 6–8 weeks after birth), and a postdischarge stage (from discharge home to approximately 1 year). Is there scientific evidence for more than one set of micronutrient requirements for infant formulas to support healthy growth and development of the preterm infant at the different stages of development? Are the micronutrient requirements for term infant formulas sufficient for thriving postdischarge preterm infants?

What is the scientific evidence of dietary essentiality for a minimum and/or maximum quantitative nutrient concentration for selenium, chromium, molybdenum, and fluoride in preterm infant formulas? What limits of intake would ensure safe and adequate exposure to these nutrients? Is there a need to specify the chemical form or other characteristics of these nutrients or their sources to ensure safety and adequacy?

Can other micronutrient interactions be identified for preterm infants besides those already established to ensure nutrient adequacy for term infants, i.e., vitamin E-to-linolenic acid, vitamin B_6-to-protein, and calcium-to-phosphorus ratios? Are there recommended ratios for metal ions? Is the evidence of interaction between these minerals sufficiently strong that the ratios should be ensured for the health of preterm infants?

Is there adequate evidence of benefit of other substances not listed above to support a requirement for their inclusion in preterm infant formulas?

The FDA requested that this report focus only on nutrition issues and not address manufacturing issues that also are relevant to preterm formula nutrient content. For example, this report does not address the important Good Manufacturing Practice issues of processing overages and nutrient stability that have a direct impact on ensuring the availability of essential nutrients throughout a formula's shelf life (Gelardi & Mountford, 1999). Although the FDA considers manufacturing issues in developing regulations, the Expert Panel also interpreted these issues to be beyond the scope of work and therefore they are not addressed in this report.

The LSRO conducted a review of published scientific studies to determine the availability of information pertaining to the questions raised by the FDA regarding nutrient specifications for preterm infant formulas. To evaluate the information gathered and to respond to the questions posed by the FDA, the LSRO convened an ad hoc Expert Panel of scientists with expertise in disciplines relevant to the study, such as neonatology, gastroenterology, trace mineral metabolism, and nutritional biochemistry. Additional information and scientific expertise were obtained from scientists who were identified by members of the Expert Panel as consultants. Public input into this process was also obtained at two open meetings held on March 26, 1997, and March 27, 1998. The LSRO staff incorporated relevant published literature identified by ad hoc reviewers, members of the Expert Panel, and electronic literature

searches during preparation of the final draft of the report in 2001. The Expert Panel considered the materials, information, and opinions obtained from all of these sources, and the LSRO staff drafted and edited the final report in consultation with the Expert Panel.

2. PRINCIPLES AND CONCEPTS USED IN DEVELOPING RECOMMENDATIONS

The Expert Panel used the following principles and concepts to arrive at its conclusions and recommendations for nutrient composition of preterm infant formula.

SCOPE AND DATA INTREPRETATION

The preterm infant is a hospitalized patient who requires medical supervision and treatment in addition to the delivery of preterm infant formula and who is often at risk of clinically critical or life-threatening sequelae. The Expert Panel reviewed data with due consideration of limitations imposed by a shortened period of gestation and the stage of development at birth. Thus, multiple problems affecting premature infants may confound nutritional issues.

The Expert Panel based its recommendations on the concept that the formula would serve as the sole source of nutrition for the preterm-low birth weight (LBW) infant. The recommendations are for formulas as fed. The use of enteral formulas as the sole source of nutrition for preterm infants is limited by the developmental stage of the gastrointestinal and renal systems (see Appendix A). The recommendations in this report are intended for infants born preterm [before 36 weeks of gestational age (GA)] and for those born small for GA (SGA; SGA infants are LBW, i.e., weigh <2500 g). Insufficient data exist to distinguish between the nutrient requirements of these two groups, and many infants fit into both categories. Recommendations in this report are appropriate for preterm-LBW infants under medical supervision during their initial hospitalization, which usually ends at a weight of 1800–2000 g (Cruz et al., 1997). Whether these recommendations will also meet the needs of infants weighing <750 g is not known, because few data concerning their nutritional requirements are available. In this report, recommendations, examples, and sample calculations that are presented are often based on a 1000-g preterm-LBW infant consuming 120 kcal/kg in 150 mL/d of an 810 kcal/L formula (Georgieff, 1999; Wessel, 2000).

The Expert Panel concluded that changes in nutrient needs consequent to a clinical condition or medical care that would not be met by changing the concentration or dilution allowable within the caloric range of a preterm formula should be the responsibility of the clinician caring for the patient. Recommendations are intended to apply to most preterm infants as supported by documented evidence, whereas the process of achieving nutritional goals for a specific preterm infant is determined on a case-by-case basis according to that infant's GA, physiological development, and clinical condition. Goals (e.g., growth rate) are to be achieved over time and can be accelerated or delayed based on the clinical judgment of the neonatologist.

Biochemical indices of deficiency, adequacy, and toxicity developed in term infants, children, and adults may have limitations when used for preterm infants. Nevertheless, extrapolations based on body weight, metabolic capacity, or nutrient load were made and served as a basis of consideration.

Human milk was not used as the primary model as a means to identify the amount of each nutrient to be contained in preterm infant formula. However, because there may be ingredients in human milk that are essential for preterm infants,

substances present in human milk were considered for inclusion in preterm infant formulas. The Expert Panel endorsed mother's milk as the preferred source of nutrition for the preterm infant, if the milk is fortified to ensure its nutritional adequacy. In all cases, the level of nutrients delivered by the fortifier and by the representative samples of expressed breast milk fed at appropriate volumes should combine to meet the LSRO nutrient recommendations for preterm-LBW infants and not exceed the maximum recommended for each nutrient. The Expert Panel considered the history of use of fortified human milk as an effective source of nutrition for preterm infants as providing evidence for the lack of adverse effects attributable to naturally occurring ingredients at levels normally present in human milk.

The Expert Panel recognized that growth curves represent observed patterns of fetal growth at various percentiles, whereas standards define an established rate of optimal development that can be used to identify clinically relevant growth deviations that entail morbid consequences (Alexander et al., 1996). Notwithstanding this caveat, the Expert Panel concluded that growth curves could be used as standards in generating its recommendations. However, the Expert Panel limited that use to determining the amounts of specific nutrients that allow this growth potential to be realized by the 50th-percentile infant. This application should not be construed to imply that this growth rate would be maintained by every infant fed a formula with a composition consistent with the levels recommended in this report. The Expert Panel agreed with the clinical practice of using intrauterine weight gain as a reference for growth and for evaluating the adequacy of formula in meeting the nutritional requirements of preterm-LBW infants.

The factorial approach for estimating nutrient requirements considers that the total requirement for a nutrient is equal to the sum of the obligatory losses (e.g., urine, feces, skin), plus the amount incorporated into newly formed tissue (Beaton et al., 1996; Sandstead & Smith, Jr., 1996). Factorial estimates of requirements were used and were based on a variety of data for preterm-LBW infants and other age groups. These estimates may have included estimates of the intrauterine accretion rate and percentage of retention. The Expert Panel is aware of the limitations of the factorial method (Beaton et al., 1996).

Investigating the nutritional needs of preterm-LBW infants is an active and dynamic field of research. For example, the neurological and immunological systems, because of the nature of their development, involve studies comparing feeding and development after discharge from the hospital, including long-term outcome in adolescence and adulthood. Limited studies are available. The Expert Panel identified research needs in various sections of this report.

CONCEPTS APPLIED IN ESTABLISHING MINIMUM AND MAXIMUM NUTRIENT LEVELS

With regard to the charge to recommend minimum and maximum levels for nutrients and substances covered in this report, the Expert Panel recognized that various factors including essentiality, stability, history of use, safety, and toxicity are involved in the determination of safe and adequate levels of nutrients for infant formula. Some or all of these factors are relevant for each nutrient. The Expert Panel sought specific evidence when its recommendations differed from the maximum and minimum recommendations presented in the earlier report on term infant formula (Raiten et al., 1998a). The Expert Panel made its recommendations for preterm formula with the expectation of periodic reassessments of the appropriateness of the range for minimum and maximum values.

Definitions of optimal intakes of individual nutrients and their relative concentrations in complete formulas are areas of active research. The Expert Panel has specified ranges rather than specific concentrations for the components of preterm formula. Within these ranges, requirements are expected to be met without adverse effects, similar to the use of ranges in the *Estimated Safe and Adequate Daily Dietary Intakes of Selected Vitamins and Minerals* (National Research Council. Food and Nutrition Board, 1989).

Clinical experience and history of use of preterm formulas and fortified human milk were accepted as evidence for recommending minimum and maximum levels of individual nutrients. The Expert Panel often considered this information during assessment of minimum nutrient levels, when seeking data related to the lowest levels fed to preterm infants without observing adverse effects or indications of deficiency. When these levels were above the lower limits recommended for term infant formula, the Expert Panel judged, unless otherwise stated, that such levels be exceeded in preterm formulas until a compelling rationale is presented for change and clinical studies of lower levels are completed.

The Expert Panel considered the basic nutrient requirements supported by the literature and allowed an adequate range in nutrient concentrations to achieve realistic goals of nutrient accretion and growth. Thus, the Expert Panel recommended minimum amounts of specific nutrients in formula to meet the estimated requirements of preterm-LBW infants. Likewise, maximum amounts were recommended to prevent daily intake of potentially harmful amounts of nutrients. The recommended range of minimum and maximum levels of a specific nutrient was intended to be adequate for catch-up growth.

Direct experimental evidence, when available or convincing clinical observations related to the requirement were used to set a minimum recommended content. For example, clinical studies were cited demonstrating that the amount of a nutrient in term formula was too low to support that nutrient's status in preterm-LBW infants. Furthermore, clinical studies of supplemental feeding of preterm-LBW infants were cited that verified that preterm-LBW infants fed the minimum amount recommended for preterm formula had superior nutritional status compared with similar infants fed the minimum amount recommended for term formula.

When data on intrauterine accretion were available, the amount of nutrient required for attaining the mean rate was used to estimate the minimum nutrient content for preterm formula. When there were adequate bioavailability data on the relative absorption of a nutrient from human milk and infant formulas, the minimum amount for absorption that resulted in a net retention rate of nutrients similar to the intrauterine accretion rate was recommended. Current use, the amount in domestic preterm formula, was also assessed. In some instances, the minimum or maximum value was calculated from a nutrient-to-nutrient ratio.

The Expert Panel's judgment in establishing a maximum content for a nutrient in preterm infant formulas depended on scientific evidence of toxicity, the potential for adverse nutrient interactions, and history of use and absence of evidence of toxicity.

The maximum level was based on the highest intake for which there was some information on the history of intake in clinical studies or current use in domestic preterm formula without adverse effects. In some instances, the maximum value currently fed was obtained from the manufacturer's product brochure (Abbott Laboratories. Ross Products Division, 2001; Mead Johnson Nutritionals, 2000), and this may underestimate actual amounts fed because the Panel did not review data

on manufacturing practices. In addition, the Panel did not review data on changes in nutrient composition during storage and administration.

3. FEEDING PRETERM-LOW BIRTH WEIGHT INFANTS

This chapter briefly reviews the current state of knowledge concerning typical feeding practices and sources of enteral nutrition for preterm-low birth weight (LBW) infants.

INITIATION AND PROGRESSION OF FEEDING

The Canadian Paediatric Society (CPS) (1995) described a transition period of about 1 week following birth and preceding the period of weight gain during hospitalization. A goal for the transition period was to provide sufficient nutrients to prevent deficiencies and substrate catabolism (wasting). During this period, the patient often requires a combination of parenteral and tube feeding, manipulation of fluids and electrolytes, and other clinical procedures requiring monitoring and clinical judgment. This period is characterized by higher risk of metabolic instability, particularly among smaller and younger infants. The Expert Panel agrees with the recommendation of an ad hoc Expert Consultation Group to the Health Protection Branch of Health Canada (Canadian Paediatric Society & Nutrition Committee, 1995) that concluded that the variability of nutrient requirements was insufficient to justify specifying compositions for different formulas intended as the sole source of nutrition for preterm infants during this early postnatal period.

Many preterm-LBW infants require total parenteral nutrition (TPN) for their initial nutritional support because their organ systems are immature, particularly because of the functional immaturity of the gastrointestinal tract and their need for respiratory ventilation (Georgieff, 1999). TPN is infused as soon as the infant is metabolically stable, typically on the second or third day of life (Georgieff, 1999; Papageorgiou & Bardin, 1999). Parenteral nutrition is associated with several complications, including the increased risk of infection, mucosal atrophy, and cholestatic jaundice (Georgieff, 1999). Therefore, the transition to full enteral feeding and the termination of TPN are accomplished as soon as feasible and safe. The requirement for TPN varies with gestational age (GA); the infants of youngest GA take longer to begin enteral feeding and convert to full enteral feeding (Thorp et al., 2000). The duration of TPN also varies among hospitals because of differences in clinical practice (Papageorgiou & Bardin, 1999). Papageorgiou and Bardin (1999) reported a mean of 40 days (range: 9–120 days) of TPN use for surviving infants who weighed less than 1000 g at birth, whereas preterm-LBW infants who spend more time in utero may require only 4–10 days of TPN (Georgieff, 1999; Lucas et al., 1992). A report from seven medical centers participating in the National Institute of Child Health and Human Development Neonatal Network between 1987 and 1988 determined that of surviving infants ($n = 1306$), those with birth weights (BWs) of 501–750 g received TPN for an average of 33 days, those weighing 751–1000 g received TPN for an average of 25 days, and those weighing 1001– 1500 g received TPN for an average of 15 days (Hack et al., 1991).

The CPS (1995) recommended fortified milk from the infant's own mother or formula designed for premature infants for infants with BWs of 500–1800 g (and possibly up to 2000 g) or with a GA of between 24 and 34 weeks (and possibly up to 38 weeks, often the age when the infant can nurse effectively). Enteral nutrition can be initiated as early as 48 hours after birth by introducing small amounts (1 mL/h or less) of preterm milk or formula via a tube into the stomach, or less frequently, directly into the small intestine (Papageorgiou & Bardin, 1999). Enteral feeding at this low rate has little nutritional value but may enhance the development of the gastrointestinal tract and for this reason is referred to as "trophic," "priming," or "minimal enteral nutrition" (Georgieff, 1999; Wessel, 2000) (See Appendix A). Schedules for advancing from the initial rate to full enteral feeding of the preterm-LBW infant have been developed on the basis of BW (Georgieff, 1999; Wessel, 2000). Some clinicians administer a 20 kcal/fl oz preterm formula and progress to a 24 kcal/fl oz preterm formula when the infant is consuming one-quarter to one-half of the total required amount (Papageorgiou & Bardin, 1999). Others prefer to initiate enteral feeding with a 24 kcal/fl oz formula (Wessel, 2000). In general, full enteral feeding can be achieved by 20–30 days of life for preterm-LBW infants (Papageorgiou & Bardin, 1999). Wilson et al. (1992) reported that, on average, enteral feeding was introduced in preterm-LBW infants, who had a mean GA of 28 ± 1.5 (SD) weeks and a BW of 1027 ± 222 (SD) g, at 7 days (range: 1–27 days) of life, yet infants did not reach full enteral feeding until 31 days (range: 7–101 days) of life. The age when full enteral feedings are achieved tends to decrease progressively as BW increases (Ehrenkranz et al., 1999). On average, infants of BW 1300-1500 g consumed more than 100 kcal/ (kg·d) by 11 days of life; whereas, this level of intake is not achieved by infants of BW 900-1000 g until 21 days of life (Ehrenkranz et al., 1999). Preterm-LBW infants receive almost all fluids via enteral formula once intravenous fluids are discontinued. Some fluid is supplied by periodic flushes of water to maintain patency of the feeding tube.

Conversion from tube feeding to feeding by bottle or breast depends on the development of coordinated and adequate sucking, swallowing, and breathing. Introduction of one oral feeding per day can begin at approximately 33 weeks of postconceptional age (Georgieff, 1999; Wessel, 2000). The frequency of oral feeding increases as tolerated by the infant. The energy required for oral ingestion can detract from weight gain (Georgieff, 1999). The infant should be periodically monitored to assess whether ad libitum, on-demand feeding will provide adequate weight gain or whether other nutritional support is required (Georgieff, 1999).

SOURCES OF EARLY ENTERAL NUTRITION

Human milk

Banking human milk donated from mothers of term infants was a common practice to supply enteral nutrition for preterm-LBW infants until the 1960s. During that period, alternative sources including expressed human preterm milk and modified cow milk protein-based formula preparations were introduced for these infants. Raiten et al. (1998a) reported the energy and nutritional composition of pooled banked human term milk. Milk banks containing human milk from donors are presently rare, in part because of problems controlling collection, storage, distribution, contamination, and safety (e.g., human immunodeficiency virus) (Lucas, 1993).

Milk produced by mothers of infants born prematurely is designated preterm milk. Typically, mothers' preterm milk is stored frozen at –20°C until needed for feeding (Lucas, 1993; Schanler et al., 1999). The macronutrient composition of preterm milk varies widely. Atkinson (1995) reported several possible sources of variability, including intra- and interindividual variation, differences in collection methods, and a wide range of GA for infants studied. The variability of the composition of preterm milk confounds its use as the sole source of

essential nutrients for premature infants. In fact, in clinical practice, the nutrient composition of the specific human milk being fed is unknown. Preterm milk varies in nutrient composition from the milk of mothers of infants born at term.

The mean energy content of preterm milk from mothers of infants of GAs of 26–36 weeks ranged from 51–58 kcal/100 mL in the first days postpartum (Anderson et al., 1981; Gross et al., 1980) to approximately 66–75 kcal/100 mL in the second to fourth week of life (Anderson et al., 1981; Gross et al., 1980; Hibberd et al., 1981; Lemons et al., 1982). The energy content of milk from mothers of term infants in the first month postpartum was 48–73 kcal/100 mL—equal to or less than that in preterm milk (Anderson et al., 1981; Gross et al., 1980; Lemons et al., 1982).

The reported mean total fat content of preterm milk collected in the first week postpartum from mothers of infants of GAs ranging from 26 to 37 weeks was 2.6–3.1 g/100 mL (Anderson et al., 1981; Bitman et al., 1983; Darwish et al., 1989; Lemons et al., 1982; Sann et al., 1981). From 8 to 30 days postpartum, fat content in preterm milk ranged from 2.5 to 4.3 g/100 mL (Anderson et al., 1981; Bitman et al., 1983; Butte et al., 1984; Darwish et al., 1989; Lemons et al., 1982; Sann et al., 1981). The fat content of milk from mothers of term infants in the first month postpartum was 2.2–3.1 g/100 mL—similar to or less than that in preterm milk (Bitman et al., 1983; Darwish et al., 1989; Sann et al., 1981).

The mean total protein content of preterm milk has been reported to range from 2.1 to 3.2 g/100 mL during the first week postpartum (Anderson et al., 1981; Chandra, 1982; Gross et al., 1980). The protein concentration of preterm milk ranged from 1.4 to 2.4 g/100 mL from 8 to 30 days postpartum (Anderson et al., 1981; Chandra, 1982; Gross et al., 1980). During the first month postpartum, term milk reportedly contains 1.3–1.9 g/100 mL, less than that found in preterm milk (Anderson et al., 1981; Chandra, 1982; Gross et al., 1980).

Atkinson et al. (1981) provided data in support of using expressed human milk from mothers giving birth prematurely. They compared growth, nitrogen balance, and biochemical indices during the first 2 weeks postpartum for infants of BWs less than 1300 g. Despite the brevity of the study, the infants fed the unfortified preterm human milk showed greater nitrogen accretion and greater net fat absorption than those fed the unfortified term milk (Atkinson et al., 1981). Moreover, fecal fat levels were two- to three-fold higher for the infants fed unfortified pooled term milk; fat intake was similar. Likewise, Gross (1983) compared the growth of preterm-LBW infants fed isocaloric diets of term milk or unfortified preterm pooled human milk until they reached 1800 g. The protein intake differed because of the higher protein concentration in preterm milk. The data confirmed earlier results of less daily weight gain and slower head and linear growth of infants fed unfortified term milk compared with infants fed unfortified preterm breast milk.

Despite the aforementioned results, Atkinson (1995) noted that there was no justification for assuming the composition of unfortified preterm milk was optimal for or adapted to the nutritional requirements of the preterm infant. Lucas (1993) identified two problems that do not support the use of unsupplemented human milk as a sole source of essential nutrients for premature infants. First, both preterm milk and banked term human milk provide insufficient amounts of energy, protein, sodium, calcium, and phosphorus as well as a number of other essential nutrients needed for rapid growth and normal development of infants as if they had remained in utero. Furthermore, unlike term infants, preterm infants cannot regulate their intake to compensate for nutrient insufficiency (Lucas, 1993). The nutritional inadequacy of human milk for the preterm-LBW infant has been reviewed

extensively (Schanler, 1989; Schanler, 1995; Schanler & Hurst, 1994). Therefore, although human milk is universally accepted as the ideal food source for healthy term infants, unsupplemented human preterm milk is inadequate for preterm-LBW infants.

Fortification of mothers' own preterm milk with additional human or bovine milk solids (protein, fat, minerals) offers a partial solution to the problems of variability in composition and nutrient inadequacy. A nutrient fortifier can provide improved infant nutriture. The composition of commercially available human milk fortifiers was recently summarized (Sapsford, 2000). The fortifier is typically added to preterm milk when the infant's intake ranges between one-quarter and one-half of the total feeding goal (Papageorgiou & Bardin, 1999). There are limitations, however, in using fortified preterm milk. For example, the use of a standardized fortifier in a fixed proportion could lead to inadequate nutrient intakes by larger infants or those having higher requirements (Moro & Minoli, 1999). Due to the high levels of protein often present in expressed breast milk produced in the first few weeks by mothers delivering prematurely, infants receiving protein-enhanced breast milk in the first month should be monitored for the effects of protein excess. Furthermore, the mother may not be able to express a sufficient volume of preterm milk to be fortified; therefore, the preterm infant's fluid and nutritional requirements may not be met. Also, the use of preterm milk is contraindicated if the mother has human immunodeficiency virus infection and/or has ingested drugs of abuse, has been exposed to toxic environmental agents associated with an effect on breast milk or has received drug therapy that is a contraindication for providing human milk to the infant (American Academy of Pediatrics. Committee on Drugs, 1994; Anderson, 1995; Berlin, 1995; Eidelman & Schimmel, 1995; Schanler & Butte, 1997). In addition to the difficulties of maintaining lactation and providing an adequate and sustained supply of milk to infants hospitalized for 10 weeks or more, the nutritional variability and inadequacy of the mothers' milk may not be totally restored by fortification. For these reasons, preterm-LBW infants require preterm infant formula as an additional source of enteral nutrition.

For several reasons, including nutritional, immunological, and psychosocial ones, feeding preterm-LBW infants their own mothers' expressed breast milk became conventional practice (American Academy of Pediatrics. Committee on Nutrition, 1998). Human milk contains cells and substances such as macrophages, lymphocytes, enzymes, and various immunological factors that could promote the health of preterm-LBW infants. Schanler et al. (1999) found that preterm-LBW infants fed fortified mother's milk and supplemented with preterm formula as needed required fewer days of oxygen therapy, had a lower incidence of late-onset sepsis, and were discharged sooner compared with similar infants fed solely preterm infant formula. McKinley et al. (2000) observed that preterm-LBW infants fed some human milk had fewer hospitalizations by 18 months (corrected age) than infants fed only formula. Although the infants fed preterm milk and supplemented with preterm formula had a better immunological response, they had decreased fat absorption; had less gain in weight [g/(kg·d)], length, and skinfold thickness; and required more treatments for mild acidosis and low serum sodium levels than did the infants fed solely preterm formula (Schanler et al., 1999). Despite these limitations, Schanler et al. (1999) promoted the feeding of fortified preterm milk, when available, because of its immunological advantages.

Infant formula

As previously mentioned, milk from mothers of term infants is inadequate to meet the nutritional requirements of preterm-LBW infants. Likewise, because the nutrient compo-

sition of term formula was modeled on mature breast milk, formulas appropriate for term infants are inadequate for premature infants (Lucas, 1993). For example, in 1985 Hillman et al. (1985a) reported that preterm-LBW infants fed standard term formula exhibited low serum levels of 25-hydroxyvitamin D and inadequate bone mineralization as assessed by radiographs of the wrist. They concluded that standard term infant formula did not provide the estimated fetal accretion rate of calcium. Cooke and Nicholds (1986) similarly concluded that preterm-LBW infants who were fed standard term formula retained only 50% or less of the calcium required to support the estimated fetal calcium accretion rate.

Lucas et al. (Lucas et al., 1990b; Lucas et al., 1998; Morley & Lucas, 2000) conducted a series of studies in the United Kingdom to examine the influence of feeding term formula or preterm formula to preterm-LBW infants until they weighed 2000 g or were discharged from the hospital. At 18 months of age, the infants who had been fed preterm formula as their sole source of nutrients in the hospital ($n = 61$) had a greater daily weight gain [g/(kg·d)], head circumference, and motor development than did infants fed term formula ($n = 58$) (Lucas et al., 1990b). At 7.5–8 years of age, the children who had been fed preterm formula in the early neonatal period scored higher on tests of intelligence (revised Wechsler I scale) than did children who had been fed term formula, particularly for boys (Lucas et al., 1998). At this age, no differences in weight, height, or other anthropometric measures were evident. Although the early 4 weeks of feeding with preterm formula was of sufficient duration to induce an effect on neonatal growth and long-term neurological development, it remains to be determined whether a longer period of formula enrichment would have had an effect on measures of growth (Morley & Lucas, 2000).

Several of the earlier sources of nutrition are presently considered inappropriate for premature infants. These include formulas containing half-skimmed cow milk (because of inadequate calories and a high renal solute load), acidified milk (because it produces metabolic acidosis), evaporated milk (because of insufficient sodium, copper, and possibly other micronutrients), and soy milk (because of decreased calcium and phosphorus absorption and an increased incidence of osteopenia and rickets) (Hillman et al., 1979; Hillman, 1990). Furthermore, the American Academy of Pediatrics Committee on Nutrition (AAP-CON) (1998) concluded that formulas derived from soy protein, rather than animal casein and whey, were unsuitable for premature infants. These discontinued sources of enteral nutrition will therefore not be discussed in this report except in the context of comparative nutritional studies.

The preterm-LBW infant formulas were developed on the basis of classic fetal body composition data by Widdowson and Spray (1951). In 1966, Mead Johnson and Company introduced an enriched enteral formula developed specifically for preterm-LBW infants. The initial preterm formulas were enriched with protein (18% whey:82% casein). By 1977, some preterm formula contained 40% medium triglycerides, and a portion of the lactose was replaced with sucrose to improve digestibility and absorption. By 1979, the composition of protein in preterm formula had changed to 60% whey and 40% casein, a ratio that was associated with less metabolic acidosis than was the casein-dominant formula (Räihä, 1994a; Shenai et al., 1986). Another compositional change at that time was the substitution of glucose polymers for sucrose to decrease the osmolality of the formula. Formulas were further modified after the publication of various reviews undertaken to evaluate the evidence regarding the nutritional requirements of preterm-LBW infants, particularly after the classic reports by Tsang et al. (1985;1993). A more detailed history of the feeding of

preterm-LBW infants is available (Abbott Laboratories. Ross Products Division, 1998).

The preterm formulas currently provided in hospitals in the United States were designed to serve as the sole source of nutrition for the preterm-LBW infant. In general, these formulas contain added whey protein, glucose polymers, medium-chain triglycerides, calcium, phosphorus, electrolytes, folate, and fat-soluble vitamins. The compositions of two preterm formulas presently available in the United States are summarized in Table 3-1 and Table 3-2 (Abbott Laboratories. Ross Products Division, 2001; Mead Johnson Nutritionals, 2000). These formulas can promote average rates of nutrient assimilation and growth that approximate the rates typical for an in utero fetus; however, because of initial weight loss after birth, the total body weight may be lower than the expected intrauterine fetal weight for the same postconceptional age (Schanler, 1999). Preterm formula is available for preterm infants whose mothers are unable to express sufficient milk for fortification. When postpartum mothers cannot provide milk, hospitalized preterm-LBW infants receive solely preterm formula for their enteral nourishment. In practice, the preterm formula is fed until, in the clinical judgment of the responsible physician, the infant can be supported nutritionally by a term formula. The decision to change from preterm formula is not based on when an infant attains a certain age or weight, but rather when it is deemed clinically appropriate.

FEEDING AT HOME

At present, preterm formulas are not usually available to the infant after discharge from the hospital and may not be appropriate for use at that time. For example, Georgieff (1999) noted that the infant's capacity for fat digestion improves after 34 weeks postconception and expressed concern that this might lead to excessive vitamin A absorption if the infant were to continue to be fed the preterm formula.

After discharge from the hospital, preterm-LBW infants might be fed one of the commercially available formulas containing nutrients at levels that are intermediary between preterm formula and term formula and have been referred to as "transitional," "expremie," "follow-up," and "postdischarge" formulas (Georgieff, 1999; Lucas et al., 1992). The use of these formulas has increased significantly since the mid-1990s (Worrel et al., 2000). In 1992, Lucas et al. (1992) reported that U. K. preterm infants ($n = 16$) fed an enriched formula until 9 months (corrected postnatal age) had greater weight gain and linear growth than did similar preterm infants ($n = 15$) fed standard term formula after discharge. In addition, the infants fed the enriched formula had greater bone mineral content at 3 and 9 months (corrected postnatal age) than did the infants fed standard term formula (Bishop et al., 1993). In contrast, there were no significant differences in weight, length, head circumference, and Brazelton assessment results (behavior) for preterm-LBW infants fed a standard term formula ($n = 10$), a preterm formula ($n = 12$), or an intermediate enriched formula ($n = 11$) for 8 weeks after discharge (Chan et al., 1994). However, infants fed the preterm formula for 8 weeks after discharge had greater bone mineral content than did infants fed the standard term formula or intermediate formula for 8 weeks after discharge (Chan, 1993). Cooke et al. (1998) determined that 16 male U. K. preterm-LBW infants fed standard preterm formula from discharge to 6 months (corrected age) had greater weight and length gains than did similar infants ($n = 11$) fed standard term formula after discharge; no differences were found for female infants ($n = 13$ and $n = 20$, respectively). On the basis of the results of Lucas

TABLE 3-1

Composition of selected preterm infant formula per 100 kcal

Constituent or variable	20 calories per fluid ounce (68 kcal/100 mL)		24 calories per fluid ounce (81 kcal/100 mL)	
	Enfamil Premature with Iron®[1]	Similac Special Care with Iron®[2]	Enfamil Premature with Iron®[1]	Similac Special Care with Iron®[2]
Volume (*mL*)	148	148	124	124
Protein (*g*)	3.0	2.7	3.0	2.7
Fat (*g*)	5.1	5.4	5.1	5.4
Linoleic acid (*mg*)	1060	700	1060	700
Carbohydrate (*g*)	11.1	10.6	11.1	10.6
Vitamin A (*IU*)	1250	1250	1250	1250
Vitamin D (*IU*)	270	150	270	150
Vitamin E (*mg*)	6.3	4.0	6.3	4.0
Vitamin K (*μg*)	8	12	8	12
Thiamin (vitamin B_1) (*μg*)	200	250	200	250
Riboflavin (vitamin B_2) (*μg*)	300	620	300	620
Vitamin B_6 (*μg*)	150	250	150	250
Vitamin B_{12} (*μg*)	0.25	0.55	0.25	0.55
Niacin (*μg*)	4000	5000	4000	5000
Folic acid (folacin) (*μg*)	35	37	35	37
Pantothenic acid (*μg*)	1200	1900	1200	1900
Biotin (*μg*)	4	37	4	37
Vitamin C (*mg*)	20	37	20	37
Choline (*mg*)	12	10	12	10
Inositol (*mg*)	17	6	17	6
Calcium (*mg*)	165	180	165	180
Phosphorus (*mg*)	83	100	83	100
Magnesium (*mg*)	6.8	12.0	6.8	12.0
Iron (*mg*)	1.8	1.8	1.8	1.8
Zinc (*mg*)	1.5	1.5	1.5	1.5
Manganese (*μg*)	6.3	12	6.3	12
Copper (*μg*)	125	250	125	250
Iodine (*μg*)	25	6	25	6
Selenium (*μg*)	—[3]	1.8	—[3]	1.8
Sodium (*mg*)	39	43	39	43
Potassium (*mg*)	103	129	103	129
Chloride (*mg*)	85	81	85	81

[1] Used with permission of Mead Johnson & Company, Evansville, IN 47721. From Enfamil Family Products Handbook, Mead Johnson Nutritionals, Mead Johnson & Company. (Mead Johnson Nutritionals, 2000)

[2] Used with permission of Ross Products Division, Abbott Laboratories, Columbus, OH 43215. From Pediatric Nutritionals Guide, March 2001, Ross Products Division, Abbott Laboratories. (Abbott Laboratories.Ross Products Division, 1999).

[3] Actual quantities not reported.

et al. (1992), the AAP-CON (1998) suggested that preterm-LBW infants who have been discharged from the hospital might benefit from a formula that is enriched with nutrients at levels above those provided by term formula. However, specific nutritional recommendations based on evidence of requirement are not available for feeding preterm-LBW infants after discharge.

CONCLUSIONS

Many preterm-LBW infants require TPN for initial nutritional support. Frequently, the youngest GA infants with the lowest BW require a prolonged course of TPN. Gradually, preterm-LBW infants receive increasing amounts of enteral

TABLE 3-2

Selected compositional characteristics of preterm infant formulas

Characteristic	20 calories per fluid ounce (68 kcal/100 mL)		24 calories per fluid ounce (81 kcal/100 mL)	
	Enfamil Premature with Iron®[1]	Similac Special Care with Iron®[2]	Enfamil Premature with Iron®[1]	Similac Special Care with Iron®[2]
Energy (*kcal/100 mL*)	68	68	81	81
Water (*g/100 kcal*)	133	133	108	109
Osmolality (*mOsm/kg* water)	260	235	310	280
Osmolarity (*mOsm/L*)	230	211	270	246

[1] Used with permission of Mead Johnson & Company, Evansville, IN 47721. From Enfamil Family Products Handbook, Mead Johnson Nutritionals, Mead Johnson & Company. (Mead Johnson Nutritionals, 2000).

[2] Used with permission of Ross Products Division, Abbott Laboratories, Columbus, OH 43215. From Pediatric Nutritionals Guide, March 2001, Ross Products Division, Abbott Laboratories. (Abbott Laboratories.Ross Products Division, 1999).

feedings until their total nutritional support is via tube feeding. When preterm-LBW infants are approximately 33 weeks of postconceptional age, they may be capable of beginning oral feeding and prepare for the transition home.

When available, mothers' preterm milk is a component of the diet of preterm-LBW infants. Because human preterm milk as the sole source of nutrients is deficient in several essential nutrients, especially protein and minerals, the Expert Panel concluded that the most appropriate source of essential nutrients is fortified preterm milk of documented nutritional composition. However, because the quantities of mothers' preterm milk are unreliable and often inadequate, formulas designed to meet the requirements of preterm-LBW infants can serve as a more uniform and adequate source of nutrients to support optimal growth.

FUTURE RESEARCH

The presently available enteral preterm formulas in the United States use cow milk protein. Some preterm-LBW infants develop an allergy to the milk protein used in preterm formula. At present, there are no alternative enteral products designed to meet the nutritional needs of these children. The type of hydrolysate or elemental amino acid formulation that would be best tolerated, absorbed, and utilized is yet to be determined. Research is needed to develop an adequate alternative to formula containing cow milk protein.

Additional definitive data are required concerning the impact of specific components of formula (e.g., glutamine) on outcomes such as morbidity, growth, and length of time in the hospital.

Crucial data should be obtained regarding the nutritional requirements of preterm-LBW infants from the time of discharge to their first birthday, including possible differences between males and females and differences related to ethnicity. The effects of various intermediary formulas fed after discharge should also be definitively assessed.

4. WEIGHT GAIN

Weight gain is one of the main indices of growth of preterm-low birth weight (LBW) infants while they are under medical supervision (American Academy of Pediatrics. Committee on Fetus and Newborn, 1998; Katrine, 2000; Napp & Fink, 2000). A sustained pattern of weight gain is associated with the adequacy of formula to meet the nutritional requirements of preterm infants and to promote improved general health. Therefore, weight gain is central to the charge of the Expert Panel to evaluate studies of LBW infants. In this chapter, we review data and concepts supporting various weight standards that may be useful in the evaluation of the components and composition of infant formula. Among the additional factors that need to be considered are the utility of

an "ideal preterm infant" in terms of gestational age (GA) and birth weight (BW) and the validity and reliability of the data used to establish a target growth rate (in terms of both body composition and body mass) for this very heterogeneous population. This chapter identifies and reviews the key issues concerning growth that formed the basis of the Expert Panel's deliberations and recommendations related to the nutrient composition of preterm infant formula.

COMPOSITION OF WEIGHT GAIN

Putet et al. (1993) suggested that knowledge of the rate of growth was insufficient to establish a diet for preterm-LBW infants without additional knowledge of the composition of that weight gain. They reasoned that optimal growth would increase lean body mass as well as fat stores and that there is probably an optimum energy-to-protein ratio above which an increase in energy intake would result in mainly fat storage.

The historical references for the composition of fetal and newborn term weight gain were the seminal studies of Fomon (1967) and Ziegler et al. (1976). The data from these studies are summarized in Table 4-1.

The standards for optimal postnatal growth by premature infants have historically been the in utero rates of increase in weight, length, and head circumference by fetuses of the same postconceptional age (Putet, 1993). A more rigorous standard would include both the rate of growth and the composition of the weight gained (American Academy of Pediatrics. Committee on Nutrition, 1998). However, a combined standard of weight and body composition for preterm-LBW infants has not been identified and would be difficult to develop on the basis of present knowledge. For example, preterm-LBW infants weighing about 1100 g who are gaining weight at a rate approaching that of a fetus of the same GA (about 29 weeks) compartmentalize 32% of that gain to fat when fed commercially available preterm infant formula (protein 1.5–1.8 g/100 mL, fat 3.6–4.3 g/100 mL) (Reichman et al., 1981). This percentage is three times the proportion of fat in weight gained by the comparable fetus [11% at 28–32 weeks (Ziegler et al., 1976)], and it approaches the proportion of fat gained by the term infant of 16 weeks of age (1967) (Table 4-1). However, it is not clear that this is undesirable, because rapid fat deposition may be an important adaptation to extrauterine life (Putet, 1993). Putet et al. (1993) suggested that a fat retention of 20–25% of weight gain may be a reasonable goal for a growing preterm-LBW infant.

ASSESSMENT OF POSTNATAL GROWTH

The most commonly used and practical method to assess growth and well-being of preterm infants is weight gain, because weight can be measured accurately, rapidly, frequently, and without expensive, sophisticated instrumentation (Katrine, 2000;

TABLE 4-1

Variations in body composition as a percentage of weight gained during different postconceptional time periods

Component of weight gained	Gestational age of fetus (Ziegler et al., 1976)				Age of infant (Fomon, 1967)
	24–28 wk	28–32 wk	32–36 wk	36–40 wk	Birth to 16 wk
Protein (%)	11	12	13	14	11
Fat (%)	8	11	14	20	41
Other: e.g., water, minerals (%)	81	77	73	66	45

Napp & Fink, 2000). However, observed extrauterine growth rates of preterm infants with uncomplicated clinical courses may not be appropriate for use as a general standard against which to measure growth because the optimal nutritional intake for these infants is not well documented.

The American Academy of Pediatrics' *Pediatric Nutrition Handbook* (American Academy of Pediatrics. Committee on Nutrition, 1998) recommended that preterm infants be provided nutrients in amounts sufficient to allow growth similar to that of a fetus of comparable postconceptional age. However, difficulties in feeding newborn preterm-LBW infants, coupled with some loss of extracellular fluid (Van der Wagen et al., 1985), cause infants to lose weight during the first few days of life (Brosius et al., 1984; Dancis et al., 1948; Shaffer et al., 1987b). During this early, critical period of an infant's life, metabolic stability rather than rate of weight gain is of primary clinical concern. Even without complications, these infants do not regain BW until about 2 weeks of age (Ehrenkranz et al., 1999; Hack et al., 1991), so their growth lags behind that of a "normal" fetus of the same postconceptional age, even if they were appropriate for gestational age (AGA) at birth (Wright et al., 1993). The first few postnatal weeks may also be critical for brain development (Heird & Wu, 1996), so that lag periods in growth should be minimized. However, there seems to be no direct evidence as to what extent catch-up growth is important in this regard. Many preterm infants are also born small for gestational age (SGA), so there is an additional need for enough catch-up growth to bring them to the size of a normal fetus of comparable GA. The amount of catch-up growth required is the difference between the AGA fetal weight and the BW observed, plus the growth deficit incurred neonatally (Forbes, 1974). Clinical factors can also affect growth. For example, in a randomized double-blinded controlled trial to test the effects of a 12-day course of early steroid therapy on chronic lung disease, those preterm-LBW infants receiving treatment had poor weight gain compared to those not receiving treatment (Anonymous, 2001). Kuschel and Harding (1999b) verified that preterm-LBW infants who are critically ill (e.g., BPD, NEC, unable to consume more than one mL of milk every four hours at seven days of age) regained body weight significantly slower than less ill infants. Lemons et al. (2001) determined that 95-97% of infants born at 1001-1500 g weighed less than the 10th percentile for expected weights at 36 weeks postmenstrual age based on the fetal growth curve described by Alexander et al. (1996).

The preterm-LBW infant typically weighs 1800–2000 g when discharged from the hospital (Cruz et al., 1997); however, the clinical decision to discharge a patient is not based on attainment of a specific weight but does consider whether the infant has a sustained pattern of weight gain (American Academy of Pediatrics. Committee on Fetus and Newborn, 1998). Although a delay of 2–4 weeks in maturation might appear very significant at 2000 g or less, it should become less important as the months pass on and should be negligible by 1 year or more of postnatal age. Casey et al. (1990) followed preterm-LBW infants from the 40th postconceptional week to 1 year later and found that these infants had a pattern of growth that was lower than published standards (National Center for Health Statistics percentiles)(Hamill et al., 1979) for term infants of the same age and sex. Moreover, after 1 year, full catch-up growth had not been achieved. A group of preterm infants who weighed less than 1500 g at birth but were AGA and who survived were examined at intervals by Ross et al. (1990). These infants were smaller than their term peers in height and weight, although not in head circumference, at 1 and 3 years of age. However, they did not differ from their term peers in any growth parameter at 7 and 8 years of age. This was not true for infants born SGA, whose somatic measures at the age of 7 years were lower than those of infants born AGA (Ounsted et al., 1984). There is some evidence to suggest that metabolic bone disease during the neonatal period may be associated with shorter stature up to 12 years of age (Fewtrell et al., 2000).

Fetal growth is used as the primary standard for evaluating growth of preterm-LBW infants (American Academy of Pediatrics. Committee on Nutrition, 1998; Katrine, 2000). Data used to construct fetal growth curves are obtained from cross-sectional studies of preterm BWs and from ultrasound studies of fetal weight gain in utero. The current limit of viability for preterm infants is considered to be approximately 24 weeks of gestation (Hoffman et al., 1974). The Expert Panel focused on studies of fetal growth during the third trimester because this represents the period most relevant to the charge of the panel.

ASSESSMENT OF INTRAUTERINE GROWTH RATE

Birth weight at various gestational ages

Cross-sectional studies of BW at different GAs have been used to estimate fetal weight gain, although such studies have certain disadvantages. Body weight can be reliably measured; however, the results of cross-sectional studies are easily confounded by errors in establishing the GA at birth or when the conceptus was spontaneously aborted (Sparks, 1984). The estimation of GA is often in error because of bleeding in the first trimester, which can be mistaken for a menstrual period (Battaglia et al., 1966; Berg & Bracken, 1992; Naeye & Dixon, 1978). Such an error would result in the calculation of an erroneously low GA and, therefore, an erroneously high ratio of fetal weight to GA.

Another problem associated with the use of BW and GA to determine intrauterine growth is that infants delivered at less than 37 weeks of gestation may not be normal (Persson & Weldner, 1986). They may be smaller than in utero fetuses of the same age because a large proportion of preterm deliveries occur after complications of pregnancy (Greisen, 1992). This concept is supported by evidence that growth charts based on data from selected low-risk pregnancies show higher weights for GA than those based on the general population (Larsen et al., 1990; Ulrich, 1982). According to Greisen (1992), infants born at 28 weeks of gestation weigh nearly 10% less than fetuses of that GA who remain in utero. This indicates that the use of charts of optimal fetal weight would result in approximately 40% of the infants who are born very preterm in being (correctly) classified as SGA.

Sparks (1984) summarized previously reported cross-sectional data in an effort to evaluate fetal growth during the third trimester. Most of the studies he examined included singleton infants born alive after spontaneous premature labor, as indicated by postmenstrual dates. Even though these study populations were diverse in ethnicity, varied in socioeconomic status, and disparate in environmental conditions (altitude, maternal nutrition), several common features emerged, as follows:

- Weight curves were a sigmoidal, curvilinearly increasing function of GA, declining abruptly near term
- Growth was nearly linear between 24 and 37–39 weeks
- Differences in infant weights among these studies were small, typically less than 10% for a given percentile at a given GA
- Nearly all the studies recorded a median or 50th percentile fetal weight of 1000–1050 g at the 27th week of gestation

Sparks (1984) concluded that for those infants who were most frequently of perinatal clinical concern (GA of 24–37

weeks), fetal growth could be approximated by nearly linear, simple exponential curves.

One of the seminal works used to estimate fetal weight gain was by Lubchenco et al. (1963) nearly three decades ago, in which the BWs of low-income infants in Denver, Colorado, were graphed as percentiles. Not only was this report the most often cited of its era (Goldenberg et al., 1989) but the data for the 10th percentile line were adopted by the American Academy of Pediatrics' Committee on the Fetus and Newborn (1967) as the standard for intrauterine growth retardation. The report by Lubchenco et al. (1963) appears to be the origin of the frequently cited but rarely referenced statement that the third-trimester fetus normally gains 15 g/(kg·d) (Bremer et al., 1987; Waterlow, 1988). Using the data of Lubchenco et al. (1963), Sparks (1984) calculated a weight gain of 1.5% of body weight per day, or 15 g/d for a 1000-g fetus. The best-fitting rate for the data of Lubchenco et al. (1963) is presented in Table 4-2. Regression analysis was used to fit linear and

exponential curves to the growth data in all studies cited when possible.

Despite widespread use of the growth curve generated by Lubchenco et al. (1963), the generality of the results is attenuated by the altitude at which the fetuses in the study were gestated (>1600 m). An inverse relationship between fetal weight and altitude is well documented (Lichty et al., 1957; Unger et al., 1988). Moreover, Battaglia et al. (1966) reported that the weights reported by Lubchenco et al. (1963) in Denver were lower and less linear (closer to an exponential curve) than values for median weight for GA in Baltimore and New York City. In particular, the weights reported by Lubchenco et al. (1963) for about the 30th week to the 36th–37th week were markedly lower compared with those from other cities. However, the intrauterine growth rate calculated for the data of Naeye and Dixon (1978) was similar to the rate calculated for the Denver data of Lubchenco et al. (1963) (Table 4-2). Naeye and Dixon (1978), like Hardy et al.

TABLE 4-2

Characteristics of selected studies used to estimate intrauterine fetal growth rate

Reference	Type[1]	n	Site	Race or ethnicity and sex	Weight (g) at 27 wk	Weight (g) at 34 wk	Rate of weight gain at 27–28 wk (g/d)	Rate of weight gain at 33–34 wk (g/d)	Rate of weight gain at 27–34 wk [g/(kg · d)]
Lubchenco et al. (1963) Pediatrics 32: 793–800	BW	5635	US	70% C 30% H	1045	2200	15.0	40.0	14.9
Babson et al. (1970) Pediatrics 45: 937–944	BW	40,000	US	95% C 5% O	1022	2298	13.7	29.0	17.1
Hoffman et al. (1974) Obstet. Gynecol. Surv. 29: 651–681	BW	1,164,871	US	7% AM 7% AF 44% CM 41% CF	1484 1605 1260 1251	2819 2725 2698 2628	11.4 20.9 18.9 24.9	23.0 17.4 29.0 32.1	13.7 11.2 15.7 15.4
Brenner et al. (1976) Am. J. Obstet. Gynecol. 126: 555–564	BW	30,772	US	51% C 49% O	990	2220	22.9	30.0	16.2
Naeye & Dixon (1978) Pediatr. Res. 12: 987–991	BW	48,239	US	46% A 46% C 8% O	—[2]	2160	—[2]	29.3	14.7
Arbuckle et al. (1993) Obstet. Gynecol. 81: 39–48	BW	1,087,629	CA	~91% C ~9% O	~1025 M ~975 F	~2350M ~2285 F	—[2]	—[2]	16.3M 16.9 F
Alexander et al. (1996) Obstet. Gynecol. 87: 163–168	BW	3,134,879	US	—[2]	1035	2667	23.0	29.9	20.0
Hadlock (1991) Radiology 181: 129–133	FW	392	US	100% C	1055	2377	22.1	30.7	16.6
Ott (1993) Am. J. Obstet. Gynecol. 168: 1710–1717	FW	1583	US	—[2]	1112	2322	20.9	28.3	15.0
Gallivan et al. (1993) Ultrasound Obstet. Gynecol. 3: 109–114	FW	70	UK	100% C	1096	2445	22.9	30.3	16.4

[1] BW, birth weight; FW, fetal weight measured by ultrasonography; US, United States; CA, Canada; UK, United Kingdom; C, Caucasian; H, Hispanic and Native Indian; A, African-American; O, other races; M, male; F, female; weeks are weeks of gestation. Weight and weight gain in g/d are from reported 50th percentile values; weight gain from 27 to 34 wk is from exponential regression of reported 50th percentile values; weight and weight gain values at various GAs were derived from subsets of the total number of subjects.

[2] Data not reported.

(1979), analyzed data collected between 1959 and 1966 from 12 hospitals in the United States, but only Naeye and Dixon (1978) included the BWs of infants who survived the first year and who did not suffer from any disorder that could have caused fetal growth retardation. Naeye and Dixon (1978) found that before 38 weeks of GA, BWs tended to cluster at intervals consistent with monthly vaginal bleeding during early pregnancy. This suggested that such bleeding was sometimes misinterpreted as a menstrual period by the pregnant woman, and the GA had been erroneously dated from that bleeding episode. To address this bias, Naeye and Dixon (1978) used only the lowest weight cluster at each GA as the database for that age, on the assumption that only this cluster represented cases without postconceptional vaginal bleeding. Therefore, BWs were lower than if this correction had not been made. Often, low growth rates were associated with high calculated or observed weights at the starting point, which was not the case in the study by Naeye and Dixon (1978). In this report, the 50th percentile and mean weights were exactly the same, to the gram, for all GAs from 28 to 38 weeks, which is not plausible for this large collection of births.

Brenner et al. (1976) surveyed births in Cleveland between 1962 and 1969. BWs were recorded for infants (from uncomplicated singleton pregnancies) who were alive at the onset of labor in the 21st to the 44th week of gestation. The rate of increase in fetal growth for the 50th percentile during weeks 26–35 was similar to mean values observed by Usher and McLean (1969) (percentile values were not reported) and calculated by Sparks (1984), at about 1.6% per day. The results of Brenner et al. (1976) corroborated the results of the earlier study by Hendricks (1964), who estimated the mean daily growth increment to be 1.6–1.8% between 22 and 34 weeks of gestation, by using an earlier (1956–1962) 11,000-birth sample from the same hospital (percentile values were not reported). In addition, the results are consistent with the data of Babson et al. (1970), whose study included 723 births in the 27th to the 34th week of GA.

Hoffman et al. (1974) started with a 50% sample of all live birth records in the United States from 1968 provided by the National Center for Health Statistics, to which they applied a number of exclusion criteria. They limited their analysis to singleton live births by Caucasian or African-American mothers in the District of Columbia and the 36 states that at that time recorded GA. Hoffman et al. (1974) and Hardy et al. (1979) did not reject implausible weights for GA from their raw data. The inclusion of the outliers produced a disproportionately large effect on weight percentiles at early GAs, at which the number of liveborn infants was limited (Alexander et al., 1996; Gruenwald, 1966; Milner & Richards, 1974; Naeye & Dixon, 1978). For example, Hoffman et al. (1974) reported a 90th percentile BW of more than 3500 g for Caucasian infants born at the 20th week, which was higher than the 90th percentile observed in this same data set at the 31st week. The weights reported by Hoffman et al. (1974) for the 50th percentile in the 27th week were higher than those in other studies (Table 4-2). The inclusion of outliers at early ages could raise the weight percentiles and decrease the apparent rate of subsequent weight gain. This probably contributes to the reason why the calculated exponential rate for growth after the 27th week for the data of Hoffman et al. (1974) is low. The cross-sectional weight percentiles in the study by Hardy et al. (1979) were not useful for estimating fetal weight gain because mean BWs for short gestations were too high to be credible and because of the large variability in BWs at different GAs. Evidently, the menstrual age data were not reliable.

The growth rates for fetuses of GAs 28-34 weeks that can be calculated from the data of Milner and Richards (1974) in the United Kingdom, who purged data of outliers, were lower than rates calculated from the data of Hoffman et al. (1974). This apparent slow growth was the result of an unusually high 50th percentile weight for infants at the 28th week (1360 g for males; 1330 g for females).

A more recent study of BWs and GA was published by Alexander et al. (1996), who used data from 82% of the singleton live births to residents of the United States in 1991. Like most of their predecessors, these researchers eliminated all combinations of BW and GA that could not be encompassed in a unimodal curve or were otherwise unrealistic yet reported high weights for GA. Alexander et al. (1996) calculated smoothed percentiles of BW for GA using the method of Himes and Hoaglin (1989), whereas most other studies used curve-smoothing techniques that were neither specified nor referenced. The rate of weight gain at the 50th percentile for the GA of 24–27 weeks was approximately 17 g/d. Between 27 and 34 weeks, the growth curve for the 50th percentile was approximately a straight line with a slope yielding a weight gain of 34.8 g/d. The weight-based [g/(kg·d)] rate of growth between 27 and 34 weeks GA is 34% higher than the growth rate obtained from the data of Lubchenco et al. (1963) (Table 4-2), whose growth curve is in present clinical use for preterm-LBW infants (Katrine, 2000). In fact, rates of growth from the data of Alexander et al. (1996) were higher than those from nearly all previous reports of infants of 27–34 weeks GA.

Data from Arbuckle et al. (1993) resulted in a linear rate of weight gain of 26.2 g/d for males and 25.9 g/d for females for a similar GA (27–34 weeks). These values are nearly 10 g/d lower (~30%) than values from the data of Alexander et al. (1996) (34.8 g/d).

Data from Wong and Scott (1972) suggested fetal growth rates of 32.2 g/d, averaged for males and females. However, this study included only 437 Canadian infants of 30–36 weeks GA born near sea level. Median and percentile values were not provided.

In Figure 4-1, fetal growth rates from the data of Alexander et al. (1996) are compared with the lower rates of fetal growth from the data of Lubchenco et al. (1963).
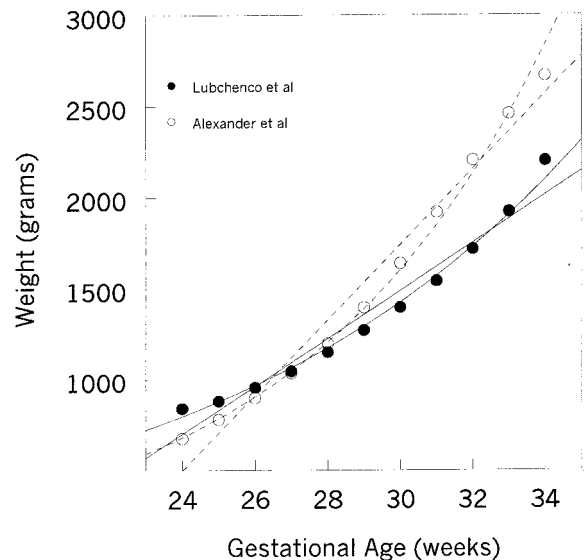
FIGURE 4-1 Linear (g/d) and exponential rates [g/(kg·d)] of fetal growth calculated from the data (50th percentile) of Alexander et al. (1996) and Lubchenco et al. (1963), showing a wide discrepancy between data from the two studies.

Except for data from Hoffman et al. (1974), Milner and Richards (1974), and Naeye and Dixon (1978), results from cross-sectional BW studies have indicated that exponential growth rates are higher than the 14.9 g/(kg·d) rate derived from the work of Lubchenco et al. (1963).

Data from cross-sectional BW studies indicate that an intrauterine weight gain of approximately 16–17 g/(kg·d) between 27 and 34 weeks GA may be a reasonable standard against which the effect of infant formula on extrauterine growth of preterm-LBW infants can be evaluated.

Ultrasound studies of fetal weight gain

Certain disadvantages of using the cross-sectional studies of BW to estimate fetal weight gain could be avoided by longitudinal studies in which the growth of the fetus is followed in utero. The only currently available noninvasive method for longitudinal study of fetal growth is ultrasonography. By this method, weight is inferred from the measured dimensions of various body parts (Hadlock, 1990) or calculated from volume on the basis of assumptions about body volume and density (Combs et al., 1993; Deter et al., 1984). Although certain disadvantages of using BW to estimate fetal weight gain are avoided by ultrasonography, this method is not without bias. Significant errors in the independent (GA) and dependent (growth) variables have occurred, which underscores the difficulty in using ultrasonography (Scott et al., 1996). In addition, ultrasonography has had limited use in research protocols performed in the United States and is impractical and expensive to apply to a large population. In other countries, however, ultrasonographic methods have been used more extensively to estimate fetal weight in the third trimester. Relatively few ultrasound studies have repeatedly measured the same fetus more than a few times, so these studies are primarily composed of cross-sectional data or use regression analysis to estimate fetal weight at the GA of interest. Studies in which both GA and fetal weight are determined ultrasonographically should be internally consistent.

In ultrasound studies, methods other than menstrual data are typically used to estimate GA. Therefore, data from these studies are often not comparable to data collected in older BW studies, which typically used the date of the last menstrual period to estimate fetal age. Secher et al. (1986) agreed with Warsof et al. (1983) that GA is more reliably determined by ultrasound studies than from menstrual data.

Nevertheless, changing the method of determining GA makes it awkward to compare the weight gains measured in the ultrasound studies to the preterm BW data. Fetal weights from the BW data are lower than those from the ultrasound studies for the same putative GA. In addition, it was reported that smaller fetuses grow more slowly in the third trimester (Gallivan et al., 1993).

Although some reports of ultrasound studies are not useful for establishing a reference curve of fetal weight gain because they lack raw data or age-dependent medians, other investigators have applied polynomial formulas to calculate expected fetal weights at various weeks of gestation. For example, in the Swedish study of Persson and Weldner (1986), ultrasound measures were obtained from 89 pregnant women within 48 hours of delivery or abortion; fetal weights were stratified by weight class from a minimum of 500 g to a maximum of 5000 g. An additional group of ultrasound measures of 19 normal fetuses was obtained an average of nine times each during pregnancy. From the data obtained, Persson and Weldner (1986) derived a third-degree polynomial equation to estimate intrauterine weight; 50th percentile values were not

reported. When applied to 27–34 weeks GA, this equation yields a rate of growth of 24.9 g/d.

Mongelli and Gardosi (1995) also generated an equation to predict fetal weight. Their longitudinal study included 226 pregnancies in a group of U. K. women of whom 95% were European. By applying their formula, an average growth rate of 27.9 g/d was calculated for 27–34 weeks GA (1021–2388 g); 50th percentile values were not reported.

Larsen et al. (1990) studied 35 Danish low-risk pregnancies four or more times each from the 19th week of gestation until spontaneous delivery and made an average of 8.7 sonographic measurements on each fetus. Weight gains "showed only insignificant non-linearity" after the 27th week, as calculated from a polynomial regression equation. Larsen et al. (1990) determined GA by both menstrual dates and sonographic criteria, but the extent of disagreement between the methods was not specified. The sonographic results apparently were used to compute the growth rates, which yielded 28.4 g/d for 22 males and 27.3 g/d for 13 females between 27 and 34 weeks GA.

Hadlock et al. (1991) observed, for predominantly middle-class women in Texas, an in utero rate of growth of fetuses that was similar to the rates derived from several of the cross-sectional BW studies (Table 4-2).

Gallivan et al. (1993) limited their study to Caucasian fetuses to avoid the ethnic and racial differences known to occur in the London clinic population (Alvear & Brooke, 1978). In addition, they excluded measures from women in whom sonographic estimations of GA were more than 7 days different from the menstrual dates. Their results suggested that growth was approximately linear for fetuses in the third trimester up to 36 weeks of gestation. Although the linear model produced wider 95% confidence limits than did more complex formulations, their data were consistent with a 50th percentile growth rate of 27.7 g/d from the 27th to 34th week. The exponential rate of fetal growth obtained from the data by Gallivan et al. (1993) was near that obtained from the data of Hadlock et al. (1991), and these rates were virtually identical to the rate that emerged from the cross-sectional data (Table 4-2).

Ott (1993) obtained BWs of 5757 infants born alive to predominantly middle- and upper-class women in suburban St. Louis, Missouri. Among these infants, 1583 were entered into an ultrasound study of fetal weight, with two examinations each (Table 4-2). Fetal weights estimated by ultrasonography ranged from 75 to 83 g higher for the 50th percentile than 50th percentile values derived from actual weight measures of live births; the difference between methods diminished from the 28th to the 34th week. Nevertheless, the rate of weight gain was nearly identical during this period, regardless of which method was used. These data were consistent with a fetal growth rate of 15.0 g/(kg·d) obtained from mean fetal weight measured in an earlier study (Jeanty et al., 1984a) of Caucasian middle-class women in Brussels that estimated fetal age from menstrual data (Jeanty et al., 1984b); 50th percentile values were not reported.

A longitudinal study, which reported rates of fetal weight gains (not fetal weights) lower than those in most other publications, was conducted in Dundee, Scotland, by Owen et al. (1996). Low-risk pregnancies of 274 women were followed with seven or eight scans each over 28-day intervals; 50th percentile values were not reported. Overall, however, fetal weight gains were approximated by a linear rate of 23 g/d or an exponential rate of 15.4 g/(kg·d), nearly as low as the growth rate described by Lubchenco et al. (1963) more than 30 years earlier. Unlike the study by Lubchenco et al. (1963), high altitude was not a factor. No explanation was apparent for this

low rate of weight gain, as the women lacked the known risk factors associated with delivering LBW infants.

De Jong et al. (1998) longitudinally followed 121 Dutch women with high-risk pregnancies that had a favorable outcome. Menstrual dates were used to ascertain GA, unless there was more than a 7-day difference from dates by ultrasound scans. The published graphs of growth appear to be linear from the 27th to the 36th week, but they lack detail. However, these investigators provided numerical data for the 30th and 34th week, which indicated an average weight gain of 27.7 g/d during that period, identical to the rate obtained with data from the study by Gallivan et al. (1993) for GA 27–34 weeks.

There are several limitations to using the fetal weight ultrasound data. First, few studies have reported data on fetuses in the United States (1991; Ott, 1993). Of the remaining available studies, those of Owen (1996) and Jeanty et al. (1984a) reported mean estimated weights rather than 50th percentile values.

Overall, an exponential rate of intrauterine weight gain of 15.0–16.6 g/(kg·d) was calculated from ultrasound studies, slightly lower than that calculated from BW studies (Table 4-2). This was because fetal weights estimated by ultrasonography at 27 weeks are higher than observed BWs at that GA [see Ott (1993)], probably for the reasons already detailed. Fetal weights determined by ultrasonography and BW measurements must converge to the same median value at term; therefore, the ultrasonographically determined growth rate must be slower.

ADDITIONAL CONSIDERATIONS

Sex differences

Among the reports of BW data, those of Freeman et al. (1970), Hoffman et al. (1974), Wong and Scott (1972), Milner and Richards (1974), and Arbuckle et al. (1993) provided separate values for males and females. An additional study, by Larson et al. (1990), provided fetal weight percentiles by gender. The first three studies reported that preterm females were heavier than preterm males; in contrast, the latter studies found the reverse. However, Freeman et al. (1970) also found that Caucasian females were smaller than males at 27 weeks but grew faster, whereas African-American females were larger than African-American males but grew slower. The sex differences for weight in both races were less than 5% after the 29th week. Likewise, Milner and Richards (1974), in the United Kingdom, found the difference in weight between sexes was approximately 4% for the 50th percentile in the third trimester; male infants were heavier. In the Canadian study by Arbuckle et al. (1993), males were 3–7% larger than females between the 27th and 34th weeks, the larger differences occurring most frequently for the younger GA infants. In the longitudinal study of Larsen et al. (1990), weights calculated from the 27th week until birth for males at the 50th percentile were 4% higher than those for females.

Therefore, in the absence of definitive data, specifying separate preterm infant growth rates for each sex cannot be justified.

Racial differences

Whether there should be separate standards for weight gain for African-American and Caucasian infants born preterm is not clear from the literature. Lubchenco et al. (1963) noted a difference in weights for African-American and Caucasian infants, and although Hoffman et al. (1974) and Hardy et al. (1979) separated racial data, neither provided enough information to answer this question adequately. The results of these

analyses were interpreted as indicating a racial difference in the BWs of preterm infants in the gestational range of 27–34 weeks. African-American infants had higher weights at 27 weeks, yet at 36 weeks or later they weighed less than Caucasian infants of the same GA. After 27 weeks GA, African-American fetuses apparently had a slower rate of weight gain (Table 4-2). Hardy et al. (1979) ascribed this effect to a near-term fall off of weight gain at an earlier GA by African-American fetuses, whose length of gestation averaged 1.3 weeks shorter than that of Caucasian fetuses. The high weights and the subsequently slower rates of weight gain could also have resulted from the inclusion of some preterm infants for whom GA was implausibly short. As might be expected, the differences between the 50th percentile weights in Hoffman et al. (1974) and weights in those studies that were purged of extreme values become smaller closer to term, because errors in GA become proportionally less significant as gestation lengthens and fetal weight gain levels off near term.

At present, the only appropriate choice is to develop reference standards that will allow the growth of an African-American premature infant of whatever BW to keep pace with the 50th percentile growth rate of Caucasian infants of the same GA. This in some cases may lead to an unwarranted concern regarding "abnormal" weight gain rates of African-American infants.

SUMMARY

Fetal weight gain from the 27th to the 34th week conforms well to simple exponential curves (Sparks, 1984), or even to straight lines (Dunn, 1985; Larsen et al., 1990). Therefore, polynomial equations with multiple terms to describe the slopes of the fetal growth curve, although available (Persson & Weldner, 1986; Larsen et al., 1990) are unnecessary.

For fetuses who were appropriate for GA (AGA; approximately the 50th percentile), it mattered little whether an exponential [g/(kg·d)] or linear (g/d) growth curve was assumed for intrauterine growth for the 27th to 34th week. The predictions did not differ sufficiently to have clinical significance. However, fetuses who are SGA gain less total weight than those who are AGA (Ehrenkranz et al., 1999; Gallivan et al., 1993). For example, an 850-g fetus at 27 weeks GA will by the 34th week weigh 311 g less than a 1000-g (AGA) fetus at the same GA, if both are growing at 15 g/(kg·d); the difference in their weights will have doubled in 7 weeks. Therefore, the use of one linear daily rate (g/d) of intrauterine fetal growth to evaluate the adequacy of infant formula could lead to the conclusion that a formula is not adequate for the smaller, younger infant; whereas, the use of a body weight-based exponential rate [g/(kg·d)] could result in the conclusion that growth was adequate. However, a linear growth expectation (g/d) could be satisfactory, and perhaps slightly more convenient, when following any premature infant who is not SGA for the purpose of evaluating infant formula.

Nevertheless, the question emerges of whether a growth rate of 15 g/(kg·d), which has been used as a minimum growth standard, is adequate. Neonatal undernutrition is hazardous to both physical and mental development. On the other hand, overfeeding is also problematic. The linear and exponential growth rates of 34.8 g/d and 20.0 g/(kg·d) calculated from the data of Alexander et al. (1996) were much higher than rates calculated from earlier and smaller studies (Table 4-2). Although no major flaw in that report has been identified, the adoption of those rates would result in a drastic change in the growth standard, requiring major alterations in feeding practices. Certainly, the risk of overfeeding preterm-LBW infants

would increase, as would the incidence of adverse effects. Arbuckle et al. (1993) proposed changing the growth standard only gradually and revising norms of weight for age every 5–10 years, and this seems to be a prudent course.

The adoption of an exponential rate slightly higher than the reference growth rates used at present would have several advantages. It would change the weight gain standard toward the higher rates found in the most recent data, while probably requiring only incremental alterations in current feeding practices.

Therefore, on the basis of both cross-sectional BW data and ultrasound fetal weight data, the Expert Panel concluded that intrauterine fetal weight gain is approximately 16–17 g/(kg·d) between 27 and 34 weeks GA. This growth rate is a reasonable standard to evaluate the adequacy of formula to meet the nutritional requirements of preterm-LBW infants of 1000 g or more in the period from 27 to 34 weeks postconceptional age. For example, for an exponential rate of 16.5 g/(kg·d), an infant born at 27 weeks, weighing 1000 g (approximate 50th percentile), would be expected to weigh approximately 2230 g 7 weeks later, at 34 weeks postconceptional age. Although daily rates of infant growth, on average, can approximate the rates typical for in utero fetuses, total body weight may be short of the expected intrauterine fetal weight for the same postconceptional age because of initial weight loss after birth. The Expert Panel recognizes that comparison of an infant's growth to intrauterine growth may be best accomplished by plotting averaged weekly weight for GA against a reference curve and tracking the pattern of weight gain over time.

Slower rates of weight gain are apparent from 24 to 27 weeks GA (Alexander et al., 1996), although the shape of the curve cannot be defined by only three points (weeks 25, 26, and 27). The best available data (Alexander et al., 1996) indicate that intrauterine fetal weight gain from the 24th to the 27th week and/or for fetuses weighing less than 1000 g is approximately 17 g/d.

FURTHER RESEARCH

Additional longitudinal ultrasound studies to measure in utero fetal growth in the United States would be of benefit for better estimation of intrauterine growth rate and for development of a clinically useful growth curve for plotting extrauterine infant progress beginning from the time of viability (23–24 weeks GA) until at least discharge from the hospital.

Whether a different postnatal growth rate should be expected for African-American premature infants than for Caucasian premature infants is an important question for further research, because the rate of infants of LBW is higher among African-Americans than among Caucasians (David & Collins, 1997; Hutchins et al., 1984). Certainly, collecting sufficient data to produce percentiles of BW for African-American preterm infants of established GA and collecting definitive data of growth of African-American fetuses would help establish better nutrient specifications for feeding LBW infants.

Additional knowledge is needed about the optimal composition of the body weight gained by preterm-LBW infants. For example, should the composition of the weight gained by growing preterm infants be compared to that of the growing fetus of the same postconceptional age? Furthermore, research is needed to determine how the composition of formula affects the composition of weight gained, particularly whether there is an optimum energy-to-protein ratio above which an increase in energy intake would result in mainly fat storage. To this end, research is needed to develop more accurate and easier to use measuring tools for composition of weight gain.

5. WATER AND POTENTIAL RENAL SOLUTE LOAD

BACKGROUND

Water-soluble waste products that require excretion by the kidneys are collectively referred to as the renal solute load (RSL). Under most circumstances, the renal solute is of dietary origin and is closely related to the nitrogen and electrolyte content of the diet. To excrete these products, a certain amount of water is required, and this affects the net balance of water. As the RSL increases or the available water for urine formation decreases, the kidneys must increase the solute concentration (osmolality) of the urine. The premature infant's ability to concentrate urine depends on the development of the kidneys and their physiological functions (see Appendix A).

Potential renal solute load (PRSL) refers to solutes of dietary origin that would need to be excreted in the urine if none were diverted into synthesis of new tissues and none were lost through extrarenal routes. Under most circumstances, urea, chloride, potassium, phosphorus, and sodium contribute more than 90% of the PRSL (Ziegler, 1991b). The following equation may be used to calculate an approximation of the PRSL that will result from an infant formula (Fomon & Ziegler, 1999):

$$PRSL = [N]/28 + Na + Cl + K + P_a$$

In this equation, the dietary intakes of solutes are expressed in millimoles (mmol, or milliosmoles, mOsm), [N] is milligrams of nitrogen, and the factor [N]/28 represents the excretion of nitrogenous substances as urea (urea contains two nitrogen atoms, atomic weight 14). Na is sodium, Cl is chloride, K is potassium, and P_a is available (nonphytate) phosphorus, which is the same as total phosphorus in milk-based formulas (Fomon & Ziegler, 1999). Nitrogen is usually calculated as 16% of the protein ingested (Fomon & Ziegler, 1993).

The PRSL of mature human milk is 14 mOsm/100 kcal (Fomon & Ziegler, 1993), whereas that of currently available premature infant formulas is approximately 26 mOsm/100 kcal (American Academy of Pediatrics. Committee on Nutrition, 1998). The Expert Panel on Assessment of Nutrient Requirements for Infant Formulas calculated that its maximum recommended levels of nutrients precluded a value for PRSL greater than 33 mOsm/100 kcal (Raiten et al., 1998a). By setting this as the maximum allowable level, the panel members signaled their conclusion that this load would not stress the renal concentrating ability of term neonates.

The Canadian Paediatric Society (1995) and Health Canada *Guidelines* (1995) have made recommendations regarding the PRSL load of premature infant formulas. They noted that the amounts of protein, sodium, potassium, chloride, and phosphorus in premature infant formulas need to be greater than those of term formulas in order to meet the recommended nutrient intakes for premature infants, and that it is important that the PRSL of these formulas not exceed the renal excretion capacity of low birth weight infants. On the basis of their recommended maximum amounts of protein and electrolytes, the maximum PRSL would be 28 mOsm/100 kcal.

Energy-dense formulas are recommended for premature infants because their tolerance for volume is low. These formulas are associated with relatively small fluid intakes, because the quantity consumed ad libitum or by the choice of caregivers is largely regulated by energy needs (Fomon & Ziegler, 1999). It is thus important to specify a maximum PRSL that is safe for formula-fed premature infants on a routine basis.

REVIEW OF THE LITERATURE

Concerns about the PRSL are based on epidemiological data indicating that term infants consuming formula with a PRSL of 39 mOsm/100 kcal were predisposed to hypertonic dehydration (Ziegler & Fomon, 1989). This information was discussed in detail when a maximum PRSL of 33 mOsm/100 kcal for term formula was recommended by the expert panel assessing nutrient requirements for term infant formulas (Raiten et al., 1998a). Although some smaller premature infants may be unable to concentrate urine above 600–700 mOsm/L early in life (see Appendix A), concern is reduced by knowledge of the substantial component of the PRSL required for newly formed tissues (Ziegler & Ryu, 1976). In fact, the rapidly growing premature infant incorporates a larger percentage of the PRSL into new tissue than does the term infant (Saigal & Sinclair, 1977). Brusilow (1991) noted that premature infants excrete on average less than 14% of their nitrogen intake, which is ordinarily more than half of the PRSL, as urea nitrogen when fed standard infant formula (Pencharz et al., 1977).

Ziegler and Ryu (1976) related the PRSL of premature infant formula to the actual RSL excreted by seven male infants between 32 and 37 weeks of gestational age (GA) who were fed 150 kcal/(kg·d) of preterm infant formula containing 80 kcal/100 mL. The PRSL values of the two different formulas fed these infants were 161 and 202 mOsm/L (20.1 and 25.3 mOsm/100 kcal), respectively. Urinary solute excretion in this study accounted for 37.8% of the PRSL, ranging from 31.8% to 50.1% while the infants were gaining 21–50 g/d, with a mean of 36 g/d (18 g/kg daily). The mean osmolality of the urine of these infants was 127 mOsm/kg water. The infants were relatively large and the sample size was small; however, there was a large margin of safety under these conditions. The investigators noted that a greater concentration of solute in the urine might be required if extrarenal fluid losses were to increase or if fluid intake were intentionally decreased (as for management of clinical problems such as chronic lung disease or patent ductus arteriosus).

In a larger and more complex study of 77 relatively large premature infants (mean birth weight of 1787 g, GA of 33 weeks) at 3 weeks of age fed human milk or a variety of formulas, De Curtis et al. (1990) confirmed that the true osmolar load of so-called adapted formulas is low for growing premature infants. Mean intakes with the formula were 39.1 mmol/(kg·d) of nitrogen and 1.8, 3.2, 2.3, and 2.3 mmol/(kg·d) of sodium, potassium, chloride, and phosphorus, respectively. The calculated PRSL for these values would be 29 mOsm/(kg·d), or 23.8 mOsm/100 kcal ingested at the observed intake of 122 kcal/(kg·d). The infants were observed to gain 15 g/(kg·d), implying uptake of an osmolar equivalent of approximately 13.5 mOsm/(kg·d) on the basis of assimilation of 0.9 mOsm solute/g of weight gained (De Curtis et al., 1990). This left only 15.5 mOsm/(kg·d) to be excreted. Urine volume was 140 mL/(kg·d), at an average osmolality of 143 mOsm/kg, or 20 mOsm/(kg·d), so 122% of the administered osmoles was accounted for. The mean urine osmolality of the formula-fed infants was higher than that of the human milk-fed control group (102 mOsm/kg), but it was far below a level that would stress an infant with normal kidneys.

DISCUSSION

The renal concentrating ability of premature infants is initially less than that of full-term infants and depends on the GA. Clinical observations suggest that any more severe limitation of the concentrating ability of premature infants weighing more than 1500 g compared with that of term infants (about 900 mOsm/L) would be transient. However, some infants weighing less than 1000 g may not be able to concentrate urine beyond 500–600 mOsm/L. Using the methods of Fomon and Ziegler (1993), the Expert Panel estimated the maximum PRSL for a 81 kcal/100 mL formula based on the water intake and water available for urine formation, as shown in Table 5-1.

This calculation of a PRSL limit is at a specific fluid intake and assumes high values for extrarenal losses (11 mL of fecal water loss and 63 mL of insensible water loss). Moreover, the calculation assumes no sequestration of solutes by growth, but a formula-fed premature infant receiving these intakes who fails to grow for more than a day or two after the first week of life is acutely or chronically ill and needs special management. Thus, the concatenation of circumstances postulated in the example is unlikely, yet possible, for it could occur if an ill premature infant failed to grow for 2–3 days yet continued to tolerate feedings, as is occasionally observed.

The PRSL can be calculated for both the maximum and minimum concentrations recommended. This is done in Table 5-2, which shows that the PRSL for a preterm formula might be as high as 35.4 mOsm/100 kcal if it contained the maximum level of nutrients recommended by the Expert Panel. Producing a formula of lower PRSL would require adding less of one or more of these components.

The Expert Panel observed that a PRSL upper limit of 32 mOsm/100 kcal would allow for substantial increases in the concentration of relevant components, such as sodium and protein, for which the recommendations in this report are higher than those in contemporary preterm formulas.

The minimum PRSL calculated (Table 5-2) from the minimum levels of its component nutrients recommended in this report is 22 mOsm/100 kcal. This number becomes important for recommending a maximum energy density for premature infant formula. For example, the actual water intake depends on caloric density because the volume consumed depends primarily on energy needs (Fomon & Ziegler, 1999). This means that a caloric density is too high if it produces energy

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TABLE 5-1

Estimation of maximum potential renal solute load based on available water for an 81 kcal/100 mL formula

- A formula of 81 kcal/100 mL provides 93% of its volume as water to meet metabolic needs and water losses. This is based on the water used in formulation plus the water produced from the metabolic oxidation of components of the formula (Fomon & Ziegler, 1993; 1989).
- Consumed at 120 kcal/(kg · d), the formula provides (0.93 × 120/0.81) = 138 mL/(kg · d) water.
- The maximum daily loss of water from the skin and lungs of a healthy term infant not exposed to extreme environmental conditions is 70 mL/(kg · d) (Fomon & Ziegler, 1993). In the absence of specific data on preterm infants, the Expert Panel assumed the maximum water loss by this route to be 90% of the maximum for term infants, or 63 mL/(kg · d) for a premature infant, leaving 75 mL/(kg · d) water available. This assumption significantly affects the estimate of available water.
- Fecal loss of water by formula-fed preterm infants is 8–11 mL/(kg · d) (Parmar et al., 1986), leaving 64 mL/(kg · d) available for urine formation.
- The great majority of preterm infants without renal disease can concentrate the urine to 600 mOsm/L (Chevalier, 1996). Therefore, a maximal potential renal solute load by this calculation (64 × 0.6/1.20) is 32 mOsm/100 kcal at 120 kcal/(kg · d).

satiety in premature infants before they have consumed enough water to provide for their insensible and fecal water losses and renal excretory requirements. In other words, the higher the energy density in formula, the lower the PRSL value should be because less water is consumed.

At the minimum PRSL achievable from the recommendations in this report [22 mOsm/100 kcal or 26.4 mOsm/(kg·d), assuming 120 kcal/d], excretion at the assumed urine concentration limit of 600 mOsm/L would require 44 mL/(kg·d) of water available for urine formation and 74 mL/(kg·d) of water available for extrarenal loss, or an intake of 118 mL/(kg·d) of water from 127 mL of formula (127 mL × 0.93 = 118 mL). This fixes the maximum acceptable energy density as 94 kcal/100 mL (120 kcal/127 mL = 0.945 kcal/mL).

All of the recommendations in this report are predicated based on the assumption that the caloric density of the administered formula would be 81 kcal/100 mL, at an average intake of 110–135 kcal/(kg·d). Higher caloric densities are possible and are sometimes used for preterm infants under close medical supervision, usually because intake of fluid is restricted. For example, Moro et al. (1984) fed a formula of 940 kcal/L, Roy et al. (1976) fed a formula of 1000 kcal/L and Friedman et al. (2000) fed formula of 1010 kcal/L. These studies are discussed in Chapter 6. The maximum tolerable energy density will depend on the water intake and RSL. Table 5-3 illustrates the relationship among these factors as calculated according to the methods of Fomon and Ziegler (1993) (see Table 5-1). No PRSL values are given for energy densities lower than 67 kcal/100 mL, because that value is the minimum energy density recommended for premature infant formula. No PRSL values are given for energy densities higher than 94 kcal/100 mL, because such densities would require a PRSL lower than 22 mOsm/100 kcal, the minimum attainable under the minimum content recommendations for relevant mineral solutes and protein.

Recommendations

Minimum. **The Expert Panel recommended that the minimum PRSL for preterm infant formula be set at 22 mOsm/ 100 kcal, resulting from the minimum recommended amounts of nutrients contributing to the PRSL.** This fixes the maximum acceptable energy density as 94 kcal/100 mL.

TABLE 5-2

Calculation of potential renal solute load resulting from the maximum and minimum nutrient contents recommended by the Expert Panel for preterm infant formula of 81 kcal/100 mL

Nutrient	Maximum content		Minimum content	
	mg/100 kcal	mOsm/100 kcal	mg/100 kcal	mOsm/100 kcal
Nitrogen (N)[1]	576	20.6	400	14.2
Sodium (Na)	63	2.7	39	1.7
Chloride (Cl)	160	4.5	60	1.7
Potassium (K)	160	4.1	60	1.5
Available Phosphorus (P)	109	3.5	84	2.7
Potential renal solute load		35.4[2]		21.8

[1] Calculated by multiplying recommended protein g/100 kcal by 160 mg N/g protein to obtain mg N/100 kcal, and then dividing by 28 mg N/mmol urea to obtain mOsm/100 kcal.

[2] Exceeds the recommended maximum potential renal solute load.

TABLE 5-3

Relationship of energy density of formula, water available for urine formation, and upper limit of potential renal solute load for formula, assuming no growth of preterm infant

Energy density (kcal/100 mL)	Formula volume [mL/(kg · d)]	Water for urine formation[1] [mL/(kg · d)]	PRSL upper limit[2] (mOsm/100 kcal)
67	179	92	46[3]
74	162	77	38[3]
81	148	64	32
87	137	53	27
94	127	44	22

[1] (Formula volume × 0.93) − 74 mL/(kg · d) extrarenal loss.

[2] PRSL: potential renal solute load.

[3] Theoretical limit because one or more nutrients would exceed the recommended maximum.

None of the components of the PRSL can be present in a formula of energy density 94 kcal/100 mL at any concentration greater than the minimum recommended in this report.

Maximum. **The Expert Panel recommended that the maximum PRSL for preterm infant formula be set at 32 mOsm/ 100 kcal for a formula containing 81 kcal/100 mL.** To comply with this maximum PRSL, some components of the PRSL (nitrogen, sodium, chloride, potassium and available phosphorus) must be provided at less than their maximum recommended level.

It is important to recognize that this calculation of permissible PRSL applies only under a specific set of assumptions. The calculation is very sensitive to these assumptions, especially to insensible fluid loss, for which there are the fewest data. For this reason, the Expert Panel urged that the recommended PRSL limit not inhibit the clinical investigation of formulas with higher concentrations of some of the solutes that contribute to the PRSL, provided that testing of such formulas includes an evaluation of the safety of the resulting PRSL.

This recommendation applies to formula for premature infants who are thriving, gaining weight, and not being subjected to extraordinary endogenous or exogenous variables such as fever or excessive radiant warming. Ill infants, overheated infants, or infants not growing because of congenital conditions or intercurrent illnesses should not be maintained solely by use of a fixed formula, regardless of its composition, and recommendations in this report are not intended to provide adequate maintenance parameters for them. Their fluid, calorie, mineral, and nitrogen balances can be maintained only by using information gained from frequent physical examinations and laboratory measurements.

6. ENERGY AND THE PROTEIN-ENERGY RELATIONSHIP

BACKGROUND

Energy balance

The energy demands for preterm infants depend on many factors, including:
- Stages of fetal and neonatal development
- Genetic differences in metabolic rate
- Differences in thermal environment
- Variation in activity and sleep states
- Differences in state of nutrition and nutrient intake
- Variations in growth rate

- Status of body composition
- Variations in postnatal development
- Metabolic demands of illnesses

The energy balance equation is defined (Sinclair, 1978) as

$$Energy_{intake} = energy_{excreted} + energy_{stored} + energy_{expended}$$

where energy$_{intake}$ represents the metabolizable energy in the food, energy$_{excreted}$ occurs primarily in the feces in the postnatal period, energy$_{stored}$ is the metabolizable energy incorporated into lean body mass (LBM) and fat, and energy$_{expended}$ consists of four components:

1. Energy used in the maintenance of body functions (e.g., respiration, maintenance of cardiac output, digestion)
2. Energy used for thermoregulation
3. Energy used in physical activity
4. Energy used for the synthesis of new tissues

Energy required for catch-up growth

The American Academy of Pediatrics' *Pediatric Nutrition Handbook* (American Academy of Pediatrics. Committee on Nutrition, 1998) recommended that premature infants be provided nutrients in amounts sufficient to allow growth similar to that of a fetus of comparable postgestational age. However, difficulties in feeding newborn low birth weight (LBW) infants, coupled with some loss of extracellular fluid (Van der Wagen et al., 1985) (see Appendix A), cause premature infants to lose weight in the first few days of life (Heird & Wu, 1996). Even without complications, they do not regain birth weight (BW) until about 2 weeks of age, so their growth lags behind that of a "normal" fetus of the same postconceptional age, even if they were appropriate for gestational age (AGA) at birth (Wright et al., 1993). In addition, many premature infants are born small for gestational age (SGA), so there is a need for enough catch-up growth to bring them to the size of a normal fetus of comparable gestational age (GA). The amount of catch-up growth required is the difference between the AGA fetal weight and the BW observed, plus the growth deficit incurred neonatally (Forbes, 1974). Whether or not there is a need for increased energy and nutrient requirements to compensate for this deficit has been a subject of debate.

Although a delay of 2–4 weeks in maturation might appear very significant at 2000 g or less, it should become less important as the months pass on and be negligible by 1 year or more of postnatal age. In fact, Wright et al. (1993) did find that premature infants in different weight classes gained weight along parallel lines from the 60th day of life through the 105th. Earlier, Casey et al. (1990) had followed similar groups of LBW infants from the 40th postgestational week to 1 year of corrected GA and found that the average growth curves of groups weighing less than 1251, 1251–2000, and 2001–2500 g paralleled each other and were only slightly lower than the National Center for Health Statistics average rate for term infants. This means that the differences among term and preterm infants became proportionately smaller with increasing age. This result confirms the observation that the height and weight of 46 infants born at less than 35 weeks GA were normal when calculated as related to postconceptional age (Forslund & Bjerre, 1985). Premature infants weighing less than 1500 g at birth (mean weight 1170 ± 207 g) who were examined at intervals by Ross et al. (1990) were smaller than their term peers in height and weight, although not in head circumference, at 1 and 3 years of age but not at 7 and 8 years. This is not true for infants born SGA (Ounsted et al., 1984). It is possible that the first few postnatal weeks may be critical for brain development (Heird & Wu, 1996), so that the lag period should be minimized. However, there seems to be no direct evidence as to what extent catch-up growth is important in this regard.

Previous recommendations for energy density and intake

The report *Assessment of Nutrient Requirements for Infant Formulas* recommended that the energy density of term infant formulas fall in the range of 63–71 kcal/100 mL. These recommendations were based on the history of use and consideration of the interactions of energy and fluid balance (Raiten et al., 1998a). It was noted that if the energy density of formula is low, the infants must consume a large volume to meet energy needs; if the energy density is high, fluid intake is decreased. The positions of some authoritative agencies regarding energy content of formulas intended for premature infants are given in Table 6-1.

The caloric density of formulas currently available for preterm infants in the United States is 81 kcal/100 mL (American Academy of Pediatrics. Committee on Nutrition, 1998).

A wide range of values has been reported for the caloric density of milk from mothers who gave birth to infants prematurely. This could be accounted for by differences in sample collection procedures and a greater degree of interindividual variability in preterm milk composition than in term milk composition (Atkinson, 1995); although the energy content of milk from mother's who gave birth to infants prematurely does not differ significantly from term milk (Butte et al., 1984). Typically, the energy content of human milk from mothers in the United States averages 670-690 kcal/L in the first few months postpartum (Butte et al., 1984; Butte et al., 1990; Nommsen et al., 1991). There is no evidence or rationale to suggest that the composition of preterm milk is adapted to the

TABLE 6-1

Existing recommendations for energy intake by preterm infants and provision of energy in preterm formulas

		American Academy of Pediatrics Committee on Nutrition, 1998	European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) Committee on Nutrition, 1991	Health Canada, Health Protection Branch, 1995	Industry Desalino Associoné (IDACE), 1996
Energy intake recommendation[1]	Minimum	105 kcal/(kg · d) (70 kcal/100 mL of formula)	98 kcal/(kg · d) (65 kcal/100 mL of formula)	105 kcal/(kg · d) (67 kcal/100 mL of formula)	98 kcal/(kg · d) (65 kcal/100 mL of formula)
	Maximum	130 kcal/(kg · d) (87 kcal/100 mL of formula)	128 kcal/(kg · d) (85 kcal/100 mL of formula)	135 kcal/(kg · d) (85 kcal/100 mL of formula)	128 kcal/(kg · d) (85 kcal/100 mL of formula)

[1] Calculated for 150 mL/(kg · d) of formula.

needs of the premature infant (Atkinson, 1995), and in this report, human milk is not being proposed as the standard for the energy content of preterm formulas.

For energy intake, the Health Canada *Guidelines for the Composition and Clinical Testing of Formulas for Preterm Infants* (1995) states: "The P-RNI [recommended nutrient intakes for preterm infants] for enteral energy intakes range from 105 to 135 kcal/kg·d, and this can be achieved with a formula with an energy density ranging from 67 to 85 kcal/100 mL, with a carbohydrate:fat energy ratio of 1:1 and a protein:energy (P:E) ratio not exceeding 3.5 g/100 kcal." The European Commission Industry Desalino Associoné (IDACE) (1996) guidelines recommended a minimum of 65 and a maximum of 85 kcal/100 mL; these were identical to the recommendations of the European Society of Paediatric Gastroenterology and Nutrition Committee on Nutrition (ESPGAN Committee on Nutrition of the Preterm Infant, 1987).

REVIEW OF THE LITERATURE

Total energy intake

The following discussion is based solely on considerations of weight gain and the tissue composition of that gain. Other indicators of growth, such as changes in body length or head circumference, are not consistently different by a magnitude useful in discriminating among various feeding formulations.

A summary of published data (Putet, 1993) indicated that premature infants can use 85–90% of energy intake after the first 2–3 days of life. The remainder is lost in stool and to a small extent as urea in the urine.

Gordon et al. (1940) found an average energy expenditure of premature infants to be 68 kcal/(kg·d), with stool losses of 18 kcal/(kg·d), for a total maintenance requirement of 86 kcal/(kg·d). More recently, Reichman et al. (1981) administered 149 kcal/(kg·d) to premature infants, of which 18 kcal/(kg·d) was lost in the stool and urine and 68 kcal/(kg·d) was stored, leaving a total maintenance expenditure of 63 kcal/(kg·d). In a typical study of 1200-g formula-fed infants receiving about 150 kcal/(kg·d), the measured maintenance requirement was 51 kcal/(kg·d) (Reichman et al., 1982). Because the energy cost of synthesis and composition of new tissue in this study was measured at 4.9 kcal/g of weight gain, it can be calculated that 83 kcal/(kg·d) above maintenance would be required for a fetus-like weight gain of 17 g/(kg·d) (see Chapter 4), and the total caloric requirement would be 134 kcal/(kg·d). These and other studies show that a caloric intake above 70–90 kcal/(kg·d) (allowing for wide variations in energy expenditure for activity) is required for weight gain by a premature infant.

Sauer et al. (1984) conducted longitudinal studies of metabolism and energy costs in 14 LBW infants (BW of 920–1850 g), in which they measured the infants' metabolic rates repeatedly up to 58 days of life. The daily resting metabolic rate after the first week was calculated as y = a ± bx, where a = 246 kJ/kg, b = 0.07, and × = age in days. At less than 100 days, the second term is certainly negligible, so the resting rate is 246/4.175 or 59 kcal/(kg·d). The mean energy used for activity was about 7 kcal/(kg·d), slowly rising during that period, and losses were estimated at 10% of intake, or 13 kcal/(kg·d). The total maintenance requirement was therefore 60 kcal/(kg·d). These infants were gaining at 17.8–19.0 g/(kg·d) after the 14th day. The investigators calculated that a gain of 17 g/(kg·d) by an infant weighing between 1000 and 2000 g would require an intake of 535 kJ/(kg·d), or 128 kcal/(kg·d). This is very close to the optimal intake of 120 kcal/(kg·d) assumed as early as Gordon et al. (1940), and close

to the upper limit of many current recommendations (see Table 6-1).

Brooke (1987) reviewed studies of the energy costs of growth conducted until that time and concluded that 10–25 kcal/(kg·d) above maintenance would be required for a weight gain of 15 g/(kg·d). Brooke estimated total energy expenditures and losses to be 105 kcal/(kg·d), leading to an estimated total requirement of 115–130 kcal/(kg·d).

Previously, Brooke (1980) studied variations in energy intake during 7–10 days in 16 infants of BWs less than 1500 g when they reached 32–39 weeks of postconceptional age. Energy intakes ranged from 133 to 187 kcal/(kg·d). There were no significant differences in weight gain, which varied from 26 to 29 g/d. Because the weights were constantly increasing, it is not possible to calculate the gain in g/(kg·d) from the data provided, but the mean at the middle of the study would have been about 15 g/(kg·d). The important point is that although the infants absorbed more food energy with higher caloric intakes, they did not grow faster. This could be due to an increase in energy expenditure with high intakes of energy (Brooke, 1985), or by counterbalancing loss of body water, perhaps in part caused by a gain in the percentage of body fat. It should be noted that because the increased energy was provided as fat, the protein-to-energy (P:E) ratio was decreasing as the caloric intake was increasing (see below).

The ability to achieve rates of weight gain and accumulation of some nutrients, which are as much as 50% greater than the intrauterine rates, has been demonstrated in a number of studies (Heird & Wu, 1996). However, the high caloric intakes necessary to support such rates lead to the deposition of a higher proportion of the weight gain as fat than occurs in utero (Bell, 1994). This phenomenon can occur even at growth rates comparable to fetal growth rates, if the P:E ratio (in g/100 kcal) is low (Reichman et al., 1981). However, it is not known whether a rapid accretion of body fat is an undesirable accompaniment of the nutrition of premature infants or an advantageous adaptation to postnatal life (Heird & Wu, 1996; Putet, 1993). In any case, rapid accretion of body fat appears to be unavoidable, although it can be altered considerably by manipulation of the P:E ratio (Bell, 1994; Kashyap et al., 1994) (see below). A fat deposition of 20–25% of weight gain has been proposed as a reasonable goal (Putet, 1993), because with this proportion "normalization" of body composition can be expected within the first few years of life (De Gamarra et al., 1987). There are, however, no long-term data to justify this limit.

The Expert Panel estimated that energy intakes for preterm-LBW infants would be in the range of 110–135 kcal/(kg·d). Unless otherwise noted, an energy intake of 120 kcal/(kg·d) was assumed when making a recommendation for minimum and maximum levels of nutrients in this report.

Energy density

The energy density of premature infant formulas currently available in the United States is 67.6 and 80.5 kcal/100 mL (Abbott Laboratories. Ross Products Division, 2001; Mead Johnson Nutritionals, 2000). The higher value of 81 kcal/100 mL seems to be an appropriate compromise between the limited ability of premature infants to tolerate volume and their limited excretory ability. As mentioned earlier, all of the recommendations in this report are predicated based on the assumption that the caloric density of the administered formula would be 81 kcal/100 mL, at an average intake of 110–135 kcal/(kg·d). Higher caloric densities are possible and are sometimes used for preterm infants under close medical supervision, usually because intake of fluid is restricted. In

Chapter 5, the rationale for recommending a maximum energy density of formula for premature infants of 94 kcal/100 mL (940 kcal/L) was presented. A formula of 94 kcal/100 mL can contain only the minimum recommended amounts of the components of the PRSL (nitrogen, sodium, chloride, potassium, and available phosphorus) in order to supply sufficient water for urine production.

Moro et al. (1984) fed formula containing 94 kcal/100 mL to 10 preterm-LBW infants (31–36 weeks GA), beginning the first day of life, and formula containing 81 kcal/100 mL to 10 other similar infants. The ratio of fat to carbohydrate was similar in the two formulas. Caloric intake was approximately 141 and 121 kcal/kg in the high and moderate calorie groups, respectively. By 28 days, linear growth and head circumference did not vary between groups; however, from the time that weight was regained until day 28, mean daily weight gain was significantly greater in the group fed the high calorie formula (32 g/d) compared with the other group (22 g/d). One limitation of this (Moro et al., 1984) study was the inability to determine the composition of the weight gained. The authors speculated that the increased daily weight gain may have been due to increased fat accretion.

Roy et al. (1976) fed a formula of 1000 kcal/L to 17 preterm-LBW infants, who consumed 150-200 mL/(kg·d) for an average of 33 days. Friedman et al. (2000) fed formula containing 1010 kcal/L to 24 preterm-LBW infants (mean 28 weeks GA, 987 g) requiring oxygen and surfactant therapy. Initially, daily goals for feeding were to provide 150 mL/kg body weight and feeding continued with this formula until within one week of discharge when body weight was 1800-1900 g. No reported adverse effects were attributed to these high-energy feedings that were administered under close medical supervision.

In the past, the infant's own mother's breast milk (typical value 67-69 kcal/100 mL) has been used for nourishing the premature infant, but it is clearly not optimal for reasons detailed in Appendix A and throughout this report. A formula of such low energy density would necessitate a daily intake of 179 mL of fluid to achieve a dose of 120 kcal/(kg·d). This can present serious difficulties with smaller premature infants. Nevertheless, one recent study found that a nutrient-supplemented premature infant formula with 67 kcal/100 mL and 3.2 g of protein/100 kcal allowed premature infants to grow as rapidly, and with greater LBM, than did a similar formula with 80 kcal/100 mL and 2.75 g of protein/100 kcal (Van Goudoever et al., 2000). For these reasons, the Expert Panel recommended the energy content of breast milk as the minimum density for preterm infant formula.

Recommendations

Minimum. **The Expert Panel recommended that the minimum energy density for preterm infant formula be 67 kcal/100 mL.**

Maximum. **The Expert Panel recommended that the maximum energy density for preterm infant formula be 94 kcal/100 mL**.

Note. **All of the recommendations in this report are predicated based on the assumption that the caloric density of the administered formula would be 81 kcal/100 mL, at an average intake of 110–135 kcal/(kg·d).**

Protein-energy relationship

An important restriction on formula composition imposed by the rapid growth of the thriving preterm neonate is specification of the protein-energy relationship. Protein and energy needs are reciprocally limiting, the intake of one affecting the

ability of the infant to assimilate the other. If the P:E ratio is not in the optimal range, there are undesirable consequences (Micheli & Schutz, 1993). If energy intake is inadequate, protein is used as an energy source and the nitrogen balance becomes less positive; increasing the caloric intake can spare protein and allow it to be assimilated. Conversely, if protein intake is low, a high caloric intake can spare protein. However, if there is a surfeit of energy with a limited protein intake, the protein gain plateaus and the excess energy can be used for only fat deposition (Micheli & Schutz, 1993). In their summary of numerous studies of the effect of energy intake on protein gain of LBW infants fed a variety of protein intakes as parenteral nutrition, human milk, and milk-based formulas, Micheli and Schutz (1993) showed the plateau point at 100 kcal/(kg·d) for protein intakes of 2.0–4.0 g/(kg·d). This would set the limits for the P:E ratio of 2–4 g/100 kcal. P:E ratios of the two formulas for premature infants available in the United States are 2.7 and 3.0 g/100 kcal (American Academy of Pediatrics. Committee on Nutrition, 1998).

Kashyap and Heird (1994) showed that the lowest mean protein intake likely to result in mean rates of growth and nitrogen retention in utero, which they took to be 15 g/(kg·d) and 300 mg/(kg·d), respectively, is about 2.75 g/(kg·d), assuming energy intake is adequate. To attain acceptable mean plasma indices of protein adequacy (transthyretin and albumin) as well, a mean protein intake of 3 g/(kg·d) appeared to be required at an energy intake of 100–150 kcal/(kg·d). By using the estimated range of energy requirement of 110–135 kcal/kg set in the section on total energy intake, the minimum P:E ratio would be 2.2–2.7 g/100 kcal. As indicated above, however, these investigators pointed out that considerably higher intakes of protein were not excessive.

An increase in the P:E ratio leads to the deposition of more of the ingested calories as LBM (Bell, 1994), but an upper limit to this increase is set by the ability of the premature neonate to assimilate protein and excrete the obligatory breakdown products of any excess. Kashyap et al. (1994) showed that the accretion of protein is greater at a P:E ratio of 3.7 than at a ratio of 3.3 g/100 kcal, but the efficiency of utilization had begun to fall off. At the higher intake, which provided 4.3 g of true protein/(kg·d), the mean plasma concentrations of all amino acids except threonine and tyrosine were within the 95% confidence limits of their cord plasma concentrations in infants born between 29 and 40 weeks GA. In another report, Kashyap and Heird (1994) summarized their data from earlier publications that described feeding formulas with different protein intakes to 12 groups of infants receiving energy intakes ranging from 100 to 150 kcal/(kg·d). The mean blood urea nitrogen level remained below 10 mg/100 mL in every group that received a protein intake of less than 4 g/(kg·d), and mean plasma concentrations of most amino acids were within the 95% confidence limits of the plasma concentrations in LBW infants who were fed their own mothers' milk. At a caloric intake as low as 110 kcal/(kg·d), this would conservatively indicate a maximum P:E ratio of 3.6 g/100 kcal (see Chapter 7).

It is generally believed that the administration of diets containing more than 120 kcal/(kg·d), even at relatively high levels of protein [3.8 g/(kg·d) (Pencharz et al., 1977),(Ziegler, 1986) which gives a P:E ratio of <3.2 g/100 kcal], will lead to a deposition of fat unlike that in the fetus. For example, when premature infants were given 149 kcal/(kg·d) at a protein intake of 3.5 g/(kg·d) (a P:E ratio of 2.3 g/100 kcal), they did not gain more weight than those given 115 kcal/(kg·d) at a protein intake of 3.6 g/(kg·d) (a P:E ratio of 3.13 g/100 kcal) (Kashyap et al., 1986). However, the rates of increase of their skinfold thicknesses were greater, indicating that they had accumulated more fat. Polberger et al. (1989) came to a similar

conclusion from their study of infants fed either preterm human milk or preterm human milk supplemented with fat and/or protein derived from mature human milk. No weight or length benefits were obtained by administering protein at more than 3 g/(kg·d) or by exceeding a total energy intake of 120 kcal/(kg·d) at a protein intake of 3 g/(kg·d) (P:E = 2.5). Moro et al. (1989) found growth no faster with a formula delivering 145 kcal/(kg·d) than with fortified human milk at 124 kcal/(kg·d) when both groups were receiving protein at 3.7 g/(kg·d) (P:E ratio = 2.6 and 3.0, respectively). Of course, because both protein intakes were in the surfeit range, the additional daily energy intake of 21 kcal/(kg·d) would be stored as just over 2 g of additional fat, which might have been difficult to detect statistically in a 2-week study involving only 20 subjects. In contrast, Fairey et al. (1997) reported nitrogen retention to be no higher with a P:E ratio of 3.2 g/100 kcal than with a ratio of 2.6 g/100 kcal when the energy intake was fixed at 120 kcal/(kg·d).

Bell (1994) reviewed 21 studies of premature infant feedings that had been carried out between 1940 and 1988 and did not cite any feeding protocol in which the P:E ratio was higher than 3.1 g/100 kcal after the pioneering work of Gordon et al. (1940), in which it was 4.0 g/100 kcal as a mean. Growth of the infants in the Gordon et al. (1940) study was the least of any in the review, at 2.3 kcal/g of weight gain [not 2.1 as reported in Bell (1994) because of a printing error], for a gain of 16 g/(kg·d), except for one of the studies reviewed by Bell et al. (1988), in which caloric storage was 2.1 kcal/g for a gain of 17 g/(kg·d). However, this study of Gordon et al. (1940) was performed with prematurely born infants whose average weight at the time was 2294 g; whereas, at present many preterm-LBW infants are discharged from the hospital weighing 1800-2000 g (Cruz et al., 1997). Nevertheless, the observations of Kashyap and Heird (1994) and Kashyap et al. (1994) have demonstrated the desirability of P:E ratios as high as 3.7 g/100 kcal, without adverse effects.

Bell's (1994) literature review led him to generalize about the P:E ratio and weight gain. One generalization was that "there is no clear relation between energy intake and weight gain" within the range of energy intake studied (92-181 kcal/kg). This surprising conclusion is not precisely correct, because there is a small relationship ($r^2 = 0.13$ for a straight line). This relationship rises significantly ($r^2 = 0.42$) if one outlying point is omitted, where caloric intake was very high but weight gain low (Brooke et al., 1979). Brooke et al. (1979) assumed 81.7 kcal/100 mL for the formula that they used, which they based on bomb calorimetry (includes nonmetabolizable energy) rather than the manufacturer's stated 62 kcal/100 mL. The caloric content calculated from an assumption of 9 kcal/g of fat, 5.65 kcal/g of protein, and 4.0 kcal/g of carbohydrate is 66 kcal/100 mL. Bell noted a relationship between caloric intake and energy stored ($r^2 = 0.56$ for a straight line), which becomes more robust ($r^2 = 0.79$) without the Brooke et al. (1979) point. This again indicates that an increase in calories without a concomitant increase in protein results in an increased percentage of growth as fat. This can be seen directly in a plot of energy stored [kcal/(kg·d)] against weight gain [g/(kg·d)]; the r^2 for this is 0.41, with a slope of 2.6. There was no correlation between the weight gain and the P:E ratio of the diet ($r^2 = 0.004$), whereas the energy stored is to some extent inversely related to the P:E ratio ($r^2 = 0.26$).

None of the studies reviewed by Bell (1994) had a P:E ratio as low as 2.0 g/100 kcal, but the study by Reichman et al. (1981) at 149 kcal/(kg·d), and that of Whyte et al. (1983) at 126–127 kcal/(kg·d), had P:E ratios of 2.1 g/100 kcal. Each of

these protocols led to high-energy tissue deposition, indicating a high percentage of fat.

Conclusions and recommendations. P:E ratios of the two formulas for premature infants available in the United States are 2.7 and 3.0 g/100 kcal (American Academy of Pediatrics. Committee on Nutrition, 1998).

Minimum. A P:E ratio as low as 2.1 g/100 kcal apparently leads to high-energy tissue storage, i.e., predominantly fat, and unless the energy intake is at least 135 kcal/(kg·d), is likely to provide insufficient protein. Although this may not be harmful, it does not have demonstrated advantages. In the series of reports collected by Putet (1993), there were nine in which a weight gain of more than 15 g/(kg·d) was composed of less than 25% fat. The lowest P:E ratio offered in any of these studies (3 of 22) was 2.4 g/100 kcal. In two others, it was 2.5–2.6 g/100 kcal. For a weight gain of 17 g/(kg·d) (see Chapter 4), and the rule of thumb described above that this weight gain comprise no more than 25% fat, the Expert Panel suggested that the reasonable recommendation for a minimum P:E ratio is 2.5 g/100 kcal. This is consistent with the estimation of the minimum acceptable protein intake derived in Chapter 7.

Maximum. Contemporary experiments that justify a P:E ratio higher than 3.1 g/100 kcal are summarized by Kashyap and Heird (1994) and Kashyap et al. (1994). There is another report of a gain of 17 g/(kg·d) at a ratio of 3.5 g/100 kcal with fortified human milk, but this was at a caloric intake of 106 kcal/(kg·d) (Putet et al., 1987). Protein intake in that study was calculated as nitrogen intake × 6.25, which means that nonprotein nitrogen would have been included. Weight gain comprised only 14% fat, which could be inadvisably low. The limited available information on the effects of high protein intakes led the Expert Panel to recommend a value of 3.6 g/100 kcal as a maximum.

Recommendation

Note. **On the basis of studies of the effects of diet on composition of weight gain, the Expert Panel recommended that the P:E ratio for preterm infant formula be 2.5–3.6 g/100 kcal.** This recommendation provides limiting parameters for a protein intake recommendation based on the provision of a quantity of protein needed to achieve fetal rates of lean tissue growth and nitrogen accretion, as considered in Chapter 7. A P:E ratio of 2.5–3.6 g/100 kcal at a caloric intake of 110–135 kcal/(kg·d) restricts the limits of protein intake to 2.8–4.9 g/(kg·d).

7. PROTEINS, AMINO ACIDS, AND OTHER NITROGENOUS SUBSTANCES

TOTAL PROTEIN

Background

The goal in estimating the protein nutritional needs of the preterm infant is to provide the quantity and quality of protein needed to achieve fetal rates of tissue growth and nitrogen accretion (Ziegler, 1986). This goal must be accomplished in the context of the level of physiological and metabolic development of the infant in order to avoid accumulation of potentially harmful protein metabolic products (Ziegler, 1986).

The ratio of protein gain to protein intake is defined as the "coefficient of protein utilization" (Micheli & Schutz, 1993) and represents the efficiency with which protein intake can be used for anabolic processes. The coefficient of protein utilization is influenced by several factors, some of which may be more important for preterm infants than for other populations, but each of which should be considered in the evaluation of the protein content of formula. These factors are

- Quality and quantity of the protein ingested
- Ratio of protein to energy intake
- Developmental maturity of the infant
- General nutritional state of the infant
- Infant's physiological state (e.g., temperature, basal metabolic rate)
- Infant's capacity for nitrogen absorption and retention

On the basis of the relative amounts of essential amino acids supplied by an adequate intake of human milk, the report *Assessment of Nutrient Requirements for Infant Formulas* recommended a minimum of 1.7 g of "true protein"/100 kcal of formula. True protein (α-amino nitrogen $\times$ 6.25) included only those nitrogenous compounds that are the major sources of amino acids for tissue growth. Not included were the nonprotein nitrogen (NPN) sources in formula. That panel recommended a maximum for crude or "total" protein (total nitrogen $\times$ 6.25) of 3.4 g/100 kcal in formulas for term infants on the basis of the potential renal solute load produced by dietary protein.

Table 7-1 summarizes the recommendations for protein content of formulas designed for preterm infants offered by several agencies and groups.

In a recent revision of its guidelines, the European Commission (1996) proposed that the minimum protein content for preterm formulas be raised from 2.2 to 2.4 g [from 2.6 to 2.9 g/kg at 120 kcal/(kg·d)] while retaining the recommended maximum of 3.1 g/100 kcal (3.7 g/d). The recommendation to increase the protein content was based principally on the study by Lucas et al. (1990) in which low birth weight (LBW) infants who were fed standard infant formula with a protein content of 2.2 g/100 kcal [2.6 g/(kg·d)] had inferior cognitive development. Eighteen months after the expected term date, these infants demonstrated lower scores on the Bayley Scales of Infant Development, especially for motor function, than did infants fed preterm formula providing 3.0 g/100 kcal [3.6 g/(kg·d)].

The European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) (ESPGAN Committee on Nutrition of the Preterm Infant, 1987) proposed guidelines for preterm infant formulas that recommended a minimum protein content of 2.25 g/100 kcal [2.7 g/(kg·d)] and a maximum of 3.1 g/100 kcal (3.7 g/kg). ESPGAN was concerned that a protein intake of 2.2 g/100 kcal might be too low when consumption is less than 130 kcal/(kg·d) and an intake of 3.1 g/100 kcal might be too high when consumption is greater than 130 kcal/(kg·d).

The report from Health Canada (1995) identified a "rea-

sonable range" of 2.5–3.0 g/100 kcal, which if fed at the mean energy requirement of 120 kcal/(kg·d) would provide between 3.0 and 3.6 g/(kg·d). The report recommended that the protein concentration of a premature infant formula using current whey-dominant proteins should not exceed 3.5 g/100 kcal, because at that concentration infants fed at 120 kcal/(kg·d) would ingest about 4.2 g/(kg·d) of protein.

The values in Table 7-1 resemble the recommendation for protein of 2.5–3.6 g/100 kcal derived from considerations of the appropriate protein-to-energy (P:E) ratio but are not identical with it. Formulas currently marketed for preterm infants in the United States contain protein concentrations of 22 and 24 g/L (2.7 and 3.0 g/100 kcal, respectively); when given at 120 kcal/(kg·d), these provide 3.2 and 3.6 g of protein/(kg·d), respectively (American Academy of Pediatrics. Committee on Nutrition, 1998).

Review of the literature

Dietary protein needs for preterm infants have been estimated by two different methods. The first, the factorial approach, considers that the requirement for a nutrient is equal to the sum of the obligatory losses (e.g., urine, feces, skin), plus the amount incorporated into newly formed tissues. The second method, the empirical approach, measures biochemical or physiological responses to graded intakes of the nutrient.

The factorial approach

This method, originally described for use in humans by Hegsted (1957), has been useful for estimating requirements and designing experiments for obtaining definitive empirical data. For the fetus and the preterm infant, protein is the predominant component of the requirements for developing new tissue. Compositional analysis of fetal tissues has been a valuable source of data for our understanding of the nutrient needs of the fetus, and by extension, those of the growing preterm infant.

Fetal accretion rates of protein have been compiled from compositional analyses of aborted fetuses or stillborn infants (Forbes, 1989). In these analyses, fetal nitrogen accretion has been expressed in terms of both gestational age (GA) (24 weeks to term) and birth weight (BW) (700–2500 g). For example, the nitrogen accretion rates in mg/d [or mg/(kg·d)] for fetuses of GAs 25, 30, and 35 weeks have been reported to be 273 [341], 435 [294], and 741 [302], respectively (Forbes, 1989). This fetal nitrogen accretion model is limited because of the large variations in the rates of fetal weight gain, particularly at early time points. Nevertheless, from such data Ziegler (1986) had composed a table of nitrogen and protein requirements derived by the factorial approach (Table 7-2). The recommended protein intakes were obtained by adding 8–10% to the estimated requirements, in part to address individual variation.

Fomon et al. (1986) noted that these allowances, even with the margin provided by the advisable intake, do not meet the protein requirements of infants for rapid catch-up growth, which may be expected to average an additional 1 g/(kg·d) or more. The advisable intakes of protein calculated on the basis of the factorial method are higher for all weight classes than the minimum acceptable P:E ratio, 2.5 g/100 kcal, and lower than the maximum acceptable ratio chosen, 3.6 g/100 kcal.

The empirical approach

This approach evaluates physiological and biochemical variables to determine protein sufficiency or excess in the growing preterm infant. Among the outcome measures used in these types of studies are the following.

TABLE 7-1

Previous recommendations for protein in preterm formula

Organization	Protein content (g/100 kcal)	
	Minimum	Maximum
European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) (1987)	2.25	3.1
Health Canada (1995)	2.5	3.0
Association of the Food Industries for Particular Nutritional Uses of the European Union (Industry Desalino Associoné (IDACE), 1996)	2.4	3.1
European Commission[1] (1996)	2.4	3.1
American Academy of Pediatrics Committee on Nutrition (1998)	2.9	3.3

[1] Specified only for preterm infants of birth weight higher than 1000 g

TABLE 7-2

Estimates of protein requirements and advisable intakes using the factorial method

Variable	Birth weight (g)			
	<1000	1000–1500	1500–2000	2000–2700
Tissue accretion (g/d)[1]	1.75	2.47	3.30	4.09
Dermal loss (g/d)[1]	0.14	0.21	0.30	0.39
Urinary loss (g/d)[1]	0.79	1.17	1.64	2.20
Required absorbed (g/d)[1,2]	2.68	3.85	5.24	6.68
Required intake				
(g/d)[1]	3.27	4.53	5.82	7.34
[g/(kg · d)][1]	3.85	3.62	3.33	3.12
Advisable intake				
[g/(kg · d)][1,3]	4.0	3.8	3.5	3.2
(g/100 kcal)[4]	3.3	3.2	2.9	2.7

[1] From Ziegler, 1994.

[2] Assumes relative absorptions of 82%, 85%, 88%, and 90%, for the respective strata (Snyderman et al., 1969, 1970).

[3] Allowing for 8–10% above requirement for individual variability; averaged for the range of weights.

[4] Assumes a caloric intake of 120 kcal/(kg · d).

Anthropometry. Variables measured have been weight, crown-heel or crown-rump lengths, head circumference, and skinfold thickness (usually in the triceps or subscapular regions).

Nitrogen balance or retention. The nitrogen balance technique accounts for the difference between nitrogen intake and excretion. Despite having a number of potential complicating variables, the nitrogen balance technique has been shown to be a reliable and consistent method (Micheli & Schutz, 1993). Nitrogen losses from skin have been estimated to be 27 mg/(kg·d) (Ziegler, 1986), but there are few data (Snyderman et al., 1969); most investigators evaluating nitrogen balance have utilized only fecal and urinary nitrogen excretion, as these are the most significant and easiest measures to quantify in small infants.

Metabolic or chemical indices. Most often, these indices provide an indirect reflection of protein synthesis. The most commonly used measures are levels of serum or plasma components such as serum albumin, total protein, immunoglobulin, retinol-binding protein, and transthyretin (prealbumin). This last variable has been proposed as a sensitive indicator of protein nutritional status because of its rapid turnover (Giacoia et al., 1984; Moskowitz et al., 1983). In some cases, biochemical markers of toxicological or adverse effects of protein excess have been measured, such as blood urea nitrogen (BUN), and pH, P_{CO_2}, and bicarbonate, to monitor for metabolic acidosis.

Amino acid analyses. Serial measurements of plasma and urinary amino acids have been included in several studies. These measures are useful for evaluating the effects of the level of protein intake and protein quality (such as differences in whey-to-casein ratios) and the metabolic pathways involved in the synthesis of nonessential amino acids.

Developmental assessment. Increased scientific interest in the effect of the quantity and quality of protein on neurological development is reflected in the incorporation of various neurometric tests, most notably the Neonatal Behavioral Assessment Scale (NBAS) and the Bayley Scales of Infant Development, in recent studies of preterm infants.

Isotope studies of whole-body nitrogen kinetics. In these studies, a ^{15}N- or ^{13}C-labeled amino acid is administered orally or parenterally and is distributed throughout the body's free nitrogen pool. Urinary isotope dilutions in ammonia or urea are then used as indirect measures of protein synthesis rates (Bier & Young, 1986).

Tissue accretion. Some of the early empirical studies of nutrition for LBW infants were obscured by a controversy about the effects of the protein and electrolyte components of the diet. After Gordon et al. (1947) demonstrated that human milk did not support as rapid a growth or retention of nitrogen by premature infants as did a skim milk formula with higher levels of protein, it was suggested that the formula increased fluid retention because of its higher ash content (Snyderman et al., 1969). However, increasing protein intake up to 4 g/(kg·d) (3.3 g/100 kcal) had a direct effect on weight gain, regardless of electrolyte intake in the range from 0.43 to 1.3 g/(kg·d). A study by Babson and Bramhall (1969), by using isocaloric feedings, evaluated growth as a function of different levels of electrolyte and protein intake. Infants of BW less than 1500 g were fed isocaloric diets that provided protein at either 2.25 or 5.25 g/(kg·d) (1.8 or 4.3 g/100 kcal, respectively) in combination with ash contents of 0.45 or 0.9 g/(kg·d) (0.37 or 0.73 g/100 kcal, respectively). Despite small sample sizes, significantly greater increases in crown-heel and tibial bone lengths were observed with the higher protein diet during the 42-day study. The diet with the higher ash content did contribute to increased weight gain, but without an effect on linear or tibial growth. Later, it was shown that a high ash formula with protein at 3.9 g/100 kcal led to better linear growth than low ash human milk at 1.6 g/100 kcal (Davies, 1977). It is probably significant, however, that the amounts of calcium and phosphorus in the formula were, respectively, three and five times as high as those in the milk.

Intravenous amino acid requirement. One approach to estimation of a standard protein intake for premature infants has been the consideration of the amounts of intravenous amino acids necessary to achieve a positive nitrogen balance. Initial studies in the immediate newborn period of preterm infants who are stable and not stressed from life-threatening illnesses seemed to indicate that about 0.6 g/(kg·d) of free amino acids would achieve this goal (Anderson et al., 1979; Duffy et al., 1981; Zlotkin et al., 1981b). More recently, Heird (1999b) suggested a higher value, such that the mean nitrogen loss by newborn LBW infants receiving no protein or amino acid intake was 180 mg/(kg·d), the infant equivalent to about 1% of body protein stores. An amino acid intake of 1.0 g/(kg·d) was needed to reverse negative nitrogen balance in the last study. Further, an amino acid intake of at least 3 g/(kg·d) is probably required to support a rate of deposition of lean body mass (LBM) approaching the intrauterine rate (Heird, 1999b).

Studies of banked human milk. Feeding banked human milk was a common practice for preterm infants until the 1960s, when alternative sources such as expressed human preterm milk and modified cow milk protein-based formula preparations began to be used. The first study comparing human milk and cow milk protein for nourishing preterm infants was conducted by Gordon et al. (1947). It showed that preterm infants (BW 1022–1621 g) fed a skimmed cow milk that provided 6 g of protein/(kg·d) had a greater weight gain during a 21-day period than those fed pooled "mature" (term) human milk that provided a protein content of 2.2 g/(kg·d). Omans et al. (1961) tested human milk and cow milk formulas (protein contents ranging from 1.7 to 7.7 g/100 kcal) at varying caloric intakes, in an attempt to relate growth to protein intake. They noted that preterm infants fed less than 2.5 g of protein/(kg·d)

gained weight poorly. Those infants fed 3–8 g/(kg·d) all gained weight equally, whereas those inadvertently receiving protein intakes in excess of 8 g/(kg·d) had diminished growth and significantly higher mortality than those fed other diets. The BUN value in all infants was proportional to the protein content of the formula, but infants receiving more than 5 g of protein/(kg·d) had elevated levels.

The failure of mature human milk to support growth adequately in LBW infants was further confirmed by Davies (1977), who evaluated 28 preterm infants of GA 28–32 weeks. Half the infants received expressed mature human milk containing 1.1 g of protein/100 mL and the remainder received a cow milk formula containing 2.7 g of protein/100 mL. Daily water and calorie intakes were similar in the two groups. The mature human milk-fed group had slower weight gain and significantly lower linear and head growth during the first month of life. The differences in growth rates were transient, however, as growth between groups during the second month of the study was similar, without evidence of catch-up growth.

It should be noted that Järvenpää et al. (1983b) found that premature infants fed 180–200 mL/(kg·d) of preterm milk could gain weight at a growth rate equal to the intrauterine rate, which they took to be 29.6 g/d [14.8 g/(kg·d), assuming an average weight of 2000 g]. Their protein intake from breast milk was presumably 1.8–2.0 g/(kg·d), or no more than 1.5 g/100 kcal. The BW of these infants averaged 1789 g, and all were less than 2200 g (GA of 31–36 weeks); they were studied until they reached 2400 g. Thus, they were apparently larger during this study than those reported by Gordon et al. (1947), Davies (1977), and others who found human milk to contain too little protein for small premature infants.

Additional reports demonstrated that mature milk provides insufficient protein to support growth rates similar to intrauterine rates, although in many trials protein was not the only variable. In the study of Svenningsen et al. (1982), 48 LBW infants received protein at 1.9 g/(kg·d) (human milk) or at 2.3 or 3.0 g /(kg·d) (formula), all at 116–118 kcal/(kg·d). The formula-fed infants gained weight and length more rapidly, but the differences were not significant. Significant differences in the gain of both weight and length occurred when premature infants were fed human milk with a protein intake of 2.4 g/(kg·d) or formula with a protein intake of 3.1 g/(kg·d) by Putet et al. (1984), but energy intake and absorption were also higher with the formula. Tyson et al. (1983) demonstrated that LBW infants fed mature human milk with a protein content of 1.8–2.1 g/100 kcal had substantially lower growth in weight, length, and head circumference than those fed premature infant formula with a protein content of 2.6 g/100 kcal. Again, in the protein-enriched formula, mineral and calorie levels were also higher than those in the milk. Nevertheless, these studies strongly suggest that mature milk or formula with protein concentrations of 1.0 g/100 mL or 1.5 g/100 kcal are insufficient for premature infants. In those studies in which premature infants were fed sufficient calories, protein concentrations of at least 2.2 g/100 kcal appeared to be necessary to support acceptable growth rates. At 120 kcal/(kg·d), this is 2.6 g/(kg·d).

Studies of preterm human milk. The use of expressed human milk from mothers delivering prematurely was prompted by the findings of Atkinson et al. (1978; 1981). They compared growth, nitrogen balance, and biochemical variables in infants of BW less than 1300 g during the first 2 weeks of life who were given feedings of banked mature milk, expressed preterm breast milk, or infant formula (670 and 810 kcal/L) (Atkinson et al., 1981). Although the study lasted only 2 weeks, feedings of preterm human milk and the higher energy formula were

associated with greater nitrogen accretion than were the other two feeding regimens. In addition, preterm infant formulas containing 2.3 g of protein/100 kcal seemed to be an adequate nutrient source and preferable to pooled mature human milk in supporting growth. These formula-fed infants were actually ingesting protein at 3.4 g/(kg·d). Subsequent to these findings, the feeding to LBW infants of expressed breast milk from their own mothers became a conventional practice for nutritional, immunological, and psychosocial reasons.

Atkinson (1995) reviewed the literature on the composition of milk from mothers who had delivered prematurely ("preterm milk") and offered several generalizations about its protein content, as follows.

Total nitrogen concentration in preterm milk is generally higher than that in milk from mothers of full-term infants ("term milk") during the first month of lactation. Total nitrogen concentration in preterm milk decreases throughout the course of lactation, as it does in term milk. The range of values for protein content in 10 different reports was 2–3 g/100 mL in colostrum, 1.3–7.8 g/100 mL in transitional milk, and 1.3–1.8 g/100 mL in later milk. For transitional milk, only one study in the review reported a concentration greater than 2.4 g/100 mL. In comparison, the protein content of term milk has been reported to decline from 1.4 to about 0.8 g/100 kcal through the first 6 months of lactation (Raiten et al., 1998a). The average proportion of total protein contributed by NPN has been reported to be similar in preterm milk and term milk, although highly variable in preterm milk. Even though this proportion remains relatively stable over the course of lactation, in preterm milk the major contributors to the NPN content, urea and free amino acids, become increasingly predominant with advancing stages of lactation.

As noted previously in this report, there is no indication that the composition of preterm milk is adaptive (Atkinson, 1995).

A study by Gross (1983) compared growth of 60 infants fed isocaloric diets of term human milk (protein content 1.0 g/100 mL), preterm human milk (protein content 1.5 g/100 mL), or whey-based cow milk formula (protein content 1.93 g/100 mL). This study confirmed earlier findings of lower weight gain and slower head and linear growth of the term milk-fed group than in the groups fed expressed breast milk and cow milk formula. The infants fed the banked term milk took an average of 19 days to regain BW, the formula and preterm milk-fed infants only 10 and 11 days, respectively. Later, however, Tudehope et al. (1986) found similar advantages in the growth rate variables of weight, length, and head circumference for infants fed preterm formula rather than their own mothers' preterm milk.

Boehm et al. (1987) thought that a protein intake of more than 2.5 g/(kg·d) created a metabolic overload, as evinced by higher urinary nitrogen losses proportional to the increased intake. At this limit, the milk of mothers delivering prematurely would be adequate for protein if the premature infant could tolerate feeding volumes of 180–200 mL/(kg·d). This is consistent with the findings of Järvenpää et al. (1983a).

Studies of supplementation (fortification) of human milk. Despite its shortcomings, human milk has several advantages over formula for feeding infants (Räihä, 1994b): protection against infections and necrotizing enterocolitis, high tolerance characteristics, and perhaps improved absorption of fat (Rönnholm et al., 1986). For these reasons, various approaches to enhancing the protein nutrient quality of banked term human milk have been evaluated. These studies provide additional insight into the levels of protein that are required to support growth at rates similar to intrauterine accretion rates.

Rönnholm et al. (1982) studied the effect of supplementing pooled term milk with human milk protein for 12 weeks on growth and plasma amino acid levels. The study was conducted with 18 LBW infants fed diets of their own mothers' milk or banked milk that averaged 0.9 g of protein/100 mL (control groups), or the same milk types supplemented with 0.8 g of protein/100 mL isolated from banked term human milk (supplemented groups). The average protein intake for the control groups was 2 g/kg·d; the supplemented groups averaged intakes of 2.6, 3.6, and 3.4 g/(kg·d) at 2, 6, and 12 weeks, respectively. No growth differences were noted among groups for the duration of the test feeding, but the study was confounded by a variable provision of protein within the control group. The lack of demonstrable differences in growth between the groups may have been due to the small numbers of subjects in each group and the inclusion of infants who received preterm milk (with higher protein levels) in the control group. An important point, however, is that the infants in the control group became hypoproteinemic at about 2 months of age, whereas the infants in the supplemented group did not. The serum concentrations of phenylalanine and tyrosine were not significantly different between the two groups, but their concentrations were correlated with the protein intake. According to a regression line for the correlation between serum urea nitrogen and protein intake, intakes of 2.8 and 3.5 g/(kg·d) would result in serum urea nitrogen concentrations of 15 and 20 mg/100 mL, respectively. The authors interpreted these observations as indicating that a maximum limit for protein intake without adverse effect would be about 3 g/(kg·d) at 2 weeks of age.

In a subsequent study, Rönnholm et al. (1984) evaluated the effect of supplementation of banked human milk with fat and/or protein on plasma amino acid concentrations. Using a design similar to their earlier one, they fed 44 LBW infants one of the following types of milk: mature human milk providing 0.9 g of protein/100 mL, mature milk supplemented with fat [medium-chain triglyceride (MCT) oil, 1 g/100 mL], mature milk with human milk protein supplementation providing 1.8 g of protein/100 mL, or mature milk with both MCT oil and protein supplementation. They found that the levels of amino acids at 2, 8, and 10 weeks of age were 1.5- to 3-fold higher in the groups that had protein supplementation. Fat supplementation alone had no effect on plasma amino acid levels.

Finally, some of these same investigators succeeded in demonstrating that intakes of human milk supplemented with human milk protein produced much greater gains in weight and length between 2 and 6 weeks of age than did isocaloric unsupplemented milk, whether preterm or banked (Rönnholm et al., 1986). Intrauterine rates of weight gain were equaled during the first 6 weeks.

Polberger et al. (1989) demonstrated in thriving premature infants of BWs about 1200 g that daily weight gain and weekly length increments correlated with protein intake up to about 2.8–3.0 g/(kg·d) at an energy intake of 120 kcal/(kg·d). Later work showed that with a protein intake of 2.6 g/(kg·d), nitrogen retention rates of infants in this BW range increased from 65% to 76% of intake between the first and sixth week ex utero (Boehm et al., 1990b). This is substantially higher than the nitrogen retentions of less than 47% seen with full-term infants fed protein at 1.8–4.0 g/100 kcal (Fomon et al., 1986; MacLaurin et al., 1975). In general, protein intake positively affects the rate of nitrogen accretion throughout the range of usual protein intakes unless caloric intake is growth limiting.

Polberger et al. (1990c) contributed the observation that levels of the plasma proteins transthyretin, retinol-binding protein, and transferrin were correlated with protein intake in LBW-appropriate for gestational age (AGA) infants at up to 3.3 or posssibly 3.9 g/(kg·d). The concentrations of the first two of these proteins were also correlated with increases in weight and length of the infants, as well as with preprandial concentrations of plasma amino acids. These data support the suggestion that that these substances can be used as indicators of protein nutritional status (Moskowitz et al., 1983). Enrichment of human milk with human milk protein to provide 3.6 g/(kg·d) (2.8 g/100 kcal) also results in significantly increased plasma concentrations of 15 of 18 amino acids, and the total essential amino acid concentration in plasma is significantly correlated with observed gain in weight and length (Polberger et al., 1990a). Moreover, in this study the plasma concentrations of essential amino acids in the protein-supplemented infants corresponded well to plasma concentrations found in breast-fed term infants at 1 and 3 months of age. The serum urea concentrations of infants receiving up to 3.9 g/(kg·d) were never higher than 25 mg/100 mL (Polberger et al., 1990b).

Neither preterm milk nor adequate supplies of human milk protein are always available. This led Putet et al. (1987) to investigate the effect of supplementation of pooled mature human milk with cow milk protein on growth and nitrogen balance. In this study, 16 LBW infants were fed either pooled human milk with a protein content of 2.3 g/100 kcal [mean intake 107 kcal/(kg·d)] or pooled human milk with cow protein supplementation that provided 3.5 g/100 kcal [mean intake 106 kcal/(kg·d)]. The growth differences did not reach significance during a 2-week period, but nitrogen retention, BUN, total protein, and individual amino acid levels were higher in the protein-supplemented group. Higher energy expenditures were noted in the supplemented group, and a higher percentage of the weight gain was due to protein accretion. Thus, this study demonstrated that providing a protein intake greater than 2.3 g/100 kcal resulted in higher nitrogen retention. The higher protein accretion coupled with the lack of difference in weight gain between groups in this study may be attributable in part to the small number of patients (i.e., insufficient power to distinguish differences) and the relatively short study period, but it could also indicate that a higher fraction of the weight was gained as LBM.

The effect of supplementing preterm human milk with protein has been studied in terms of weight gain, nitrogen retention, and metabolic responses (Kashyap et al., 1992). In this study, one group of infants received their own mothers' expressed breast milk, a second group received the same kind of feeding with a protein supplement (whey-to-casein ratio of 60:40) prepared from cow milk, and a third group received pasteurized term breast milk plus the same supplement. The mean protein intakes of the three groups were, respectively, 2.5, 3.2, and 2.8 g/(kg·d) (1.9, 2.4, and 2.4 g/100 kcal). The supplement also contained calcium, phosphorus, and sodium. The unsupplemented group gained weight at 16.4 g/(kg·d) (thought to be the fetal rate) and the supplemented group gained weight at 20.5 g/(kg·d), although caloric intakes of the two groups were virtually the same, with mean values of 129 and 131 kcal/(kg·d). Moreover, nitrogen retention rates were higher in those infants provided with supplementation [353 mg/(kg·d)] than in the unsupplemented group [270 mg/(kg·d)], and their mean plasma serum albumin and transthyretin levels were higher. The infants fed protein-supplemented milk gained a smaller proportion of the weight as fat (26%) than did the unsupplemented group (34%).

The results of these supplementation studies indicated that LBW infants receiving human milk have improved growth

when provided protein intakes above 2.1 g/100 kcal. Additional protein intake results in higher levels of putative indicators of protein nutritional status, higher levels of plasma amino acids, and possibly a greater accretion of LBM.

In a study intended to evaluate protein quality (see below), Moro et al. (1995) fed 12 premature infants (BWs of 900–1500 g) fortified human milk at 3.7–4.0 g/(kg·d) at a P:E ratio of about 3 g/100 kal. No untoward effects could be ascribed to this feeding, but growth was not better than that of infants receiving 3.4–3.7 g/(kg·d).

Protein supplementation of infant formula. Snyderman et al. (1969) showed that nitrogen retention was higher in preterm infants fed 9 g of total protein/(kg·d) ($n = 11$) than in those infants receiving 2 g/(kg·d) ($n = 15$); however, no differences in growth were noted between the groups. Other studies at about the same time, however, showed that weight gain is higher with protein intakes of 4.0–5.25 g/(kg·d) than with 2.0–2.25 g/(kg·d) (Babson & Bramhall, 1969; Davidson et al., 1967).

Goldman et al. (1969) conducted one of the first studies of the effect of different levels of protein intake from formula on the growth of LBW infants. In this study, incremental increases in weight and changes in biochemical variables (e.g., pH, albumin, and urea) were compared in 304 LBW infants fed isocaloric cow milk-based formulas containing either 2.5 or 5 g of protein/100 kcal, which provided 3.0–3.6 and 6.0–7.2 g of protein/(kg·d), respectively. Despite significantly different protein intakes, no differences in weight gain were observed between the groups. Those fed the higher level of protein intake had higher plasma protein levels and less edema. However, the possibility that adverse effects could result from protein intakes substantially higher than those provided in human milk was raised by the observation that infants fed the higher protein intake had an increased incidence of fever, lethargy, and poor feeding than did those fed the lower level of protein.

Schanler et al. (1985a) evaluated protein balance of LBW infants of GA 28–30 weeks by comparing isocaloric diets of either commercial formula designed for preterm infants ($n = 14$) or expressed human milk from mothers delivering prematurely ($n = 17$). The preterm milk was modified by adding skim milk and cream components derived from donor mature human milk, so that nitrogen and energy levels were similar to those in the formula. During the first of two study periods, infants were fed diets containing 100 kcal/100 mL for about 2.5 weeks, with intake increasing according to each infant's tolerance up to 125 kcal/(kg·d). In the second period, lasting until the infants reached 1800 g (about 8 weeks of age), they received diets containing 80 kcal/100 mL. Growth and biochemical parameters were measured, and 96-hour balance studies were conducted when the infants were 2 and 6 weeks of age. Protein intakes (calculated as reported nitrogen values × 6.25) during the first period were 2.9 g/(kg·d) for the fortified preterm human milk and 2.8 g/(kg·d) for the formula. During the second study period, protein intakes were 2.9 g/(kg·d) for the fortified milk group and 3.4 g/(kg·d) for the commercial formula group. Caloric intakes were 128–134 kcal/(kg·d) with all the feedings.

Both the breast milk and formula preparations led to gains in weight and length similar to intrauterine rates during the full 8 weeks of the investigation. The total nitrogen retention was higher for both groups during the second study period compared with the first, although the percentage of protein retention did not differ. These findings indicated an ability of the premature infant to utilize available nitrogen when provided at 3.4 g of protein/(kg·d), or 2.7 g of protein/100 kcal.

Brooke et al. (1982) evaluated growth, nitrogen retention, and energy balance during a 2-week period in 37 LBW infants who were fed expressed mature human milk; a standard term infant formula; a standard formula with added protein; or a special adapted preterm formula modified to provide increased protein, minerals, and energy (76 kcal/100 mL instead of 67 kcal/100 mL). These feedings contained protein contents at 1.2, 2.1, 2.7, and 2.8 g/100 kcal, respectively. The infants fed the premature "adapted" formula had greater mean gains in weight, length, head circumference, multiple skinfold thicknesses, and nitrogen retention than did the infants fed the other diets.

In another study, conducted for 3.5 weeks, Brooke et al. (1987) evaluated growth, nitrogen retention, and energy balance in two groups of LBW infants, who weighed less than 1300 g or greater than 1300 g at the beginning of the investigation, nourished with expressed preterm human milk (protein content 1.8 g/100 kcal) or preterm infant formula (protein content 2.7 g/100 kcal). Although caloric intakes were similar among the groups, a greater rate of weight gain was observed in both groups fed the preterm formula. Nitrogen intake and retention were also higher in those infants fed the preterm formula, thus indicating that protein intake up to a level of at least 3.7 g/(kg·d), or 2.7 g/100 kcal is beneficial to preterm infants.

A similar finding was reported by Lucas et al. (1984), who studied the growth of 62 infants weighing less than 1850 g who were fed either preterm infant formula with a protein content of 2.5 g/100 kcal or banked mature human milk with a protein content of 2.3 g/100 kcal (1.07 g of protein and 46 kcal/100 mL). Infants fed the preterm infant formula had significantly greater gains in weight, length, and head circumference. The result is flawed, however, because the pooled sample of 340 individual collections of milk for banking contained only 46 kcal/100 mL, apparently a result of the collection of drip breast milk, which has a low fat content.

A second trial in this study evaluated the same diets used as supplements for 132 infants who were also being fed expressed breast milk (preterm milk). Growth differences were again noted, with the rate of increase in weight gain and length being greater in those infants supplemented with preterm formula. These growth effects were most prominent in infants with a BW less than 1200 g. Moreover, the group of infants who were fed exclusively banked milk required 3 weeks longer to reach a discharge weight of 2000 g than did those fed preterm formula or expressed breast milk with preterm formula supplementation. It may be important that the high protein preterm formula also provided a higher energy density and a greater mineral content.

Later on, members of this same research group found that despite the differences in growth of premature infants fed differently during their initial hospitalization, the nature of the feedings at that time, which differed in protein, energy, and a wide range of mineral, micronutrient, and non-nutrient (breast milk) factors, did not result in dissimilarities in body size or fatness at 9 months, 18 months, or 7.5–8.0 years of age (Morley & Lucas, 2000). This was in contrast to the findings with respect to cognitive function, which demonstrated that increased nutrition in the neonatal period had beneficial effects on long-term neurodevelopment (Lucas et al., 1998) (see below). The mean values for height and weight of the premature infants were about 0.5 SD lower than recent norms for the national population.

Some attempts have been made to evaluate the effect of variations in protein intake to nitrogen retention and increases in LBM. Kashyap et al. (1986) tested three formulas

that provided, respectively, actual daily intakes of (1) 2.24 g of protein/kg and 115 kcal/kg (1.9 g/100 kcal); (2) 3.62 g of protein/kg and 114 kcal/kg (3.2 g/ 100 kcal); and (3) 3.5 g of protein/kg and 149 kcal/kg (2.34 g/100 kcal). These formulas were administered to infants of BW 900–1750 g (GA of 27–37 weeks), beginning as soon as oral feedings were tolerated and continuing until weight reached 2200 g.

Weight gain was significantly less in the first group fed 1.9 g/100 kcal than in the other 2 groups, as was the growth in head circumference. Nitrogen retention was higher than 420 mg/(kg·d) in the latter two groups, compared with 268 g/(kg·d) in the first group. Weight gain was 20% greater in the last group, fed 2.34 g/100 kcal, than in the second group fed 3.2 g/100 kcal, but the difference did not reach statistical significance. In contrast, fat formation (as judged by skinfold thicknesses) was much greater in the last group, which received almost as much protein as the second group but considerably more calories. Chemical indicators of protein intake adequacy (i.e., plasma albumin and transthyretin) were better at higher protein intakes, and mean BUN values were below 3.0 mg/100 mL. The investigators concluded that the lowest protein intake was inadequate, that higher protein intakes were well tolerated, and that a marked increase in calories at the higher protein intake would only lead to fat deposition.

Some of the same investigators later conducted a similar trial with a different set of values for the variables (Kashyap et al., 1988). This time the daily intakes were (1) 2.8 g of protein/kg and 119 kcal/kg (2.36 g/100 kcal); (2) 3.8 g of protein/kg and 120 kcal/kg (3.17 g/100 kcal); and (3) 3.9 g of protein/kg and 142 kcal/kg (2.75 g/100 kcal). The weight gain and nitrogen retention on even the lowest protein intake were greater than intrauterine rates, and plasma indicators of protein nutritional status were considered to be "adequate." The mean BUN value was 8.1 mg/100 mL in the second group fed 3.17 g/100 kcal and 5.5 mg/100 mL in the last group fed 2.75 g/100 kcal, a difference that was thought to reflect a favorable influence of increased calories on nitrogen retention. These relatively high values were not thought to represent toxicity, because BUN values in excess of 10 mg/100 mL were uncommon and no infant developed acidosis. The ratio of protein stored to fat stored in each group reflected the protein contribution to the total energy intake in that group.

In another study in this series, the infants received daily protein and calorie intakes of (1) 3.3 g of protein/kg and 98 kcal/kg (3.36 g/100 kcal); (2) 4.3 g of protein/kg and 117 kcal/kg (3.7 g/100 kcal); and (3) 4.2 g of protein/kg and 142 kcal/kg (2.95 g/100 kcal) (Kashyap et al., 1994). This time, weight gains in the three groups were, respectively, 16.7, 21.7, and 24.0 g/(kg·d); the amounts of protein stored were, respectively, 2.28, 2.75, and 2.80 g/(kg·d); and the fat accretions were, respectively, 2.42, 3.56, and 5.54 g/(kg·d). The ratio of protein deposition to fat deposition was 1.0 in the first group, 0.8 in the second group, and 0.5 in the third group. Apparently, increasing calories above about 120 kcal/(kg·d) would not facilitate protein deposition, even at a high protein intake. Because Kashyap and Heird (1994) surmised that 5 g/(kg·d) would not be tolerated, it appeared that a maximum was being approached. Once again, there was no indication of protein toxicity on these intakes.

These reports, taken together, suggested that adequate protein intake for LBW infants can be provided satisfactorily, but not optimally, by formula with a protein content of approximately 2.5 g/100 kcal, and that little benefit and potential adverse effects are observed when they are fed formula with a protein content greater than 3.6 g/100 kcal [4.3 g/(kg·d) at an energy intake of 120 kcal/(kg·d)].

Neurodevelopment. Several investigators have specifically addressed the effect of protein intake levels on the neurodevelopment of premature infants. Tyson et al. (1983) measured behavioral pattern differences among 76 LBW infants who were fed either mature human milk with a protein content of 1.8 g/100 kcal [intake 118 kcal/(kg·d)] or preterm formula with protein content of 2.6 g/100 kcal [intake 143 kcal/(kg·d)]. The NBAS (Brazelton & Nugent, 1995) was used at a postconceptional age of 37 weeks to measure the infants' coping capacities and adaptive strategies. Higher scores for the orientation scales of the NBAS were noted in the group of infants fed preterm infant formula, whereas no differences were observed in the auditory or visual components of the scales.

Bhatia et al. (1991) evaluated early neurobehavioral effects in 15 healthy preterm infants of a BW less than 1550 g who were fed formulas with contents of low protein (2.2 g/100 kcal), mid protein (2.7 g/100 kcal), or high protein (3.2 g/100 kcal) for 2 weeks. Within 5 days of completing the feeding study, the infants were administered six of the seven items of the NBAS (Lester et al., 1990) by a psychologist who was blinded to the diet assignment. Infants fed the mid protein or high protein intakes achieved significantly higher scores on the orientation, habituation, and stability clusters, although not in the regulation, range, or motor clusters. Associations were also observed between specific behavior clusters and individual plasma essential amino acid levels of these infants. Positive correlations were reported for the orientation, habituation, and stability clusters with plasma levels of valine, isoleucine, leucine, and the sum of large neutral amino acids. These small-scale, short-term studies have not been confirmed or extended. Furthermore, the NBAS scores have been shown to be influenced by a variety of confounding variables (Lester et al., 1990).

Lucas et al. (1990b) used the Bayley Scales of Infant Mental and Psychomotor Development (Bayley, 1969) to evaluate development at 18 months after the term date of 377 AGA infants and small for gestational age (SGA) infants with BWs less than 1850 g. These scales are generally recognized as broadly based measures of developmental functioning in infancy up to the age of 42 months (Bayley, 1993). When applied to infants of ages 18–24 months, the Bayley Mental Development Index (MDI) has been shown to have a small correlation with IQ measured later in childhood.

The first part of the Lucas et al. (1990b) study involved infants of mothers who had elected not to provide breast milk. The infants were randomized to receive term infant formula (2.1 g of protein/100 kcal) or preterm formula (2.5 g of protein/100 kcal) until they weighed 2000 g or were discharged from the neonatal unit, whichever came first. Infants receiving the formula with the higher protein content were found to have a later advantage of 15 points in the MDI scores; the difference was most pronounced in the SGA subset of the study—23 points, almost 1.5 SD. Moreover, there was half the incidence of moderate developmental impairment (Bayley score <86, when 100 is normal) in those receiving the higher protein formula, both in the MDI and in the Psychomotor Development Index.

In the second part of the study, infants were fed their own mothers' expressed breast milk, with the addition of either term infant formula or preterm formula. The diet-related effects on Bayley scores were not as striking as those observed in the first trial. Nevertheless, the developmental test scores also tended to be higher for those infants fed expressed human milk with added preterm formula than in those with added term formula, most significantly in those infants that received for-

mula as more than 50% of total intake. As noted by the authors, the study was designed to investigate outcome differences dependent on total diet, not individual nutrients. Although the protein and energy contents of the preterm formulas were, respectively, 38% and 18% higher than those of the term formula, the differences in mineral intake were even more substantial. Additional possible confounding variables, such as socioeconomic status and other parental factors, were ruled out by the study design or the analyses.

Some of these same children were later followed up with IQ tests at age 7.5–8 years (Lucas et al., 1998). There was a marked sex difference in the impact of the diet, males being much more affected. Males fed the higher protein (and otherwise supplemented) formula had a 12-point advantage in verbal IQ. More infants of both sexes fed the term formula had a verbal IQ less than 85 (31% versus 14%), and they had a higher incidence of cerebral palsy.

These neurodevelopmental studies provide an additional reason for giving a higher protein formula to premature infants compared with term infants. Although these studies do not provide the quantitative information that the growth studies furnish, they do demonstrate that the diet administered to preterm infants in just the first 3 or 4 weeks postpartum has a significant effect on neurodevelopmental status months or years later. The data support the hypothesis (Dobbing, 1974; Heird & Wu, 1996) that a critical neonatal period in brain development of premature infants depends on nutrition.

Protein turnover. Preterm neonates have a higher rate of protein turnover compared to full-term infants (Denne et al., 1996; Poindexter et al., 2001). The effects of graded amounts of protein feeding on rates of proteolysis were not considered in determining recommendations for the protein content of preterm formula. This is an area of research that would benefit from definitive studies.

Toxicity. There seems to be a limit to the neurodevelopmental benefit provided by increased protein intake. Long before the work of Bhatia and Lucas and their respective coworkers, Goldman et al. (1974) demonstrated a significantly increased incidence of low IQ scores in infants with a BW below 1300 g who received high protein diets [6–7.2 g/(kg·d)]. There was also a higher incidence of strabismus in children born at less than 1700 g who received the high protein diet, not remarked upon in other reports.

Toxicity of high protein intakes from formula or supplemented human milk given to preterm infants has not been a major focus of recent clinical studies. A report of higher mortality from an extremely high protein intake [>8 g/(kg·d)] was made by Omans et al. (1961). Previously mentioned were the reports of Goldman et al. (1969) and Goldman et al. (1974) of increased incidences of fever, lethargy, poor feeding, and low IQ in small premature infants receiving protein at 6–7.2 g/(kg·d). Reports of elevated BUN levels with protein intakes greater than about 3–3.5 g/(kg·d) have been described in previous sections and have also been noted by others (Räihä et al., 1976). The last-named study found BUN levels to be 8–20 mg/100 mL with protein intakes of 3.8 g/100 kcal, which was 4.4 g/(kg·d), whereas Kashyap et al. (1988) found BUN levels always below 10 mg/100 mL with protein intakes of 3.8 g/(kg·d). Because the former study used nondialyzable nitrogen to assay protein in the diet and the latter study used total nitrogen, which would misleadingly decrease the apparent difference between the calculated protein intakes, the results are in general agreement. Snyderman et al. (1970) noted that when high protein diets are given to premature infants, biochemical immaturity of metabolic pathways can result in the accumulation of certain amino acids.

In most situations in adult humans or experimental animals, urea production increases with dietary protein intake (Morris, 1992), but this is not established in premature infants. Activities of urea-synthesizing enzymes in human fetal liver develop early but are lower than those in the adult, and the in vivo rates and maturation rates of these enzymes are not known (Boehm et al., 1991). The overall urea-synthesizing capacity is limited in the premature infant, especially those of less than 31 weeks GA (Boehm & Kirchner, 1988). On the eighth day of life in those infants (but not the more mature ones), the urea-synthesizing capacity was not higher with fortified human milk [3 g of protein/(kg·d)] than with unfortified human milk [2.1 g of protein/(kg·d)] (Boehm et al., 1988). Urea-synthesizing capacity seems to rise between the third and eighth weeks of life, although it does not reach adult values during this time (Boehm et al., 1990a; Boehm et al., 1991). Indeed, transient hyperammonemia (values twice normal) associated with low blood levels of arginine and ornithine has been found in many preterm infants fed proprietary formula (Batshaw et al., 1984), but it does not seem to be associated with neurological or developmental problems at 18 months of age and its relationship to protein ingestion is unclear. The hyperammonemia responds to administration of oral arginine (Batshaw et al., 1984).

A more serious form of nongenetic neonatal hyperammonemia (values 50–100 times normal), associated with lethargy and coma, can occur in the first 2 days of life (Ballard et al., 1978). Because it can start as early as 4 hours after birth, it is presumably unrelated to feeding and may result from shunting of blood away from the portal circulation and into the systemic circulation, resulting in a lack of ammonia removal (Tuchman & Georgieff, 1992). Protein hydrolysates administered parenterally can cause hyperammonemia with symptoms in premature infants, especially those who have sepsis (Thomas et al., 1982). So, too, can crystalline amino acid solutions if the arginine content of the mixture is low.

Toxicological issues regarding individual amino acids are addressed in the section on amino acids (below). Menkes et al. (1972) reported about learning disabilities in infants who had developed tyrosinemia with an intake of formula with 3.9% protein (Menkes & Avery, 1963), containing 4.8 g/100 kcal. At 120 kcal/(kg·d), this protein intake was 5.8 g/(kg·d).

Conclusions and recommendations

Almost all of the studies cited in this section considered total nitrogen rather than α-amino nitrogen as representative of the protein content. Some nonprotein nitrogenous compounds are thus included. Nevertheless, this report is concerned with formula derived from animal milk, usually cow, in which the total NPN content is only about 5% of the total nitrogen content (Alston-Mills, 1995). The Expert Panel, therefore, made its recommendations in terms of total protein, i.e., total nitrogen × 6.25.

The factorial and empirical data clearly indicate that mature human milk (derived from mothers delivering term infants) cannot serve as a standard for establishing the minimum protein content for preterm infant formula. Many studies have indicated that insufficient growth is achieved when such milk is the sole source of nutrition (Atkinson et al., 1981; Davies, 1977; Gross, 1983; Lucas et al., 1984; Putet et al., 1984; Tyson et al., 1983). Protein concentrations in milk from mothers delivering prematurely are higher than those in milk from term infants' mothers but also are probably inadequate to support a rate of growth like that of the fetus, especially for infants of BW less than 1000 g (Tudehope et al., 1986).

Under these circumstances, the Expert Panel examined empirical and factorial approaches to determining the appro-

priate content of protein for preterm infant formulas. The empirical approach comprises in part a series of clinical studies that have evaluated growth and a variety of biochemical variables with levels of protein intake ranging from 2 to 8 g/(kg·d). In many cases, these studies of protein were confounded by variations in calorie and mineral content as well. Nevertheless, with the assumption that the quality of cow milk formula protein used in these studies is approximately equivalent to that of human milk, the conclusions of the investigators as listed in the next paragraph appear valid. They are supported by other studies cited in foregoing sections.

Protein intake at

- 2.1 g/(kg·d) is insufficient (Tyson et al., 1983)
- 2.2 g/(kg·d) is still insufficient (Kashyap et al., 1986)
- 2.5 g/(kg·d) produces poor weight gain (Omans et al., 1961)
- 2.5 g/(kg·d) gives a lower nitrogen retention than 3.7 g/(kg·d) (Putet et al., 1987)
- 2.6 g/(kg·d) is minimal (Tyson et al., 1983)
- 2.8 g/(kg·d) is adequate but not optimal (Kashyap et al., 1988)
- 3.0 g/(kg·d) seems minimal (Kashyap & Heird, 1994)
- 3.1 g/(kg·d) from formula gives better weight gain than 2.4 g/(kg·d) from human milk (Putet et al., 1984)
- 3.2 g/(kg·d) from supplemented preterm milk improves weight gain (Kashyap et al., 1992)
- 3.3 g/(kg·d) increases the level of plasma protein markers of protein nutritional status (Polberger et al., 1989)
- 3.4 g/(kg·d) seems adequate (Atkinson et al., 1981)
- 3.4 g/(kg·d) can be used for growth (Schanler et al., 1985a)
- 3.6 g/(kg·d) increases growth and plasma markers of protein nutritional status (Kashyap et al., 1986)
- 3.6 g/(kg·d) still increases plasma essential amino acids, the levels of which correlate with growth (Polberger et al., 1990a)
- 3.8 g/(kg·d) does not produce elevated BUN values at 120 kcal/(kg·d) (Kashyap et al., 1988)
- 3.9 g/(kg·d) may be beneficial in terms of plasma markers of protein nutritional status (Polberger et al., 1990c)
- 3.9 g/(kg·d) does not elevate the serum urea value above 4.1 mmol/L, corresponding to a serum urea nitrogen value of 11 mg/100 mL (Polberger et al., 1990b)
- 3.9 g/(kg·d) does not produce elevated BUN values at 142 kcal/(kg·d) (Kashyap et al., 1988)
- 3.7–4.0 g/(kg·d) for 3 weeks seems to be without adverse effect but does not improve growth when compared with 3.4–3.7 g/(kg·d) (Moro et al., 1995)
- 4.3 g/(kg·d) begins to show diminishing benefit in terms of ratio of protein to fat deposited, plasma amino acid concentration is increased (i.e., tyrosine) but it does not elevate mean BUN values above 10 mg/100 mL (Kashyap et al., 1994)
- 5 g/(kg·d) would probably be excessive (Kashyap & Heird, 1994)
- 5.25 g/(kg·d) gives better weight gain than 2.25 g/(kg·d) (Babson & Bramhall, 1969)
- 5.8 g/(kg·d) can produce undesirable hypertyrosinemia (Menkes & Avery, 1963)
- 5–6 g/(kg·d) raises the BUN value to undesirable levels (Omans et al., 1961)
- 6 g/(kg·d) does not give significantly greater weight gain than 4 g/(kg·d) (Davidson et al., 1967)
- 6–7.2 g/(kg·d) is associated with an increased incidence of fever, poor feeding, lethargy (Goldman et al., 1969), and low IQ scores (Goldman et al., 1974)
- greater than 8 g/(kg·d) is unnecessary and probably toxic (Omans et al., 1961)

Some reports, however, do not agree with this formulation. Protein intake at

- greater than 2.5 g/(kg·d) leads to a proportional increase in urinary loss of nitrogen (Boehm et al., 1987)
- 3 g/(kg·d) may raise the BUN value to an undesirable level (Rönnholm et al., 1982)
- greater than 3 g/(kg·d) provides no weight gain benefit (Omans et al., 1961)
- 3.5 g/(kg·d) would increase the BUN value to 20 mg/100 mL (Rönnholm et al., 1982)

The other component of the empirical approach is the evidence indicating that intakes of 2.1–2.2 g of protein/100 kcal result in poorer neurodevelopment than do intakes of 2.5 or 2.7 g/100 kcal (Bhatia et al., 1991; Lucas et al., 1990b; Lucas et al., 1998). Some of these data led to the recommendations of ESPGAN and the European Commission to consider minima of 2.25 and 2.4 g/100 kcal, respectively (Table 7-1), which would provide 2.7–2.9 g of protein/(kg·d) at an energy intake of 120 kcal/(kg·d).

Minimum. Most of the studies reviewed in this section had calculated the administered protein in terms of g/(kg·d), as summarized in the bulleted lists above. On the basis of these studies, the Expert Panel believed that the minimum protein intake for premature infants should be set at 3.4 g/(kg·d). This corresponds to a minimum intake of 2.5 g/100 kcal at the recommended maximum caloric intake of 135 kcal/(kg·d), the same as the minimum P:E ratio calculated from data on body composition changes during growth discussed earlier. At 120 kcal/(kg·d), the minimum intake would be 2.8 g/100 kcal, which is approximately the P:E ratio delivered by currently available preterm infant formulas in the United States (American Academy of Pediatrics. Committee on Nutrition, 1998). Heird and Wu (1996) calculated that the expected mean rate of weight gain of infants receiving a protein intake of 3.0 g/(kg·d) at an energy intake of 120 kcal/(kg·d) (2.5 g/100 kcal) is 17.7 g/(kg·d), or a little more than the fetus-like rate of growth [16.5 g/(kg·d)] established as a standard.

The factorial approach to evaluating protein requirements yields suggested protein intake levels about 30% higher than these for infants weighing less than 1000 g (3.2–3.3 g/100 kcal) and about 10–15% higher for those weighing more than 1000 g (2.7–2.9 g/100 kcal, Table 7-2). The discrepancy between results with the two approaches results in part from the inclusion in the factorial estimate of an 8–10% margin to provide for individual differences. The Expert Panel gave more credence to the empirical approach.

Maximum. The Expert Panel recommended a maximum protein concentration of 3.6 g/100 kcal in view of the data discussed in Chapter 6. On the basis of the experimental evidence detailed above, the Expert Panel concluded that a protein intake of 4.3 g/(kg·d) appears to be without adverse effect, whereas intakes of 5.0 g/(kg·d) and higher are associated with undesirable consequences. However, at the recommended minimum energy intake of 110 kcal/(kg·d), 3.6 g of protein/100 kcal would equate to a protein intake of 4.0 g/(kg·d), and at the typical energy intake of 120 kcal/(kg·d) it equates to a protein intake of 4.3 g/(kg·d). At the highest recommended energy intake, 135 kcal/(kg·d), a maximum protein concentration of 3.6 g/100 kcal would imply an allowable protein intake of 4.9 g/(kg·d), which is beyond the range that has been studied extensively but below the levels associated with undesirable consequences. However, because an energy intake of 135 kcal/(kg·d) will further enhance use of the ingested protein, the Expert Panel judged that the protein would likely be better utilized at this caloric intake. The Expert Panel noted that the volume of premature infant for-

mula of 81 kcal/100 mL required to supply 135 kcal/(kg·d) is greater than that usually provided by caregivers, although not necessarily greater than that consumed by larger premature infants fed ad libitum. Whether such a high caloric intake with this protein content should be given to the smaller premature infants, who are susceptible to toxicity from protein, is a matter for clinical judgment.

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of protein in preterm infant formula be 2.5 g/100 kcal, specifying this value to refer to total protein (g total nitrogen × 6.25).**

Maximum. **The Expert Panel recommended that the maximum concentration of protein in preterm infant formula be 3.6 g/100 kcal, specifying this to refer to total protein (g of total nitrogen × 6.25).**

Note. **The Expert Panel noted that the scientific evidence is not sufficient to identify the optimal protein content for preterm infant formulas. Detailed studies of appropriate protein-energy intake relationships should be explored to facilitate an understanding of the unique metabolic and physiological characteristics of the premature infant during this period of rapid growth and nutrient accretion. The Expert Panel considered this a high-priority area for future research.**

PROTEIN QUALITY

The discussion that follows does not include consideration of soy protein, because the American Academy of Pediatrics has deemed formula based on soy protein to be inappropriate for LBW infants (American Academy of Pediatrics. Committee on Nutrition, 1998).

Background

Two quality criteria have been specified by the U. S. Food and Drug Administration (FDA) for infant formula: bioavailability and support of healthy growth (Food and Drug Administration, 1996). Bioavailability encompasses the capabilities of being transported (absorbed) and of being utilized for metabolic functions (Southgate, 1989). Healthy growth means normal physical growth during the first 4 months of life when the formula is used as the sole nutrient source (Food and Drug Administration, 1996).

The FDA currently requires that a formula (except those intended for specific inborn errors of metabolism) support adequate growth in the weanling rat protein efficiency ratio (PER) bioassay. The PER of a protein expresses the weight gain of the animals in g/d, divided by protein intake of the animals in g/d, with the test substance used as the sole protein source. Values are assigned relative to a set of control rats maintained with casein (Rupnow, 1992). Although the PER for the control group can vary from 1.8 to 3.3, the control value is arbitrarily set as 2.5 (Satterlee et al., 1977). The PER of any protein can then be described as a percentage of the PER of casein ["casein" refers to the fraction of milk protein that is insoluble at a certain acid pH, 4.6 at 20°C for cow milk and 4.3 in the presence of $CaCl_2$; "whey" refers to the fraction soluble under these conditions (Jensen et al., 1995c)].

The FDA (1996) concluded that this rat bioassay is necessary to establish not only that the essential amino acids are present in the protein source (which could be determined by a chemical analysis), but also that adequate amounts and proportions of these amino acids are available for digestion and absorption by infants. Chemical determination of amino acid composition is insufficient to serve as a quality factor, because

it does not assess this bioavailability issue. This is "particularly important when a [formula] manufacturer is using a novel protein source . . . , a new protein mixture, a new processing method . . . , or a formulation that provides an amount of protein near the minimum required level . . . ." (Food and Drug Administration, 1996).

The PER has been criticized because it does not properly evaluate proteins that are acceptable for maintenance but do not support growth (McLaughlan et al., 1980); this criticism is not relevant to protein quality evaluations for infant formula. More important, however, the PER may be misleading when applied to infant formula because it correlates closely with the content of sulfur-containing amino acids in the test protein. Rats require large amounts of sulfur-containing amino acids, in part to support hair growth, but the need for these substances by the human infant is much less (Fomon & Nelson, 1993a). An additional complication is the limited ability of the weanling rat to hydrolyze lactose, requiring adjustment of the test product (Fomon & Nelson, 1993a). Other criticisms of the PER standard have been its lack of accuracy, poor reproducibility, and high cost (McLaughlan et al., 1980; Sarwar et al., 1989a; Sarwar et al., 1989b).

The Subcommittee on Nutrition of Preterm Infants of the American Academy of Pediatrics Committee on Nutrition (1988) reaffirmed the PER definition of protein. The subcommittee acknowledged the difficulty of defining the appropriate means to assess protein quality and made the following recommendations: "Experimental animals should not be used for the evaluation of whole formulas, given our present state of knowledge about the physiology of the available animals. Animals should only be used for evaluation of components of the formulas, such as for evaluation of protein quality. [Nevertheless], precise analyses of amino acid composition of new proteins . . . are essential for the prediction of nutritional efficacy and patient tolerance of new formula components." (American Academy of Pediatrics. Committee on Nutrition. Subcommittee on Nutrition of Preterm Infants, 1988).

Health Canada *Guidelines* (1995) gave no specifications for considering protein quality. They expressed concern, however, about the practice of adding cow whey protein to cow milk protein in formula to make the whey-to-casein ratio more human-like (60:40, compared with 70:30 in human milk). Although the whey-predominant protein may contain more appropriate levels of aromatic and sulfur-containing amino acids, it may also produce elevated blood levels of threonine (see below). However, infants fed whey-predominant formula generally have metabolic indices and plasma levels of amino acids other than threonine that are closer to those of infants fed pooled mature human milk (American Academy of Pediatrics. Committee on Nutrition, 1998).

Conclusions of the Association of the Food Industries for Particular Nutritional Uses of the European Union (IDACE) (1996) about standards for protein quality included an additional specification: "For an equal energy value, the formula must contain an available quantity of each essential and semi-essential amino acid at least equal to that contained in the reference protein (breast milk). In contrast to [the practice with] standard infant formula, the concentrations of methionine and cystine cannot be added together for calculation purposes." The IDACE also asserted that the main criterion for protein quality is the chemical index, but that if new sources of proteins are to be used the PER and net protein utilization should be evaluated before starting clinical investigations in humans. The PER and net protein utilization must be at least equivalent to those of casein.

Additional IDACE "explanatory remarks" regarding nitro-

gen sources describe the amino acid pattern of breast milk as the standard for infant formula, both term and preterm, citing Rassin (1994). The IDACE warned that the formula content of each amino acid should be based on its content per 100 g of breast milk protein rather than per 100 kcal, as is the case with term infant formula. Otherwise, specific amino acid deficiencies might be masked, because the protein and energy contents of preterm formula should be higher than those of breast milk.

The Expert Panel on the Review of Infant Formula Nutrient Requirements for Preterm Infants recommended that the PER be supplemented by an amino acid score based on the pattern of essential amino acids found in mature human milk (See Table 5-1 in Raiten et al., 1998). The minimum and maximum values for acceptable content of each amino acid are to be obtained by multiplying its fractional content in human milk protein by the minimum and maximum protein allowances recommended by the Expert Panel, 2.5 and 3.6 g/100 kcal, respectively. This amino acid score can be used only as a preliminary indicator of protein quality, not as a substitute for clinical evaluation, because it would not reflect any alteration in protein digestibility. This would require use of an amino acid score corrected for protein digestibility (Joint FAO/WHO Expert Consultation, 1990). The Expert Panel was also aware that an essential amino acid score ignores the importance of amino acids that may be conditionally essential, and that it does not take into account the possible adverse effects of antinutritional factors such as gossypol in cottonseed or lysinoalanine formed during heat processing. Therefore, the clinical testing of infant formulas is still essential for new sources of protein or for major alterations in methods of protein source processing.

Review of the literature

Some of the major issues about protein quality of preterm formula that differ from those for term infant formulas are as follows:

- Whether premature infants should be fed formulas with the whey-to-casein ratio adjusted to be similar to that of human milk (70:30) rather than that of the protein source (18:82 for cow milk)
- How to account for changes in protein digestibility as the preterm gastrointestinal tract matures
- How to account for immaturity of amino acid biosynthetic and catabolic pathways in preterm infants
- Whether amino acid intakes should produce blood levels that reflect the changing intrauterine plasma amino acid patterns during gestation or those of the growing term infant

Whey-to-casein ratio

Whey-predominant infant formula produces concentrations of plasma free amino acids that are more like those in infants fed pooled human milk than does casein-predominant formula (Rassin et al., 1977a). It has a higher concentration of cystine than does casein-predominant formula, which may make it advantageous for the preterm infant (Fomon et al., 1973). It appears that it is less likely to produce lactobezoars than are casein-predominant formulas (Schreiner et al., 1982). Formulas with higher percentages of casein are more likely to lead to the development of metabolic acidosis (Räihä et al., 1976) and to produce higher plasma tyrosine and phenylalanine concentrations, which may be undesirable for the premature infant (Rassin et al., 1977b). In some animals, whey protein (lactalbumin) is more effective in promoting growth and leads to a higher retention of absorbed nitrogen (Berger et al., 1979). A discussion of the whey-to-casein ratio issue with

respect to metabolic acidosis in premature infants is included in Appendix A.

Several studies have evaluated growth and biochemical factors in response to feedings comprising different cow milk protein compositions. Berger et al. (1979) measured growth, biochemical variables (e.g., pH, albumin, and urea), and nitrogen balances of preterm LBW and term SGA infants who were fed either a "curd" formula with whole cow milk protein (82% of the nitrogen as casein) or a "curd and whey" formula (41% of the nitrogen as casein). The formulas had the same total protein content (2.2 g/100 kcal) and were isocaloric (67 kcal/100 mL). The investigators found that the preterm LBW infants fed the curd and whey formula had greater weight gain during the first 3 weeks of the study than those fed curd formula; there were no differences between the SGA term infants fed the two formulas. Nitrogen absorption was higher and urea excretion lower in those preterm infants fed the curd and whey formula. The investigators concluded that the whey fraction of cow milk provided advantages in terms of protein balance, but they pointed out that the advantages were marginal and temporary.

Kashyap et al. (1987) compared some of the same variables, as well as plasma and urinary amino acids, between infants fed two cow milk-based premature infant formulas containing similar protein and energy contents but differing in amino acid composition. Casein-predominant (whey-to-casein ratio of 18:82) formula or whey-predominant (whey-to-casein ratio of 60:40) formula was fed to 20 infants from the time they reached full feedings until body weight reached 2200 g. No differences were observed in growth, nitrogen retention, BUN, or acid-base status. As Rassin et al. (1977a) have observed, plasma tyrosine concentrations were higher in infants fed the casein-predominant formula, and plasma cyst(e)ine and threonine concentrations were higher in infants fed the whey-predominant formula. Whey-predominant formula provides higher intakes of cysteine (Rassin et al., 1977b), which could be beneficial to LBW infants, whose biosynthetic capability for cysteine may be low (see below).

Tikanoja et al. (1982a; 1982b) also evaluated the acute effects of protein sources with differences in the whey-to-casein ratio on the amino acid profiles of LBW infants. In their study, 31 infants were fed pooled human milk (protein content of 0.77 g/100 mL), a casein-predominant formula (protein content of 1.45 g/100 mL), or a whey-predominant formula (protein content of 1.5 g/100 mL); amino acid levels were assessed at intervals after a single feeding. Total plasma amino acid levels were higher in the infants fed either formula, reflecting the differences in protein content of the formulas and human milk. The analysis revealed that the branched-chain amino acid levels had the highest increase postprandially and that glycine levels decreased after feedings. The investigators suggested that for long-term studies, samples should be taken immediately before feeding, but that postprandial amino acid measurements might be a useful way to test an infant's ability to handle a protein or amino acid load.

Protein quality has also been evaluated in clinical studies that provided various sources of protein for supplementation of mature or expressed human milk. For example, the study by Moro et al. (1991) evaluated growth, biochemical effects, and plasma amino acid profiles of infants fed human milk fortified with either protein from mature human milk (n = 9) or a cow milk protein-peptide-amino acid preparation computer-designed to mimic the amino acid composition of the nutritionally available human milk proteins (n = 12). The two kinds of fortified milk provided similar total protein contents (2.6–2.8 g/100 kcal) during a 2-week study period. There was little

difference in protein intake between the cow milk-fortified or human milk-fortified groups [3.3 and 3.6 g/(kg·d), respectively]. Growth patterns (weight, length, and head circumference) for both groups approximated intrauterine accretion rates, and there were no differences between the groups. Biochemical analyses revealed no differences in BUN, total protein, albumin, pH, or amino acid profiles.

Some of these same investigators evaluated the growth and biochemical effects associated with different levels of cow milk protein fortification of expressed human milk (Moro et al., 1995). The human milk was fortified with mature human milk protein, a fixed content of cow milk protein, or cow milk protein fortification level that was adjusted twice weekly on the basis of BUN levels. Diets were continued and growth variables were measured until the time of hospital discharge (when the infants weighed ~2200 g). The protein intake levels for the human milk-fortified and fixed-content cow milk-fortified fed infants were similar [3.35 and 3.67 g/(kg·d), respectively], whereas the adjusted fortification group had higher protein intakes [3.73–4.0 g/(kg·d)]. Weight, head circumference, and length measurements were similar among the study groups throughout the study, as were the biochemical markers. Plasma amino acid profiles were similar in the human milk-fortified and fixed-content cow milk-fortified groups, with two exceptions: proline levels were higher in the human milk-fortified group, and taurine levels were higher in the fixed-content cow milk-fortified group. Most plasma amino acid levels were significantly higher in the group fed cow milk-fortified milk with adjusted protein intake levels. In general, the increased levels of these amino acids reflected the increased protein intake of this group. In this study, protein intake levels of up to 4.0 g/(kg·d) did not have any adverse effects. However, as indicated in this study and others, this level of protein intake also did not promote greater growth, so no benefit was demonstrated.

At least three different standards for the plasma amino acid profile have been proposed for feedings provided to LBW infants (Micheli & Schutz, 1993):

1. The amino acid concentrations of cord blood, which are stable over the last trimester of pregnancy (Hanning & Zlotkin, 1989)
2. Plasma amino acid levels of rapidly growing preterm infants receiving their own mother's milk (Atkinson & Hanning, 1989)
3. Plasma amino acid levels of healthy breast-fed term infants (Micheli & Schutz, 1993)

The Expert Panel concluded that considerable data indicate that the amount of protein provided to LBW infants by banked mature human milk is insufficient to support growth rates that approximate the intrauterine accretion rates or to provide support for normal psychomotor development. Similarly, it may not follow that the protein quality of infant formulas can be based on the amino acid composition of human milk. A variation suggested by Polberger et al. (1990b) is that the standard for protein quality should be that which produces a plasma amino acid pattern that reflects the pattern in optimally growing LBW infants fed only human milk proteins.

SPECIFIC AMINO ACIDS

In studies of protein quality, the assessment of the protein and amino acid compositions of various formula preparations is usually conducted by comparing them to human milk. This assessment must consider that cow milk formula contains more protein per unit volume than human milk and that there are differences in the composition of both whey and casein from the two species. The result is a marked difference in the plasma amino acid patterns of infants fed human milk or formula based on cow milk protein (Rassin, 1994).

The amino acid pattern of preterm milk is generally similar to that of term milk. Preterm milk and term milk provide more taurine and cysteine than does formula (Atkinson et al., 1981), and the relative amounts of threonine and lysine, amino acids for which the catabolic capacity of very preterm infants may be low, are less in preterm milk than in whey-predominant formula (Atkinson & Hanning, 1989).

Basing values for desirable amino acid intakes of preterm infants on values from the composition of preterm human milk is inadvisable. First, the absorption of amino acids present in human milk may be incomplete because of nonuniform hydrolysis of various proteins by the preterm infant (Atkinson & Hanning, 1989). Second, the total amino acid content of preterm milk is limiting for preterm infants after the first 2 weeks of life (see the section on total protein, above). Third, appropriate amino acid composition of protein and amino acid intake requirements may change throughout the neonatal period from the initiation of feedings to the conversion to term formula later in infancy. Last, as has been noted before, there is no reason to believe that the composition of preterm milk is adaptive to the needs of the preterm infant.

The classically defined essential amino acids have been supplemented by a group deemed "conditionally essential" in preterm infants, because the temporary metabolic and physiological immaturity of these infants often leads to a delayed onset of adequate endogenous synthesis.

Classically essential amino acids based on growth and nitrogen balance data in adults (Rassin, 1994) include

- Threonine
- Valine
- Isoleucine
- Leucine
- Phenylalanine
- Lysine
- Methionine
- Tryptophan

Conditionally essential amino acids during early development because of the biochemical immaturity of the preterm infant (Rassin, 1994) include

- Cysteine
- Taurine
- Tyrosine
- Histidine
- Arginine
- Glycine

In addition, almost every amino acid classified as nonessential in adults has been at some time proposed as conditionally essential in preterm infants [see, for example, Jackson et al. (1997) and Miller et al. (1995)].

To reduce the risk of cow milk protein sensitivity in preterm infants, the use of protein hydrolysate formulas has sometimes been proposed. However, the nutrient value of a hydrolysate may not be equivalent to that of the native protein (Rigo & Senterre, 1994). In particular, whey hydrolysate or whey-casein hydrolysate formula may produce less growth and less nitrogen absorption and retention than conventional formula or human milk, as well as lower plasma protein concentrations, increased plasma threonine concentrations, decreased plasma phenylalanine and tyrosine concentrations, and other plasma amino acid perturbations (Rigo et al., 1995). Enzymatic or chemical hydrolysis destroys the physical structure of the protein, making many more amino acids available for chemical reaction, and heat treatment used to denature proteins may induce reaction products that reduce the bioavailability

of amino acids (Lee, 1992). Nutritional studies of target infants are necessary to establish that specific protein hydrolysate formulas are as effective as native protein-based formulas.

Plasma amino acid patterns of LBW infants may be influenced not only by the quantity and quality of protein ingested (Kashyap et al., 1987; Polberger et al., 1990a; Scott et al., 1985) but also by the energy supply, GA (Rigo & Senterre, 1987), rate of growth (Scott et al., 1985), time of sampling with respect to feeding (Tikanoja et al., 1982a; Tikanoja et al., 1982b), and maturation of enzymes that synthesize and metabolize amino acids (Räihä, 1974). Another consideration is the contribution to the plasma amino acid pattern of concomitantly administered parenteral nutrition.

Cysteine

A summary of human milk composition derived from a number of studies appeared in the LSRO report: *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a). The mean concentration for cysteine was 19.3 mg/g of total protein, or 49 mg/100 kcal. Despite the listing of cysteine in the table of indispensable amino acids in Raiten et al., no specific recommendation was made for its concentration in formula. However, for the purposes of assessing protein quality, the report recommended that cysteine and methionine values be combined at 58 mg/1.7 g of true protein (34 mg/g), which would be 85–123 mg/100 kcal at 2.5–3.6 g of protein/100 kcal. The content of cysteine in milk-based formulas marketed at that time was reported to be 25–32 mg/100 kcal and the methionine content was 55–62 mg/100 kcal (Raiten et al., 1998a). The cystine (disulfide formed from two molecules of cysteine) requirement for normal growth by full-term infants fed a soy-isolate formula has been reported to be less than 25 mg/100 kcal on a diet of 1.62 g of protein/100 kcal (Fomon et al., 1973).

ESPGAN (1987) did not make a specific recommendation about a cysteine requirement for preterm infants but stated that the amino acid content of the protein in the formula should not fall below that of breast milk. Presumably this means each amino acid. Health Canada (1995) also made no specific recommendation for cysteine except to refer to "concerns raised with respect to possible delayed endogenous synthesis of certain amino acids such as cysteine." IDACE (1996) recommended that the concentration of cysteine in formula for LBW infants be comparable to that of breast milk, which was taken to be 13 mg/g of protein, rather than that of cow milk, taken to be 9 mg/g. At a protein concentration of 1.1 g/100 mL in human milk, this is 21 mg/100 kcal.

Review of the literature. The initial suggestion that cysteine might be required in the diet of premature infants came from Snyderman (1971). She found that the nitrogen retention and rate of weight gain of 2- to 4-month-old prematurely born infants were lower when cysteine was removed from an otherwise adequate synthetic diet, and plasma cyst(e)ine (cysteine plus cystine) concentrations were lower. According to her observations, 85 mg/(kg·d) was adequate to support growth but 66 mg/(kg·d) was not. The essential nature of cysteine in the diet of premature infants has been understood since then to be a result of inadequate enzymatic activity of cystathionase, a critical enzyme in its synthesis (Gaull et al., 1972).

Cysteine is synthesized in the body by a pathway beginning with the essential amino acid methionine (Figure 7-1). In animal experiments, cyst(e)ine has been found capable of sparing (that is, partially replacing) a large fraction of the methionine requirement (Finkelstein et al., 1988; Womack & Rose, 1941). The last enzyme in the transsulfuration pathway for the conversion of methionine to cysteine, cystathionase, is
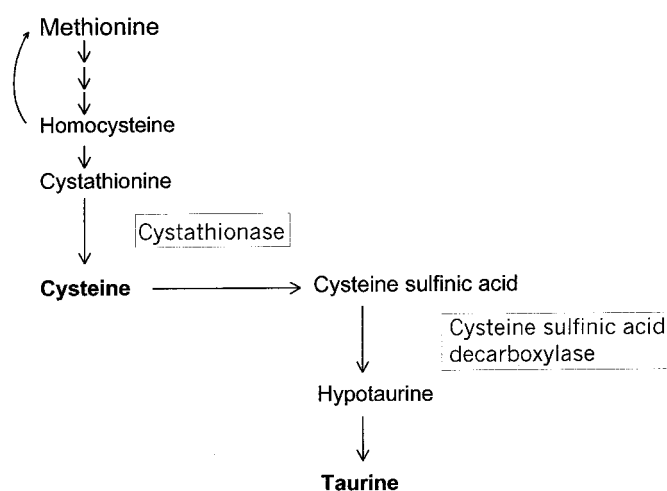

FIGURE 7-1 Transsulfuration pathway.

expressed at very low levels in fetal liver (Räihä, 1974). The low cystathionase activity is correlated with higher levels of methionine. Infants being supported with glucose-electrolyte solutions during the first 2 days of life because of prematurity or respiratory distress syndrome (RDS) quickly developed low blood cystine levels (Pohlandt, 1974). When the infants were treated with a mixture of synthetic amino acids containing methionine but lacking cystine, the cystine concentrations did not rise despite elevated methionine concentrations. It could be concluded that cysteine formation was limited by catalyst rather than by substrate.

Cystathionase activity increases rapidly in the liver after birth and reaches mature levels at about 3 months of age. Activity is also high in the adrenals and kidney. When Zlotkin and Anderson (1982) demonstrated this, they postulated that given adequate methionine, preterm infants might be able to synthesize sufficient cysteine to meet their needs. This would not be consistent with the results of Snyderman (1971) in prematurely born infants of 2–4 months of age, described above. Moreover, plasma cystine concentration decreases markedly in healthy adults fed intravenously with casein hydrolysate solutions that are free of cystine but rich in methionine, suggesting that the synthesis of cysteine in extrahepatic tissues is limited even in adults (Stegink & Besten, 1972) or that intravenous methionine is metabolized differently from oral methionine. It is important to recognize, however, that the control of flux through tissues cannot be defined by looking at one step in isolation (Kacser & Burns, 1995).

The plasma amino acid imbalance produced by inadequate dietary cysteine was demonstrated by measurements of plasma and urinary methionine and cystine in preterm infants (GA of 28–36 weeks) who were receiving pooled human milk (presumably containing about 1 g of protein/100 mL) or cow milk formulas with low (1.5 g/100 mL) or high (3 g/100 mL) protein content, either casein-predominant or whey-predominant formulas (Gaull et al., 1977b). The mean plasma and urinary methionine and cystathionine levels of infants fed any of the formulas were much higher than those of the infants fed pooled human milk, generally reflecting the amount of protein ingested.

The whey-predominant formula had a cystine concentration more than twice that of breast milk or any of the other formulas, but feeding of this formula at the low protein level produced the same blood cystine concentrations as did feeding breast milk. However, feeding it at the high protein level

raised the blood cystine concentrations about 50%. The casein-predominant formula had a mean cystine concentration slightly lower than that of breast milk but a methionine concentration almost three times higher; feeding of this formula at a low protein level also produced the same blood cystine concentration as did feeding breast milk. This suggested that the conversion of methionine to cysteine was not rate limited by substrate. Feeding of this formula at the high protein level raised the plasma cystine concentration, but only after the second week of life. The investigators interpreted this as suggesting maturation of the cystathionase function, so that at 2–3 weeks of life methionine can be converted to cysteine to a greater extent than it can be earlier (Gaull et al., 1977b). This is consistent with the report of Zlotkin and Anderson (1982) cited previously. Determination of plasma cystine alone underestimates the total cyst(e)ine content, as about half of it is contained in a mixed disulfide with plasma proteins (Malloy et al., 1984).

A stable isotope tracer study of week-old infants of a mean GA of 30 weeks failed to demonstrate any incorporation of label into plasma cysteine from D-[U-^{13}C]glucose (200 mg/kg infused during 4 hours) (Miller et al., 1995), but it is possible that compartmentation of cysteine synthesis prevented labeling of free cysteine in plasma (Keshen et al., 1997; Miller et al., 1996). Nevertheless, recent work supports the idea that metabolic flux through the transsulfuration pathway is inadequate to meet the cysteine requirements of premature infants (Viña et al., 1995). Plasma cystathionine concentrations are higher in premature infants of less than 33 weeks GA than in older premature or full-term infants, and plasma cysteine concentrations are lower in both groups of premature infants than in full-term infants. Moreover, the concentrations of plasma glutathione are apparently limited, like those of cysteine, as a result of a requirement for the latter as a component of the former. In support of this idea, the directly observed synthesis of glutathione from methionine by red blood cells (RBCs) from the smaller premature infants was slower than that in RBCs from the premature infants of GA greater than 32 weeks or the full-term infants. However, although reduced glutathione concentrations are much lower in LBW infants than in older children and adults, oxidized glutathione concentrations are correspondingly higher (Smith et al., 1993). This result does not provide information on any difference in the rate of incorporation of cysteine into glutathione in premature infants.

Nevertheless, the cysteine requirement of most premature infants may not be as high as that suggested by the work of Snyderman (1971). It was calculated by Uauy et al. (1993) that premature infant formulas constructed from modified cow milk proteins (60% whey proteins and 40% casein) without cysteine supplementation would provide only 45–55 mg/(kg·d) of cyst(e)ine at 3–3.6 g of protein/(kg·d). Despite this, however, these formulas seem to support adequate growth. Uauy et al. (1993) noted that Snyderman did not report the methionine intake of the infants studied; if this were less than the 75–90 mg/(kg·d) supplied to infants fed modern formulas, this could explain the higher apparent cysteine requirement she observed. This implies that there is sufficient cystathionase activity to provide some of the cysteine requirement from the methionine pathway if intake of methionine is generous (Zlotkin & Anderson, 1982).

The plasma concentration of cyst(e)ine was maintained within normal limits in postsurgical preterm infants (GA of 34.5 ± 2.4 weeks, postnatal age 19 ± 18 days) by the addition of cysteine [77 mg/(kg·d)] to their intravenous nutrition regimen (Helms et al., 1995). The infants in this study were receiving at least 2.5 g/(kg·d) of other amino acids and more than 60 kcal/(kg·d). But similar supplements in comparable groups of parenterally fed infants failed to produce changes in nitrogen retention, weight, or length during 6-day periods beginning at 4–15 days of postnatal life (Zlotkin et al., 1981a); the only suggestively favorable result was an increase in 3-methylhistidine excretion, perhaps indicating an increased muscle mass but possibly increased muscle catabolism instead. However, the observation periods may have been too short and/or the infants too large to demonstrate growth effects: cystathionase activity increases with postnatal age and possibly also with GA (Gaull et al., 1972; Gaull et al., 1977a). Alternatively, a low parenteral tyrosine intake may have obscured any positive effect of cysteine supplementation on growth and nitrogen retention (Uauy et al., 1993).

Toxicity related to cysteine administration in preterm infants has been associated only with the acid load provided when the hydrochloride salt was added to parenteral fluid regimens, especially in infants of BW less than 1250 g (Uauy et al., 1993). Appropriate counteracting base intake can alleviate this problem (Laine et al., 1991). No reports of toxicity from cysteine in formula have been found.

Conclusions and recommendations

It is very difficult to be specific about a cysteine requirement that seems to change rapidly with postnatal age. The critical experiments on cysteine deprivation performed in the 1960s (Snyderman, 1971) are not now considered permissible and cannot be repeated. Nevertheless, those experiments seemed to prove that a partial dietary requirement persists in premature infants until at least 2–4 months of age. Uauy et al. (1993) suggested an intake of 0.2–0.5 mmol of cysteine equivalents/(kg·d) in LBW infants fed human milk or conventional formulas. This amounts to 24–61 mg/(kg·d), or 20–51 mg/100 kcal at 120 kcal/(kg·d). A controlled study of cysteine addition to protein-supplemented human milk fed to premature infants has not been found by this Expert Panel.

Recommendations for cysteine content in preterm formula are presented with methionine in Table 7-3.

Threonine

This amino acid is generally accepted as an essential amino acid for humans of all ages. As with all essential amino acids, its minimum intake by preterm infants should be at least equivalent to the amount present in 1 g of human milk protein [45.2 mg/g (Raiten et al., 1998a)], multiplied by the recommended minimum intake of protein, in g (2.5 g/100 kcal; see above). This computation leads to 113 mg/100 kcal.

Concern about threonine intake does not center on the uncertainty about the minimum required amount but rather on the possibility of its toxicity as a result of immature processes for its degradation (Rigo & Senterre, 1980). High concentrations of single amino acids can inhibit uptake of other amino acids into the brain and interfere with protein synthesis there (ESPGAN Committee on Nutrition, 1977), but in most animal studies threonine seems to be one of the less toxic amino acids (Peng et al., 1973; Sauberlich, 1961), perhaps in part because of an induction of the threonine degradation capacity (Kang-Lee & Harper, 1978). This is true even though the increase in brain threonine in relation to its plasma concentration is higher for threonine than for most other essential amino acids (Gustafson et al., 1986). Nevertheless, a high threonine diet for rats leads to a decrease in food intake and growth (Muramatsu et al., 1971). Also, a recent study in young rats suggests that increasing threonine in rat plasma by diet

raises brain glycine levels, thereby affecting the neurotransmitter balance; this could have undesirable effects on brain development during early postnatal life (Boehm et al., 1998).

Infants fed cow whey- or casein-predominant formulas, especially the former, have higher plasma threonine levels than those fed human milk (Rassin et al., 1977a). This is due to use of the sweet whey fraction, which contains high threonine glycomacropeptide (Boehm et al., 1998). Rigo and Senterre (1980) studied the effect of different protein sources on threonine levels in 163 LBW infants who were fed human milk, parenteral nutrition, or various "adapted" formulas. Positive correlations were found between the intake of threonine and plasma levels of threonine, especially in infants of lowest GA. Threonine oxidation capacity may be a little higher in infants fed breast milk (24% of intake) than in those fed formula containing comparable amounts of that amino acid (17% of intake) (Darling et al., 1999). The plasma threonine levels were lower in infants fed preterm milk than in those fed formula, although there were only four or five subjects in each group.

Whey-predominant formula produces a higher plasma concentration of threonine than does casein-predominant formula (Kashyap et al., 1987). In addition, although plasma amino acids showed a number of differences when term infants given a whey-predominant formula (60:40 whey-to-casein ratio) were compared with those given a purely whey hydrolysate formula, all values were in the normal range except for plasma threonine concentrations in infants given the hydrolysate (Hauser et al., 1997). Average plasma threonine values at 2.5–3 hours after a feeding were 224 μmol/L in the whey hydrolysate group, compared with 159 μmol/L in the whey-predominant formula group. Both groups had greater plasma threonine concentrations than the 24–174 μmol/L reported for breast-fed term infants. Growth in weight and length were the same in both groups. There was no report of toxicity, but 20% of the infants rejected the hydrolysate. Likewise, no untoward effects were noted when the serum threonine concentration rose to 259 $\pm$ 88 μmol/L in premature infants fed human milk fortified with a cow whey protein (Polberger et al., 1999).

Little direct evidence provides support for the idea that threonine might be toxic to premature infants at the levels that likely could be achieved by protein fortification. However, when Rigo and Senterre (1980) found a correlation between threonine intake and serum threonine concentration, they also noted that levels were highest in infants of the lowest postconceptional age. They suggested that because of reports of toxicity in experimental animals and in infants with disorders of threonine metabolism (Reddi, 1978), threonine intake of preterm infants should be limited to 1200 μmol (143 mg)/(kg·d) (119 mg/100 kcal). However, feeding studies with this amount of threonine were not conducted, and there was wide variation in plasma concentrations of threonine at all levels of feeding.

Recommendations for threonine content in preterm formula are presented in Table 7-3.

Tyrosine

Phenylalanine, the metabolic precursor of tyrosine, is one of the universally recognized essential amino acids of mammals. In the mammalian liver, phenylalanine is converted to tyrosine by phenylalanine hydroxylase. On the basis of some early studies, it was surmised that tyrosine is essential in the diet of premature infants, but the activity of phenylalanine hydroxylase is well developed in human fetal liver, reaching

near adult levels well before birth (Räihä, 1973), and phenylalanine tolerance tests are usually normal (Menkes et al., 1966). A direct in vivo test of the conversion of phenylalanine to tyrosine showed that there is no immaturity of this process in infants as young as 26 weeks GA (Denne et al., 1996). Moreover, the enzyme activity increases normally in response to provision of substrate (Kilani et al., 1995). Increasing the parenteral phenylalanine input to neonates beyond its usual concentration in protein can increase the formation of tyrosine, but it may also lead to an inadvisable elevation of the phenylalanine level (Roberts et al., 1998).

Deeply entrenched is the idea that tyrosine is a conditionally essential amino acids for most premature and some full-term infants (Uauy et al., 1993). This idea apparently arose from the publication by Snyderman (1971) of data on a single 10-day-old full-term infant whose plasma tyrosine level, nitrogen retention, and rate of weight gain all decreased moderately during 30 days of a tyrosine-free diet. All these variables returned to their previous level after tyrosine was added to the diet at 50 mg/(kg·d). The Expert Panel could find no other data helpful in specifying a minimum intake.

In contrast, tyrosine intake to excess may be toxic. One of the most common human disorders of amino acid metabolism is transient neonatal tyrosinemia (Scriver & Rosenberg, 1973). It is manifested by the increased excretion of several metabolic products and by-products of tyrosine metabolism (Levine et al., 1941). Premature infants are most often affected. The condition is thought to result from a lag in the development of the activities of enzymes involved in tyrosine degradation.

The first two enzymes in the degradation of tyrosine are tyrosine aminotransferase and *p*-hydroxyphenylpyruvic acid oxidase. The latter enzyme is thought to be late to mature in the fetus and newborn and becomes rate limiting for the catabolism of phenylalanine and tyrosine with high intakes of protein (Scriver & Rosenberg, 1973). Some patients respond to ascorbic acid (100 mg four times daily), which is thought to protect the oxidase against substrate inhibition, or to reduction of the protein intake to 2–3 g/(kg·d) (Goldsmith & Laberge, 1989). A few cases may be due to delayed maturation of tyrosine aminotransferase (Mitchell et al., 1995). Tyrosine levels may exceed 20 mg/100 mL, but typically resolve by 1 month of age (Mathews & Partington, 1964). Protein intake levels below 5 g/(kg·d) seem to be without adverse effect (Mathews & Partington, 1964).

No unequivocal ill effects were found in hypertyrosinemic infants at the time of the gross disturbance in tyrosine metabolism or at follow-up studies of physical development or developmental quotient up to age 4 years (Menkes et al., 1966; Partington et al., 1968). Nevertheless, it is possible that hypertyrosinemia is associated with subsequent learning disabilities (Mamunes et al., 1976; Menkes et al., 1972; Rice et al., 1989). The formula fed to the hypertyrosinemic infants in the study by Mamunes et al. (1976) delivered 5.4 g of protein/(kg·d). In Menkes et al. (1972), formula provided 4.8 g/100 kcal (Menkes & Avery, 1963), which would be 5.8 g/(kg·d) at an energy intake of 120 kcal/(kg·d). Because cow milk protein is 5.6% tyrosine (Swaisgood, 1995), 302 mg/(kg·d) of tyrosine was fed in the study by Mamunes et al. (1976) and 325 mg/(kg·d) in the study by Menkes and Avery (1963). In Rice et al. (1989), the composition of the formula was not stated, but the investigators suggested that neonatal protein intakes be limited to 3 g/(kg·d).

The Expert Panel noted that the maximum recommended concentration of protein in formula, 3.6 g/100 kcal, could provide as much as 4.9 g of protein/(kg·d) on an energy intake

of 135 kcal/(kg·d). This would probably still provide a sufficient margin for tyrosine intake in the occasional occurrence of delayed maturation of tyrosine aminotransferase.

Conclusions. In summary, the Expert Panel found no unequivocal evidence for a tyrosine requirement of premature infants whose phenylalanine intake is adequate. The Expert Panel noted that the cow milk proteins commonly used in the preparation of preterm formula contain 5–6% tyrosine by weight, approximately equally distributed between whey and casein components (Swaisgood, 1995), compared with 5% of each of the major whey and casein proteins in human milk (Atkinson et al., 1995). Kashyap et al. (1987) reported the tyrosine of the protein in whey-predominant cow milk formula, meaning that the whey-to-casein ratio was 60:40, to be 4.3%. Thus, preterm infants receiving formula containing protein in the concentration range recommended by the Expert Panel (2.5–3.6 g/100 kcal) will receive tyrosine intakes of 108–155 mg/100 kcal, or 119–209 mg/(kg·d). Even the least of these numbers is greater than the 50 mg/(kg·d) used by Snyderman (1971) to correct "tyrosine deficiency." Whether tyrosine is truly essential in newborn premature infants needs to be studied directly (Roberts et al., 1998).

Recommendations for tyrosine content in preterm formula are presented with phenylalanine in Table 7-3.

Arginine

Arginine is a key participant in the urea cycle, a process necessary for the excretion of ammonia. The metabolism of arginine in preterm infants is important from the perspective of both deficiency and toxicity. Arginine is an essential amino acid in many animals (Costello et al., 1980; Ha et al., 1978), which may suffer poor growth, tremors, excessive salivation, orotic aciduria, and hyperammonemia when consuming arginine-deficient diets.

Studies by Snyderman et al. (1970) indicated that premature infants fed protein at 2 g/(kg·d) had plasma arginine levels that were more than 1 SD below those of similar infants fed protein at 3 g/(kg·d). It would thus seem that premature infants have a higher dietary requirement for arginine than do term infants, perhaps because their initially low protein intake diminishes the activity of argininosuccinate lyase (Stephen & Waterlow, 1968). It is known that the activities of the five hepatic enzymes of the urea cycle in several species of animals respond to changes in the protein content of the diet (Nuzum & Snodgrass, 1971).

Information on the arginine requirement of term infants could establish a floor above which to estimate the requirement of preterm infants. In addition to the data of Snyderman et al. (1970) (cited above), Janas et al. (1987) made observations on the plasma amino acid concentrations in term infants receiving human milk or formulas of various whey-to-casein ratios at 1.8 g of protein/100 kcal and 67 kcal/100 mL. When the infants were 4 and 8 weeks of age, plasma arginine concentrations (about 100 μmol/L) did not vary with the type of feedings, all of which provided about 350 μmol/(kg·d) of arginine (51 mg/100 kcal). Brooke et al. (1987) found plasma arginine levels to be only about half as high in premature infants fed premature infant formula (2.5 g of protein/100 kcal) as in those fed expressed breast milk (2.1 g of protein/100 kcal). Except for taurine, no other amino acid was significantly lower in plasma from formula-fed infants. Tikanoja et al. (1982a) also reported a two-fold higher plasma arginine concentration before feedings in breast-fed premature infants than in formula-fed premature infants. That group of researchers

found that formula contained 0.18 mmol of arginine/g of protein, and human milk, 0.25 mmol/g.

Other evidence that arginine is conditionally essential came from a report by Heird et al. (1972) of hyperammonemia associated with seizures in infants given parenteral mixtures of crystalline amino acid solutions that were relatively low in arginine. This hyperammonemia was corrected by arginine infusions and could be prevented by arginine supplementation of the parenteral nutrition solution. It was not due to preformed ammonia in the infusate, which may have caused some other hyperammonemic events in infants (Johnson et al., 1972). Hyperammonemia occurring in adults receiving intravenous mixtures containing all the essential amino acids was responsive to arginine (Fahey, 1957). Although the protein hydrolysates used at that time may have contained ammonia, the use of NH_4Cl to treat metabolic acidosis, in amounts equivalent to the free NH_3 in the hydrolysates, does not cause hyperammonemia. This makes it reasonable that low arginine intake was the responsible agent in these cases.

One of the two forms of transient hyperammonemia of the newborn not related to overt dietary arginine deficiency is asymptomatic (Batshaw & Brusilow, 1978). More than 50% of infants of BW less than 2500 g demonstrate asymptomatic hyperammonemia, with plasma ammonium levels two to three times normal (Batshaw et al., 1984). Concentrations of several amino acids are at low preprandial levels in the plasma, particularly arginine and ornithine, but not below the normal range. Arginine supplementation [1–2 mmol/(kg·d)] for 1–3 weeks can normalize the ammonia level, reportedly within 24 hours (Batshaw, 1984), and raise the arginine and ornithine levels above normal. Early elevated plasma ammonium levels in these patients, treated or not, did not correlate with neurological function (auditory response and habituation) during the first month of life or with IQ scores at 30 months. The investigator concluded that the data did not support a need for treatment of transient asymptomatic hyperammonemia of the premature newborn. It is not certain whether the study had an adequate number of subjects or a sufficient duration of follow-up to detect subtle alterations of brain function.

Symptomatic transient hyperammonemia of the newborn is associated with plasma ammonium levels 100 times higher than normal. It leads to neurological sequelae dependent on the duration of hyperammonemic coma and does not respond to arginine (Batshaw, 1984). Hyperammonemia resulting from inborn errors of urea synthesis results in mental retardation and cerebral palsy unless the hyperammonemia is prevented or treated rapidly by dialysis (Batshaw, 1984).

Arginine also has an important metabolic role as the substrate for nitric oxide (NO) synthesis. Plasma arginine is decreased on the third day of life in premature infants with RDS. There is an inverse correlation between the oxygenation index (which increases with increasing severity of RDS) and the arginine concentration, but no similar correlation between the oxygenation index and the total plasma amino acid concentration. This suggests either that arginine is consumed by the production of large amounts of NO in the pulmonary circulation or that a relative lack of arginine may interfere with NO production and contribute to an increased severity of RDS (Zamora et al., 1998).

Plasma arginine levels of infants with necrotizing enterocolitis are decreased on the third day of life, but ammonia concentrations are increased (Zamora et al., 1997). Intestinal ischemia or just the presence of nutrients in the premature gut may increase permeability, favoring the penetration of bacteria

or endotoxins through the mucosa. NO is part of the normal response to this—regulating intestinal blood flow, protecting the mucosa, and modulating the inflammatory response. The arginine concentration could be decreased by the combination of a limited arginine intake, a limited arginine production, and an increased metabolic demand for NO synthesis (Zamora et al., 1997). In light of these findings, the potential benefit of arginine supplementation deserves evaluation (Zamora et al., 1997).

However, arginine excess can also have adverse consequences. One possible mechanism is an increase in inducible nitric oxide synthase (Peters et al., 1999), which produces tissue injury. Hyperargininemia caused by congenital hepatic arginase deficiency is associated with mental retardation, growth failure, and neurological abnormalities (Brusilow & Horwich, 1995). These patients do not always develop persistent or acute hyperammonemia, perhaps because of a second arginase locus, expressed primarily in the kidney (Iyer et al., 1998). Moreover, neurological damage associated with hyperargininemia may precede the hyperammonemia. Blood arginine concentrations in arginase deficiency can be as high as 1500 μM, but other diamino acids are also increased in plasma.

Conclusions and recommendations.

Minimum. The studies cited in this section, limited as they are, suggest that it would be prudent to treat arginine as a conditionally essential amino acid for preterm infants and recommend a minimum value for its intake. The observations of Heird et al. (1972), Heird and Winters, (1975) and Batshaw (1984) indicate that an intake of 0.5–1 mmol/(kg·d) can eliminate hyperammonemia caused by arginine inadequacy and restore normal plasma arginine levels. This is reasonably consistent with what would be provided by the amount in human milk adjusted to the greater protein requirement of the premature infant compared with the term infant. The arginine content of pooled human milk is 0.28 mmol/100 kcal [calculated from Tikanajoa et al. (1982a) assuming an energy content of 670 kcal/L] and total protein in human term milk ranges 1.7-1.8 g/100 kcal (Raiten et al., 1998a). So applying the 0.28 mmol arginine: 1.7 g protein ratio of 0.165 to the minimum protein intake of 2.5 g/100 kcal suggested for preterm formula, the allowance would be 0.41 mmol arginine/100 kcal or 72 mg/100 kcal. At an energy intake of 120 kcal/(kg·d), this would be 86 mg/(kg·d), or 0.5 mmol/(kg·d), consistent with the findings of Heird et al. (1972) as an amount that can help to prevent the hyperammonemia associated with arginine deficiency.

Maximum. A recommendation for the maximum level of arginine in formula is an even more difficult problem, even though arginine in large amounts is certainly toxic to an otherwise normal infant (Brusilow & Horwich, 1995). The observations of congenital argininemia by Snyderman et al. (1977) do not allow calculation of a toxic level, because plasma arginine levels of these hyperactive, spastic, retarded patients were nearly eight times as high as those of premature infants overfed protein at 9 g/(kg·d) (Snyderman et al., 1970). The mean plasma arginine level of the latter infants was only twice that of premature infants fed protein at 2 g/(kg·d), indicating normally good control of the level of arginine synthesis and catabolism. The Expert Panel, therefore, resorted to recommending a maximum allowance based on the maximum protein intake recommended in aprevious section, 3.6 g/100 kcal. By a calculation similar

to that carried out for the minimum intake (3.6 g maximum protein multiplied by the ratio value of 0.165), the recommendation is for 0.594 mmol/100 kcal or 104 mg/100 kcal.

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of arginine in preterm infant formula be 72 mg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum concentration of arginine in preterm infant formula be 104 mg/100 kcal.**

Intakes of essential amino acids

With the exception of arginine, the Expert Panel did not find sufficient evidence to differ from the minimum and maximum levels for the conditionally essential amino acids recommended for term infant formula, relative to total protein. Accordingly, the Expert Panel has accepted the recommended levels for term infant formula as adjusted for the different protein intake recommended within this report. There being no recommendation for arginine within the term infant formula report, the recommendation for arginine is based on the same principles used to establish the recommendations for the other essential amino acids.

Recommendations

TABLE 7-3

The essential amino acid content of preterm infant formula recommended by the Expert Panel

	Content (mg/100 kcal)[1]	
Amino acid	Minimum	Maximum
Histidine[2]	53	76
Isoleucine	129	186
Leucine	252	362
Lysine	182	263
Methionine + cysteine[3]	85	123
Phenylalanine + tyrosine[3]	196	282
Threonine	113	163
Tryptophan	38	55
Valine	132	191
Arginine	72	104

[1] Except for arginine, the minimum and maximum values in this table are adapted from the values specified in Assessment of Nutrient Requirements for Infant Formulas (Raiten et al., 1998a). They have been adjusted to the range of protein concentrations recommended by the Expert Panel for premature infants, 2.5–3.6 g/100 kcal. When an individual amino acid is added to a formula, the maximum for that amino acid is not to exceed 1.5 times the minimum value.

[2] The values for histidine are adapted from the minimum and maximum values specified by in an erratum to Assessment of Nutrient Requirements for Infant Formulas (Raiten et al., 1998b). They have been adjusted to the range of protein concentrations recommended by the Expert Panel for premature infants, 2.5–3.6 g/100 kcal.

[3] Although the sulfur-containing amino acids (methionine and cysteine) and the aromatic amino acids (phenylalanine and tyrosine) are listed in combination, it should be noted that in no case should the requirement be met with only one of the respective constituents. Because the ratio of each of these combinations of amino acids is approximately 1:1 in human milk, ratios that exceed 2:1 or 1:2 are probably unbalanced and should not be used without appropriate testing for adequacy.

OTHER NITROGENOUS SUBSTANCES

Taurine

Background. Taurine (2-aminoethanesulfonic acid, $^+NH_3$-CH_2-CH_2-SO_3^-) is an amino acid with a sulfonic acid instead of a carboxyl group. Synthesized from cysteine, it is a major intracellular amino acid in most mammalian tissues, but it is not incorporated into protein and participates in few metabolic reactions.

A number of biochemical roles have been identified for taurine, including participation in the detoxification of retinol, iron, and xenobiotics (Emudianughe et al., 1983); calcium transport (Dolara et al., 1973; Huxtable, 1980; Huxtable et al., 1980); myocardial contractility (Grosso & Bressler, 1976); and osmotic regulation (Trachtman et al., 1988a; Trachtman et al., 1988b). Taurine also plays a well-recognized role in fat digestion via its conjugation with bile acids (Wasserhess et al., 1993), and taurine conjugates predominate over glycine conjugates in early infancy (Brueton et al., 1978). A deficiency of dietary taurine during formula feeding was reported not to influence the formation of taurine conjugates by term infants during the first month of life, as compared with breast-fed infants (Wahlén & Strandvik, 1994). However, the range of bile acid excretion was very large and the number of infants in each group was small, which may have obscured a significant effect. Taurine does increase bile acid excretion in smaller preterm infants (Wasserhess et al., 1993).

Human milk concentrations of taurine have been reported to range from 34 to 80 mg/L (Harzer et al., 1984; Rana & Sanders, 1986; Rassin et al., 1978), whereas cow milk has very low taurine levels, about 1.25 mg/L (Rassin et al., 1978). Supplementation of term infant formula was begun in Europe in 1981 because of retinal abnormalities in infant monkeys deprived of taurine and in patients who were nourished with parenteral nutrition solutions lacking taurine and cysteine (Sturman & Chesney, 1995). Since the FDA approval for supplementation in 1984, commercial infant formulas manufactured in the United States have been supplemented with taurine to compensate for the low amounts provided by dairy milk. The most recent information available is that formulas for preterm infants marketed in the United States contain 48–57 mg/L, or 5.9–7.0 mg of taurine/100 kcal (Mountford, 1999).

The expert panel assessing nutrient requirements for term infant formulas concluded that the nutritional advantage of adding taurine had not been demonstrated convincingly and therefore recommended a minimum taurine level of zero (Raiten et al., 1998a). That panel considered that the maximum level should be no greater than that measured in human milk, which contains 4.5–12 mg/100 kcal, and therefore recommended a maximum taurine content for infant formula of 12 mg/100 kcal. The IDACE (1996) recommendations for taurine for preterm infant formulas were that the "content shall be at least 42 μmol/100 kcal (3.75 mg/100 kcal)." Health Canada *Guidelines* (1995) indicated that "data lend support to the addition of taurine to infant formula, which is considered to be a prudent measure although evidence of clinical benefits is limited . . . [T]aurine content in preterm formula, if added, should not exceed 0.48 mmol/L (6 mg/dl)" (9 mg/100 kcal).

Review of the literature. The two lines of evidence that support an essential role for taurine in the diet of premature infants are animal deficiency models and biochemical and physiological responses of preterm infants provided taurine-free diets. Taurine is found in high concentration in the brains of mammals of all ages, where it has a well-established osmoregulatory function (Chesney et al., 1998a; Chesney et al.,

1998b; Nagelhus et al., 1993). Taurine is also found at high concentrations in the inner ear, localized to the supporting cells of the organ of Corti, where it again may be involved in the maintenance of osmotic equilibrium (Horner & Aurousseau, 1997). Concentrations in brain in many animals are several times higher in the neonate than in the adult (Sturman, 1988), suggesting a role for taurine in neurological development. A decline in brain taurine occurs in many animals during the natural period of weaning, probably because of the higher concentration of taurine in milk than in other foods for most species (Sturman & Hayes, 1980).

Taurine is an absolute dietary requirement for cats (Knopf et al., 1978), which develop a dilated cardiomyopathy when depleted of taurine (Pion et al., 1987). Cats lack appreciable amounts of the hepatic cysteine sulfinic acid decarboxylase needed for the last enzyme-catalyzed step in taurine biosynthesis (Jacobsen et al., 1964) (Figure 7-1). Female cats deprived of taurine have poor reproductive performance. When reproductively successful, their milk is low in taurine (10% of normal) and they rear offspring with a poor survival rate, a poor growth rate, and numerous neurological abnormalities, including abnormal cortical development (Sturman & Chesney, 1995). Cats raised on casein, which is low in taurine and its precursor cysteine, as the sole protein source have lower concentrations of taurine in plasma and retina and develop retinal degeneration (Hayes et al., 1975), as do rats (Hageman & Schmidt, 1987).

Rhesus monkeys also depend on dietary taurine to maintain a normal retinal structure, at least until 6 months of age (Imaki et al., 1993), and its omission is associated with a diminished visual acuity (Neuringer & Sturman, 1987). The retinal degeneration is at least partially reversible by taurine, but there are also abnormalities in the visual cortex that are not reversed by feeding formula with taurine from age 6 to 12 months (Palackal et al., 1993). Retinal cone receptor cells degenerated in rhesus monkeys raised for their first 26 months given a human infant protein hydrolysate formula containing taurine at 1 μmol/100 mL (Sturman et al., 1984), as they did after 3 months taking a taurine-free soy protein-based formula (Neuringer & Sturman, 1987). In vitro, taurine is a retinal rod-promoting factor (Altshuler et al., 1993).

Cow milk, the source of the protein in most infant formula, has the lowest taurine concentration of milk of any animal studied, about 1 μmol/100 mL after the first week of lactation (Rassin et al., 1978). Taurine is also at very low concentrations in formula based on cow milk protein, especially casein-predominant formula (Gaull et al., 1977a; Gaull et al., 1977b). There is at least one report that taurine-free formula does not support normal growth in some (nonrhesus) species of monkeys (Hayes et al., 1980). However, in LBW infants of 31–36 weeks GA, supplementation of their formula with taurine at 4.6 mg/100 kcal until they reached 2400 g did not affect any indicators of growth (Järvenpää et al., 1983b), although it normalized plasma and urinary concentrations of taurine (Rassin et al., 1983). Nevertheless, taurine could have a role in maturation of the central nervous system (Sturman, 1988; Sturman & Chesney, 1995), which might not be reflected in overall growth.

Gaull et al. (1977a; 1977b) measured plasma and urinary levels of taurine in preterm infants fed human milk, which is relatively rich in taurine (Rassin et al., 1978), or infant formulas with little taurine. The formulas had whey-to-casein ratios of 60:40 and 18:82 and were each fed at protein concentrations of 1.5 and 3.0 g/100 mL, or 1.9 and 3.8 g/100 kcal. The concentration of taurine in the urine of infants fed human milk [taurine intake 45 μmol/(kg·d)] was two to eight times

higher than that in the urine of infants fed formulas, except for those infants fed the high-protein, whey-predominant formula. This formula had a higher taurine content than any of the others but provided an intake of only 4.5 μmol/(kg·d); this was not high enough to account for the higher plasma and urine taurine concentrations. Thus, it is likely that a high cysteine intake results in an increased endogenous synthesis of taurine.

Plasma taurine levels were also higher in infants fed breast milk than in those fed formula, except for the high protein whey-predominant preparation. These results suggested to Gaull et al. (1977b) a relative lack of the capacity to convert even a high intake of cysteine to adequate taurine in premature infants. This idea is consistent with measured low levels of cysteine sulfinic acid decarboxylase in fetal and newborn tissues (Gaull et al., 1977b; Sturman & Hayes, 1980), because the product of this enzyme, hypotaurine is the immediate precursor of taurine. When preterm infants are fed unsupplemented cow milk formula, taurine is the only plasma amino acid that is present at lower concentrations than in plasma of infants fed human milk (Rassin et al., 1983). Thus, taurine may be conditionally essential in the preterm infant.

Older children given long-term parenteral nutrition containing methionine but lacking taurine and cysteine developed lower levels of taurine (but not cystine) in platelets, plasma, and urine (Vinton et al., 1987). This suggests a rate-limiting step between cysteine and taurine. When receiving less than 25% of their calories from gastrointestinal absorption, taurine-deprived parenteral nutrition patients show evidence of a mild electroretinographic abnormality, reversed by taurine (Geggel et al., 1985), and a marked granularity of the retinal pigmented epithelium, visible funduscopically (Vinton et al., 1990). Because plasma concentrations of methionine and cyst(e)ine were almost normal in these children, it seems likely that the relative block in taurine synthesis present at the step catalyzed by cysteine sulfinic acid decarboxylase can be pathologically significant in preterm infants (Sturman & Hayes, 1980). Some evidence points to an effect of taurine supplementation on maturation of auditory brainstem-evoked responses in infants of less than 1301 g BW tested at the 37th week of postmenstrual age (Tyson et al., 1989a), although results from this study have been criticized as inconclusive (Perlman, 1989; Tyson et al., 1989b).

Plasma taurine levels were likewise lower in LBW infants (<1000 g) maintained with taurine-free parenteral nutrition solutions than in infants of the same size fed human milk or taurine-supplemented formula (Zelikovic et al., 1990). These levels returned to normal when oral feeding was begun. Mean fractional taurine excretion levels were elevated (38–56%) regardless of whether infants of this size were administered total parenteral nutrition lacking taurine or taurine-fortified formula, as compared with levels in healthy, full-term infants fed formula (16 ± 3%). Zelikovic et al. (1990) concluded that the premature kidney has a limited ability to conserve taurine by increasing reabsorption, which could account for some of the need for taurine in the diet. Later on, it was found that the fractional excretion of taurine in premature infants is inversely proportional to BW (Helms et al., 1995). Taurine is virtually unique among amino acids as being subject to adaptive reabsorption in more mature individuals (Chesney et al., 1998a; Chesney et al., 1998b); other amino acids are almost completely reabsorbed at any plasma level.

Gastrointestinal findings include the observation that with taurine-free formula, vitamin D absorption is compromised in infants of mean GA of 32 weeks, compared with that in infants taking a taurine-supplemented formula (Zamboni et al., 1993). Heird et al. (1987) reported that taurine supplementation during the administration of total parenteral nutrition may reduce the incidence and degree of cholestasis (impairment of bile secretion) in infants. It reduces cholesterol synthesis and raises bile acid excretion and fatty acid absorption in preterm infants of GA less than 33 weeks, although not in older preterm or term infants (Wasserhess et al., 1993); this could be related to an expanded body taurine pool at a higher GA (Chesney et al., 1998a; Chesney et al., 1998b). Taurine-conjugated bile acids favor the formation of mixed micelles, promoting cholesterol and fatty acid absorption (Wasserhess et al., 1993). Taurine deficiency may increase the less-soluble glycine conjugates of bile acids and favor cholestasis (Howard & Thompson, 1992).

Conclusions and recommendations. Chesney et al. (1998a; 1998b) provided an excellent summary of taurine effects and cogent arguments for considering it to be an essential nutrient in preterm infants. Plasma levels of taurine fall if infants are nourished with taurine-free formula or parenteral nutrition solutions. Urinary excretion also falls, suggesting a response by the kidneys toward conservation of taurine. Because of renal functional immaturity, the smallest preterm infants cannot conserve taurine efficiently by tubular reabsorption, and the fractional excretion of taurine is high. Dietary taurine insufficiency in preterm infants also results in an impairment in bile acid secretion, which is reversed by taurine supplementation.

In the brain and renal medulla, taurine is involved actively in the cell volume regulatory process: cells shrink or swell in response to osmolar changes but return to their previous volumes according to the uptake or release of certain osmolytes (Law, 1991). Chesney et al. (1998a; 1998b) suggested that because of failure of renal taurine conservation, immature infants deprived of taurine may be unable to respond well to hyper- or hypo-osmolar stress without large changes in neuronal volume, with obvious clinical significance. Moderate impairment of osmoregulatory function might be hard to distinguish from other sorts of cerebral pathophysiology in ill premature infants yet might have permanent consequences.

For all these reasons, taurine must be considered "conditionally" essential for preterm infants. The Expert Panel found the circumstantial evidence to be strong enough to justify the addition of taurine to preterm formula. It is true that no overt disease caused by taurine deprivation was identified in infants after decades of use of taurine-free formula (Sturman & Chesney, 1995). However, it may be that the taurine-dependent osmoregulatory and digestive functions are not stressed to their limits in most infants fed only formula, especially term infants. These functions could, however, be taxed beyond capacity in small premature infants with low taurine stores and multiple intercurrent illnesses. Also, the effects of taurine deprivation on development of vision or hearing in humans may result in only a delay rather than a loss, but such a delay could have a permanent effect should it occur during a critical period of cognitive development. Finally, the Expert Panel found no report of toxicity from unconjugated taurine added to formula.

Minimum. Few data are available to inform the choice of a minimum value for taurine intake. Essentially no dose-ranging studies were done before it became ethically dubious to withhold taurine from premature infants. Among the clinical investigations, Zamboni et al. (1993) found normalization of vitamin D absorption by an intake of 93 μmol (11.6 mg)/(kg·d) at an energy intake of 120 kcal/kg, or 9.7 mg/100 kcal. Rassin et al. (1990) found formula with added taurine at 8 mg/100 kcal to normalize plasma concentrations of taurine, but some of these same investigators had previously reported

that normal values of taurine in plasma and urine were supported by supplementation at 4.6 mg/100 kcal (Järvenpää et al., 1983b; Rassin et al., 1983). In the study of Gaull et al. (1977b) plasma and urinary concentrations of taurine were virtually the same on a whey-predominant formula providing taurine at a little less than 0.5 mg/100 kcal as on a human milk mixture containing 10 times as much taurine. With this formula, taurine intake was only 2.5 times what it would have been with cow milk [10 μmol/L (Rassin et al., 1978), or 0.19 mg/100 kcal]. However, cystine intake was quite high, which might have pushed endogenous taurine synthesis to some extent. In a review of the requirements of conditionally essential nutrients, Uauy et al. (1993) concluded that the lowest advisable intake of taurine should be the minimum amount commonly found in human milk, or 30 μmol (3.75 mg)/100 kcal.

The Expert Panel saw no reason to consider the minimum acceptable intake of taurine to be the lowest intake demonstrated to normalize plasma and urinary concentrations (Gaull et al., 1977a; Gaull et al., 1977b). It is possible, for instance, that this normalization is not a strict enough criterion for dietary sufficiency; moreover, there is no evidence of taurine toxicity in premature infants. The Expert Panel accepted the lowest level commonly found in human milk as a reasonable minimum, which they rounded up to 5 mg/100 kcal. This level is consistent with the levels in formulas marketed for preterm infants in the United States, and consistent with clinical experience and a history of use.

Maximum. There is every reason to consider taurine to be without adverse effect at the highest concentration usually found in human milk. There are no reports of toxicity from taurine in preterm infant formula.

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of taurine in preterm infant formula be 5 mg/100 kcal.**

Maximum**. The Expert Panel recommended that the maximum concentration of taurine in preterm infant formula be 12 mg/100 kcal.**

Carnitine

Background. In the discussion that follows, carnitine refers to L-carnitine. The nonphysiological isomer D-carnitine does not participate in the normal metabolic processes described for carnitine; rather, it is metabolized in animals primarily to trimethylaminoacetone, whereas the physiological isomer is mainly excreted by the kidneys (Bremer, 1983) or metabolized by enteric bacteria (Rebouche & Seim, 1998; Seim et al., 1985). Carnitine is not degraded by enzymes from mammalian sources (Rebouche & Seim, 1998).

Carnitine (β-hydroxy-γ-trimethylaminobutyrate) is a quaternary amine, an N-substituted amino acid that cannot be a component of a peptide chain. It has an important function in the transport of long-chain fatty acids into the matrix of the mitochondria for β-oxidation. Coenzyme A-linked fatty acids, which cannot cross membranes, are transesterified to carnitine in the mitochondrial membrane. In this form they can enter the mitochondria, where the process is reversed. The carnitine compound preserves the high-energy state of the molecule and allows its delivery to the site of β-oxidation.

Because medium-chain fatty acids are activated by coenzyme A within the mitochondrial matrix in the liver but outside it in other tissues, carnitine is not necessary for the oxidation of these acids in liver but is necessary in cardiac and skeletal muscle (Borum, 1995). Provision of a large percentage of formula fat as MCT thus does not obviate the need for carnitine, because under those circumstances much of the fatty acid oxidation occurs in carnitine-requiring extrahepatic tissues. Direct evidence for the need for carnitine for this process in term infants was obtained by Rebouche et al. (1990).

Carnitine may also be critical for the transport of other carboxylic acids, either toxic or metabolic (Borum, 1995); acylated compounds of exogenous origin esterified to carnitine can be transported in the plasma, catabolized by the liver, or excreted in the urine (Giovannini et al., 1991). It is also supposed to have regulatory effects independent of its role as substrate for carnitine acyltransferase and has been reported to protect against free radical damage (Rebouche, 1992). Carnitine is supposed to ameliorate ammonia toxicity in animals, but whether the nonphysiological D-isomer is as effective as L-carnitine in that regard is controversial (Matsuoka & Igisu, 1993; Ohtsuka & Griffith, 1991).

On the basis of the documented capacity of adults to produce some carnitine endogenously from lysine and methionine, no recommended dietary allowance was established for carnitine in the 1989 revisions (National Research Council. Food and Nutrition Board, 1989). So little lysine is required for this process that diets producing signs and symptoms of lysine deficiency do not elicit manifestations of carnitine deficiency (Rebouche, 1992). Carnitine is not included in the U. S. Code of Federal Regulations CFR 107.100. Since 1986, however, all commercial formulas based on soybean protein manufactured in the United States have been supplemented with carnitine to a concentration similar to that in human milk (Rebouche, 1992). In general, cow milk has approximately twice the carnitine concentrations of human milk (Penn et al., 1987), and formulas based on cow milk contain amounts of carnitine comparable to concentrations in human milk (Borum, 1995).

Most reports of the total carnitine (free plus acyl-carnitine) concentration in human milk after 4 weeks of lactation are within the range reported by Penn et al. (1987) of 40–104 μmol/L, or about 0.8–2.1 mg/100 kcal. Carnitine in human milk, mostly in the whey fraction, would thus provide 1–2.5 mg/(kg·d) to the nursing infant (Borum, 1983; Penn et al., 1987). One publication reported a fall from a mean of 58.7 nmol/mL (1.4 mg/100 kcal at 670 kcal/L) during the first 21 days of lactation to 35.2 nmol/mL (0.8 mg/100 kcal) at 40–50 days (Sandor et al., 1982), although the mothers' mean serum concentration remained constant. Carnitine concentrations in milk of mothers delivering prematurely are not different than those found in mature milk (Innis, 1993).

Health Canada *Guidelines* (1995) for preterm infant formula stated that "if carnitine is added, supplementation should provide for levels of carnitine within the range found in human milk, up to 15 μmol/100 kcal" (2.4 mg/100 kcal). However, they also stated that clinical testing would be required for a new preterm infant formula with a carnitine concentration of more than 0.1 mmol/L, or approximately 2 mg/100 kcal. ESPGAN (1991), citing the range of carnitine levels found in breast milk, recommended a minimum for LBW infant formula of 7.5 μmol/100 kcal (or 1.2 mg/100 kcal). The IDACE (1996) recommendation concurred with the ESPGAN minimum level of carnitine of 1.2 mg/100 kcal. The minimum level recommended by Tsang et al. (1993) is 2.4 mg/100 kcal for enterally fed infants. Commercial formulas were reported in that same reference (in table A.3) to contain 1.7–5.9 mg/100 kcal, but only 1 of 11 formulas listed contained more than 2 mg/100 kcal. A carnitine concentration of 2 mg/100 kcal provides an intake of 2.4 mg/(kg·d) at an energy intake of 120 kcal/(kg·d).

Although the evidence that dietary carnitine is essential for the term infant is not convincing, biochemical changes are noted when infants are fed a carnitine-free diet. There are also several reports of abnormal clinical manifestations associated with diets low in carnitine. Infants nourished with soy protein-based formula with low carnitine content demonstrate differences in plasma and urinary carnitine levels and evidence of altered lipid metabolism, without a significant difference in rates of growth (Raiten et al., 1998a). The functional significance of these metabolic differences in normal term infants is not known. Nevertheless, in view of the long history of use of carnitine in infant formula, the expert panel assessing nutrient requirements for term infant formula concluded that formula levels comparable to those of human milk would seem reasonable for term infants. It recommended minimum and maximum carnitine concentrations in infant formulas of 1.2 and 2.0 mg/100 kcal, respectively (Raiten et al., 1998a).

Review of the literature. The metabolic pathway for the biosynthesis of carnitine begins with the trimethylation of lysine in various proteins, particularly in muscle (Rebouche, 1992) and liver (Shenai & Borum, 1984). Trimethyllysine (TML) freed by protein turnover is hydroxylated, split by an aldolase with the release of glycine, oxidized to an acid, and hydroxylated again. Ascorbic acid, iron, vitamin B_6, niacin, and α-ketoglutarate are cofactors in these reactions.

The last enzyme in the biosynthesis of carnitine, γ-butyrobetaine hydroxylase, has been shown to be regulated developmentally in the liver, but not in the kidney (Olson & Rebouche, 1987). However, activity of this enzyme is not rate limiting for carnitine biosynthesis, even at the relatively low levels present in newborn liver (Olson & Rebouche, 1987). A recent study suggested that in premature infants the rate-limiting step is located at the second transformation in the pathway, the conversion of ε-N-TML to β-hydroxy-ε-N-TML (Melegh et al., 1996).

Borum (1995) and others have contended that carnitine should be considered a conditionally essential nutrient for infants, that is, a dietary source required under certain circumstances. According to Rebouche (1992), the biosynthetic capability of both adults and infants is limited, and carnitine homeostasis is maintained by supplementation of synthesis with dietary intake and efficient conservation by the kidney (Rebouche et al., 1993). If the exogenous supply of carnitine is stopped to individuals whose previous intake has not been limited, plasma carnitine concentrations drop significantly within 21 days, but this does not necessarily provide information on the carnitine concentration in critical tissues (Rebouche, 1992). Carnitine levels in blood and urine do not necessarily reflect the content of different tissue pools (Borum, 1995) but rather depend on recent intake (Giovannini et al., 1991). The concentration of carnitine in human skeletal and cardiac muscle is normally 50 times higher than that in plasma (Rebouche & Seim, 1998), which indicates tissue uptake against a concentration gradient.

Urinary and plasma carnitine levels are substantially lower in long-term lacto-ovovegetarians as well as strict vegetarians, probably because the carnitine content of vegetables and fruits is less than 1% that of meat, and that of cereals less than 5% (Lombard et al., 1989). In omnivorous individuals, carnitine synthesis needs to provide from one-half to one-eighth of the total carnitine available, whereas in strict vegetarians it must provide more than 90% (Rebouche, 1992). It has been noted that low carnitine synthetic ability in poorly nourished individuals might result from subclinical deficiencies of the cofactors involved in the in vivo synthesis of carnitine (Khan-Siddiqui & Bamji, 1980).

During the newborn period, plasma and tissue carnitine levels are low and biosynthetic capabilities may be particularly limited (Borum, 1986; Giovannini et al., 1991). Normally, carnitine is accumulated in adipose tissue shortly after birth and has been shown to increase the oxidation and esterification of fatty acids in adipocytes of newborns (Schmidt-Sommerfeld et al., 1978). It enhances glycerol release (indicating lipolysis) from newborn subcutaneous adipose tissue fragments, although not from similar adult fragments unless the β-adrenergic stimulator isoproterenol is present (Novak et al., 1975).

Studies of term infants. Because all infant formula now contains carnitine, clinical studies of the effect of its absence from nutrient mixtures either were conducted with soy-based formula before 1985, when supplementation of those formulas began, or involve infants (primarily premature infants) nourished parenterally. As of 1995, most parenteral nutrition solutions lacked carnitine (Borum, 1995).

The clinical presentation of carnitine deficiency reported to occur in some infants nourished with soy-based formulas included failure to thrive, nonketotic hypoglycemia, hypotonia, and cardiomyopathy (Slonim et al., 1981; Winter et al., 1987). Although there have been relatively few reports of an overt deficiency among the large number of infants receiving soy-based formulas, these reports suggest that the mandated supplementation of soy-based formulas is indeed necessary. This in turn forces a conclusion that carnitine must be considered an essential nutrient for infants (Raiten et al., 1998a).

Studies considering the essentiality of dietary carnitine have often evaluated plasma carnitine and lipid levels in response to various diets rather than assessing de novo production (Rebouche, 1992). Novak et al. (1983) compared two small groups of healthy full-term infants receiving on or before 2 days of life commercial soy-based formula without carnitine ($n = 5$) or the same formula supplemented with "50 nM/mL" carnitine ($n = 7$). Presumably this means 50 nmol/mL, or 1.2 mg/100 kcal, because the investigators specified that the concentration used was comparable to that in human milk. The test diets served as the sole source of carnitine for the first 3–4 months of life; other solid foods were discouraged but not forbidden, except for meat products. Plasma carnitine and acylcarnitine levels were lower and plasma triglyceride and very low density lipoprotein levels were higher in the unsupplemented group. Despite the small sample size, the differences at 3 months seemed clinically significant. Novak et al. (1983) interpreted the results as indicating that carnitine supplementation increases the uptake of free fatty acids for subsequent oxidation. They considered that the data confirmed the importance of carnitine in fat metabolism and the possible metabolic significance of an absence of a dietary source of carnitine. In a parallel study, they showed that a larger amount, 250 nmol/mL (6 mg/100 kcal), resulted in no further elevation in plasma carnitine and acylcarnitine concentrations but did increase the renal loss of carnitine (Novak et al., 1983).

In another study, Novak et al. (1983) compared plasma and urinary concentrations of free carnitine and acylcarnitine in infants given three different formulas beginning at 3–7 days of life: a group receiving a carnitine-free soy-based formula ($n = 13$) and two groups receiving the same formula supplemented with either 1.2 or 6 mg/100 kcal ($n = 13$ and $n = 6$, respectively). The infants were followed for 3 months with the same dietary restrictions as previously. Novak et al. (1983) reported that with the exception of greater concentrations of urinary acylcarnitine in the groups receiving 6 mg/(kg·d), no significant differences in plasma and urinary values were found between the two supplemented groups. It might be concluded

from these results that carnitine at 6 mg/100 kcal is more than is necessary but is not toxic.

Olson et al. (1989) confirmed the observation of Novak et al. (1983) that serum concentrations of free fatty acids are higher in soy-fed infants not given carnitine supplementation than in those provided with supplementation. They also demonstrated an elevated excretion of the dicarboxylic acids adipic, suberic, and sebacic (C_6, C_8, and C_{10}, respectively) in the unsupplemented group. This was interpreted as indicating oxidation of fatty acids by the microsomal ω-oxidation pathway, which occurred as the result of a bottleneck in the carnitine-dependent mitochondrial β-oxidation pathway. They pointed out that human disease states that present with dicarboxylic aciduria are associated with defective β-oxidation ["non-ketotic dicarboxylic aciduria" (Mortensen, 1984)]. Perhaps activation of this microsomal "salvage" pathway explains why Olson et al. (1989) detected no effect of carnitine supplementation on growth rate or caloric efficiency of weight gain for 112 days.

Some have advocated the use of the ratios acylcarnitine to free carnitine (elevated levels indicate low carnitine availability, or "carnitine insufficiency") and free carnitine to total carnitine (depressed values indicate "carnitine deficiency") as indicators of carnitine nutritional status. Using these ratios as standards, Campoy et al. (1999) observed that unsupplemented term infants fed typical cow milk formula developed abnormalities by the end of the first month of life, and that addition of carnitine to formula at 3.3 mg/100 kcal normalized the values. The infants given supplemented cow milk formula were receiving a total carnitine of 4.1 mg/100 kcal. Campoy et al. (1999) did not report results with other concentrations of carnitine.

Studies of preterm infants. A significant accretion of carnitine by muscle tissue takes place during the last trimester of gestation, correlated with GA and with body dimensions (Shenai & Borum, 1984). Plasma and RBC carnitine concentrations are higher in cord blood of premature infants (GA of <34 weeks) than in cord blood of term infants (Giannacopoulou et al., 1998; Shenai et al., 1983), reflecting the concentration in maternal arterial plasma (Novak et al., 1981). However, concentrations are lower in the tissues of premature infants than in those of term infants (Penn et al., 1985; Shenai & Borum, 1984). This has been interpreted to indicate that the mechanisms for tissue uptake of carnitine against a concentration gradient (Scaglia et al., 1999) are immature before term (Shenai et al., 1983).

Concentrations of carnitine in skeletal muscle and liver continue to increase postnatally in breast-fed infants (Nakano et al., 1989). Cederblad and Svennignsen (1986) monitored breast milk intake and urinary and plasma carnitine levels of 10 healthy LBW infants (GA of 27–32 weeks) during the first 2 weeks of life. Breast milk carnitine levels ranged from 2.7 to 23.8 mg/L (0.4–3.6 mg/100 kcal), with a mean of 10.6 mg/L. Daily carnitine intake rose from an average of 0.2 μmol/kg to 6–7 μmol/kg (1.5 mg/100 kcal) in the second week, whereas the total plasma carnitine level remained unchanged at 28–31 μmol/L. Breast milk is evidently effective in maintaining plasma carnitine levels while increasing tissue stores. Most studies have used metabolic responses to breast milk as the standard for comparing formula sufficiency with respect to carnitine.

At least one study suggested, however, that even the amount of carnitine in breast milk may not be adequate to fill tissue stores optimally (Melegh et al., 1987). In this work, 20 infants of BW 1200–1880 g who had been maintained with pooled human milk were studied for 7 days, beginning at about

3 weeks of life. For that period, the milk, presumably at 0.8–2.1 mg carnitine/100 kcal (see above), was supplemented with an additional 7.2 mg/100 kcal. Increases in plasma carnitine levels, both free and esterified, above the levels seen in the pre- and postsupplementary periods, indicated that the carnitine supplement entered intermediary metabolism. A decrease in plasma triglyceride and an increase in β-hydroxybutyrate levels during supplementation suggested enhanced fat oxidation. Nitrogen balance was affected favorably, as if fat oxidation were sparing the use of protein for energy.

Plasma carnitine levels fall rapidly in the first 3 days of life of prematurely born infants unless exogenous carnitine is given (Novak et al., 1981) and continue to fall until the infants are given carnitine-containing feedings (Smith et al., 1988). Several studies of preterm infants fed exclusively parenterally have also shown that plasma carnitine levels remain very low after birth if no carnitine supplementation is given (Schiff et al., 1979; Schmidt-Sommerfeld et al., 1983). In parenterally fed carnitine-deprived premature infants, plasma and RBC concentrations at 3 weeks of age are only one-third those found in the cord blood of term neonates (Borum, 1995). Carnitine supplementation at a dose of 50 μmol (8 mg)/(kg·d) for 1 week followed by 100 μmol (16 mg)/(kg·d) increased the plasma concentrations at 3 weeks to a level 30% higher than that in the breast-fed full-term infants at 12 weeks. It should be noted that this is a large intake of carnitine when compared with the amount present in human milk.

Depletion of tissue stores of carnitine has been demonstrated if parenteral nutrition is continued for more than 15 days (Penn et al., 1981). In addition, a number of studies in premature infants have shown an impaired ability to utilize long-chain fatty acids infused intravenously (Borum, 1995) and impaired ketogenesis (Schmidt-Sommerfeld et al., 1983; Warshaw & Curry, 1980) in the absence of exogenously supplied carnitine. Schmidt-Sommerfeld et al. (1991), for example, tested fat tolerance (1 g/kg of an intravenous lipid emulsion given during 4 hours) in 29 premature infants of GA less than 34 weeks at 6–10 days of life. The infants were receiving total parenteral nutrition because of feeding intolerance. The ratio of free fatty acids to β-hydroxybutyrate was lower, and the increase of acylcarnitine greater, in those infants who had received carnitine at 10 mg/(kg·d) intravenously than in unsupplemented control infants.

A group of 14 infants (including 9 of GA <37 weeks) who required parenteral nutrition for gastrointestinal indications (e.g., necrotizing enterocolitis, volvulus, tracheoesophageal fistula, failure to thrive) were randomized to receive carnitine at 50 μmol/(kg·d) (10 mg/kg) or placebo by enteral tube feeding (Helms et al., 1986). Plasma and urinary carnitine concentrations were, respectively, 3- and 10-fold higher in the group receiving carnitine. When plasma triglyceride and free fatty acid concentrations were measured at 2-hour intervals after a lipid infusion (0.5 g/kg), no changes were observed. However, the carnitine-supplemented infants showed higher plasma concentrations of β-hydroxybutyrate and acetoacetate. This result was taken to reflect increased ketogenesis, indicative of enhanced fatty acid oxidation.

Subsequently, Helms et al. (1990) investigated the effects of intravenous carnitine supplementation in 43 infants (mean GA of 32 weeks) receiving more than 84% of their protein and calorie intake parenterally, using a similar fat-infusion protocol. These infants were given 50 μmol/(kg·d) (10 mg/kg) for 7 days, followed by 100 μmol/(kg·d) (20 mg/kg) for another 7 days and compared with unsupplemented infants. Nitrogen assimilation was improved by 20% in the supplemented group [300 mg/(kg·d) versus 250 mg/(kg·d)], and mean carnitine

balance became positive. Weight gain was increased during the second week, from 6 to 19 g/(kg·d). There was a suggestion of increased fat utilization at the end of the second week, as indicated by a decrease in free fatty acid concentrations and an increase in the ratio of plasma ketone bodies to free fatty acids.

In more recent studies with smaller infants, postnatal carnitine supplementation at 50 μmol/(kg·d) (10 mg/kg) was given to two groups of parenterally fed premature infants, BW of 750–1000 g and 1001–1500 g, and comparable control groups (Bonner et al., 1995). After 2 weeks, levels of β-hydroxybutyrate were maintained in both supplemented groups while decreasing from baseline in the control groups. Thus, ketogenesis appeared to be more adequate when carnitine was supplied. For infants in the higher weight range, those supplemented tolerated 20% more fat (as judged by serum triglyceride levels) than the unsupplemented ones and showed a greater weight gain during the first 2 weeks of life, 6.9 ± 5.9 g/d versus 1.3 ± 7.1 g/d ($P < 0.05$).

These would be key studies except for one difficulty: the carnitine dose administered is not "the minimal amount normally found in human milk," as stated in Bonner et al. (1995) but rather is about 10 times this amount (Penn et al., 1987). It is approximately the carnitine content of 1 L of breast milk (see above). Moreover, gastrointestinal absorption of carnitine by humans is only 54–87% of intake (Rebouche & Seim, 1998), and carnitine in formula may show less bioavailability than carnitine in breast milk (Warshaw & Curry, 1980). Thus, the possibility exists that the investigators were observing a pharmacological effect of carnitine at this parenteral dosage.

Most studies do not show an effect of carnitine supplementation on weight gain. A recent randomized placebo-controlled trial of carnitine as a nutritional supplement [25 mg/(kg·d), or 21 mg/100 kcal; see French et al. (1982)] involved study of 86 preterm infants of GA 28–34 weeks given the test diets until they reached 1800 g, and then until 6 months after the expected date of delivery (Shortland et al., 1998). At this rather substantial dose, there were no differences between the placebo and carnitine groups in weight, length, skinfold thicknesses, head circumference, or incidence of episodes of hypoglycemia. The investigators concluded that the failure to observe an effect suggested that the tissue values of carnitine in the placebo group were not low enough to have a clinically significant effect on energy metabolism. Alternatively, it is possible that a carnitine intake of this magnitude was beyond the nutritionally effective range and produced a toxic effect.

An even greater supplementation of 12 parenterally fed preterm infants, 48 mg/(kg·d) on days 4–7 of life, elevated the plasma total carnitine (free carnitine plus acylcarnitine) concentration 13-fold (Sulkers et al., 1990). Because the caloric intake was only 75 kcal/(kg·d), this was equivalent to 64 mg/100 kcal. The mean plasma concentration of 197 μmol/L was far higher even than normal adult levels (25–70 μmol/L). The respiratory quotient was lower and fat oxidation higher by day 7 in the supplemented group, but protein oxidation was also higher than in an unsupplemented control group. Time to regain BW was increased by supplementation from a mean of 7 to a mean of 9 days. The investigators recognized the potential advantages of carnitine in stimulating fat oxidation and promoting ketone body formation, thus providing an alternative substrate for an otherwise glucose-dependent brain. Nevertheless, they thought that the observed lower protein accretion might outweigh these advantages. Although there is no consensus as to the optimal fat content of a growing premature infant, they considered the protein and weight observations to be adverse effects. Whether lower doses of carnitine could in-

crease fat oxidation without the undesirable changes in metabolic rate, protein oxidation, and growth warrants investigation.

Role of renal immaturity in carnitine requirement. At one time it was suspected that a carnitine requirement of the premature neonate was a function of excessive renal loss rather than inadequate biosynthesis of carnitine in the absence of intake (Zamora et al., 1995). It is true that when supplied with a carnitine-deficient diet in the first week of life, premature infants of GA 26–31 weeks had a somewhat lower reabsorption of carnitine (94%) than did older premature infants or term infants (98% and 99%, respectively). However, reabsorption correlates with GA, increasing at the same rate as other known indices of tubular function (Zamora et al., 1995). Patients with medium-chain acylcarnitine transferase deficiency or other organic acidemias often develop a secondary carnitine deficiency as a result of excessive excretion of the carnitine ester of the accumulating metabolite (Borum, 1995). No such mechanism is operating in infants, in whom renal losses of carnitine do not account for the low plasma carnitine levels seen with ingestion of carnitine-free formula (Olson & Rebouche, 1989).

Conclusions and recommendations. Only a few observations are available that speak to the concentration of carnitine appropriate for fortification of preterm formula. The recommendation of the expert panel studying term infant formula was for a carnitine concentration between 1.2 and 2 mg/100 kcal (Raiten et al., 1998a). The average level present in breast milk (1.6 mg/100 kcal) is adequate to maintain plasma levels while increasing tissue stores (Cederblad & Svenningsen, 1986) but may not optimally fill tissue stores (Melegh et al., 1987). An intake of 2 mg/100 kcal blocks the ω-oxidation salvage pathway, probably indicating an adequate amount of β-oxidation of fatty acids (Olson et al., 1989). At a value between 2.75 and 5.5 mg/100 kcal, an increased fractional excretion of carnitine begins (Olson & Rebouche, 1989). One preterm formula the United States with a carnitine content of 5.9 mg/100 kcal (Tsang et al., 1993) has been fed to preterm infants for over 7 years with no known adverse effects. Intakes of 6 mg/100 kcal are effective and without adverse effect, if probably unnecessary (Novak et al., 1988). Short-term intakes of 8.1 mg/100 kcal increased ketogenesis (Helms et al., 1986), and 8.1 mg/100 kcal administered intravenously for 1 week followed by 16 mg/100 kcal administered intravenously for 1 week increased weight gain (Helms et al., 1990). An intravenous dose of 11 mg/100 kcal was reported to have a beneficial effect on weight gain during 2 weeks of supplementation (Bonner et al., 1995), but this intake is so high as to suggest a pharmacological effect. A month-long placebo-controlled trial of 21 mg/100 kcal failed to show a favorable effect on growth (Shortland et al., 1998). Finally, administration of 48 mg/(kg·d) intravenously seemed to have undesirable side effects (Sulkers et al., 1990). There is no unequivocal indication of a toxic effect of carnitine at intakes lower than 48 mg/(kg·d) intravenously (Sulkers et al., 1990).

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of carnitine in preterm infant formula be 2.0 mg/100 kcal.** This is higher than the average amount present in breast milk but seems to be necessary to support β-oxidation adequately.

Maximum. **The Expert Panel recommended that the maximum concentration of carnitine in preterm infant formula be 5.9 mg/100 kcal.**

Choline

Background. In humans, choline [(β-hydroxyethyl) trimethylammonium, $(CH_3)_3\text{-}N^+\text{-}CH_2\text{-}CH_2OH)$] is required for synthesis of phospholipids and the neurotransmitter acetylcholine (Zeisel, 1994). It is also an important source of transferable methyl groups. The only nondietary source of choline for humans is via de novo synthesis of phosphatidylcholine (lecithin) from phosphatidylethanolamine in liver, brain, mammary glands, and most other tissues, by using methyl groups donated from methionine via S-adenosylmethionine (Blusztajn et al., 1985; Ridgway & Vance, 1987; Zeisel, 1981). Free choline can then be produced, at least in rat brain, by the cleavage of choline from phosphatidylcholine (Blusztajn & Wurtman, 1981).

In vivo choline is oxidized to betaine, a donor of methyl groups. Betaine can transfer the methyl group to homocysteine to re-form methionine (Chan, 1984; Finkelstein et al., 1988). The need for choline as a precursor of the methyl donor betaine is considered a major factor in the pathological changes observed in the liver during conditions of choline deficiency (Zeisel, 1994).

Only a small proportion of dietary choline intake is required for the synthesis of acetylcholine (Wecker, 1990; Zeisel, 1981), but alterations in dietary choline influence brain levels of this neurotransmitter (Chan, 1984). Under normal conditions, the exogenous supply of choline is sufficient to maintain acetylcholine synthesis and release. Animal experiments, however, have demonstrated that when the demand for choline is increased by the administration of drugs that stimulate the release of acetylcholine, or the dietary supply of choline is restricted, endogenous synthesis cannot maintain acetylcholine levels (Wecker, 1988; Wecker, 1990). In addition, substantial evidence indicates that choline itself has a role in brain development and function: for example, in rodents it plays a facilitatory role in prenatal and postnatal development of spatial memory capacity (Loy et al., 1991; Meck et al., 1988; Meck et al., 1989).

ESPGAN (1991) specified no recommendation for the amount of choline that should be present in LBW infant formula. IDACE (1996) recommended a minimum of 7.0 mg/100 kcal. Health Canada (1995) made no specific recommendation for a minimum content of choline but indicated that levels up to 20 mg/100 kcal, near the upper limit of the choline content of human milk, are acceptable. The American Academy of Pediatrics Committee on Nutrition (1998) made no recommendation for choline but quoted a consensus recommendation of 12–23.4 mg/100 kcal.

The Expert Panel on Assessment of Nutrient Requirements for Infant Formulas recommended a minimum choline content in term infant formulas of 7 mg/100 kcal (Raiten et al., 1998a). The rationale for this recommendation was that a dietary requirement for choline has been demonstrated for several species of mammals (although not for humans), and it seems likely that the dietary choline need of infants growing rapidly might be greater than that of older individuals because choline is an important component of membranes. In the absence of empirical data on the dietary requirement of infants for choline, the minimum content was specified on the basis of the limited available information as to the lower end of the range of the choline content of human milk.

That expert panel also recommended a maximum content of choline in term infant formula of 30 mg/100 kcal based on extrapolation of safe intake by adults. This maximum value is 2.5–3 times the label claim of the amount of choline in infant formulas marketed currently (American Academy of Pediatrics. Committee on Nutrition, 1998). Per unit of BW, however, it would be well below the intake of choline used therapeutically in neurological illnesses [up to 20 g/d; (Davis & Berger, 1978)] or to replenish stores in choline-depleted adults [5–6 g/d, or 72–86 mg/(kg·d) for a 70-kg individual; (Chawla et al., 1989)]. Total dietary choline intake by normal adults is probably 630–1000 mg/d or more (Zeisel, 1981).

The free choline content of human milk averages 80-200 μmol/L (9-22 mg/L) (Holmes et al., 2000; Zeisel, 1981; Zeisel et al., 1986). However, other forms of choline in aqueous (phosphocholine and glycerophosphocholine) and lipid (phosphatidylcholine and sphingomyelin) fractions raise the average total choline in mature milk to 1.28-1.35 mmol/L (Holmes-McNary et al., 1996; Holmes et al., 2000). The IOM (1998) uses a total choline concentration in human milk of 1.5 mmol/L (160 mg/L or 23 mg/100 kcal for milk with an energy content of 690 kcal/L) to calculate adequate intake for term infants. Choline levels in cow milk are reported to be 43–285 mg/L, or from 6 to 43 mg/100 kcal (Alston-Mills, 1995).

Review of the literature. Specific dietary requirements for choline have been demonstrated in some animal species (Zeisel, 1994). Kaminski et al. (1980) reported that rats and patients given choline-free parenteral nutrition for 10 days or more developed increased fat deposits in the liver, which were reversed in the rats by choline repletion. Several other investigators have demonstrated that rats fed choline-free diets had reduced levels of carnitine in liver, heart, and skeletal muscle, which were attributed to a deficiency of available methyl groups required for carnitine synthesis (Carter & Frenkel, 1978). A similar carnitine depletion in choline deficiency was observed to be associated with a diminished rate of long-chain fatty acid oxidation by tissue homogenates (Corredor et al., 1967). Choline or carnitine given normalized the oxidation rate in vivo, but only carnitine was effective in vitro. In addition, choline-deficient animals are more likely to develop liver cancer, especially with low methionine intakes (Chandar & Lombardi, 1988; Ghoshal et al., 1983; Ghoshal & Farber, 1984; Newberne & Rogers, 1986). Although the mechanisms for the development of hepatic carcinoma in dietary choline deficiency are unknown, liver cell necrosis with regeneration (Ghoshal et al., 1983), enhanced cellular proliferation (Shinozuka & Lombardi, 1980), increased levels of lipid peroxidation in the nucleus (Rushmore et al., 1984), and undermethylation of DNA (Dizik et al., 1991) have all been reported.

Zeisel (1994) has summarized the lines of evidence that suggest that choline is an essential nutrient for humans:
- Human cells grown in culture have an absolute requirement for choline
- Healthy humans fed choline-deficient diets have decreased plasma choline concentrations
- Malnourished humans have diminished plasma or serum choline concentrations
- In other mammals, including the monkey, choline deficiency results in severe liver dysfunction
- Humans fed intravenously with solutions containing little choline develop liver dysfunction that is similar to that seen in other choline-deficient mammals.

Inasmuch as the precursors for choline biosynthesis phosphatidylcholine and phosphatidylethanolamine are abundant in the diet, free choline itself has not been considered as an essential nutrient for humans. Whether de novo synthetic capabilities of adults or infants are sufficient to obviate the need for a dietary source is not known.

The review of literature in the report Assessment of Nutrient Requirements for Infant Formulas (Raiten et al., 1998a) did not describe any nutritional studies of term infants related to

choline requirements. The present Expert Panel has found no literature on choline metabolism and biochemistry in preterm infants. There seem to have been no studies demonstrating toxic effects of choline. Health Canada (1995) asserted that no adverse effects have been reported with current Canadian levels of choline in formula (12 mg/100 kcal).

Conclusions and recommendations. Despite suggestive evidence that choline is a conditionally essential nutrient for humans of all ages, there is apparently no relevant literature to inform a recommendation as to the optimal intake of choline by premature infants. Nevertheless, the Expert Panel thought that it would be inconsistent and illogical not to provide formula guidelines for premature infants when such guidelines have already been recommended for full-term infants (Raiten et al., 1998a). There being no basis on which to recommend any alteration in minimum level of choline recommended for term infant formula, the Expert Panel chose to accept the term level of 7 mg/100 kcal as a minimum for premature infant formula as well. However, Expert Panel members recommended a maximum of 23 mg/100 kcal, closer to the upper limit of choline content in human milk (Health Canada Health Protection Branch, 1995; Holmes-McNary et al., 1996; Holmes et al., 1996; Holmes et al., 2000) than the maximum of 30 mg/100 kcal recommended for term infant formula (Raiten et al., 1998a).

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of choline in preterm infant formula be 7 mg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum concentration of choline in preterm infant formula be 23 mg/100 kcal.**

Nucleotides, nucleosides, and other nucleotide precursors

Background. Nucleotides are small compounds of low molecular mass (300–350 Da) that represent up to 20% of the nonprotein nitrogen in human milk. A nucleotide contains one of the purine bases adenine and guanine, or one of the pyrimidine bases cytosine, thymine, and uracil, to which a phosphorylated pentose sugar is attached. Nucleosides are nucleotide precursors that lack the phosphorylation. Other nucleotide precursors include ribonucleic acids, deoxyribonucleic acids, purine and pyrimidine bases, and nucleoproteins. These entities in dietary sources are collectively referred to as total potentially available nucleotides.

There are two mechanisms by which intracellular nucleotide pools are maintained. One is the de novo pathway, which involves the generation of new nucleotides from the precursor amino acids glutamine, glycine, and aspartate. The second is the salvage pathway, which utilizes dietary sources or nucleic acid breakdown products to synthesize new nucleotides. There is some evidence in support of the idea that purines and pyrimidines are conditionally essential nutrients, but there is no evidence of a dietary deficiency state (Uauy et al., 1993). Cells that are dividing rapidly, such as lymphoid and intestinal epithelial cells, depend on the salvage pathway (Savaiano & Clifford, 1981; Uauy et al., 1993).

Among the recognized biological functions of nucleotides is their participation in nucleic acid synthesis. They are also believed to have a role in functions of the immune system. Congenital deficiencies of adenosine deaminase and nucleoside phosphorylase, enzymes of nucleotide metabolism, produce clinically significant impairments of T and B cell function (Carson et al., 1977; Giblett et al., 1975). Animal studies and observations on transplant recipients support a role for nucleotides in macrophage activation, cytokine production, natural killer cell activity, and resistance to bacterial and fungal infections (Kulkarni et al., 1986; Kulkarni et al., 1994; Quan et al., 1990). Studies of in vitro human cells indicate an influence of nucleotides on immunoglobulin production (Jyonouchi et al., 1993). Many aspects of nucleotide-mediated effects on the immune system that have been observed in animal model systems have not yet been confirmed in humans.

Nonimmune functions of nucleotides may include the enhancement of iron absorption (Quan et al., 1990), stimulation of small intestine development (Uauy et al., 1994b), and modification of intestinal microflora (Gil & Uauy, 1995). Nucleotides favor the growth of *Bifidobacterium* species in vitro (Tanaka & Mutai, 1980), and Gil et al. (1986) reported the same effect on formula-fed term infants in vivo. Bifidobacteria normally grow avidly in the intestinal environment of the breast-fed infant, where they contribute to a lowering of the pH of the intestinal contents by hydrolyzing various sugars. This action might be expected to provide protection against the growth of acid-intolerant strains of intestinal pathogens (Gil & Uauy, 1995). Balmer et al. (1994), however, could not confirm the reported favorable effect of nucleotide salts on growth of bifidobacteria (Gil et al., 1986). They reported that these compounds actually decreased the percentage of formula-fed term infants who became colonized by bifidobacteria and increased the percentage of those who became colonized with *Escherichia coli*.

Nucleotides and nucleosides ("total nucleic acids") in milk may not actually have any adaptive function but may simply be derived from milk-borne cells that have been disrupted during secretion (Atkinson & Lönnerdal, 1995);(Jensen et al., 1995c). Whether this is plausible quantitatively does not seem to have been calculated and reported.

The nucleic acids in milk comprise a complex mixture, with concentrations varying among individuals and stages of lactation, and a total concentration range of 118–720 μmol/L (Gil & Uauy, 1995). Leach et al. (1995) found the total potentially available nucleosides (the preferred form for absorption in the small intestine), either native or derived from enzymatic catabolism of nucleotides, to be 240 μmol/L of milk at 1 month postpartum ("early mature milk") and 202 μmol/L at 3 months postpartum ("late mature milk"). Allowing for the different molecular mass of the different nitrogenous bases and the different relative amounts of their nucleotides in milk (Leach et al., 1995), we calculate a corresponding mean for the total potentially available nucleoside content of 61 mg/L in human early milk and 51 mg/L in late milk. In milk providing 670 kcal/L, these values are 9.1 and 7.6 mg/100 kcal, respectively.

The Expert Panel on Assessment of Nutrient Requirements for Infant Formulas concluded that the nutritional advantage of adding nucleotides to infant formulas has not been demonstrated convincingly and therefore recommended a minimum nucleotide content of zero (Raiten et al., 1998a). Nevertheless, because there are potential benefits derived from adding nucleotides to infant formulas, the panel urged a continuation of research in this area. The recommendation for a maximum content of nucleotides and nucleotide precursors (polymeric nucleic acids) in term infant formula was 16 mg/100 kcal, a value similar to the upper limit of nucleotide equivalents reported for human milk (Thorell et al., 1996).

IDACE (1996) recommended that the inclusion of nucleotides in LBW formulas be optional. The maximum total potentially available nucleotides in LBW formula was not to exceed 22 mg/100 kcal. IDACE converted the micromolar nucleoside values in Leach et al. (1995) to mg/100 kcal of nucleotides, because this is the form used for fortification, and

recommended specific maxima of 3.7, 6.7, 6.5, and 5.2 mg/100 kcal for uridine monophosphate, cytidine monophosphate, guanosine monophosphate, and adenosine monophosphate, respectively. Health Canada (1995) concluded that additional preclinical and clinical studies would be necessary to justify the addition of nucleotides to term or preterm infant formulas.

Review of the literature. The report *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a) detailed the relevant studies on nucleotides in formula that have been performed with term infants, and that information will not be presented here. The following summarizes other important studies relevant to preterm infants.

Dietary nucleotides were found to alter the fatty acid profile of RBC phospholipids and the lipid content of the RBC membrane in premature infants during the first month of life (Pita et al., 1988). The infants were fed preterm human milk, usual preterm formula, or preterm formula with nucleotides added at 1.8 mg/100 kcal. The nucleotide concentration of the preterm human milk was not determined. After 30 days, the phospholipids of the formula-fed infants not receiving nucleotides had more linoleic acid and less n-6 polyunsaturated fatty acids longer than 18 carbons than did the human milk-fed infants. Nucleotide supplementation altered the values in the direction of those in the breast-fed infants. Membrane cholesterol levels were higher in infants fed nucleotide-free formula than in those receiving mother's milk or nucleotide-supplemented formula. Pita et al. (1988) suggested that the lower unsaturation index and increased cholesterol-to-phosphorus molar ratio found in the unsupplemented preterm infants could decrease fluidity of the RBC membrane. The resulting diminished deformability of the RBC might compromise the circulation to some tissues and lead to cell damage. However, Woltil et al. (1995) could not confirm the reported stimulation of the production of long-chain polyunsaturated fatty acids by nucleotide supplementation of formula in preterm infants.

Sanchez-Pozo et al. (1994) provided a total nucleotide content of 1.5 mg/100 kcal in a preterm infant formula fed for 7 days to 10 infants of mean GA 34 weeks. The serum from these infants was analyzed for lipoproteins, and the results were compared with those in serum from 10 preterm infants fed a similar formula without nucleotide supplementation. Nucleotide supplementation increased the concentrations of all lipoproteins measured. It did not change the cholesterol content of the lipoproteins, but the ratio of cholesterol ester to unesterified cholesterol rose. The changes observed in lipoprotein concentration were mainly due to an increased apolipoprotein content. Nucleotide-induced changes in AGA term infants were not as marked (Sánchez-Pozo et al., 1994), but those in SGA term infants resembled those in premature infants (Morillas et al., 1994).

Sanchez-Pozo et al. (1995) pursued these observations to develop a possible mechanism for the nucleotide effects based on the activity of lecithin-cholesterol acyltransferase (LCAT), an important enzyme in the metabolism of plasma lipoprotein phospholipids and cholesterol. The proposed mechanism depends on an observed effect of nucleotides in promoting intestinal maturation, thereby increasing the production of both the high-density lipoprotein, which is the LCAT substrate, and the apolipoproteins A-I and A-IV, which are its activators.

Neither Pita et al. (1988) nor Sanchez-Pozo et al. (1994) observed effects on growth in these relatively short-term studies. The premature infants studied in both investigations were mostly near the upper end of the range for preterm infants, GA of 35–37 weeks at birth and mean BW of about 2000 g.

The supposed immunological effects of dietary nucleotides are explained by their influence on natural killer cell function (Carver et al., 1991) and the maturation of helper-inducer T cells (Gil & Uauy, 1995). Preterm infants receiving a formula supplemented with nucleotides for the first 3 months of life have recently been reported to exhibit higher plasma levels of immunoglobulin IgM antibodies at 1 and 3 months of age (Navarro et al., 1999). The IgA level was also higher at 3 months, but IgG was not affected. Two years earlier, however, Martinez-Augustin et al. (1997) had reported that the serum concentrations of IgG antibody to β-lactoglobulin were higher in premature infants fed nucleotide-supplemented formula through the first month of life than in control infants not receiving the supplement. Intestinal absorption of β-lactoglobulin was not affected by the supplementation, so the effect was apparently not on presentation of the antigen but on the humoral immune response to it.

Conclusions and recommendations. No authoritative individuals or groups have categorically recommended the addition of nucleotides to infant formula. This Expert Panel found no reason to do differently. The evidence that nucleotides are important nutrients to premature infants is supportive but preliminary (Uauy et al., 1993). The effects on gastrointestinal maturation, immune function in vivo, LCAT activity, and plasma lipoproteins are weak, of short duration, and/or in conflict with other reports (Hamosh, 1997). There is a theoretical possibility of toxicity, either from hydroxyl radicals generated during conversion of the purine breakdown product hypoxanthine to uric acid, or from the latter compound itself. However, this has not been observed with the use of formula supplemented with nucleotides to levels similar to those found in human milk (Quan et al., 1990; Uauy et al., 1993). At higher levels, there is a possibility of toxicity from adenine or from heat-induced degradation of pyrimidine nucleotides in formula to orotic acid (Quan et al., 1990).

Recommendation

Note. **The Expert Panel made no recommendation about the level of nucleotides appropriate for preterm infant formula.**

8. CARBOHYDRATES

TOTAL CARBOHYDRATE

Background

Several expert committees have recommended that the postnatal growth of a prematurely born infant should approach the rate of growth that would be achieved in utero by a normal fetus of the same postconceptional age. This is possible with properly constituted preterm infant formulas for infants born at more than 1000 g. However, a discrepancy between extrauterine and intrauterine growth curves exists with more preterm neonates, so that the growth of preterm infants (born at less than 27 weeks of gestation) often lags behind in utero rates (Ehrenkranz et al., 1999). Adequate intake of energy, provided as protein, carbohydrate and fat, supports growth.

Data were reviewed (see Appendix A) that suggested that fractional fat absorption in preterm infants fed formula was approximately 90% (Kien et al., 1982; Kien et al., 1990a). In addition, evidence will be presented in this chapter that indicates that some of the potential dietary carbohydrate energy ingested by preterm infants is also not available to the infant because of incomplete digestion of lactose in the small intestine. As will be discussed, carbohydrate is apparently efficiently fermented in the colon of preterm infants so that little

of this source of dietary energy is found in the feces (although some is lost as heat during fermentation) (Kien et al., 1982).

Review of the literature

Need for carbohydrate and limits of intake. Carbohydrates are a major source of energy for children and adults. The principal circulating carbohydrate in the body is glucose. It is the primary source of energy for the brain and is an important carbon source for de novo synthesis of fatty acids and several nonessential amino acids. The majority of exogenously administered carbohydrates during low energy intake or starvation have been shown to have nitrogen-sparing effects in adult humans (Gamble, 1946). However, a relationship between carbohydrate intake (as distinguished from caloric intake) and nitrogen balance in children has not been evaluated systematically.

The acceptable upper and lower limits of carbohydrate intake in infants and children have been reviewed (Bier et al., 1999; Kalhan & Kilic, 1999). Although very few studies have examined the whole-body metabolism of glucose in the human preterm infant, a few inferences can be drawn from the published data (Kalhan, 1994). For example, some studies have quantified the rates of production and oxidation of glucose, the quantitative contribution of glucose to energy expenditure, the impact of exogenous glucose administration, and the estimated rate of utilization of glucose by the brain, the major glucose-dependent organ.

The rates of endogenous production of glucose in preterm and term infants are presented in Table 8-1. From several published reports, it can be inferred that the preterm infant has a higher rate of basal glucose production than does the full-term infant [8–9 mg/(kg·min) compared with 5 mg/(kg·min)] (Bier et al., 1977; Cowett et al., 1983; Denne & Kalhan, 1986; Hertz et al., 1993; Kalhan, 1994; King et al., 1982; Patel & Kalhan, 1992; Sunehag et al., 1993; Van Goudoever et al., 1993). In addition, respiratory exchange data indicate that 65–70% of the glucose produced is oxidized to carbon dioxide in full-term infants (Denne & Kalhan, 1986). At this rate of oxidation, only approximately 50% of the total energy needs will be met, perhaps accounting for most of the energy requirement of the brain. No data are available for preterm infants, in part because clinical and ethical considerations make it impossible to study them in the basal state.

The preterm infant has the capacity to oxidize large amounts of glucose to meet energy demands, so that at high rates of glucose infusion nearly all energy expenditure comes from oxidation of glucose or other carbohydrates (Sauer et al., 1986).

TABLE 8-1

Rates of endogenous glucose production and oxidation in newborn infants and children[1]

Variable	Unit	Preterm infant	Term infant	Child
Rate of glucose production	mg/(kg · min)	~8.9	5.0	4.0
	g/(kg · d)	11.5–12.9	7.2	5.8
Rate of glucose oxidation	g/(kg · d)	—[2]	4.7	—[2]
Percentage of glucose produced that is oxidized	%	—[2]	65	—[2]

[1] Data are averages from several studies (see text for details and references). Oxidation was calculated from respiratory gas exchange.
[2] Indicates that data were not available.

Glucose utilization by the brain. The rate of glucose consumption by the brain can be calculated from the published data on the brain's oxygen consumption. The average rate of oxygen consumption by the brain in children is 235 μmol/(min·100 g of brain weight), indicating oxidation of 39 μmol of glucose/(min·100 g). With these data for a full-term newborn with a brain weight of 399 g, the rate of glucose consumption by the brain will correspond to 37 g/d or 11.5 g/(kg·d). Thus, to meet the glucose requirement of the brain, the full-term infant should receive a minimum 11.5 g of carbohydrate (glucose)/(kg·d) (Kalhan & Kilic, 1999). Data for the preterm infant are not available. However, recent studies using positron emission tomography have suggested a lower rate of glucose uptake by the brain in the neonate, particularly immediately after birth. In addition, regional differences in the local cerebral metabolic rate of glucose have been published (Chugani, 1993; Kinnala et al., 1996; Suhonen-Polvi et al., 1993). The reasons for the difference between the positron emission tomography and the arteriovenous gradient data are not clear.

Carbohydrate supplementation to promote growth. Kuschel and Harding (1999a) reviewed several published neonatal and perinatal databases to determine whether carbohydrate supplements added to human milk would lead to improved growth and neurodevelopmental outcome in preterm infants without significant adverse effects. They found no studies that specifically evaluated the addition of carbohydrates alone for the purpose of improving growth or neurodevelopmental outcome. All published trials had used carbohydrate as only one component of a multicomponent fortifier.

Glucose uptake by the gut. Glucose is the major biological carbohydrate, an intermediate on the final pathway for the metabolism and oxidation of dietary carbohydrates. Free glucose is present in human milk at very low concentrations, i.e., less than 300 mg/L (Newburg & Neubauer, 1995).

Glucose is absorbed by the intestinal tract via a specific sodium/glucose cotransporter that handles all hexoses. D-Glucose and D-galactose are the natural substrates for this transporter (Wright, 1993). This cotransporter has been identified as a 73-kDa integral membrane protein; the gene has been cloned and sequenced. Indirect evidence suggests that gut absorption of glucose is present in fetuses of a gestational age (GA) of 12 weeks, but ontogenic changes have not been demonstrated (Jirsová et al., 1966; Levin et al., 1968). Other indirect measurements of glucose absorption have not demonstrated significant differences between preterm and term infants (McNeish et al., 1979).

The absorption kinetics of glucose by the gut in preterm infants has been examined in 16 infants with a birth weight (BW) of 1120 ± 89 g and a GA of 27.7 ± 0.5 weeks (mean ± SEM) (Shulman, 1999). The infants were studied at 32 ± 0.6 days (28–38 days) after birth, when the orogastric intake of formula or human milk was fully established. There was a positive correlation between both the maximum velocity V_{max} and Michaelis-Menten constant K_m and the postnatal age of the infants at the time of study (K_m: $r = 0.6$, $P = 0.015$; V_{max}: $r = 0.64$, $P = 0.008$). The K_m was also positively related to the amount of preterm infant formula that the infant received. There was a correlation between GA and $\log V_{max}$ for glucose ($r = 0.52$, $P = 0.039$) (Shulman, 1999). This study also demonstrated that at equal glucose loads, measured in mg/(min·cm), glucose absorption was greater when the infusion was delivered at a higher rate and lower concentration than when the infusion was delivered at a lower rate and higher concentration. This finding may have resulted from the difference in osmolarities of the infusates. Finally, there was some

indication that antenatal glucocorticoids given to the mother might enhance the $V_{\max}$ for glucose in the infants, but the results in this study were not significant. These data are from the only carefully conducted investigation of glucose absorption by preterm infants in the literature. However, the postnatal age of the infant and the duration of enteral feeding appear to have been the predominant determinants of K_m and $V_{\max}$, rather than the GA of the infant. The K_m values reported by Shulman are comparable to those obtained from studies in adults (Modigliani et al., 1973).

Thus, the data from these studies suggest that absorption of glucose is well developed in enterally fed preterm infants, that it appears to change with postnatal age (or duration of enteral feeding), and that it is influenced by type of feeding. The administration of glucocorticoids might also have a positive impact on glucose absorption by the infant; however, this hypothesis requires further testing. Glucose has not been used as the exclusive carbohydrate in infant formula because it would increase the osmolarity presented to the intestine; moreover, its nonenzymatic reaction with protein when heated can lead to the Maillard reaction (Raiten et al., 1998a).

Upper and lower limits of carbohydrate intake. On the basis of the above-mentioned data, acceptable upper and lower limits of carbohydrate intake can be estimated. These limits are theoretical, because the impact of carbohydrate intake at such limits on whole-body metabolism, growth, and nitrogen sparing has not been evaluated in infants and children, neither in the basal state nor under defined experimental conditions (Bier et al., 1999; Kalhan & Kilic, 1999).

The upper limit of carbohydrate intake should be related to the minimal need for the other macronutrients: protein and fat. Although total energy needs of an infant or an adult can be met by carbohydrates, there is an obligatory need for protein and fat to provide for growth and essential nutrients. Thus, the upper limit of carbohydrate intake can be calculated as the glucose equivalent of the total energy expenditure minus the calories from the minimum requirements for protein and fat.

The minimum protein concentration in formula recommended in this report is 2.5 g/100 kcal, or approximately 10% of calories. The minimum lipid concentration in formula recommended in this report is 4.4 g/100 kcal, or approximately 40% of calories. The maximum caloric intake from carbohydrate is therefore 100% minus 10% (for protein) minus 40% (for fat), or 50% of total caloric intake. This is approximately 12.5 g of carbohydrate/100 kcal.

In contrast to the upper limit, the lower limit for carbohydrate intake can be defined on the basis of that necessary to meet the energy requirements of the brain and other glucose-dependent organs, that which can minimize the irreversible loss of protein and nitrogen and minimize gluconeogenesis, and that which prevents ketosis. For the preterm infant, such estimates can be made only on the basis of the minimum endogenous rate of glucose production, or 11.5 g/(kg·d) (See Table 8-1). These estimates assume the preterm infants to be a uniform homogeneous group and do not separate the very preterm infants from the less preterm infants. Further studies are needed to extend existing data on rates of glucose production and utilization by the preterm-LBW infant. The recommended minimum of 11.5 g/(kg·d) for preterm-LBW infants is the same amount needed to meet the glucose requirement for the brain of the newborn full-term infant (Kalhan & Kilic, 1999). Preterm formulas containing 9.6 g carbohydrate/100 kcal provide 11.5 g/(kg·d) at an energy intake of 120 kcal/(kg·d). The minimum content of preterm infant formulas

should not be lower than 9.6 g carbohydrate/100 kcal to support normal neural function.

Recommendations

Minimum. **The Expert Panel recommended that the minimum carbohydrate content (lactose or nutritionally equivalent di-, oligo- or polysaccharides) of preterm infant formula be 9.6 g/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum carbohydrate content (lactose or nutritionally equivalent di-, oligo- and polysaccharides) of preterm infant formula be set on the basis of the minimum fat and protein requirements as 12.5 g/100 kcal.**

LACTOSE

Absorption and metabolism

Intestinal lactase activity is measurable in the human fetus at 10–12 weeks of gestation. There is a gradual increase in lactase activity with advancing gestation, but the enzyme activity remains low until about 36 weeks GA, when it reaches the levels of full-term neonates (Auricchio et al., 1965). On the basis of the low lactase activity in early gestation and the estimated length of the bowel, in a preterm infant weighing 1300–1400 g, 50–70% of the ingested lactose might pass unabsorbed into the colon (Watkins, 1982). These estimates are similar to those of Kien et al. (1987; 1996) who studied 14 preterm infants (GA of 26–31 weeks), at 31–37 weeks postconceptional age, using stable isotope tracers of glucose and lactose to assess lactose digestion and breath hydrogen excretion to assess carbohydrate fermentation. They demonstrated that lactose digestion was 79 ± 26% (mean ± SD); the percentage of lactose that was not absorbed, but fermented in the colon, averaged 35 ± 27%.

Whether premature birth or early exposure to lactose-containing nutrients can induce intestinal lactase is unknown. Shulman et al. (1998) examined whether the timing of initiation of feeding affected the development of intestinal lactase activity. In a randomized trial, 135 infants (GA of 26–30 weeks) were assigned to begin feedings either early (4 days) or late (15 days). The intestinal lactase activity was evaluated by measuring the urinary excretion ratios of lactulose to lactose after the two sugars were administered. The data suggest that early feeding increases intestinal lactase activity in preterm infants. In addition, the age at which full feedings were attained was inversely related to lactase activity ($r = -0.34$; $P = 0.001$).

One study has suggested that elimination of lactose from formula ameliorates feeding intolerance (Griffin & Hansen, 1999). These investigators reported a prospective, randomized double-blinded controlled trial involving 306 preterm infants. The study compared whey-predominant, cow protein formulas that differed in the type of carbohydrate. Although the original publication did not provide some details on composition of the formulas fed, through personal communications and the published response to a letter to the editor (Kien, 2001), the Expert Panel was able to provide in this report additional information concerning the study. Specifically, the carbohydrate in the control formula was 41% lactose and 59% corn syrup solids, whereas the low lactose experimental formula was composed of 35.1% maltose, 64% corn syrup solids, and less than 1% lactose. The infants received one of these formulas for an average of 4 weeks (J. W. Hansen, personal communication). The intention-to-treat groups and treatment-received groups were distinguished by the fact that despite assignment to one of the two formulas, the former also received some

human milk or solely human milk. In both of these groups, markedly reducing (effectively eliminating) lactose from the formula increased weight gain and formula intake and reduced the average gastric residuals (mL/d) as well as the number of days to attain full enteral feedings [115 kcal/(kg·d)]. None of these individual outcome variables were statistically significant between formula groups, although by using the multivariate rank sum test to analyze all these outcome variables (as well as some additional variables), there was a statistically significant improvement in feeding outcome in the infants receiving the low lactose formula. These results are somewhat consistent with the findings of Shulman et al. (1998) that indicated that the time to full enteral feedings was inversely correlated with lactase activity, according to the urinary lactulose-to-lactose ratio technique.

Fate of lactose or other dietary carbohydrates reaching the colon: possible clinical significance

As noted above, a fraction of ingested lactose (and possibly other dietary carbohydrates) is fermented in the colon of preterm infants, where much of the constituent energy can be absorbed as short-chain fatty acids and lactate (Kien et al., 1987; Kien et al., 1989; Kien et al., 1990b; Kien, 1990; Kien et al., 1992a; Kien, 1996). This process of colonic salvage or retrieval of carbohydrate energy compensates, at least in part, for the inefficiency of dietary energy utilization resulting from inefficient small intestinal digestion of lactose by preterm infants (Kien et al., 1992b; Kien et al., 1996). However, there is controversy concerning whether the production of short-chain fatty acids such as butyric acid is advantageous to the preterm infant or is toxic to the colon or the small intestine if excessive fermentation occurs at that site (Argenzio & Meuten, 1991; Butel et al., 1998; Kien, 1990; Roediger, 1982; Szylit et al., 1998).

On the positive side, butyric acid is an important substrate for colonocytes (Roediger, 1982). In adults, colitis and mucosal atrophy, in a diverted segment of colon has been treated with instillation of short-chain fatty acids (Harig et al., 1989). Moreover, short-chain fatty acids produced via fermentation in the colon may have trophic effects on the small intestine (Frankel et al., 1994).

In contrast, butyric acid has proinflammatory effects when rapidly infused into the bovine rumen (Sakata & Tamate, 1978), and high luminal concentrations of lactate, acetate, and other short-chain fatty acids acidify the colonic lumen, which in turn can lead to local irritant effects and even ulceration of the colon as well as increased cell proliferation (Argenzio & Meuten, 1991; Lupton et al., 1985). In addition, some have suggested that the colonic luminal concentration of butyric acid may be a factor in the etiology of necrotizing enterocolitis (Butel et al., 1998; Szylit et al., 1998). Lactose is the primary carbohydrate in human milk; its concentration in milk is approximately twice that found in presently available preterm infant formulas (Kien, 1996). Therefore, it is difficult to accept, a priori, how lactose and its subsequent fermentation could play an important etiologic role in necrotizing enterocolitis. Also, the model for necrotizing enterocolitis used by Butel et al. (1998) is the quail, which lacks intestinal lactase. Their model for butyric acid toxicity (to the cecum) involves inoculation followed by colonization of the cecum with clostridial species, which are thought to produce butyric acid at high levels. When bifidobacteria species were cointroduced into the cecum, the colonization of the cecum by clostridial species was suppressed, cecal luminal butyric acid concentrations were markedly lowered, and cecal toxicity was eliminated (Butel et al., 1998). Because feeding human milk to term neonates tends to stimulate colonization of the gut with bifidobacteria species and suppresses colonization by clostridia (Mackie et al., 1999), it is possible that, with the high lactose concentration of human milk compared with that of preterm infant formulas, ingestion of human milk represents a special case in which lactose malabsorption may play a minor role in causing gut toxicity or inflammation.

Lactose malabsorption that occurs even in older, term infants (Barr et al., 1984) may serve a beneficial, "fiber-like" function for preterm infants undergoing rapid intestinal and colonic development. However, one should be cognizant that "overfermentation" of carbohydrate may cause colonic damage (and/or small intestinal damage if "bacterial overgrowth" and abnormal fermentation occur at that site) as well as necrotizing enterocolitis (Argenzio & Meuten, 1991; Butel et al., 1998; Kien, 1990). Clearly, data to evaluate either of these two hypotheses sufficiently are lacking, especially for the preterm infant.

Lactose and calcium bioavailability

Data from animal studies, particularly the rat model, have provided evidence that lactose has beneficial effects on intestinal calcium absorption and calcium retention in bone (Cochet et al., 1983; Lengemann et al., 1959). This beneficial effect may be related to the resistance of lactose to enzymatic degradation in the rat, because the presence of other nonabsorbable sugars can also promote calcium absorption in the rat small intestine (Brommage et al., 1993). However, it has been surmised that the effect of sugars to promote calcium absorption in the jejunum may also be related to the process of sugar absorption and thus water absorption, resulting in an increase in calcium concentration at the site of its absorption (Schuette et al., 1989). In lactose-tolerant normal healthy adults, lactose, when administered as an aqueous solution, has been found to have either a positive effect (Cochet et al., 1983) or no effect (Zittermann et al., 2000) on calcium absorption (determined, respectively, using a dual radiolabeled calcium technique or a stable strontium-loading test). However, in healthy, young adults, characterized as lactase deficient based on hydrogen breath testing, the administration of lactose in an aqueous solution reduced calcium absorption (Cochet et al., 1983), whereas lactose administered as a milk formula increased calcium absorption in similar subjects (Griessen et al., 1989).

As with data from studies in adults, the data on the effect of lactose on calcium absorption on newborn term infants are also conflicting. In six healthy Caucasian term infants (BW > 2500 g) fed soy formula and studied during the first few months of life, Ziegler and Fomon (1983) observed a significant and sustained absorption-promoting effect of lactose on calcium and other minerals. However, the mean calcium intake was significantly higher and fecal excretion of calcium only "somewhat less" [61 ± 30 versus 66 ± 25 mg/(kg·d), mean ± SD] in the infants fed formula containing lactose than in the infants fed formula containing sucrose and cornstarch hydrolysate. Moya et al. (1992) also showed 20% improvement in fractional calcium absorption in term infants when lactose, as opposed to glucose polymers, was the source of carbohydrate in a cow milk formula. In a similar later study designed apparently to evaluate a new lactose-free formula, Moya et al. (1999) did not observe a significant effect of lactose on calcium absorption. In this later study, calcium retention was greater with the lactose-free formula and was ascribed by the authors to the higher calcium concentration of the lactose-free formula (and thus higher calcium intake).

Stathos et al. (1996) studied preterm infants ($n = 14$, BW of 960–2230 g, GA of 29–36 weeks) by using a stable isotope of calcium as a tracer; they observed an approximate 21-fold higher rate of calcium absorption with glucose polymers than with a similar amount of lactose. Calcium absorption correlated positively with water and carbohydrate absorption; however, the authors did not indicate whether lactose impaired calcium absorption in any infants who may have had equivalent absorption of lactose and glucose polymers. It should be underscored that the study of Stathos et al.(1996) used the triple-lumen catheter perfusion method, and hence the calcium absorption kinetics were measured only in the proximal intestine; the absorption in the distal gut was not quantified. During fetal life, lactase activity is higher in the jejunum than in the duodenum (Antonowicz et al., 1974; Auricchio et al., 1965). This gut location issue could also be relevant because it is believed that lactose affects calcium absorption by the non-vitamin D-dependent, paracellular uptake process thought to be facilitated by nonhydrolyzed lactose. Apparently, this uptake occurs throughout the small intestine, especially distal to the duodenum (Zittermann et al., 2000). Wirth et al. (1990) found that increasing the lactose content of a "standard" preterm infant formula (from 50% to 100%) had no affect on absorption of minerals.

The data for adults and term infants suggest that lactose does not have a clear beneficial effect on calcium bioavailability in lactose-tolerant subjects. Likewise, in lactose-intolerant adult subjects, the presence of nonhydrolyzed lactose in the distal bowel may either increase or impair calcium absorption. Although for the preterm infant the only available data suggest that lactose either has no effect or impairs calcium absorption, there is a significant level of uncertainty about these data.

Conclusions and recommendations

On the basis of data from published studies, preterm infants will probably not exhibit clinical signs indicative of lactose intolerance when they ingest the amount of lactose present in either human milk or commercial preterm infant formulas available in the United States. Moreover, despite the data reviewed above indicating that some preterm infants fed experimental formulas containing the amount of lactose present in human milk digest less than 50% of the lactose in the small intestine, they do not exhibit clinically significant problems with growth. Although it is true that some data in the literature raise the suspicion that lactose feeding in preterm infants may impair, rather than enhance, calcium absorption, the inevitable technical limitations of such studies prevent the Expert Panel from concluding that feeding lactose even as the sole or predominant disaccharide in the diet would be harmful. Similarly, although the authors of one recent study (Griffin & Hansen, 1999) suggested that removal of lactose might improve feeding tolerance, their conclusion was based on a multivariate statistic, the clinical significance of which is unclear. Because the majority of studies that have attempted to address the risk versus the benefit of reducing or eliminating lactose from the diet of preterm infants have shown only potential adverse effects of lactose feeding, it is tempting to argue that data are insufficient to justify any minimum level of lactose in preterm infant formulas. However, the Expert Panel was strongly influenced by the conservative position that lactose is the only source of dietary galactose (see the section on galactose below), and, except for some oligosaccharides, it is the only source of carbohydrate in human milk. Furthermore, the very fact that lactose is probably not fully digested in many

preterm and term infants may indicate that it serves as a source of nutrition for beneficial bacterial flora in the colon, which, through the process of fermentation, not only facilitate colonic water and electrolyte absorption but also stimulate cell turnover of both the colon and the small intestine. Although the Expert Panel does not wish to summarily dismiss the interesting data in the avian model demonstrating potential colonic toxicity from lactose malabsorption and butyric acid, these data are simply not sufficient to support restriction of lactose intake on this basis. Thus, the Expert Panel presently concludes, in the absence of much stronger evidence supporting the elimination of lactose from preterm infant formulas, that the experience derived from many years of successful enteral feeding of preterm infants using human milk or cow milk formulas containing lactose should not be dismissed and that lactose should be included as a constituent of preterm infant formulas.

Recommendations

Minimum. **On the basis of available data in the literature, the Expert Panel was not able to strongly justify a specific recommendation for a minimum amount of lactose in preterm infant formulas, but because the literature does document extensive clinical experience with feeding preterm infants both human milk and commercial formulas, the Expert Panel recommended that formulas contain at least 4 g of lactose/100 kcal or 40% of the carbohydrate intake (the approximate minimum value found in commercial preterm infant formulas).**

Maximum. **The maximum level recommended is that necessary to meet the entire carbohydrate requirement, namely 12.5 g/100 kcal.**

GALACTOSE

Although most human infants and the newborns of several other mammalian species ingest large quantities of galactose, the requirements and benefits of galactose feeding remain unknown (Kliegman & Sparks, 1985). The major source of galactose for the human newborn is lactose, which is the predominant carbohydrate in human milk and infant formula. Hydrolysis of lactose at the intestinal brush border results in the release of glucose and galactose, both of which are readily absorbed. Most of the enterally absorbed galactose is taken up by the liver during the first pass (Goresky et al., 1973; Kaempf et al., 1988), so that feeding causes only a minimal change in plasma galactose concentration. Galactose in the liver is either converted to glucose or deposited as glycogen. In the isolated perfused liver preparation and in in vivo studies of newborn animals, galactose has been shown to augment hepatic glycogen synthesis and to activate hepatic glycogen synthase (Kliegman et al., 1981; Kunst et al., 1989; Sparks et al., 1976a; Sparks et al., 1976b).

Galactose administered parenterally is also rapidly cleared from the plasma (Kliegman & Sparks, 1985). The rate of clearance appears to be similar in preterm and term neonates and in older infants. Although galactose has been used for the treatment and prevention of hypoglycemia and hyperglycemia in newborn infants, the nutritional requirement for galactose has not been examined. However, a significant rate of endogenous synthesis of galactose has been observed in normal healthy adults, as measured by stable isotope dilution methods (Berry et al., 1995). Thus, there does not appear to be an obligatory requirement for galactose in the normal healthy adult human.

Recommendation

Note. **The Expert Panel found no evidence to justify a recommendation for galactose in preterm infant formula.**

OLIGOSACCHARIDES

Human milk contains significant quantities of carbohydrates other than lactose in the form of oligosaccharides (Brand Miller & McVeagh, 1999). Most of these oligosaccharides are formed from the addition of monosaccharides to the molecule of lactose by specific glucosyl transferases of the mammary gland (Coppa et al., 1999). At least 130 different oligosaccharides have been identified in human milk (Brand Miller & McVeagh, 1999). Their presence and quantity are determined by genetic and environmental factors (Erney et al., 2000). The concentration of oligosaccharides represents the third largest solute load to the intestinal tract from mature milk, 9 g/L, after lactose and fat, and is more than that contributed by protein (Coppa et al., 1999; Newburg & Neubauer, 1995). Cow milk and infant formula contain very little oligosaccharide other than disaccharides or glucose polymers (Brand-Miller et al., 1998). The concentration of oligosaccharides in human milk also changes with the duration of lactation, being highest in the colostrum at 20–23 g/L, about 20 g/L on day 4 of lactation, and 9 g/L on day 120 of lactation (Coppa et al., 1993). Nakhla et al. (1999) showed that concentrations of 10 neutral oligosaccharides in preterm milk are similar to those in term milk. The concentrations of other oligosaccharides may be higher in preterm milk than in term milk (Brand Miller & McVeagh, 1999).

The biological role of oligosaccharides in human milk remains to be investigated. Some data show that breast-fed infants have a higher concentration of sialic acid in their saliva than do formula-fed infants (Tram et al., 1997). These investigators suggested that higher sialic levels were due to the higher concentration of sialyated oligosaccharides in human milk.

Little is known concerning the absorption and metabolism of oligosaccharides in preterm and term infants. Human milk-fed preterm infants have been observed to excrete lactose-derived oligosaccharides in the urine, suggesting some absorption of intact oligosaccharides (Rudloff et al., 1996). Brand-Miller et al. (1998), by using the lactulose hydrogen breath test, have demonstrated that most human milk oligosaccharides resist digestion in the small intestine of breast-fed infants, undergo fermentation in the colon, and may be the source of some of the breath hydrogen in breast-fed infants (but not formula-fed infants)(Barr et al., 1984). In the large bowel, the oligosaccharides may be involved in the maintenance of normal gut flora and in the inhibition of the growth of pathogenic bacteria; their fermentation products, short-chain fatty acids, provide nutrition and energy for the colonocytes and the body as a whole. Other evidence suggests that human milk oligosaccharides may have anti-infection roles in the intestinal, respiratory, and urinary tracts. Finally, sialic acid is a structural and functional component of brain gangliosides and could play a role in neurotransmission and memory. Animal data suggest that early supplementation with sialic acid improves brain ganglioside sialic acid concentration and learning ability (Brand Miller & McVeagh, 1999).

In summary, the role for oligosaccharides in the nutrition of preterm infants, although suggestive, has not been definitively evaluated. Therefore, the Expert Panel decided that no specific recommendation could be made for their use in the nutritional support of the preterm infant.

Recommendation

Note. **The Expert Panel found no evidence to justify a recommendation for oligosaccharides in preterm infant formula.**

NONLACTOSE DIETARY CARBOHYDRATES: GLUCOSE POLYMERS AND MALTOSE

Glucose polymers are pure carbohydrates prepared by controlled acid or enzyme hydrolysis of cornstarch. They consist of polymers of glucose of various chain lengths, although the majority are of medium (6–10 glucose units) chain length. The hydrolysates contain a small amount of free glucose, usually less than 2%. The glucose polymers are mainly linear, with the glucose residues attached to each other by α-1,4-glucosidic bonds. They have been used as nutritional supplements for adults and infants because their lower osmolality per kilocalorie than glucose or other hexose solutions may allow more rapid gastric emptying and because they are not as sweet as monosaccharides and disaccharides such as fructose and sucrose.

Glucose polymers are hydrolyzed by salivary, pancreatic, and intestinal amylases and maltases to free glucose. In the newborn, glucose polymers are primarily digested via intestinal glucoamylase and isomaltase (Gray, 1992; Kien et al., 1989). The absorption and oxidation of glucose polymers of different lengths were examined by Shulman et al. (1995) in 12 healthy 1-month-old infants. Their data show that long-chain glucose polymers are not absorbed as completely as short-chain glucose polymers are by some infants and that the colonic bacterial flora plays a major role in salvaging the carbohydrate energy not absorbed in the small intestine. However, there was a wide variation among subjects in the measured colonic fermentation of unabsorbed carbohydrate. Studies by Kien et al. (1982) with preterm infants suggested that only about 10% of energy derived from lactose or a combination (50:50) of lactose and glucose polymers was excreted in the feces. In a later study, Kien et al. (1987) presented evidence for extensive colonic fermentation of carbohydrates in infants fed a combined lactose and glucose polymer formula; however, doubling the lactose concentration of the formula (and eliminating glucose polymers from the formula) caused a doubling of the expired hydrogen value.

The absorption of lactose, glucose polymers, and a mixture of lactose and glucose polymers by preterm infants was compared by Shulman et al. (1995), using a double-lumen perfusion catheter placed in the duodenum-proximal jejunum. Twenty-one low BW infants (GA of 33 ± 3 weeks, mean ± SD) who were receiving nasogastric tube feedings were studied at a mean postnatal age of 19.3 ± 9 days (range: 9–39 days) (in a personal communication, Shulman indicated that the paper incorrectly presented the postnatal age in weeks). Absorption of lactose was significantly less than that of either the glucose polymers alone or the mixture of lactose and glucose polymers [0.18 ± 0.24 mg/(min·cm) versus 0.51 ± 0.45 and 0.57 ± 0.59 mg/(min·cm), respectively; $P < 0.005$]. In addition, lactose absorption was not related to postnatal age at the time of study or to the total duration of enteral feeding before the study. In contrast, absorption of glucose polymer alone or the mixture of lactose and glucose polymers was significantly correlated with postnatal age and the total duration of enteral feeding before the study. However, the correlation may have been excessively weighted by just two data points in the older age group (Figure 2 of the publication). Moreover, fetal lactase activity, although rather uniformly distributed along the small intestine, is lower in the

duodenum, the site where these perfusion studies were carried out (Antonowicz et al., 1974; Auricchio et al., 1965). A more extensive discussion of the digestion of glucose polymers appears in Appendix A.

As noted above, Griffin and Hansen (1999) showed that elimination of lactose from the formula and replacing it with maltose improved feeding tolerance in preterm infants. Although these investigators did not specifically address the merits of glucose polymer feeding, their results are germane to the issue of whether reduction or elimination of lactose in preterm infant formula may be relatively advantageous. Current commercial preterm infant formulas contain glucose polymers as a replacement for lactose. Because glucose polymers in present preterm infant formulas are probably digested in a manner similar to maltose (Gray, 1992; Kien et al., 1989), it is plausible that similar results to those of Griffin and Hansen (1999) would be observed if glucose polymers and not maltose were substituted for lactose. In fact, as pointed out in a letter to the editor (Kien, 2001), in a series of small, brief studies comparing a preterm infant formula containing 50% of the carbohydrate as lactose and glucose polymers with a similar formula containing lactose as the sole carbohydrate, weight gain was lower in the 100% lactose group (although not significantly so)(Kien et al., 1982; Kien et al., 1990a; Kien et al., 1998). However, in contrast to the Griffin and Hansen paper (1999), energy intake was also higher (but also, $P > 0.05$), implying that energy utilization could have been impaired.

Also, as discussed above, data are contradictory on the effect of lactose on calcium absorption, but it does appear that substitution of glucose polymers for lactose in preterm infants with defective lactose digestion may result in an improvement in calcium absorption. On the basis primarily of the results of Griffin and Hansen (1999), but supported apparently by casual observations on weight gain made in the course of studies of lactose digestion and absorption (Kien, 2001), reduction or elimination of lactose and replacement with more readily digestible carbohydrate such as maltose or glucose polymers may facilitate better feeding tolerance in preterm infants.

In summary, glucose polymers appear to be rapidly hydrolyzed and absorbed by the neonate, and the carbohydrate energy not absorbed in the small intestine is rapidly salvaged by colonic bacteria. In the lactase-deficient preterm infant, substitution of glucose polymers for lactose may enhance calcium absorption. Finally, substitution of lactose in formulas with maltose (and by inference, based on the mode of digestion, also glucose polymers) may improve feeding intolerance in preterm infants.

Recommendations

Note. **The Expert Panel found no evidence to justify a specific recommendation for glucose polymers or maltose per se in preterm infant formula. However, the use of these carbohydrates (or potentially other more readily digestible carbohydrates) as a partial alternative to lactose may have beneficial effects.**

MYO-INOSITOL

Background

myo-Inositol (inositol) is a six-carbon, cyclic sugar-related alcohol that is abundant in mammalian tissues. In plants and animals, *myo*-inositol exists in its free form, as phosphorylated lipid derivatives (phosphoinositides), and as glycosylphosphatidylinositol, anchors of membrane lipids (Aukema & Holub, 1994). A novel glycoprotein containing *myo*-inositol has been identified in rat brain and may serve as a recognition molecule on neurons during development (Holub, 1992; Uauy et al., 1993). In addition to containing free *myo*-inositol, human milk contains a disaccharide form of *myo*-inositol, 6-β-galactinol (Holub, 1986; Holub, 1992; Uauy et al., 1993). Scientific interest in *myo*-inositol derivatives, particularly the polyphosphoinositides, has been heightened by the discovery of a major role for these compounds as cellular mediators of signal transduction and intracellular calcium regulation (Aukema & Holub, 1994; Holub, 1986).

In rats, endogenous synthesis of *myo*-inositol from D-glucose occurs in testis, brain, liver, and kidney (Hauser, 1963). Clements and Diethelm (1979) demonstrated that the human kidney also has the ability to synthesize *myo*-inositol. Despite this, tissue concentrations of *myo*-inositol in rodents are still sensitive to fluctuations related to dietary intake (Holub, 1986). After 2 weeks of life, serum *myo*-inositol concentrations in preterm-low birth weight (LBW) infants were correlated with *myo*-inositol intake (Bromberger & Hallman, 1986).

In mammals, dietary sources of *myo*-inositol are transported through the intestinal epithelium by energy-dependent and sodium-dependent mechanisms (Vilella et al., 1989). Once absorbed, *myo*-inositol is distributed to the brain, liver, spleen, kidney, thyroid, and reproductive systems (Lewin et al., 1978). Serum *myo*-inositol level and the *myo*-inositol pool in humans are regulated primarily by renal metabolism and clearance mechanisms (Clements & Diethelm, 1979). The homeostasis of serum *myo*-inositol levels in neonates has been shown to be primarily influenced by renal clearance (Lewin et al., 1978).

Rats have markedly elevated triacylglycerol and esterified cholesterol levels when fed *myo*-inositol-deficient diets (Andersen & Holub, 1976; Burton et al., 1976; Hayashi et al., 1974). Growing rats fed diets deficient in *myo*-inositol have been reported to exhibit fatty infiltration of the liver, presumably as a result of increased hepatic fatty acid synthesis or the mobilization of fatty acids from adipose tissues (Beach & Flick, 1982; Hayashi et al., 1974). In contrast, neonatal rats fed *myo*-inositol-restricted diets from 6 days of age and *myo*-inositol-free diets from 16 to 72 days of age exhibited no fatty infiltration of the liver and no impairment of central nervous system myelination, despite diminished plasma and liver *myo*-inositol concentrations (Burton et al., 1976). Growth was suboptimal in both the experimental and the control groups. These investigators concluded that *myo*-inositol synthetic abilities of newborn rats are sufficient to maintain proper cellular and organ function despite dietary deficiency.

As the synthesis and degradation of *myo*-inositol are regulated in vivo, only under unusual circumstances does the clinician give particular attention to the patient's intake of exogenous *myo*-inositol. These circumstances include certain clinical situations in which *myo*-inositol synthesis is impaired (Aukema & Holub, 1994). Diabetes mellitus and chronic renal failure are examples of conditions in which altered *myo*-inositol metabolism and excretion may result from specific organ system dysfunction (Clements & Diethelm, 1979; Haneda et al., 1990; Holub, 1986; Olgemöller et al., 1990).

An estimated one or two infants per 1000 births in North America are born with a neural tube defect (Rosenberg, 1997). Approximately 70% of these defects can be prevented by adequate maternal folate intake and status during the first month of pregnancy. The remaining 30% of cases occur independent of maternal folate status (van Straaten & Copp, 2001). The curly tail strain of mice has been considered a genetic model of human folate-resistant neural tube defects because of the following similarities: form and structure of the defects, axial location, sex bias, and elevated concentration of α-fetoprotein in amniotic fluid (Rosenberg, 1997; van

Straaten & Copp, 2001). In curly tail mice, a deficiency of *myo*-inositol increases the incidence of exencephaly, whereas administration of exogenous *myo*-inositol ameliorates this effect (van Straaten & Copp, 2001). Furthermore, injecting dams with *myo*-inositol during gestation reduces the frequency of spina bifida (Rosenberg, 1997). Some have suggested that the effects of *myo*-inositol may be mediated through increased action of protein kinase C (Rosenberg, 1997; van Straaten & Copp, 2001) and up-regulation of expression of retinoic acid receptor β in the hindgut (Rosenberg, 1997).

Dietary sources of myo-inositol

Most foodstuffs from both plant and animal sources are rich in free and phosphorylated forms of *myo*-inositol (Holub, 1986). In plants, the predominant forms of *myo*-inositol are phytates, which are compounds known to form complexes with divalent cations; this is mostly relevant for soy-based formulas that are not recommended for premature infants.

The concentration of *myo*-inositol in human milk is three to four times greater than that in cow milk (Indyk & Woollard, 1994; Ogasa et al., 1975). Myo-Inositol concentration in human milk up to 4 months postpartum varies widely, with studies reporting the following concentrations:

- 490 μmol of free *myo*-inositol/L (~13 mg/100 kcal) (Indyk & Woollard, 1994)
- 830 μmol of total inositol/L (~22 mg/100 kcal) (Ogasa et al., 1975)
- 1450 μmol of free *myo*-inositol/L (~38–39 mg/100 kcal) (Pereira et al., 1990)
- 1800 μmol of *myo*-inositol/L (~47–48 mg/100 kcal) (Bromberger & Hallman, 1986)

On average, 24-hour milk samples collected at 14 (n = 99), 42 (n = 99), and 89 (n = 25) days postpartum contained 642, 766, and 830 μmol of *myo*-inositol/L (~17–22 mg/100 kcal), respectively (Huisman et al., 1996). In summary, the concentration of *myo*-inositol in human milk ranges from about 13 to 48 mg/100 kcal.

The variation of *myo*-inositol concentrations in milk reported in the literature has been attributed to differences in analytical methodologies (Raiten et al., 1998a). As explained by Raiten et al. (1998a), earlier studies used enzymatic fluorimetric (Pereira et al., 1990), microbial, and gas-liquid chromatographic (Bromberger & Hallman, 1986) (Ogasa et al., 1975) assays. More recent studies have utilized high-performance liquid chromatography because of its high level of accuracy (Indyk & Woollard, 1994). A new method of ion chromatographic determination of *myo*-inositol in liquids was recently published (Tagliaferri et al., 2000).

The concentration of *myo*-inositol in human milk is similar for mothers of term and preterm infants up to 4 months of lactation (Bromberger & Hallman, 1986; Pereira et al., 1990) and decreases with time during the first 6 weeks postpartum (Ogasa et al., 1975; Pereira et al., 1990). Phosphatidylinositol content, both total concentration and as a percentage of phospholipid, was similar for mature milk from mothers of term and preterm infants (Bitman et al., 1984; Bromberger & Hallman, 1986).

For term infants, the *myo*-inositol content of infant formulas available in the Netherlands reportedly varied from 22 to 346 μmol/L (4–62 mg/L) (Huisman et al., 1996). The infant formulas available in New Zealand contain 23–65 mg of free *myo*-inositol/100 g (~1275–3600 μmol/L) and 38–77 mg of total inositol/100 g (~2100–4275 μmol/L) (Indyk & Woollard, 1994). Milk-based term infant formulas available in Switzerland contain 30–150 mg of total inositol/100 g (~1670–8300 μmol/L), similar to the range measured in soy-based infant formula, in which total inositol refers to the sum of free

myo-inositol, inositol monophosphate, and *myo*-inositol derived from lecithin (Tagliaferri et al., 2000). The preterm formulas in the United States contain 5.5–17 mg/100 kcal (~270–760 μmol of *myo*-inositol/L) (Abbott Laboratories. Ross Products Division, 2001; Mead Johnson Nutritionals, 2000). An amino acid-based, hypoallergenic formula, not designed for preterm-LBW infants but sometimes used in the United States for such infants with food protein intolerance or allergy, contains 23.3 mg of *myo*-inositol/100 kcal (~5400 μmol of *myo*-inositol/L) (SHS North America, 2000).

Current recommendations for myo-inositol

The Code of Federal Regulations (CFR) specified a minimum of 4 mg of *myo*-inositol/100 kcal for non-milk-based infant formulas (Food and Drug Administration, 1985); no maximum concentration was indicated. The minimum was based on a recommendations of the American Academy of Pediatrics Committee on Nutrition (AAP-CON) (1976; 1985b), set in 1976 as 4 mg/100 kcal (22 μmol/100 kcal), to represent an average value of *myo*-inositol concentration in milk-based formulas at that time. Because evidence of *myo*-inositol deficiency was lacking, milk-based formula rather than human milk was used as the reference to set the minimum recommendation (American Academy of Pediatrics. Committee on Nutrition, 1976). Although the AAP-CON (1976) did not specify a maximum for *myo*-inositol, amounts equal to the amount found in human milk were permitted in infant formula.

The expert panel compiling the report *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a) recommended a minimum *myo*-inositol content of 4 mg/100 kcal for term formula. That expert panel was unaware of any new evidence that would shed light on the question of the essentiality of *myo*-inositol, particularly for healthy term infants, and therefore accepted the 1985 specification of the CFR. The same expert panel recommended a maximum content of 40 mg of *myo*-inositol/100 kcal (~1500–1600 μmol/L) for term infant formulas. This upper limit was within the range of *myo*-inositol reported for mature human milk.

The European Society of Paediatric Gastroenterology and Nutrition (1991) made no recommendations for *myo*-inositol. The Association of the Food Industries for Particular Nutritional Uses of the European Union (1996) recommended that the minimum *myo*-inositol content of LBW formulas be 4.0 mg/100 kcal. Health Canada (1995) *Guidelines* made no recommendations for a minimum *myo*-inositol content but indicated that levels in formula should not exceed those found in mature human milk (2500 μmol/L or 65-67 mg/100 kcal).

Investigators have also recommended that preterm infant formula contain amounts of *myo*-inositol similar to that found in human milk (Hallman et al., 1992, Uauy et al., 1993), 1000–2500 μmol of *myo*-inositol/L (Uauy et al., 1993) (26–67 mg/100 kcal, assuming an energy content of 670–690 kcal/L).

Tissue concentrations of myo-inositol

The in vivo concentrations of *myo*-inositol in human cerebellum, as measured by magnetic resonance, did not differ among 12 preterm infants of 27–42 weeks postconceptional age, 8 preterm and term infants of 31–45 weeks postconceptional age, and 6 adults (Hüppi et al., 1991).

The concentration of free *myo*-inositol in cerebrospinal fluid of both term and preterm infants is elevated in the perinatal period (Burgi & Caldwell, 1975). The concentrations then decline to levels similar to adults by 12 months of age.

Blood concentrations of *myo*-inositol are inversely corre-

lated with gestational age (Hallman et al., 1985); plasma concentrations of *myo*-inositol are significantly higher during the first week of life in infants born at less than 31 weeks of gestational age than in infants of longer gestation (Carver et al., 1997). After birth, the plasma concentration of *myo*-inositol declines, at least until the preterm infant can tolerate 70 mL/kg or more of enteral formula (~15–28 days of life) (Carver et al., 1997). Plasma concentrations of *myo*-inositol were not significantly different between this time and hospital discharge (Carver et al., 1997). Plasma *myo*-inositol concentrations 2 months after discharge from the hospital were significantly lower than at discharge (Carver et al., 1997). Others (Friedman et al., 2000; Hallman et al., 1985; Hallman et al., 1987; Hallman et al., 1992) reported similar progressive declines in serum concentrations of *myo*-inositol in preterm infants from birth until 60 days of life, with substantial declines typically evident between birth and 3 days of life.

Some have speculated that high *myo*-inositol levels in cord blood might indicate a higher requirement for *myo*-inositol in prenatal development (Raiten et al., 1998a). Because serum samples from preterm infants contain *myo*-inositol levels that are higher than levels in adults and term infants and *myo*-inositol levels in human milk are relatively high, questions have also been raised about the neonatal requirement (Burton et al., 1976). Whether preterm-LBW infants have a higher requirement than term infants for endogenous and perhaps exogenous *myo*-inositol for optimal development is not known (Raiten et al., 1998a). If this were true, the perinatal period would be a period of potential susceptibility to dietary *myo*-inositol deficiency (Aukema & Holub, 1994).

The serum *myo*-inositol level, adjusted for gestational age, was significantly higher during the first 12 hours of life for infants with respiratory distress syndrome compared with those infants with no lung disease (Hallman et al., 1985). Yet among the infants with respiratory distress syndrome, those with lower serum *myo*-inositol tended to have more severe respiratory distress (Hallman et al., 1985). Hallman et al. (1992) determined that the serum *myo*-inositol concentration averaged 298 μmol/L in the first week postpartum for preterm infants who developed lung or eye disease and/or died. In contrast, the serum *myo*-inositol value averaged 496 μmol/L in the first week postpartum for preterm infants without serious morbidity. Similarly, Friedman et al. (2000) reported that 37 preterm infants who developed retinopathy had an average serum *myo*-inositol concentration of 280 μmol/L during the first 3 days of life compared with an average of 415 μmol/L for 51 infants who did not develop retinopathy. Hallman et al. (1992) suggested that serum *myo*-inositol concentrations lower than 380 μmol/L in the first week of life were associated with an increased risk of retinopathy, respiratory failure, and death. Using regression techniques to adjust for *myo*-inositol intake, duration of oxygen therapy, and birth weight, Friedman et al. (2000) determined that each decrease of 100 μmol/L in serum *myo*-inositol concentration produced greater than a four-fold increase in the odds of developing severe retinopathy of prematurity.

The blood concentrations of free *myo*-inositol in neonates are influenced by diet (Bromberger & Hallman, 1986; Pereira et al., 1990). Five preterm infants fed human milk containing an average of 1456 μmol of *myo*-inositol/L (~38–39 mg/100 kcal) had serum *myo*-inositol levels of 232–250 μmol/L during the third to fifth weeks of life (Pereira et al., 1990). These values were significantly higher than the 125–150 μmol/L reported for five preterm infants fed formula containing 420 μmol/L (~9 mg/100 kcal).

Myo-inositol supplementation

Special interest has been aroused by the knowledge of the role played by *myo*-inositol in the synthesis of phosphatidylglycerol, a primary component of lung surfactant. The synthesis and secretion of surfactant are major regulatory features of lung development. A disorder of these processes leads to respiratory distress syndrome, a significant impairment of respiratory function commonly associated with prematurity in humans.

myo-Inositol supplementation has had beneficial effects on respiratory distress syndrome and bronchopulmonary dysplasia, particularly in conjunction with other therapies (Anceschi et al., 1988; Hallman et al., 1986; 1987; 1990; Hallman et al., 1992) (Howlett & Ohlsson, 2001).

The incidence of retinopathy, particularly severe retinopathy, is significantly reduced by *myo*-inositol supplementation ranging from 80 mg/kg for the first 5 days of life to about 68 mg/kg daily until within 1 week of discharge from the hospital, delivered in formula containing 44.4 mg/100 kcal (Hallman et al., 1990; Hallman et al., 1992, Howlett & Ohlsson, 2001). Preterm-LBW infants with a serum *myo*-inositol concentration of less than 215 μmol/L after 30 days postpartum were four to six times more likely to develop severe retinopathy if they consumed infant formula containing 242 μmol of *myo*-inositol/L (5.4 mg/100 kcal) during hospitalization than if their formula contained 2500 μmol/L (44 mg/100 kcal, at an energy intake of 30 kcal/fl oz) (Friedman et al., 2000). Because of potential benefit for the retinal and respiratory conditions, Hallman et al. (1992) recommended that formula for preterm infants provide amounts of *myo*-inositol equal to that in breast milk (13-48 mg/100 kcal).

Howlett and Ohlsson (2001) conducted a systematic review and meta-analysis of studies that tested the effect of *myo*-inositol on respiratory distress syndrome in preterm infants. The occurrence of intraventricular hemorrhage, grade III-IV, was significantly decreased (RR 0.55, 95% CI 0.32, 0.95; RD −0.09, 95% CI −0.17, -0.01) and mortality was also significantly reduced (RR 0.48, 95% CI 0.28, 0.80; RD −0.131, 95% CI −0.218, -0.043) in those preterm-LBW infants supplemented with *myo*-inositol. In contrast, sepsis and necrotizing enterocolitis appear to be unaffected by dietary intake of *myo*-inositol (Howlett & Ohlsson, 2001). Future multicenter randomized controlled trials of *myo*-inositol supplementation are encouraged to confirm reported benefits (Howlett & Ohlsson, 2001).

Toxicity

Uauy et al. (1993) speculated that a large intake of *myo*-inositol might cause diuresis, leading to fluid loss and, potentially, dehydration. However, indices of hematological, renal, and liver function did not change after oral administration of 12 g of *myo*-inositol/d for 4 weeks to 13 adults diagnosed with major depressive disorder or bipolar affective disorder-depressed and to 12 physically healthy adults diagnosed with chronic schizophrenia (Levine et al., 1995; Levine, 1997). One patient complained of nausea and one of flatus (Levine, 1997). Benjamin et al. (1995) reported that an oral dose of 6 g of *myo*-inositol twice a day for 4 weeks resulted in minimal side effects in adults (2 of 21 adults complained of sleepiness). In nine children, an average of 5.6 years of age, who had a diagnosis of infantile autism, oral administration of 100 mg/kg of body weight twice a day for 4 weeks did not lead to any reported side effects (Levine et al., 1997). Specifically, there were no reported changes in appetite, no nausea, and no diarrhea (Levine et al., 1997). In 11 children older than 4 years with attention deficit disorder, oral administration of

myo-inositol at 100 mg/kg of body weight twice a day for 4 weeks did not lead to serious side effects, although a trend for aggravation of the syndrome was noted (Levine, 1997). Therefore, *myo*-inositol supplementation in physically healthy adults and children has been well tolerated.

Levine et al. (1995) and others (Holub, 1986) cautioned that conditions leading to severe hyperinositolemia, particularly renal failure, may be associated with reduced peripheral nerve conduction. Furthermore, is not known whether *myo*-inositol supplementation might increase fetal resorption in pregnant women.

Renal immaturity may impair the preterm neonate's ability to filter, catabolize, and excrete *myo*-inositol. Lewin et al. (1978) determined that preterm-LBW infants were capable of excreting in urine as much or more *myo*-inositol than they had ingested when intakes ranged from 5 to 36 mg of *myo*-inositol/d. However, renal excretion by preterm-LBW infants does not keep pace with daily *myo*-inositol intakes of 205 mg/kg. Under these circumstances, renal excretion of *myo*-inositol represents, on average, about 53% of intake (Hallman et al., 1987). Hallman et al. (1986) administered 40 mg of *myo*-inositol/kg intragastrically, or 75% of that amount intravenously when the enteral route was inaccessible, four times per day to preterm-LBW infants with adequate renal function beginning 12–48 hours postpartum and continuing for 10 days without any adverse effects. Friedman et al. (2000) fed preterm infants 44 mg of *myo*-inositol/100 kcal (68 mg/150 mL) until they weighed 1800–1900 g. They noted that there were no deleterious effects associated with this level of *myo*-inositol feeding.

Conclusions and recommendations

Minimum. The Expert Panel found no publications that identified a requirement for *myo*-inositol by preterm-LBW infants. The CFR specified a minimum of 4 mg of *myo*-inositol/100 kcal for non-milk-based infant formulas (Food and Drug Administration, 1985). Although current domestic preterm formulas are milk-based ones, hypoallergenic non-milk-based formulas may be developed for this population. The Expert Panel recommended the minimum *myo*-inositol concentration for preterm infant formula of 4 mg/100 kcal, as had previously been recommended for term infant formula (Raiten et al., 1998a).

Maximum. Because preterm-LBW infants with serum *myo*-inositol concentrations less than 215 μmol/L after 30 days postpartum were four to six times more likely to develop severe retinopathy if they consumed infant formula containing *myo*-inositol at 242 μmol/L (5.4 mg/100 kcal) during hospitalization than if their formula contained 2500 μmol/L (44 mg/100 kcal) (Friedman et al., 2000), supplementing formula with amounts of *myo*-inositol comparable to that found in human milk may be beneficial. On average, measures of human milk range from about 13 to 48 mg of *myo*-inositol/100 kcal.

Friedman et al. (2000) fed preterm infants 44 mg of *myo*-inositol/100 kcal (68 mg/150 mL) until they weighed 1800–1900 g without reports of adverse effects.

The Expert Panel recommended a maximum concentration of 44 mg/100 kcal for preterm infant formula, increased from the 40 mg/100 kcal as had previously been recommended for term infant formula (Raiten et al., 1998a). Further studies are needed to determine how much, if any, dietary *myo*-inositol leads to an improved outcome for preterm-LBW infants.

Recommendations

Minimum. **The Expert Panel recommended that the minimum *myo*-inositol content of preterm infant formula be 4 mg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum *myo*-inositol content of preterm infant formula be 44 mg/100 kcal.**

9. FAT

TOTAL FAT

Background

The absolute requirement of the human species for fat is limited to the amount of essential fatty acids necessary to ensure optimal fatty acid composition and function of growing tissues and for normal eicosanoid synthesis. At most, this requirement is no more than 5% of total energy intake. However, fat accounts for approximately 50% of the nonprotein energy content of both human milk and currently available infant formulas. This is thought to be necessary to ensure that total energy intake is adequate to support growth as well as optimal utilization of dietary protein (Fomon, 1993b).

In theory, the energy usually supplied by fat could be supplied as carbohydrate, from which all fatty acids except the essential fatty acids and their anabolic products can be synthesized. In practice, however, it is difficult to ensure sufficient energy intake without a fat intake considerably in excess of the requirement for essential fatty acids. Moreover, metabolic efficiency is greater if the total nonprotein energy intake is achieved with a mixture of dietary fat and carbohydrate rather than predominately carbohydrate. In addition, if the carbohydrate content of a high carbohydrate formula is supplied as simple carbohydrates (i.e., mono- and disaccharides), the resulting high osmolality is likely to produce diarrhea. Dietary fat also serves a useful role in facilitation of the absorption, transport, and delivery of fat-soluble vitamins, and it is an important satiety factor (Fomon, 1993a).

The total fat content of human milk, including that of mothers who deliver prematurely, varies considerably, but it usually accounts for 45–60% of the total energy content of the milk (Jensen, 1999). Fat also accounts for approximately 50% of the nonprotein energy content of current preterm infant formulas. These formulas contain a variety of vegetable oils as well as medium-chain triglyceride (MCT) to enhance fat absorption. In general, the mixture of oils in these formulas does not provide the same proportions of saturated, monosaturated, and polyunsaturated fatty acids as those found in human milk.

However, because the fatty acid pattern of human milk fat is highly dependent on maternal diet, the amounts of constituent fatty acids in human milk are quite variable (Jensen, 1999). Moreover, because medium-chain fatty acids rarely account for more than 10% of the total fatty acid content of human milk, inclusion of up to 50% of total fat as MCTs, as is the case with currently available preterm infant formulas, virtually ensures that the fatty acid pattern of these formulas will not mimic the pattern of human milk. Nevertheless, there is no evidence that the fatty acid patterns of currently available preterm infant formulas, other than perhaps the absence of long-chain polyunsaturated fatty acids (LCPUFAs), are problematic.

Review of the literature

Studies have shown that term infants older than 6 months of age grow normally with a diet providing only approximately 30% of energy as fat (Niinikoski et al., 1997b; Niinikoski et

al., 1997a). Younger infants have also been said to grow appropriately with diets providing total fat intakes of only 30% of total energy (Fomon, 1993a). However, data concerning growth of term infants fed the lower intakes are few, and there are no data concerning growth of preterm infants fed such intakes. The report *Assessment of Nutrient Requirements for Infant Formulas* recommended a minimum fat content of 4.4 g/100 kcal for term infant formulas, about 40% of total energy, and a maximum fat content of 6.4 g/100 kcal, slightly less than 60% of total energy (Raiten et al., 1998a). The fat content of human milk (4.6–7.8 g/100 kcal) encompasses the recommended range.

Conclusions and recommendations

Minimum. The Expert Panel was unaware of data concerning growth or other outcomes of preterm infants fed fat intakes different from those of human milk or currently available preterm infant formulas. The Expert Panel was also unaware of data suggesting that the appropriate fat content of formulas intended for preterm infants is different from that of formulas intended for term infants. The Expert Panel therefore recommended that the minimum fat content of preterm infant formulas be the same as that recommended for term infants in *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a), i.e., 4.4 g/100 kcal, or 40% of total energy.

Maximum. Recommending a maximum fat content for preterm infant formulas is more problematic. A preterm infant receiving the maximum recommended energy intake, 135 kcal/(kg·d), from a formula containing the maximum fat content recommended for term infant formulas, 6.4 g/100 kcal, will receive a total fat intake of 8.6 g/(kg·d). In view of the somewhat limited fat digestion and absorption of preterm infants (see Appendix A), this amount of fat could be excessive for some infants. Thus, a lower maximum fat content than that recommended for term infant formulas seems desirable for preterm infant formulas. The preterm formulas currently available in the United States have a fat content of 5.1–5.4 g/100 kcal. If fed at the maximum recommended energy intake, these formulas provide a fat intake of 6.9–7.3 g/(kg·d). Although definitive data are not available, the currently available preterm infant formulas have a long history of use and, even at high-energy intakes, do not appear to result in intestinal fat malabsorption.

The recommendation for the maximum fat content of preterm infant formula was determined after consideration of the minimum amounts recommended for protein (2.5 g/100 kcal) and carbohydrate (9.6 g/100 kcal). The caloric contribution of 2.5 g of protein/100 kcal is 10 kcal/100 kcal and a contribution of 9.6 g of carbohydrate/100 kcal is 38.4 kcal/100 kcal, for a total of 48.4 kcal/100 kcal of formula. Thus, the maximum possible fat composition of preterm formula would be 51.6 kcal/100 kcal or 5.7 g/100 kcal (i.e., 51.9/9 = 5.7). This would provide about 52% of energy as fat.

Recommendations

Minimum. **The Expert Panel recommended that the minimum fat content of preterm infant formula be 4.4 g/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum fat content of preterm infant formula be 5.7 g/100 kcal.**

ESSENTIAL FATTY ACIDS

Background

All fatty acids have common names [e.g., palmitic acid, oleic acid, linoleic acid (LA), α-linolenic acid (ALA)]. How-ever, the preferred nomenclature is based on a system designating the number of carbon atoms and double bonds in the chain as well as the position of the first double bond from the methyl end of the fatty acid chain (Innis, 1991). For example, palmitic acid is described as 16:0 (i.e., 16 carbons with no double bonds), oleic acid is described as 18:1n-9 (i.e., 18 carbons and one double bond located between the 9th and 10th carbons from the methyl terminal); LA is described as 18:2n-6 (i.e., 18 carbons and two double bonds with the first double bond located between the 6th and 7th carbons from the methyl terminal); and ALA is described as 18:3n-3 (i.e., 18 carbons and three double bonds with the first double bond located between the 3rd and 4th carbons from the methyl terminal). The position of the first double bond from the methyl, or omega (ω), end of the carbon chain is often designated as $\omega 9$, $\omega 6$, or $\omega 3$ rather than n-9, n-6, or n-3.

Because the human species cannot insert double bonds at the n-3 and n-6 positions (Innis, 1991), fatty acids with double bonds in these positions cannot be synthesized endogenously. Thus, either specific n-3 and n-6 fatty acids or the precursor of each series (with double bonds at the n-3 and n-6 positions), i.e., ALA (18:3n-3) and LA (18:2n-6), must be provided as a component of the diet. Both 18:3n-3 and 18:2n-6 are metabolized by a series of desaturation and elongation reactions to the longer chain, more unsaturated fatty acids of the two series (see Figure 9-1). Important metabolites of these two fatty acids include 20:5n-3, or eicosapentaenoic acid (EPA); 22:6n-3, or docosahexaenoic acid (DHA); 18:3n-6, or γ-linolenic acid (GLA); 20:3n-6, or dihomo-γ-linolenic acid (DHLA); and 20:4n-6, or arachidonic acid (AA).

The precursors of the n-3 and n-6 fatty acid series, 18:3n-3 (ALA) and 18:2n-6 (LA), are found in storage lipids, cell membrane phospholipids, intracellular cholesterol esters, and plasma lipids and include triglycerides, phospholipids, cholesterol esters, and nonesterified fatty acids. The longer chain, more unsaturated fatty acids of both series are major components of specific cell membrane phospholipids. EPA (20:5n-3), DHLA (20:3n-6), and AA (20:4n-6) are precursors for the synthesis of biologically active, oxygenated metabolites termed eicosanoids (Hamosh, 1988; Innis, 1991). Each of these fatty acids is the precursor of a different series of eicosanoids, and those of each series exert different biological activities and/or functions.

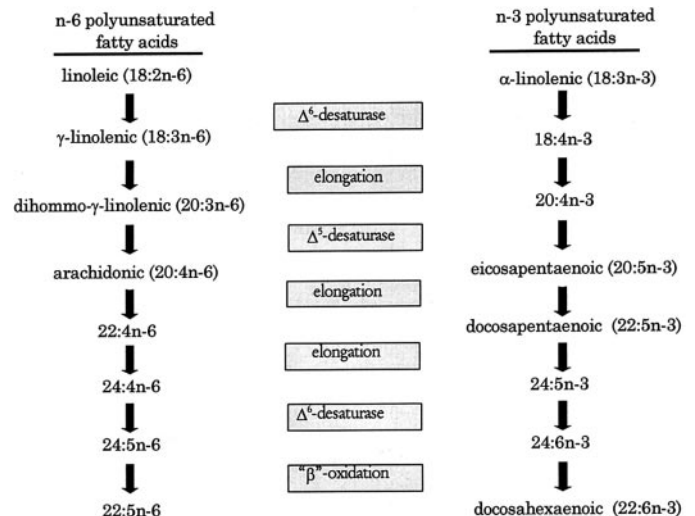The same series of desaturases and elongases are involved in

FIGURE 9-1 Metabolic pathways for polyunsaturated fatty acids.

desaturation and elongation of the n-3 and n-6 as well as the n-9 series of fatty acids. The substrate preference is in the order of n-3 > n-6 > n-9. Competition between the n-9 fatty acids and either the n-3 or n-6 fatty acids is rarely an issue. However, if the concentrations of LA and/or ALA are low, as occurs in deficiency states, oleic acid is readily desaturated and elongated. The plasma lipid content of eicosatrienoic acid (20:3n-9), a desaturated, elongated derivative of oleic acid, is a diagnostic index of n-6 essential fatty acid deficiency, particularly if considered in relation to the plasma lipid content of AA. Whether the concentration of 20:3n-9 is increased with isolated ALA deficiency is not clear. The ratio of 20:3n-9 to 20:4n-6, i.e., the triene-to-tetraene ratio, of plasma lipids is usually less than 0.1 in healthy adults, children, and infants but is higher in those with essential fatty acid deficiency. A ratio of more than 0.4 is usually cited as indicative of n-6 essential fatty acid deficiency (Hamosh, 1988; Innis, 1991), but some believe that an even lower value, 0.2, might be more clinically relevant.

LA has been recognized as an essential nutrient for the human species for more than 60 years (Burr & Burr, 1929; Hansen et al., 1962). Symptoms of deficiency include poor growth and scaly skin lesions. These are usually preceded by an increase in the triene-to-tetraene ratio of plasma lipids. More recently, it has become clear that ALA is also an essential nutrient. In animals, deficiency of this fatty acid results in visual and neurological abnormalities (Benolken et al., 1973; Neuringer et al., 1984; Neuringer et al., 1986; Wheeler et al., 1975). Neurological abnormalities that were corrected by provision of ALA have been documented in a human infant who had been maintained for a prolonged period with a parenteral nutrition regimen lacking ALA (Holman et al., 1982), as well as in elderly nursing home residents who were receiving intragastric feedings of an elemental formula with no ALA (Bjerve et al., 1987). Although symptoms related to deficiency of the two series of fatty acids seem to differ, it should be noted that many studies on which the description of n-6 fatty acid deficiency is based employed a fat-free or very low fat diet rather than a diet deficient in only LA. Thus, there may be some overlap in symptoms of LA versus ALA deficiency. The clinical symptoms of n-6 polyunsaturated fatty acid deficiency can be corrected with LA or AA; those related to ALA deficiency can be corrected with ALA, EPA, or DHA. LA usually comprises between 8% and 20% of the total fatty acid content of human milk, and ALA usually comprises between 0.5% and 1% (Jensen, 1999). Human milk also contains a number of longer chain, more unsaturated metabolites of both LA and ALA, primarily AA and DHA but also other intermediates of LA and ALA metabolism (see Figure 9-1).

The concentrations of all fatty acids in human milk are influenced greatly by maternal diet. The concentration of DHA in the milk of women consuming a typical North American diet is generally in the range of 0.1–0.3% of total fatty acids; the level of AA ranges from 0.4% to 0.6% of total fatty acids (Jensen, 1999). The milk of vegetarian women contains less DHA (Sanders & Reddy, 1992), and DHA level of milk of women whose dietary fish consumption is high or who take DHA supplements is higher (Henderson et al., 1992; Innis, 1992; Jensen et al., 2000; Makrides et al., 1996). The AA content of human milk is less variable and less dependent on maternal AA intake, which may reflect the relatively high LA intake of most populations.

The polyunsaturated vegetable oils commonly used in the manufacture of infant formulas include corn, safflower, and soybean oils. All of these oils contain abundant amounts of LA (from 45% to 75% of total fatty acids), but only soybean oil contains an appreciable amount of ALA (6–9% of total fatty acids). Canola oil, a component of many formulas available in Europe and other parts of the world but not the United States, contains somewhat less LA and more ALA. Until recently, little emphasis has been placed on the ALA content of infant formulas, and many with virtually no ALA were available as recently as a decade ago. The preterm infant formulas currently available in the United States contain approximately 2% ALA and approximately 20% LA.

The report *Assessment of Nutrient Requirements for Infant Formulas* recommended minimum and maximum LA contents of 8% and 35% of total fatty acids, respectively (Raiten et al., 1998a). Previous recommendations specified a minimum LA content of only 4–5% of total fatty acids (Food and Drug Administration, 1985). The minimum and maximum ALA contents recommended in this report were 1.75% and 4% of total fatty acids, respectively, with the additional stipulation that the ratio of LA to ALA not be more than 16:1 nor less than 6:1 (Raiten et al., 1998a). These recommendations are similar to those of other advisory panels and regulatory groups (Codex Alimentarius Commission. Joint FAO/WHO Food Standards Programme, 1994; ESPGAN Committee on Nutrition, 1991).

Review of the literature

Biochemistry and metabolism. A number of studies have demonstrated that the DHA and AA contents of plasma and erythrocyte lipids of breast-fed infants are higher than those of formula-fed infants (Carlson et al., 1986; Innis et al., 1997; Jørgensen et al., 1996; Ponder et al., 1992). Because human milk contains these fatty acids and other long-chain polyunsaturated n-3 and n-6 fatty acids but formulas do not, these observations have been interpreted to indicate that the infant, particularly the preterm infant, cannot synthesize enough of these fatty acids to meet ongoing needs. Concurrent observations of higher cognitive function in breast-fed versus formula-fed infants (Lucas, 1997; Lucas et al., 1999; Morrow-Tlucak et al., 1988; Rogan & Gladen, 1993), including preterm infants fed human milk versus those fed formula during hospitalization (Lucas et al., 1990a; Lucas, 1997), focused attention on the possibility that the lower cognitive function of formula-fed infants might be related to inadequate intake of LCPUFAs. Indeed, DHA and AA are the major n-3 and n-6 fatty acids of neural tissues (Clandinin et al., 1980a,b; Martinez, 1992) and DHA is a major component of retinal photoreceptor membranes (Martinez, 1992). Furthermore, a gradient between maternal and fetal plasma concentrations of these LCPUFAs suggests that the major supply of these fatty acids to the fetus during development is from the maternal plasma (Berghaus et al., 1998; Dutta-Roy, 2000). Thus, the need for these fatty acids by the preterm infant who is born early in the third trimester of pregnancy and therefore receives a limited supply of LCPUFAs before birth is thought to be greater than the need for these fatty acids by the term infant.

Postmortem studies from both Great Britain (Farquharson et al., 1992; Jamieson et al., 1994) and Australia (Makrides et al., 1994) have shown that the cerebral content of DHA, but not AA, is minimally but significantly lower in formula-fed term infants who died suddenly during the first year of life than in breast-fed infants who died of similar causes. Only the Australian study examined the DHA content of the retina, which did not differ between breast-fed and formula-fed infants (Makrides et al., 1994). It should be noted that the content of this fatty acid in retina reaches adult levels at approximately term gestation, whereas the adult level in the cerebrum is not reached until much later (Martinez, 1992).

The British study suggests that the cerebral DHA content of formula-fed infants may be related to the ALA content of formula (Jamieson et al., 1994). This is consistent with studies of piglets showing that ALA intakes greater than 0.7% of total energy result in normal brain levels of DHA (Innis, 1991). Interestingly, the formula-fed infants included in the Australian study received a formula with a relatively low content of ALA (Makrides et al., 1994). Unfortunately, similar data are not available for preterm infants. However, if maternal transfer of LCPUFA to the fetus during the last trimester of pregnancy is as important as is generally thought, prematurely born infants who do not receive an adequate supply of these fatty acids may be at greater risk of developing retinal and central nervous system dysfunction. However, the amounts of these fatty acids deposited daily in the brains of the premature and term infant are almost identical (Martinez, 1992).

Certain studies have demonstrated that both term and preterm infants can convert ALA to DHA and LA to AA (Carnielli et al., 1996; Demmelmair et al., 1995; Salem, Jr. et al., 1996; Sauerwald et al., 1996; Sauerwald et al., 1997; Uauy et al., 2000). In these studies, the precursor fatty acids labeled with stable isotopes of either carbon (^{13}C) or hydrogen (^{2}H) were administered to the infants, and blood levels of the labeled fatty acids as well as labeled metabolites of each were measured by gas chromatography/mass spectrometry. The studies of Sauerwald et al. (1996; 1997) and Uauy et al. (2000) suggest that the overall activity of the desaturase/elongase system may be greater in preterm than in term infants. No data are available, however, concerning the amounts of LCPUFAs that either term or preterm infants can synthesize. This is because measurements of enrichment have been limited to plasma, which represents only a small fraction of the total body pool of both the precursor and the product fatty acids and may not represent fatty acid pools in other tissues.

The studies of Sauerwald et al. (1996) indicated that the amount of labeled precursor found as the labeled product in plasma lipids depends on intake of the precursor. In this study, term infants fed a formula containing 16% of total fatty acids as LA and 3.2% as ALA appeared to synthesize more DHA (based on measurements in plasma lipids) than infants fed a formula with the same content of LA but either 1% or 0.4% as ALA. As expected from the known competition between n-3 and n-6 fatty acids for the enzymes involved in desaturation and elongation of the precursors, infants fed the formula with the highest ALA content appeared to synthesize less AA.

Despite obvious conversion of ALA to DHA, the content of DHA in plasma lipids of formula-fed preterm infants, including those who receive a relatively high intake of ALA, is lower than that observed in plasma lipids of infants fed human milk or formulas supplemented with DHA (Carlson et al., 1986, 1991, 1994b; Uauy et al., 1990, 1994a). Thus, it seems that the amounts of products formed by infants fed formulas with only the precursors of the n-3 and the n-6 fatty acids, particularly the amount of DHA formed, may be less than the amounts contained in human milk or supplemented formulas. This raises the crucial question of whether the concentrations of individual fatty acids in plasma, particularly the long-chain polyunsaturated n-3 and n-6 fatty acids, reflect the content of these fatty acids in tissues.

In this regard, animal studies have shown that the contents of long-chain polyunsaturated n-3 and n-6 fatty acids in plasma are much more highly correlated with the contents of these fatty acids in erythrocytes and liver than with the contents in brain (Rioux et al., 1997). This suggests that the contents of LCPUFAs in brain lipids may not be reflected by their contents in plasma lipids. In contrast, the postmortem studies of human infants demonstrated a statistically significant correlation between the plasma and brain contents of DHA, but the correlation between the content of this fatty acid in plasma and the contents of other tissues was not reported.

One study of primates showed that preformed DHA is transferred from the plasma to the brain (Su et al., 1999). In this study, ALA and DHA, both uniformly labeled with ^{13}C, were administered concurrently to the animals, and the relative enrichment in the brain of [U-^{13}C]DHA (i.e., preformed DHA) was compared with enrichment of DHA synthesized from [U-^{13}C]ALA (i.e., endogenously synthesized DHA). The amount of preformed DHA in brain was approximately seven times that of the amount of endogenously synthesized DHA.

These data appear to answer the relevant nutritional question of the equivalency of DHA and ALA as sources of brain DHA. However, interpretation of these data may not be quite as straightforward as first appears. The study did not take into account the relative enrichment or specific activity of the labeled fatty acids in plasma. For example, because a large proportion of administered precursor (i.e., ALA) is not desaturated and elongated to DHA, the plasma enrichment (specific activity) of the administered preformed product is likely to be considerably higher than that of the endogenously synthesized product. Taking the different plasma enrichments into account leads to the conclusion that a greater proportion of endogenously synthesized DHA versus preformed DHA in plasma enters the brain. This latter interpretation is consistent with studies of isolated cell systems showing that precursors of DHA are taken up by astrocytes and converted to DHA, which is subsequently transferred from astrocytes to neurons (Moore et al., 1991; Moore, 1993). This issue of how DHA reaches the developing central nervous system may be particularly critical for interpreting some of the functional differences between breast-fed and formula-fed infants as well as differences between infants fed supplemented and unsupplemented formulas.

Long-chain polyunsaturated fatty acid intake and visual function. Early studies of rodents established the importance of n-3 fatty acids for normal retinal function (Benolken et al., 1973; Wheeler et al., 1975) and subsequent studies confirmed this result for primates (Neuringer et al., 1984; Neuringer et al., 1986). More recently, studies have focused on the effect of n-3 fatty acids on retinal function and overall function of the visual system of human infants. However, although the abnormal retinal and visual function of n-3 fatty acid-deficient animals was clearly produced by an inadequate intake of ALA, studies of infants have focused primarily on the effects of DHA intake on retinal and/or visual function.

Four controlled clinical trials have addressed differences in visual acuity of LBW infants who were fed formulas supplemented with DHA or unsupplemented formulas. These studies utilized one or more methods of assessing visual acuity and function, i.e., preferential looking acuity, acuity as determined by visual evoked potential (VEP), and/or electroretinography.

The preferential looking tests of visual acuity are based on the innate tendency to look toward a discernible pattern rather than a blank field (Dobson, 1983; Dobson & Teller, 1978; Dutta-Roy, 2000; McDonald et al., 1985). The test involving Teller Acuity Cards, a rapid measure of resolution acuity that combines forced-choice preferential looking and operant preferential looking procedures, is usually used. The test is performed by showing the infant a series of cards that contain stripes (gratings) of different widths on one side and observing the looking behavior of the infant through a peephole in each card. Scoring or evaluation of visual acuity is

based on the finest grating that the infant can resolve, i.e., the finest grating toward which the infant looks preferentially.

The VEP test measures the activation of the visual cortex in response to visual information processed by the retina and transmitted along the geniculostriate pathway to the visual cortex (Norcia & Tyler, 1985). The presence of a reliable evoked response indicates that the stimulus information was resolved up to the point in the visual pathway where the response is processed. Use of VEPs to assess visual acuity requires measuring the electrical potentials of the visual cortex in response to patterns of contrast reversal with vertical square wave gratings or checkerboards. During the presentations, the frequency of the gratings or checkerboards is decreased from low to high (large to small), and the visual acuity threshold is estimated by linear regression of the VEP amplitudes versus the frequency, or size, of the grating or checkerboard stimulus (Uauy et al., 1992). A rapid VEP method (sweep VEP) has been developed for use in infant populations (Norcia & Tyler, 1985; Sokol et al., 1983); data are recorded as the log_{10} of the minimum angle of resolution (logMAR), with smaller logMAR values indicative of better visual acuity.

Electroretinography measures the summed activity of the retina in response to a flash of light. The primary components of the electroretinogram are the a-wave, which is produced by hyperpolarization of the photoreceptor, and the b-wave, which reflects the subsequent activation of retinal neurons. Performance is quantified by a number of parameters, some measured directly and some calculated. These include the threshold (the minimal intensity of light necessary to elicit a small amplitude), the implicit time or peak latency (the time from the presentation of a brief flash of light to the response peak), the maximal amplitude, and the sensitivity (the intensity of light that elicits a response of half the maximal amplitude) (Hood & Birch, 1990; Naka & Rushton, 1966).

The first randomized trial evaluating the effect of LCPUFAs on visual acuity (Uauy et al., 1990, 1994a) included three groups of formula-fed preterm infants plus a reference group of infants fed human milk (birth weight of 1000–1500 g). The formula-fed groups received a corn oil formula, a soybean oil formula, or a soybean oil formula supplemented with marine oil (0.35% of total fatty acids as DHA and 0.65% as EPA) from shortly after birth through 57 weeks of postconceptional age. Preterm formulas were fed through 36 weeks of postconceptional age, and term formulas were fed thereafter. The corn oil formula contained very little ALA. The soybean oil formula had about the same amount of LA as the corn oil formula but a higher content of ALA. The ALA content of the formulas supplemented with marine oil was somewhat lower than that of the soybean oil formulas, but the total content of n-3 fatty acids, i.e., ALA, EPA, and DHA, was almost the same as that of the soybean oil formula. Electroretinograms were obtained, and visual acuity was assessed by sweep VEP at postconceptional ages of 36 and 57 weeks. Visual acuity was also assessed at 57 weeks of postconceptional age by forced preferential looking.

At 36 weeks of postconceptional age, the infants fed the formula supplemented with marine oil and the human milk reference group had higher electroretinographic a-wave and b-wave amplitudes as well as a lower b-wave threshold than either of the other two formula-fed groups (Uauy et al., 1990). However, the difference between these two groups and the soybean oil group was not statistically significant, and no differences among groups were noted at 57 weeks of postconceptional age. There were no differences in VEP acuity among groups at 36 weeks of postconceptional age; at 57 weeks of postconceptional age, however, infants fed the formula sup-

plemented with marine oil had better VEP acuity than either of the other formula-fed groups (Birch et al., 1992). No difference among the three formula-fed groups was detected with the forced preferential looking procedure at 57 weeks of postconceptional age (Birch et al., 1992).

Carlson et al. reported two randomized trials in LBW infants (1992; 1993b; 1994a; 1996). In the first trial (Carlson et al., 1993b; Carlson et al., 1994a), infants (birth weight of 725–1400 g) were randomly assigned to receive either a control formula or the same formula supplemented with marine oil through 9 months of corrected age (months after 40 weeks of postconceptional age). Preterm formulas were fed until weight reached 1800 g, and standard term formulas were fed thereafter. The control formula fed until the infants' weight reached 1800 g contained approximately 20% LA and 3.1% ALA. That fed after the weight reached 1800 g contained approximately 35% LA and 4.9% ALA. The supplemented preterm and term formulas contained the same amounts of LA and ALA plus 0.2% of total fatty acids as DHA and 0.3% as EPA.

The second trial by Carlson et al. (1996) was also conducted with infants who weighed between 725 and 1400 g at birth. However, in this study, the supplement used was a low EPA fish oil providing 0.2% of total fatty acids as DHA and a negligible amount of EPA. In addition, the DHA-supplemented formula was fed only through 2 months of corrected age (48 weeks of postconceptional age) rather than through 9 months of corrected age as in the first study. During this time, both DHA-supplemented infants and unsupplemented infants received a nutrient-enriched preterm formula with approximately 20% of total fatty acids as LA and 3.1% as ALA. The formula fed after 48 weeks of postconceptional age was the same as that used in the first study after the infants' weight reached 1800 g (approximately 35% of total fatty acids as LA and 4.9% as ALA).

In the first study, infants fed the supplemented diet had better forced preferential looking acuity at 2 and 4 months of corrected age but not at 6, 8, 10, or 12 months (Carlson et al., 1992; Carlson et al., 1993b; Carlson et al., 1994b). It was also noted that most infants, regardless of group assignment, had normal forced preferential looking acuity at all ages evaluated; exceptions included two infants who had abnormal acuity at 2 months of age but not before or after that age (whether these two infants received the control or the experimental formula is not stated). In the second study, infants fed the supplemented diet had better forced preferential looking acuity at 2 months of corrected age but not at 4, 6, 9, or 12 months (Carlson et al., 1996). It appears probable that visual acuity of both groups in the second study was also within normal limits at all times tested; however, this was not explicitly stated.

The second study of Carlson et al. (1996), unlike the first, included infants who required oxygen therapy during hospitalization, some of whom subsequently developed bronchopulmonary dysplasia. Interestingly, the infants with bronchopulmonary dysplasia did not benefit from marine oil supplementation. At some ages, in fact, supplemented infants with bronchopulmonary dysplasia had lower acuity than unsupplemented infants with or without bronchopulmonary dysplasia. Furthermore, in the total population (i.e., those with and without bronchopulmonary dysplasia), the visual acuity of supplemented versus unsupplemented infants did not differ at any age.

A recent study also shows the advantages of LCPUFA supplementation on visual function (O'Connor et al., 2001). In this study, preterm infants fed formula with 0.42% of fatty acids as AA and 0.25% of fatty acids as DHA until term and a formula with 0.4% of fatty acids as AA and 0.15% as DHA

(either as a combination of fish and fungal oils or a combination of egg triglyceride and fish oil) thereafter had better sweep VEP acuity at 6 months of corrected age than did infants fed a control formula without LCPUFA supplementation. Posthoc analysis, but not the preplanned repeated measures analysis, also showed that infants fed the supplemented formula (egg triglyceride and fish oil only) had a higher mean behavioral acuity score at 4 months of corrected age but not at 2 or 6 months.

Thus, in contrast to studies of term infants, some studies showing an advantage of DHA supplementation on visual acuity as measured by either forced preferential looking or sweep VEP and some studies showing none (Raiten et al., 1998a), these four studies of preterm infants all show some advantage of DHA supplementation on visual acuity. However, these advantages are modest and do not persist throughout the period of study. Moreover, the meaning of differences in visual acuity between two groups with "normal" visual acuity is not clear. The minimal differences in retinal function and visual acuity between infants fed a soybean oil formula and those fed a similar formula supplemented with marine oil (Birch et al., 1992; Uauy et al., 1990) suggest that DHA supplementation may not be necessary if ALA intake is adequate. However, ALA intake was undoubtedly adequate in both studies of Carlson et al. (1992; 1993b; 1994a; 1996). The most disturbing finding is the suggestion that DHA supplementation may actually be detrimental in infants with bronchopulmonary dysplasia (Carlson et al., 1996). The potential importance of this possibility is underscored by the fact that supplemented formulas, if available, will be fed to all formula-fed infants, not only to those who might benefit from them.

A metaanalysis of data (Birch et al., 1992; Carlson et al. 1992, 1993b, 1994a, 1996; Uauy et al., 1990) indicates advantages of DHA-supplemented over unsupplemented formulas on both behaviorally based and electrophysiologically based measurements of visual acuity (SanGiovanni et al., 2000). Supplemented formula resulted in advantages of 0.47 ± 0.14 octaves at 2 months of corrected age and 0.28 ± 0.08 octaves at 4 months of corrected age with behaviorally based methods and an advantage of 0.83 ± 0.20 octaves at 4 months of corrected age with electrophysiologically based measurements. The range of normal acuity values for term infants based on behavioral assessments is 1.6 octaves at 4–6 weeks of age to 0.81 octaves at 16–19 weeks of age (Mayer & Dobson, 1997).

This discussion would be incomplete without noting that the visual acuity of infants is difficult to assess. Acuity as determined by sweep VEP is generally better than that determined by forced preferential looking, perhaps because the latter depends on both visual and motor pathways, whereas the former requires only an intact visual pathway. For example, in the preferential looking procedure, it is necessary for the infant to know which side of the card contains the grating (i.e., to see the grating) and to be able to look in that direction. Neither method differentiates retinal problems from problems with projection of the image from the retina to the visual center of the occipital cortex. The effect of DHA supplementation on the latency component of the transient VEP, meaning the time between presentation of a stimulus and appearance of the potential that should reflect the rate of neural transmission, has not been studied. This may be unfortunate because the rate of neural transmission is a logical outcome variable that may be affected by an abnormal fatty acid composition of synapses.

Prager et al. (1999) evaluated the test-retest reliability of the two most commonly used methods for assessing visual acuity of infants and also compared visual acuity as assessed by different methods. The mean test and retest values for visual acuity were quite similar with both methods, but the mean difference between individual test and retest values was large. This suggests that either method is appropriate for assessing visual acuity of a group of infants, as has been done in most studies, but that neither method reliably predicts the visual acuity of an individual infant. Correlations between the methods were quite poor, i.e., those with better acuity as assessed by one method did not necessarily have better acuity as assessed by the other method.

Long-chain polyunsaturated fatty acid intake and cognitive/behavioral development. DHA and AA are the predominant LCPUFAs in the central nervous system. They are present primarily in the nonmyelin membranes, particularly in the cortical synaptic terminals. Accretion occurs during the brain growth spurt from the beginning of the third trimester until approximately 2 years of age (Clandinin et al., 1980a,b; Martinez, 1992). The rate of accretion may slow somewhat around term but remains appreciable until the end of the brain growth spurt. Because of this and the possibility that endogenous synthesis of DHA and AA from ALA and LA, respectively, may not be sufficient to provide the amounts needed for normal rates of brain accretion, there has been considerable interest in the importance of DHA and AA intake for normal cognitive/behavioral development. Although the better cognitive/behavioral development of breast-fed infants than formula-fed infants has been attributed to the presence of DHA and AA in breast milk but not formulas (Lucas, 1990), the many other differences between breast milk and formulas as well as between mothers who elect breast-feeding and those who elect formula feeding preclude such a conclusion. The effects of these fatty acids on cognitive/behavioral development can be determined only by comparing infants fed supplemented versus unsupplemented formulas.

The Bayley Scales of Infant Development (SID) and the Fagan Test of Infant Intelligence (FTII) have been used to assess the cognitive/behavioral development of infants fed DHA-supplemented versus unsupplemented formulas. The Bayley SID, which provide indices of both mental development [Mental Development Index (MDI)] and psychomotor development [Psychomotor Development Index (PDI)], have been used for years and are considered the "gold standard" for assessing global abilities of infants from birth to approximately 3 years of age. Within-age test-retest reliability is excellent, i.e., 80% or better, but the relationship between either the MDI or the PDI of the Bayley SID and later cognitive function is poor, particularly for "normal" or low-risk infants (McCall & Mash, 1997).

The FTII assesses novelty preference (Fagan, III & Singer, 1983). In this test, the infant is shown a single stimulus (usually a face) for a standardized time period based on age (i.e., a longer period at younger ages) and then is shown this stimulus along with a novel one. If the infant has "learned" the original stimulus during the time it was made available before the novelty test (i.e., the familiarization phase), the typical response is to look selectively toward the novel versus the "familiar" image. Scores on this test during infancy are somewhat more predictive of later cognitive function than the Bayley SID; however, the internal consistency (reproducibility) of the test is relatively poor (Colombo, 1997). More recently, look duration during the familiarization phase and the paired comparison phase of the FTII have been shown to be modest predictors of both concurrent performance on tasks within infancy and later intelligence (Colombo, 1997). In this test, shorter look durations during the familiarization part of

the FTII predict better concurrent as well as later cognitive performance.

The Bayley SID were administered to infants enrolled in both studies of Carlson et al. (1993b; 1994a; 1996) at 12 months of corrected age, and the Fagan TII was administered at different times between 6 and 12 months of corrected age. Neither the MDI nor the PDI score of the Bayley SID differed significantly between supplemented and unsupplemented groups in either study (Carlson et al., 1994a). In the first study, infants fed the supplemented formula had lower MDI and PDI scores but, in the second study, infants without bronchopulmonary dysplasia who were fed the supplemented formula had higher scores. The differences in both cases, if at all predictive of subsequent intelligence, could have been biologically important but were not statistically significant. This apparent discrepancy was attributed to the fact that infants participating in the first study received a term formula from discharge until 12 months of corrected age, whereas infants participating in the second study received a nutrient-enriched formula through 48 weeks of postconceptional age followed by the term formula until 12 months of corrected age (this, of course, was true also for the control group). In both studies, supplemented infants, as compared with unsupplemented infants, had a definitely stronger preference for the novel than for the familiar image and also had shorter look durations during the familiarization phase (Carlson & Werkman, 1996; Werkman & Carlson, 1996).

A recent study (O'Connor et al., 2001) of infants fed formulas supplemented with 0.25% DHA and 0.4% AA from a combination of egg triglyceride and fish oil or a combination of fish oil and fungal oils until discharge from the hospital and 0.15% DHA and 0.4% AA from the same source from discharge to 12 months of corrected age showed that infants receiving the combination of egg triglyceride and fish oil had a better novelty preference score at 6 months of corrected age and that infants who weighed less than 1250 g at birth and received the combination of fish and fungal oil had a higher Bayley PDI score at 12 months of corrected age. Of note, better novelty preference was observed only for infants who received a combination of egg triglyceride and fish oil, whereas the better Bayley PDI score was noted for those who received a combination of fish and fungal oils.

These are the only data concerning the effect of LCPUFA supplementation on cognitive/behavioral development of preterm infants. A number of studies have been conducted with term infants; some showed positive effects of supplementation, some showed no effects, and at least one showed negative effects. These findings were reviewed in the report *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a). As a group, these studies were criticized by consultants to the Expert Panel for including too few infants, failing to control adequately for confounding factors, failing to assess function at multiple times, failing to examine individual differences in development, and failing to follow the infants for a sufficiently long period. For example, none of the studies cited in the report and none of the studies in preterm infants cited above included data beyond 1 year of age.

Since publication of the report *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a), four randomized trials in term infants that included Bayley MDI and PDI scores beyond 12 months of age have been published (Birch et al., 2000; Lucas et al., 1999; Makrides et al., 2000; Auestad et al., 2001). The first of these (Lucas et al., 1999) compared a formula supplemented with both DHA and AA (0.32% and 0.3% of total fatty acids, respectively, from purified egg phospholipid and triglyceride fractions) with an un-

supplemented formula, both fed for the first 6 months of life. Although the LA and ALA contents of the two formulas differed somewhat, the LA-to-ALA ratios were similar (11.4 and 11.3). The mean Bayley MDI score of the supplemented formula group ($n = 125$) at 18 months of age was 95.5 ± 1.2 (SEM), that of the control group ($n = 125$) was 94.5 ± 1.2, and that of a breast-fed reference group ($n = 104$) was 96.0 ± 1.0. Mean Bayley PDI scores of the supplemented, control, and reference groups, respectively, were 96.4 ± 0.9, 95 ± 0.8, and 94.4 ± 1.20. There obviously were no statistically significant differences among groups.

The second, more recently published study (Makrides et al., 2000) included a group of term infants fed a formula supplemented with DHA (0.35% of total fatty acids as tuna oil; $n = 23$) and a group fed a formula supplemented with both DHA and AA (0.34% of total fatty acids as each from egg yolk phospholipid; $n = 24$) as well as a control or placebo group (no LCPUFA, $n = 21$) and a breast-fed reference group ($n = 46$). In this study, the formulas were fed through 1 year of age. Bayley MDI and PDI scores of the four groups did not differ at 12 months of age. Bayley MDI and PDI scores of the three formula groups also did not differ at 24 months of age, but the MDI score of the breast-fed group was significantly higher than that of any of the formula groups.

The third recently published study of term infants (Birch et al., 2000) also included three formula-fed groups, all fed the assigned formula through 4 months of age, but no concurrent breast-fed reference group. The formula groups included a control group (no LCPUFA; $n = 20$), a group fed a DHA-supplemented formula (0.35% of total fatty acids as a triglyceride derived from a unicellular organism; $n = 17$), and a group fed a formula supplemented with both DHA and AA (0.36% and 0.72% of total fatty acids, respectively, as triglycerides from unicellular organisms; $n = 19$). In this study, the mean Bayley MDI score of the group fed the formula supplemented with DHA and AA was 7.3 points higher at 18 months of age than that of the control group [105.6 ± 11.8 (SD) versus 98.3 ± 8.2, $P < 0.05$]. The mean Bayley MDI score of the group supplemented with DHA was 4.1 points higher than that of the control group (102.4 ± 7.5 versus 98.3 ± 8.2) but did not differ significantly from the mean MDI of either the control group or the group receiving the formula supplemented with both DHA and AA. Bayley PDI scores of the three groups did not differ.

The most recently reported study (Auestad et al., 2001) included reference groups of breast-fed infants ($n = 165$) weaned to formulas with or without AA and DHA (0.46% and 0.14% of total fatty acids, respectively) as well as groups fed a control formula ($n = 77$) or one of two formulas with the same contents of AA and DHA from either egg triglyceride ($n = 80$) or a combination of fish and fungal oil ($n = 82$) for the first year of life. At 12 months of age, there were no differences among groups in visual acuity (see above), information processing (FTII), general development (Bayley SID), language development (MacArthur Communicative Development Inventories), or temperament (Infant Behavior Questionnaire).

Reasons for the discrepant results among these studies (and others) are not clear. Possibilities include different LCPUFA sources, different durations of treatment, different amounts of AA, and different ratios of AA to DHA. There also were some differences in the LA and ALA contents of the control and experimental formulas; the LA:ALA ratios ranged from 8.5 to 16. In addition, the studies were conducted on three different continents: Europe (Lucas et al., 1999), Australia (Makrides et al., 2000), and North America (Birch et al., 2000). The variance in Bayley MDI and PDI scores also differed among

studies, and it may be noteworthy that the variance was smallest in the study that showed a statistically significant difference in Bayley MDI scores between infants who received a formula supplemented with DHA plus AA and those who received an unsupplemented formula.

Even fewer studies of preterm infants fed supplemented versus unsupplemented formulas are available, and these are subject to the same criticisms as those in term infants. However, the available data, including those from a recently reported large and comprehensive study (O'Connor et al., 2001), suggest that preterm infants are more likely to benefit from supplementation than are term infants. In the latest study, infants weighing between 750 and 1800 g at birth were assigned randomly, before initiation of enteral feeding, to receive one of three formulas until term: a control formula (n = 144); a formula with AA and DHA (0.46% and 0.26% of total fatty acids, respectively, as a combination of fish and fungal oils (n = 140); or a formula with the same amounts of AA and DHA from a combination of egg triglyceride and fish oil (n = 143). From term through 12 months of age, the AA and DHA contents of the formulas were 0.42% and 0.16% of total fatty acids, respectively, from the same sources. Infants fed human milk exclusively through term served as a reference group. The effects of the supplemented formulas on visual acuity are described above. Scores on the Fagan Test of Novelty Preference were higher at 6 months in the group receiving the supplement of the combination of egg triglyceride and fish oil than in the control group or the group receiving fish and fungal oils. There were no differences among groups at 9 months of age. There was no difference among groups on the Bayley MDI score at 12 months of age, but the Bayley PDI score of infants who weighed less than 1250 g at birth was higher in the group assigned to the fish and fungal oil supplement than in the control group. The Bayley PDI score of the group receiving the supplement with egg triglyceride and fish oil was not different from either the control or the other supplemented group. When twins and infants from Spanish-speaking families were excluded, infants receiving supplemented formula had better vocabulary comprehension at 14 months of corrected age than did the control group; however, without these exclusions, there was no difference in vocabulary comprehension among groups.

Sources for long-chain polyunsaturated fatty acid supplementation. Available sources for LCPUFA supplementation include egg yolk lipid, phospholipid, and triglyceride (all of which contain n-6 as well as n-3 LCPUFAs), fish oils, and oils produced by single-celled organism (i.e., microalgal and fungal oils). Aside from the possible effect of marine oil on growth of infants (see below), few untoward effects of the available supplements have been noted. In vitro and animal studies of toxicity have also revealed minimal if any toxicity of any of these sources (Arterburn et al., 2000a; Arterburn et al., 2000b; Arterburn et al., 2000c; Boswell et al., 1996; Burns et al., 1999; Hempenius et al., 1997; Hempenius et al., 2000; Streekstra, 1997; Wibert et al., 1997). In fact, the Food and Drug Administration announced that it has no objection to the manufacturer's self-declared generally recognized as safe (GRAS) status for algal and fungal sources of DHA and AA, respectively, for use in infant formulas (Food and Drug Administration, 2001).

Concern has been expressed about the bioavailability of phospholipid versus triglyceride sources of fatty acids, but studies of both term and preterm infants have shown that fatty acids from the phospholipid source are absorbed as well or better than fatty acids from the triglyceride source (Carnielli et al., 1996). On balance, therefore, current evidence suggests that the doses and sources of DHA and AA used in preterm infant formula fed in recent studies produced no adverse effects.

Adverse effects of long-chain polyunsaturated fatty acids. The observation that infants receiving supplemented formula in both studies of Carlson et al. (1992; 1996) weighed less or had lower weight for length at various times during the first year of life than infants assigned to the control formulas has generated considerable concern. This effect was more marked in the first study, in which the long-chain n-3 fatty acid supplement was marine oil containing EPA (0.3% of total fatty acids) as well as DHA (0.2% of total fatty acids). In that study, weight at a corrected age of 12 months was shown subsequently to be correlated with plasma phospholipid AA content at various times during the first year of life (Carlson et al., 1993a), i.e., the better the AA status, the higher the weight at 12 months of corrected age. A similar relationship was not apparent in the second study, in which the effect on growth was less marked but there was a correlation between weights at some ages and the plasma phospholipid ratio of AA to DHA. The long-chain n-3 fatty acid supplement used in this study was a low EPA fish oil (0.2% of total fatty acids as DHA and 0.06% of total fatty acids as EPA).

As discussed above, infants who receive intakes with a lower LA:ALA ratio as well as infants who receive supplements of long-chain polyunsaturated n-3 fatty acids have lower plasma phospholipid concentrations of AA than do infants who receive diets with a higher ratio or no LCPUFAs. Thus, the lower weight observed by Carlson et al. (1992; 1996) in infants receiving supplemented formula is likely to have been correlated with poorer AA status, even if the two effects were totally independent. Also of note is the fact that the ALA content of the formulas used in both studies was reasonably high. This suggests that the observed adverse effects on weight might be related to the total intake of n-3 fatty acids rather than to a single n-3 fatty acid. Indeed, a smaller study in which more of the same (or similar) marine oil supplement used in the first study of Carlson et al. (1992) was added to a formula with a lower content of ALA did not show differences in growth between supplemented and unsupplemented groups (Uauy et al., 1994a).

Of interest in this regard is the finding of Jensen et al. (1997) that term infants fed a formula with an LA:ALA ratio of 4.8 (15.4% LA and 3.2% ALA) for the first 4 months of life weighed approximately 750 g less at 4 months of age than infants fed a formula with a ratio of 40 (16% LA and 0.4% ALA). Jensen et al. (1995a) also reported similar differences between weights of LBW infants fed formula with an LA:ALA ratio of 4.8 and those fed a formula with a ratio of approximately 16 (3.2% and 1% of fatty acids as ALA, respectively, both with ~16% of total fatty acids as LA). Although the range of LA:ALA ratios studied by Jensen et al. (1995a; 1997) has not been studied by other investigators, other studies in which different ratios were fed have not shown differences in rates of weight gain secondary to the LA:ALA ratio of formulas (Clark et al., 1992; Makrides et al., 2000). However, in some of these studies, infants were followed for rather short periods, and in others the different ratios were achieved by differences in the content of both LA and ALA.

Ryan et al. (1999) observed lower rates of gain in weight, length, and head circumference in preterm male, but not female, infants fed a formula with a moderately high content of ALA plus the same low EPA fish oil supplement (0.2% of total fatty acid as DHA and 0.06% of total fatty acids as EPA) used in the second study of Carlson et al. (1996) versus a control formula from shortly before hospital discharge until 59

weeks of postmenstrual age. In this study, the plasma phospholipid AA content of the infants assigned to the supplemented formula was lower through 59 weeks of postmenstrual age than observed in those fed the control formula, but there was no correlation between plasma phospholipid AA content and any aspect of growth. Rather, rates of increase in weight and length of male infants during various intervals were inversely correlated with plasma phospholipid DHA content at either the beginning or the end of the interval of observation. There was no statistically significant correlation between plasma phospholipid DHA content and increase in head circumference.

In contrast to these observations of an apparent adverse effect of total n-3 fatty acid intake or the intake of a specific n-3 fatty acid, Diersen-Schade et al. (1998) observed no difference in growth of preterm infants fed a DHA-supplemented formula (0.34% of fat as an algal oil) versus a control formula for at least 28 days before hospital discharge and followed until 48 weeks of postmenstrual age. Moreover, the investigators observed somewhat better growth than that observed in either of these groups in a third group fed a formula supplemented with both DHA (0.33% of fat as an algal oil) and AA (0.6% of fat as a fungal oil). Foreman-van Drongelen et al. (1996) found no difference in growth between preterm infants fed a formula supplemented with DHA and AA (0.3% and 0.61% of fat as algal and fungal oils, respectively) versus a control formula from roughly 33 through 52 weeks of postmenstrual age. Finally, Vanderhoof et al. (1999; 2000) also found no difference in weight, length, head circumference, or middle arm circumference at either 40 or 92 weeks of postconceptional age between preterm infants fed a formula supplemented with DHA and AA (0.35% and 0.5% of fat as algal and fungal oils, respectively) versus a control formula from shortly after birth until 48 weeks of postconceptional age. Growth of both groups during the period of study was somewhat greater than that of a reference breast-fed group studied concurrently.

Finally, in the most recent and largest study of preterm infants reported to date (O'Connor et al., 2001), few and inconsistent differences were found in absolute weight, length, and head circumference and in rates of increase in weight, length, and head circumference from the beginning of the study to term, 4 months of corrected age, or 12 months of corrected age between infants fed the control formula and those fed formulas supplemented with DHA and AA (0.25% and 0.4% of fatty acid, respectively) either as a combination of fish and fungal oils or as a combination of fish oil and egg triglyceride. Moreover, the few inconsistent differences in rates of growth were not seen when data from infants who consumed more than 50% of intake as human milk were excluded or when the statistical analyses were limited to infants who received at least 80% of intake as the assigned study formula (in this study, infants were randomly assigned to study formula before enteral intake was initiated but were allowed to consume human milk if it was available).

The reasons for the observed differences in rates of growth between infants who received formula supplemented with long-chain n-3 fatty acid versus unsupplemented formula in three studies are not clear. Suggested reasons include inhibition of desaturation and elongation of LA to AA by the n-3 fatty acids and/or inhibition of eicosanoid synthesis from AA by the intake of preformed EPA as a component fish oil or endogenous synthesis of EPA from a moderately high intake of ALA. However, regardless of the reasons for this effect, it is important to note that no study in which DHA supplementation was accompanied by AA supplementation has shown an adverse effect on any aspect of growth.

In addition to concerns about adverse effects of n-3 fatty acids on growth, a number of theoretical concerns about the safety of both n-6 and n-3 LCPUFA supplements have been raised (Heird, 1999a). Among these is the possibility that supplementation with highly unsaturated oils will increase the likelihood of oxidant damage. Because peroxidation occurs at the site of double bonds, membranes with more unsaturated fatty acids are likely to be more vulnerable to oxidant damage. If so, supplementation with LCPUFAs, which increases the membrane content of LCPUFAs, may increase the incidence of common neonatal diseases thought to be related to oxidant damage (e.g., necrotizing enterocolitis, bronchopulmonary dysplasia, and retrolental fibroplasia). There is also concern that unbalanced supplementation with n-3 and n-6 LCPUFAs will result in altered eicosanoid metabolism with potential effects on a variety of systems (e.g., blood clotting and infection). Furthermore, more polyunsaturated fatty acids in muscle cell membranes has been related to enhanced insulin sensitivity (Borkman et al., 1993; Pan et al., 1994; Storlien et al., 1991), and specific LCPUFAs have been shown to inhibit as well as to enhance transcription of a variety of genes (Clarke & Jump, 1996).

All of these theoretical concerns are related to known biological effects of relatively large amounts of LCPUFAs. However, there are few data to either support or refute these concerns with respect to the small amounts likely to be added to infant formulas. One exception is the finding that bleeding time, although within normal limits in both supplemented and unsupplemented infants, was somewhat longer in infants who received a marine oil supplement (Uauy et al., 1994a). The second study of Carlson et al. (1996) included data concerning the incidence of bronchopulmonary dysplasia, sepsis, and necrotizing enterocolitis in supplemented versus unsupplemented infants. There was no statistically significant difference in the incidence of any of these conditions between the two groups; however, the incidence of both necrotizing enterocolitis and sepsis was higher in the supplemented than in the unsupplemented group. Further, as noted by Lucas (1997), the combined incidence of necrotizing enterocolitis and sepsis [which was not examined by Carlson et al. (1996) but which might be relevant] was significantly higher in the supplemented group.

In contrast, Carlson et al. (1998), in a more recent study, found that the incidence of necrotizing enterocolitis was markedly and significantly lower in infants fed a formula supplemented with egg yolk phospholipid than in those fed an unsupplemented formula. Whether this was a chance finding or a result of either the AA or the choline content of the egg yolk phospholipid is not clear.

With respect to the effects of LCPUFA supplementation on eicosanoid metabolism, Huang and Craig-Schmidt (1996) found that the tissue content of eicosanoids derived from AA relative to those derived from EPA was lower in pigs receiving only DHA supplementation (as algal oil) than in pigs receiving both DHA and AA (as fungal oil). One study of infants also showed that the ratio of eicosanoid metabolites derived from AA and EPA was not deranged in infants receiving supplementation with both DHA and AA (Stier et al., 1997).

Three recent relatively large studies in preterm infants (Diersen-Schade et al., 1998; O'Connor et al., 2001; Vanderhoof et al., 1999) have shown that the incidence of bronchopulmonary dysplasia, necrotizing enterocolitis, and other neonatal conditions is not greater in infants receiving supplements of either DHA or both AA and DHA from a variety of sources (e.g., oils from single-celled organisms, low EPA fish oil, or egg yolk triglyceride) than in infants receiving unsupplemented

formula. Furthermore, as discussed above, no difference in growth of the various formula groups was observed. Together, these studies included 769 infants assigned to supplemented (n = 487) or unsupplemented (n = 282) formulas. Similar findings have been reported in smaller studies focusing on outcomes other than the incidence of common neonatal conditions but including information concerning the incidence of these problems.

Thus, despite the relative absence of data (i.e., definitive data) concerning the validity of a number of the specific theoretical concerns of harmful effects that have been raised (Heird, 1997), the fact that supplementation of formulas with the amounts of DHA and AA used in the studies of preterm infants cited above did not result in a greater incidence of conditions thought to be related etiologically to the theoretical concerns suggests that the amounts of the sources of LCPUFA used in these studies are without reported adverse effects. The effect of fish oil on growth of preterm infants, as reported by Carlson et al. (1992) and Ryan et al. (1999) remains a concern. However, it is important to note that the growth rates of the affected infants were not significantly less than the growth rates of normal term infants (Fomon & Nelson, 1993b; Heird, 1997). Furthermore, no study has shown an effect of balanced supplementation with both DHA and AA on growth of either preterm or term infants.

Conclusions and recommendations

Linoleic acid

Minimum. The Expert Panel recommended a minimum all-*cis* LA content of preterm infant formula of 8% of total fatty acids. This is identical to the recommendation in the report *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a). The Expert Panel is not aware of data indicating that the minimal LA content of preterm infant formulas should be less than that for term infant formulas. With a minimum fat content of 4.4 g/100 kcal, the minimum LA content of formulas would be 352 mg/100 kcal, or 3.2% of energy. This is well within the range of requirements for LA established decades ago for term infants, i.e., 2–4% of energy. The LA content of human milk varies considerably, largely reflecting the LA content of the maternal diet, but a content of less than 8% of total fatty acids is unusual (Jensen, 1999).

Maximum. The Expert Panel recommended that the maximum LA content of preterm infant formulas not exceed 25% of total fatty acids. This is about 28% lower than the maximum content recommended recently for term infant formulas (Raiten et al., 1998a). However, the rationale for the recommendation for term infant formulas was based largely on the fact that term infant formulas containing more than 35% of fatty acids as LA have been available for years and have not been associated with recognized adverse effects as well as observations that the recommended maximum is within the limits that have been reported for individual human milk samples.

In arriving at the recommendation for a lower maximal LA content for preterm infant formulas, the Expert Panel notes that the LA content of human milk only rarely exceeds 25% of total fatty acids. Moreover, perhaps because most current preterm infant formulas contain MCTs, the LA content of these formulas rarely exceeds 25% of total fatty acids. The preterm infant formulas currently available in the United States contain from 13% to 21% of fatty acids as LA (~6.5–10.5% of energy). Thus, unlike the situation for term infants, there are no historical data concerning use of preterm infant formulas with an LA content as high as 35% of total fatty

acids. Furthermore, there is no evidence that an LA content as high as 35% of fatty acids, which is several times higher that the apparent requirement, is beneficial for either the term or the preterm infant. For the maximum fat content of 5.7 g/100 kcal, the maximum LA content recommended for preterm infant formulas is 1425 mg/100 kcal (25% of 5.7 g) or 12.8% of energy.

Recommendations

Minimum. **The Expert Panel recommended that the minimum LA content of preterm infant formula be 8% of total fatty acids.**

Maximum. **The Expert Panel recommended that the maximum content of LA in preterm infant formula not exceed 25% of total fatty acids.**

α-Linolenic acid

The Expert Panel, being unaware of data indicating that the ALA content of preterm infant formulas should be different from that of term infant formulas, recommended the same minimum and maximum ALA contents for preterm infant formulas as recommended in the report *Assessment of Nutrient Requirements for Infant Formulas:* a minimum content of 1.75% of total fatty acids and a maximum content of 4% of total fatty acids (Raiten et al., 1998a). The Expert Panel also endorsed the additional stipulation that the ratio of LA to ALA not be more than 16:1 nor less than 6:1. In making these recommendations, the Expert Panel was aware that the absolute requirement for ALA has not been established for either term or preterm infants and that the recommendations report on infant formulas relied heavily on data from studies of animals. Nonetheless, it is clear that an ALA-deficient diet, in the absence of other n-3 fatty acids, results in deranged visual function and other abnormalities in both rodents (Benolken et al., 1973; Wheeler et al., 1975) and primates (Neuringer et al., 1984; Neuringer et al., 1986) as well as neurological derangements in both infants (Holman et al., 1982) and adults (Bjerve et al., 1987). Furthermore, it is not clear that these derangements can be prevented by provision of other n-3 fatty acids. Thus, the recommendations in the report *Assessment of Nutrient Requirements for Infant Formulas* were considered reasonable and, in the absence of data to indicate that the ALA requirement for preterm infant formulas should be different from that of term infant formulas, were adopted by the Expert Panel on Assessment of Nutrient Requirements for Preterm Infant Formula.

With the minimal recommended total fat content of preterm infant formula, 4.4 g/100 kcal, the minimum ALA content will be 77 mg/100 kcal (1.75% of 4.4 g), or approximately 0.7% of energy. Animal studies have shown that formulas containing less ALA result in lower brain levels of DHA (Innis, 1991). For the maximal recommended fat content of 5.7 g/100 kcal, the maximum recommended content of ALA in preterm infant formula will be 228 mg/100 kcal (4% of 5.7 g), or approximately 2.1% of energy.

The recommended upper limit for the ratio of LA to ALA (16:1) is intended to prevent an inappropriate combination of high LA content with low ALA content, which could interfere with the formation of longer chain n-3 PUFA. Thus, at the highest recommended LA content, 1425 mg/100 kcal, the lowest recommended ALA content is 89 mg/100 kcal rather than 77 mg/100 kcal. The recommended minimum ratio of LA to ALA (6:1) is intended to prevent the combination of the minimal recommended LA content with the maximal recommended ALA content, which could interfere with production of LCPUFAs of the n-6 series. Because this combination of the

lowest recommended LA content (352 mg/100 kcal) and the highest recommended ALA content (228 mg/100 kcal) will result in a ratio of LA to ALA of 1.5:1, it is incompatible with the recommendation. At the minimum recommended LA content (352 mg/100 kcal), the maximum ALA content must be 59 mg/100 kcal. Because this is less than the minimum amount of ALA recommended (179 mg/100 kcal), this combination, also, is incompatible with the recommendation. At the minimum recommended ALA content (77 mg/100 kcal), the minimum LA content must be 462 mg/100 kcal rather than 352 mg/100 kcal. Similarly, the maximum ALA content recommended (228 mg/100 kcal) must be accompanied by an LA content of at least 1368 mg/100 kcal.

Recommendations

Minimum. **The Expert Panel recommended that the minimum ALA content of preterm infant formula be 1.75 % of total fatty acids.**

Maximum. **The Expert Panel recommended that the maximum ALA content of preterm infant formula not exceed 4% of total fatty acids.**

Ratio. **The Expert Panel also recommended that the ratio of LA to ALA not be less than 6 nor more than 16.**

γ-Linolenic acid

The report *Assessment of Nutrient Requirements for Infant Formulas* noted that no convincing data were found to indicate that the conversion of LA to GLA is a limiting factor in the n-6 elongation pathway or that addition of GLA to infant formulas results in appreciably greater concentrations of n-6 LCPUFAs (Raiten et al., 1998a). Although data are limited, this is likely to be equally true for preterm infants.

Recommendations

Note. **The Expert Panel concluded that there is no demonstrated benefit of adding GLA to preterm infant formulas.**

Arachidonic, docosahexaenoic and eicosapentaenoic long-chain polyunsaturated fatty acids

Minimum. In reaching a decision about LCPUFAs, the Expert Panel noted that the observed advantages of LCPUFAs on visual development and/or neurodevelopment have been minimal and/or transient. Moreover, despite the beneficial effects of n-3 LCPUFA supplementation on visual acuity, most infants who were evaluated, whether they received the supplemented or the unsupplemented formula, had normal visual acuity. The Expert Panel also noted that the effects of supplementation may not be the same for all infants and, in fact, may be negative for some (e.g., infants with bronchopulmonary dysplasia).

Maximum. In contrast, preterm infants, in theory, are likely to be more vulnerable to inadequate intake of these important fatty acids, and all studies of preterm infants have shown transient and/or small positive effects of LCPUFA-supplemented versus unsupplemented formula on visual development and/or neurodevelopment. Furthermore, the few supplements that have been studied do not raise important issues of deleterious effects. Recent studies involving a relatively large number of infants suggest that supplementation of preterm infant formulas with both DHA and AA from single-celled sources, egg lipid and/or low EPA fish oil (0.25–0.35% of total fatty acids as DHA and 0.4–0.6% as AA) did not result in an increased incidence of diseases that have been linked etiologically to known biological effects of these powerful mediators of metabolism. These n-3 and n-6 LCPUFA supplements, when used together, also did not affect growth

adversely and did not result in other adverse effects. These important observations plus the possibility that small differences in early visual and/or cognitive function may be important on a population basis or may be important for subsequent visual and/or cognitive function entered into the Expert Panel's decision to recommend a maximum content of DHA and AA for preterm infant formulas.

The designation of maximal contents of DHA and AA provides an option for inclusion of these acids in preterm infant formulas at levels that are not likely to result in adverse effects. The Expert Panel also recommended that formulas not be supplemented with only DHA or only AA but rather that both fatty acids be used. The ratio of AA to DHA should be between 1.5 and 2.0, but the maximum amounts of each should not exceed the amounts recommended. This stipulation with respect to the ratio of AA to DHA is intended to help guard against imbalances in eicosanoids synthesized from n-6 versus n-3 fatty acids.

Recommending a maximum content of DHA of 0.35% of total fatty acids is more than the amount shown by Carlson et al. (1992; 1996) and O'Connor et al. (2001) to be beneficial but less than the maximum amount that has been studied and found to be without obvious adverse effects (Clandinin et al., 1997). Recommending a maximum amount of AA of 0.6% of total fatty acids is also less than the maximal amount studied and found to be without obvious adverse effects. However, a higher intake at the recommended maximum intake of DHA could exceed the usual ratio of AA to DHA in human milk, and such ratios have not been studied extensively. In addition, because fish oil with a high content of EPA has been implicated as a possible cause of growth failure (see above), the Expert Panel recommended that the content of this fatty acid in preterm formulas not exceed the amount present in formulas studied recently and found not to result in adverse effects on growth or other outcomes, i.e., about 30% of the content of DHA.

Future clinical studies of the effects of supplementing infant formulas with LCPUFAs should not repeat the now-recognized shortcomings of many of the important earlier studies: small sample size, failure to adjust for all potentially confounding variables, and inadequate follow-up to permit assessment of long-term effects. Although admittedly difficult to execute and interpret, studies of preterm infant populations typical of those likely to receive formulas with DHA and AA are needed to further our understanding of the optimal amount for feeding. Finally, although studies of animals clearly cannot define the requirements for the human infant, such studies could help clarify some outstanding issues (e.g., transport of LCPUFAs to the central nervous system) or suggest mechanisms for some of the molecular effects that have been observed (e.g., the effect of LCPUFAs on gene transcription).

Recommendations

Minimum. **The Expert Panel did not recommend a minimum content of AA, DHA or EPA for preterm infant formulas.**

Maximum. **The Expert Panel recommended that the maximum concentration of AA be 0.6% of total fatty acids, that the maximum concentration of DHA in preterm infant formulas be 0.35% of total fatty acids, and that the maximum concentration of EPA be 30% of the concentration of DHA.**

Ratio. **The Expert Panel also recommended that the final ratio of AA to DHA in any supplemented preterm formula be 1.5-2.0.**

Relationship of polyunsaturated fatty acids to vitamin E

Membranes enriched with polyunsaturated fatty acids are particularly susceptible to oxidative damage. Thus, a concern about the use of LCPUFAs in infant formulas is the possible need for a higher vitamin E intake. Indeed, animals fed large amounts of marine oil have higher levels of lipid peroxidation as measured by higher levels of thiobarbituric acid-reactive products in various tissues; addition of vitamin E to the diet reduces the level of these products (Garrido et al., 1993; Leibovitz et al., 1990). Signs of vitamin E deficiency in adipose tissue of rats fed diets containing fish oil without supplemental vitamin E have also been reported.

There is no evidence that a high level of LCPUFAs in the diet specifically leads to vitamin E deficiency in human infants. In a recent study in which fish oil supplementation (6 g/d) of nursing mothers resulted in high levels of n-3 LCPUFAs in breast milk, no change was noted in infant plasma tocopherol levels (Henderson et al., 1992). However, the duration of the supplementation was only 3 weeks, and the number of subjects who received this high dose of fish oil was small ($n = 5$). Similarly, Decsi and Koletzko (1995) found that increased AA levels secondary to supplementation of commercial formula with evening primrose oil, a source of GLA, did not alter plasma retinol or tocopherol levels. Furthermore, as noted above, recent studies show no evidence of an increased incidence of diseases thought to be related to oxidant stress in infants who received LCPUFA-supplemented formula versus unsupplemented formula. Nonetheless, because of the known relationship between the polyunsaturated fatty acid content of membranes and oxidant damage, the Expert Panel recommended that the vitamin E content of preterm infant formulas be based on the polyunsaturated fatty acid content (See Chapter 13). One limitation of this report is that no recommendations were made for the total polyunsaturated fatty acid content of preterm infant formula; recommendations were provided for individual LCPUFAs.

Recommendation

Note. **The Expert Panel recommended that the vitamin E content of preterm infant formulas be based on the polyunsaturated fatty acid content (See Chapter 13).**

OTHER FATTY ACIDS AND RELATED SUBSTANCES

Myristic acid and lauric acid

Myristic acid (14:0) and lauric acid (12:0) are found in human milk, each usually constituting from 2% to 12% of total fatty acids (Jensen, 1999). Currently, although both are present in formulas containing MCTs, there are no recommendations for either minimum or maximum levels of these fatty acids in preterm or term infant formulas available in the United States. The Commission of the European Communities has indicated that the maximum level of myristic acid in formulas should not exceed 15% of the total fatty acid content (Raiten et al., 1998a); the content of lauric acid has not been addressed by any regulatory body except as the content of MCTs affects the content of this fatty acid.

This Expert Panel, as in the report *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a), did not recommend adding myristic or lauric acids to formulas for preterm infants. Although lauric acid seems to have more bactericidal activity than some other fatty acids (Jamieson et al., 1994), there are no data to indicate a specific role for either myristic or lauric acid as a dietary nutrient. However, these fatty acids are components of some oils used in preterm infant formulas, and the Expert Panel does not wish to proscribe the use of such oils, which have a long history of being efficacious without reports of adverse effects. Because no data are available on which to base a recommendation, the Expert Panel recommended that the maximum levels of myristic and lauric acid in preterm infant formulas not exceed the content of each of these fatty acids in human milk, i.e., about 12% total fatty acids. For example, at the minimum fat content recommended of 4.4 g/100 kcal this would provide for a maximum content of myristic acid of 528 mg/100 kcal.

Recommendations

Minimum. **The Expert Panel did not recommend a minimum content of myristic or lauric acid in preterm infant formula.**

Maximum. **The Expert Panel recommended that the maximum content of myristic acid in preterm infant formula be 12% of total fatty acids. The Expert Panel recommended that the maximum content of lauric acid in preterm infant formula be 12% of total fatty acids.**

Medium-chain triglycerides

MCT oil, usually prepared from coconut oil, contains a mixture of fatty acids of chain lengths between 6 and 12 carbons, more than 90% of which are 8:0 and 10:0. Currently available preterm formulas contain 40% or 50% of total fat as MCTs. The rationale for including these triglycerides is to improve intestinal fat absorption. However, the concept that intestinal fat absorption of preterm infants is severely compromised seems to be based primarily on earlier studies of the absorption of butterfat. The vegetable oil blends currently used in infant formulas seem to be well absorbed by preterm infants. In fact, modern studies of fat absorption of preterm infants fed formulas with or without MCTs show minimal, if any, difference (Hamosh et al., 1989; Sulkers et al., 1992). Moreover, any advantage of including MCTs on fat absorption is not accompanied by improved rates of growth (Hamosh et al., 1989; Okamoto et al., 1982). Thus, in the absence of data indicating a distinct advantage of MCTs in feeding preterm infants, the Expert Panel did not recommend the addition of MCT oil to premature infant formula.

The predominant metabolic pathway for medium-chain fatty acids is catabolism to acetyl-CoA. A number of studies have shown that these fatty acids are more readily oxidized than are long-chain fatty acids (Sulkers et al., 1989); hence, they may serve a useful purpose in infants with limited energy intake. However, in adults, the administration of MCTs has been associated with alterations in tissue prostaglandin synthesis (Katz & Knittle, 1987). Moreover, the serum concentrations of ketones and the urinary excretion of dicarboxylic acids increase in a linear fashion with increases in MCT intake (Bach & Babayan, 1982). Whether this is a problem, as suggested by some, or simply a normal response to intake of MCTs is not clear. Certainly, impaired function has not been demonstrated with moderate intakes of MCTs.

Medium-chain fatty acids make up as much as 8–10% of the total fatty acids of human milk, the actual amount being related to maternal carbohydrate intake (Jensen, 1999). Despite this, the Expert Panel did not recommend a minimum content of MCT for preterm infant formulas. However, MCTs account for 40–50% of the total fat content of currently available preterm infant formulas, and these formulas have not been associated with adverse effects related to their content of MCTs. The Expert Panel therefore recommended that the maximum MCT content of preterm infant formulas not exceed 50% of total fat content.

Recommendation

<u>Minimum.</u> **The Expert Panel did not recommend the addition of MCT oil to premature infant formula. Therefore, the Expert Panel did not recommend a minimum content of MCT for preterm infant formulas.**

<u>Maximum.</u> **The Expert Panel recommended that the maximum MCT content of preterm infant formula be 50% of total fat content. This would be 2.2–3.0 g/100 kcal, depending on the total fat concentration.**

Trans-fatty acids

The chemical properties of *trans*-fatty acids differ markedly from their respective *cis* isomers (e.g., higher melting points). Most dietary *trans*-fatty acids originate from industrial hydrogenation of unsaturated vegetable oils. Small amounts of these acids are also present in animal fats, including dairy fats (Senti, 1985). Potential sources of *trans*-fatty acids in infant formulas are partially hydrogenated vegetable oils and the isomerization of *cis* isomers during the formula-manufacturing process. Koletzko and Bremer (1989) reported that the *trans*-fatty acid content of formulas produced in Europe range from 0.2% to 4.6% of total fatty acids. In general, levels of these fatty acids in human milk are higher than those in formula and reflect the *trans*-fatty acid content of the maternal diet (Carroll, 1989). Craig-Schmidt et al. (1984) reported that 4.8% of the 18:1n-9 content of human milk from a population in the United States (30–35% of total fatty acids) is the *trans* isomer, i.e., 1.4–1.7% of total fatty acids. On average, *trans* isomers of unsaturated fatty acids account for 4.4% of the total fatty acid content of the milk of German mothers (Koletzko et al., 1988a).

A number of untoward biological effects have been attributed to *trans*-fatty acid consumption (Carlson et al., 1997). At the cellular level, *trans*-fatty acids may impair microsomal desaturation and chain elongation of LA and ALA (Mahfouz et al., 1980, 1981). It is not known whether this occurs in vivo, but Koletzko (1994) expressed concern about the possibility that it does. This concern is reinforced by identification of measurable amounts of *trans*-fatty acids in plasma triglycerides, sterol esters, and phospholipids of preterm infants on the fourth day of life when enteral feeding had just started (Koletzko, 1994). *Trans*-fatty acids are also thought to increase serum lipoprotein levels (Mensink et al., 1992; Mensink & Katan, 1993). No evidence of teratogenicity or carcinogenicity has been reported.

No upper limit or lower limit for the content of *trans*-fatty acids in infant formula was specified by the Committee on Nutrition of the European Society of Paediatric Gastroenterology and Nutrition (1991) or the Codex Alimentarius Commission (1994). The Commission of the European Communities, however, recommended that the *trans*-fatty acid content of infant formula not exceed 4% of the total fat content (Raiten et al., 1998a).

The Expert Panel recommended that hydrogenated oils, the major source of *trans*-fatty acids in infant formulas, not be used in the manufacture of infant formulas. Although no specific deleterious effects of dietary *trans*-fatty acids have been identified in premature infants, the Expert Panel noted that there are potential short- and long-term deleterious effects and, also, that no known nutritional benefits of these substances have been identified. Thus, the Expert Panel recommended that the content of these substances in infant formulas be limited to the minimum amount feasible. If hydrogenated oils are not used as fat components of infant formulas, the content of *trans*-fatty acids in formulas will be limited to the amount resulting from the manufacturing process, which although not known with certainty, is probably small.

Recommendation

<u>Note.</u> **The Expert Panel recommended that the content of *trans*-fatty acids in preterm infant formula be limited to the minimum amount feasible.**

CHOLESTEROL

The report *Assessment of Nutrient Requirements for Infant Formulas* did not recommend addition of cholesterol to term infant formulas (Raiten et al., 1998a). That report discussed the rationale for possibly including cholesterol in infant formulas. It also summarized data from studies of infants fed human milk, which contains cholesterol, versus formula as well as data from studies of infants fed cholesterol-supplemented versus unsupplemented formulas and animal studies relevant to this issue. The present Expert Panel was not aware of any additional data that would lead to a different recommendation for preterm infant formulas.

Recommendation

<u>Note.</u> **The Expert Panel did not recommend addition of cholesterol to formulas intended for preterm infants.**

10. MINERALS: CALCIUM AND PHOSPHORUS

CALCIUM

Background

More than 99% of the total body calcium is bound to the structural matrix of bone (osteoid). Two-thirds of the weight of cortical bone is mineral (Greer, 1991). Calcium constitutes 30% of the weight of apatite, $Ca_5(PO_4)_3OH$, which is the major mineral constituent of bone; phosphorus constitutes 14%. Another mineral constituent was thought to be amorphous calcium phosphate (Arnaud & Sanchez, 1996), but recent animal studies cast doubt on its existence in significant amounts in bone (Boskey, 1997).

Only about 50% of the circulating calcium is in the physiologically active, ionized form (Ca^{2+}) (Arnaud & Sanchez, 1996); the remainder is protein bound or chelated. The physiological functions of calcium were reviewed in the report *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a).

Review of the literature

<u>Calcium accumulation by the fetus.</u> Mineral accretion rates in utero increase exponentially between 24 and 37 weeks of gestational age (GA) (Steichen et al., 1980). As a result, about 80% of the mineral content in the full-term newborn [30 g of calcium and 16 g of phosphorus (Campbell & Fleischman, 1988)] is accumulated rapidly in the third trimester [See Heaney and Skillman (1971)]. Mineral accretion rates are nearly three times as high in the last trimester as in the period immediately after term birth. This means that when a premature birth occurs at a GA of 24–34 weeks, the separation of the infant from the placental active transport of calcium affects subsequent bone mineralization disproportionately to body size [see Forbes (1976), discussed below].

Current estimates of the calcium needs of prematurely born infants are based on the intrauterine accretion of nutrients, as derived from the chemical composition of fetal tissue at various GAs (Widdowson & Spray, 1951) and adapted to birth weight (BW) curves for North American infants (Ziegler et al., 1976). Greer and Tsang (1985) and Greer (1991) critically reviewed many of the earlier studies of fetal calcium accretion between 25 and 36 weeks GA and found all of them flawed. Nevertheless, the results showed general agreement that the

daily rate of accretion rises from about 120 mg/kg at 26 weeks GA to 130–140 mg/kg at 36 weeks. The daily accretion rate is 150–155 mg/kg at 36–38 weeks of GA (Steichen et al., 1980). By assuming that body calcium content is a power function of BW, Forbes (1976) used some of the older data to demonstrate a linear relationship on a logarithmic plot:

$$\text{Calcium (g)} = (1.168 \times 10^{-3}) \times \text{fetal weight in grams}^{1.2408}$$

The correlation coefficient for this relationship in the data used by Forbes (1976) was 0.993.

An intrauterine bone mineral accretion rate has been obtained by measuring the bone mineral content (BMC) of newborn infants of various GAs with photon absorptiometry (Chan, 1992; Greer et al., 1983). Mineral content at the one-third distal radius increases from 25 mg/cm at 26 weeks to 65 mg/cm at 36 weeks, but body size more than doubles.

Calcium content of milk. Even though human milk is not being used as a standard in this report, it is pertinent to review its calcium content. Atkinson et al. (1995) summarized the data from 24 studies of the calcium content of human milk. They reported that it rises from about 4 mmol (160 mg)/L in initial colostrum to 6.4 mmol (256 mg)/L by the third day and then plateaus there for the first 3 months of nursing. Thereafter it declines slowly to 4.9 mmol (176 mg)/L by 1 year. Milk from mothers giving birth prematurely ("preterm milk") is not significantly different in calcium content from term milk during the first month of nursing (Atkinson et al., 1980). The calcium content of preterm infant formulas available in the United States is 1340–1460 mg/L.

Calcium accumulation by the premature infant. Abundant evidence indicates that the concentration of calcium in breast milk or in formula designed to mimic breast milk is insufficient to maintain the fetal bone mineral accretion rate in the preterm-low birth weight (LBW) infant. Hamilton (1922) showed almost 80 years ago that the total amount of human milk that can reasonably be consumed by a premature infant in the first few months of life does not contain enough calcium to permit its deposition at a rate proportional to skeletal and total body growth, even if it were 100% assimilated. Steichen et al. (1980) estimated that an intake of 200–400 mg/(kg·d), or 1–2 L of human milk/d, would be required. By using current numbers for the growth rate of premature newborns (see Chapter 4), the fetal calcium accretion rate (Forbes, 1976), and the calcium content of human milk (Atkinson et al., 1995), we can estimate the amount of milk required to maintain the fetal bone mineral accretion rate. For example, if a fetus of 500 g at 22 weeks GA gains 1500 g to reach 2000 g by 33 weeks GA (Arbuckle et al., 1993), total body calcium content increases by 12 g (from 2.6 to 14.6 g) (Forbes, 1976). This would require the calcium in 46.9 L of human milk, assuming that it contained 6.4 mmol calcium (256 mg)/L and was 100% absorbed and assimilated. However, this would also require an average intake of 610 mL/d of human milk for 11 weeks, which is clearly unlikely for preterm-LBW infants, whose typical daily consumption is 150 mL/kg (Georgieff, 1999; Wessel, 2000).

A further restriction on the ability of premature infants to mineralize bone is imposed by their incomplete assimilation of ingested calcium. Greer and Tsang (1985) summarized previous studies as showing absorption of ingested calcium ranging from 17% to 79% of intake, and retention ranging from 16% to 71% of intake. Atkinson (1985) attributed these wide variations to the large number of factors affecting calcium absorption and retention, especially the nature and quantity of other components of intake and/or medication (e.g., phosphorus, fat, lactose, phytate, protein, and diuretics).

Later, metabolic balance studies indicated that net calcium absorption from supplemented or unsupplemented banked human milk or from preterm formulas fed to 1-month-old premature infants was a linear function of intake in the range of 40–120 mg of calcium/(kg·d) (Bronner et al., 1992). Overall absorption for all types of feeding averaged 58 ± 9% (SEM). There was no indication that the higher dietary calcium intakes used exceeded the maximum solubility of food calcium in infant luminal fluids. One limitation of the study was that all subjects might not have received the same amount of phosphorus per kilogram.

The calcium-to-phosphorus ratio in the diet is an important determinant of calcium retention, a minimum amount of phosphorus being critical. The calcium-to-phosphorus ratio of human milk is 2.0 in terms of mass and 1.6 in terms of moles. The ratios are the same in currently available preterm infant formulas in the United States, although the absolute amounts are quite different (American Academy of Pediatrics. Committee on Nutrition, 1998). In addition, the bioavailability of the calcium provided in the diet will affect the ratio of calcium to phosphorus absorbed. Sometimes, different mineral salts added to cow milk-based formulas have different coefficients of absorption (Schanler & Abrams, 1995).

Measurements of net absorption of calcium account for failure of intestinal uptake and for fecal excretion, but they do not equate with calcium assimilation because of urinary losses. In adults and older children, the urinary losses are about the same as gastrointestinal (GI) excretion, but the amount of endogenous GI excretion has only recently been measured in premature infants. Thus, how closely absorption reflects assimilation has been uncertain.

Mass spectrometric methodology using stable isotopes has shed light on this issue. Abrams et al. (1991) and Hillman et al. (1993) measured true dietary absorption, endogenous fecal excretion, urinary loss, and net calcium retention by administering two different isotopes of calcium, one given orally and the other intravenously. Because 99% of body calcium is in bone, total retention is virtually identical to bone assimilation. In 12 preterm infants (mean BW of 1426 g, mean weight at time of study of 1693 g) studied by Abrams et al. (1991), the net retention on intakes of 189–226 mg/(kg·d) (calcium-to-phosphorus mass ratio was 2.0) averaged 103 ± 38 mg/(kg·d) (SD)(48%). Endogenous GI excretion was equal to about 7% of intake, and urinary excretion, about 2% of intake. Hillman et al. (1993) found higher and more variable values for fecal and urinary excretion. Nevertheless, these results, together with those of Bronner et al. (1992) cited above, showing a linear increase in calcium uptake with increasing calcium dose at lower intakes of 40–120 mg/(kg·d), support the use of formulas containing high calcium levels (at least up to 1520 mg/L) in an attempt to achieve fetal rates of calcium retention in preterm-LBW infants. For example, a daily feeding of 120 kcal/kg and 226 mg of calcium/kg requires 149 mL/kg of a formula containing 810 kcal/L and 1520 mg of calcium/L.

Hillman et al. (1993) found that in infants of BW less than 1500 g and GA less than 33 weeks, studied at 2–3 weeks of age, the mean percentage of calcium retention per kilogram was not different between human milk and preterm formula, with or without calcium fortification, even though absorption, urinary excretion, and endogenous fecal excretion varied considerably. However, calcium-fortified formula (1340 mg/L) yielded a 41% greater average total retention of calcium [82 mg/(kg·d)] than did formula containing less calcium [940 mg/L; calcium retention of 58 mg/(kg·d)]. It was stated that retention did not change with BW or GA down to 810 g and 27 weeks, respectively, in the 40 infants studied. This work extends the linear relationship for retention found by Bronner

et al. (1992) to indicate a linear increase in assimilation at intakes of up to 200 mg/(kg·d) [assuming an intake of 150 mL/(kg·d) of fortified human milk or formula].

A challenge to the general consensus as to the beneficial effect of mineral supplementation on bone mineralization of premature infants was published recently (Faerk et al., 2000). This relatively large trial randomized 127 premature infants (<32 weeks GA) to receive feedings of human milk supplemented with phosphorus; human milk supplemented with protein, calcium, and phosphorus; or preterm infant formula. The sources of human milk in the first two groups were the infants' mothers' milk, high protein banked milk, or a mixture of these. The formula-fed group included some infants who received their own mothers' milk, with or without added formula, and some who received formula alone, making nine subgroups. The study followed the infants from age 1–36 weeks of postconceptional age; the target intake was 200 mL/(kg·d) at full feeding [caloric intake of 140 kcal/(kg·d)]. Actual mean intakes were 184–198 mL/(kg·d) in the different subgroups. The formula-fed infants gained more weight and had a higher BMC than did the others, but the BMC corrected for body size was not increased. This could be a result of an undernutrition proportional for a number of nutrients in the infants not formula fed. No other significant differences were observed.

This study included variables that might have confounded the ability to detect significant differences related to supplementation. First, only 16 of 51 infants randomized to receive preterm formula actually did receive that feeding; the others (21 of 51) received either their own mother's milk or formula and their own mother's milk (14 of 51) in various proportions. Also, the average weight of the infants in each of the subgroups was above 1100 g, whereas infants of BW less than 1000 g have demonstrated the maximum effect of supplementation. The control (milk-fed) groups received phosphorus supplementation, which by itself has a marked effect on bone density (see below), thereby increasing the difficulty of getting statistically significant differences between the groups. Finally, the calcium and phosphorus supplementations increased the intakes by relatively small amounts, bringing them to one-half to two-thirds the minima recommended in this report on the basis of other studies.

Effect of inadequate calcium assimilation by the premature infant. The result of insufficient assimilation of calcium (or phosphorus) by the preterm-LBW infant is metabolic bone disease. This condition is characterized by a failure of complete mineralization of osteoid and encompasses disturbances ranging from mild undermineralization (osteopenia) to severe bone disease with fractures (rickets) (Campbell & Fleischman, 1988). Metabolic bone disease is particularly common in infants of GA less than 28 weeks and a BW less than 1000 g, among whom the incidence has been reported to be 50% or more (Campbell & Fleischman, 1988). Rickets is characterized by the accumulation of unmineralized osteoid, which interrupts the mineralization of the growth plate. It is therefore a childhood disease. Other forms of osteopenia occur in a variety of other medical situations.

Complications and efficacy of calcium-fortified formulas in premature infants. Some investigators (Campbell & Fleischman, 1988) advised against attempting to maintain the fetal accretion rate for bone mineral in the postnatal period because of concern that this could produce hypercalciuria and potentially lead to nephrocalcinosis. However, Abrams et al. (1994), using the dual-tracer method in premature infants fed fortified human milk or formula with a high mineral content, found no significant relationship between dietary and urinary calcium. They showed that in almost all subjects receiving these preparations, urinary calcium was derived from tissue (bone) cal-

cium rather than the diet. They did find that some premature infants had persistent hypercalciuria [defined as excretion of more than 4 mg/(kg·d)] that was unrelated to inadequate mineral intake or calcium absorption. Rather, it resulted from losses of both ingested and tissue (bone) calcium.

Steichen et al. (1980) showed that supplementation to increase neonatal calcium accretion in premature infants can be accomplished without adverse effect. They measured BMC with photon absorptiometry and demonstrated that given enough calcium supplementation, infants of 28–35 weeks GA would achieve a mean BMC within the 95% confidence limits for the BMC of fetuses of the same postconceptional age up to 40 weeks. No significant side effects were seen in 13 infants receiving calcium at 220–250 mg/(kg·d) from the 2nd to the 12th week of postnatal life. Rowe et al. (1987) studied retention by LBW (<1500 g) infants ingesting calcium at 219 ± 36 mg/(kg·d) and phosphorus at 116 ± 20 mg/(kg·d). At about 1–1.5 months of age, retention of calcium and phosphorus was 1.3 and 1.2 times the intrauterine accretion rate, respectively, without significant side effects. Others have demonstrated improved although less complete bone mineralization (Greer & McCormick, 1988) or mineral retention (Rowe et al., 1989).

Schanler and Abrams (1995) reported that by supplementing human milk with calcium phosphate [123 mg/(kg·d)], intrauterine accretion rates for calcium could be approached in 1000-g infants. With a more milk-soluble calcium gluconate-glycerophosphate (CaGP) preparation, retention rates averaged 2.6 ± 0.9 mmol/(kg·d) [104 mg/(kg·d)]. However, it was the greater intake of calcium and phosphorus in the CaGP preparation [184 mg/(kg·d)], not an improved bioavailability, that led to the improved net retention. Schanler and Abrams found the CaGP preparation to be without adverse effect in the smallest LBW infants, but these authors were concerned that the large volumes of the CaGP-supplemented milk required to meet targeted growth rates might not be tolerated by ill premature infants whose fluid intakes needed to be restricted. In addition, the minerals (at high amounts) in the feedings can combine with fatty acids and promote fecal fat excretion, which in turn can adversely affect mineral retention (Katz & Hamilton, 1974). As expected with a higher concentration of minerals, the efficiency of fat absorption was lower with the CaGP formulation. The formation of insoluble soaps by minerals and fatty acids in the intestinal lumen may explain why the mean percent absorption of calcium was no greater with CaGP (60 ± 13%) than it was with calcium phosphate (59 ± 19%).

Insight as to how the adverse effect of fat on the absorption of calcium from formula, and the reverse, might be prevented was provided by a randomized trial of synthetic triglycerides that contained 74% of the palmitate in the 2-position (Lucas et al., 1997). These triglycerides thus more closely mimicked the structure of triglycerides in breast milk than did the control formulas, which contained less palmitate in that position (Jensen et al., 1995b). The synthetic triglycerides decreased the formation of insoluble soaps in the stool and increased calcium absorption from 42% to 57%. They also had a favorable effect on the absorption of palmitic and stearic acids.

It should be noted that many of the fortifiers used in some of these studies included several other substances in addition to calcium, particularly phosphorus, magnesium, and protein. More recently, Narbona et al. (1998) used dual-energy densitometry to measure the BMC and bone mineral density (BMD) in infants (BW of <1896 g) after 1 month of postnatal life who received formulas different only in calcium and phosphorus content. Groups of 15 infants were fed mean calcium

intakes of 122 or 149 mg/(kg·d) at mean phosphorus intakes of 80 or 93 mg/(kg·d), i.e., calcium-to-phosphorus ratios 1.5 and 1.6, respectively. The energy and protein intakes and the rates of increase in weight and length were similar in the two groups. Intake of calcium and phosphorus correlated with the BMC ($r = 0.65$) and BMD ($r = 0.49$). In the group with the higher intake of calcium and phosphorus, Narbona et al. (1998) found mean BMC and BMD values virtually equivalent to those measured at birth in a control group of 15 preterm infants whose mean GA was 4 weeks greater than that of the experimental group, thus indicating an intrauterine-like rate of increase in this experimental group. In the group receiving the lower intake of calcium, the BMC was 39% lower than in controls and the BMD was 8% lower. Narbona et al. (1998) concluded that the calcium content of preterm formula should not be lower than 120 mg/100 kcal, the amount given to the higher intake group.

Optimal calcium-to-phosphorus ratio. The importance of the phosphorus concentration in determining both the solubility of calcium in the formula (Bhatia & Fomon, 1983) and its assimilation into bone (Senterre et al., 1983) makes it important to specify a ratio of calcium to phosphorus in the diet. This will necessarily influence the individual recommendations for the two elements. The calcium-to-phosphorus ratios (by mass) of intakes in supplementation studies of Rowe et al. (1987) and of Schanler and Abrams (1995), were in the range of 1.8-2.0, respectively. However, when calcium supplementation leads to a high intake, in the range of 220 mg/(kg·d), increasing the phosphorus intake to lower the calcium-to-phosphorus mass ratio below approximately 2.0 does not further increase calcium retention (Mize et al., 1995); it may, however, lead to an increased nonosseous tissue uptake of phosphorus and an improvement in gain in body length (Mize et al., 1995) (see the section on phosphorus, below). However, too low a calcium-to-phosphorus ratio may precipitate "late neonatal hypocalcemia" with seizures (Specker et al., 1991).

An earlier experiment similar to that of Mize et al. (1995) produced important information about the lowest calcium-to-phosphorus ratio in calcium-fortified human milk or formula that is likely to be effective in stimulating bone mineral accretion. Chan et al. (1988) studied 30 preterm infants (BW of <1600 g) until they reached 1900 g, along with six similar control infants who received their own mothers' milk. The experimental group was fed one of three diets: a commercial premature infant formula (810 kcal/L) containing 948 mg of calcium/L and 474 mg of phosphorus/L (117 and 58.5 mg/100 kcal, respectively), the same formula with a higher phosphorus content (664 mg/L, or 82 mg/100 kcal), or the same formula with a higher concentration of both calcium (1134 mg/L) and phosphorus (664 mg/L) (140 and 82 mg/100 kcal, respectively). The calcium-to-phosphorus mass ratios of these diets were 2.0, 1.4, and 1.7, respectively. Bone mineralization rates as measured by photon absorptiometry were above the fifth percentile of the reference intrauterine rate for the first and third formulas only. So in this experiment, a calcium-to-phosphorus ratio of 1.7 in the formula was adequate at 948 mg of phosphorus/L (82 mg/100 kcal), whereas a ratio of 1.4 at the same calcium concentration was not.

An assessment of requirements for formulas for full-term infants (Raiten et al., 1998a) recommended a calcium-to-phosphorus mass ratio of between 1.1 and 2.0 on the basis of the Code of Federal Regulations (21 CFR 107.100) requirement. The recommendation of the Canadian Paediatric Society (CPS) (1995) for the calcium-to-phosphorus ratio of premature infants in the stable growing period is a molar ratio of 1.6-2.0, which is 2.1-2.6 on a mass basis. This is not consis-

tent with the recommendation in the same publication for feedings of 20–30 mmol/L of calcium and 16–20 mmol/L of phosphorus, which would allow a molar ratio range of 1.0–1.9 and a mass ratio range of 1.3–2.5. Formulas for premature infants currently available in the United States have calcium-to-phosphorus mass ratios of 1.8–2.0 (Abbott Laboratories. Ross Products Division, 1999; American Academy of Pediatrics. Committee on Nutrition, 1998).

Current recommendations

The CPS (1995) stated that a consensus from available studies indicates 20–30 mmol/L of calcium (and 16–20 mmol/L of phosphorus) in feedings, which would provide 4.0–6.0 mmol/(kg·d) of calcium at the CPS-recommended maximum formula intake of 200 mL/(kg·d). At 810 kcal/L, this is 133–200 mg of calcium/100 kcal. Wauben et al. (1998) found that a more conservative calcium supplementation [to 2.7–3.3 mmol/(kg·d), or 108–130 mg/(kg·d)] at a phosphorus intake of 3.3–3.4 mmol/(kg·d) [104–108 mg/(kg·d)], from the time of full enteral feedings to 40 weeks postmenstrual age, was sufficient to bring the total BMC per unit of body length and lean body mass into the low normal range for 5-day-old term infants. Because the breast-fed premature infants were taking feedings at 164–177 mL/(kg·d), their calcium intake was 2.5–3 times what it would have been with their mothers' milk [assuming that to be 6.4 mmol/L (Atkinson et al., 1995)].

The American Academy of Pediatrics Committee on Nutrition (1998) recommended 175 mg of calcium/100 kcal, which calculates to 1418 mg/L of formula at a caloric concentration of 810 kcal/L. With the recommendation for phosphorus of 91.5 mg/100 kcal, the recommended calcium-to-phosphorus ratio would be 1.9. The Committee on Nutrition of the Preterm Infant of the European Society of Paediatric Gastroenterology and Nutrition (1987) recommended 70–140 mg of calcium/100 kcal and 50–90 mg of phosphorus/100 kcal. These ranges are limited in practice by an additional recommendation that the calcium-to-phosphorus mass ratio be within 1.4–2.0.

Summary of supplementation studies

Table 10-1 lists some of the studies of intakes of calcium-supplemented breast milk and formula that have been demonstrated to be without adverse effect and at least partially effective in stimulating bone mineralization to near in utero rates. Also shown for each study are the calculated amounts of calcium that would have been ingested per 100 kcal at a caloric intake of 120 kcal/(kg·d), the concentration of calcium in the formula that would be required for such an intake at 810 kcal/L, and the calcium-to-phosphorus ratio in the efficacious feedings used in the study.

None of these trials reported any significant toxicity of the supplements, but in most of them the number of subjects was small. Current formulas designed for premature infants, which have calcium concentrations of 1340–1450 mg/L and calcium-to-phosphorus ratios of 2.0 and 1.8 (Abbott Laboratories. Ross Products Division, 1999; American Academy of Pediatrics. Committee on Nutrition, 1998), are without any known adverse effect. Koletzko et al. (1988b) reported three cases of calcium-soap bezoars in premature infants receiving formula containing 40.6 mmol/L, or 1840 mg/L. In two of these cases, serious ileal obstruction led to intestinal perforation (Koletzko et al., 1988b).

Table 10-1 demonstrates that calcium concentrations as low as 1000 mg/L have been satisfactory at calcium-to-phosphorus ratios of 2.0. To our knowledge, no recent studies have used a higher ratio in formula at a calcium concentration this high. Pohlandt (1994) succeeded in obtaining intrauterine rates of bone mineral accretion by individualizing calcium and phospho-

TABLE 10-1

Trials of calcium supplementation successful in increasing bone mineralization in premature infants above that obtained with mother's milk

Investigators	Calcium intake			Intake ratio (mass) Ca:P
	mg/(kg · d)	mg/100 kcal	mg/L	
Steichen et al. J Pediatr 1980;96:528–534	237	188	1260	2.0
Rowe et al. J Pediatr 1987;110:581–585	219	194	1350	1.9
Chan et al. J Pediatr 1988;113:225–229	138	117	948	2.0
	165	140	1134	1.7
Greer & McCormick J Pediatr 1988;112:961–969	136	136	915	2.0
	146	164	1100	1.8
Schanler & Abrams J Pediatr 1995;126:441–447	123	98	755	1.8[1]
	184	136	1070	2.0[1]
Mize et al. Am J Clin Nutr 1995;62:385–391	220–230	198–208	1224	1.5–2.0
Wauben et al. Am J Clin Nutr 1998;67:465–472	108–130	81–109	659–734	1.0–1.2
Narbona et al. Early Hum Dev 1998;53:S173–S180	149	120	Not available	1.6

[1] Ratio of calcium-to-phosphorus in the human milk fortifier studied.

rus intakes at an average calcium-to-phosphorus mass ratio of 2.4. Chan et al. (1988) found a ratio of 1.7, but not 1.4, to be adequate at a phosphorus concentration of 664 mg/L. Schanler and Abrams (1995) found better mineral accretion with a calcium-to-phosphorus mass ratio in the feeding of ~2.0 than with a ratio of ~1.8, but the phosphorus intake with the former was 127 mg/(kg·d), compared with only 65 mg/(kg·d) for the latter. Another difficulty with the interpretation of the results is that the former ratio was in a formula containing CaGP and the latter in a formula containing calcium phosphate. Mize et al. (1995) concluded that the optimal ratio was 1.6–1.8, based on a need for uptake of phosphorus by soft (nonosseous) tissue in the presence of rapid growth induced by high calcium intake.

Among these investigators, only Wauben et al. (1998) reported satisfactory mineralization with a lower calcium-to-phosphorus mass ratio of 1.03. This was accomplished by feeding human milk supplemented to contain concentrations of calcium and phosphorus at 658 and 638 mg/L, respectively, until 40 weeks postmenstrual age. This calcium fortification was less than that used by most other investigators (see Table 10-1). Some infants received their mother's milk with a multinutrient supplement and some with only a CaGP supplement. The internal control group received formula containing 750 mg of calcium/L, so there was no control group receiving a high calcium and phosphorus intake. The investigators concluded that mineralization was satisfactory because there were no differences among the groups, and whole-body bone mineral content of the infants was at the low end of normal as measured in term infants within 5 days of birth, presumably reflecting the intrauterine accretion rate for bone density and body length at the time of discharge. However, Greer and McCormick (1988) had previously shown that BMC was improved by fortification of both mother's milk and preterm formula to calcium contents of 136 and 164 mg/100 kcal, respectively (915 and 1100 mg/L, respectively).

Conclusions and recommendations

Salle (1991) advised that the amount of calcium added to human milk not exceed 300 mg/L (so as to provide a total of 580 mg/L), "to avoid elevating the osmolality of the milk." But at least three studies have fed amounts of calcium in formula up to 1200 mg/L or more without adverse effect (Table 10-1). Preterm infant formulas presently in use in the United States contain calcium at 1340 or 1460 mg/L (American Academy of Pediatrics. Committee on Nutrition, 1998).

The Expert Panel was aware that it is possible to manage many or most of the larger preterm-LBW infants (BW of 1400–1500 g or more) without levels of calcium and phosphorus supplementation beyond those necessary to support optimal growth. Under careful monitoring of their bone mineral status, occasional difficulties could be detected and treated. Current neonatology involves care of many infants of BW 500–800 g, receiving long-term parenteral nutrition, diuretics, and other drugs, who often suffer from complications such as bronchopulmonary dysplasia. Many of these infants develop rickets even while receiving well-supplemented formulas. Because the supplementation does not seem to be harmful to the larger infants, the advantages of simplification of management and the desire not to require staging of formula based on BW convinced the Expert Panel to recommend fortifying the formula for all preterm-LBW infants at the indicated levels.

The advantage cited by Wauben et al. (1998) for more conservative calcium and phosphorus supplementation of formulas for premature infants was "to place less stress on their developing digestive and metabolic systems." Higher intakes of these minerals were associated with biochemical findings suggestive of phosphorus depletion (Lucas et al., 1996), perhaps as a manifestation of accelerated growth (Carey et al., 1987). However, in the absence of evidence of clinical toxicity at higher levels, the Expert Panel saw no advantage to such low levels of fortification. Most other investigators have found a calcium-to-phosphorus ratio of 1.3 to be suboptimal.

For these reasons, the Expert Panel recommended that the concentration of calcium in formulas intended for preterm-LBW infants range between 1000 and 1500 mg/L (123–185 mg/100 kcal for an energy content of 810 kcal/L). Future studies may provide a justification for lowering the minimum to 100 mg/100 kcal. A calcium concentration of 1000 mg/L has repeatedly been shown to be efficacious and without adverse effect.

Recommendations

Minimum. **The Expert Panel recommended that the minimum calcium concentration of preterm infant formula be 123 mg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum calcium concentration of preterm infant formula be 185 mg/100 kcal.**

Calcium-to-phosphorus ratio

The maximum for this ratio cannot be set higher than 2.0 because no studies have been found that investigated mineralization at a higher ratio with high calcium concentrations (>1000 mg/L). Evidence to establish a satisfactory minimum ratio is incomplete, but ratios of less than 1.5 have often seemed to be too low, even at calcium concentrations of more than 1000 mg/L. A ratio of 1.7 has been found to be satisfactory under several different circumstances of fortification.

Recommendations

Minimum. **The Expert Panel recommended that the minimum calcium-to-phosphorus ratio (by mass) of preterm infant formula be 1.7:1.**

Maximum. **The Expert Panel recommended that the maximum calcium-to-phosphorus ratio (by mass) of preterm infant formula be 2.0:1.**

Note. **Establishing parameters for the calcium-to-phosphorus ratio defines a range for phosphorus in formulas for preterm-LBW. However, other considerations might restrict the range for phosphorus further, so the Expert Committee developed an independent rationale for the recommendation for phosphorus, as follows.**

PHOSPHORUS

Background

The calcium-to-phosphorus mass ratio in bone and the nitrogen-to-phosphorus mass ratio for the total body are constant for all ages from fetus to adult at 2:1 and 17:1, respectively (Salle et al., 1993). Fetuses weighing more than 1000 g accumulate about 74 mg of phosphorus/(kg·d) (Ziegler, 1985), or about 7 g during the last trimester. According to Salle et al. (1993), 75% of this is retained in bone and 25% in soft tissues.

Review of the literature

The recommendations for calcium intake (123–185 mg/100 kcal) and the calcium-to-phosphorus ratio (1.7–2.0) established in the calcium section above restrict the recommendation for phosphorus in formula to the range of 62–109 mg/100 kcal (580–882 mg/L for an energy content of 810 kcal/L). Thus, unsupplemented term or preterm human milk containing only 140 mg/L is not nearly adequate for the phosphorus requirement of a preterm-LBW infant. It is important to recognize the rate-limiting nature of phosphorus intake in the assimilation of calcium and phosphorus and the maintenance of adequate mineralization. Formulas designed for premature infants that are presently in use in the United States contain phosphorus at 670 or 806 mg/L (83 or 100 mg/100 kcal, respectively) (Abbott Laboratories. Ross Products Division, 1999; American Academy of Pediatrics. Committee on Nutrition, 1998).

Human breast milk contains adequate phosphorus to support skeletal growth in full-term infants, up to about 29 mg/(kg·d) (Mayne & Kovar, 1991). However, in premature infants fed unsupplemented human milk, hypercalciuria appears, metabolic bone disease is common, and rickets is often detected between the second and third months of life (Campbell & Fleischman, 1988). The infants become depleted of phosphorus (Lyon & McIntosh, 1984; Hillman et al., 1985b) and urinary excretion of phosphorus is minimal. In these infants, hypophosphatemia can be severe and rickets is of the hypophosphatemic type (Greer, 1994; Oppenheimer & Snodgrass, 1980).

In one study, premature infants of GA 31–32 weeks received, during the second month of life, 186–194 mL/(kg·d)

of human milk that provided phosphorus at 22–23 mg/(kg·d). Among these infants, hypercalciuria was reduced when milk was supplemented with an additional 15 mg of phosphorus/(kg·d) and was eliminated when milk was supplemented with 26 mg of phosphorus/(kg·d) (Sann et al., 1985). The total intake of the infants receiving the larger supplement was thus 49 mg/(kg·d) (39 mg/100 kcal), or more than twice the intake of the unsupplemented control group. The doubling of the phosphorus intake evidently resulted in some deposition of calcium and phosphorus in bone, yet it did not provide enough for an intrauterine-like rate of phosphorus assimilation.

A little earlier, Senterre et al. (1983) had carried out 3-day balance studies of 30 appropriate for gestational age (AGA) male infants, at 21 days of life, who had BWs <1500 g. Equal numbers had been fed from birth with pooled human milk, the same feeding plus 30 μg (1200 IU) of vitamin D, or that same feeding with vitamin D plus phosphorus at 9 mg/100 mL (13 mg/100 kcal). Calcium intakes were not significantly different. Although calcium absorption was facilitated by vitamin D, calcium retention was significantly improved only in the phosphorus-supplemented group.

Even after supplementation, phosphorus intake in both of these studies was considerably smaller than the calculated requirement. One problem with increasing the supplementation of phosphorus is that fractional retention begins to decrease. For instance, when human milk is supplemented with 90 mg/L to make a total concentration of 230 mg/L (34 mg/100 kcal), phosphorus is more than 90% absorbed but urinary excretion increases significantly (Senterre et al., 1983). Nevertheless, total retention is higher with supplementation.

The bone mineral accretion rate of thriving premature infants could be increased to the in utero rate or more by individually adjusting their mineral supplementation until low concentrations (1–2 mmol/L) of both calcium and phosphorus appeared in the urine (Pohlandt, 1994). The dosage range at which this was achieved was large for both ions [96–340 mg/(kg·d) for calcium and 50–233 mg/(kg·d) for phosphorus], and this method is probably not practical for the routine feeding of preterm-LBW infants. However, it may be important that the median calcium-to-phosphorus mass ratio for intake was 2.4 for the infants in whom the maneuver was successful and 3.1 for the infants in whom it was not.

Mize et al. (1995) performed a relatively recent study of varying phosphorus intakes at a constant high calcium intake. Thirty-five AGA male infants of BW greater than 1511 g were randomly assigned to three groups: "standard phosphorus": 90 mg/100 kcal with a calcium-to-phosphorus ratio of 2.0, "moderate phosphorus": 106 mg/100 kcal with a calcium-to-phosphorus ratio of 1.7, and "high phosphorus": 120 mg/100 kcal with a calcium-to-phosphorus ratio of 1.5.

The calcium intake of all groups was 180 mg/100 kcal, near the maximum of 185 mg/100 kcal proposed in the previous section. The concentration of phosphorus in the standard group was near the maximum available in present formulas in the United States and was at the maximum that this Expert Panel recommended. The calcium-to-phosphorus ratio in the moderate group was at the minimum this Expert Panel recommended. The calcium-to-phosphorus ratio in the high group was below the recommended minimum.

Calcium retention was adequate to meet the intrauterine accretion rate in all groups. Phosphorus retention in all groups was 55–60% of intake, and absolute retention was correlated linearly with intake. Phosphorus retention in bone did not differ among groups, but phosphorus retention in soft tissue was markedly dependent on intake; it was calculated to be

adequate for accumulation of 1.0–1.1 mg/g of soft tissue only in the moderate and high phosphorus groups. The calculated amounts indicated a need for 20–25 mg of phosphorus/(kg·d) beyond the phosphorus required by the 2:1 mass ratio of calcium and phosphorus for bone mineralization. This would suggest that an optimal formula would contain a calcium-to-phosphorus ratio of about 1.6. For example, a recommendation of 123–185 mg/100 kcal for calcium translates to 148–222 mg/(kg·d). If absorption fractions of calcium and phosphorus are comparable, this implies 74–111 mg/(kg·d) for the phosphorus required for bone growth. The addition of 20–25 mg/(kg·d) of phosphorus for soft tissue growth gives a total requirement of 94–136 mg of phosphorus/(kg·d). Then, 148/94 = 1.57 and 222/136 = 1.63. Consideration of the greater limitation in calcium absorption than in phosphorus absorption led Mize et al. (1995) to recommend a calcium-to-phosphorus mass ratio of 1.6–1.8.

Fomon and Nelson (1993a) reported that, for term infants, absorption of phosphorus from unsupplemented human milk is 85% and that from milk-based formula is 79%. It seems to be higher for preterm infants. Salle et al. (1986) observed absorption of more than 90% of dietary phosphorus at an intake of 45–53 mg/(kg·d) from supplemented human milk by premature infants of 21–27, or 41–45 days of age, with 60–65% retention. The calcium-to-phosphorus mass ratio of the intake was 0.9–1.1. Retention was 67–75% from preterm infant formula providing phosphorus at 58–59 mg/(kg·d) at a calcium-to-phosphorus ratio of 1.6–1.9 (Salle, 1991). Narbona et al. (1998) found mean phosphorus absorptions of more than 95% and retentions of 74% and 87% by preterm infants fed formula at phosphorus intakes of 80 and 93 mg/(kg·d) and calcium-to-phosphorus ratios of 1.5 and 1.6, respectively. The variations in absorption were large for calcium (± 20% and 44%), but for phosphorus were only ± 12% and 7% (SD) in the two feeding groups. In both groups, phosphorus retention correlated with intake (r = 0.95).

Conclusions and recommendations

The accretion of phosphorus in utero in the last trimester occurred at a rate of 74 mg/(kg·d) (Ziegler, 1985). According to Mize et al. (1995), about 73% of ingested phosphorus was retained by premature infants given a moderate intake (106 mg/100 kcal), and observed retention was greater with a higher phosphorus intake. A value of 73% retention is consistent with earlier reports. Hence, an ingestion of 101 mg/(kg·d) or 84 mg/100 kcal [74 mg/(kg·d), with 73% retention] would be expected to achieve an intrauterine-like amount of retention. This is relatively consistent with the reports of Chan et al. (1986; 1988) that commercial formula providing phosphorus at 82 mg/100 kcal (664 mg/L, calcium-to-phosphorus ratio of 1.7) supported an intrauterine rate of bone mineralization, whereas a formula with 59 mg of phosphorus/100 kcal (478 mg/L, calcium-to-phosphorus ratio of 2.0) did not.

Hyperphosphatemia was commonly observed when premature infants were fed formulas prepared from evaporated or undiluted cow milk (Barltrop & Oppé, 1970; Gittleman & Pincus, 1951). On average, cow milk contains a phosphorus concentration of 920 mg/L and a calcium concentration of 1200 mg/L (American Academy of Pediatrics. Committee on Nutrition, 1998). This calcium-to-phosphorus ratio of 1.3 would not be acceptable under the Expert Panel's recommendations for preterm formula. The maximum permissible concentration of phosphorus, occurring at a calcium concentration of 1500 mg/L of formula and a calcium-to-phosphorus ratio of 1.7, would be 882 mg/L (109 mg/100 kcal). No toxicity

is known at these conditions. The recommended range of bioavailable (nonphytate) phosphorus concentrations is therefore defined as 82–109 mg/100 kcal.

Recommendations

Minimum. **The Expert Panel recommended that the minimum phosphorus content of preterm infant formula be 82 mg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum phosphorus content of preterm infant formula be 109 mg/100 kcal.**

Note. **Recommendations are for bioavailable (nonphytate) phosphorus.**

11. MINERALS: SODIUM, CHLORIDE, AND POTASSIUM

SODIUM

Background

Sodium is the major cation in the extracellular fluid (ECF) and is essential for regulating its volume. It is involved in the regulation of blood pressure and in the absorption of amino acids, small peptides, and monosaccharides (Finberg & Beauchamp, 1994). Sodium also seems to have roles in the growth and development of bone and nervous tissue, roles that are separate from its osmotic function in the ECF (Haycock, 1993).

Across a range of sodium intakes, from minimum to maximum tolerated, there is an approximately linear relationship with ECF volume. Increasing the salt intake therefore leads to a temporary weight gain, but this cannot be interpreted as a growth-promoting effect, because reducing salt intake to its original level leads to loss of the retained ECF. However, experiments in growing animals, summarized by Haycock (1993), demonstrated a diminished nitrogen retention in response to sodium deficiency. In rats, severe sodium restriction [1.5 mmol/(kg·d)] during a period of rapid growth (3–7 weeks) produced permanent stunting, whereas adult rats were unaffected by such a restriction. With the same food intake during a 15-day study period, sodium-restricted weanling rats were 37% lighter than controls, although their ECF space was less by only 4%. Thus, both intracellular and extracellular growth depend on sodium.

The sodium content of human milk varies diurnally but not as a result of a short-term sodium load (Ereman et al., 1987). It diminishes over early normal lactation from 18.5 mmol/L (426 mg/L) at 3–5 days to 13.9 mmol/L (320 mg/L or 46-48 mg/100 kcal) at 7–10 days and remains relatively constant thereafter. The concentration in preterm milk is usually reported to be higher than that in term milk by 3–5 mmol/L. For example, Atkinson et al. (1983) reported 17.4 mmol/L at 6–8 days postpartum, 12.5 mmol/L at 13–15 days, and 11.2 mmol/L at 26–28 days. In a larger series, Koo and Gupta (1982) had found 70.9 mmol/L in colostrum, 17.3 mmol/L at 7 days, and 13.1 mmol/L (44-45 mg/100 kcal) at 8–14 days. According to the American Academy of Pediatrics Committee on Nutrition (AAP-CON) (1998), mature (established lactation) human milk contains 180 mg/L (7.8 mmol/L or 26-27 mg/100 kcal) and cow milk 480 mg/L (21 mmol/L). Sodium supplementation of milk or term-type formula can lead to increased weight and length gain (Al-Dahhan et al., 1984; Chance et al., 1977). Current domestic formulas for term infants contain 7–10 mmol/L sodium (24–34 mg/100 kcal), whereas those designed for premature infants contain 13.9–15.0 mmol/L (39–43 mg/

100 kcal; see Table 3-1) (American Academy of Pediatrics. Committee on Nutrition, 1998).

The report *Assessment of Nutrient Requirements for Term Infant Formulas* recommended sodium at 25–50 mg/100 kcal (Raiten et al., 1998a). The consensus recommendation from Tsang et al. (1993) is that premature infants should receive 38–58 mg/100 kcal. The recommendation of the AAP-CON (1998) is for 48–67 mg/100 kcal. The Canadian Paediatric Society (CPS) (1995) recommended 2.5–4 mmol/(kg·d), which at an energy intake of 120 kcal/(kg·d) would be 48–76 mg/100 kcal.

Review of the literature

Studies varying the sodium intake of premature newborns have rarely been one-variable, well-controlled experiments. The data of most significance to the Expert Panel were from studies concerned with the sodium requirements after neonatal renal maturation had occurred, that is, after the first 10 days of life. Assessing requirements is complicated by the changing physiological status of these infants as well as the changing composition of their mother's milk. Premature infants have difficulties with sodium regulation in the first 2 weeks of life (see Appendix A). Infants with a birth weight (BW) higher than 1000 g [appropriate for gestational age (AGA) at 27 weeks] can be maintained satisfactorily with 3 mmol (69 mg)/(kg·d) in the first week and up to 5 mmol (115 mg)/(kg·d) in the second week (Herin & Zetterström, 1994). Smaller infants usually need regulation of their serum sodium level by intakes adjusted on the basis of urinary losses (Engelke et al., 1978), or more commonly today, on the basis of repeated serum measurements; this means that they cannot be maintained through the first week only by intake of a fixed formula.

Hyponatremia that occurs in the first week of life was ascribed by Aperia et al. (1979b) to a tubular unresponsiveness to aldosterone. Inefficient absorption of sodium in the gastrointestinal tract may also be a factor (Al-Dahhan et al., 1983b). Others have suggested that despite the evidence for a negative sodium balance, early hyponatremia is probably due to water retention and the syndrome of inappropriate secretion of antidiuretic hormone (Rees et al., 1984). In small AGA premature infants (BW of <1000 g) kept in high humidity to reduce insensible fluid loss, attempts to keep the serum sodium level in the normal range led to high weight losses, averaging 22%, although without evidence of long-term harm (Takahashi et al., 1994). This suggests that losing salt in the first week of life is unavoidable.

Late hyponatremia (plasma sodium value of <130 mmol/L) is an entity occurring in smaller (BW of <1000 g) infants between 2 and 6 weeks of age (Day et al., 1976) and may recur after initial treatment with sodium. Normally, body fluid volume regulation has priority over osmotic regulation, even in premature infants (Sulyok, 1988). Shaffer et al. (1987a) confirmed that ECF volumes were lower in infants with hyponatremia than in normonatremic infants of the same age and BW. This is due to a true sodium deficit, not a dilutional phenomenon (Shaffer et al., 1987a). Shaffer et al. (1987a) ascribed the hyponatremia to an overshoot of the normal neonatal diuresis, especially in infants administered large fluid intakes. The sodium balance in these patients was positive with intakes of either 1.6–1.7 or 2.8-3 mmol/(kg·d) (Roy et al., 1976). Positive sodium balance was observed in infants of this postnatal age [BW of <1300 g, gestational age (GA) of 30 weeks] with intakes as low as 1.6 mmol/(kg·d) (26 mg/100 kcal) as calculated on the basis of intake minus urinary excretion (Roy et al., 1976) and assuming that absorption was 90% (Al-Dahhan et al., 1983a) and retention was only 0.5 mmol/(kg·d) with this intake. However, there was 19-41% incidence

of hyponatremia (plasma sodium less than 130 mmol/L) in the infants fed 1.6-1.7 mmol/(kg·d) (26 mg sodium/100 kcal) yet no hyponatremia in the infants fed 2.8-3 mmol/(kg·d) (38-46 mg sodium/100 kcal) during the second to fourth week of the study (Roy et al., 1976). Studies of younger infants reported negative sodium balances with intakes of less than 2.3 mmol/(kg·d) (Aperia et al., 1985), but this was probably a manifestation of the renal immaturity and loss of fluid because of the contraction of extracellular space. Preterm-LBW infants remained in negative sodium balance when fed 35 mg sodium/(kg·d) (29 mg/100 kcal if fed 120 kcal/d) but achieved positive balance when fed at least 46 mg/(kg·d) (38 mg/100 kcal if fed 120 kcal/d) (Al-Dahhan et al., 1984). Sulyok et al. (1979b) related late hyponatremia to a tubular unresponsiveness to aldosterone rather than to any deficiency in the renin-angiotensin-aldosterone system. This suggests that late hyponatremia should be preventable by providing additional salt, although perhaps at the cost of vasopressin-mediated water retention and volume expansion (Sulyok et al., 1993).

As mentioned, the study of Roy et al. (1976) involved 2- to 6-week-old premature infants fed 1.6–1.7 or 2.8–3.0 mmol/(kg·d) at an energy intake of 141–169 kcal/(kg·d). The incidence of hyponatremia (plasma sodium concentration of <130 mmol/L) was 31% at the start of the balance period. The plasma sodium concentration was significantly greater in the group receiving greater amounts of sodium ($P < 0.001$). This study demonstrated that feeding a formula simulating the lower end of concentrations of sodium in pooled human milk (7.6 mmol/L or 26 mg/100 kcal) will not produce a fetal acquisition rate of sodium but that increasing the sodium intake to 2.8-3.0 mmol/(kg·d) (38-46 mg/100 kcal) can prevent late hyponatremia and improve sodium retention to 1.5–2.0 mmol/(kg·d), equal to the intrauterine retention rate of 1.8 mmol/(kg·d) for fetuses of this size (Ziegler et al., 1981).

Several groups have reported on sodium intakes that nearly supported the fetal retention rate of 1.6-2.1 mmol/(kg·d) from 27-34 weeks GA (Ziegler et al., 1981). For example, Shenai et al. (1980) found that a formula containing sodium at 16 mmol/L (45 mg/100 kcal) produces a retention of 1.4 mmol/(kg·d) in infants of GA 28–31 weeks and mean BW 1310 g during the third and fourth weeks of life; serum sodium concentrations ranged 135-141 mmol/L. Similarly, Huston et al. (1983), in a study comparing formulas of different fat composition in infants of about the same BW as those in the study of Shenai et al. (1980), found a sodium retention rate of 1.5 mmol/(kg·d) on an intake of 2.3–2.4 mmol/(kg·d) from formula with 45 mg/100 kcal. Fairey et al. (1997) found a retention rate of 1.1-1.2 mmol/(kg·d) in infants of GA 30 weeks fed, at 4 weeks of age, two formulas of different protein content, both containing sodium at about 43-46 mg/100 kcal. In these three studies, the infants were, on average, about 20% larger than those in the studies of Roy et al. (1976) and Atkinson et al. (1983). Shenai et al. (1980) found no incidence of late hyponatremia in the research subjects, whereas the other two studies did not report measures of serum sodium concentration.

Although infants fed banked milk achieve positive sodium balances by 2–3 weeks of age (Aperia et al., 1979a), it had been noted much earlier that term milk and formula designed to mimic it are not high enough in sodium or other macrominerals to permit accretion of these minerals at an intrauterine rate (Fomon et al., 1977). By using a factorial method, Fomon and coworkers (1977) calculated a requirement for sodium of 3.4 mmol/(kg·d) (2.8 mmol or 64 mg/100 kcal) for infants of GA 28–32 weeks. Mature term or preterm human milk at 12 mmol/L provides 276 mg/L (1.8 mmol or 41 mg/100 kcal. The sodium intake at an energy intake of 120 kcal/(kg·d) then would be 2.2 mmol/(kg·d) [51 mg/(kg·d)], or 79% of the requirement suggested by Fomon et

al. (1977). Later, Ziegler et al. (1981) estimated that total daily requirements of sodium were 3.22 and 4.08 mmol for infants of 800–1200 and 1200–1800 g, respectively. They set the corresponding "advisable intakes" for these two groups at 3.5 and 3.0 mmol/(kg·d). At a mean intake of 120 kcal/(kg·d), this computes to an average of 2.7 mmol/100 kcal, or 62 mg/100 kcal.

Atkinson et al. (1983) theorized that the milk from the preterm infant's mother might be a more appropriate source for sodium than banked human milk. They carried out sodium balance studies during the first, second, and fourth weeks of life in premature infants (BW of <1300 g) receiving their own mothers' milk and intravenous sodium or infant formula and supplemental sodium (Atkinson et al., 1983; Atkinson, 1985). They were able to show that sodium retention from preterm milk was 1.25 mmol/(kg·d) in the second week of life when infants were fed 43 mg/100 kcal [12.5 mmol sodium/L, 180 mL/(kg·d)], better than the retention from formula [26 mg/100 kcal, 174 mL/(kg·d)] by about 0.8 mmol/(kg·d), although not equal to the accretion rate of 1.6 mmol/(kg·d) for fetuses of this size reported by Ziegler et al. (1981). Supplementation of formula during the fourth week of life to provide a total sodium intake of 2.7-3 mmol/(kg·d) [52-57 mg/100 kcal, 185 mL/(kg·d)] resulted in retention of approximately 1.7 mmol/(kg·d), similar to the 1.7 mmol/(kg·d) retention of infants fed mothers' milk [176 mL/(kg·d), 11.2 mmol sodium/L, 38 mg/100 kcal] (Atkinson et al., 1983; Atkinson, 1985). Thus, the nature of the feeding does not seem to be as important to retention as the concentration of sodium in it, at least after the first 3 weeks of life.

Engelke et al. (1978) studied the sodium balance of 17 neonates of BW less than 1200 g. They noted differences between newborns of less than 30 weeks GA (mean BW of 956 g), who were seemingly AGA, and those of more than 30 weeks GA (mean BW of 1002 g), most of whom were small for gestational age. The more mature preterm infants could maintain a sodium balance with sodium intakes of 3.3 mmol/(kg·d) at 3 days of age to 1.4 mmol/(kg·d) at 8 days of age (or 63 and 27 mg/100 kcal, assuming 120 kcal/d), whereas less mature infants required much more, especially if critically ill. The only way to estimate the needs of the latter for water and salt was by measuring urinary losses (Engelke et al., 1978).

In contrast, one study of blind sodium supplementation (without monitoring urinary losses) of 4–5 mmol/(kg·d) for 22 premature infants of 27–34 weeks GA during days 4–14 of life found persisting positive sodium balances and a significant boost in weight gain, without adverse effects (Al-Dahhan et al., 1984). The added growth was not lost after supplementation ended, indicating that undesirable fluid retention was not the cause of the extra weight gain. The infants in this study averaged near 1400 g in BW, larger than those in the report (<1200 g) described above (Engelke et al., 1978). There is known to be a considerable difference in sodium excretion during first week of life in newborns of GA 26-29 weeks [mean 3.7 mmol/(kg·d)] and in those of GA 31–34 weeks [mean 2.6 mmol/(kg·d)] (Herin & Zetterström, 1994). The former would be at the 50th percentile AGA at approximately 900–1400 g and the latter at 1900–2700 g (Alexander et al., 1996).

Other investigators have also observed significantly increased weight gain as well as minor increases in longitudinal growth of premature infants of BW less than 1300 g given formula supplemented with sodium, when intake was increased to only 2.7 mmol/(kg·d) (Chance et al., 1977). It may be relevant that breast milk contains four times as much sodium during the first 10 days after delivery than it does later in lactation (Aperia et al., 1979b). The sodium level is also higher in milk from mothers of preterm infants than in milk from those of full-term infants (Gross et al., 1980; Lemons et al., 1982).

Others have tried the opposite approach, that of restricting sodium from the enteral intake of premature infants (mean BW of 850 g) during the first 3–5 days of life (Costarino et al., 1992). Control maintenance infants received 3-4 mmol/(kg·d) and restricted infants, an average of 0.85 mmol/(kg·d). Sodium excretion and urine volumes were similar in the two groups, the maintenance group remaining nearly in sodium balance, whereas the restricted group showing an average net loss of 4 mmol/(kg·d). Two of eight infants in the maintenance group developed hypernatremia, and two in the restricted group developed hyponatremia. Weight loss data were not provided.

In summary, apparently healthy newborns weighing more than 1000 g at birth (AGA for a GA of 27 weeks) can be nourished in the first week by the provision of sodium at 3 mmol/(kg·d), and at up to 5 mmol/(kg·d) without adverse effect during the second to fourth weeks (Herin & Zetterström, 1994). Smaller infants may need regulation of the serum sodium level by intakes adjusted on the basis of the urinary losses. Moreover, sodium requirements can vary substantially because of the disorders of circulation, respiration, and renal function (Herin & Zetterström, 1994). Therefore, medical management may include supplemental sodium and water in addition to a standard preterm infant formula.

There are few data on which to base a recommendation for a maximum limit for daily sodium intake. Hypernatremia and dehydration may also be a problem in very low birth weight (LBW) infants. Small size and excessive solute administration are among the predisposing factors for hypertonic dehydration (Finberg, 1973). Many instances of hypernatremia have been ascribed to elevated insensible fluid loss in premature infants (Butterfield et al., 1960; Rees et al., 1984). However, preventing significant insensible water loss by a high-humidity environment does not eliminate hypernatremia, implicating renal dysfunction as a factor (Takahashi et al., 1994). There are insufficient data to address the theoretical possibility of predisposition to later hypertension from excessive salt administration (Ziegler, 1991a). Establishing the relationship of hypernatremia to outcomes is often difficult. For example, its relationship to intraventricular hemorrhage in preterm infants may be in dispute (de Courten & Rabinowicz, 1981; Lupton et al., 1990; Mitchell & O'Tuama, 1980).

The Expert Panel found little recent literature on toxic effects of excess sodium intake from formula or intentional supplementation in premature infants. Al-Dahhan et al. (1984) found no adverse effects from feeding premature infants sodium at 4–5 mmol/(kg·d), but the trial was small, early in postnatal life (days 4–14 of life), and of short duration. Currently available formulas provide up to 43 mg/100 kcal, whereas the recommendation of the AAP-CON (1998) is for a maximum of 67 mg/100 kcal. It is not clear that this latter concentration, which implies a sodium concentration of 24 mmol/L in formula, has ever been used in practice. Transitional milk contains nearly this concentration (see above), but the amount fed is small and transitory. Other studies indicate, however, that 5 mmol/(kg·d), about 95 mg/100 kcal, or 33.3 mmol/L in formula can be fed without adverse effects (Herin & Zetterström, 1994). The Expert Panel therefore selected a maximum one-third lower, 63 mg/100 kcal, an amount known to have been fed to preterm-LBW infants, although only briefly (less than 5 days) (Engelke et al., 1978).

Conclusions and recommendations

In view of the number of different research centers, the variations in formula composition, and the use of a variety of research protocols, the studies in the judgment of the Expert Panel are separable (by the range of concentration of sodium in formula) into two groups: group A (26-29 mg/100 kcal):

hyponatremia (Roy et al., 1976), variable balance (Al-Dahhan et al., 1984; Atkinson et al., 1983; Atkinson, 1985; Engelke et al., 1978; Roy et al., 1976) and inadequate retention (Atkinson et al., 1983; Atkinson, 1985); and, group B (38-63 mg/100 kcal): low incidence of hyponatremia (Atkinson et al., 1983; Roy et al., 1976; Shenai et al., 1980), a positive sodium balance (Al-Dahhan et al., 1984; Atkinson et al., 1983; Atkinson, 1985; Engelke et al., 1978; Huston et al., 1983; Roy et al., 1976; Shenai et al., 1980) and achievement of a fetal sodium accretion rate of 1.6 mmol/(kg·d) for some infants fed 38-46 mg/100 kcal (Atkinson et al., 1983; Atkinson, 1985; Roy et al., 1976) or fed 52-57 mg/100 kcal (Atkinson et al., 1983; Atkinson, 1985) but not for other infants fed 43-46 mg/100 kcal [range of accretion: 1.1-1.5 mmol/(kg·d)] (Atkinson et al., 1983; Atkinson, 1985; Fairey et al., 1997; Huston et al., 1983; Shenai et al., 1980). There are no known adverse effects produced from feeding current domestic preterm formulas that have a sodium content of 39 and 43 mg/100 kcal (1.7 and 1.87 mmol/100 kcal). Some researchers (Kashyap et al., 1986) suggested that the requirement for sodium depends on the amount of protein fed and the resulting rate of growth. Because feeding 3.5 g protein/(kg·d) (3.3 g protein/100 kcal) supported a rate of weight gain by preterm-LBW infants that exceeded the intrauterine rate of weight gain, these researchers suggested that at this level of protein intake the rate of sodium accretion might increase relative to nitrogen accretion. The nutritional needs of the preterm-LBW infant must be assessed on a case-by-case basis and when there are increased physiological demands for sodium (e.g., stage of renal maturity, use of diuretics or glucocorticoids drugs, etc.) an individualization of the prescription for sodium by giving supplemental sodium may be appropriate.

Recommendations

Minimum. **The Expert Panel recommended that the minimum content of sodium in preterm infant formula be 39 mg/100 kcal.**

Maximum. **The Expert panel recommended that the maximum content of sodium in preterm infant formula be 63 mg/100 kcal.**

CHLORIDE

Background

Chloride as a nutrient and its function as a component of extracellular tissue are discussed in the report *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a). The need for a certain minimum chloride content in formula was made manifest by an incident in which formula based on a soy protein contained as little as 0.3 mmol/100 kcal, and some infants became symptomatic at intakes below 0.76 mmol/100 kcal. The deficiency of dietary chloride produced hypokalemic hypochloremic metabolic alkalosis, with failure to thrive, decreased growth in body length and head circumference, anorexia, weakness, and delayed neurological development (Grossman et al., 1980; Roy, III & Arant, 1981). In another series of such patients, hypercalcemia, hypercalciuria, and hyperphosphatemia were observed and also attributed to the hypochloremia, despite the decrease in renal calcium excretion ordinarily occasioned by metabolic alkalosis (Rodriguez-Soriano et al., 1983). Inadequate chloride intake by growing infants leads to plasma chloride concentrations below 97 mmol/L (Arant, 1993).

Roy and Arant (1981) found that samples of one formula contained chloride at less than 2 mmol/L, although the product information specified 6 mmol/L. The chloride content of human milk and other formulas available at that time was 10–18 mmol/L. The infants with symptoms were ingesting chloride at 0.2–0.3 mmol/(kg·d). According to these investigators, human milk has chloride in excess of sodium, with a (molar) ratio of 1.7 [or 1.5 (American Academy of Pediatrics. Committee on Nutrition, 1998)], whereas in the defective formula it was no more than 0.1 (Roy, III & Arant, 1981). Follow-up studies of children who suffered hypochloremic alkalosis as a result of ingesting low chloride formula indicated successful catch-up growth but possible cognitive retardation [See Raiten et al. (1998a)].

A review of the literature on this subject led a previous expert panel to recommend a minimum chloride content for term formula of 50 mg/100 kcal, or 1.28 mmol/100 kcal (Raiten et al., 1998a). The recommended maximum chloride content of term formula was 160 mg/100 kcal, based on the 90th percentile of the U. S. Food and Drug Administration analysis of infant formulas. The discussion in this report will focus on whether these recommendations need to be different for premature infants.

The CPS (1995) recommended chloride for premature infants at 1–3 mmol/(kg·d) [or 30–89 mg/100 kcal at an energy intake of 120 kcal/(kg·d) during the first week of life and 2.5–4 mmol/(kg·d) (74–118 mg/100 kcal) thereafter. The European Society of Paediatric Gastroenterology and Nutrition Committee on Nutrition (ESPGAN-CON) (1987) recommended 57–89 mg/100 kcal. The AAP-CON (1998) most recently made no recommendation for the enteral intake of chloride by premature infants but quoted a consensus recommendation of 59–89 mg/100 kcal (Arant, 1993). Earlier, the AAP-CON (1993) had recommended 2.4 mmol/100 kcal (85 mg/100 kcal) for infants weighing 800–1200 g and 2.0 mmol/100 kcal (71 mg/100 kcal) for infants weighing from 1200 to 1800 g. These previous recommendations were based on calculations by the factorial method of an "advisable intake" by Ziegler et al. (1981). Earlier, Ziegler et al. (1976) had found the daily retention of chloride by a fetus of 30 weeks GA to be 1.3 mmol/d.

Review of the literature

The urinary excretion of sodium and that of chloride are usually closely correlated except in isolated chloride deficiency. A low level of chloride in the urine (<10 mmol in adults) is a characteristic sign of chloride deficiency (Harrington & Cohen, 1975). In 26 infants reported to have alimentary chloride deficiency, the urinary chloride concentration was less than 2 mmol in 18 of them, and less than 7.1 mmol in all (Manz, 1985).

The pathogenic effects of isolated chloride deficiency are thought to begin with incipient contraction of the ECF and substitution in the urine of poorly reabsorbable anions, such as phosphate and, later, bicarbonate, for the more readily reabsorbable chloride. Because homeostatic processes tend to defend body fluid volume at the expense of tonicity, sodium is reabsorbed in the distal nephron, and negative potassium and hydrogen ion balances develop (Grossman et al., 1980). The result is hypokalemic hypochloremic alkalosis.

In a study by Atkinson et al. (1983), 14 premature infants (BW of <1300 g) were fed their own mothers' milk or formula (670 and 810 kcal/L, respectively) during 3-day balance periods in the first, third, and fourth weeks of life. Chloride concentrations in the milk averaged 18.9, 14.0, and 12.8 mmol/L during the three periods, respectively, and 11.0 and 12.7 mmol/L in the two formulas. Chloride concentrations in plasma did not fall below 99 mmol/L and were similar to those previously reported for normal children and thriving LBW infants, 100–110 mmol/L (Roy et al., 1976). Chloride intakes

during the three periods can be calculated to have provided 68–100 mg/100 kcal for the infants receiving milk (with the usual assumption of 67 kcal/100 mL) and 54–58 mg/100 kcal for the infants receiving formula. Assays of chloride balances were not performed, but Atkinson et al. (1983) concluded that milk from mothers delivering preterm generally contains adequate chloride to meet the requirements of growing premature infants.

Although the concentration of chloride in preterm human milk is 3–5 mmol higher than the concentration in term milk for the first 30 days of lactation, it drops to about 12 mmol/L thereafter, similar to that of term milk (American Academy of Pediatrics. Committee on Nutrition, 1998; Atkinson et al., 1995). Moreover, this is a mean value; there are at least two separate reports in the literature on chloride deficiency developing in term infants fed exclusively breast milk after 6 months of age (Asnes et al., 1982; Wack & Roscelli, 1994). In the four reported cases, the chloride concentrations in the mother's milk were, respectively, less than 2.0, 4.2, 6.0, and 8.4 mmol. In this regard, at least, a formula providing consistently sufficient chloride may be superior to unassayed mother's milk of variable chloride content.

Few published studies have investigated chloride intake as a variable with constant sodium intake. Mosca et al. (1989) fed two different formulas to 21 premature infants (BW of 1100–2000 g) from 2 to 6 weeks of life. These were standard formulas for the time, with protein at 1.5 g/100 mL (whey-to-casein ratio of 60:40), energy at 67 kcal/100 mL, phosphorus at 39 to 42 mg/100 mL, and sodium at 25 mg/100 mL. In formula A, the concentrations of potassium, calcium, and chloride were respectively 53, 51, and 15 mg/100 mL, and in formula B, they were correspondingly 103, 75, and 32 mg/100 mL. For chloride, this is 4.2 mmol for formula A and 9.0 mmol for formula B, both below the usual concentration in human milk. The calcium and phosphorus intakes were very low by current standards (76 and 58–63 mg/100 kcal, respectively, with formula A and were probably at least partially rate limiting for growth, possibly confounding the observed putative chloride effect. However, the phosphorus intake was high enough to eliminate hypercalciuria [see Sann et al. (1985)].

The mean chloride intake of infants fed formula A was 0.90 mmol/(kg·d), and that of infants fed formula B, 1.85 mmol/(kg·d). The results of the study included an observation that the urinary excretion of chloride was extremely low in all infants fed formula A, at 0.34–8.4 mmol, as well as in some infants fed formula B, although the latter group had a higher mean value for chloride excretion. Serum values for sodium, potassium, chloride, calcium, and phosphorus were not different between the two groups, with chloride always at 109–110 mmol/L of serum. Most interesting, the premature infants fed formula B showed a higher weight gain (31 versus 28.2 g/d), a higher rate of increase in middle upper arm circumference (0.31 versus 0.24 cm/wk), and a lower net acid excretion [1.24 versus 1.92 mmol/(kg·d)]. The difficulty with this result, as was noted by Mosca et al. (1989), is that these observations do not distinguish between the effects of the increased intakes of potassium and chloride. Nevertheless, this study indicated that an intake for chloride of 0.9 mmol/(kg·d), or 23 mg/100 kcal at an energy intake of about 140 kcal/(kg·d) (formula A), is inadequate, and that twice this amount (formula B) may still be insufficient.

Excessive intake of chloride (usually with sodium) has been associated with hypertension in adult humans and experimental animals (Raiten et al., 1998a), but there has been no report of chloride toxicity in premature newborns.

Conclusions and recommendations

No evidence is available to indicate that the chloride requirement of premature infants is any different from that of term infants, for whom the recommendation is 50–160 mg/100 kcal (Raiten et al., 1998a). The higher sodium requirement of premature infants, however, may make a higher minimum chloride intake necessary. As the sodium recommendation derived in the previous section is for a minimum formula sodium content of 1.7 mmol/100 kcal, the recommendation for a minimum chloride concentration must be 1.7 mmol/100 kcal to ensure that the molar chloride-to-sodium ratio is 1.0 or higher. This sets the minimum formula concentration for chloride at 60 mg/100 kcal. The maximum set for term infant formula, 160 mg/100 kcal, is left unchanged. In the United States, preterm infant formulas currently contain chloride at 81–85 mg/100 kcal (Table 3-1).

Recommendations

Minimum. **The Expert Panel recommended that the minimum chloride content of preterm infant formula be 60 mg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum chloride content of preterm infant formula be 160 mg/100 kcal.**

POTASSIUM

Background

Potassium is the major intracellular cation, found in cells at 140–150 mmol/L; extracellular concentrations are 3.5–5.0 mmol/L. Physiological functions of potassium include maintenance of transmembrane electrical potentials and intracellular ionic strength. These functions are crucial for the transmission of nerve impulses, regulation of the contractility of cardiac and skeletal muscle, and maintenance of normal blood pressure.

Potassium is absorbed in the proximal small intestine and transported through the mucosal epithelium by passive diffusion through tight junctions. It is absorbed in the colon by active transport mechanisms involving Na^+,K^+-ATPase activity. Potassium excretion occurs predominantly through the urine and, to a lesser degree, through the gastrointestinal tract and skin.

The concentration of potassium in term milk is about 470–550 mg/L after the first 2 weeks of lactation (Atkinson et al., 1995), and it is not significantly different in preterm milk (Gross et al., 1981; Lemons et al., 1982). The concentration of potassium in the fetus rises from 4.1 mg/100 g of fat-free weight at 26 weeks to 4.6 mg/100 g at term, while the fat content is increasing from 1.5% of the BW to 11.2%, and the BW itself is increasing from 880 to 3450 g (Ziegler et al., 1976). It can be computed from this that the fetus gains 4.2 g of potassium over the last trimester, an average gain of 43 mg/d. This would require an intake of about 100 mL/d of human milk, a volume that should be easily manageable by a thriving premature infant. The factorial calculations of the amount of potassium required for a fetus-like rate of growth in a preterm infant are thus consistent with observations that mother's milk contains enough potassium to prevent hypokalemia (Herin & Zetterström, 1994).

Recommendations from the AAP-CON (1998), ESPGAN-CON (1987), and consensus of Tsang et al. (1993) for the potassium content of formulas for premature infants are 65–100 mg/100 kcal. The CPS (1995) recommended 2.5–3.5 mmol/(kg·d), which would be 81–114 mg/100 kcal at an energy intake of 120 kcal/(kg·d). The reasons for these minor differences are not apparent. Currently available formulas for

preterm infants in the United States contain 21–27 mmol/L (American Academy of Pediatrics. Committee on Nutrition, 1998), or approximately 100–130 mg/100 kcal.

The recommendation of the AAP-CON (1985a) for term infants was 80–200 mg/100 kcal, whereas the report *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a) recommended 60–160 mg/100 kcal.

Review of the literature

Potassium homeostasis seems to be less of a problem for preterm infants than is sodium homeostasis. One concern has been the occurrence of electrocardiographic abnormalities, especially prolonged conduction times, resulting from hyperkalemia in the first 3 days of life. Between 30 and 40 weeks of GA, neonatal plasma potassium concentrations gradually decrease from 6.5 to 5.1 mmol/L (Sulyok et al., 1979a). Although hyperkalemia (potassium concentration of >7 mmol/L) was at first reported to occur in 1500-g premature infants with respiratory distress syndrome (Usher, 1959), it was later found to be common in much smaller infants (BW of <1000 g) (Gruskay et al., 1988) without correlation to respiratory illness or potassium intake (Leslie et al., 1990). However, this is evidently a problem of a perinatal shift of potassium between fluid compartments (Sato et al., 1995), which need not be considered in the formula design and will not be further discussed here.

The potassium balance in preterm infants is near zero in the first few days of life (Arant, 1993; Engle & Arant, 1983; Engle & Arant, 1984), in contrast to that of sodium and chloride (see above). The balance becomes positive soon after (Day et al., 1976), presumably as growth begins. In premature infants born at 30–32 weeks GA and in those born at 39–41 weeks GA, potassium excretion and positive balance were found, at 1 week of age, to be the same, with a constant intake of potassium per unit of body weight (Sulyok et al., 1979a). With an intake of 2.5–3.3 mmol/(kg·d), mean retention of potassium by those infants was 80–90% [about 2.3–2.7 mmol/(kg·d)]. However, Arant (1993) interpreted a series of studies by several investigators to indicate that a more typical retention by thriving infants receiving a range of intakes is 1.0–1.5 mmol/(kg·d), about the same as fetal accretion [see above and Ziegler et al. (1976)].

When neonates are deprived of potassium or chloride (see above) or are treated with diuretics, hypokalemia often develops (Engle & Arant, 1984). For this reason, potassium intake should begin as soon as the infant has become stabilized and the plasma potassium content is in the normal range (4.5–5.5 mmol/L) (Arant, 1993). Concentrations of potassium in formulas that are higher than that provided by human milk [2–3 mmol/(kg·d)] have not been reported to produce untoward effects unless renal function or mineralocorticoid production is impaired.

Mosca et al. (1989) conducted a study varying the potassium intake (along with the calcium and chloride intake) in 21 healthy premature infants of 28–36 weeks GA from the 10th to the 32nd day of life. Potassium intakes were 79 and 154 mg/100 kcal. Growth was better with the higher intakes, although as explained in a previous section, it is not clear whether the chloride or potassium or both were responsible. Nevertheless, it seemed that potassium at the higher level of intake was without adverse effect.

Conclusions and recommendations

In the absence of specific data that the potassium requirements of premature infants differ from those of term infants, the Expert Panel recommended that the parameters for potassium in preterm infant formula be the same as those previously recommended for term infants (Raiten et al., 1998a).

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of potassium in preterm infant formula be 60 mg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum concentration of potassium in preterm infant formula be 160 mg/100 kcal.**

12. MINERALS: TRACE ELEMENTS

IRON

Background

Iron is one of the most paradoxical elements in human nutrition. On the one hand, it is essential for normal organ growth and development, because it is involved in critical cellular events that regulate such basic processes as cell division and oxidative metabolism. On the other hand, iron is potentially extremely toxic as an oxidant stressor. Perhaps at no other time of life is the human more vulnerable to oxidant stress than in the perinatal period, when the antioxidant defense systems involving vitamin E, selenium, and superoxide dismutase (SOD) are most immature. The premature infant is at a higher risk for oxidant stress than the term infant because of lower iron-binding capacity in the serum (Chockalingam et al., 1987) and more immature antioxidant defenses.

Determination of the iron content in formulas prepared for preterm infants must take into account several physiological differences between preterm and term infants. Preterm-low birth weight (LBW) infants have (1) lower iron stores at birth (Jansson et al., 1979; Rios et al., 1975), (2) a higher incidence of exposure to iron-containing blood products, (3) a higher fraction of dietary iron absorption (Ehrenkranz et al., 1992), and (4) a greater iron need because of faster growth rates and a higher rate of blood volume expansion during the first year of life (Siimes & Järvenpää, 1982). It is not surprising that premature infants have a wide range of iron status values by the time of hospital discharge. Some investigators have reported that ferritin concentrations greater than 500 μg/L at discharge are associated with a higher rate of retinopathy of prematurity (Inder et al., 1997), whereas others have reported a higher risk of iron deficiency in preterm infants, particularly if they do not receive iron supplements (Lundström et al., 1977). The recent introduction of therapeutic recombinant human erythropoietin (rhEpo) to prevent anemia of prematurity (Carnielli et al., 1992; Shannon et al., 1995) increases the iron needs of the preterm infant (Bader et al., 1996; Carnielli et al., 1998; Meyer et al., 1996; Widness et al., 1997).

As remarked above and in the report *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a), normal iron status is critical for growth and development. Iron is found predominantly either in hemoproteins or in proteins containing iron-sulfur clusters. The predominant biological functions of iron revolve around its roles in oxygen transport (by hemoglobin and myoglobin) and cellular energetics (cytochromes) (Dallman, 1986). Iron is also integral in enzymes that control cell division (ribonucleotide reductase), myelination (Δ^9-desaturase complex), and dopamine metabolism (tyrosine hydroxylase). Predictable decrements in organ growth and development occur during iron deficiency states in animal models and include myopathy (Mackler et al., 1984), cardiomyopathy (Blayney et al., 1976), neurocognitive effects (Felt & Lozoff, 1996), gastrointestinal (GI) motility disturbances,

and ultimately anemia, with its attendant signs and symptoms. It is important to recognize that the sequelae of iron deficiency at the tissue level (myopathy and neurocognitive deficits) are not caused solely by anemia but are primarily due to the loss of function of iron-containing enzymes in the tissues. In the fetus and neonate, the losses of tissue iron and iron-dependent enzyme function precede the appearance of anemia because iron is prioritized to the red blood cell (RBC) mass at the expense of the liver, heart, and brain (Guiang et al., 1997). Thus, iron deficiency anemia is an end stage of the failure of iron supply to meet iron demand.

As compelling as the evidence is for a need to prevent iron deficiency in the preterm infant, there is also cause for concern about iron overload (Jansson, 1990). Premature infants have lower concentrations of total iron-binding capacity and transferrin than do term infants (Chockalingam et al., 1987; Lackmann et al., 1998). A direct relationship exists during the third trimester between the serum concentration of transferrin and gestational age (GA) (Chockalingam et al., 1987; Lackmann et al., 1998). Several investigators have expressed concern that large doses of enteral or parenteral iron may overwhelm the iron-binding capacity of preterm infant serum, resulting in oxidative stress of cell membranes (Jansson, 1990). These studies have shown the presence of oxidation products in the plasma of preterm infants (Lindeman et al., 1995). Others have tried to relate the iron status of the preterm infant to diseases with presumed oxidative pathophysiologies, including bronchopulmonary dysplasia (BPD) (Cooke et al., 1997) and retinopathy of prematurity (Hesse et al., 1997; Inder et al., 1997). Most of these studies appear to be hopelessly confounded by the multifactorial nature of the diseases in question; infants enrolled in the studies were exposed to multiple factors in addition to iron that could have contributed to the disease state. Nevertheless, the possibility that enteral iron can lead to oxidative stress in the preterm infant needs to be considered in determining the maximum safe concentration of iron in preterm formula.

It is important to know the iron status of the preterm infant at birth to determine subsequent iron requirements. The fetus maintains a fairly constant concentration of 75 mg of elemental iron/kg of body weight throughout the third trimester. The greatest part of this (55 mg/kg) is found in the RBC mass as hemoglobin. Smaller amounts are found in storage pools (12 mg/kg), predominantly in the liver, and in other, nonstorage tissues (8 mg/kg). The human fetus and neonate thus have little reserve with which to protect themselves in situations in which iron supply does not meet iron demand. On the basis of the 75 mg/kg value, the term (3300 g) newborn may be calculated to have about 250 mg of iron, whereas the 500-g premature infant of 24 weeks GA has but 37.5 mg. To achieve a weight of 10 kg by 1 year of age (50th percentile), the term infant needs only to triple the birth weight (BW), whereas the 500-g infant needs a 20-fold increase in weight. There is a commensurate increase in the blood volume, the hemoglobin mass, and the iron component of hemoglobin associated with that growth. Because nutrients are ingested primarily on a per kilogram of body weight basis, the smallest preterm infants will need to acquire far more iron, both absolutely and per kilogram of body weight, to remain iron sufficient during this catch-up growth. Even in the intensive care period, when infants are likely to be fed preterm formula, the growth rate [g/(kg·d)] is higher than in the term infant. Iron needs will follow suit.

Previous recommendations

Previous recommendations for iron intake in preterm infants have ranged from 2 to 6 mg/(kg·d), in contrast to recommendations for term infants of 0.27 mg/d [0.04 mg/(kg·d)] (Institute of Medicine, 2001d) to 1 mg/(kg·d) (American Academy of Pediatrics. Committee on Nutrition, 1985a; Carnielli et al., 1998; Ehrenkranz, 1994; Siimes, 1982). The wide range of recommendations in premature infants is because of differences in the degree of prematurity and whether the infant has been treated with rhEpo. The American Academy of Pediatrics Committee on Nutrition (AAP-CON), writing in the pre-rhEpo era, recommended that iron supplementation of 2 mg/(kg·d) should be started at no later than 2 months of chronological age (American Academy of Pediatrics. Committee on Nutrition, 1985a). By that time, most infants born at a GA of 28 weeks or later will be ready for discharge from the hospital or will have already been discharged and will no longer be fed preterm infant formula. Ehrenkranz (1994) reconfirmed these recommendations in his 1994 review, although he suggested that it would be prudent to begin iron supplementation once enteral feeds have been established in the neonatal period.

Once preterm-LBW infants double their BW or reach 2 months of age, the current recommendation for iron supplementation is for 2–4 mg/(kg·d), up to a maximum of 15 mg of total iron/d (American Academy of Pediatrics. Committee on Nutrition, 1998). A further recommendation is to supplement preterm infants with elemental iron for the entire first postnatal year (American Academy of Pediatrics. Committee on Nutrition, 1998). Currently, preterm infant formulas in the United States are provided in two forms, one with iron at 2–3 mg/L and the other with 12.2–14.5 mg/L (Abbott Laboratories. Ross Products Division, 2001; Mead Johnson Nutritionals, 2000). These deliver iron intakes of 0.3–0.45 and 2.2 mg/(kg·d), respectively, when fed at 150 mL/(kg·d).

Review of the literature

The ability to make recommendations about appropriate iron content for preterm formulas is confounded by many factors, including:

- Lack of a standard. Preterm human milk is very low in iron (Lemons et al., 1982; Mendelson et al., 1982) and cannot be used as the standard for establishing minimum iron levels. There is a high prevalence of iron deficiency in human milk-fed premature infants.
- Bioavailability of iron. The reported rates of iron absorption in preterm infants fed preterm formula based on cow milk are generally much higher than the rates of absorption in term infants fed such formula. The etiology of this is unclear, but it may result either from a relatively high iron need in the preterm infant or from a poorly regulated or immature intestinal iron uptake system. The latter two possibilities raise concern about iron toxicity.
- Relative paucity of data on iron status at the time of discharge from the hospital. The apparently wide variation in iron status in preterm infants, combined with the significant risks to health attributed to both iron deficiency and iron overload, makes it difficult to determine the optimal maximum and minimum contents for preterm formula.
- Risk of iron deficiency.
- Risk of excessive iron intake from both formula and blood transfusions in the neonatal period.
- Difficulty of predicting iron status in physiologically stressed premature infants from conventional indices such as serum ferritin and transferrin levels.

It is important to recognize that formulas given to preterm infants are most often changed to term infant formulas after discharge of the infants from the hospital, typically at 36–37

weeks of postconceptional age. At that time, infants will receive formulas containing iron between 4.5 and 12 mg, providing them with the amount of iron typically prescribed for the term infant [1 mg/(kg·d)].

Iron deficiency: incidence and impact on health

As detailed in the report *Assessment of Nutrient Requirements for Term Infant Formulas*, there is extensive literature on the prevalence and functional changes that occur with iron deficiency in infants born at term (Raiten et al., 1998a). In the United States in the modern era, 9% of toddlers between ages 1 and 2 years have evidence of iron deficiency, whereas 3% have iron deficiency anemia (Looker et al., 1997). Infants as young as 6 months of age have been documented to have iron deficiency (Roncagliolo et al., 1998). The pathophysiology in developed countries results from a dietary iron intake that does not meet the iron demands of growth. Infants fed unfortified infant formulas or human milk without iron supplementation appear to be at greatest risk. In developing countries, iron loss through blood in the stool caused by chronic intestinal infections tips the balance more rapidly in the direction of iron deficiency. The process of iron deficiency develops over time, as the term infant can rely on stored iron and iron from a relative reduction of the RBC mass in the first 4 months after birth. Ultimately, the depletion of iron stores leads to compromise of tissue iron content and a decrease in circulating hemoglobin concentration.

Comparable iron deficiency prevalence data in toddlers who were born preterm are scarce. The duration of availability of iron in storage pools in preterm infants with the shortest GA has been estimated to be approximately 2 months, or half that of term infants (Siimes, 1982; Siimes & Järvenpää, 1982). In one of the few studies that assessed iron status at 1 year of age in preterm infants of GA 30–36 weeks, Borigato and Martinez (1998) determined a 36 and 74% prevalence rate of iron deficiency anemia at 12 months, depending on whether iron or aluminum pots were used to prepare infants' feedings, respectively. It is unclear how representative this rate is, as it was determined in a group of low-socioeconomic status Brazilian infants fed formula supplemented with iron at 2 mg/(kg·d) under conditions of inconsistent compliance. Iwai et al. (1986) compared the iron status of preterm infants who received either human milk without supplements or an iron-fortified proprietary formula without other iron supplements. They found high rates of iron deficiency by 6 months of age in both groups, although the prevalence was much higher in the breast-fed group (86%) than in the formula-fed group (38%). The breast-fed infants' iron status began to deteriorate significantly after 4 months of chronological age. The authors recommended additional iron supplementation from 2 months of age. The overall finding that the preterm infants' postdischarge iron status tends toward deficiency is supported by higher intestinal iron absorption rates seen in longitudinally followed preterm infants than in term infants (Dauncey et al., 1978; Ehrenkranz et al., 1992).

The potential sequelae of iron deficiency in infants and toddlers remain an area of research interest. Besides anemia, the loss of tissue iron in skeletal muscle, heart, and brain contributes to the symptoms of the iron deficiency syndrome. The long-term cognitive and shorter term motor sequelae of iron deficiency are particularly notable and have been summarized by Nokes et al. (1998) and in the report *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a). On the basis of studies by Lozoff et al. (1987; 1991; 1998), Oski et al. (1983), and Walter et al. (1989), the report of the International Nutritional Anemia Consultative Group

concluded that iron deficiency probably has a specific effect on brain anatomy and function regardless of other potential confounding variables such as socioeconomic status and maternal behavior (Nokes et al., 1998). The reversibility of the neuro-cognitive sequelae is controversial, with Lozoff et al. (1991) documenting some long-term irreversibility and Idjradinata and Pollitt (1993) documenting reversibility. Differences in conclusions regarding reversibility may have to do with timing of the insult and vulnerability of the brain to other nutritional and non-nutritional insults. From a public health perspective, as long as there is a question of long-term neurocognitive deficits it would seem to be prudent to ensure the maintenance of an iron-sufficient state during early childhood (Nokes et al., 1998).

Although at least 40 studies have assessed the effect of iron deficiency on various aspects of neurocognitive outcome (Nokes et al., 1998), none addresses the role of iron deficiency in the generally poorer cognitive outcome of premature infants compared with that of term infants.

Iron stores, absorption, and bioavailability in preterm infants

To determine the appropriate iron dosage for the preterm infant and thus the range of iron content appropriate for preterm infant formula, it is important to know the amount of storage iron in the preterm infant, the rate of utilization of those iron stores if no supplements are given, and the bioavailability of iron supplements given to preterm infants. Fortunately, nascent iron stores, neonatal iron absorption rates, bioavailability of ingested iron, and the preterm neonate's response to iron treatment have been studied extensively (Dauncey et al., 1978; Ehrenkranz et al., 1992; Friel et al., 1998; Hall et al., 1993; Lundström et al., 1977; Moody et al., 1999; Salvioli et al., 1986; Shaw, 1982; Widness et al., 1997; Zlotkin et al., 1995b).

Preterm infants have lower iron stores than term infants, as determined directly by whole-body composition (Ziegler et al., 1976) and indirectly by cord serum ferritin concentrations (Lackmann et al., 1998; Rios et al., 1975). Serum ferritin concentrations decline more rapidly in preterm infants than in term infants between birth and 3 months of age (Messer et al., 1980). Preterm infants in Finland with BWs between 1000 and 2000 g receiving human milk or low iron formulas without iron supplementation became iron deficient (serum ferritin level of <7 μg/L) by 3 months of age (Lundström et al., 1977). Moreover, these infants became anemic before they had completely exhausted their iron stores, which suggests that their rate of hepatic iron mobilization was slow compared with their rate of blood volume expansion (Lundström et al., 1977). Studies utilizing the stable isotope ^{58}Fe also suggested a hepatic sequestration of iron, with late release to the bone marrow (Widness et al., 1997). Supplementation of the infants in the Lundstrom et al. (1977) study with 2 mg/(kg·d) of iron as ferrous sulfate in drops between feedings or in formula starting at 2 weeks of age prevented iron deficiency (mean ferritin concentration of >30 g/L) and iron deficiency anemia (mean hemoglobin concentration of >12 g/100 calories) at 6 months of age. Assuming a formula intake of 150 mL/(kg·d) and an energy intake of 120 kcal/kg, a dose of 2 mg/(kg·d) from formula would require a formula iron content of 13.3 mg/L (1.67 mg/100 kcal of an 810 kcal/L formula).

Several nonisotopic iron balance studies performed by Shaw (1982), Dauncey et al. (1978), and Salvioli et al. (1986) confirmed the need for supplemental iron to avoid negative iron balance and continued iron accretion at intrauterine rates. Dauncey et al. (1978) studied six breast-fed, unsupplemented infants of GA 29 weeks during their first 30 postnatal

days and found an iron balance of −0.1 mg/(kg·d). Iron supplementation resulting in an intake of 5–6 mg/(kg·d) restored iron accretion rates to those expected in utero. The intestinal iron absorption rate was calculated to be 34%, a value very similar to that later determined by Ehrenkranz et al. (1992) using [58]Fe. Again assuming an intake of 150 mL/(kg·d), a dose of 5 mg/(kg·d) would indicate a formula iron content of 33 mg/L. However, it should be noted that the Dauncey et al. (1978) study involved only six preterm infants and that these infants were allowed to have a negative iron balance before initiation of therapy at 30 days postpartum. Thus, the dosages that the authors used might be more appropriate for treatment rather than for prevention of iron deficiency.

Shaw estimated a negative iron balance of 0.24 mg/(kg·d) for 1000-g infants fed human milk without iron supplementation (Shaw, 1982). Assuming that preterm infants absorb approximately 40% of enteral iron, he estimated a daily requirement of 0.6 mg/(kg·d) to start shortly after birth to prevent iron deficiency. This dose would require a formula content of 4 mg/L. Salvioli et al. (1986) studied the effect of iron supplementation during the first year of life in 30 preterm infants with BWs between 820 and 2000 g. These infants were fed an infant formula designed to supply 1 mg/(kg·d) of iron. Fourteen of these infants received an additional 2 mg/(kg·d) of iron as ferrous sulfate for the first 5 months of life. On follow-up at 6–12 months of age, the iron status of the supplemented infants was better than that of the unsupplemented infants: hemoglobin concentrations 12.1 versus 11.3 g/100 mL and ferritin concentrations 21.4 versus 13.1 g/L. In addition, none of the supplemented infants was anemic, whereas 37% of the unsupplemented infants were anemic. This study was primarily concerned with iron supplementation in the post-hospital discharge period and is therefore not completely relevant to the issue of iron content in preterm infant formulas. Nevertheless, it is important to note that a dose of 1 mg/(kg·d), which would require a formula content of 6–7 mg/L at an intake of 150–180 mL/(kg·d), was not sufficient to maintain iron status.

Both Shaw (1982) and Dauncey et al. (1978) recognized that RBC transfusions significantly affect iron needs and iron absorption. Dauncey et al. (1978) noted that iron absorption decreased after blood transfusion and that iron balance became positive in two infants only after the hemoglobin concentration fell below 12 g/dL. RBC transfusions are far less commonly administered in the modern era of neonatology (Strauss, 1991) and are used primarily for replacing blood that has been lost as a result of phlebotomy rather than for keeping the hemoglobin level high for oxygen-carrying purposes. Intestinal iron uptake depends on the iron status of the individual (Andrews, 1999). Thus, transfusion of neonates in the current age may have little effect on iron absorption, because the infant will always be relatively iron deficient; that is, the blood iron being administered replaces blood iron that has already been withdrawn. Growing preterm infants rarely have hemoglobin concentrations greater than 12 g/dL and are more likely to have values between 7 and 10 g/dL (Shannon et al., 1995; Strauss, 1991).

Hall et al. (1993) performed a more definitive study that compared preterm infant formulas with two different iron contents. In a randomized, double-blind trial, premature infants of less than 1800 g BW received preterm infant formula with either 3 or 15 mg of elemental iron/L. A third group received fortified human milk supplemented with iron to a content of 1.7 mg/L. At hospital discharge, the infants consuming the formula with the lower iron content had higher rates of iron deficiency anemia, defined as a hemoglobin level of <9 g/100 mL (70% versus 26%), and lower ferritin concentrations (79% versus 26%) compared with the infants receiving the formula with the higher iron content. At the time of discharge, the infant formula was changed to one containing 12 mg/L of iron and those infants who were breast-fed were given a supplement of 10 mg/d of iron. Two months after discharge, the prevalence of iron deficiency remained higher and the mean corpuscular volume lower in the low iron group. At that time, however, there were no statistically significant differences in mean hemoglobin concentration (high dose: 10.8 g/100 mL; low dose: 10.4 g/100 mL) or ferritin concentration (high dose: 28 μg/L; low dose: 14 μg/L).

These data suggest that a formula iron content of 3 mg/L is not sufficient to maintain iron status in preterm infants of BW less than 1800 g. A formula containing 3 mg of elemental iron/L would provide 0.45 mg of iron/(kg·d) if fed at 150 mL/(kg·d), whereas a formula containing 15 mg/L would provide 2.25 mg/(kg·d). This study was in the pre-rhEpo era, which makes it unlikely that these infants had higher iron needs because of rhEpo treatment. The research subjects were relatively healthy infants, because the exclusion criteria eliminated very ill infants. As a result, these infants probably did not receive multiple transfusions, thereby increasing their demand for dietary iron intake. Because prenatal use of steroids and postnatal surfactant therapy have decreased the relative number of infants with severe illness in neonatal intensive care units, the infants in this study probably represent the typical premature infant who would be fed a preterm formula starting at an early postnatal age. At least one-quarter of infants fed the formula with an iron content of 15 mg/L had anemia at the time of discharge from the hospital. Therefore, formula with an iron content of 15 mg/L may not be adequate for all preterm-LBW infants.

Another approach to measuring dietary iron bioavailability in the preterm infant has been to use stable isotopes of iron. The end points of studies utilizing stable isotopes have been either intestinal iron absorption or RBC iron incorporation. Ehrenkranz et al. (1992) used this method to establish that preterm infants absorb 42% of dietary iron. Widness et al. (1997), who utilized [58]Fe methodology, reported iron absorption rates in preterm infants receiving rhEpo or placebo to be 30% and 34%, respectively. These values are remarkably similar to values established in the classic balance studies by Dauncey et al. (1978). Actual RBC incorporation of iron from preterm infant formula ranges from 4.7% to 12.0% (Ehrenkranz et al., 1992; Friel et al., 1998; Moody et al., 1999; Stack et al., 1990; Widness et al., 1997). Ehrenkranz et al. (1992) calculated that only 29% of absorbed iron is incorporated relatively rapidly into the RBCs, implying that the major portion goes into storage pools. These results sharply contrast with those in adults, in whom 90% of absorbed iron is in RBCs (Larsen & Milman, 1975).

Treatment with rhEpo substantially increases iron demand in preterm infants (Carnielli et al., 1998; Maier et al., 1998; Meyer et al., 1996; Shannon et al., 1995). Therefore, the rhEpo-treated premature infant is especially vulnerable to iron deficiency (Carnielli et al., 1998; Ehrenkranz, 1994; Maier et al., 1998; Meyer et al., 1996; Shannon et al., 1995). In the national collaborative trial of rhEpo, 6 mg of elemental iron/kg was sufficient to maintain normal albeit somewhat low ferritin concentrations throughout the study (Shannon et al., 1995). In contrast, Bader et al. (1996) demonstrated that ferritin concentrations and iron levels decreased markedly in preterm infants treated with 900 units/(kg·wk) of rhEpo for 4 weeks in spite of 6 mg/(kg·d) of iron supplementation. These investi-

gators and Meyer et al. (1996) also reported that although elemental iron (as ferrous lactate) at 12 mg/(kg·d) appeared to support a robust erythropoietic response to rhEpo in 1500-g infants, their RBCs became hypochromic and their ferritin concentrations decreased. The robustness of the erythropoietic response to rhEpo in preterm infants is clearly a function of the amount of iron available (Carnielli et al., 1998). However, providing 6 or 12 mg/(kg·d) of iron in a preterm formula would require an iron content of 40 or 80 mg/L. Given that rhEpo is not widely used clinically, because of its high cost and inconvenient mode of delivery, the Expert Panel did not recommend fortifying preterm formulas to meet the iron needs of the infant receiving rhEpo. Rather, infants receiving rhEpo should be supplemented with medicinal iron to meet their increased needs.

Nutrient interactions

For all divalent cations, there is concern about nutrient interactions when iron fortification is used. Friel et al. (1998) showed that zinc supplementation of formula at a ratio of 4:1 with iron did not interfere with RBC incorporation (and presumably, with intestinal absorption) of ^{58}Fe. Iron supplementation at a dose of 2 mg/(kg·d) through the first year of life does not affect serum zinc levels (Salvioli et al., 1986). It is not yet clear, however, whether higher iron doses in preterm-LBW infants receiving rhEpo affect zinc or copper balances.

Risk of iron overload and undesirable sequelae of iron dosing in preterm infants

Iron is a potentially toxic element with a narrow therapeutic range. Just as iron deficiency is a concern for preterm infants, iron overload must be considered a potential problem, particularly given the high dietary requirements of term infants. Because iron is a strong oxidant and premature infants have low iron-binding capacities and poorly developed antioxidant defenses, researchers have been concerned about the potential toxicity of large doses of iron given to preterm infants (Berger et al., 1997; Cooke et al., 1997; Hesse et al., 1997; Inder et al., 1997). In particular, they worry that large amounts of iron by either the enteral or the parenteral route may temporarily overwhelm the relatively low iron-binding capacity of the preterm infant, thereby exposing cell membranes to a potent oxidant stress. Jansson (1990) has argued that the lung, the eye, and the RBC are particularly vulnerable to disease caused by oxygen-derived free radicals. Indeed, preterm infants are clearly at greater risk than are term infants of diseases in which oxygen and oxygen radicals might have a role, including retinopathy of prematurity and BPD. However, clinical studies of the role of iron, especially enterally derived iron, in precipitating these diseases have produced unconvincing results.

In most studies, attempts to find a direct association between iron exposure and oxidative diseases in preterm neonates have assessed the relationship of RBC transfusions and either BPD (Cooke et al., 1997) or retinopathy of prematurity (Hesse et al., 1997; Inder et al., 1997). Dietary iron was not assessed in any of the studies. Cooke et al. (1997) hypothesized that frequent blood transfusions may produce changes in iron status, leading to oxygen-derived free radical generation and oxidative injury in the form of BPD (chronic lung disease). They studied classic iron indices (serum iron and ferritin levels, transferrin saturation), free iron status (bleomycin-detectable iron), and free radical-reactive substances [thiobarbituric acid-reactive substance (TBARS)] in the first 28 days of life in 73 preterm infants. The 30 infants who developed chronic lung disease received twice the number of transfusions

in the first month of life than did those who did not develop the disease, but they were also gestationally younger and probably sicker (thus requiring more transfusions). Not surprisingly, these infants had serum ferritin concentrations directly related to the number of transfusions. In addition, infants with higher ferritin concentrations (and more transfusions) were more likely to have free iron in the plasma, but, interestingly, this finding was not associated with a higher level of TBARS. The authors concluded that in the absence of documentable free radical formation, blood transfusion probably does not contribute to the risk of chronic lung disease in preterm infants.

Similarly, two other studies evaluated the role of iron load on the incidence of retinopathy of prematurity (Hesse et al., 1997; Inder et al., 1997). Hesse et al. assessed the effect of the volume of RBC transfusions administered to 114 preterm infants, BWs of 500–1500 g, on their iron metabolism and retinopathy status. Iron metabolism was evaluated by serum iron, transferrin, and ferritin concentrations in the first 6 weeks of life. These investigators found, after adjusting for the degree of prematurity and oxygen and ventilator exposure, a significant relationship between the volume of transfusion and the development of retinopathy. However, they found no association between the infant's iron status and retinopathy. They concluded that LBW infants exposed to more than 15 mL/kg of transfused blood have a six-fold increased risk of developing retinopathy of prematurity, but that this relationship was not mediated by an increased iron load. Likewise, Inder et al. (1997) showed a relationship among the number of transfusions, the serum ferritin concentration, and the risk of retinopathy of prematurity. The infants in that study who showed an association between retinopathy and number of transfusions had extremely high ferritin concentrations (500 μg/L), well in excess of values reported in studies utilizing enteral iron doses as high as 6 mg/(kg·d) (Carnielli et al., 1998; Shannon et al., 1995).

The following calculations can be made to try to put into perspective the potential iron load of a RBC transfusion with respect to oral iron intake. Packed RBCs are usually administered, with a hematocrit of 70% and an assumed hemoglobin concentration of 25 g/100 mL. Utilizing the conversion factor of 3.4 mg of elemental iron/g of hemoglobin represents an iron dose of 85 mg/100 mL of packed cells. RBCs are usually transfused as a bolus of 10–15 mL/kg. On the basis of the bleomycin data in the study by Cooke et al. (1997), the concern is that the dose of iron acutely overloads the relatively limited total iron-binding capacity of the preterm infant. The Ehrenkranz et al. (1992) study suggested that preterm infants are fully capable of loading iron into ferritin when the iron is administered slowly, as is the case when it is ingested.

If the entire transfusion were completely hemolyzed in the infant's serum (a very unlikely event), it would represent an acute load to the infant of 8.5–12.8 mg of elemental iron/kg. A preterm infant formula would have to be fortified with 140–200 mg of iron/L to present a similar load via the enteral route, assuming an intestinal absorption rate of 40%. In comparison, the iron load presented to a preterm infant in a formula containing 13.3 mg/L [2 mg of iron/(kg·d) when fed formula at 150 mL/(kg·d)] is 0.8 mg/kg during a 24-hour period, assuming the same 40% absorption rate. That iron delivery is consistent with slowly hemolyzing approximately 10% of a packed RBC transfusion dose. No reliable figures are available for the average percent hemolysis of an RBC transfusion in preterm infants. Thus, inferences about enteral overload from these studies on parenterally administered iron by RBC transfusion are difficult and of dubious value.

Nevertheless, it should be noted that there likely exists a threshold dose of enteral iron above which the iron-binding capacity of the preterm infant's serum is exceeded and oxidative stress could become a problem. For example, large doses of enteral iron [8–10 mg/(kg·d)] administered without supplemental vitamin E result in lower hemoglobin concentrations in preterm infants of BW less than 1500 g than do the same doses administered with vitamin E (Gross & Melhorn, 1972). These investigators concluded that the lower hemoglobin value in the iron-only group was due to RBC hemolysis from oxidative stress, although oxidative markers were not measured. It is also important to note that detection of free iron in the serum, as indicated by a positive bleomycin test, is not necessarily associated with detection of free radicals as indicated by a positive TBARS test (Cooke et al., 1997). This implies that other serum factors mitigate the potential oxidant effect of free iron.

Berger et al. (1997) assessed this issue in preterm infant plasma containing bleomycin-reactive (free) iron that was incubated with ascorbic acid. They did not detect lipid hydroperoxide formation as long as ascorbic acid concentrations remained high. When iron was added to plasma devoid of ascorbic acid, lipid hydroperoxides were formed, whereas endogenous and exogenous ascorbic acid delayed the onset of iron-induced lipid peroxidation in a dose-dependent manner. The authors interpreted these findings as demonstrating that in iron-overloaded plasma, ascorbic acid acts as an antioxidant toward lipids. They also stated that the combination of high plasma concentrations of ascorbic acid and free iron, or free iron alone, does not cause oxidative damage to lipids and proteins in vivo. Because the ascorbic acid scavenging system is not mature until 2 weeks after premature birth, the authors suggested delaying significant iron loading of preterm infants until after 2 weeks of age.

A literature search for enteral iron intake, premature infants, and oxidant stress revealed that no studies have assessed the issue of enteral iron doses and the appearance of reactive oxygen species in the serum or urine.

Conclusions and recommendations

Minimum. Because of the high rate of iron deficiency in premature infants demonstrated in multiple studies and the lack of evidence that enteral iron causes iron overload syndromes in preterm infants, the Expert Panel recommended that preterm infant formula be supplemented with iron. The current recommendations of 1.7–2.5 mg/100 kcal by the AAP-CON (1985a) [equivalent to 2–3 mg/(kg·d) at an energy intake of 120 kcal/(kg·d)] and 2 mg/(kg·d) by Ehrenkranz (1994) are supported by trials demonstrating an unacceptable negative iron balance when preterm infants are fed formulas designed to deliver only 0.5 mg/(kg·d) (0.4 mg/100 kcal) (Hall et al., 1993) or 1 mg/(kg·d) (0.8 mg/100 kcal) (Salvioli et al., 1986) when fed at 150 mL/(kg·d).

Supplementation of LBW infants by Lundstrom et al. (1977) with iron at 2 mg/(kg·d) starting at 2 weeks of age prevented iron deficiency and iron deficiency anemia at 6 months of age. For a formula intake of 150 mL/(kg·d) and an energy intake of 120 kcal/(kg·d), a dose of 2 mg/(kg·d) would require a formula iron content of 13.3 mg/L (1.67 mg/100 kcal of an 810 kcal/L formula). Hall et al. (1993) indicated that a formula with 15 mg of iron/L, which would provide 1.85 mg/100 kcal at 810 kcal/L, resulted in a higher serum ferritin concentration and less incidence of anemia (hemoglobin value of <9 g/dL) among preterm-LBW infants in the United States than formula supplying 0.4 mg/100 kcal. Hall et al. (1993) suggested that even 15 mg/L [2.3 mg/(kg·d) when fed at 150

mL/(kg·d)] resulted in 33% of the infants' having microcytosis, 26% having anemia, and 67% having low iron stores at discharge from the hospital. Salvioli et al. (1986) found that during the first 6 months of life, there was less iron deficiency and anemia for those premature infants fed 3 mg of iron/(kg·d) (2.5 mg/100 kcal) than those fed 1 mg/(kg·d) (0.8 mg/100 kcal). The Expert Panel recommended a minimum iron content of 1.7 mg/100 kcal. This would provide 2 mg/(kg·d) when fed at 120 kcal/(kg·d).

Maximum. Determining the upper limit of preterm infant formula iron content is more problematic, as there is little consensus about this issue. Oral iron supplements providing 2–3 mg/kg were reportedly well tolerated by 40 preterm infants in South Africa, who were not receiving rhEpo (Meyer et al., 1994). No adverse effects were reported related to a total iron intake of 3–6 mg/kg by 80 preterm-LBW infants, who were not receiving rhEpo, at 11 medical centers in the United States (Shannon et al., 1995). If only formula were fed, this would be equivalent to a formula containing 2.5–5 mg/100 kcal. Melhorn and Gross (1971; 1972) suggested the occurrence of significant stress on RBC integrity at supplemental doses of 6.7–10 mg/(kg·d) (equivalent to 5.6–8 mg/100 kcal) in preterm-LBW infants. The administration of a large dose of iron with vitamin E apparently impaired the bioavailability of both nutrients and was influenced by gestational immaturity (1972). Although the studies of infants receiving rhEpo demonstrated an iron requirement for 6 mg/kg or more to support serum ferritin and normochromatic cells (Meyer et al., 1994), rhEpo is not standard treatment. Given the lack of toxicity studies, it seems unwise to fortify preterm infant formula to the level needed to support iron status and erythropoiesis in infants receiving rhEpo. The Expert Panel recommended that the maximum iron content in preterm formula be 3.0 mg/100 kcal, equivalent to an amount of iron fed to preterm-LBW infants without reported adverse effects.

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of iron in preterm infant formula be 1.7 mg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum concentration of iron in preterm infant formula be 3.0 mg/100 kcal.**

ZINC

Background

The ubiquity of zinc in biology, the crucial roles of this micronutrient in cellular growth and differentiation, and the wealth of data from animal models attesting to the extraordinary importance of zinc in pre- and postnatal growth and development (Hambidge, 2000) all serve to emphasize the exceptional importance of this mineral to the premature infant. The practical relevance of zinc to premature infants has been highlighted by recognition of their increased susceptibility to severe zinc deficiency states (Atkinson et al., 1989; Zimmerman et al., 1982), but there is cause for even greater concern about the potential clinical implications of milder, subclinical zinc deficiency states. Without adequate attention to dietary zinc intake, these subclinical deficiencies could occur frequently in this susceptible population.

There are several possible reasons why zinc requirements for the premature infant may differ from those for the term infant of comparable postnatal age, and why there is likely to be an array of requirements for premature infants depending both on GA at delivery and on postnatal age. These include the

immaturity of the GI tract; the difference in postnatal growth rate compared with that of the infant delivered at term, which is sufficient to affect zinc requirements for several months postpartum; the more limited hepatic fetal stores of zinc than for other minerals (Casey & Robinson, 1978); and the potential for other constituents of premature infant formulas to impair zinc bioavailability.

Iron and zinc interaction

Human studies of an interaction between iron and zinc have included both adults and infants. In an investigation involving adults, Solomons and Jacob (1981) used plasma zinc concentration as an index of zinc absorption, although this may not be an accurate index. Plasma zinc concentration was only slightly decreased when adults were given equivalent oral doses of inorganic iron and zinc (25 mg each) but was markedly depressed when the dose of iron was increased to 50 or 75 mg but the dose of zinc remained at 25 mg. Thus, at Fe:Zn ratios of 2:1 and 3:1, zinc absorption was significantly inhibited. On the basis of these data, the authors speculated that infant formulas having a high Fe:Zn ratio may have a decreased zinc bioavailability. After this report and the concern raised regarding Fe:Zn ratios in infant formula, a study involving 291 one-year old infants was designed to determine whether iron supplementation compromised zinc nutriture; serum zinc levels served as the index of zinc status (Yip et al., 1985). The infants were randomized to receive a 3-month treatment of placebo or 30 mg of iron/d as ferrous sulfate before a meal. Serum zinc concentration was not different between the treatment groups before or after the iron treatment. It was concluded that administering iron supplements as the sole mineral supplement did not compromise zinc nutriture in healthy, well-nourished 1-year-old infants. However, the authors recognized that serum zinc level is not a reliable sole index of total zinc nutriture. Thus, the effect of lowering the plasma zinc concentration by administering ferrous sulfate in ratios of up to 3:1 Fe:Zn to adults (Solomons & Jacob, 1981) was not confirmed in infants. The difference in results between the two studies was hypothesized (Yip et al., 1985) to be due to the high pharmacological doses of zinc and iron given to the adults. However, on a per kilogram basis the 30 mg of iron given to the 1-year-old children (estimated 3 mg/kg) would be higher than the 75 mg given to the adults (estimated 1.2 mg/kg). However, a more plausible reason could be that in the adult study the subjects received the doses of iron and zinc after an overnight fast (Solomons & Jacob, 1981), whereas the infants were given the dose of iron approximately 30 minutes before a meal (Solomons & Jacob, 1981). Thus, the study involving infants who were given the iron dose soon after a meal has greater relevancy to the levels of supplementary iron and zinc in preterm infant formula.

Nevertheless, the area of interaction should receive higher priority to definitively determine whether the present recommended levels of iron and zinc, as well as other micronutrients in preterm formulas, yield interactions that may compromise absorption of essential nutrients, thus jeopardizing the health of the infant. The present recommendations of the Expert Panel provide an Fe:Zn ratio of 2:1, e.g., using the maximum of 3.0 mg of iron to the maximum of 1.5 mg of zinc/100 kcal. The results of the studies of Yip et al. (1981) described above with infants would suggest no adverse effect of iron on zinc absorption, assuming that the serum zinc concentration accurately reflected zinc absorption and status.

Many different methods could contribute to our understanding of the zinc requirements of premature infants. These include measurements of tissue concentrations of zinc; observations of zinc-responsive growth impairment; clinical evidence of zinc deficiency; factorial estimates of zinc requirements based on kinetic data and data from zinc tracer studies and perhaps from results of traditional zinc metabolic balance studies; estimates of zinc pool sizes (Miller et al., 1994); and compartmental modeling (Wastney et al., 1999). Each of these approaches has its limitations, which are partly inherent and partly due to the limited research that has been reported, despite the availability of techniques that could rapidly expand the body of useful knowledge.

Data from these sources are integrated in this section in order to estimate requirements and to derive a range for the optimal zinc content of premature infant formulas. In deriving these estimates, it is necessary not only to minimize the risk of zinc deficiency but also to avoid excessive levels that could ultimately prove to have undesirable consequences. The incomplete but growing amount of evidence for a relatively narrow optimal range of zinc in the cerebral cortex (Frederickson et al., 2000) serves as a reminder of the need to be conservative in setting upper limits.

Review of the literature

Factorial estimates of zinc requirement

The factorial approach is the most informative, despite the lack of extensive high-quality data. The zinc requirement as estimated by the factorial approach is the minimum intake necessary to replace endogenous zinc losses and to achieve sufficient retention to meet the needs of new tissue.

Dietary requirement. The minimum endogenous zinc excretion (when intake is within the optimal range) plus the zinc required for new tissue minus the zinc contributed by hepatic stores acquired in utero is referred to as the physiological requirement. The physiological requirement divided by the fractional absorption of exogenous zinc is the dietary requirement. The major variables in this equation are the fractional absorption of dietary zinc in the intestine and the intestinal excretion of endogenous zinc. Fractional absorption depends on the quantity of zinc ingested and factors that affect its bioavailability. The intestine provides the major route for excreting endogenous zinc (Hambidge et al., 1986). Because the regulation of this excretion has a major role in maintaining of zinc homeostasis, the GI tract is the location of the most important mechanisms for regulating whole-body zinc metabolism, absorption, and excretion.

Zinc homeostasis can be more or less maintained over a wide range of intakes by regulation of the fraction of zinc absorbed and the quantity of endogenous zinc excreted. Homeostasis can be maintained or re-established at remarkably low zinc intakes, at least in adults, but this is achieved only at the cost of some diminution of body zinc levels (Sian et al., 1996), which may well have subtle but important biological and clinical implications. Typically, there is a strong positive correlation between the quantity of absorbed zinc and the quantity of endogenous zinc excreted via the intestine (Sian et al., 1996). This relationship complicates the factorial approach to a quantitative understanding of how zinc homeostasis is maintained. Fractional absorption and intestinal excretion of zinc are considered in detail below.

Another factor that adds to the complexity of the factorial approach in the young infant is the store of zinc accumulated in utero (Widdowson et al., 1974; Zlotkin & Cherian, 1988). When optimal zinc retention by premature infants in early postnatal life is calculated, there is no advantage in attempting to mimic the same accumulation of hepatic zinc stores that are acquired in utero at corresponding postconceptional ages. In

contrast to the situation with respect to other minerals, postnatal zinc nutrition for the premature infant does not have as its goal the fetal rate of zinc accretion. This is because by 32–36 weeks of postconceptional age, the infant can ingest adequate zinc to keep up with growth needs. There seems to be no need to supplement beyond this, and there are possible disadvantages to attempting to do so.

Little is known about the rates of release and utilization of fetal zinc stores, which may depend in part on physiological need. The latter, in turn, depends principally on the quantity of exogenous zinc available for absorption and the rate and composition of postnatal growth. The occurrence of severe acquired zinc deficiency by 2 months of postnatal age in premature infants whose postnatal supply of exogenous dietary zinc is low (Zimmerman et al., 1982) suggests that zinc stores can be exhausted quickly. To the extent that requirements for new tissue can be met by released hepatic zinc stores, the necessary retention of exogenous dietary zinc is correspondingly less.

Growth requirement, and intestinal conservation of zinc. Growth is the largest single factor in determining the physiological requirement for zinc in the premature infant. Although the extremely LBW infant may not achieve intrauterine growth rates for many weeks, these rates provide the most appropriate guide to zinc requirements for growth.

According to revised estimates by Widdowson et al. (1988), the fetus accumulates 850 μg of zinc/d between 24 and 40 weeks of gestation. This figure corresponds to a concentration of zinc in new lean body mass of from 35 to 40 μg/g, which is substantially higher than the more generally accepted figure of approximately 30 μg/g for older infants and adults (Krebs & Hambidge, 1986). The difference may be attributable primarily to a substantial increase in the concentration of zinc in skeletal muscle (Widdowson et al., 1988) and a hepatic accumulation of zinc (Widdowson et al., 1974; Zlotkin & Cherian, 1988).

If we assume that there is no need (and possibly no mechanism) to mimic the fetal accumulation of hepatic zinc stores in the premature infant of the same postconceptional age, this contribution to intrauterine accumulation rates may be excluded in calculating necessary postnatal retention by the premature infant. The average hepatic zinc concentration at term has been reported to be 140 μg/g of wet weight of tissue, compared with 60 μg/g for children older than 1 year of age (Zlotkin & Cherian, 1988). The difference indicates that of hepatic zinc stores, as much as 80 μg/g of wet weight of liver may be utilized in the first year. The weight of the liver at term is 140 g (Stocker & Dehner, 1992); therefore, the calculated hepatic store of zinc at 40 weeks of gestation is 80 μg of zinc/g $\times$ 140 g = 11 mg of zinc.

The zinc concentration has been reported to be exceptionally high in livers from premature infants (of unspecified GA), averaging 230 μg/g, or 170 μg/g higher than in livers from children older than 1 year of age (Zlotkin & Cherian, 1988). The liver samples from preterm infants whose average GA was 30.6 $\pm$ 2.9 (SD) weeks may have included some at 28 weeks of gestation. As total liver weight at this stage of fetal development is approximately 50 g, the calculated hepatic zinc store at this stage is approximately 170 μg of zinc/g $\times$ 50 g = 8 mg of zinc. These calculations suggest that the major part of the hepatic stores accumulate either in the second and/or in the early third trimester and that deposition of hepatic stores of zinc between 28 and 40 weeks (84 days) of gestation is only 11 minus 8 mg of zinc = 3 mg of zinc, or approximately 40 μg of zinc/d. This still leaves 850 minus 40 or approximately 810 μg

of zinc/d accumulated by the fetus between 28 and 40 weeks of gestation in sites other than the liver.

For the younger fetus of GA 24–28 weeks, lean body mass accrues at an average rate of approximately 17 g/d (Alexander et al., 1996). With the revised rate for accumulation of zinc (Widdowson et al., 1988), the average concentration of zinc in tissue gain during this interval is 850 μg of zinc/17 g of lean body mass = 50 μg of zinc/g. Presumably, this high figure is attributable to rapid accumulation of hepatic stores at this stage of gestation, although the evidence on this point is not consistent. From a combination of the data in Widdowson et al. (1988) and in Zlotkin and Cherian (1988), it appears that increases in hepatic stores of zinc account for approximately 15 μg/g of new tissue, or 255 μg/d. Therefore, the LBW infant of 24–28 weeks of postconceptional age requires 850 – 255 μg, or ~600 μg/d for new tissue, excluding stores, for growth comparable to that of a fetus of 40 weeks of postconceptional age.

Release of hepatic zinc stores. It appears that hepatic zinc stores accumulated in utero by term infants are released in early postnatal life. If one assumes that most of this release is in the first 2 months, the daily rate of release from the 11 mg hepatic storage will be 11,000/60 = 183 μg of zinc/d for the term infant, and 8000/60 = 133 μg of zinc/d for the preterm infant from the 8 mg hepatic storage. Therefore, the average release for the period of 28–40 postconceptional weeks in the preterm infant of BW 1000 g is approximately 150 μg of zinc/d. It is important to note, however, that for the extremely LBW infant, hepatic zinc stores will be lower and are likely to be depleted earlier, perhaps by 32–36 weeks. This is even more true when delivery is as early as 24 weeks. For this reason, factorial calculations include two estimates for the release of body stores for 2 months postpartum: high = 130 μg of zinc/d (more than approximately 26–28 weeks gestation), and low = 0 (GA of less than approximately 26–28 weeks).

Calculated dietary zinc that needs to be retained to meet optimal growth requirements of premature infants. For a GA of 28–40 weeks at delivery, the dietary zinc requirement (nonhepatic retention minus release from hepatic storage) is 800 μg of zinc – 130 μg of zinc released from stores, or approximately 650 μg of zinc/d. For a GA of 24–28 weeks at delivery, the dietary requirement with high store release is 600 – 130 = 470 μg of zinc/d; with low store release, it is 600 – 0 = 600 μg of zinc/d.

On a BW basis, these figures equate to retention of approximately 500 μg/kg of BW at 1000 g (27 weeks of postconceptional age), approximately 400 μg/kg at 1500–2000 g (30–32 weeks of postconceptional age), and 200–300 μg/kg at 2500–3500 g (35–40 weeks of postconceptional age). Beyond 40 weeks after conception, the quantity of exogenous zinc that needs to be retained for growth declines rapidly, especially when expressed on a body weight basis: 80 μg/kg of body weight at 6 kg (53 weeks after conception), 50 μg/kg at 8 kg (66 weeks after conception), and 25 μg/kg at 10 kg (92 weeks after conception).

Theoretically, the zinc requirement for the age-adjusted premature infant beyond 48 weeks is the same as that for the term infant of corresponding postconceptional age. Between 40 and 48 weeks, however, the term infant will still have access to hepatic zinc stores, whereas the preterm infant will not.

Endogenous zinc excretion. The intestine provides the major route for excreting endogenous zinc. Moreover, regulation of the quantity of exogenous zinc excreted by this route has a major role in maintaining of zinc homeostasis. In term breastfed infants of ages 2–4 months, endogenous excretion by this route averages 50 μg/(kg of body weight·d) (Krebs et al., 1996). Stable isotope tracer studies of intestinal endogenous

TABLE 12-1

Calculated dietary zinc requirements

Weight (g)	Retention required [mg/(kg · d)]	Endogenous zinc excretion [mg/(kg · d)]	Physiological requirement [mg/(kg · d)]	Dietary requirement [mg/(kg · d)]	
				F[1] = 0.3	F[1] = 0.4
<1000	0.50	0.09	0.60	2.0	1.5
1000–2000	0.40	0.09	0.50	1.7	1.2
2000–3500	0.30	0.09	0.40	1.3	1.0

[1] F = fractional absorption.

losses have not been reported for premature infants, whose dietary intake of zinc is relatively low. It is therefore uncertain whether the intestinal tract of the premature infant, and especially the infant of BW less than 1000 g, has the same ability to conserve endogenous zinc as does that of the term infant.

Nevertheless, measurements of intestinal excretion of endogenous zinc have been undertaken in LBW infants at 33 weeks of postconceptional age who were fed high zinc preterm infant formulas. Fecal excretion of endogenous zinc approximated 210 μg of zinc/kg of body weight (Jalla et al., 1997). This appeared appropriate for the quantity of zinc absorbed and did not provide vital information on the limits of the ability of the premature intestinal tract to conserve endogenous zinc. For some infants the excretion rate was less than 100 μg/(kg·d), suggesting that their ability to conserve endogenous zinc at 33 weeks after conception might be similar to that of term infants. In another study, intestinal excretion of endogenous zinc measured in just one premature infant fed a preterm infant formula with a high zinc level (12 mg/L) was reported to be as low as 11 μg/(kg·d) (Wastney et al., 1999). Accepting this surprisingly low level will require confirmation, but it underscores the importance of such studies. In contrast to the suggestions from earlier balance studies (Jalla et al., 1997), the LBW premature infant may have excellent potential for intestinal conservation of endogenous zinc at an early stage of postnatal life. In the absence of any indication to the contrary, the average value of 50 μg of zinc/(kg·d) estimated for term breast-fed infants (Krebs et al., 1996) will be used to derive a tentative minimum intestinal excretion of zinc by premature infants whose zinc status is within an optimal range. Lower losses are to be expected when zinc status is compromised.

Nonintestinal zinc excretion. In contrast to the intestine, the adult kidneys are insensitive to changes in zinc intake and status, and it is only with severe dietary zinc restriction that the urinary zinc value declines (Baer & King, 1984; Johnson et al., 1993). On a body weight basis, urinary zinc losses are relatively high in the premature infant in the first several weeks of postnatal life, at about 35 μg/(kg·d) (Ehrenkranz et al., 1984), again with no evidence that excretion is sensitive to zinc intake of the enterally fed infant.

Integumental zinc losses in the premature infant have not been measured and probably will not be. Although body surface area is relatively large in the premature infant, sweating is limited. It seems reasonable to use the value of 7 μg of zinc/(kg·d) derived from data for adults (Johnson et al., 1993).

In summary, it appears that despite earlier concerns and the need for more data, the intestine of the premature infant has an ability to conserve endogenous zinc similar to that of the term infant. Therefore, it is assumed that minimum losses of endogenous zinc via the intestine in premature infants whose

zinc status is optimal are no higher than those of term breast-fed infants, or 50 μg/(kg·d). This gives a calculation for total minimal endogenous zinc excretion in early postnatal life of 90 μg/(kg·d), a value that will be used in subsequent calculations.

Physiological requirement for zinc. The physiological requirement is the sum of the zinc required for retention and required to replace minimal endogenous zinc losses in subjects who are not even minimally depleted of zinc. These calculations are given in mg of zinc/(kg of body weight·d) (see Table 12-1).

Fractional absorption of dietary zinc. As with intestinal conservation of endogenous zinc, fractional absorption of zinc varies widely, depending on dietary zinc intake, the bioavailability of the ingested zinc, and zinc nutritional status. There is no evidence either to support or to refute the possibility that host factors related particularly to the LBW infant also affect absorption.

Currently available data for preterm infants are consistent with data for term infants and adults and suggest that fractional absorption varies with zinc intake over the same range as it does in term infants (Ehrenkranz et al., 1984; Ehrenkranz et al., 1989; Jalla et al., 1997; Wastney et al., 1999). Almost all available data on infants relate to subjects fed high zinc special preterm infant formulas or to those fed human milk with a high zinc fortifier. At these relatively high levels of intake, the data suggest that adequate quantities of zinc are absorbed without the necessity of achieving higher fractional absorption. Indeed, these fractional absorption data probably reflect the result of regulation to ensure that excessive quantities of zinc are not absorbed. Therefore, the data from these studies do not provide any special insight into the ability of the LBW infant to adapt to lower zinc intakes. However, the few data available suggest that in preterm infants the fractional absorption of zinc from unfortified human milk may also be similar to that achieved by term infants (Ehrenkranz et al., 1989).

Methodological issues have added to the complexity of data interpretation. Specifically, in three of the four reported studies (Ehrenkranz et al., 1984; Ehrenkranz et al., 1989; Wastney et al., 1999), all the oral stable isotope tracer was given with only one feeding. An unfortunate result of this technique was that the quantity of tracer exceeded the quantity of zinc in the milk. It is uncertain whether absorption of this extrinsic tracer reflects absorption of the intrinsic zinc in the formula in these circumstances, but the (obviously impossible) calculated negative values for fecal excretion of endogenous zinc in one of these studies (Ehrenkranz et al., 1989) could be explained by a lower fractional absorption from the one large test meal, an effect that should have been anticipated. In one of these studies, the fractional absorption was calculated from model-based compartmental analysis rather than being measured (Wastney et al., 1999).

Mean fractional absorption of zinc in three independent

studies (Ehrenkranz et al., 1989; Jalla et al., 1997; Wastney et al., 1999) has been reported to be 0.32, 0.36, and 0.22, for an overall average of 0.30. However, fractional absorption is likely higher when lower quantities of zinc are ingested, as has been documented for term infants fed cow milk-based formula (Ziegler et al., 1989). Therefore, calculated dietary requirements at a fractional absorption of 0.4 are also included in Table 12-1. Although we do not have the benefit of the specific data needed for premature infants delivered at different GAs, the Expert Panel thought that it would be more reasonable to use the 0.4 figure for fractional absorption when calculating dietary requirements.

Calculated dietary zinc requirement. The calculated dietary zinc requirement (the estimated average dietary requirement for individuals) equals the physiological requirement divided by the fractional absorption.

The dietary requirements at different fractional absorptions are shown in Table 12-1.

Calculated zinc concentration required in infant formula. With an intake of 150 mL/(kg·d) for the infant fed a cow milk-based formula and a fractional absorption of 0.4, the calculated average zinc concentration required in premature infant formula for infants born before 40 weeks postconception would be 7–8 mg of zinc/L (0.86–0.99 mg of zinc/100 kcal at 810 kcal/L). This value requires correction to achieve a population mean (World Health Organization, 1996d). As the variation in intake of formula is likely to be small for any one combination of postconceptional/postnatal age, and as low formula intake is likely to be associated with diminished growth rate and correspondingly diminished requirements, the variance for the population mean intake (volume) is set at 5%. Therefore, the zinc concentration required in formulas for the premature infant population will equal 7–8 mg + (2 × 5%), or 8–9 mg of zinc/L (1–1.1 mg/100 kcal at 810 kcal/L).

Calculated zinc requirements of premature infants fed human milk. The concentration of zinc in mature human milk is reported to be 1.2 mg/L (American Academy of Pediatrics. Committee on Nutrition, 1998). However, two factors reduce the calculated physiological and dietary zinc requirements for premature infants fed human milk: a higher fractional absorption (approximately 0.6) (Ehrenkranz et al., 1989), and a 15% slower absolute rate of weight gain (Schanler et al., 1999). This slower rate appears to be clinically acceptable to some neonatologists, as it is associated with less pathology and earlier discharge from the hospital. Also, premature infants taking breast milk have been reported to receive a higher intake volume, 180 mL/(kg·d). If a similar rate of fractional absorption were assumed, the calculated required zinc concentrations in fortified breast milk would range downward from 5 mg/L for infant weights less than 1000 g to 3 mg/L for weights of 2500–3500 g. However, this assumption of the rate of absorption may not be valid.

Empirical estimates of zinc requirements

Tissue, plasma, and serum zinc concentrations. Several factors seriously limit the value of plasma and serum zinc concentrations. One is the lack of adequate sensitivity of this widely used biomarker of zinc nutritional status (Wood, 2000); another is the possibility that there are normal variations in plasma and serum zinc with postnatal age in premature infants (Altigani et al., 1989); a third is the unexplained interlaboratory variation in normal values. In addition, cytokine stimulation lowers plasma zinc levels.

The changes with postnatal age have been documented by Altigani et al. (1989), among others. Serum or plasma zinc levels, which start in the normal adult range, reach a nadir of 65 μg/100 mL at 6 weeks. Levels of plasma zinc are correlated with growth rate but not with dietary zinc intake. However, the variation in intake observed by Altigani et al. (1989) was quite narrow and the range considerably lower than requirements calculated by the factorial approach. A similar decline in serum or plasma zinc levels has been noted by others in early postnatal weeks, but again with dietary intakes lower than those calculated as required by the factorial method. These findings may result from unidentified physiological or pathophysiological factors, such as low levels of carrier proteins, or they may be indicative of suboptimal zinc nutritional status. In the latter case, these data are consistent with the requirements estimated by the factorial approach.

Much more severe hypozincemia was reported among 26 LBW infants (GA of 23–32 weeks, median of 27 weeks) at a median postnatal age of 10 weeks (Obladen et al., 1998). Approximately half of these infants received at least 50% of their feedings with their own mothers' milk or with pooled human milk. The breast milk was fortified to provide a total of 3.7 mg of zinc/L and was supplemented as necessary with a preterm formula containing 8.3 mg of zinc/L; hence, the average zinc intake from the mix of human milk and preterm formula was about 6 mg of zinc/L (equivalent to approximately 0.7 mg/100 kcal of an 810 kcal/L formula). This intake was clearly inadequate to maintain optimal zinc status. Although it was compatible with the requirements calculated for formula-fed infants, it did not support optimal zinc status during the first 10 weeks of life in extremely LBW infants.

It should be noted that these infants received very little zinc through intravenous infusions in early postnatal life, which presumably contributed to their perilous zinc status 2 months later. Nevertheless, these findings are compatible with the factorial calculations of zinc requirements for infants receiving mothers' milk plus human milk fortifier.

Hair zinc concentrations. The results of hair zinc assays have been used as evidence as to the adequacy of different feeding regimens for the premature infant (Friel et al., 1984; Gibson & DeWolfe, 1980a; Wauben et al., 1999). For example, Wauben et al. (1999) reported that the concentrations of zinc in hair from LBW infants (born at an average GA of 29 weeks) at 6 months corrected age were as high for infants receiving their own mothers' milk with a non-zinc-containing fortifier (zinc intake at 35 weeks of postconceptional age = 0.76 mg/(kg·d)] as for infants receiving their own mothers' milk with a zinc-containing fortifier (zinc intake at the same age = 2.21 mg/(kg·d)]. Both were higher than concentrations in hair from infants receiving a preterm formula providing 1.46 mg of zinc/(kg·d). However, the zinc intake from human milk in this study was exceptionally high, and zinc absorption was close to the physiological requirement calculated in Table 12-1. From the composite of literature data on the zinc content of preterm versus term human milk (Atkinson et al., 1997; Butte et al., 1984; Friel et al., 1999a), however, it seems that this level of intake cannot be achieved without some additional zinc in the fortifier. The findings of Wauben et al. (1999) are compatible with the factorial calculations in this report.

The interpretation of hair zinc data is difficult. It is possible, for example, that differences in hair growth rates or morphology between formula-fed and breast-fed infants could affect hair zinc concentrations.

Clinical features and growth. Severe zinc deficiency, manifesting clinically as acrodermatitis enteropathica, has been documented in numerous case reports of premature infants. Typically, these have been either early reports of infants fed intravenously without added zinc in the infusate (Arakawa et

al., 1976; Bonifazi et al., 1980; Sivasubramanian & Henkin, 1978) or reports of infants fed by mothers whose milk zinc concentrations were abnormally low (Aggett et al., 1980; Atkinson et al., 1989; Murphy et al., 1985; Zimmerman et al., 1982). These extreme cases in milk-fed infants are not particularly helpful in establishing optimal requirements for daily zinc, but they do serve to illustrate the effects of a severely zinc-deficient diet, i.e., intakes of 0.75 mg of zinc/d or less from breast milk.

Results of two studies of growth rates in relation to dietary zinc intake (Friel et al., 1993b; Haschke et al., 1985) do not correlate well, perhaps because the study populations were different. One study focused on the more mature male premature infants, with a BW of 1800–2500 g (Haschke et al., 1985). Feeding a formula containing 4 mg of zinc/L was not associated with any greater growth velocity than was feeding with a formula providing only 1.4 mg of zinc/L. These data are not compatible with the factorial estimate of the requirement and suggest a much lower figure for the requirement of growth.

The more recent study involved LBW infants (mean BW of 1100 g, GA of 29 weeks) who were all fed a formula containing 12 mg of zinc/L until entry into the study with an average weight of 1850 g (Friel et al., 1993b). They were then randomized to receive formulas containing 6.7 or 11 mg of zinc/L for 6 months. Linear growth rate and motor development scores were significantly higher in infants fed the formula with the higher zinc content. Although these data are not completely incompatible with dietary zinc requirements calculated from the factorial approach, they imply benefit from a relatively high intake and certainly give a different message than does the study of Haschke et al. (1985) that was summarized in the previous paragraph. Additional well-designed intervention trials of zinc nutriture are needed.

Recent recommendations

The recent dietary reference intake (DRI)-adequate intake (AI) (Institute of Medicine, 2001d) for zinc intake for term infants 0–6 months of age was 2 mg/d (0.3 mg/kg, assuming a body weight of 7 kg). This value was based on the intake of infants fed human milk exclusively. The Institute of Medicine (IOM) (2001d) set a DRI-tolerable upper intake level (UL) of 4 mg/d (0.6 mg/kg) for term infants of age 0–6 months. The DRI-UL was based on a clinical study of term infants fed 5.8 mg of zinc/L (4.5 mg/d) for 6 months that uncovered no adverse effects, including alterations in serum copper and cholesterol concentrations. Current domestic term formula contains 0.75–1.0 mg/100 kcal, providing 5.2–4.0 mg/d, assuming intake of 780 mL/d (Abbott Laboratories. Ross Products Division, 2001; Mead Johnson Nutritionals, 2000). If this DRI-UL were appropriate for preterm-LBW infants, preterm infant formula could not exceed a concentration of 0.5 mg/100 kcal. The minimum recommendation for term infant formula (Raiten et al., 1998a) of 0.4 mg/100 kcal was based on the zinc concentration in mature human milk at 1 month postpartum minus 1 SD. The recommendation of 1.0 mg/100 kcal for the maximum concentration in term formula was based on the 90th percentile of the U. S. Food and Drug Administration (FDA) analyses of infant formula (Raiten et al., 1998a).

Conclusions and recommendations

Feeding 6 mg of zinc/L (approximately 0.7 mg/100 kcal of an 810 kcal/L formula) was inadequate to maintain optimal zinc status in preterm-LBW infants (Obladen et al., 1998). Despite the extreme care required in interpreting data and the few data from which calculations are derived, the factorial approach provides the most comprehensive and effective strategy for calculating the optimal zinc concentration of preterm formula. With the exception of some growth data, other sources of information are compatible with the conclusions derived from a factorial approach. On the basis of this approach, the recommended minimum zinc concentration for preterm formulas for use until 40–48 weeks postconception is 8–9 mg/L (1.0–1.1 mg/100 kcal, for formula at 810 kcal/L, assuming a formula intake of 150 mL/d). This provides 1.2–1.4 mg of zinc/d. Whether the minimum recommended is adequate or optimal has not, to our knowledge, been definitively resolved.

Preterm infant formulas in the United States provide 12.2 mg of zinc/L (1.5 mg/100 kcal) (American Academy of Pediatrics. Committee on Nutrition, 1998). Little information is available on what the toxic enteral dose of zinc might be, but no noxious effects have been reported at the presently used concentration. Tyrala (1986) demonstrated that feeding 1.5 mg/100 kcal results in a positive zinc balance in preterm-LBW infants. Furthermore, at this level of intake, zinc balance and serum zinc levels do not differ for copper intakes between 110 and 260 μg/100 kcal (Tyrala, 1986).

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of zinc in preterm infant formula be 1.1 mg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum concentration of zinc in preterm infant formula be 1.5 mg/100 kcal.**

COPPER

Background

The principal recognized biological role of copper is as an electron transfer intermediate in reduction-oxidation reactions. There are numerous enzymes with important reduction-oxidation activity that contain copper as an essential cofactor for catalytic activity. These include cytochrome-c oxidase, the terminal enzyme in the electron transport chain; ceruloplasmin, with its ferroxidase activity that is necessary for the release of iron from stores and iron binding to transferrin; amine oxidases; protein-lysine 6-oxidase; dopamine hydroxylase; and SODs (Uauy et al., 1998).

Copper deficiency

Reports of human copper deficiency are quite limited, but disease has been documented in several special circumstances (Cordano, 1998). Cow milk (Fransson & Lönnerdal, 1983) has a substantially lower copper content than does human milk (Casey et al., 1985; Dewey & Lönnerdal, 1983; Fransson & Lönnerdal, 1984; Higashi et al., 1982; Vaughan et al., 1979; Vuori et al., 1980; Vuori & Kuitunen, 1979) and the bioavailability of this copper compares poorly with that in human milk. Nevertheless, occurrence of clinical features of copper deficiency requires inappropriate and prolonged feeding of cow milk in infancy, without other dietary sources of copper, unless other contributory etiologic factors exist (Levy et al., 1985). These factors include rapid growth (Uauy et al., 1998), prolonged diarrhea (Castillo-Durán & Uauy, 1988; Rodriguez et al., 1985), intestinal malabsorption syndromes (Cordano & Graham, 1966), and, in early infancy, low hepatic stores (Beshgetoor & Hambidge, 1998). Copper absorption is impaired by cation-chelating agents and large quantities of oral alkali (Williams, 1983a). Of greater practical concern is the effect of high intakes of iron and zinc in reducing copper

absorption (Barclay et al., 1991; Botash et al., 1992; Haschke et al., 1986; Polberger et al., 1996).

Clinical and laboratory evidence of copper deficiency in the premature infant has been well documented to include anemia, neutropenia, skeletal abnormalities, and possibly disorders of central nervous system function (Cordano, 1998; Danks, 1988; Uauy et al., 1998). However, cases of deficiency have been rare in North America since this problem was identified and addressed in the 1970s and earlier (Josephs, 1931; Lahey & Schubert, 1957; Manser et al., 1980; Sturgeon & Brubaker, 1956).

The premature infant is at enhanced risk of copper deficiency because of the limited hepatic stores accumulated in utero in comparison with those of the term infant (Aggett, 1998; Shaw, 1980; Widdowson et al., 1974; Widdowson & Dickerson, 1964), and because of a more prolonged period of rapid postnatal growth. Although immaturity of intestinal function might also be expected to contribute, absorption studies with stable isotopes of copper have failed to support this idea (Ehrenkranz et al., 1989).

Hepatic copper stores

Any consideration of copper requirements in the young infant has to include the role of hepatic copper stores, accumulated by the fetus, in meeting early postnatal needs for synthesis of new tissue and replacement of obligatory losses. Although the magnitude of these stores depends on GA at delivery, significant neonatal hepatic copper stores are present even in the most premature infants. Copper deficiency in the premature infant has not been documented before 1 month postpartum, with an average postnatal age of approximately 3 months (Uauy et al., 1998).

The fetus accumulates copper at a rate of 50 μg [0.8 μmol)/(kg of body weight·d] (Aggett, 1998). Of total fetal copper, 50–60% is in the liver, which contains approximately 3 mg of copper at 26 weeks of GA and 10–12 mg of copper at 40 weeks. This increase during the third trimester is related to some increase in copper concentration per unit of liver weight and a nearly three-fold increase in liver size. Hepatic copper concentrations in the fetus are at least five times those in the adult, suggesting that at least 80% of hepatic copper at birth can be regarded as storage copper. This amounts to 2.5 mg at 26 weeks and 9 mg at term.

Our poor understanding of the mechanisms of release and utilization of these stores makes it difficult to estimate how these stores contribute to meeting postnatal copper requirements. This lack of knowledge applies not only to quantitative information but also to the basic physiology involved. Fetal hepatic copper is bound principally to metallothionein and is localized in the lysosomal fraction (Rydén & Deutsch, 1978). Results of studies using mammalian models have provided only partial evidence for the efficient utilization of this hepatic copper. Neonatal piglets retained systemically administered ^{64}Cu in the liver, attached to metallothionein, from where it was released slowly into the circulation as ceruloplasmin-bound copper (Aggett, 1998). In contrast, in the guinea pig there was a 50% reduction in hepatic copper by 4 days after delivery. This was associated with a marked increase in biliary copper output, which was greater than the increase in biliary flow (Srai et al., 1986).

In the adult human, very little of the copper secreted in the bile is reabsorbed. Indeed, the bile provides the major route for copper excretion and hence has a major role in the maintenance of copper homeostasis. However, in the suckling rat pup, intestinal uptake of copper of biliary origin exceeds 75%, declining to 8% after weaning (Aggett, 1998). This decline

can be induced by steroid administration, an observation that could have implications for the premature infant. Thus, although it is possible to make theoretical calculations of rates and efficiency of utilization of stores based on the above values, which fit well with what is known about the timing of the onset of postnatal copper deficiency, the uncertainties are too great to rely on such calculations. To err on the safe side, it would thus be prudent to ignore the contribution of hepatic stores when calculating the dietary copper requirements of the premature infant entering the stable growth period. The copper stores, however, do appear likely to meet the minimal postnatal needs in the perinatal transitional period, as defined as the period immediately following birth when there is water loss and inadequate energy intake.

There is no reason to attempt to reproduce the intrauterine accumulation of fetal hepatic copper stores in the premature infant of corresponding postconceptional age. This goal is unnecessary if dietary intake is designed to ensure meeting postnatal needs for new tissue and to match endogenous losses (other than stores). Moreover, it is unclear whether this goal is attainable, and attempts to achieve it may risk copper toxicity.

Review of the literature

Factorial calculations

If the input from and the need for hepatic copper stores are ignored in estimating dietary copper requirements for the premature infant after the transitional period, it is possible to achieve reasonable calculations of dietary copper requirements via a factorial approach.

Copper retention required by the premature infant is the difference between total copper retention by the fetus at a corresponding postconceptional age [approximately 50 μg/(kg·d)] and that portion of retention that is incorporated into hepatic stores [approximately 20 μg/(kg·d)]. This equals the extrahepatic accumulation [approximately 50 μg/(kg·d) $\times$ 0.4] plus the additional hepatic copper not in stores [approximately 10 μg/(kg·d)], or 20 + 10 = 30 μg/(kg·d). Endogenous excretion of copper in adults, in whom metabolism is not confounded by secretion or excretion of neonatal stores, is primarily via the intestine (Turnlund, 1998). As with zinc, excretion of copper by this route varies with the quantity absorbed and appears to have a major role in maintaining copper balance (Turnlund, 1998). Minimal intestinal excretion of copper in adults is approximately 3 μg/(kg·d). Urinary copper excretion in premature infants fed their own mothers' milk has been reported to be 6 μg/(kg·d). Therefore, minimal total excretion of endogenous copper is estimated at approximately 10 μg/(kg·d). The factorial estimate of the minimal physiological requirement under conditions of total absorption is thus 30 + 10 = 40 μg copper/(kg·d).

Fractional copper absorption values for infant formulas in infant rhesus monkeys ranged from 0.5 to 0.7, whereas the fractional absorption (6-hour liver uptake) in a suckling rat pup model was only about 0.25. Fractional absorption data are, unfortunately, technically difficult to obtain because of the lack of a suitable isotope for tracer studies. The one reported human study of premature infants (GA of 29 weeks, BW of 1300 g, postnatal age of 3–5 weeks) gave average figures for the fractional absorption of tracer of 0.7 for mothers' milk and 0.4 for preterm formula with a copper concentration of 0.61 mg/L, providing an intake nearly three times that for breast milk (Ehrenkranz et al., 1989). It should be noted that the tracer was given with only one feeding in a quantity that was approximately 18 times that of the endogenous copper in that

feeding. Although this raises serious questions about the interpretation of the data, it is reassuring that they are comparable to other less direct data for term infants (Lönnerdal, 1998).

Fractional copper absorption varies inversely with intake (Turnlund, 1998), and this regulatory mechanism is of sufficient magnitude to account for the difference in fractional absorption between human milk and preterm formula. In addition, however, absorption may be greater from breast milk, independent of intake (Lönnerdal, 1998).

With a value of 0.4 for fractional copper absorption from preterm formula, the calculated dietary requirement is 40/0.4 = 100 μg/(kg·d). Results of copper metabolic balance studies of premature infants on a variety of feeding regimens have typically shown negative retention (Cavell & Widdowson, 1964; Dauncey et al., 1977; Tyrala, 1986). This has been reported even when the fractional absorption is strongly positive and is attributable to large fecal losses (Dauncey et al., 1977; Lönnerdal, 1996). These results are consistent with substantial excretion of hepatic stores released into the intestinal lumen in biliary secretions. These data support taking a conservative approach to the role of hepatic stores in factorial calculations of copper requirements.

Laboratory indices of copper status

Laboratory indices of copper status are unsatisfactory because of a lack of specificity. The most widely used indices have been assays of serum ceruloplasmin and serum copper, which depend heavily on ceruloplasmin levels. This is especially evident in the young infant, particularly the premature infant. Ceruloplasmin levels, and therefore serum copper concentrations, are remarkably low in the neonate, independent of copper status (Halliday et al., 1985; Hillman, 1981; Lönnerdal, 1988; McMaster et al., 1983; Salmenperä et al., 1986; Sutton et al., 1985). This is evidently a physiological phenomenon reflecting limited ceruloplasmin synthesis, as indicated by low immunoreactive ceruloplasmin levels in cord blood. Ceruloplasmin levels are directly correlated with postconceptional age. The lower the GA at delivery, the lower the neonatal ceruloplasmin and copper levels are in the neonate, including serum copper concentrations as low as 20 μg/100 mL (3 μmol/L). These levels steadily increase to reach adult values by about 3 months corrected age.

Increasing copper concentrations of infant formulas from 2- to 10-fold, up to a concentration of 1–2 mg/L, has not been associated with any increase in these very low concentrations of ceruloplasmin and copper in plasma (Hillman, 1981; L'Abbé & Friel, 1992). Likewise, increasing the quantity of copper administered intravenously does not affect levels (Lockitch et al., 1983). Hence, these low levels must be accepted as physiological and are apparently adequate for delivery of copper to the brain and other organs. There are insufficient data to define the utility of these plasma assays in detecting copper deficiency in premature infants, even if control values are carefully adjusted for GA. Lower plasma copper levels have been observed in premature infants with the most rapid weight gain, suggesting that copper intake may have been suboptimal for rapid growth, but this report did not provide a figure for copper intake that would permit any conclusion about the level of dietary intake below which copper status is impaired (Manser et al., 1980).

Another index of copper status is RBC SOD, but its value in the premature infant is uncertain. For example, blood transfusions may affect values (Burns et al., 1991). Higher levels in later infancy have been reported for premature infants receiving a copper-fortified formula early in postnatal life (L'Abbé & Friel, 1992), and again an inverse correlation was observed with rate of weight gain.

Overall, these biomarkers have contributed little to our understanding of copper requirements for the premature infant, although they have served to provide a cautionary note that suboptimal copper status, without any of the laboratory and clinical features of copper deficiency, may occur in premature infants fed formulas with low copper concentrations as judged by modern domestic standards.

Copper in human milk

The copper content of milk in the first week of lactation is approximately 600 μg/L, declining to 220 μg/L by 5 months (Casey et al., 1989). A premature infant consuming 150-180 mL of breast milk/(kg·d) would initially be receiving 90-108 μg/(kg·d), or about 87-90 μg/100 kcal, assuming an energy content of 670-690 kcal/L.

Effects of iron and zinc on copper absorption

Both iron and zinc, when administered in sufficient quantities, impair copper absorption. These interactions become of practical importance with iron or zinc therapy. Furthermore, animal studies suggest that ascorbate may potentiate the inhibitory effect of iron (Lönnerdal, 1988; van den Berg & Beynen, 1992). However, there is no evidence that fortification of formulas with currently recommended quantities of iron and zinc has an effect on copper absorption, unless the copper content of the formula was unusually low compared with human milk or with the quantities of copper present in domestic formulas for preterm infants (American Academy of Pediatrics. Committee on Nutrition, 1998).

With a low copper formula (44 μg/100 kcal), iron fortification to a total of 10.2 mg/L was associated with a reduction in copper absorption by half, compared with absorption from the same formula containing only 2.5 mg of iron/L (Haschke et al., 1986). Premature infants who received 13.7 mg of iron supplement daily for 4 months had depressed SOD activity in RBCs at 5 months of postnatal age (Barclay et al., 1991).

Feeding rhesus monkeys a formula with a low zinc-to-copper ratio was associated with a higher plasma copper concentration, but there are no corresponding observations for premature infants. Therapeutic quantities of zinc are documented to cause copper deficiency (Botash et al., 1992), and zinc has been used with some success as a therapeutic agent in Wilson's disease (Polberger et al., 1996). It has been recommended that zinc-to-copper molar ratios in infant formulas not exceed 20 (Hambidge & Krebs, 1989). With the suggested 20:1 zinc-to-copper ratio, the amount of zinc currently in domestic formula of 1.5 mg/100 kcal would necessitate a copper concentration of no less than 74 μg/100 kcal (9 μmol/L).

Conclusions and recommendations

Minimum. Zlotkin and Buchanan (1983) provided evidence that intravenous administration of copper at a rate of 47 μg/(kg·d) may be insufficient for preterm infants because these infants only achieved 68% of the 50 μg/(kg·d) intrauterine rate of copper accretion. Manser et al. (1980) provided evidence that enteral feeding of 41-89 μg of copper/(kg·d) (34-74 μg/100 kcal), similar to the minimum recommended in term formula, may be inadequate for preterm-LBW infants. The serum copper concentration progressively decreased for these infants who were fed an average of 66-77 μg copper/(kg·d) (55-62 μg/100 kcal), and some infants developed frank copper deficiency that was corrected by copper supplementation. Both factorial calculations and breast milk copper concentrations in

early lactation suggest an individual copper requirement of approximately 100 μg of copper/(kg·d). Several factors contribute to some uncertainty about the variance of this value. The traditional approach of adding 2 SDs [20 μg/(kg·d)] was used in estimating the population requirement, giving a total value of 120 μg/(kg·d).

Thus, the estimated population dietary requirement is 120 μg of copper/(kg·d). With an intake of 150 mL/(kg·d) [120 kcal/(kg·d) of a formula containing 810 kcal/100 mL], this corresponds to a minimum copper concentration for premature infant formulas of 0.8 mg of copper/L (100 μg/100 kcal). Whether this amount is adequate or optimal for preterm-LBW infants has yet to be definitively resolved. Tyrala (1986) fed formula containing 110 μg/100 kcal to preterm-LBW infants; the intake achieved was 121 ± 30 μg/kg (SD). Feeding another group of similar infants 260 μg of copper/100 kcal resulted in no further benefit to copper balance or accretion or serum copper or ceruloplasmin levels (Tyrala, 1986). Some, but not all, infants in each group achieved a positive copper balance and the in utero accretion rate for copper (Tyrala, 1986).

The requirement for copper in the transitional period might be met by the recommended concentration because of the presence of copper stores, slow growth rate, and potential for toxicity if liver function is impaired. Requirements for the first 6 months of the postdischarge period will drop slowly as the rate of growth decreases. Copper stores, like those of the term infant for the first 4–6 months of life, are not available to the preterm infant of the same postconceptional age. Accordingly, there is a correspondingly greater dependence on an exogenous source. Therefore, it is probably best that a minimum copper concentration of 0.8 mg/L continue to be provided until 6 months corrected age.

The zinc-to-copper ratio would range from 11:1 to 15:1 for the recommended minimum and maximum zinc concentrations at the minimum recommended copper concentration of 100 μg/100 kcal. This is well below the suggested maximum zinc-to-copper molar ratio of 20:1 (Hambidge & Krebs, 1989).

Maximum. Acute copper toxicity occurs only when very high levels are ingested. Even in Indian childhood cirrhosis, which occurs with moderately elevated intakes and is thought to have a genetic component, calculations indicate a threshold for toxicity of 490 μg/(kg·d). This value is four times higher than calculated requirements. Current domestic preterm formulas contain 125 or 250 μg/100 kcal (Mead Johnson Nutritionals, 2000) (Abbott Laboratories. Ross Products Division, 2001). Tyrala (1986) fed formula containing 260 μg of copper/100 kcal and 1.56 mg of zinc/100 kcal to preterm-LBW infants without any negative effect on zinc balance or serum zinc levels and without any other reported adverse effect. Provision of copper is restricted to any infant with cholestatic liver disease (Hambidge & Krebs, 1989).

The zinc-to-copper ratio would range from 11:1 to 15:1 for the recommended minimum and maximum zinc concentrations at the minimum recommended copper concentration of 100 μg/100 kcal. This is well below the suggested maximum zinc-to-copper molar ratio of 20:1 (Ghitis & Tripathy, 1970).

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of copper in preterm infant formula be 100 μg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum concentration of copper in preterm infant formula be 250 μg/100 kcal.**

MAGNESIUM

Background

Magnesium is the second most abundant intracellular mineral and functions as a cofactor for more than 300 enzymes. See Raiten et al. (1998a), Shils (1999), and Caddell (1991) for reviews. The total body magnesium content of preterm infants (GA of 24–36 weeks) is 17.5–19 mg/100 g of fat-free mass, with an approximate calcium-to-magnesium mass ratio of 35 (Ziegler et al., 1976).

The highest concentration of magnesium in the body is deposited in bone. Preterm infants are generally assumed to need higher amounts of the minerals found in bone than do full-term infants (Venkataraman & Tsang, 1995). This assumption is based on data that preterm infants have a cumulative deficit of bone minerals because they are not in utero during the period when major mineralization occurs (Chen et al., 1995). Poor bone mineralization occurs in more than 30% of preterm infants with a BW of less than 1000 g (Koo & Steichen, 1998), and they are at risk for osteopenia (rickets of prematurity).

Caddell (1991) considered infant prematurity to be one possible cause of magnesium deficiency. Atkinson et al. (1983) concluded that neither preterm mothers' milk nor term formula provides adequate amounts of all macrominerals, including magnesium, if the infant is growing at the normal intrauterine rate. In addition, there was no significant increase in the preterm infants' plasma magnesium concentrations when the mothers' milk was fed during a 4-week period, in contrast to an approximate 20% increase when a modified formula higher in magnesium and other minerals was fed (Atkinson et al., 1983). It is recognized, however, that neither plasma concentrations nor intrauterine accretion rates may be accurate indices of true magnesium status. For example, retention can be elevated after a magnesium load tests, indicating magnesium deficiency even when plasma magnesium is within the normal range.

Previous recommendations

The report *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a) recommended a minimum magnesium content of 4 mg/100 kcal and a maximum of 17 mg/100 kcal for term infants. The AAP-CON (1993) made no formal recommendation for a range of minimum and maximum but listed advisable intakes as 7.5 mg/100 kcal, assuming an energy intake of 130 kcal/ (kg·d) for infants with body weights less than 1201 g. Koo and Tsang (1993) recommended that infant formula provide 6.6–12.5 mg/100 kcal based on an energy intake of 120 kcal/(kg·d). Schanler and Rifka (1994) suggested, on the basis of a review of metabolic studies, that the magnesium content of formula for LBW infants should be higher than 4.9 mg/(kg·d) (4.1 mg/100 kcal). The European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) (1987) recommended a range similar to the recommendation of Koo and Tsang (1993), 6–12 mg/100 kcal. The IOM (1997) made no recommendation for magnesium intake of preterm infants. However, a DRI-AI of 30 mg/d has been recommended for term infants up to 6 months of age (Institute of Medicine. Food and Nutrition Board, 1997). This would indicate an approximate intake of 8.6 mg/(kg·d) for a 3500-g infant. If this amount were appropriate for a preterm-LBW infant, preterm infant formula would need to contain 7.1 mg/100 kcal for an energy intake of 120 kcal/ (kg·d).

Review of the literature

Breast milk

Atkinson et al. (1983) reported that magnesium intakes from mothers' milk were inadequate to provide magnesium retentions equal to normal intrauterine accretion rates of about 2.6 mg/(kg·d) for premature infants of BW less than 1300 g. The subjects were fed their own mothers' milk during a 4-week period. Schanler and Abrams (1995) concluded from a study involving 11 LBW infants, mean GA of 28 weeks, who were fed fortified human milk without added magnesium, that net retention of magnesium approximated the estimated intrauterine accretion rate. On average, milk intake by these infants was approximately 166 mL/(kg·d) (Schanler & Abrams, 1995).

Breast milk magnesium concentrations range from 25 to 35 mg/L (Atkinson et al., 1983; Institute of Medicine. Food and Nutrition Board, 1997; Moser et al., 1988). Two reports indicate that bioavailability of magnesium is 25% lower from preterm infant formula than from breast milk (1993; Schanler & Rifka, 1994): it is 73% from unfortified human milk and 48% from formula (Schanler & Rifka, 1994).

Intrauterine accretion rates

Intrauterine accretion estimates for magnesium during weeks 26–36 of gestation averaged 3.1–3.3 mg/(kg·d), according to an estimate by Koo and Steichen (1998) on the basis of data from Shaw (1973) and Ziegler et al. (1976). For the lowest estimate for intrauterine accretion of 3.1 mg/(kg·d), a retention of 40% would yield a need for an intake of 6.5 mg/(kg·d) [5.4 mg/100 kcal at an energy intake of 120 kcal/(kg·d)].

Metabolic balance studies

Recommendations can be based on metabolic balance studies that determine the daily retention from breast milk and infant formulas varying in magnesium levels. In 17 of 18 balance studies published since 1983, involving 250 preterm infants fed human milk or cow milk-based preterm infant formula, reported retentions were clustered in the range of 3–5 mg/(kg·d) (Atkinson et al., 1983; Cooke et al., 1988; Giles et al., 1990; Lapillonne et al., 1994; Moya & Doménech, 1982; Rigo & Senterre, 1994; Rodder et al., 1992; Salle et al., 1993; Schanler et al., 1985b; Schanler et al., 1988; Schanler & Garza, 1988; Wirth et al., 1990). This retention averaged approximately 40% of daily dietary intake (Koo & Steichen, 1998). Therefore, to provide a net retention of 4 mg/kg, a daily intake of 10 mg/kg would be required, or 8.3 mg/100 kcal for an energy intake of 120 kcal/(kg·d). The balance technique, however, has been criticized as inadequate for determining requirements (Greer, 1989). Also, the majority of the balance studies of preterm infants have been for durations of only 72–84 hours, too short to allow for equilibration and adaptation (Mertz, 1987).

Toxicity

No UL for magnesium intake has been established for infants by the IOM (1997) because of the lack of available data on toxicity in infants or children. The UL for children 1–3 years old is 65 mg/d (5 mg/kg). Wirth et al. (1990) fed 19 mg/100 kcal for 6 days to preterm-LBW infants without reports of adverse effects. Caddell (1988; 1991) administered 24 mg of magnesium during a 24-hour period, orally as the chloride, usually for 2 weeks, to 200 premature infants with apnea neonatorum. She reported that none of the infants showed signs of magnesium toxicity. Greer (1989) recommended an upper limit of magnesium content of 18 mg/100 kcal for term infant formulas on the basis of the magnesium concentration of cow milk.

Conclusions and recommendations

In at least one clinical study (Atkinson et al., 1983), preterm-LBW infants fed their own mothers' milk containing 3.5–4.0 mg of magnesium/100 kcal were unable to achieve a retention rate of 2.7 mg/(kg·d), lower than the estimated intrauterine accretion rate of 3.1 mg/(kg·d). Therefore, a minimum recommendation based on the minimum magnesium concentration in term milk is inadequate. The estimate for the amount of magnesium required to meet the intrauterine accretion rate (5.4 mg/100 kcal) was derived from fetal analyses and estimates of percent retention from metabolic balance studies. Schanler and Garza (1988) demonstrated that preterm-LBW infants fed 6 mg of magnesium/100 kcal were able to retain an average of 2.8 and 4.2 mg/(kg·d) in two, 96-hour balance studies, approximating the intrauterine accretion rate. Giles et al. (1990) fed 6.7 mg/100 kcal to preterm-LBW infants who were able to achieve positive magnesium balance, yet most did not achieve the estimated intrauterine accretion rate. The Expert Panel considered that one domestic preterm formula contains 6.8 mg/100 kcal. Whether this amount is adequate or optimal for preterm-LBW infants has yet to be definitively resolved; however, there is no known adverse effect from feeding domestic preterm infant formula.

In setting the maximum recommendation for preterm formula, there was no evidence to differ from the recommendation of 17 mg/100 kcal set for term formula (Raiten et al., 1998a). It is similar to the upper limit recommended by Greer (1989) for term infant formula. Wirth et al. (1990) fed 19 mg/100 kcal for 6 days to preterm-LBW infants without reports of adverse effects.

<u>*Recommendations*</u>

<u>*Minimum.*</u> **The Expert Panel recommended that the minimum concentration of magnesium in preterm infant formula be 6.8 mg/100 kcal.**

<u>*Maximum.*</u> **The Expert Panel recommended that the maximum concentration of magnesium in preterm infant formula be 17 mg/100 kcal.**

SELENIUM

Background

Selenium functions mainly in association with proteins or as a constituent of them, including enzymes such as glutathione peroxidase (GSHPx) (Rotruck et al., 1973). To date, 11 selenoproteins have been identified, including four types of GSHPx, which play important roles in maintaining health by preventing free radical formation (Tyrala et al., 1996) and oxygen toxicity (Huston et al., 1991). Other selenoenzymes or selenoproteins include thioredoxin reductase, three iodothyronine deiodinases, two selenophosphate synthetases, and selenoproteins P and W, whose biological functions are not established. It is estimated that numerous additional biologically active selenoproteins will be identified in animal tissues (Levander & Burk, 1996). An AI for selenium was established in 2000 with a recommendation of 15 μg/d (2.1 μg/kg) for infants up to 6 months of age (Institute of Medicine, 2000c).

Full-term infant formula based on cow milk marketed in the United States in the 1970s was reported to contain from 7 to 19 μg of selenium/100 kcal (Zabel et al., 1978). This variation apparently reflects the cow's dietary selenium content and its bioavailability. Smith et al. (1982) reported that the selenium

content of eight infant formulas marketed in the United States in the early 1980s ranged from 5.1 to 9.2 μg/L, compared with an average of 16 μg/L in human milk. The concentration of selenium in cow milk from central Illinois was about one-half that of human milk from mothers living in the same region. Litov et al. (1989) reported a content of 13 μg of selenium/L in whey-based unfortified term formula produced in the United States. One preterm infant formula currently marketed in the United States is fortified with sodium selenite and contains 14.5 μg/L (1.8 μg/100 kcal) (Abbott Laboratories. Ross Products Division, 2001). The endogenous selenium content of an unfortified preterm formula is not listed on the composition label but was reported to be 1.34 μg/100 mL (1.7 μg/100 kcal, assuming 810 kcal/L) (Ehrenkranz et al., 1991).

Review of the literature

General reviews of the biochemistry, function, and human nutriture of selenium have been published (Casey & Hambidge, 1985; Gibson, 1990; Institute of Medicine, 2000c; Levander & Burk, 1996; Litov & Combs, 1991; Raiten et al., 1998a; Rayman, 2000; Reifen & Zlotkin, 1993; Yang et al., 1989).

Human selenium deficiency has been reported as caused by an inadequate intake, either enterally or via total parenteral nutrition (TPN), in infants, children, childbearing women, and other adults (Keshan Disease Research Group, 1979; Kien & Ganther, 1983; Levander & Burk, 1996; Litov & Combs, 1991; Sluis et al., 1992; van Rij et al., 1986; Yang et al., 1987; Yang et al., 1989). The major indices available to assess selenium status of premature infants include total daily intake, plasma and serum selenium concentrations, RBC selenium level, plasma and RBC GSHPx values, and urinary excretion (Daniels et al., 1997; Gibson, 1990; Smith et al., 1991). Data are limited for developing normal biochemical and other assessment indices for preterm infants.

Preterm infants are at high risk of oxidative stress-related conditions such as BPD, retinopathy of prematurity, and intraventricular hemorrhage (Sluis et al., 1992). Poor selenium status has been suggested to be a risk factor for BPD associated with prematurity (Sluis et al., 1992). Amin et al. (1980) reported a rapid and dramatic decline in serum selenium concentration within 2 weeks after birth in eight preterm infants who received no oral or parenteral selenium and who developed respiratory distress syndrome. Such patients are usually treated with oxygen therapy that can itself promote oxidative stress. As a component of GSHPx, selenium can function as an antioxidant (Levander & Burk, 1996) and thus could be protective. Lockitch et al. (1989) also reported that preterm infants with chronic lung disease who received no selenium supplementation showed marked decreases in plasma selenium concentrations, with 7 of the 16 infants exhibiting values below the minimum detection level (5 μg/L). However, it has not yet been established whether inadequate selenium status, as reflected by decreasing plasma levels, is causally related to respiratory outcome, intraventricular hemorrhage, and/or retinopathy of prematurity or whether it is secondary to poor general nutritional status associated with respiratory disease or other complications of prematurity, including sepsis (Daniels et al., 1997).

A prospective observational study by Darlow et al. (1995) in New Zealand, a country in which soil and food are low in selenium as compared with those in the United States, examined the relationship of selenium status to chronic lung disease or BPD in 79 preterm infants with BW less than 1500 g or of GA less than 32 weeks. The plasma selenium level fell 30% after birth in the 28 days of the study and was associated with

an increased respiratory morbidity. Moreover, plasma selenium concentrations were significantly lower ($P < 0.001$) in the infants with oxygen dependency at 28 days.

Comparison of selenium in infant formula and breast milk

The selenium intake of infants fed cow milk-based formula has been reported to be markedly lower than the intake of those receiving breast milk in the United States (Smith et al., 1982) and other countries (Lombeck et al., 1978; von Stockhausen et al., 1988). In one study, term infants fed preterm formula commercially available in Germany, Spain, the United Kingdom, or Venezuela had lower serum selenium levels by 50% or more than those fed breast milk (Brätter et al., 1991).

There are conflicting data regarding whether plasma selenium concentrations of newborn preterm infants are similar to those of full-term infants (Daniels et al., 1996; Sluis et al., 1992) or are lower (Daniels et al., 1997; Lockitch et al., 1989). However dramatic declines in plasma or serum selenium concentrations have been reported for to up to 70 days after birth, apparently because of an intake inadequate to meet the needs of the rapidly growing infant (Amin et al., 1980; Casey & Hambidge, 1985; Huston et al., 1982; 1987; Lockitch et al., 1989; Sluis et al., 1992; Tubman et al., 1990; Tyrala et al., 1996). Daniels et al. (1997) observed that plasma selenium levels declined at a more rapid rate in preterm infants who developed chronic lung disease. Lockitch et al. (1989) also reported decreased plasma GSHPx in unsupplemented preterm infants.

Simple selenium deficiency disease has seldom been seen in infants, except for Keshan disease in China and infants receiving long-term TPN therapy without selenium (Gross, 1976; Huston et al., 1987; Kien & Ganther, 1983). Nevertheless, prolonged inadequate intakes can impair biochemical functions that predisposes individuals to illnesses associated with metabolic stress without the development of overt disease (Institute of Medicine, 2000c).

Gross (1976; 1979) reported significant progressive declines in different indices of selenium status in preterm infants fed cow milk-based formulas that had an average content of 14 μg of selenium/L. Although the formula was rich in iron and adequate in vitamin E, the infants developed hemolytic anemia during the study, which continued for more than 60 days. An interaction between selenium and vitamin E is well established in vertebrates, and dietary selenium affects the plasma vitamin E concentration if the concentrations of vitamin E levels are marginal (Thompson & Scott, 1969). Vitamin E supplements have been reported to have no effect on serum selenium concentrations in healthy preterm infants (Amin et al., 1980). Gross (1979) reported an antioxidant synergistic relationship between GSHPx and vitamin E in preterm infants. Casey and Hambidge (1985) suggested that there was no evidence that selenium-vitamin E interrelationships were of practical importance to human nutrition. However, this relationship has not been adequately elucidated in preterm infants, in whom there is a potentially greater need for antioxidants to combat metabolic stress.

Previous recommendations

The report *Assessment of Nutrient Requirements for Infant Formulas* recommended a minimum selenium intake of 1.5 μg/100 kcal (Raiten et al., 1998a) for term infants. This level approximates the estimated mean minus 1 SD of selenium concentration in human milk from mothers in countries in which symptomatic selenium deficiency has not been reported in breast-fed infants.

On the basis of original research and a review of the literature, including measurements of fetal tissues (Casey et al., 1982a; Meinel et al., 1979) and blood indices of selenium status in response to different diets (Amin et al., 1980; Smith et al., 1982), Casey and Hambidge (1985) recommended a minimum selenium requirement of 1 μg/(kg·d) for preterm infants. In a later evaluation, Casey and Walravens (1988) retained this recommendation for the daily requirement for term infants during the first year of life. Later, Hambidge (1989) recommended 3 μg/(kg·d) for preterm infants. More recently, Reifen and Zlotkin (1993) recommended a minimum level of 1.3 μg/(kg·d) (1.1 μg/100 kcal) for the first 14 days of life. This recommendation was later expanded to 1.3–3.0 μg/(kg·d) for the stable and post-hospital discharge growth periods (Zlotkin et al., 1995a).

The AAP-CON (1998) made no recommendation regarding the enteral intake of selenium for preterm infants on the grounds that no deficiency of selenium had been reported in healthy preterm infants fed human milk. The consensus recommendation was from 1.08 to 2.5 μg/100 kcal (Tsang et al., 1993), and the Canadian Paediatric Society (CPS) (1995) recommendation was 2.7–3.9 μg/100 kcal. ESPGAN (1987) also had no recommendations. Perhaps this decision should be reconsidered, because preterm infants fed unsupplemented formula based on cow milk would be receiving considerably less selenium than those fed human milk (Ehrenkranz et al., 1991; Smith et al., 1982). The World Health Organization (1996c) recommended 3 μg/d as the lower limit of selenium intake for infants 0–4 months of age (0.6 μg/kg assuming a body weight of 5000 g). This recommendation was based mainly on extrapolating by weight the lowest mean daily intake of adults living in areas of China where selenium deficiency (Keshan disease) was not evident. No recommended dietary allowance (RDA) was made by the IOM (Institute of Medicine, 2000a) for preterm-LBW infants. However, the current DRI-AI of the IOM (Institute of Medicine, 2000a) for term infants from birth through 6 months of age is 2.1 μg/(kg·d), based on a mean selenium concentration of 18 μg/L in breast milk of unsupplemented but well-nourished mothers and an average milk intake for this age group of 780 mL/d.

Fetal accretion

Although fetal accretion has been used to estimate many of the mineral requirements of preterm infants, no data from direct chemical analysis of selenium of whole fetuses are available. However, Casey and Hambidge (1985) estimated the third-trimester fetal accretion to be about 1 μg/(kg·d), based on extrapolating downward from the adult body content that was determined by compartmental analysis of normal New Zealand women (Stewart et al., 1978) and the selenium concentration of specific tissues obtained from New Zealand fetuses (GA of 22–42 weeks). Based on these data, Casey and Hambidge (1985) estimated the minimum requirement for absorbed selenium for preterm-LBW infants in New Zealand to be 1 μg/(kg·d). This recommendation may be low for preterm-LBW infants in the United States because the soil and food of New Zealand are lower in selenium and thus the selenium status of childbearing women of New Zealand is lower that that of similar women in the United States (Darlow et al., 1995).

Extrapolation from adult requirements

Extrapolation may underestimate the needs of the preterm-LBW infants because they generally have higher nutrient requirements per unit of body weight than either adults or full-term infants (Morley & Lucas, 2000; Schanler & Atkinson, 1999; Venkataraman & Tsang, 1995). Preterm infants also apparently have lower body selenium stores (Aggett, 2000; Bayliss et al., 1985; Smith et al., 1991) and are more subject to postnatal stress and catabolic responses than term infants (Aggett, 2000). Furthermore, they undergo rapid catch-up growth (Hack et al., 1996). Oxidative stress related to oxygen therapy may also increase the turnover of GSHPx.

Breast milk selenium content

Recommendations based on human milk may be difficult to make because of the relatively wide geographic differences in selenium content. Shearer and Hadjimarkos (1975) reported that the mean selenium content of mature milk from 241 subjects living in 17 states in the United States varied more than two-fold among regions. The overall mean was 18 μg/L, with three outliers; the highest outlier was 60 μg/L. These differences have been attributed to variations in maternal dietary intake and selenium status (Brätter et al., 1991; Kumpulainen et al., 1985). In 1989, Levander (1989) summarized the selenium content of breast milk from six countries. It ranged from 2.6 μg/L (in a Keshan disease area in China) to 283 μg/L (in an endemic selenosis area of China). Preterm breast milk from New Zealand mothers was reported to contain 20 μg/L (Sluis et al., 1992), similar to that for mature milk of mothers in the United States, 18 μg/L (Shearer & Hadjimarkos, 1975). However, this preterm breast milk selenium concentration was much higher than values of 7.6 μg/L (Williams, 1983b) or 13 μg/L (Sluis et al., 1992) reported by others for mature breast milk of New Zealand mothers. The authors attributed the higher values to increased blood levels, possibly because of greater consumption of an imported wheat that was higher in selenium content than their domestic crop (Sluis et al., 1992). The CPS (1995) estimated that 120–200 mL/(kg·d) of preterm mothers' milk would be needed to meet the Canadian preterm recommendation of 3.2–4.7 μg/(kg·d).

Clinical studies of supplemental selenium

In 1987, Huston et al. (1987) reported that low serum selenium levels did not improve for 2 weeks after full-feeding volumes with 1.1 μg of selenium/(kg·d) (formula: 0.011 μg/mL, or 1.4 μg/100 kcal) were established. Several of the preterm infants had blood selenium concentrations of less than 10 ng/mL, levels associated with Keshan disease (Huston et al., 1987). Blood concentrations alone should not be relied on for accurate reflection of selenium status, although undetectable levels may signify deficiency (Huston et al., 1987). The activity of GSHPx may be influenced by variations in selenium intake (Tyrala et al., 1996). Friel et al. (1993a) studied the selenium intake and status of 82 preterm-LBW infants, mean GA of 29 of weeks. Finding that RBC GSHPx activity increased when the infants increased their intake of selenium, Friel et al. (1993a) suggested that formula containing 1.2 μg/100 kcal was inadequate. On the basis of their results, they suggested that formula should provide selenium at 20–25 μg/L, or 3–4 μg/(kg·d).

Darlow et al. (2000) conducted a large multicenter, randomized, double-blind placebo-controlled investigation. It was designed to determine the effect of selenium supplementation on the clinical outcome of 534 preterm infants of BW less than 1500 g at 28 days and 36 weeks of postmenstrual age. The specific aim was to determine whether selenium supplementation sufficient to increase the plasma concentrations to be comparable to those of full-term breast-fed infants in the same region was associated with improved clinical outcome. The treatment group received 7 μg of selenium/(kg·d) when fed parenterally or 5 μg of selenium/(kg·d) added to breast milk or infant formula. Plasma selenium and GSHPx values were

significantly lower for the unsupplemented group at 28 days and 36 weeks. There were no significant differences between the supplemented and the placebo groups in the major indices of clinical outcome, including retinopathy of prematurely or positive pressure ventilation. However, lower maternal and infant pre-randomization plasma selenium concentrations were associated with increased oxygen dependency or death. Moreover, the selenium-supplemented infants given steroids had fewer episodes of sepsis after the first weeks of life. However, the relevance of this study suggesting that selenium-supplemented infants experience less sepsis is unclear. These infants were a very select group, and there are many confounders for the incidence of sepsis. Limitations of this study include its location in New Zealand, an area low in selenium intake, and lack of measures of total selenium intake.

These results are in contrast to the results of Smith et al. (1991), who fed 46 preterm infants, BW of 720–1690 g, human milk providing a mean selenium intake of 3.4 μg/(kg·d) (2.8 μg/100 kcal), unfortified preterm formula providing 1.15 μg/(kg·d) (1.0 μg/100 kcal), or fortified formula supplying 4.9 μg/(kg·d) (4.1 μg/100 kcal). Plasma and RBC selenium levels as well as plasma and RBC GSHPx values were not different among the three groups at the end of the 3-week study. However, the interpretation of these results is limited by the significantly lower plasma and RBC GSHPx values at baseline in the group fed fortified formula. All selenium indices measured in preterm infants within all groups were lower than those reported for term infants fed human milk. Evidently the chosen blood variables did not reflect selenium intake. Smith et al. (1991) suggested that the lack of a relationship between selenium intake and plasma selenium indices resulted from low stores, which could have resulted in an immediate need for selenium by the rapidly growing tissue and its quick removal from circulation. Urinary selenium levels did not fall in the group receiving fortified formula, although it did fall significantly in the other two groups.

Tyrala et al. (1996) reported a controlled, randomized, blinded study in the United States that compared the growth and selenium status indices of preterm infants of GA of 30 weeks, or a BW of about 1300 g. Seven infants were fed selenium-fortified preterm formula, 28.4 μg/L (3.5 μg/100 kcal), in the hospital, followed by fortified term formula with selenium at 17.6 μg/L (2.6 μg/100 kcal) after discharge from the hospital. The latter concentration approximates the mean level in breast milk in the United States. Ten infants received unfortified preterm formula containing 10 μg of selenium/L (1.2 μg/100 kcal) in the hospital, and term unfortified formula, 8.6 μg/L (1.3 μg/100 kcal), when at home. The length of the study was 12 weeks. There were no differences in growth between the two groups, although plasma and RBC selenium concentrations were higher for the fortified infants. The plasma selenium level decreased only in the group fed the unfortified formula, whereas the RBC selenium content of both groups declined. Plasma GSHPx showed a significantly greater increase in the fortified group, but not until the final 12th week, indicating that this index reflects long-term status, whereas plasma selenium in this study responded more quickly to supplementation. The investigators concluded that selenium fortification of infant formula improved the selenium status of preterm infants. They suggested that 1.2–1.3 μg of selenium/100 kcal was inadequate for meeting the nutritional needs of some of preterm-LBW infants. One preterm infant formula currently available in the United States is fortified with selenium at the level of 1.8 μg/100 kcal (Abbott Laboratories. Ross Products Division, 1999). Bender et al. (1996) randomly assigned severely ill preterm-LBW infants with BPD in the United States to either selenate-supplemented formula

containing 4.4 μg of selenium/100 kcal or domestic preterm infant formula for 4 weeks beginning on the first day the infants were able to tolerate 120 kcal/kg of formula. At study day 28, plasma selenium and plasma GSHPx values were significantly higher in the infants fed the supplemented formula.

Metabolic balance studies

Few data are available concerning the direct measurement of absorption or retention of selenium in premature infants. Ehrenkranz et al. (1991), who studied 20 preterm infants weighing 720–1630 g, GA of 26–33 weeks, reported 91% absorption of ^{74}Se as selenite added to the commercially available unfortified preterm formula, compared with 86% absorption from product fortified with sodium selenite ($P < 0.05$). A similar amount of ^{74}Se absorbed, about 95%, was retained from both formulas. Employing the traditional metabolic balance technique with no stable isotope, the percent retention of selenium was the same for the unfortified and fortified formulas. The data do suggest that a mean intake of 3.1 μg/(kg·d) is adequate to maintain a positive balance for LBW infants. However, the relevancy of the data to recommendations is questionable, because only a single selenium intake per group was offered and the balance was well above equilibrium for either formula. Mertz (1987) and Greer (1989) have criticized the metabolic balance technique as inadequate for determining requirements for minerals.

Forms of selenium

The most commonly used forms of supplemental selenium in infant formula are sodium selenite and sodium selenate (Mead Johnson Nutritionals, 2000) (Abbott Laboratories. Ross Products Division, 2001) (1996). One preliminary report suggested that selenate may maintain higher blood concentrations in preterm-LBW infants than formula supplemented with selenite (Huston et al., 1996). A preliminary study using rats compared the apparent absorption and retention of selenate to those of selenite from infant formula. The data indicate significantly greater absorption and retention by the animals fed the selenate form of selenium (Mason & Borschel, 1991).

Excessive intakes

Only 3 of 241 breast milk samples from 17 states throughout the United States contained 52–60 μg of selenium/L (approximately 7.5-9.0 μg/100 kcal) (Shearer & Hadjimarkos, 1975). The highest concentration measured, 60 μg/L (45 μg/d), was selected as the no observed adverse effect level (Institute of Medicine, 2000a). However, there was no assessment of the selenium status or general health of the infants fed this high selenium milk to determine whether selenosis or other adverse effects were evident. Considering the no observed adverse effect level, a DRI-UL of 45 μg/d (7 μg/kg) for term infants 0–6 months old was set by the IOM (Institute of Medicine, 2000a). If this UL were appropriate for preterm-LBW infants, preterm infant formula would contain no more than 5.4 μg/100 kcal. Extrapolating from the toxic intakes for Chinese adults reported by Yang et al. (1989), Levander (1989) suggested that 133–160 μg/d would be toxic for a 6000-g infant. Further extrapolation would yield an estimate of about 25 μg/d for a 1000-g infant. However, preterm-LBW infants may be less capable of tolerating high selenium intakes because of immature liver and kidney function.

The margin between deficiency and toxicity has been reported to be narrower for selenium than for many other trace minerals (Olson, 1986). Darlow et al. (2000) fed preterm-

LBW infants more than 4.2 μg/100 kcal from the time enteral feeding was initiated up to 36 weeks of postmenstrual age. No adverse effects, including rash, garlic odor, or diarrhea, from selenium supplementation were evident. As reported in an abstract by Bender et al. (1996), severely ill preterm-LBW infants with BPD were fed 4.4 μg of selenium/100 kcal for 4 weeks beginning on the first day that the infants were able to tolerate a formula intake of 120 kcal/kg. No adverse effects of supplementation were reported.

Conclusions and recommendations

Huston et al. (1987) demonstrated in preterm-LBW infants that 1.4 μg/100 kcal, similar to the minimum amount set for term formula, resulted in serum selenium concentrations associated with Keshan disease and therefore was inadequate. The recommendation for the minimum concentration in preterm infant formula is based on the current amount reported for preterm infant formula, 1.8 μg/100 kcal (Abbott Laboratories. Ross Products Division, 2001). The DRI-AI for term infants 0–6 months old was set at 15 μg/d, or approximately 2.1 μg/(kg·d) (Institute of Medicine, 2000a). Providing this amount for a preterm-LBW infant would necessitate a formula content of selenium of 1.8 μg/100 kcal. Sufficient selenium should be provided to the preterm-LBW infant, because marginal selenium intakes combined with oxygen therapy are thought to impair biochemical functions and predispose to illnesses associated with oxidative stress, such as BPD (Institute of Medicine, 2000c). This recommendation is also within the range suggested by the consensus, 1.08–2.5 μg/100 kcal (Tsang et al., 1993). The CPS (1995) recommended 2.7–3.9 μg/100 kcal. The Expert Panel recommended that the minimum selenium concentration for preterm infant formula be 1.8 μg/100 kcal. Whether this amount is adequate or optimal for preterm-LBW infants has not been definitively established.

There is no evidence that the maximum concentration of selenium in preterm formula should differ from that of term formula, 5 μg/100 kcal (Raiten et al., 1998a). This would provide 6 μg/(kg·d) at an energy intake of 120 kcal/(kg·d), much less than the estimated 47 μg/d intake from breast milk in high selenium areas of the United States, where there has been no reported human selenosis. The recommendation is also similar to the 5 μg/(kg·d) suggested by Olson (1986), who reviewed comprehensively the chronic toxicity of selenium for farm and experimental animals as well as for humans with chronic selenosis. Darlow et al. (2000) fed preterm-LBW infants more than 4.2 μg/100 kcal up to 36 weeks of postmenstrual age. No adverse effects, including rash, garlic odor, or diarrhea, from selenium supplementation were evident. Bender et al. (1996) fed 4.4 μg of selenium/100 kcal to severely ill preterm-LBW infants. No adverse effects of supplementation were reported.

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of selenium in preterm infant formula be 1.8 μg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum concentration of selenium in preterm infant formula be 5.0 μg/100 kcal, similar to that recommended for term infants.**

IODINE

Background and review of the literature

For a review of the assessment of iodine requirements for term infant formula, see Raiten et al. (1998a). A more recent review was published with the DRIs for iodine (Institute of Medicine, 2001c). For specific reviews of the biochemistry, function, and human nutriture relevant to preterm infants, see Casey and Hambidge (1985), Delange et al. (1993), Aggett (2000), Fisher (1998), CPS (1995), and Semba and Delange (2001).

Few data related to establishing the dietary iodine requirements for preterm infants are available. Moreover, the majority of research focused on this subject has been conducted in foreign countries, such as Belgium, where iodine deficiency is endemic and there is no food fortification program.

The deleterious effects of maternal iodine deficiency are especially critical for fetal tissue maturation (Hetzel, 1988; World Health Organization, 1996a), with the brain being particularly sensitive and affected by the sixth intrauterine month (Liu et al., 1983). When there is maternal iodine deficiency, preterm infants have lower serum concentrations of tri- and tetraiodothyronine (T_3 and T_4, respectively) and suffer a higher prevalence of transient hypothyroidism than do term infants (Adams et al., 1995; Fisher, 1998; 1999; Rooman et al., 1996). This condition may contribute to impaired neurodevelopment and school performance problems that have been reported to persist during early childhood (den Ouden et al., 1996; Lucas et al., 1988; Meijer et al., 1992; Reuss et al., 1996). Reuss et al. (1996) reported that severe hypothyroxinemia in preterm infants is associated with a more than four-fold greater risk of disabling cerebral palsy ($n = 463$) and a nearly seven point reduction in the mental development score ($n = 400$) measured at age 2 years. The historical cohort study included preterm infants whose BWs were 500-2000 g and who ranged in GA from 22 to 33 weeks. The recent prevalence of transient primary hypothyroidism in the United States has been 0.12% for LBW infants and 0.41% for LBW infants with the least body weight (Fisher, 1999).

Preterm hypothyroidism resulting in glandular immaturity, although usually related to maternal iodine deficiency, can also be caused to a lesser degree by iodine toxicity in the mother during pregnancy (Bona et al., 1998; Casey & Walravens, 1988). Regardless of whether the condition results from deficient or excessive maternal iodine intake, the treatment for preterm infant hypothyroidism is hormonal and not dietary alterations in the infant's enteral iodine intake.

It is well established that maternal and fetal iodine status is mainly a reflection of the mother's dietary intake, and to a lesser degree exposure to topically applied iodine or radioactive iodine fallout and subsequent thyroid uptake. Dietary intake is associated with the endogenous iodine content of the soil and water and thus the iodine content of foodstuffs and iodine-fortified salt. More than 1.5 billion people globally are at risk for iodine deficiency disorders. These include inhabitants of Europe, Africa, Southeast Asia, the eastern Mediterranean, the western Pacific, and the Americas (Pharoah, 1991; World Health Organization, 1996a). Small regions of United States have iodine-poor soil, but frank deficiency has been rare since the establishment of iodine fortification of salt. As a result, the iodine status of mothers in the United States is considered high, based on breast milk concentrations, compared with that of mothers living in goitrogenic areas outside the United States.

There is no report of preterm infants in the United States becoming iodine deficient when fed formula presently available that contains a minimum of 6 or 25 μg/100 kcal (Abbott Laboratories. Ross Products Division, 2001; Mead Johnson Nutritionals, 2000). However, preterm infants living in low-iodine, goitrogenic countries apparently have a lower iodine status than do preterm infants in the United States. For

example, Delange et al. (1984) reported that preterm infants in Belgium excreted markedly less urinary iodine than did similar infants in California ($P < 0.001$). Moreover, the iodine content of the thyroid glands of 17 preterm Belgian infants who died 1–10 days after birth was 92 μg/g, one-third the value of 270 μg/g for a similar group in California ($P < 0.01$). In addition, a 3-day metabolic balance study of 29 preterm infants and 20 full-term controls in Belgium reported that 40% of the preterm infants had a negative iodine balance even when intake was 17–25 μg of iodine/100 kcal (Delange et al., 1988). However, the relevancy of these data to preterm infants in the United States is questionable because the Belgian mothers were probably of lower iodine status. In addition, there are serious limitations associated with attempting to determine the iodine requirements based solely on metabolic balance studies, especially when their short durations do not allow establishment of equilibrium (Institute of Medicine, 2001c). Nevertheless, balance data were used for estimating the recent iodine AI in children (Institute of Medicine, 2001c). In the United States, no metabolic balance studies involving iodine have been published for preterm infants.

Breast milk iodine content

The iodine content of breast milk from North American women has been reported to range between 140 and 180 μg/L, reflecting the high maternal dietary intakes, and exceeds the dietary requirements of term infants (Reifen & Zlotkin, 1993). Recently, the IOM (2001c) found the iodine content of breast milk to average 146 μg/L (22 μg/100 kcal). The mean iodine concentration of human milk from nongoitrogenic regions throughout the world is lower than that from mothers in the United States and ranges from 59 to 178 μg/L (8.8–26.5 μg/100 kcal) (1998a).

Infant formula iodine content

The preterm infant formulas available in the United States at present contain 6 or 25 μg of iodine/100 kcal (Abbott Laboratories. Ross Products Division, 2001; Mead Johnson Nutritionals, 2000). Ares et al. (1994) reported the iodine content of 127 different preterm formulas used in Europe, Asia, Canada, and the United States. Their data indicated that approximately 50% of the preterm formulas analyzed provided less than the minimum 10 μg/100 kcal recommended by ESPGAN (1987). In addition, the 24-hour iodine intake supplied by preterm infant formulas failed to provide the above recommended intakes for infants of GA 27–33 weeks.

Recommendations for daily enteral intake of iodine by term infants

The report *Assessment of Nutrient Requirements for Infant Formulas* recommended a minimum iodine level of 8 μg/100 kcal (Raiten et al., 1998a) for term infants. This level was extrapolated from the 1989 RDA (National Research Council. Food and Nutrition Board, 1989) of 40 μg/d for 5000-g infants up to 6 months of age. The same report on term infant formula recommended a maximum iodine content of 35 μg/100 kcal (Raiten et al., 1998a). The value was based on the 75th percentile of the FDA analyses of the term infant formulas available. The current U. S. Code of Federal Regulations maximum of 63 μg/100 kcal for term infant formula was considered to be too high. Recently, the DRI-AI for term infants, 0–6 months of age, was set at 110 μg/d [16 μg/(kg·d)] by the IOM (2001c) based on the intake from human milk (22 μg/100 kcal) and urinary excretion. This recommendation represents a 100% increase in the recommended daily intakes for infants 0–6 months of age compared with the 1989 RDA

of 8 μg/(kg·d) (National Research Council. Food and Nutrition Board, 1989).

Previous recommendations for daily enteral intake of iodine by preterm infants

A DRI-AI has not been set for preterm infants. There is an extremely wide range of recommendations for intake of iodine by preterm infants. For example, Hambidge (1989) recommended 4 μg/(kg·d) (3.3 μg/100 kcal), based on an estimated fetal iodine accretion during the third trimester of 1 μg/(kg·d), a 50% retention calculated from term data, and a margin of safety. Reifen and Zlotkin (1993) recommended 30–60 μg/(kg·d), based on several factors: a review of the literature, a personal communication with Delange in Belgium whose balance studies suggested more than 30 μg/(kg·d), and recognition that the iodine content of mature milk of European mothers approximates one-half or less of that of mothers in the United States. Delange et al. (1988) indicated, based on studies in Belgium, that some premature infants may have a negative iodine balance with intakes of less than 25 μg/100 kcal.

A recent study in the United Kingdom involved a randomized trial with 121 preterm-LBW infants (GA of <30 weeks) who were fed preterm formula containing either 68 or 272 μg of iodine/L (8.4 to at least 33.6 μg/100 kcal) (Rogahn et al., 2000). There were no differences in the thyroid hormone levels between groups or reports of adverse effects in infants fed for 10 or more weeks. The authors concluded that increasing iodine content in preterm infant formula to above 68 μg of iodine/L (8.4 μg/100 kcal) was not justified on the basis of their data. Apparently this recommendation was limited to the infant formula manufactured in the United Kingdom. The CPS (1995) recommended 32–64 μg/(kg·d) (27–53 μg/100 kcal), based on the metabolic balance data of Delange et al. (1988). The CPS suggested that if a preterm infant is exclusively breast-fed, an iodine supplement would be needed, because an unlikely amount, 190–375 mL/(kg·d), of preterm mothers' milk would be needed to achieve the recommended intake (Canadian Paediatric Society & Nutrition Committee, 1995). The French Pediatric Society Nutrition Committee recently recommended 20 μg/100 kcal, based on data available in France (Beaufrère et al., 2000). Also cited was the concern that thyroid uptake of environmental radioactive iodine, creating an increased risk of thyroid cancer, would be increased if iodine status were inadequate. ESPGAN (1987) recommended 10–45 μg/100 kcal. The consensus recommendation was 25–50 μg/100 kcal (Tsang et al., 1993), whereas the AAP-CON (1998) recommendation was much lower (5 μg/100 kcal). Higher recommendations for indigenous populations outside the United States may reflect their relatively lower intake of dietary iodine and comparatively low iodine status.

Toxicity

The current recommendation of the AAP-CON (1998) states that an intake higher than "50 mg/100 kcal" (presumably a typographical error for 50 μg/100 kcal) may cause toxic effects in preterm infants. Similarly, ESPGAN (1987) recommended 45 μg/100 kcal as the maximum level. No UL for iodine intake by preterm or term infants 0–12 months of age has been established (Institute of Medicine, 2001c). The UL for children 1–3 years of age is 200 μg/d (Institute of Medicine, 2001c).

Conclusions and recommendations

One domestic preterm formula was reported to contain 6 μg of iodine/100 kcal (Abbott Laboratories. Ross Products

Division, 2001). Ares et al. (1994) reported the concentration of preterm formula used in Canada as approximately 7.5 μg/100 kcal. Given the history of use of domestic preterm formula, the Expert Panel recommended a minimum concentration of 6 μg of iodine/100 kcal. The Expert Panel did not find sufficient evidence specific to preterm infants in the United States to change the maximum level from 35 μg/100 kcal, i.e., that recommended for term infants. Rogahn et al. (2000) fed at least 34 μg/100 kcal to preterm-LBW (GA of <30 weeks) infants for 10 or more weeks and reported no adverse effects related to iodine supplementation. In comparison, the consensus maximum recommendation is 50 μg/100 kcal and that of ESPGAN (1987) is 45 μg/100 kcal.

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of iodine in preterm infant formula be 6 μg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum concentration of iodine in preterm infant formula be 35 μg/100 kcal.**

MANGANESE

Background and review of the literature

Several key functions of manganese are particularly important for the growth and well-being of the preterm-LBW infant. Manganese is a cofactor in mitochondrial SOD, an antioxidant (Morton et al., 1999). In addition, manganese activates the glycosyltransferase that is involved in the synthesis of mucopolysaccharides necessary for the growth and maintenance of connective tissue, cartilage, and bone (Pleban et al., 1985; Zlotkin et al., 1995a).

Manganese homeostasis

Manganese homeostasis is regulated through variable absorption of dietary manganese (Nielsen, 1999; Weigand et al., 1986). Absorption efficiency declines as intake increases (Nielsen, 1994; Weigand et al., 1986). Although the main excretory route for manganese is via secretion in bile (Casey & Walravens, 1988), which appears to be relatively unaffected by dietary intake (Nielsen, 1999), a minute amount of manganese is lost in urine. Adults lose approximately 4 μg of manganese/d (approximately 0.06 μg/kg) or less in urine (Freeland-Graves et al., 1988; Mena, 1980). Healthy, breast-fed term infants (n = 10) excreted an average of 0.07 μg of urinary manganese/kg on the sixth to the eighth day of life (Widdowson, 1969). Daily urinary excretion of manganese in surgically stressed term infants (n = 3) was higher, averaging 2.7–8.9 μg/kg during the first 11–12 days of life (Sampson et al., 1983). Preterm-LBW infants (n = 11) infused with 40 μg/kg of manganese in TPN, however, excreted an average of 0.35 μg of manganese/d in urine, little more than the 0.1 μg/d excreted by those receiving TPN that was not supplemented with manganese (n = 2) (Friel et al., 1988). In general, renal excretion does not serve a regulatory function in manganese homeostasis (Weigand et al., 1986).

Manganese deficiency

Manganese deficiency in young animals mainly affects the structure of cartilage and bone and neurological function (Casey & Walravens, 1988). Depleting animals of manganese impairs skeletal development and produces an abnormality of the inner ear. This results in stunted growth and ataxia in the newborn (Aggett, 1998; Nielsen, 1994). In addition, alter-

ations in glucose tolerance and insulin secretion have been reported (Aggett, 1998). Animals that were deficient in manganese accumulated lipid in the liver and kidney, which was accompanied by hypocholesterolemia (Aggett, 1998). In the rat, deprivation of manganese is associated with abnormal electroencephalographic measures and increased susceptibility to convulsions (Aggett, 1998). Animals are most vulnerable to the effects of manganese deficiency during gestation and early infancy (Casey & Walravens, 1988).

The first reported human case of manganese deficiency was of an adult subject fed an experimental diet deficient in both vitamin K and manganese (Doisy, Jr., 1974). The diet contained 345 μg of manganese/d (approximately 5 μg/kg) (Doisy, Jr., 1974) or 15% of the DRI-AI of 2.3 mg of manganese/d for adult men (Institute of Medicine, 2001d). This deficient diet resulted in weight loss and abnormal signs and symptoms, some of which were atypical of vitamin K deficiency. Biochemical signs of manganese deficiency included decreased levels of serum manganese (55% decrease), total triglycerides, serum phospholipids and cholesterol (decreased from 206 to 80 mg/dL). By observation, his black hair reddened, the growth of hair and nails slowed, and he developed scaly dermatitis. Interestingly, vitamin K supplementation as intravenous phylloquinone did not reverse blood-clotting abnormalities until a diet containing adequate manganese was also provided. Other abnormalities also resolved after he received a normal diet; fecal and urinary manganese excretion increased.

Other cases of human manganese deficiency have since been reported. Seven healthy young men were fed an experimental diet containing 1.1–1.8 μg of manganese/kg (Friedman et al., 1987) that produced negative manganese balances for most of the 39-day depletion period. Biochemical measures indicated bone resorption, and five of seven subjects developed a transient scaly dermatitis (Freeland-Graves & Turnlund, 1996; Friedman et al., 1987). One case of pediatric manganese deficiency was reported for a child aged 4 years who had received TPN deficient in manganese from 9 days of age. Although her short stature and brittle bones may have resulted from several factors, a low serum manganese concentration was evident. In support of the relationship between bone growth and manganese, bone density and longitudinal growth improved after manganese supplementation (Freeland-Graves & Turnlund, 1996). The likelihood of manganese deficiency occurring in preterm-LBW infants is unknown, and the signs of manganese deficiency in these infants remain to be defined.

Blood concentrations of manganese

For infants, concentrations of various measures of blood manganese were two to three times greater than the concentrations for adults. Specifically, measures of manganese in RBCs for preterm-LBW infants (Pleban et al., 1985) and term infants (Hatano et al., 1985), plasma for preterm-LBW infants (Wilson et al., 1992), and whole blood for term infants (Spencer, 1999) are greater than those for adults.

RBC manganese. The mean (± SD) RBC manganese concentration in preterm-LBW infants (n = 48; BW of <1500 g) was 127 ± 62 ng/g of hemoglobin, whereas it was 52 ± 18 ng/g of hemoglobin in adults (Pleban et al., 1985). Studies in Japan, which measured relatively higher concentrations of RBC manganese overall, demonstrated a similar pattern between the concentrations of infants and adults. RBC manganese was two to three times greater in cord blood from term infants and in blood from infants at 1–5 weeks of age and at 6–16 weeks of age (Hatano et al., 1985) than in blood from 13 women 20–40 years of age (Hatano et al., 1983). The mean concentration of

RBC manganese was similar in cord blood and in infant blood collected at 1–5 weeks of age but the concentration of RBC manganese decreased thereafter (Hatano et al., 1985).

Plasma manganese. The mean (± SD) plasma manganese concentration in the cord blood of premature infants (n = 10, GA of 27–36 weeks) was 91.0 ± 20.0 nmol/L, similar to the 100.1 ± 27.3 nmol/L concentration in the cord blood of term infants (n = 46) (Wilson et al., 1991a). The concentration of plasma manganese in cord blood was not correlated with paired maternal blood samples collected at delivery (Wilson et al., 1991a). The mean (± SD) plasma manganese concentration in the mothers of the preterm infants (n = 10) was 78.3 ± 32.8 nmol/L, which was similar to that of mothers of term infants (n = 46; 76.4 ± 34.6 nmol/L) but higher than that measured in nonpregnant women (values not reported) (Wilson et al., 1991a). The reference range for plasma manganese concentration in adults, developed by Rükgauer et al. (1997) from blood donors (n = 68) in Germany, was 14.3 ± 1.4 nmol/L, lower than that reported for women at delivery in Northern Ireland (Wilson et al., 1991a). No sex-dependent differences for manganese were detected in plasma of adults or in serum of children (Alarcon et al., 1996; Rükgauer et al., 1997). Similarly, Wilson et al. (1992) found no difference in the concentration of plasma manganese between male (n = 25) and female (n = 15) preterm-LBW infants on first day postpartum, whose average GA was 28 ± 1.5 weeks (SD) and BW was 1027 ± 222 g (SD) (Wilson et al., 1992). For these infants, the average concentration of plasma manganese at birth was 65.5 nmol/L (Wilson et al., 1992). Subsequent concentrations, measured every 2 weeks up to 12 weeks of life, were not significantly different; these values were higher than the 20.0 ± 10.7 nmol/L (SD) measured in adult volunteers (n = 9) (Wilson et al., 1992).

Furthermore, mean plasma manganese values did not differ between preterm-LBW infants fed maternal breast milk and infants fed preterm formula (Wilson et al., 1992). In that study, manganese intake was not correlated with plasma manganese concentrations (Wilson et al., 1992).

Blood measures of manganese can be influenced by blood transfusion in addition to age. Plasma manganese measures in preterm-LBW infants (n = 20) increased from 69.2 ± 27.3 to 109.2 ± 41.9 nmol/L (SD) by 72 hours after transfusion (Wilson et al., 1991b).

Serum manganese. Stastny et al. (1984) reported that the concentration of serum manganese was not significantly different between healthy term infants (3 months of age) fed human milk (n = 8; 80.1 nmol/L) or formula (n = 16; 85.5 nmol/L), even though the infants fed formula consumed significantly more manganese daily than those fed human milk (183.2 and 0.4 μg/kg of body weight, respectively) (Stastny et al., 1984). The serum values reported by Stastny et al. (1984) were higher than those reported for infants by Rükgauer et al. (1997) and Alarcon et al. (1996).

The reference range for serum manganese developed by Rükgauer et al. (1997) was 23.7–53.7 nmol/L for children aged younger than 6 months (n = 13) and 24.6–52.4 nmol/L for children aged 6 months to 1 year (n = 18). Rükgauer et al. (1997) determined that for children (n = 137, aged 1 month to 18 years), serum manganese concentration exhibited an age-dependent linear decrease, with the highest values in the group younger than 1 year old. From birth to 12 months, there is also an age-dependent decrease in serum manganese value (Alarcon et al., 1996).

Whole-blood manganese. Despite hemodilution during pregnancy, the maternal whole-blood manganese concentration increased significantly from the first prenatal examination at 10–20 weeks GA (n = 33; 150.4 nmol/L) to 25 weeks GA (n = 29; 171.6 nmol/L) to 34 weeks GA (n = 23; 230.0 nmol/L) (Spencer, 1999). Whole-blood manganese concentration in healthy term infants at 3–4 days of age (n = 22; 737.7 nmol/L) was similar to that in cord blood at term (n = 10; 732.3 nmol/L) but was three times higher than that collected from mothers at 34 weeks GA (n = 23; 230.0 nmol/L) (Spencer, 1999).

Summary of blood concentrations of manganese. These studies indicate that, in general, blood manganese levels increase during pregnancy (Spencer, 1999) for both mothers of term and preterm-LBW infants (Wilson et al., 1991a), and at delivery the concentration is increased further in cord blood (Spencer, 1999; Wilson et al., 1991a). Furthermore, the blood manganese concentration in cord blood is similar for preterm-LBW infants and term infants (Wilson et al., 1991a). In addition, the elevated blood manganese concentrations at birth are apparently maintained through 5 weeks of age in term infants (Hatano et al., 1985) and up to 12 weeks of age in preterm-LBW infants (Wilson et al., 1992), regardless of whether the infant was provided with breast milk or formula (Stastny et al., 1984; Wilson et al., 1992). Furthermore, there is an age-dependent decrease in serum manganese as the child grows (Rükgauer et al., 1997).

Factors such as developmental status, age (Rükgauer et al., 1997), blood transfusion (James & MacMahon, 1970; Wilson et al, 1991b), and iron status (Pleban et al., 1985) (Mena et al., 1969) may alter the manganese concentration in blood. Of these, developmental status is of particular interest. As mentioned, blood manganese concentrations increase during pregnancy and result in high concentrations in the fetus, which are maintained for several months in the preterm-LBW infant. This suggests that the preterm-LBW infant may have a relatively higher requirement for manganese, or that excretion is impaired and/or accumulation of manganese is an opportunistic consequence of an iron deficit (See below).

Manganese concentration in solid tissues and fetal accretion

In the fetus (n = 40; GA of 22–43 weeks), the concentration of manganese is highest in the liver compared with tissue from kidney, brain, heart, lung, skeletal muscle, and bone (Casey & Robinson, 1978). Similarly, the greatest tissue concentration of manganese in adults is in liver (Casey et al., 1982b; Schroeder et al., 1966). Widdowson et al. (1972) determined that the mean concentration of 0.13 mg of manganese/100 g of liver (range: 0.06–0.23 mg/100 g) in the fetus was the same throughout gestation (n = 30; GA of 20–41 weeks); likewise, concentration was similar to the mean of 0.18 mg of manganese/100 g of liver in adults (n = 5). Studies in New Zealand also found that the concentration of manganese in fetal tissues did not vary with GA during the last trimester of pregnancy (Casey & Robinson, 1978). There was no difference between concentrations in fetal and adult liver (Casey et al., 1982b; Casey & Robinson, 1978). In contrast, the mean concentration of manganese in fetal lung tissue was approximately twice that of adults (Casey et al., 1982b; Schroeder et al., 1966). Similarly, concentrations of manganese in the heart, skeletal muscle, and bone were significantly greater in the fetus than in the adult (Casey et al., 1982b). Interestingly, fetal skeletal muscle (n = 40; GA of 22–43 weeks) had a greater concentration of manganese than did skeletal muscle of children (n = 11) aged 1 week to 4 years (Casey et al., 1982b). These results suggest that manganese accumulates in several growing fetal tissues rather than in one storage pool. In New Zealand, Casey (1976) analyzed for manganese concentration in tissue from 38 infants who were

stillborn or survived less than 24 hours. Using her estimates of total body manganese, average rates of daily manganese accretion by the fetus were calculated for the following periods: 6.8 μg/d between measures taken from 22–25 weeks GA to 26–29 weeks GA; 7.1 μg/d between measures taken from 26–29 weeks GA to 34–37 weeks GA; and 10.8 μg/d between measures taken from 34–37 weeks GA to 38–41 weeks GA. The average daily rate of manganese accretion was similar for the approximate time periods of 28–32 weeks GA (7.3 μg/d) and 32–36 weeks GA (6.9 μg/d), despite body weight increases of 450 g and 750 g, respectively. Body weights for the GAs of these infants, obtained in the early 1970s, were generally lighter than the BW of infants born alive 10 or more years later (Arbuckle et al., 1993). Therefore, daily rates of accretion derived from Casey (1976) may underestimate the current rate of manganese accretion by the fetus.

Manganese concentration in human milk

Although the concentration for manganese in human milk varies considerably, ranging from 1.4-28 μg/L (Casey et al., 1989; Krachler et al., 2000; Stastny et al., 1984; Vaughan et al., 1979), on average, mature breast milk from women who gave birth to term infants in the United States and Europe contains 3.2-6.6 μg manganese/L (0.5-1.0 μg/100 kcal, assuming an energy content of 670-680 kcal/L) in the first months postpartum (Casey et al., 1985; Dörner et al., 1989; Krachler et al., 2000; Stastny et al., 1984; Vuori et al., 1980).

Perinatal manganese absorption, excretion, and retention

Maternal absorption and retention.   In animals, manganese absorption, excretion, and retention were altered during gravidity and lactation (Kirchgessner et al., 1982a). Specifically, the apparent absorption and total retention of manganese increased during gravidity in sows, and jejunal absorption increased in the last third of gravidity and during the first 10 days of lactation in dams (Kirchgessner et al., 1982a).

Manganese balance by term infants.   The degree of maturity of the GI system may influence the absorption and retention of manganese by infants. At 1 week of age, 11 healthy term infants fed breast milk excreted at least five times more manganese in the feces than they consumed in milk, leading to a negative balance (Widdowson, 1969). Likewise, surgically stressed term infants ($n = 3$) remained in negative balance (–14.7 to –63.2 μg/kg) for at least the first 11–12 days postpartum, excreting two to four times as much manganese in feces as consumed orally (Sampson et al., 1983). At about 2 weeks postpartum, 43% (three of seven) of manganese balance studies in healthy, breast-fed term infants were negative (Dörner et al., 1989). For three term infants who had phenylketonuria, 30% (3 of 10) of balance studies had negative results from 2 to 16 weeks postpartum, despite a median daily consumption by the infants of 78.4 μg of manganese/kg (range: 55–110 μg/kg) in special formula (Sievers et al., 1990). Overall, eight healthy term infants who were fed formula with approximately 14 μg of manganese/kg had 23% negative (7 of 30) balance studies during the first 2–16 weeks postpartum (Dörner et al., 1985; Dörner et al., 1989). In contrast, 11 healthy term infants fed breast milk containing even less manganese ($\sim$ 1.1 μg manganese/kg body weight) than term formula had only 5% (2 of 42) negative balance studies during the first 2–16 weeks postpartum (Dörner et al., 1985; Dörner et al., 1989).

Manganese balance by preterm infants.   Manganese balance studies were more likely to be negative during the first 2–16 weeks postpartum for LBW infants than for term infants (Dörner et al., 1985). For six LBW infants (GA of 34–36 weeks) who were fed formula with 15.0 μg of manganese/kg, a high excretion of fecal manganese caused 48% (10 of 21) of balance studies conducted during the first 2–16 weeks postpartum to be negative (Dörner et al., 1989). The amount of manganese actually absorbed or lost via endogenous secretions by infants in these studies cannot be quantified. The authors suggested that the negative manganese balances among preterm-LBW infants were caused by a high fecal excretion of manganese (Dörner et al., 1989).

Enterohepatic circulation and excretion.   Widdowson (1969) attributed the excessive loss of manganese in the early neonatal period to a diminished ability by infants to reabsorb manganese secreted into the GI tract with bile, perhaps because the manganese remained in a bound form. Widdowson (1969) assumed that neonatal infants would be capable of secreting a relatively large amount of manganese in bile despite less synthesis and lower concentration of bile in the intestines than adults (Watkin et al., 1975). In rats, bile flow rates [mL/(kg·h)] were 50–75% lower for 14-day-old animals than for adult animals (Ballatori et al., 1987). Preterm-LBW infants have even less bile synthesis and concentration of bile in the intestines than do term infants relative to body weight and body surface area (Watkin et al., 1975).

Other investigators (Miller et al., 1975) have suggested a different perspective than that of Widdowson (1969). In a study by Miller et al. (1975), 7-day-old mice were injected intraperitoneally with ^{54}Mn. Excretion of ^{54}Mn was determined indirectly by comparing the whole-body ^{54}Mn activity obtained at 4 hours after injection with subsequent levels of radioactivity; ^{54}Mn in excrement and in mother's milk was not measured. The investigators suggested that neonatal mice had an avid retention of manganese for the first 17 days of life, perhaps because of an inability to excrete manganese, at least in sufficient quantities necessary to be detected. However, because the pups were housed with dams until the time of weaning, the dams may have consumed ^{54}Mn while grooming the pups or through coprophagy. Therefore, ^{54}Mn might have been excreted by the pups and recirculated back to the pup through milk. Yet, Ballatori et al. (1987) determined that dams ingested only minimal amounts of ^{54}Mn in this manner.

More important, in support of Widdowson (1969), Ballatori et al. (1987) demonstrated that within 1 day of intravenous infusion, intraperitoneal injection, or GI gavage of larger amounts of manganese than that given by Miller et al. (1975), the neonatal rat (postnatal day 8–9) could eliminate manganese. Ballatori et al. (1987) concluded that the neonatal rat had the capacity to avidly retain manganese when dietary intake was low and excrete manganese by a saturable process when intake was in excess of need. Because the process of excretion was saturable, toxicity from an overload of manganese was possible, although not evident (Ballatori et al., 1987). Altogether, these studies raise the question of whether developmental immaturity has an impact on the initial absorption and enterohepatic circulation of manganese in the preterm-LBW infant and what effect, if any, this has on the need and/or tolerance for dietary manganese.

Age and absorption of manganese.   For young rodents, the rate of manganese absorption is known to be highest in the early postnatal period (Kostial et al., 1978; Kostial et al., 1980). In rats, the absorption of manganese from dam milk that was incubated with ^{54}MnCl$_2$ depended on age, decreasing from 8 to 13 days of life (Raghib et al., 1986). Furthermore, there was a sharp decline in jejunal absorption by the rat from 18–24 days of life (Kirchgessner et al., 1982b). Knudsen et al. (1995) determined that rat pups absorbed 85.5 $\pm$ 1.3% (SEM)

of manganese from preterm infant formula labeled with ^{54}Mn by incubation.

The absorption of ^{54}Mn from infant formula fed to human adults ranges from 0.8% to 16.0% (Davidsson et al., 1989b) and averages approximately 6–8% (Davidsson et al., 1989b, Sandstrom et al., 1986). After reviewing two reports published in German, Rükgauer et al. (1997) suggested that manganese absorption in neonates might be higher than that in adults. Compared with adults, both preterm and term infants have shown increased total retention of manganese (Mena, 1980). Ten days after ingestion of 0.5 μCi of ^{54}MnCl$_2$ with a carrier of 10 μg of ^{55}MnCl$_2$, total body retention of manganese was 15.6 $\pm$ 3.3% (SEM) in preterm infants (GA of 32–34 weeks), 8.0 $\pm$ 2.0% (SEM) in newborn term infants, and 1.6 $\pm$ 0.17% (SEM) in adults (Mena, 1980). Mena suggested (1981) that preterm infants had a greater percentage of intestinal absorption of manganese than did term infants and adults, increasing their susceptibility to manganese toxicity. Whether absorption of exogenous manganese is more efficient in preterm-LBW infants than in term infants, whether they have greater requirements for manganese, or whether secretion and reabsorption of endogenous manganese are less efficient remains to be clarified.

Minimal requirement of absorbed manganese for balance. Two preterm-LBW infants who received manganese only as a contaminant in TPN (0.8 and 2.1 μg/d) were able to maintain a neutral or slightly positive manganese balance (+0.7 and +2.0 μg/d, respectively) (Friel et al., 1988). Their actual body weights were not reported but were classified as "very low BW"; the mean BW for other infants in the report was 909 g. Measures of these infants suggest that the requirement for absorbed (or infused) manganese may be about 1.0 μg/d for preterm-LBW infants in the first 2–3 weeks of life. This is further supported by another study, in which 10 of 13 infants (7 preterm-LBW) were able to maintain a positive manganese balance when receiving 0.8 $\pm$ 0.5 μg of manganese/kg as a contaminant in TPN (Zlotkin & Buchanan, 1986). The recommendation for manganese in TPN for preterm-LBW infants is 1 μg/kg of body weight; however, supplementation is withheld when cholestatic liver disease is present (Fell et al., 1996; Greene et al., 1988; National Advisory Group on Standards and Practices for Parenteral Nutrition, 1998).

Manganese absorption in relation to other minerals

Relation to iron. In mice and rats, dietary iron deficiency increases the duodenal and jejunal absorption of manganese (Flanagan et al., 1980; Thomson et al., 1971). In humans, manganese absorption was 2.5–5.4 times greater in adult patients with iron deficiency compared with those with normal iron stores (Mena et al., 1969; Sandstrom et al., 1986; Thomson et al., 1971). Anemic adults have significantly higher RBC manganese concentrations than do healthy adults (Mena et al., 1969). Pleban et al. (1985) reported one case of an anemic human neonate whose RBC manganese concentration was more than twice the upper limit of normal and decreased to normal after iron supplementation. Under conditions of iron deficiency in rats, the addition of supplemental iron was associated with a significant reduction in manganese absorption compared with control rats without supplementation (Thomson et al., 1971). Thomson et al. (1971) suggested that iron competitively inhibited the absorption of manganese. The effect of iron supplementation on manganese absorption may depend on age. For example, Kostial et al. (1980) demonstrated that supplemental iron added to cow milk decreased the retention of an oral dose of ^{54}Mn in 6-week-old rats but not in 6-day-old rats. Although iron deficiency increases man-

ganese absorption in both rats and humans, there was no difference in retention of manganese 11 days after dosing in rats and after 10 days in humans because of increased excretion of manganese (Thomson et al., 1971). Therefore, there may be no long-term impact of iron nutriture on manganese status in adults, at least in manganese-replete subjects (Thomson et al., 1971). Clearly, iron status and iron supplementation have an influence on the uptake of manganese, but the implications for preterm-LBW infants are not known. In a report (Health Canada Health Protection Branch, 1995) of an Ad Hoc Expert Consultation (AHEC) to the Health Protection Branch, Health Canada (HPB-HC), it was recommended that the ratio of iron (in mg) to manganese (in mg) be 30:1 to 120:1 in preterm infant formula, assuming a range of iron concentrations of 0.15–3.0 mg/100 kcal. For a range of 1.7–3.0 mg of iron/100 kcal, a ratio of 120:1 would translate into a manganese concentration of 14.2–25 μg/100 kcal.

The effect of iron supplementation on manganese absorption and retention may depend on the overall iron status of the individual. In studies of adults given an extrinsically labeled test meal, a change in manganese absorption could not be detected by whole-body counting after the addition of 5 mg of iron to wheat bread (Davidsson et al., 1991), nor was change in manganese absorption in adults detected between whey-predominant term formula and the same formula fortified with additional iron (Davidsson et al., 1989a). However, among women whose intake was recorded for 124 days, those who had a relatively high intake of nonheme iron had lower concentrations of serum manganese and lymphocyte manganese SOD and higher urinary manganese excretion than women with relatively low intake of nonheme iron (Davis et al., 1992a). This suggests that, over time, nonheme iron consumption can have a negative effect on manganese status. Weanling rats (Davis et al., 1992b) that were fed high intakes of iron for 7 weeks exhibited reduced absorption of manganese. In that study and in one (Keen et al., 1984) of weanling mice that were fed high amounts of iron for 4 weeks, the concentration of manganese in liver was reduced, possibly indirectly by competitive inhibition of manganese by iron, decreasing uptake into the mucosal cells (Davis et al., 1992b). On the basis primarily of results from healthy term infants, Dörner et al. (1989) concluded that iron-supplemented formula (10.1 mg/L) did not alter manganese retention when compared with unsupplemented formula (1.1 mg/L). However, among the preterm-LBW infants in that study, those consuming iron-supplemented formula (n = 3; 15 balance studies) showed a negative retention of −0.62 $\pm$ 6.63 μg/kg (SD) of manganese compared with a positive 1.74 $\pm$ 3.20 μg/kg (SD) retained by those fed unsupplemented formula (n = 3; 6 balance studies) (Dörner et al., 1989). Small subject numbers limit the interpretation of these results, as do the wide variations in manganese retention, lower manganese intake by the group consuming the iron-supplemented formula, and a lack of direct measure of manganese absorption. Whether the current iron-enriched preterm infant formulas impede the achievement of optimal manganese status in preterm-LBW infants is not known.

Relation to calcium. Calcium is another factor that may interfere with manganese absorption and alter the manganese requirement. Supplementation of 80 mg of calcium/100 mL of a test meal of banked human milk (32 mg of calcium/1 μg of manganese) resulted in a significant decrease in manganese absorption by adults (n = 9) compared with diets containing 5.8 mg of calcium/1 μg of manganese (Davidsson et al., 1991). In animals, high intakes of calcium increase the requirement for manganese (Schroeder et al., 1966). Because the calcium

concentration (per 100 kcal) in current preterm infant formulas is more than double that of term formula (Abbott Laboratories. Ross Products Division, 2001; Mead Johnson Nutritionals, 2000), the manganese in preterm formula may be less bioavailable, and its concentration may need to be increased over that in term formula. In one study (Lönnerdal et al., 1994), the absorption (% of dose) of manganese by suckling rats from preterm formula was lower than that from cow milk-based term formula and was similar to that from soy milk-based formula. In another study (Knudsen et al., 1995), absorption (% of dose) was similar for preterm formula and fortified- and unfortified milk from mothers of preterm infants. Overall, for suckling rats, the bioavailability of manganese from infant formulas is comparatively high (Knudsen et al., 1995; Lönnerdal et al., 1994). The minimum recommended concentrations of calcium and manganese for term formula are 50 mg/100 kcal and 1 μg/100 kcal, respectively, a 50 mg:1 μg ratio (Raiten et al., 1998a). Achieving this ratio or lower in preterm formula, for which the minimum recommended calcium concentration is 123 mg/100 kcal, would necessitate a minimum concentration of 2.5 μg of manganese/100 kcal.

Relation to nickel. When the normal 60:1 ratio of manganese to nickel in the diet of pigs was changed by supplementing nickel to a ratio of 1:1, manganese secretion in bile was reduced by approximately 50%, despite a significant increase in bile flow rate (Kirchgessner et al., 1990). The antagonistic effect between nickel and manganese may have occurred at the luminal site of absorption because parenteral administration of nickel had no effect and nickel was not detectable in bile (Kirchgessner et al., 1990). Nickel is not added to preterm infant formula (Abbott Laboratories. Ross Products Division, 2001; Mead Johnson Nutritionals, 2000). Therefore, nickel interactions with manganese were not considered in making recommendations for preterm formula.

Clinical factors affecting requirements

Oxygen therapy. Preterm-LBW infants, particularly the youngest infants, often require oxygen therapy (Hack et al., 1991). Pleban et al. (1985) hypothesized that manganese deficiency might predispose an infant on oxygen therapy to increased tissue damage from superoxide radicals because manganese is required for manganese SOD. In baboons, lung manganese SOD activity increases throughout the third trimester of gestation (Morton et al., 1999). When exposed to 100% oxygen, premature baboons showed increased expression of lung manganese SOD mRNA. However, the manganese SOD specific activity in the lung did not change, possibly because of posttranslational modification of the protein (Morton et al., 1999). Strange et al. (1990) determined that expression of manganese SOD in lung tissue was similar for preterm infants and adults, whether or not the infants developed respiratory distress with or without BPD. There was no correlation between exposure to oxygen and lipid peroxidation in lung tissue (Strange et al., 1990). It is not known definitively whether oxygen therapy increases the requirement for manganese in preterm-LBW infants.

Biliary function. Because bile is the main excretory route for manganese and because neonatal infants synthesize less bile (Watkin et al., 1975), preterm-LBW infants may be less able to excrete excess manganese than older children and adults. Infants with extrahepatic biliary atresia accumulate more than twice the reference concentration of manganese in hepatic tissue (Bayliss et al., 1995). Therefore, infants with biliary obstruction or other liver disorders are at increased risk of toxicity from an elevated intake of manganese (Bayliss et al.,

1995; Casey & Hambidge, 1985; Fell et al., 1996; Greene et al., 1988).

Dietary forms of manganese

Very little is known about the bioavailability of various chemical species of manganese (U.S. Environmental Protection Agency, 2001). In human milk, manganese is in the trivalent form bound to lactoferrin, allowing for some regulation of absorption. In infant formulas, manganese is in the divalent state, and its absorption is not regulated through lactoferrin receptors. (U.S. Environmental Protection Agency, 2001). Aside from manganese introduced to formula through naturally occurring incidental contamination, preterm infant formula (Abbott Laboratories. Ross Products Division, 2001) and formula sometimes used for preterm-LBW infants in the United States, although not designed for this purpose (SHS North America, 2000), have been supplemented with manganese as manganese sulfate.

Adverse effects of manganese exposure

Effects on iron status. Whether or not a high concentration of manganese in infant formula might influence iron absorption was considered. Manganese supplementation at a manganese-to-iron ratio (mg:mg) of 2.5:1 in a test meal has been shown to significantly reduce iron absorption in healthy adults ($n = 98$), possibly by direct competitive inhibition at common binding sites in the gut mucosa (Rossander-Hultén et al., 1991). Increasing the manganese-to-iron ratio from 2.5:1 to 9.9:1 had no further impact on iron absorption, hematocrit, or concentration of iron in tissues of weanling rats fed a marginal amount of iron (Davis et al., 1992b). Supplemental manganese was shown to cause anemia (low hemoglobin, serum iron, and hepatic iron values) in young lambs (Hartman, 1955). In addition, supplemental manganese retarded the regeneration of hemoglobin in lambs that were bled to cause anemia (Hartman, 1955). If the maximum concentration of manganese in preterm formula were 0.1 mg/100 kcal, which is the recommended maximum for term formula, and the minimum amount of iron in preterm formula were 1.7 mg/100 kcal, the manganese-to-iron ratio would be 0.06:1, far less than that known to inhibit iron absorption. Therefore, an effect of manganese concentration in preterm formula on iron absorption does not appear to be a practical concern.

Effects on rodents. Differences in route of exposure can lead to profound differences in metabolism and toxicity of chemicals. Chronic inhalation of manganese is known to affect the development of mice. Mice exposed to manganese dust (MnO_2) produced offspring that were growth restricted and had significantly lower scores on activity tests than did control mice (Lown et al., 1984). In 6-week-old mice that were fed a diet containing high amounts of manganese, weight gain was reduced from normal by 9 months of treatment and growth remained depressed as long as 12 months of treatment (Komura & Sakamoto, 1992). Excessive oral intake of manganese is also known to alter the distribution of manganese in suckling rats. The manganese concentration in the brain of rat pups was significantly increased by 15 days in those pups whose dams had received excess manganese daily by gastric intubation from the second day of lactation (Seth et al., 1977). In addition, significant alterations in the enzymatic activity in the brain of rat pups suggested that exposure to excess manganese could cause functional impairment at neurotransmitter levels (Seth et al., 1977), thus affect behavior (Chandra et al., 1979). Similarly, in the young rat treated with manganese by gastric intubation, the manganese concentration increased in the brain with time up to postintubation day 7 (Keen et al.,

1986) and postnatal day 21 (Dorman et al., 2000). Rats older than 15 days at treatment did not display a similar uptake of manganese by the brain (Keen et al., 1986). These results suggested that the developing central nervous system of neonatal rats may be particularly susceptible to the effects of an excessive intake of manganese (Dorman et al., 2000; Keen et al., 1986). Two-week-old suckling rats had lower median lethal dose values for manganese per kg of body weight compared with older rats (Kostial et al., 1978). Therefore, Kostial et al. (1978) suggested that neonatal rats had an increased risk of toxicity if exposed to high levels of manganese.

Long-term exposure in adults. In general, homeostatic mechanisms are thought to protect adults from exposure to excess oral intake of manganese. However, older persons living in areas where the manganese concentration is elevated in drinking water score worse on neurological tests compared with those living in areas having lower concentrations of manganese in drinking water (Kondakis et al., 1989). Using the Kondakis et al. (1989) study, Velazquez and Du (1994) calculated the lowest observed adverse effect level and no observed adverse effect level for daily manganese intake in drinking water as 60 and 5 μg/kg, respectively. However, because of limitations with estimates of dietary consumption of manganese, the Environmental Protection Agency opted not to include the Kondakis et al. (1989) study among those used to determine a quantitative dose-response relationship for the toxicity of manganese in humans (U.S.Environmental Protection Agency, 2001). The Environmental Protection Agency set the no observed adverse effect level for the total oral intake of manganese at 140 μg/(kg·d) for a 70-kg adult (U.S. Environmental Protection Agency, 2001). A lowest observed adverse effect level for manganese was not set (U.S. Environmental Protection Agency, 2001).

Chronic exposure to manganese ore during mining can lead to brain lesions and irreversible Parkinson-type deficits (Mena, 1981). Some classic signs of manganese intoxication in adults are mental lethargy, physical rigidity, slowness of movement (bradykinesia), loss of postural reflexes, and impairment of gait and balance (Mena, 1981).

Toxicity of parenteral manganese. Manganese toxicity has been reported in children as young as 4 and 5 months after they received 54.9 μg of manganese/kg via TPN for 2 months or more (Fell et al., 1996). Elevated whole-blood manganese concentration was associated with cholestatic liver disease in some (Fell et al., 1996; Reynolds et al., 1994) but not all (Quaghebeur et al., 1996) of these types of pediatric cases. Some children also had abnormal posturing, generalized seizures, and evidence of damage to the basal ganglia after long-term intravenous administration of manganese (Fell et al., 1996; Komaki et al., 1999; Quaghebeur et al., 1996; Reynolds et al., 1994). Those children who received at most 33 μg/kg daily via TPN for more than 2 years were clinically asymptomatic, yet they had evidence of abnormal manganese deposition in the basal ganglia and/or subthalamic region of the brain accompanied by elevated whole-blood manganese values (Quaghebeur et al., 1996). Friel et al. (1988) suggested that provision of manganese at 40 μg/kg daily in TPN might be excessive for preterm-LBW infants.

In adults, abnormal high signal intensity on T1-weighted magnetic resonance images of the brain were reversibly and reproducibly related to the intravenous administration (1.1 mg/d) or withdrawal of manganese; whole-blood manganese values followed a similar pattern (Takagi et al., 2001).

Implications for preterm formula. The uptake of manganese by body tissues may vary by route of feeding (oral or parenteral) (Davidsson et al., 1989c; Mena, 1980). However, without conclusive evidence to the contrary, we should consider that an intestinal absorption of high amounts of manganese by preterm-LBW infants might lead to the adverse effects seen in pediatric cases after manganese overload in TPN. Additional key considerations are the relatively high absorption (% of dose) of manganese from preterm infant formula demonstrated by suckling rats (Knudsen et al., 1995) and the immaturity of biliary function in early life (Ballatori et al., 1987; Watkin et al., 1975). Therefore, it may be prudent to set the maximum concentration in formula so that it will not exceed 40 μg/kg (33.3 μg/100 kcal for a 1000-g infant consuming up to 120 kcal/d). This amount would provide sufficient manganese to achieve realistic goals of nutrient accretion and growth rate in preterm-LBW infants and appears to be within a range of intake that is without known adverse effect.

Previous manganese recommendations

The DRI-AI for manganese for healthy, 0- to 6-month-old infants was set at 3 μg/d (0.4 μg/kg), reflecting the mean manganese intake of term infants fed human milk as their principal food (Institute of Medicine, 2001d). The recommended minimum manganese content of term formula is 1 μg/100 kcal (Raiten et al., 1998a), which if applied to preterm formula would provide 1.2 μg/(kg·d) to infants consuming 120 kcal/d. For preterm infant formula, the AAP-CON (American Academy of Pediatrics. Committee on Nutrition, 1998) recommended more than 5 μg of manganese/100 kcal; a consensus of other experts recommended 6.3 μg/100 kcal (Tsang et al., 1993); ESPGAN (American Academy of Pediatrics. Committee on Nutrition, 1998) recommended 1.5–7.5 μg of manganese/100 kcal. In June of 1995, the CPS (Canadian Paediatric Society & Nutrition Committee, 1995) advised use of between 10 and 20 nmol/kg, or 0.5–0.9 μg of manganese/100 kcal. Later that year, the AHEC HPB-HC (1995) recommended 0.1–0.45 μmol/100 kcal, or 5.5–25.0 μg of manganese/100 kcal. The AHEC HPB-HC acknowledged that naturally occurring incidental contamination of manganese would most likely provide the minimum amount of manganese suggested. They based the maximum value on data from preliminary reports of a study (Atkinson & Shah, 1991) in which preterm infants had a positive manganese balance after consuming about 22 μg/(kg·d) (Zlotkin et al., 1995a) (or approximately 21–26 μg of manganese/100 kcal, assuming 810 kcal/L) (Wauben et al., 1998) and fecal losses were about 11 μg/(kg·d). At present, preterm infant formulas provide 6.3–12.0 μg/100 kcal, which would provide 7.6–15.6 μg/d for infants consuming 120–130 kcal/d (Abbott Laboratories. Ross Products Division, 2001; Mead Johnson Nutritionals, 2000). An amino acid-based, hypoallergenic formula, not designed for use with preterm-LBW infants but sometimes used in the United States for such infants with food protein intolerance or allergy, contains 90 μg of manganese/100 kcal (SHS North America, 2000). From a 24-hour dietary recall provided by mothers, Gibson and Dewolfe (1980b) calculated that 37 preterm-LBW infants (16% breast-fed infants) consumed an average of 9 μg of manganese/100 kcal (15 μg/kg) at 1 month of age.

Conclusions and recommendations

The overall goal for formula-fed preterm-LBW infants is that they absorb and retain manganese in amounts that support optimal growth and development. Indices such as RBC manganese concentrations indicate that preterm-LBW infants may have an increased retention of manganese, similar to that of fetal growth in the third trimester. Because calcium concentration influences the absorption of manganese, the Expert

Panel considered providing at least the same ratio of calcium to manganese (50 mg of calcium to 1 μg of manganese) as provided by the minimum recommendations for term formula (Raiten et al., 1998a). The minimum calcium recommendation of 123 mg/100 kcal in preterm formula implied a concentration of at least 2.5 μg of manganese/100 kcal to achieve a ratio of 50 mg of calcium to 1 μg of manganese. This would also provide the DRI-AI of 3 μg manganese/d recommended for term infants of 0–6 months. Because of naturally occurring incidental contamination, it is recognized that preterm formulas will most likely contain higher concentrations of manganese. For example, one preterm formula (Mead Johnson Nutritionals, 2000) currently available does not contain any added manganese yet contains 6.3 μg/100 kcal derived from naturally occurring incidental contamination in whey, calcium salts, and ferrous sulfate (Lönnerdal, 1989). Infants with an energy intake of 120 kcal/d would receive approximately 7.6 μg manganese/d from this formula. It is not known whether the amount of manganese absorbed from a preterm infant formula not supplemented with manganese is adequate to support the fetal manganese accretion rate, estimated to be approximately 7 μg/d (Casey, 1976). The Expert panel based the minimum recommendation for manganese on the lowest level currently provided by domestic preterm formulas with no known cases of manganese deficiency.

The recommendation of a maximum level of manganese in preterm formula included consideration of evidence of increased absorption by neonatal rats; increased retention by preterm-LBW infants; elevated whole-blood manganese levels and abnormal manganese deposition in the brain in children exposed to chronic intravenous infusions of 33 μg of manganese/kg; and abnormal posturing and seizures after long-term exposure to 55 μg of intravenous manganese/kg. The recommendation for the maximum concentration in preterm formula was set at 25 μg /100 kcal, an amount associated with positive manganese balance (Atkinson & Shah, 1991; Zlotkin et al., 1995a) with no reported adverse effects (Zlotkin et al., 1995a). The maximum set for the concentration in preterm formula is lower than the maximum of 100 μg/100 kcal set for term formula (Raiten et al., 1998a).

Future research

Studies should be undertaken to determine the requirement for manganese by preterm-LBW infants. Further studies are required to determine whether the amount of manganese absorbed from preterm infant formulas not supplemented with manganese is adequate. Furthermore, whether gestational immaturity compromises the homeostatic control mechanisms for manganese, thus increasing the risk of toxicity for preterm-LBW infants, needs investigation.

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of manganese in preterm infant formula be 6.3 μg/ 100 kcal.**

Maximum. **The Expert Panel recommended that the maximum concentration of manganese in preterm infant formula be 25 μg/ 100 kcal.**

FLUORIDE

Background and review of the literature

Minimal intake of fluoride

Fluoride is considered to be a beneficial trace element but not essential (Nielsen, 1999). No reported evidence from human studies of overt clinical signs of fluoride deficiency exist. No specific diagnostic clinical or biochemical indices have been related to fluoride deficiency.

Fluoride is not added to TPN (Krug, 2000). In Europe, parenteral solution was contaminated with fluoride when electrolytes (e.g., potassium chloride) that had been stored in glass ampules were used to compound the solution (Montero et al., 1995). Whether prolonged use of TPN without fluoride during the postnatal period predisposes the preterm-LBW infant to future dental defects is not known.

Fearne et al. (1990) pointed out that the developmental period for the primary dentition is relatively long. For example, calcification of the human primary incisors is evident as early as 15 weeks GA and continues up to several months postpartum (Fearne et al., 1990; Sunderland et al., 1987). The mineralization of the crowns of the permanent first molars normally begins shortly after birth in term infants. The AAP-CON concluded that the systemic effects of fluoride on teeth are exerted in utero and continue up to 6 years of age (American Academy of Pediatrics. Committee on Nutrition, 1986).

Dental enamel

Birth weight and dental enamel. At about 4 years of age, BW was not a factor in the prevalence of dental caries (Lai et al., 1997). However, in those children who had LBW, the enamel defect hypoplasia with opacity was strongly associated with dental caries at 44 and 52 months of age (Lai et al., 1997). Among children whose BW was less than 2000 g, more dental defects occurred in children who were seriously ill, requiring TPN for more than 7 days and/or required more than 72 hours of ventilator support and/or required drugs for apnea (Fearne et al., 1990). Among LBW infants, Fearne et al. (1990) found no significant difference in the prevalence of enamel defects at five years of age of children with a BW less than 1500 g (n=60) compared with those with a BW of 1501–2000 g (n=50). In contrast, Seow et al. (1987) reported that the prevalence of enamel defects (opacity and hypoplasia) in children 9–42 months of age varied significantly by BW, in that dental defects were greatest for those with a BW less than 1500 g (n=77), moderate for those with a BW of 1500–2500 g (n=33), and least for those with a BW greater than 2500 g (n=47).

Compared with term infants, LBW infants have significantly greater incidence of enamel defects in the primary dentition up to 5 years of age (Fearne et al., 1990; Lai et al., 1997). Both systemic factors (e.g., hypocalcemia) and local factors (e.g., endotracheal intubation) during the neonatal period might contribute to the later development of dental defects (Seow et al., 1987).

Fluoride and dental enamel. The fluoride concentration in tooth enamel represents fluoride incorporated into enamel during development and before eruption of teeth (Fejerskov et al., 1994). Casey and Walravens (1988) suggested that teeth erupt in the preterm-LBW infant at the same postnatal time as in the term infant. Therefore, less time is available for mineralization of the preterm-LBW infants' teeth before eruption (Casey & Walravens, 1988). After reviewing available evidence, Phipps (1996) suggested that systemic fluoride during the preeruptive stage of tooth development could be incorporated into the developing enamel hydroxyapatite crystal and thereby may have the potential to reduce later enamel solubility and caries after eruption of the teeth. This hypothesis remains controversial. Fejerskov et al. (1994) emphasized that there was no evidence that fluoride induced enamel hypoplasia at the time of eruption of the teeth and that dental fluorosis does not reflect hypoplastic enamel.

Hellstrom (1976) investigated the relationship between fluoride content in enamel, dentin, and bone from the jaw (with mineralizing teeth) and ribs in LBW and term infants from Swedish cities with low (<0.8 ppm) and high (≥0.8 ppm) amounts of fluoride in the drinking water. In term infants (0–9 days postpartum), the fluoride concentration was significantly greater in enamel, dentin, jaw bone, and rib bone of infants from cities where water had a higher concentration of fluoride. For infants born in these cities, the fluoride content in enamel of term infants was significantly greater than that of LBW infants. Among LBW infants (0–4 days postpartum), the fluoride content of peripheral jaw bone was significantly less for those born in cities where water had a lower concentration of fluoride. There was no correlation between fluoride and calcium content in tissues, and calcium content did not vary by water fluoride level or BW (Hellstrom, 1976). In a small study, Glenn et al. (1997) compared incisors extracted from a 5-month human fetus whose mother had consumed prenatal fluoride supplements with those of three age-matched fetuses from areas where water was similarly nonfluorinated (<0.3 ppm). The incisors of the fetus whose mother took prenatal fluoride supplements were more developed, particularly the inner enamel epithelium, the ameloblasts, and the odontoblasts, compared with the control incisors (Glenn et al., 1997). Bawden et al. (1992) determined that fluoride uptake in developing fetal enamel of the guinea pig was linearly related to maternal consumption of doses up to 6 ppm of fluoride in drinking water but was not increased further at a higher dose (8 ppm). The mean fetal enamel fluoride was approximately an order of magnitude less than the paired maternal uptake at each dose (Bawden et al., 1992). These results suggest fetal enamel fluoride level is related to maternal intake, but there may be maternal limits on the amount of fluoride available to the developing fetus.

Human milk

Human breast milk contains variable amounts of fluoride. Early measures of fluoride in breast milk were high, confounded by problems with methodology (Dirks et al., 1974; Ericsson & Ribelius, 1971; Forsman, 1977). Analysis of term milk samples from Sweden (Ekstrand et al., 1981; Ekstrand et al., 1984; Spak et al., 1983), Italy (Aquilio et al., 1996), Finland (Esala et al., 1982), and Turkey (Koparal et al., 2000) indicated that average concentrations ranged from 7 to 19 μg/L. Concentrations as low as 2–4.5 μg/L have been reported in human milk (Ekstrand et al., 1981; Ekstrand et al., 1984; Esala et al., 1982). There was no significant difference in fluoride concentration between term and preterm milk during the first few weeks postpartum (Aquilio et al., 1996).

Chemical forms of fluoride and interactions

The absorption of soluble fluoride, such as sodium fluoride, is fairly rapid (50% within 30 minutes), and up to 90% is absorbed (Nielsen, 1999). Less than 50% of the fluoride in bone meal is absorbed (Nielsen, 1999). In the past, oral supplements containing sodium fluoride have been administered to term infants after 78 days of age (Ekstrand et al., 1994).

At birth, baby pigs of sows fed diets containing high levels of calcium and phosphorus have reduced fluoride concentrations in bone compared with baby pigs whose mothers were fed low levels of calcium and phosphorus (Forsyth et al., 1972). Forsyth et al. (1972) suggested that an interaction of dietary calcium, phosphorus, and fluoride influenced the fluoride accumulation in the offspring. Whitford (2000) suggested that the absorption of fluoride is reduced 20–35% by food high in calcium.

Dental fluorosis

The U. S. Environmental Protection Agency (1989) considers dental fluorosis to be a cosmetic effect (mottling) of excess fluoride intake, not a toxic or adverse health effect. Nevertheless, a no observed adverse effect level was set at 60 μg/(kg·d) for total daily fluoride intake (U.S. Environmental Protection Agency, 1989). This value was based on data from 12- to 14-year-old children assumed to weigh 20 kg and consume water at 1 L/d and 10 μg of dietary fluoride/kg of body weight (U.S. Environmental Protection Agency, 1989). Fejerskov et al. (1994) and others (Whitford, 2000) similarly concluded that there is a strong linear relationship between the daily dose of fluoride and the manifestation of dental fluorosis in communities. Furthermore, Fejerskov et al. (1994) suggested that there was no threshold value for fluoride intake below which the effect of fluoride on dental enamel would not be evident in the community. In rare instances of no known excessive fluoride exposure, dental mottling similar to that caused by fluoride is evident (Fejerskov et al., 1990). Based on earlier data (Dean & Elvove, 1937), an average intake of 50 μg of fluoride/kg (range: 30–90 μg/kg) was expected to provide near-maximum protection against dental caries in children and result in mild fluorosis in less than 10% of children with developing teeth. This is why the soluble fluorine concentration of community drinking water was set at 1.0 ppm (1.0 mg/L), a level that would provide intakes of about 50 μg fluoride/kg in children (Dean & Elvove, 1937; Whitford, 2000).

One study (Aasenden & Peebles, 1978) compared the prevalence of fluorosis in children in the United States, 7–12 years of age, who were from nonfluoridated communities and who had consumed fluoride supplements with children from a similar area who had not received fluoride supplements. The average daily dose of fluoride from supplements was estimated to be 56 μg/kg (range: 42–70 μg/kg) (Baelum et al., 1987). The prevalence of enamel fluorosis was about twice as high in the group receiving fluoride supplements; however, the degree of fluorosis was relatively mild, and in no case was any discoloration or pitting of the enamel observed. In contrast, 54% of children who had consumed fluoride supplements had permanent dentition that were caries free compared with 5.4% of children who had not received fluoride supplements. A proportion of children from each of these groups were examined approximately 4.5 years later, at an average age of 14.3 years (Aasenden & Peebles, 1978). The prevalence and severity of fluorosis were reduced in the follow-up study compared with observations of these same children in the first study. The authors hypothesized that the relative dose of fluoride per body weight was higher during the early formation period of first teeth than during development of permanent teeth, increasing the risk of fluorosis in the younger children. Furthermore, they hypothesized that affected areas of enamel may have gradually remineralized in later years. Consistent with the results of the first study (Aasenden & Peebles, 1974), children who had received fluoride supplements had less caries damage to enamel than children who had not received fluoride supplements (Aasenden & Peebles, 1978).

Some infants have been exposed to high amounts of fluoride in infant formula. The relatively high fluoride intake via infant formula made from milk powder reconstituted with fluorinated water may have increased the risk of mild enamel fluorosis in primary teeth (Larsen et al., 1988). Use of infant formula in the form of powdered concentrate during the first

year of life was significantly associated with mild to moderate fluorosis on enamel surfaces that began to form in the second year of life (Pendrys & Katz, 1998). Therefore, Pendrys and Katz (1998) suggested that an early exposure to fluoride has the potential to affect enamel surfaces that do not begin to form until after that exposure has occurred. They hypothesized that fluoride taken up into the bone during fluoride exposure was later released, with detrimental consequences during later enamel development (Pendrys & Katz, 1998). Bardsen (1999) noted that the start and the duration of the period of mineralization vary among different categories of teeth, so the period of maximum susceptibility to fluoride would vary accordingly.

Infant formula

The manufacturers of ready-to-feed infant formula now use defluorinated water. These formulas contain fluoride at less than 0.3 ppm (American Academy of Pediatrics. Committee on Nutrition, 1986) and range from 0.09 to 0.20 ppm ($\leq$30 μg/150 mL, or $\leq$25 μg/100 kcal) (Whitford, 2000). The use of ready-to-feed infant formulas in the United States after 1979 has not been associated with enamel fluorosis (Pendrys & Katz, 1998).

Toxicity

There have been at least three documented case reports of fatal acute fluoride ingestion (Whitford, 2000). One 3-year-old child died within 3 hours of swallowing an amount between 24 and 35 mg of fluoride/kg. A second 3-year-old child died after swallowing approximately 16 mg/kg as fluoride tablets. Last, a third child, 27 months of age, died after a dose estimated to be slightly below 5 mg/kg. Thus, an acute fluoride dose of approximately 5 mg/kg or more appears to be fatal in children (Nielsen, 1999; Whitford, 2000).

Previous recommendations

Term infants. According to the IOM (1997), the quantity of fluoride obtained by term infants from human milk does not increase the risk of dental caries; therefore, the fluoride concentration found in human milk was deemed as adequate to set recommendations for term infants. The DRI-AI for infants 0–6 months of age was set at 10 μg/d (1.4 μg/kg) (Institute of Medicine. Food and Nutrition Board, 1997). This would be equivalent to a minimum of 1.2 μg/100 kcal for an infant weighing 1000 g consuming 120 kcal/d of formula.

Recommendations of 60 μg/100 kcal for the maximum concentration of fluoride in formula for term infants was based on the value in current usage, including term infant formulas made from concentrate and fluorinated water (Raiten et al., 1998a).

A DRI-UL of 0.1 mg/(kg·d) for infants ages 0–6 months was set by the IOM (1997), assuming a weight of 7000 g. If this amount were appropriate for preterm-LBW infants, this would necessitate that preterm infant formula contain at most 83 μg/100 kcal. The World Health Organization (1996d) suggested that intake of 1-year-old infants be limited to 0.5 mg/d, with no more than 75% in the form of the highly soluble fluorides; assuming a body weight of 10 kg would yield a maximum intake of 50 μg/d, or 43 μg/100 kcal.

Preterm infants. Neither the AAP-CON (1998) nor the AHEC-HBP-HC (1995) made recommendations for fluoride intake by preterm infants. The ESPGAN (1987) did not make specific recommendations on the desirable level of fluoride intake by preterm infants but assumed that the amount of fluoride present in human milk and formula was probably sufficient to meet the minimal requirement for bone and teeth formation.

Conclusions and recommendations

The fluoride content in the tooth enamel of LBW infants is significantly lower than that of term infants within the first 9 days of life (Hellstrom, 1976). The LBW infants have a significantly greater incidence of enamel defects in the primary dentition up to 5 years of age (Fearne et al., 1990; Lai et al., 1997). Fluoride is not now a required ingredient of infant formula [Federal Food, Drug and Cosmetic Act Pub. L. No. 75-717, 52 Stat. 1040 (1938), as amended 21 U.S.C. §§ 301, SEC. 412. [350a]). Requirements for infant formulas]. The Expert Panel recognized that there may be a narrow therapeutic or nutritional range of fluoride of benefit (American Academy of Pediatrics. Committee on Nutrition, 1986). This must be weighed against the potential for excess fluoride intake during the preeruptive stage of tooth development and resulting dental fluorosis if formula is supplemented with fluoride (American Academy of Pediatrics. Committee on Nutrition, 1986). No information is available on renal clearance of fluoride by preterm-LBW infants. A minimum fluoride concentration reflecting the lower end of the range measured in human milk, approximately 4 μg/L, could be considered. This is equivalent to 0.6 μg/100 kcal (0.7 μg/kg), assuming an intake of 150 mL/d and an energy intake of 120 kcal/(kg·d) and is 50% of the IOM (1997) recommendation of 1.4 μg/kg for term infants 0–6 months of age . However, the Expert Panel determined there was not enough evidence to recommend a minimum concentration of fluoride in preterm infant formula. Research is needed to establish whether fluoride-supplemented formula can decrease the incidence of dental defects in preterm-LBW infants without later development of fluorosis.

The use of ready-to-feed infant formulas in the United States after 1979 has not been associated with fluorosis. Fluoride concentrations in domestic ready-to-feed infant formulas are 25 μg/100 kcal or less because of the use of defluorinated water in manufacturing. Therefore, the Expert Panel recommended a maximum concentration of 25 μg/100 kcal in preterm infant formula.

Recommendations

Minimum. **The Expert Panel did not find sufficient evidence to recommend a specific minimum concentration of fluoride in preterm infant formula.**

Maximum. **The Expert Panel recommended that the maximum concentration of fluoride in preterm infant formula be 25 μg/100 kcal.**

CHROMIUM

Background and review of the literature

Trivalent chromium III, probably the only form present in biological tissues, is considered to be an essential nutrient. A recommended safe and adequate range for dietary chromium was first established more than 20 years ago (National Research Council. Food and Nutrition Board, 1980). The major role of chromium is to potentiate the action of insulin; thus, it is important in maintaining normal glucose metabolism (Mertz, 1969; Mertz, 1993). Roles for chromium in lipid metabolism and the immune response are less clear (Stoecker, 1996).

For reviews, see IOM (2001b), Anderson (1987; 1998), Anderson et al. (2000), Offenbacher and Pi-Sunyer (1988), Stoecker (1996), Mertz (1969; 1993), and Vincent (2000). Issues of chromium analysis are a major problem for determining contamination and verifying accuracy. This area has been reviewed by Veillon and Patterson (1999).

Deficiency and requirements for chromium

Three adult patients who received long-term TPN were reported to respond clinically to chromium (Brown et al., 1986b; Freund et al., 1979; Jeejeebhoy et al., 1977). Infants suffering from kwashiorkor or protein-energy malnutrition showed improvement in impaired glucose tolerance after they received chromium administered as an oral supplement (Gurson & Saner, 1971; Hopkins, Jr. et al., 1968). However, an earlier report found no effect of chromium supplementation for similar subjects (Carter et al., 1968). Another report involving malnourished children given a chromium supplement indicated a significantly increased rate of growth compared with similar controls given no chromium (Gurson & Saner, 1973). There have been no reports of response to chromium supplementation in infants fed breast milk or preterm infant formula presently in use.

Plasma and urinary chromium concentrations

Bougle et al. (1992) found no evidence of a difference in chromium status during the first month of life based on comparison of plasma and urinary concentration between preterm infants of GA 28–36 weeks and term newborns living in France; however, the plasma chromium concentration was statistically greater in the preterm infants when measured at the second and third months of life. Moreover, there was a negative correlation between plasma chromium concentration and GA between 26 and 42 weeks.

Breast milk chromium content

Based on an extensive review of values reported in the literature, the IOM (Institute of Medicine, 2001b) estimated the average chromium concentration of human term milk to be 250 ng/L. With an intake of 150 mL/day, a preterm infant fed exclusively breast milk would have a chromium intake of approximately 40 ng/(kg·d), or 36-37 ng/100 kcal, assuming an energy intake of 120 kcal/(kg·d).

Recommendations of other organizations

The consensus (Tsang et al., 1993) enteral recommendation for clinically stable and growing preterm infants is 83–420 ng of chromium/100 kcal. The CPS (1995) recommended that preterm infants receive 52–98 ng/kg (1.0–1.9 nmol/kg), or 43–82 ng of chromium/100 kcal, assuming an energy intake of 120 kcal/(kg·d). Recently, the IOM (2001b) recommended a DRI-AI of 29 ng of chromium/(kg·d) for healthy term infants 0–6 months of age. If this amount were also appropriate for preterm-LBW infants, this would necessitate that formula contain 24 ng of chromium/100 kcal, assuming an energy intake of 120 kcal/(kg·d).

Chromium content of infant formula

The chromium content of domestic preterm infant formula currently in use in the United States is not reported on the label because no supplemental chromium is added. The chromium present is inherent as well as that leached from contact with stainless steel and other items yielding chromium during the manufacturing process. However, Patterson et al. (1985), analyzed two brands of milk-based formulas in the United States for LBW infants and reported concentrations of 7.5 (Mead Johnson & Company, Evansville, Indiana 47721) and 18 μg/L (Ross Products Division, Abbott Laboratories Inc., Columbus, Ohio 43215), equal to 1100–2650 ng/100 kcal, assuming 680 kcal/L. (The brands were identified by a written communication with Dr. Patterson, USDA, Bldg 307 Rm 227, Beltsville, MD 20705). They applied a sophisticated isotope dilution/mass spectrometry method and used special precau-

tions to prevent contamination, including the use of a class 100 clean room with filtered air. Both the room and the materials used in the procedures were devoid of stainless steel and other metals. National Bureau of Standards (now National Institute of Standards and Technology)-certified reference materials were included in the analysis to verify accuracy.

Similar data were reported by Deelstra et al. (1988), who analyzed milk-based infant formulas for therapeutic use. That is, the chromium concentration of a hypoallergenic protein hydrolysate product that is sometimes fed to preterm infants, although not produced for that purpose, was 8.1 μg/L (1200 ng/100 kcal), assuming a formula intake of 680 kcal/L. Using unvalidated methods to test infant formulas, the inherent chromium concentration was reported to be less than 7.7 μg/L in milk-based term formulas, approximately 20–40 μg/L for soy-based term formulas, 142–174 μg/L for casein hydrolysate-based formulas, and 8–22 μg/L for milk-based preterm formulas (Ross Products Division of Abbott Laboratories, 1998). One FDA-approved amino acid-based infant formula (SHS North America, 2000) developed for infants with food allergies contains 1600 ng of chromium/100 kcal. Thus, there is good agreement that the inherent chromium concentration in both term and preterm formulas greatly exceeds that found in breast milk. That is, the range of chromium in preterm formulas is 7.5–22 μg/L (1100–3235 ng/100 kcal, assuming a caloric intake of 680 kcal/L) (Patterson et al., 1985; Ross Products Division of Abbott Laboratories, 1998), and the average breast milk concentration is 250 ng/L (37 ng/100 kcal, assuming a caloric value of 670 kcal/L)(Institute of Medicine, 2001b). Thus, the chromium concentration of domestic preterm infant formulas is approximately 30–90 times higher than that in term human milk. Unfortunately, no data on the bioavailability of chromium from human milk, infant preterm formula, or term formula exist (Institute of Medicine, 2001b; Ross Products Division of Abbott Laboratories, 1998).

Toxicity

There have been no established and universally recognized adverse effects of chromium derived from food or supplemental chromium III salts (Institute of Medicine, 2001b). However, chromium VI is considered a carcinogen, mutagen, and clastogen. For term and preterm parenteral nutrition solutions, Greene et al. (1988) recommended 200 ng/(kg·d). Likewise, this level was cited in a table entitled "Trace element daily requirements for pediatrics" (National Advisory Group on Standards and Practices for Parenteral Nutrition, 1998). However, Moukarzel et al. (1992) studied 15 children with a median age of 10 years (range: 1.3–18 years) receiving TPN therapy and compared them with 15 well-nourished control children without TPN, matched for age and sex. The median duration of TPN therapy was 9.5 years (range: 1.3–14 years). The results indicated a mean daily infusion of 150 ng of chromium/(kg·d), less than the 200 ng/(kg·d) recommended. Alarmingly, the serum chromium concentration averaged 20 times higher than that of the control group ($P < 0.0001$). In 1986, Kein et al. (1986) reported markedly elevated serum concentrations and daily urinary excretions of chromium for a 10 yr old boy who had received 16 months of TPN without supplemental chromium. Apparently the increased serum and urinary chromium was the result of contamination of TPN. In the study of Moukarzel et al (1992), even after discontinuation of chromium supplemented TPN solutions for one year, serum concentrations remained significantly higher than that of the control group, p<0.01; apparently this also was due to the analytically documented contaminating chromium in the TPN solutions, fat emulsion and drinking water. More re-

cently, Hak et al. (1998) determined the quantity of chromium in unsupplemented TPN solutions compounded for infants. They calculated that a child weighing < 10 kg could possibly receive up to 700 ng/(kg·d) in contaminating chromium from TPN components. This amount greatly exceeds the AI of 29 ng/(kg·d) recently established by the IOM (2001b) for infants 0-6 months of age. No published reports of chromium toxicity were found for term or preterm infants administered TPN solutions or enteral formula, with or without supplemental chromium. However, the signs and symptoms of excessive chromium ingestion such as renal and liver failure (Institute of Medicine, 2001b) are not commonly known among clinicians. There were insufficient definitive data for the IOM (2001b) to establish a DRI-UL for chromium. Interestingly, Neocate (SHS North America, 2000) contains nearly 150 times the amount recommended for term infants on a per kilogram basis (Institute of Medicine, 2001b). As indicated previously, manufacturers of the domestic preterm formulas currently in use in the United States do not report in their product brochures the amount of chromium in formula, so the quantity routinely ingested by preterm infants is unknown.

Interactions and chemical forms of chromium

Several dietary components influence chromium absorption. Vitamin C has been reported to promote absorption of chromium in both humans and rats (Offenbacher, 1994; Seaborn & Stoecker, 1992). In a 1994 study by Offenbacher (1994), three women were given 1 mg of chromium as chromium chloride with 100 mg of vitamin C. The plasma chromium level averaged approximately three times higher compared with levels when they were not given vitamin C. Likewise, rats given vitamin C in combination with chromium showed significantly higher 24-hour urinary excretions of chromium compared with control rats (Seaborn & Stoecker, 1992). Dowling et al. (1990) reported that including amino acids in a test meal enhanced chromium III transport, as determined by a rat small intestine vascular perfusate model. Dietary starch increased tissue uptake of chromium (both inherent and ^{51}Cr) compared with fructose, glucose, or sucrose, with both obese and normal control mice (Seaborn & Stoecker, 1989). Likewise, absorption increased in zinc-deficient rats but was reduced after zinc administration (Hahn & Evans, 1975). The effects of oxalates and other chelators were reported to increase uptake and excretion of chromium in a rat model; in contrast, high levels of phytate decreased absorption (Chen et al., 1973).

Medications have been reported to enhance or inhibit chromium absorption and/or retention. Those reported to increase these indices in animal models include aspirin (Mills & Davis, 1987) and indomethacin (Kamath et al., 1997). In contrast, an analog of prostaglandin E_2 (Kamath et al., 1995) and different antacids reduced ^{51}Cr in blood and tissues compared with values in control rats (Davis et al., 1995; Seaborn & Stoecker, 1990).

Trivalent soluble chromium compounds were considered safe by Mertz (1969) and have been used as a supplement in several studies (Mertz, 1993). Chromium III as picolinate has also been used as a chromium supplement in human studies (Campbell et al., 1997) and is commercially available to the public. One report indicates that picolinic acid, per se, reduced iron uptake in rat kidney cells (Fernandez-Pol, 1977). Thus, there is a need to determine more definitively the safety and efficacy of various forms of chromium compounds for use in preterm infant formulas and supplements.

Conclusions and recommendations

The average concentration of chromium in mature term breast milk was recently estimated by the IOM (2001b) to be 33 ng/100 kcal. Recommendations by the CPS (1995) for preterm infants range from 43 to 82 ng/100 kcal. The consensus recommendation is 83–420 ng/100 kcal (Tsang et al., 1993), and the DRI-AI recommendation of the IOM (2001b) is 24 ng/100 kcal for term infants 0–6 months of age. The highest concentration found in current domestic preterm infant formulas was 2650 ng/100 kcal, determined in 1985 by using validated methods (Patterson et al., 1985). The Expert Panel found insufficient data on which to recommend minimum or maximum levels of chromium in preterm infant formulas. The recommendations by the authoritative organizations and the markedly greater amount present in current domestic formula, with no reports of toxicity, provide a range that would provide an adequate but nontoxic level of chromium for preterm infants.

Recommendation

Note. **The Expert Panel found insufficient data on which to recommend minimum or maximum levels of chromium in preterm infant formula.**

MOLYBDENUM

Background and review of the literature

The essentiality of molybdenum is based on its chemical properties of being capable of readily changing its oxidation state and its action as an electron transfer agent. Thus, it serves as a cofactor for several enzyme systems that catalyze oxidation-reduction systems. These enzymes include xanthine oxidase, sulfite oxidase, and aldehyde oxidase (Institute of Medicine, 2001a). For reviews, see IOM (2001d), Nielsen (1999), World Health Organization (1996b), and Mills and Davis (1987).

Requirements

Intrauterine accretion of molybdenum during the last trimester has been estimated to be 1 μg/(kg·d), with the caution that this may be an overestimation (David & Anast, 1974). Meinel et al. (1979) compared fetal and adult concentrations of molybdenum in liver. The fetal-to-adult ratio was 0.13, with an inverse relationship noted between copper and molybdenum.

The World Health Organization (1996b) recommended adults consume 24 μg molybdenum/d, or 0.4 μg/(kg·d); however, a caution was included that there were insufficient data to give an estimate for infants. Balance studies involving adults in a controlled metabolic research facility suggested a minimum requirement of 25 μg/d (Turnlund et al., 1995a; 1995b). Friel et al. (1999b) conducted a study in Canada involving 16 LBW infants whose mean BW was 1336 g and GA was 30 weeks. Each infant was fed human milk or preterm formula or both for 30 days. The formula contained 3.2 μg of molybdenum/100 kcal, compared with 0.7 μg of molybdenum/100 kcal in the milk. They speculated that 4–6 μg of molybdenum/(kg·d) (3.3–5 μg/100 kcal) would be adequate.

Recently, Sievers et al. (2001a) reported 72-hour balance studies that involved 14 preterm infants fed a median molybdenum intake of 10.4 μg/(kg·d) [range: 6.1–19.6 μg/(kg·d)] excreted a median of 53% (range: 13–81%) via the urinary route and only a median of 9% (range: 3–23%) in feces. Although the median retention was 4.4 μg/(kg·d), the range was extremely wide, 0.99–7.77 μg/(kg·d), with a median urinary excretion of 76%; none of the infants had a negative

molybdenum balance. This is unlike the situation when the same infants were fed only 2.27 μg/(kg·d) and the median balance was negative, -0.08 μg/(kg·d). A third subsequent balance study in which the infants were fed a still lower median intake, 1.38 μg/(kg·d), yielded a positive median balance of 0.13 μg/(kg·d), but the median urinary excretion was slightly lower, 76%. These data are difficult to interpret and could not be used as a basis for a requirement or recommendation for the amount of molybdenum in preterm infant formulas. However, they do suggest that preterm infants excrete most molybdenum via the kidneys and that urinary excretion apparently varies with the level of intake. Thus, the kidneys of these neonates appear to be actively regulating molybdenum retention. A second study involving balance studies by Sievers et al. (2001b) also indicated that urinary molybdenum was correlated with intake ($P < 0.01$) and that renal function was adequate to handle relatively high intakes of molybdenum, e.g., 27 μg/(kg·d).

Molybdenum deficiency

There are no known cases of dietary molybdenum deficiency in healthy humans, although molybdenum cofactor deficiency, an inborn error of metabolism, had been observed in 47 patients by 1993 (Institute of Medicine, 2001a). One case of apparent molybdenum deficiency in an man receiving TPN for 18 months without molybdenum supplementation was reported (Abumrad et al., 1981; Abumrad, 1984).

Serum concentrations of preterm infants have been reported to be significantly lower than those in term infants, both at day 1 and at day 21 of life (Aquilio et al., 1996). However, there are no specific functional indices for molybdenum status that reflect a response to dietary molybdenum in infants, making it difficult to definitively identify a deficiency (Institute of Medicine, 2001a).

Molybdenum concentrations in milk and bioavailability

Breast milk molybdenum concentrations vary widely; six studies cited by the IOM (2001a) show a range of 1.4–17.0 μg of molybdenum/L. On the basis of these data, the IOM (2001a) estimated the mean concentration in milk to be 2.0 μg/L, or 0.3 μg/100 kcal, assuming a caloric intake of 670 kcal/L. Bougle et al. (1988) reported that preterm milk was lower in concentration compared with term milk at every stage of lactation; this difference was significant in the early stages. More recently, Aquilio et al. (1996) confirmed the significantly lower concentrations (approximating one-half) in preterm milk compared with term milk, by using sophisticated methodology and sample handling. Bougle et al. (1992) compared the molybdenum intake, plasma and urinary molybdenum concentrations, and uric acid excretion of eight preterm infants (GA of 33 weeks) fed preterm formulas with 10 others fed breast milk. The daily molybdenum intake of the formula-fed infants was more than 60 times greater compared with those fed breast milk, whose intake was estimated without direct measurement. The results demonstrated that the preterm infants fed breast milk tended to have higher plasma molybdenum concentrations (not significantly higher), urinary levels of molybdenum ($P < 0.05$), and uric acid excretions (not significantly higher) compared with the formula-fed neonates. The study indicates that the bioavailability of molybdenum from the breast milk is higher than that in the preterm formula. However, the main weakness of the study is that the daily intake of breast milk was not measured but estimated from a previous study. Using stable molybdenum isotope studies in a metabolic research setting, Turnlund et al. (1999) reported near 87% absorption from kale by 12 women

compared with 57% from ground soybeans. Earlier, Alexander et al. (1974) had reported an 80% absorption of molybdenum from human milk and cow milk-based infant formula. More recently, Cantone et al. (1997), using a single healthy subject given solutions of stable isotopes of molybdenum (^{95}Mo and ^{96}Mo) orally, reported that when an aqueous solution of the molybdenum was given alone, nearly all was absorbed (95%); in contrast, absorption was decreased to 51% when the labeled trace element was mixed with infant formula to simulate a meal, before ingestion.

Interactions

An interaction among sulfate, molybdenum, and copper has been reported in ruminants (Mills & Davis, 1987) but may be of no significance to humans (Institute of Medicine, 2001a). Deosthale and Gopalan (1974) reported that high intakes of molybdenum by humans ingesting sorghums in the range of 540–1500 μg of molybdenum/d resulted in an increase in urinary copper compared with a molybdenum intake of 160 μg/d. However, the increase in urinary copper excretion could not be confirmed in a controlled human study using the same molybdenum intake (1500 μg/d) (Turnlund & Keyes, 1999). Seelig (1973) proposed a role of an interaction between copper and molybdenum in iron deficiency and iron storage diseases.

Chemical forms of molybdenum

Molybdenum is found in five oxidation states (numbers 2 to 6) and does not exist in the pure metallic form. Of the various forms, the more soluble ones are less toxic compared with those less soluble or insoluble (Institute of Medicine, 2001a). Sodium molybdate is the form currently used to fortify a special domestic lactose-free formula for term infants (Mead Johnson Nutritionals, 2000) as well as one amino acid-based formula for allergic infants (SHS North America, 2000). Most forms, water-soluble and water-insoluble forms as well as dietary sources, are readily absorbed (Turnlund et al., 1995a). However, insoluble molybdenum sulfate apparently is less well absorbed than are other insoluble compounds, including molybdenum trioxide and calcium molybdate (Mills & Davis, 1987).

Infant formulas and other sources of molybdenum

Only one domestic specialty formula includes molybdenum fortification with a label claim of 1.25 μg of molybdenum/100 kcal (Mead Johnson Nutritionals, 2000). Friel et al. (1999b) reported that three preterm formulas from companies in the United States contained 23–26 μg of molybdenum/L (2.8–3.2 μg/100 kcal, assuming an energy content of 810 kcal/L). Biego et al. (1998) reported from France that six purchased commercial infant formulas, unidentified as to brand but assumed to be for term infants, contained an average of 18 μg of molybdenum/L (2.2 μg/100 kcal, assuming an energy content of 810 kcal/L). The hypoallergenic amino acid-based formula available from the United Kingdom has a label claim of 3–3.5 μg of molybdenum/100 kcal (SHS North America, 2000). Using state-of-the art methodology and precautions for preventing contamination, Dabeka and McKenzie (1992) reported the mean concentration of 36 samples of Canadian milk-based formulas to be 0.033 μg of molybdenum/g of formula; this would approximate 33 μg/L, or 4.1 μg/100 kcal, assuming a caloric intake of 810 kcal/L. Nondetectable amounts of molybdenum are obtained in medications commonly used for preterm-LBW infants in the intensive care unit (Friel et al., 1999b).

Toxicity

Most of the toxicity data relate to animals, namely ruminants, with limited information for humans (Institute of Medicine, 2001a). No harm was reported for adult humans having a daily dietary intake of 1500 μg (Deosthale & Gopalan, 1974); increased urinary copper excretion was noted in one instance during elevated intake (Deosthale & Gopalan, 1974), yet this was not confirmed by a controlled metabolic study (Turnlund & Keyes, 1999). Because of the lack of sufficient data and the concern about the infant's ability to handle excess amounts of molybdenum, no DRI-UL was established for infants (Institute of Medicine, 2001a).

Previous recommendations by other organizations

The CPS (1995) recommended a range of 0.2–0.4 μg/(kg·d) (0.17–0.34 μg/100 kcal) for preterm-LBW infants. The AAP-CON (1998) made no recommendation. The IOM (2001a) recommended 2 μg/d [0.3 μg/(kg·d)] for term infants 0–6 months old. If this recommendation were appropriate for preterm-LBW infants, this would imply that the preterm infant formula contain at least 0.25 μg/100 kcal. This is similar to the amount of 0.3 μg/(kg·d) previously recommended by Reifen and Zlotkin (1993), on the basis of human milk intake of preterm infants.

Conclusions and recommendations

The average concentration of molybdenum in mature breast milk was recently estimated by the IOM (2001b) to be 2 μg/L. If the IOM (2001a) recommendation of 2 μg/d for term infants were appropriate for preterm-LBW infants, this would necessitate that preterm infant formula contain at least 0.25 μg/100 kcal. As indicated above, others recommended a range from 0.17 to 0.34 μg/100 kcal for preterm formula. Molybdenum is not an added supplement in current domestic preterm formulas (Abbott Laboratories. Ross Products Division, 2001; Mead Johnson Nutritionals, 2000). However, reported analysis indicated molybdenum levels approximating 3 μg/100 kcal, apparently present by inherent contamination. It has been suggested that nearly all infant formula in the United States currently contain more than 15 μg/L (1.85 μg/100 kcal) (Ross Products Division of Abbott Laboratories, 1998). The Expert Panel found insufficient data on which to recommend minimum and maximum levels of molybdenum for preterm infants. The recommendations by the authoritative organizations and the markedly greater amount present in current domestic formula, with no reports of toxicity, provide a range that would provide an adequate but nontoxic level of molybdenum.

Recommendation

Note. **The Expert Panel found insufficient data on which to recommend minimum and maximum levels of molybdenum in preterm infant formula.**

13. VITAMINS: FAT-SOLUBLE VITAMINS

BACKGROUND

The metabolism and functions of many of the fat-soluble vitamins are related directly to the availability of many other nutrients and the functions of specific organs. For example, vitamin D metabolism is directly influenced by the availability of calcium and phosphorus, and it requires the function of at least two organs, liver and kidney, to convert it to its active metabolites. The vitamin E requirement is influenced by the amount of polyunsaturated fatty acids (PUFAs) and iron in the diet, and possibly by the adequacy of other nutrients, such as

vitamin C and selenium. The extent of the use of parenteral nutrition to minimize the depletion of a precarious state of nutrient reserve and the clinical practices of vitamin A prophylaxis for chronic lung disease or daily multivitamin supplementation for infants receiving enteral feeding are also important considerations when estimating the nutritional requirements for the fat-soluble vitamins A, D, and E. The following discussion is based on the assumptions that preterm infants will receive standard parenteral nutrition support beginning within 24 hours of birth and continued until at least 75% of the desired enteral intake is being taken, without the use of enteral vitamin supplements.

VITAMIN A

Background

Vitamin A and its biologically active derivatives, retinal and retinoic acid, together with a large repertoire of synthetic analogues, are collectively referred to as retinoids. Naturally occurring retinoids regulate growth and differentiation of a wide variety of cell types and play crucial roles in the physiology of vision, integrity of the immune system, and morphogenesis during embryonic development (Ross, 1999). Retinol (vitamin A alcohol) is the prototype of all other natural retinoids. It is a dietary component present in the form of retinyl esters in food sources of animal origin and is also formed in vivo from its precursor β-carotene (a carotenoid), which is present in food sources of plant origin.

The commonly used nutritional unit for vitamin A is the μg retinol equivalent (μg RE). Biological activity of 1 RE (μg) is assumed generally to equal 1 μg of all-*trans*-retinol, 6 μg of all-*trans*-β-carotene, or 12 μg of other provitamin A carotenoids. The United States Pharmacopeia (USP) unit or the international unit (IU) is also used to quantify vitamin A activity in pharmaceutical preparations. One IU equals 0.3 μg of retinol, 0.344 μg of retinyl acetate, or 0.55 μg of retinyl palmitate, all as their all-*trans* isomers. In other words, 3.33 IU equals 1 μg RE.

Dietary retinyl esters and β-carotene are processed by a complex but coordinated series of physical and chemical events in the bowel, requiring pancreatic and other enzymes as well as bile salts. Retinol is a common intracellular product from the cleavage of vitamin A precursors in the enteric mucosa. It is then largely re-esterified with long-chain fatty acids and transported as chylomicrons via intestinal lymphatics and the circulation to the liver for further processing.

Retinol is distributed to the target tissues in the form of a complex of retinol with retinol-binding protein (RBP) bound to transthyretin. Target tissue uptake is via a specific membrane receptor, and biological activity is mediated through intracellular transport and nuclear binding to specific receptors that belong to the superfamily of steroid hormone zinc-finger receptors controlling gene expression.

Cord blood retinol and RBP concentrations are generally about 50–70% of maternal values; values are lower in preterm infants. Liver is the major storage organ for vitamin A; limited data in term infants show storage may be 15–40% of the value for adults and even less in preterm infants. Thus, the only way for a preterm infant to meet nutritional needs for vitamin A is through exogenous enteral or parenteral sources.

More than 90% of the vitamin A in human milk is in the form of retinyl esters contained in milk fat globules. Vitamin A content is highest in colostrum and decreases gradually during the first few months of lactation. Mature milk from mothers of term infants has 33–77 μg RE/100 mL and milk from mothers of preterm infants, 83–100 μg RE/100 mL.

Details of the physiological and nutritional aspects of vitamin A are reviewed elsewhere (Ross, 1999; Shenai, 1993).

The recommendation for the vitamin A intake of term infants in the report *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a) was 200–500 IU/100 kcal (60–150 μg RE/100 kcal). The recommendation of the American Academy of Pediatrics Committee on Nutrition (1998) for the vitamin A intake of premature infants was 75–225 IU/100 kcal. Shenai (1993) recommended 1250–2333 IU/100 kcal. The European Society of Paediatric Gastroenterology and Nutrition Committee on Nutrition (1987) recommended 90–150 μg RE/100 kcal.

Review of the literature

It is generally accepted that in children, in the absence of inflammation, a plasma retinol concentration of less than 0.70 μmol/L (20 μg/100 mL) is indicative of marginal vitamin A status (hyporetinolemia), and a plasma retinol concentration of less than 0.35 μmol/L (10 μg/100 mL) is indicative of vitamin A deficiency. However, antenatal and postnatal exposures to corticosteroid therapy, a common occurrence in small preterm infants, can transiently raise plasma retinol and RBP concentrations, regardless of the true nutritional status.

Almost all preterm infants are hyporetinolemic soon after birth, especially those who require total parenteral nutrition (TPN). However, except for the potential role of vitamin A deficiency in the prevention of bronchopulmonary dysplasia (BPD), none of the other problems commonly experienced by preterm infants have been convincingly found to be responsive to vitamin A. Hypovitaminosis A is thought to contribute to the development of BPD because of the role of vitamin A in epithelial differentiation and maturation processes. A recent multicenter trial demonstrated a reduced relative risk (0.85, $p<0.03$) of chronic lung disease, defined as oxygen dependence at 36 weeks of postmenstrual age, in low birth weight (LBW) infants (<1000 g) given an intramuscular (IM) dose of 1500 μg RE as (as retinyl palmitate) three times weekly for 4 weeks, beginning between 24 and 96 hours after birth (Tyson et al., 1999). In contrast, the relative risks of death and outcomes not associated with chronic lung disease were not significantly improved for those infants receiving vitamin A supplementation. However, preterm infants with low values for plasma retinol, RBP, and retinol-to-RBP molar ratios can grow as well as those with higher values (Koo et al., 1995b), and a reduction in the amplitude in the electroretinogram, an early sign of vitamin A deficiency in children and adults, shows no correlation with low circulating levels of retinol in preterm infants (Mactier et al., 1988). Furthermore, there are many factors critical to lung growth and development (Chytil, 1992), and the multifactorial etiology of chronic lung disease (Jobe, 1999) suggests that the reduction in its incidence with vitamin A treatment should be regarded as a pharmacological effect rather than a nutritional benefit.

In-hospital needs. Vitamin A status, as indicated by plasma retinol and RBP concentrations, is often reported to be inadequate in preterm infants, particularly in infants receiving TPN. It is estimated that no more than 38% of the administered amount of vitamin A is delivered to the infant if the parenteral multivitamin preparation is added to a dextrose-amino acid solution (Shenai, 1993). This is because of photodegradation of vitamin A and loss from adsorption to the nutrient delivery system.

In one study of LBW infants (≤1500 g) receiving 455 μg of retinol daily from TPN, those with a birth weight (BW) less than 1000 g showed a significant decline from baseline retinol concentrations of 14.8 ± 4.6 μg/100 mL (SD) by the second week after birth, and values remained low during TPN administration (Greene et al., 1987). During serial measurements for up to 4 weeks of TPN, 14 of 24 infants in this group had at least one plasma retinol concentration of less than 10 μg/100 mL, a level associated with signs of retinol deficiency in older children. In infants who subsequently tolerated enteral feedings of preterm infant formula providing 200–300 μg of retinol/d, the retinol concentrations increased to 13.7 ± 7.7 μg/100 mL and maintained this level for up to 4 weeks of enteral feeding. In the same study, in 17 infants with a BW of 1000–1500 g, circulating retinol concentrations at baseline were 13.5 ± 2.9 μg/100 mL (SD) and remained stable through up to 4 weeks of TPN and 4 weeks of enteral feeding.

In another study of healthy infants with a BW less than 1500 g, with baseline serum retinol and RBP concentrations of 18.3 μg/100 mL and 1.9 mg/100 mL, respectively, 61 infants were randomized to receive vitamin A at 104, 208, or 369 μg RE/100 kcal (1 RE = 3.3 IU of vitamin A activity). Actual intakes were 102, 225, and 337 μg RE/100 kcal. After an average of 1 month of feeding, infants fed the formula with the lowest vitamin A content had significantly lower serum retinol and RBP concentrations than did the intermediate and high vitamin A intake groups. All infants in the lowest vitamin A intake group had hyporetinolemia (retinol concentration of <20 μg/100 mL) at the end of the study period, whereas only six infants in each of the intermediate and high intake groups had hyporetinolemia. The average molar retinol-to-RBP ratio at baseline was 0.74 and did not vary statistically among the groups, although there was a trend toward an increase in the highest intake group. All infants remained well during the study, and there was no difference in daily gain in weight (~30 g), length (~0.17 cm), or head circumference (~0.16 cm) among the three groups (Koo et al., 1995b).

In a study of formula-fed preterm infants (BW of <1990 g), plasma retinol concentrations did not increase significantly with daily enteral supplements of 3000 IU or 1500 IU of vitamin A for at least 2 weeks (Woodruff et al., 1986). In the high vitamin A supplement group, the mean plasma retinol concentration at the end of the study was 17.2 μg/100 mL. In another report of infants with a BW less than 1501 g, a daily supplement of vitamin A of 1500 μg RE enterally or 600 μg RE intramuscularly between 2 and 32 days after birth raised plasma retinol and RBP concentrations in both groups (Landman et al., 1992). Enteral feedings, which included various proportions of human milk and milk formula with vitamin A contents of 90 μg RE/100 kcal, were started on the third day (range: 2–7 days). The enteral group actually received an average of 4564 IU daily, the IM group, an average of 2531 IU daily (60% of it enterally). At the end of the study, plasma retinol and RBP concentrations were 24.9 μg/100 mL and 2.4 mg/100 mL, respectively, in the enterally supplemented infants, and 26.3 μg/100 mL and 2.4 mg/100 mL, respectively, in the intramuscularly supplemented infants. There was no sign of vitamin A toxicity.

Post-hospital discharge needs. Several reports have assessed vitamin A status in preterm infants after hospital discharge. One study followed LBW infants (750–1398 g) who received variable durations of parenteral nutrition and were fed preterm formula with retinyl palmitate at 195 μg RE/100 kcal until hospital discharge (Peeples et al., 1991). Thereafter they were fed standard term formula containing 44% of the vitamin A content of the preterm formula. Plasma retinol and RBP concentrations and the retinol-to-RBP molar ratio reached nadirs near 40 weeks of postmenstrual age, with 32 of 67 infants having plasma retinol concentrations indicative of deficiency (<10 μg/100 mL). Vitamin A status was inversely related to the duration of parenteral nutrition. These values increased steadily during the next 12 months, although hyporetinolemia (retinol concentration of <20

μg/100 mL) was still present in 16% of infants up to 4 months after the term date.

In the study of Koo et al. (1995b), a follow-up of 19 newborn infants at 6 months or later showed an average increase in serum retinol and RBP concentrations of ~60% compared with baseline values while they were receiving standard cow milk formula with 100 μg RE/100 kcal. In another study of 63 LBW infants of similar size who received enteral feedings of preterm infant formula (vitamin A content 204 μg RE/100 kcal) beginning on the third day, there was a progressive increase in plasma retinol concentrations once full feeding was achieved, and all infants, including infants with BPD, had normal plasma retinol concentrations (>20 μg/100 mL) when reassessed at 2 months post-term (Carlson et al., 1995).

Apparently, then, a large proportion of seemingly healthy preterm infants, with or without oral vitamin A supplementation, may have hyporetinolemia. In infants with a BW less than 1500 g, early introduction of enteral feeding of appropriately fortified preterm formula, coupled with early use of parenteral nutrition until full feeding, appears to be critical in minimizing further deterioration of vitamin A status. The initial decrease or persistently low plasma retinol concentration in preterm infants may be due to a lack of vitamin A intake from a decreased delivery of vitamin A in TPN, which can be overcome by providing it in a lipid emulsion, or to a lack of adequate enteral intake of vitamin A. It is also possible that some absorbed vitamin A is directed to replenishing tissue stores or utilized to meet the potential increased need for vitamin A during oxygen therapy. In addition, there may be a maturational lag in vitamin A absorption and metabolism, because plasma retinol concentrations increase after term corrected age despite a relatively low vitamin A intake. It would appear that based on unit weight or energy intake, the vitamin A requirement after hospital discharge is probably lower than that during the initial hospitalization.

Toxicity. Hypervitaminosis A from acute or chronic overconsumption of preformed vitamin A (not carotenoids) in older individuals can have a variety of manifestations, including symptoms and signs of raised intracranial pressure (e.g., headache, nausea, vomiting, double vision), bone and joint pain with or without radiographic changes, dryness of mucous membranes, desquamation, hepatomegaly and hepatic injury, hypercalcemia, and hematological abnormalities (Persson et al., 1965; Ross, 1999). In this condition, the plasma vitamin A value generally exceeds 100 μg/100 mL, the ratio of retinyl ester to free retinol is elevated, and assays of fasting plasma show esterified retinol. Plasma RBP concentrations usually remain normal (Smith & Goodman, 1976). In adults, even healthy users of vitamin and mineral supplements in quantities twice the recommended dietary allowance of vitamin A for adults have significantly increased fasting plasma retinyl ester levels (Krasinski et al., 1989), an early indicator of hypervitaminosis A (Ross, 1999).

Vitamin A toxicity was reported in five infants who received 800–3200 μg RE/(kg·d) for 1–3 months (Persson et al., 1965). By extrapolation from various toxicological data, it has been suggested that maximum dietary vitamin A intake should be 225 μg RE/100 kcal during infancy for infants born at term, but vitamin A toxicity can occur at intakes only two- or three-fold higher (Hathcock, 1989). In the multicenter trial of IM vitamin A supplementation for infants with a BW less than 1000 g (Tyson et al., 1999), the average daily intake of vitamin A from all sources was about 4000 IU/kg, with parenteral and enteral dietary intake accounting for about 750 IU/kg. At the end of 4 weeks of supplementation, the mean serum retinol concentration was double that in unsupplemented controls. In the subgroup of infants who received glucocorticoid therapy within 2 weeks of sampling, the average serum retinol concentration was 46 μg/100 mL, but the 95th percentile value was 89 μg/100 mL. The average serum retinyl ester concentration was three-fold greater in the vitamin A-supplemented group and more than six-fold greater in the glucocorticoid-treated subgroup than in the controls. The investigators reported no clinical manifestation of vitamin A toxicity in the supplemented group. It is conceivable that the serum retinol and retinyl ester concentrations could have been even higher in some infants, because the peak serum retinol concentration occurs within 1 week after steroid therapy (Georgieff et al., 1989), whereas the blood sampling in the multicenter trial was performed at various intervals after the steroid therapy.

Conclusions and recommendations

Many clinically healthy preterm infants have hyporetinolemia until after term corrected age, although they continue to have adequate growth. The minimum formula content of vitamin A should be associated with relatively few hyporetinolemic infants at the time of hospital discharge (Carlson et al., 1995; Koo et al., 1995b).

In one study (Koo et al., 1995b), actual intakes by preterm infants were 102, 225, and 337 μg RE/100 kcal. After an average of 1 month of feeding, the infants fed formula containing the lowest amount of vitamin A had significantly lower serum retinol and RBP concentrations than did the intermediate and high vitamin A intake groups. All infants in the lowest vitamin A intake group had hyporetinolemia (retinol concentration of <20 μg/100 mL) at the end of the study period, whereas only six infants in each of the intermediate and high vitamin A intake groups had hyporetinolemia. In another study of 63 LBW infants who received enteral feeding of preterm formula (vitamin A content 204 μg RE/100 kcal) beginning on the third day, there was a progressive increase in plasma retinol concentrations once full feeding was achieved. All infants, including those with BPD, had normal plasma retinol concentrations (<20 μg/100 mL) when reassessed at 2 months post-term (Carlson et al., 1995). Therefore, the Expert Panel recommended that preterm infant formula contain a minimum of 204 μg RE/100 kcal.

The maximum recommended vitamin A content should be at least the highest level of vitamin A content of infant formula studied without reported adverse effect, 337 μg RE/100 kcal (Koo et al., 1995b). At this level of intake, the average daily intake for a 1000-g infant consuming 120 kcal/kg would be 404 μg RE (1335 IU). This amount of vitamin A is comparable to the intake of preterm infants fed their own mothers' milk fortified with one of the commercial fortifiers, allowing for biological variability in milk vitamin A content. There are no data to indicate that a higher amount of vitamin A is needed for nutrition. However, higher daily intakes have been provided via supplements without reports of adverse effect (Landman et al., 1992; Woodruff et al., 1986). Moreover, one of the two formulas in the United States for premature infants contains even more vitamin A, 374 μg RE/100 kcal (American Academy of Pediatrics. Committee on Nutrition, 1998) and is not known to give rise to hypervitaminosis A. Therefore, the Expert Panel recommended a maximum of 380 μg RE/100 kcal.

In a multicenter vitamin A supplementation trial, an average total vitamin A intake of 1200 μg RE (4000 IU)/(kg·d) was fed for the first 4 weeks to infants (Tyson et al., 1999). A few infants in the vitamin A-supplemented group in that study had plasma retinol concentrations close to 100 μg/100 mL, a concentration reported to be associated with vitamin A toxicity. Also, serum retinyl ester concentrations were at least three-fold higher in the supplemented than in the unsupplemented group, although none of the infants showed clinical

vitamin A toxicity. Continuous monitoring for vitamin A toxicity is needed for infants receiving IM vitamin A prophylaxis for chronic lung disease.

It is likely that vitamin A needs are lower in infants during the second half of infancy-6-12 months of age. If vitamin A provided by premature infant formula with the maximum recommended content is the only source of vitamin A, then it is still well below the lowest vitamin A intake reported to be associated with toxicity (Persson et al., 1965). Decreased dependence on infant formula as the sole source of nutrition also decreases the likelihood of toxicity.

Recommendations

Minimum. **The Expert Panel recommended that the minimum vitamin A concentration of preterm infant formula be 204 µg RE (700 IU)/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum vitamin A concentration of preterm infant formula be 380 µg RE (1254 IU)/100 kcal.**

VITAMIN D

Background and review of the literature

Vitamin D metabolites have numerous potential physiological and pharmacological actions, but their principal physiological function is maintaining serum calcium and phosphorus concentrations in a range that supports cellular processes, neuromuscular function, and bone mineralization. Vitamin D (1 µg = 40 IU) is derived from plants as ergocalciferol (vitamin D_2) and from animals as cholecalciferol (vitamin D_3). In humans, vitamin D_3 can be synthesized endogenously from 7-dehydrocholesterol in the skin after exposure to the ultraviolet (UV) B spectrum (290–315 nm) of sunlight. Vitamins D_2 and D_3 appear to be metabolized along the same pathway, with little functional difference between their metabolites. They are referred to collectively as vitamin D.

Vitamin D is hydroxylated to 25-hydroxyvitamin D (25-OHD) in the liver. Quantitatively, 25-OHD is the most abundant vitamin D metabolite in the circulation and is a useful index of vitamin D reserve. It is bound to vitamin D-binding protein in the circulation and is transported to the kidney, where it is hydroxylated again to 1,25-dihydroxyvitamin D [1,25(OH)$_2$D], the most active vitamin D metabolite. This 1,25(OH)$_2$D acts primarily on intestine, kidney, and bone to increase the absorption and retention of minerals, thereby preventing rickets and osteomalacia.

Vitamin D metabolism is strongly influenced by calcium and phosphorus needs and by calcitropic hormones (parathyroid hormone, calcitonin, and other factors). Target tissue action is mediated classically through binding of the active metabolite to a specific nuclear receptor that belongs to the superfamily of steroid-hormone zinc-finger receptors, although there are also rapid nongenomic actions on intracellular calcium, phosphatidylinositol, and cyclic guanosine triphosphate metabolism, and on intestinal calcium absorption. There are at least 40 other vitamin D metabolites, with and without putative functions, produced in various organs.

The recommendation in the report *Assessment of Nutrient Requirements for Infant Formulas* for the vitamin D intake of term infants was 40–100 IU/100 kcal (Raiten et al., 1998a). For premature infants, the AAP-CON (1998) recommended 270 IU/100 kcal. The Association of the Food Industries for Particular Nutritional Uses of the European Union (1996) recommendation was 1–10 µg/100 kcal (40–400 IU/100 kcal). Koo and Tsang (1993) recommended 150–400 IU/kg daily, which would be 125–333 IU/100 kcal on an energy intake of 120 kcal/(kg·d).

ESPGAN-CON (1987) recommended "the same intake as [that of] babies receiving breast milk." According to that document, this would be the endogenous amount in milk plus a supplement of 20–40 µg/d (800-1600 IU/d). It was recommended that the vitamin D content of a formula not exceed 3 µg (120 IU)/100 mL, which would be 3.7 µg (148 IU)/100 kcal in a formula with 810 kcal/L.

When given to preterm infants as early as the first week after birth, vitamin D and several of its metabolites can raise the circulating levels of the corresponding compound or 25-OHD. Furthermore, elevation of serum 1,25(OH)$_2$D concentrations in response to low calcium and phosphorus intakes is well documented in LBW infants. This occurs regardless of race (black or white), mode of nutrient intake (enteral or parenteral), and with or without the presence of fractures or rickets (Koo & Steichen, 1998; Koo & Tsang, 1993). Thus, mechanisms for the absorption and metabolism of vitamin D compounds appear to be functional in LBW infants. The sources of vitamin D for infants include stores from transplacental transfer, primarily as 25-OHD, endogenous synthesis of vitamin D with exposure to UV irradiation in sunlight, and enteral and parenteral intakes.

Cord blood concentrations of vitamin D metabolites in preterm infants are similar to those for term infants, even though their vitamin D stores are probably lower than those of term infants. This is not only because of the shortened gestational age (GA), but also because of a smaller amount of fat and muscle, the major vitamin D storage sites. In any case, serum 25-OHD concentrations of healthy infants living in temperate climates fall to levels characteristic of vitamin D deficiency within about 8 weeks of birth. There is limited information on the endogenous production of vitamin D after exposure to UV irradiation from sunlight exposure during infancy, but this is of no clinical significance for LBW infants, whose exposure to sunlight, especially during hospitalization, is minimal. Human milk total vitamin D activity (primarily accounted for by the vitamin D parent compound and 25-OHD) is low, averaging about 13 IU/L IOM (1997; Lammi-Keefe, 1995) and varying with race (higher in whites) and season (higher in summer), but not with duration of lactation or GA at delivery. Also, the daily intake of vitamin D from human milk is minimal, so the most reliable way of supplying vitamin D to preterm infants is by direct administration or by fortification of the milk, usually with the parent compound. Details of the physiology and nutritional aspects of vitamin D are reviewed elsewhere (Koo & Tsang, 1993; Koo & Tsang, 1997).

In LBW infants, the need for vitamin D for intestinal calcium absorption is probably low. Standard balance methodology (Bronner et al., 1992) and stable isotope studies (Ehrenkranz et al., 1985) showed that calcium absorption is a linear function of the daily calcium intake in the range from 40 to 142 mg/kg and is independent of vitamin D supplementation of up to 2000 IU daily. Thus, most calcium absorption in preterm infants is probably a passive diffusion process, with the vitamin D-regulated mechanism not expressed during early infancy, even though a response of the intestine to vitamin D had been demonstrated earlier (Senterre et al., 1983).

Prevention of neonatal hypocalcemia by use of vitamin D or its active metabolite is due to a pharmacological rather than a nutritional effect (Koo & Tsang, 1999). Hypocalcemia has not been reported in healthy preterm infants receiving preterm infant formula with a high mineral content, with or without an additional vitamin D supplement. In preterm infants fed unfortified human milk, hypophosphatemia with hypercalcemia is the typical manifestation (Koo & Tsang, 1999). Development of radiographic osteopenia or rickets in LBW infants was not prevented by a 6-week course of daily vitamin D supplementation of 2000

IU or 400 IU (Evans et al., 1989). These infants were fed various proportions of human milk or standard cow milk- or soy-based infant formula, all with low calcium and phosphorus contents. The median serum 25-OHD concentration in the low vitamin D intake group was 24 ng/mL, well within the normal range, and in the high vitamin D intake group was 68 ng/mL, close to the upper limit of the normal range (Evans et al., 1989).

Vitamin D metabolism is intimately related to the availability of substrates, including vitamin D itself, calcium, and phosphorus. Early studies of vitamin D status in preterm infants fed milk with low calcium and low phosphorus contents have been reviewed elsewhere (Koo & Tsang, 1993). In states of calcium and phosphorus deficiency, vitamin D metabolism is increased, with an increased production of $1,25(OH)_2D$. The latter, in turn, increases the metabolic clearance of 25-OHD and further lowers its serum concentration (Clements et al., 1987). This process may explain early reports of vitamin D deficiency, as indicated by low serum 25-OHD concentrations, in preterm infants fed low calcium and low phosphorus mixtures such as human milk or standard cow milk formula, along with a low vitamin D intake.

After the initial report by Steichen et al. (1980), there were numerous studies supporting the need for much higher calcium and phosphorus intakes than those provided by human milk or standard cow milk formula designed for term infants (Koo et al., 1998). The role of vitamin D in preventing osteopenia, rickets, and fractures is probably second in importance to that of the quantity of mineral intake (Koo et al., 1998). Nevertheless, it seems prudent to supply enough vitamin D to maintain serum 25-OHD concentrations in the normal range, because there are many other potential physiological roles for vitamin D metabolites. The vitamin D requirement for preterm infants in this report will therefore be assessed on the basis of an assumption of dietary calcium and phosphorus intakes much higher than those achievable with human milk.

In-hospital needs. Almost all reports of the vitamin D status of LBW infants involved the use of vitamin D supplementation of human milk. There are at least eight reports of a total of 115 hospitalized preterm infants receiving 200–2000 IU of vitamin D supplementation daily in addition to an estimated vitamin D intake of 100–700 IU from the formula. The average total daily vitamin D intake of the infants in these studies was between 450 and 2100 IU. No study reported any disturbances in growth or biochemistry, although none made a systematic assessment of vitamin D status (Koo & Tsang, 1993). In one report, LBW infants were randomized to receive one of three milk formulas with the same calcium and phosphorus contents of 180 and 90 mg/100 kcal respectively, but with levels of vitamin D (cholecalciferol) at 74, 148, or 329 IU/100 kcal (Koo et al., 1995b), and no other vitamin D supplementation. There were no significant differences among the three groups in average daily gains in weight, length, and head circumference; in serum levels of calcium, phosphorus, magnesium, alkaline phosphatase, osteocalcin, 25-OHD, or $1,25(OH)_2D$; in urinary calcium-to-creatinine or magnesium-to-creatinine ratios; or in standard radiographs of both wrists (Koo et al., 1995a). In these reports, the lowest average daily vitamin D intake of a 1000-g infant with a daily energy intake of 120 kcal/kg would be about 90 IU (Koo et al., 1995a), and the highest average daily vitamin D intake would be about 2100 IU (Koo & Tsang, 1993). Because most studies give the same dose of vitamin D supplement to infants of all ages, the average daily intake per kilogram of body weight of vitamin D would decrease as the infant grew. For a 1000-g infant consuming 150 mL/kg of fortified human milk daily, the vitamin D intake from the commercial powdered fortifiers would be 180–315 IU/d.

Post-hospital discharge needs. The only longitudinal assessment of vitamin D status in LBW infants followed 71 infants with an average BW of 1001 g, of whom 22 had radiographically confirmed rickets or fractures (Koo et al., 1989). Sequential serum concentrations of calcium, magnesium, phosphorus, 25-OHD, $1,25(OH)_2D$, and vitamin D-binding protein, along with radiographs of the forearms including wrists, were determined at 3, 6, 9, and 12 months. These infants received supplementation of vitamin D of 400 IU/d, except for five infants (three with rickets or fractures) who received an additional 400–2400 IU/d for 7–94 days (median 38 days). Parents of approximately half of the subjects voluntarily discontinued the administration of supplemental vitamin D after 6 months. Vitamin D status, as indicated by serum 25-OHD concentrations, was in the range for normal healthy infants. These concentrations were similar in infants with or without rickets or fractures, although serum phosphorus concentrations were lower and $1,25(OH)_2D$ concentrations were higher at 3 and 6 months in the rickets/fractures group, which is not surprising given the role of mineral deficiency in infants with this disease. It would appear that on the basis of weight or energy intake the vitamin D requirement after hospital discharge is probably lower than that for in-hospital needs.

Toxicity. Vitamin D toxicity is characterized by anorexia, vomiting, failure to thrive, polyuria, ectopic calcification, hypercalcemia, hypercalciuria, and grossly elevated serum 25-OHD concentrations. Toxicity has been reported in infants and adults who received milk with accidental gross overfortification of vitamin D ($>$200,000 IU/L) (Jacobus et al., 1992). In one report, all infant formulas tested had vitamin D content two to three times greater than the label claims of 400 IU/L (Holick et al., 1992), presumably reflecting a practice by the infant formula manufacturers of adding extra amounts of nutrients (overage) to compensate for expected breakdown during storage, so as to meet the label claim near the end of the labeled shelf life. Vitamin D toxicity was reported in infants after high-dose vitamin D (600,000 IU of ergocalciferol every 2–3 months) for the prevention of rickets (Markestad et al., 1987).

Many of the nonspecific manifestations of vitamin D toxicity, including anorexia, vomiting, failure to thrive, and ectopic calcification, have been reported in various combinations in preterm infants with chronic lung disease who received numerous medications that may affect vitamin D, calcium, and phosphorus metabolism, although there are no convincing data attributing these manifestations to vitamin D toxicity. Elevated serum 25-OHD concentrations and hypercalcemia were reported in a 16-month-old infant with chronic lung disease, failure to thrive, and a requirement for multiple medications (Nako et al., 1993). This infant had a BW of 816 g and received milk formula with modest amounts of calcium and phosphorus (653 and 405 mg/L, respectively) and a vitamin D content of 2700 IU/L for 15 months. Estimated daily total vitamin D intake, including the vitamin D supplement, was 1200 IU. Thus, this infant had probably received a daily vitamin D intake between 414 IU/kg and 1480 IU/kg on the basis of his BW and his weight of 2900 g at 1 year. The roles of chronic lung disease, chronic diuretic therapy, and failure to thrive in contributing to this situation are not known, although serum parathyroid hormone levels were normal on several occasions.

Conclusions and recommendations

The vitamin D stores of preterm-LBW infants are probably lower than those of term infants. This is not only because of the shortened GA, but also because of a smaller amount of fat and muscle, the major vitamin D storage sites. The minimum recommended vitamin D content is also based on the evidence

that calcium absorption in LBW infants is related to calcium intake rather than to vitamin D intake (Bronner et al., 1992; Ehrenkranz et al., 1985) and that a daily intake of 90 IU/kg [75 IU/100 kcal at 120 kcal/(kg·d)] can maintain adequate vitamin D status (Koo et al., 1995b). Furthermore, with the typical vitamin D overage in milk formulas, this amount should be more than sufficient to meet the daily vitamin D requirement for clinically stable preterm infants receiving formula with adequate amounts of calcium and phosphorus.

The maximum recommended content was based on the amount provided by commercial human milk fortifier, which has been used without documented vitamin D toxicity since its introduction more than 10 years ago. In addition, one current domestic preterm formula contains 270 IU/100 kcal. Furthermore, at least one clinical study fed up to 329 IU/100 kcal without report of an adverse effect (Koo et al., 1995b). This amount is significantly lower than the 2000 IU of vitamin D experimentally administered to preterm infants fed formulas containing low calcium and low phosphorus contents and the same supplement given to limited numbers of preterm infants receiving formulas with higher calcium and phosphorus contents.

There are no data to suggest that the vitamin D content of milk formula should be different throughout the first year after birth. With a daily feeding of formula with high calcium and high phosphorus contents at 120 kcal/kg and the recommended range of vitamin D fortification in mother's milk, no additional vitamin D supplementation should be given to preterm infants unless there is documented vitamin D deficiency.

Recommendations

Minimum. **The Expert Panel recommended that the minimum vitamin D content of preterm infant formula be 75 IU/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum vitamin D content of preterm infant formula be 270 IU/100 kcal.**

VITAMIN E

Background

Vitamin E is the collective name for molecules that exhibit the biological activity of α-tocopherol. That includes all eight naturally occurring forms of the vitamin–four tocopherols and four tocotrienols. All have similar chromanol structures: trimethyl (α), dimethyl (β or γ), and monomethyl (δ). Tocotrienols differ from tocopherols in having an unsaturated side chain. The most biologically active form of vitamin E is the naturally occurring *RRR-α*-tocopherol (*d*-α-tocopherol). Chemically synthesized α-tocopherol has eight stereoisomers, collectively known as all-*rac*-α-tocopherol (racemic α-tocopherol). They include both *R* and *S* stereoisomers, including *RRR-α*-tocopherol.

Vitamin E acts as a chain-breaking antioxidant that prevents propagation of free radical damage in biological membranes. It is a potent peroxy radical scavenger. It especially protects PUFAs within phospholipids of biological membranes and in plasma lipoproteins. As a result, the vitamin E requirement is proportional to the amount of PUFA that is consumed and incorporated into membranes. Iron administration may also increase vitamin E need, because iron catalyzes the oxidation of cellular lipids through generation of free radicals. Iron also interferes with vitamin E absorption by increasing its destruction in the gut. The tocoperoxy radical (formed after reaction of tocopherol with other organic peroxy radicals) prevents further oxidation of lipids. In addition, α-tocopherol shows structure-specific effects on several enzyme activities

and membrane properties. For example, it down-regulates vascular smooth muscle cell proliferation, decreases protein kinase C activity, suppresses arachidonic acid metabolism via phospholipase A_2 inhibition, and enhances the degradation of the enzyme hydroxymethylglutaryl-coenzyme A reductase, which catalyzes the rate-limiting step in cholesterol biosynthesis. In preterm infants, the potential benefit of vitamin E in various disease processes, including retinopathy of prematurity, BPD, and intracranial hemorrhage, remains controversial. Such a benefit is probably due to a pharmacological action, and reports of associated toxicity (see below) have dampened enthusiasm for its use.

Regeneration of tocopherol from the tocopheroxy radical may occur in the presence of ascorbate (vitamin C) or thiols, especially glutathione, as hydrogen donors. Therefore, the vitamin E requirement also depends on the adequacy of other reducing agents, such as vitamin C. It further depends on selenium, which is essential for the activity of glutathione peroxidase. The flux through the cyclic radical pathway may be much larger than the flux through the pathway of degradation.

The relative biological activities of the different forms of tocopherol are as follows: 1 IU of vitamin E equals 1 mg of all-*rac*-α-tocopheryl acetate, 0.67 mg of *RRR-α*-tocopherol, or 0.74 mg of *RRR-α*-tocopheryl acetate. Vitamin E intake is calculated as *RRR-α*-tocopherol or *RRR-α*-tocopherol equivalents (α-TE). The relative efficiency of different vitamin E stereoisomers as substitutes for α-tocopherol has been reported to be 50% for β-tocopherol, 30% for tocotrienol, and 10% for γ-tocopherol. However, these forms of vitamin E are not functionally equivalent to tocopherol, as the eight different isomers of synthetic vitamin E (all-*rac*-α-tocopherol) have equivalent antioxidant activities but different biological activities.

All dietary forms of vitamin E are absorbed with similar efficiencies. Pancreatic esterases are required for hydrolytic cleavage of tocopheryl esters. Vitamin E absorption also requires bile acids, fatty acids, and monoglycerides for micelle formation. Vitamin E is secreted into lymph after incorporation into triglyceride-rich lipoprotein-containing chylomicrons. During chylomicron metabolism in the circulation, some of the newly absorbed vitamin E may be transferred to tissues directly; some is transferred indirectly through binding to high-density lipoprotein (HDL) and subsequent exchange to other circulating lipoproteins. Vitamin E rapidly exchanges among lipoproteins and between lipoproteins and membranes.

After partial delipidation by lipoprotein lipase and acquisition of apolipoprotein E, chylomicron remnants containing a major portion of the absorbed vitamin E are taken up by liver parenchyma. In the liver, α-tocopherol transfer protein preferentially incorporates *RRR-α*-tocopherol (which constitutes about 80% of the total tocopherol) into nascent very low density lipoprotein (VLDL). Thus, the liver, but not the intestine, discriminates between tocopherols. VLDL delipidation in the circulation by lipoprotein lipase and hepatic triglyceride lipase results in the preferential enrichment of HDL with *RRR-α*-tocopherol. HDL can then transfer vitamin E to all other lipoproteins.

There are at least two major routes by which tissues are likely to acquire to vitamin E, through lipoprotein lipase-mediated lipoprotein catabolism and via the low-density lipoprotein (LDL) receptor. Specific α-tocopherol-binding proteins have been described for certain tissues, including liver, heart, and RBCs, that might regulate cellular uptake or tissue metabolism of vitamin E. Kinetic studies show that some tissues, such as liver, spleen, and RBCs, undergo rapid equi-

librium with plasma α-tocopherol. Other tissues, such as heart, muscle, and spinal cord, have slower α-tocopherol turnover. The brain has the slowest αα-tocopherol turnover, although peripheral nerve is the most responsive part of the nervous system to vitamin E concentrations in the diet. In general, the vitamin E content of the nervous system is spared during vitamin E depletion.

More than 90% of the human body pool of α-tocopherol is in fat droplets of adipose tissue rather than in membranes. It is estimated that 2 years or more is required for ratios of α-tocopherol to γ-tocopherol to reach new steady-state levels in response to changes in dietary intake. It is uncertain whether vitamin E in human adipose tissues is readily available.

Absorbed vitamin E undergoes further metabolism, primarily by oxidation, and the end products are excreted into bile or urine. Skin and sebaceous glands contain various forms of dietary vitamin E and thus may be important sources of antioxidant in the protection of cutaneous lipids, as well as a route for vitamin E excretion. The fractional disappearance rate for circulating RRR-α-tocopherol is similar to that of other vitamin E stereoisomers (half-life of 13 hours), although its preferential incorporation into hepatic VLDL returns it to the circulation and results in a longer apparent half-life (48 hours).

Recommendations by other organizations. Dietary recommendations for α-TE recognize the interrelationship among vitamin E, PUFAs [linoleic acid (LA) and α-linolenic acid], and iron (Canadian Paediatric Society & Nutrition Committee, 1995). The 1995 minimum recommendation from the Canadian Paediatric Society for the vitamin E intake of preterm infants was no less than 0.5 mg α-TE/100 kcal (Canadian Paediatric Society & Nutrition Committee, 1995). The Canadian Paediatric Society recommended a ratio greater than 1 mg α-TE /g of PUFA. For premature infants, the AAP-CON (1998) recommended that intake of vitamin E exceed 1.1 IU/100 kcal. Gross (1993) recommended a vitamin E intake of 0.7 IU/100 kcal and 1.0 IU/g of LA. ESPGAN-CON (1987) recommended minima of 0.6 mg/100 kcal and 0.9 mg/g of PUFA. The 2000 dietary reference intake-tolerable upper intake level (DRI-UL) was set at 21 mg α-TE/d for premature infants of 1500 g (Institute of Medicine, 2000a). This would be equivalent to 14 mg α-TE/d for a premature infant of 1000 g. The DRI-UL for premature infants was derived from the DRI-UL for adults, and the work of Phelps et al. (1987) was cited by the Institute of Medicine to support this value.

Review of the literature

The concentrations of α-tocopherol and the tocopherol-to-LA ratio in human milk decrease with progression of lactation. Milk from mothers delivering preterm infants is reported to contain 1.9 IU/100 kcal, with a vitamin E-to-PUFA ratio (mg of d-α-tocopherol/g of LA) of 2.0 during the first week postpartum. This content decreases to 0.6 IU/100 kcal with a vitamin E-to-PUFA ratio of 0.7 during the fourth week postpartum (Gross & Gabriel, 1985). Cow milk vitamin E content averages 0.04 mg αof α-tocopherol/100 g. Preterm infant formulas in the United States are fortified with all-rac-α-tocopheryl acetate to a vitamin E content of 4.0–6.3 IU /100 kcal (2.7-4.2 mg α-TE/100 kcal), an LA content of 0.7–1.06 g /100 kcal, and therefore a vitamin E-to-LA ratio of 3.83–3.98. Commercial powdered human milk fortifiers at the recommended dilution provide 4.8–6.9 IU/100 kcal. Assuming human milk to contain 0.86 g of LA (or 0.99 g of total PUFA)/100 kcal (Innis, 1993) would make the vitamin E-to-LA ratio 3.7–5.4, and the vitamin E-to-total PUFA ratio

3.3–4.7, if the variable amount of endogenous tocopherol content in milk is excluded from the calculation.

The adequacy of vitamin E intake is often estimated by measurement of plasma α-tocopherol, although the influence of circulating lipids on tocopherol concentrations makes it preferable to express plasma α-tocopherol concentration relative to β-lipoprotein, cholesterol, or total lipid concentration (Horwitt et al., 1972). The 5th to 95th percentiles of plasma α-tocopherol from the U. S. National Health and Nutrition Examination Survey III survey of subjects between 6 and more than 80 years of age were 14.2–44.2 μmol/L (6.1–19.0 mg/L). When adjusted for cholesterol, they were 3.41–7.67 μmol/mmol (3.76–8.53 μg/mg) (Ross, 1999). Normal plasma α-tocopherol values are generally considered to be higher than 11 μmol/L (5 mg/L), or higher than 0.8 mg of α- tocopherol/g of total lipid, or 2.8 mg of α-tocopherol/g of cholesterol.

Preterm infants with limited adipose tissue also have a limited amount of vitamin E. Plasma concentrations of αα-tocopherol in infants at birth (regardless of GA) average less than half the normal adult values and are only 20–30% of the corresponding maternal values; thus, almost all infants have plasma α-tocopherol values that would cause them to be considered at high risk for vitamin E deficiency. However, when expressed as a ratio to lipid, the values are similar for neonates and adults. Details of the physiological and nutritional aspects of vitamin E are reviewed elsewhere (Gross, 1993; Traber, 1999).

Deficiency of vitamin E. It is well documented that deficiency of vitamin E can result in progressive neuronal damage in both children and adults with various fat malabsorption syndromes, such as chronic cholestatic hepatobiliary disease or pancreatic disorders, or as a result of genetic abnormalities that cause in an absence or a functional defect in α-tocopherol transfer protein, or from defects in lipoprotein synthesis, including hypo- or abetalipoproteinemia. In the general population, dietary deficiency of vitamin E is rare because of the ubiquitous distribution of tocopherol in foods, especially in oils and fats. Soybean oil emulsions used widely in parenteral nutrition contain high levels of γ- but not α-tocopherol, so a separate source of vitamin E is needed in patients receiving long-term TPN.

In vitro hemolysis of RBC with hydrogen peroxide was increased within a few days of birth for preterm-LBW infants fed fat-free formula that was deficient in vitamin E or fat-free formula that was deficient in vitamin E plus 5% of calories as LA (Panos et al., 1968). The administration of vitamin E increased serum vitamin E concentration and concomitantly decreased the percentage of hemolysis and increased RBC half-life survival for both groups. In LBW infants, measures of hemolytic anemia are the only manifestation of dietary vitamin E deficiency reported after feeding formula with low vitamin E content (1 mg α TE /100 kcal), a vitamin E-to-PUFA ratio of 1.3, and iron supplementation of 1.8 mg/100 kcal (Williams et al., 1975). In vitro hemolysis of RBC with hydrogen peroxide was significantly greater for those infants fed formula containing 32% of fat as LA compared with those fed formula containing 13% of fat as LA (Williams et al., 1975). This abnormal tendency toward RBC hemolysis has not been reported after fortification of vitamin E to the level present in current preterm infant formulas (2.7-4 mg α TE/100 kcal).

In the late 1960's, preterm-LBW infants fed formula with a vitamin E content of 0.20-0.35 mg α-TE/100 mL developed edema and anemia, which were corrected with vitamin E supplementation (Hassan et al., 1966; Ritchie et al., 1968). Prior to supplementation, the feeding contained ratios of 0.20-0.27 mg α-TE/g of PUFA (Ritchie et al., 1968) or 0.19-0.37

mg α-TE/g of LA (Hassan et al., 1966). Interestingly, preterm-LBW infants developed anemia if fed formula with a vitamin E content of 0.77 mg α-TE/100 mL and an iron content up to 1.2 mg/100 mL when the feeding provided 0.52 mg α-TE /g of LA (1.49 g LA/100 mL, 43% of fat) but not when the feeding provided 1.68 mg α-TE /g of LA (0.46 g LA/100 mL, 13% of fat) (Gross, 1979). Similarly, preterm-LBW infants developed anemia and borderline-low serum vitamin E concentration if fed formula with a vitamin E content of 0.67 mg α-TE/100 mL (1.0 mg α-TE/100 kcal), an iron content of 1.2 mg/100 mL (1.8-2.0 mg/100 kcal) and 1.3 mg α-TE /g of PUFA when the feeding provided 5.9 mg α-TE /g of LA (1.13 g LA/100 mL, 33% of fat) but not when the feeding provided 15 mg α-TE/g of LA (0.45 g LA/100 mL, 13% of fat) (Williams et al., 1975). Therefore, when the amount of vitamin E fed is borderline-adequate, the amount of LA (PUFA) fed has a clinically significant impact on vitamin E status.

In-hospital needs. One report of preterm infants (average BW of 1439 g) fed their own mothers' milk showed a progressive increase in α- and γ- tocopherol in milk and plasma as well as in cells. This was true of cells with LDL receptors (e.g., buccal mucosal cells, monocytes, and neutrophils) as well as those without (e.g., RBCs and platelets), during the first 6 weeks after birth (Kaempf & Linderkamp, 1998). The tocopherol contents of all types of cells were similar to the contents of cells from older healthy infants, and plasma α-tocopherol concentrations and tocopherol-to-lipid ratios were within the normal ranges after full enteral feeding was achieved (>7 days). Mean milk α-tocopherol content was 325–500 μg/100 mL (~590 μg α-TE/100 kcal, assuming an energy content of 700 kcal/L) in the period from 14 to 42 days after delivery. These infants did not receive vitamin E supplements.

In another study, LBW infants (BW of 735–1500 g) were fed milk from mothers who delivered term infants, milk from mothers who delivered preterm infants, or infant formula, during the first 6 weeks after birth (Gross & Gabriel, 1985). LA averaged 25% of total fat in formula, 22% in term milk, and 16% in preterm milk. All infants received a daily supplement of 4.1 mg α-TE as d-α-tocopherol succinate. The iron content of the formula was 0.15 mg/100 kcal. One-half of all infants had 2 mg/(kg·d) iron, as ferrous sulfate, added their diet (total ~2.3 mg/d or ~1.7 mg iron/100 kcal) beginning in the second week. No infant receiving human milk had supplements with other fortifiers. The formula contained 1.3 mg of α-tocopherol/100 mL (1.9 mg of α-tocopherol/100 kcal), with an α-tocopherol-to-LA ratio of 1.54. The total α-tocopherol intake, including supplement, was 6.4 mg/d (4.6–4.9 mg/100 kcal when formula was fed at 180 mL/kg). Serum α-tocopherol concentrations and vitamin E-to-total lipid (mg/g) ratios were higher in all groups at 6 weeks than during the first week. At 6 weeks, serum α-tocopherol concentrations were highest in those infants fed preterm milk and lowest in those fed formula. Serum vitamin E-to-total lipid ratios were also lowest in formula-fed infants. For groups receiving added iron, mean serum α-tocopherol concentrations were significantly lower yet remained within the normal range. By the sixth week postpartum, the group fed formula with iron supplementation had the lowest mean serum α-tocopherol concentration (4.5 mg/dL) and serum vitamin E-to-lipid ratio (0.79 mg/g). These mean values were borderline-low, suggesting some cases of suboptimal vitamin E status. The total vitamin E intake was similar to that provided by currently available preterm infant formulas alone and somewhat lower than that provided by commercial human milk fortifier.

Another report compared larger preterm infants (average BW of 1925 g) fed their own mothers' milk or either of two low iron formulas (0.9 or 0.08 mg/100 mL) with vitamin E contents of 1.3 and 2 mg/100 mL and vitamin E-to-PUFA ratios of 1.7 and 2.8 mg/g (Van Zoeren-Grobben et al., 1998). Within the first week after full enteral feeding, regardless of the type of feeding, there was a rapid normalization of plasma α-tocopherol concentrations, and plasma α-tocopherol-to-total lipid ratios were normal for most infants. However, the RBC tocopherol content and the RBC tocopherol-to-PUFA ratio, measured in a sub-group of 13 infants (type of feeding not reported), remained below the adult values even at 6 weeks, when the study ended.

It thus appears that preterm-LBW infants fed their own mothers' milk or preterm infant formula currently available in the United States at 120 kcal/(kg·d) can achieve adequate vitamin E status without additional vitamin E supplementation, although vitamin E intake should be provided to infants receiving parenteral nutrition until full enteral feeding is achieved. Preterm infants receiving a daily intake of between 2.8 and 3.5 mg of α-tocopheryl acetate through parenteral nutrition are usually able to maintain adequate plasma α-tocopherol concentrations of 1–2 mg/100 mL (Greene et al., 1991).

Neal et al. (1986) determined that oral administration of 25 mg/kg of vitamin E (tocopherol acetate) to preterm-LBW infants every six hours beginning on the first day of life [total: 100 mg/(kg·d)], frequently resulted in serum tocopherol concentrations exceeding 3.5 mg/dL compared to adult physiologic ranges reported to be 0.7-2.0 mg/dL. Whereas, oral supplementation of 60 mg α-tocopherol/d (40 mg/100 kcal, 22 mg of α- tocopherol/g LA) beginning on the third day of life resulted in mean plasma vitamin E concentrations of 1.2 mg/dL or less for preterm-LBW infant, measured weekly over a 20 week period (Hassan et al., 1966).

An intravenous preparation containing 25 USP units of dl-α tocopherol acetate per mL solubolized in polysorbate was marketed in the early 1980s as a vitamin supplement. (Bove et al, 1985, Balistreri et al, 1986). No clinical trial concerning safety or efficacy were conducted prior to introduction. After widespread distribution to neonatologists throughout the country, there were reports of unexplained symptoms in LBW infants, including hypotension, thrombocytopenia, renal dysfunction and other life threatening conditions (Bove et al. 1985, Arrowsmith et al, 1989, Martone et al, 1986). At least 38 deaths in 11 states were reported with 43 other serious adverse events among LBW infants. It was concluded that this vitaminE-polysorbate preparation was associated with the increased morbidity and mortality among the exposed LBW infants (Arrowsmith et al., 1989). Moreover, a dose response relationship was noted with toxicity evident in LBW infants receiving doses greater than 20 USP units/(kg·d), (Martone et al, 1986). Unfortunately, no definitive data were available to determine which constituent was responsible for the extreme toxicity or whether a cumulative effect was operative (Bove et al., 1985). Animal tests have shown toxic effects from polysorbate (Balistreri, 1986).

Phelps et al. (1987) conducted a randomized, double-blind study of preterm-LBW infants in the United States. The infants in the treatment group were administered intravenous (IV) doses of 20 mg of all-rac-α-tocopherol/kg on 2 or 3 days in the first few days postpartum. Oral, IV or IM doses of vitamin E continued up to 1 year of age, with the goal of achieving a plasma tocopherol level of at least 2.5 mg/dL. The infants in the treatment group had more retinal hemorrhages than those in the placebo group. Furthermore, grades 3 and 4 intraventricular hemorrhage occurred more frequently in the infants in the treatment group whose BWs were less than

1000 g. The results of this study indicate that pharmacological doses of vitamin E to preterm-LBW infants increases their risk of hemorrhagic complications.

Post-hospital discharge needs during first year. In a 15-month follow-up study of 51 infants with a BW of less than 1521 g (17 of them small for gestational age) and GA of 27–36 weeks, plasma α-tocopherol concentrations decreased when daily oral vitamin E supplementation (1.6 mg d-α-tocopherol succinate on day 1, increased up to 10 mg daily by day 14, and continued at that level thereafter) was discontinued at 12 weeks after birth. By 15 months, the plasma α-tocopherol level reached a mean concentration of less than 8 mg/L (the 10th percentile for adult values), although α-tocopherol-to-β-lipoprotein ratios remained within the normal range for adults (Rönnholm et al., 1989). These infants were fed a variety of milks (own mother's milk, banked human milk, or formula) during hospitalization. After hospital discharge, breast-feeding was continued in 20 infants for 3 months, in 15 infants for 6 months, and in 8 infants for 1 year. The infants who were not breast-fed received formula with an average α-tocopherol content of 10.1 mg/L. Thus, it is possible that some preterm infants may require a milk formula with higher vitamin E content than 10.1 mg/L after hospital discharge. For d-α-tocopherol and 810 kcal/L, this would be 1.9 IU/100 kcal. Vitamin E deficiency has not been reported in preterm infants fed term infant formulas that contained 2–3 IU of vitamin E/100 kcal.

Conclusions and recommendations

At full enteral intake, preterm infants fed their own mothers' milk with a total tocopherol content of about 1.2 mg/100 kcal have adequate vitamin E status without additional vitamin E supplementation (Kaempf & Linderkamp, 1998). A vitamin E intake of 1.3 or 2.0 mg/100 mL from preterm formula was adequate to maintain sufficient plasma vitamin E concentration for most infants when the vitamin E-to-LA ratio (mg/g) was 1.7 or 2.8, respectively, and the iron concentration was 0.9 or 0.08 mg/100 mL, respectively (Van Zoeren-Grobben et al., 1998). In LBW infants, hemolytic anemia results after feeding formula with a vitamin E content of 1.5 IU/100 kcal, a vitamin E-to-LA ratio of 1.3, and iron supplementation of 1.8 mg/100 kcal (Williams et al., 1975). However, an intake of 4.1 mg of vitamin E/d in addition to formula (total $\sim$4.9 mg α-TE/100 kcal and α-tocopherol-to-LA ratio $\sim$3.9) with iron supplementation of 2 mg/kg (total $\sim$2.3 mg/d or $\sim$1.7 mg iron/100 kcal) resulted in borderline-low serum α-tocopherol concentrations and α-tocopherol-to-total lipid ratios (Gross & Gabriel, 1985; Van Zoeren-Grobben et al., 1998). Therefore, the Expert Panel recommended that the minimum vitamin E content of premature infant formula be 2 mg α-TE/100 kcal. The vitamin E-to-PUFA ratio (mg of α-tocopherol/g of total PUFA) should exceed 1.5 mg/g. Note that if preterm infant formula contained the maximum recommended amount of LA (25% of total maximum fat, 1.4 g LA/100 kcal), the vitamin E-to-LA ratio (mg of α- tocopherol/g LA) would only be 1.4 mg/g.

The results of the study by Phelps et al. (1987) indicate that pharmacological doses of vitamin E to preterm-LBW infants increased their risk of hemorrhagic complications. The 2000 DRI-UL was set at 21 mg α-TE/d for premature infants of 1500 g (Institute of Medicine, 2000a). This would be equivalent to 14 mg α-TE/d for a premature infant of 1000 g, or 12 mg/100 kcal. In one Finnish study (Rönnholm et al., 1989), preterm-LBW infants fed human milk during hospitalization were administered a water-soluble vitamin E at 10 mg/d and iron at 3–4 mg/kg at 2 weeks of age. Vitamin E and iron supplements were not administered simultaneously. Supple-

mentation continued daily up to 12 weeks for vitamin E and up to 15 months for iron. The preterm-LBW infants achieved the adult level of normal plasma α-tocopherol concentration, and no adverse effects were reported (Rönnholm et al., 1989). However, no additional benefit was reported either. It is possible that vitamin E needs are lower during later infancy, but there is no known toxicity with vitamin E intake at the maximum recommended level. A maximum concentration of 8 mg α-TE/100 kcal ($\sim$10 mg/d) was recommended for preterm infant formula.

Recommendations

Minimum. **The Expert Panel recommended that the minimum vitamin E content of preterm infant formula be 2 mg α-TE/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum vitamin E content of preterm infant formula be 8 mg α-TE/100 kcal.**

Ratio. **The vitamin E-to-PUFA ratio (mg of α-tocopherol/g of total PUFA) should exceed 1.5 mg/g.**

VITAMIN K

Background

Vitamin K is the generic descriptor for 2-methyl-1,4-naphthoquinone and those of its derivatives that qualitatively exhibit the antihemorrhagic activity of phylloquinone (Combs, Jr., 1992). There are three biologically active forms of vitamin K: vitamin K_1, or phylloquinone, present in green plants; vitamin K_2, or menaquinones, products of bacterial synthesis; and vitamin K_3, or menadione, the synthetic form of vitamin K (Suttie, 1996). Vitamin K is required for the post-translational conversion of glutamic acid to γ-carboxyglutamic acid in several mammalian proteins, which are therefore referred to as vitamin K dependent. The γ-carboxyglutamic acid residues serve as calcium-binding moieties (Olson, 1999). Without vitamin K, the unmodified proteins cannot bind calcium and are inactive. The vitamin K-dependent proteins include prothrombin; plasma clotting factors VII, IX, and X; and anticoagulant proteins C and S, all present in plasma. In addition, there are two vitamin K-dependent proteins in bone, osteocalcin and matrix protein.

Vitamin K deficiency in the newborn is rare in the United States because of vitamin K prophylaxis; it remains a problem worldwide. The specific deficiency is characterized mainly by hemorrhagic disease of the newborn (HDN), manifested as excessive bleeding, caused by abnormal clotting factors. For reasons described below, premature infants may be more susceptible to vitamin K deficiency than are full-term infants (Olson, 1999).

In 1961, the AAP-CON (1961) recommended that vitamin K be administered to both term and preterm infants at birth to prevent HDN. They recommended vitamin K_1 as the drug of choice. Prophylactic doses of vitamin K_1 are currently standard procedure in the United States (American Academy of Pediatrics. Committee on Nutrition, 1998). The current recommendation by the AAP-CON (1998) specifies IM prophylactic doses of 0.3 mg of phylloquinone for preterm infants weighing less than 1000 g and 1.0 mg for those weighing more than 1000 g. An earlier recommendation by the AAP-CON (1993) specified that all preterm (and term) infants should receive 0.5-1.0 mg vitamin K at birth. Similarly, the Fetus and Newborn Committee (1998) of the Canadian Paediatric Society and the Committee on Child and Adolescent Health of the College of Family Physicians of Canada recommended that vitamin K should be given as a single IM dose of 0.5 mg for

infants of BW 1500 g or less and 1.0 mg for those of BW greater than 1500 g. For a single oral dose option, 2.0 mg was recommended.

The Code of Federal Regulations (21 CFR 107.100) (Senti, 1985) specified that term infant formula should have a minimum of 4 μg/100 kcal. It was suggested that this supplement be in the form of phylloquinone (vitamin K_1).

In 1993, Fomon and Suttie (1993) estimated that 1.5 μg/100 kcal would be adequate. The present DRI-adequate intake for term infants 0–6 months of age is 2 μg/d or 0.3 μg/kg, assuming a 7-kg infant (Institute of Medicine, 2001d). This value was based on the average concentration of human milk of 2.5 μg/L and an average intake of 780 mL/d. The recommendations for term infants (Raiten et al., 1998a) were for a minimum vitamin K_1 content of 1 μg/100 kcal and a maximum of 25 μg/100 kcal.

For preterm infants, the present enteral recommendation by the AAP-CON (1998) is 4 μg/100 kcal. The consensus recommendation from Tsang et al. (1993) quoted in AAP-CON (1998) for enteral vitamin K intake of preterm infants is 6.6–8.33 μg/100 kcal. To date, no maximum level has been recommended for preterm infants by these groups. ESPGAN (1987) recommended 4–15 μg/100 kcal for preterm infant formula. The Ad Hoc Expert Consultation to the Health Protection Branch, Health Canada, recommended a minimum and maximum of 4 and 26 μg/100 kcal, respectively, for preterm formula (Health Canada Health Protection Branch, 1995).

Review of the literature

Deficiency. Without supplementation, preterm infants are at higher risk for vitamin K deficiency. This is because of immaturity of organ systems, low tissue stores, low serum vitamin K and vitamin K-dependent coagulation proteins, and an increased risk of infections (Greer, 1999). In addition, in a study (Goldschmidt et al., 1988) designed to measure the activity of vitamin K-dependent clotting factors in infants requiring TPN and IV antibiotics, preterm infants had lower factor II activity as measured functionally and antigenically than did full-term infants. Furthermore, the preterm infants required a second dose of prophylactic vitamin K_1 5–7 days after the initial dose because of decreased factor II activity (Goldschmidt et al., 1988). However, HDN is relatively rare in preterm infants in the United States because vitamin K is administered prophylactically, but it is a significant cause of morbidity and mortality in other countries (Loughnan & McDougall, 1993). Breast-feeding is a risk factor for HDN, perhaps because the intestinal microflora of breast-fed infants do not synthesize menaquinones (originally named vitamin K_2), unlike the Gram-negative microbes of the intestinal tract of formula-fed infants (Greer, 1999), although absorption in the lower gastrointestinal tract is very limited (Suttie, 1995). A summation of data from 15 countries indicated that among 131 cases of HDN, including 11 in the United States, described in 36 publications between 1967 and 1992, only 2 were in premature infants, neither of these "very LBW." Eighty-nine affected infants (68%) did not receive any prophylactic vitamin K (Loughnan & McDougall, 1993).

The Institute of Medicine (2001d) estimated an average concentration of human milk of 2.5 μg of phylloquinone/L on the basis of a review of seven studies published since 1982, conducted during 8 days to 6 months postpartum, which included subject numbers ranging from 9 to 60. The vitamin K content of human milk without fortification is inadequate to meet the recommended intake of 4 μg/100 kcal for the preterm infants (American Academy of Pediatrics. Committee on

Nutrition, 1998). The phylloquinone content of milk from six healthy mothers of preterm infants, GA range of 2–0 weeks, was 3 μg/L (Bolisetty et al., 1998).

Estimated requirements. In 1961, the AAP-CON (1961) estimated on the basis of the limited data available at that time that the minimal effective oral dose of vitamin K to prevent coagulation abnormalities in newborn infants was 1–5 μg/d. In 1984, Miller and Chopra (1984) suggested that the "absolute requirement" for a specific vitamin may be greater for the premature infant than for the full-term one. Schanler and Atkinson (1999) indicated that the nutritional needs of the preterm infant are greater than at any other time of the life cycle, presumably on a per kilogram basis. Preterm infants are often subjected to the stresses of supplementary oxygen and ventilatory support, which potentially increase the basal metabolic rate. Also, catch-up growth may increase the requirement (Hack et al., 1996).

The amount of a vitamin, including vitamin K, to be added to full-term infant formula has usually been based on the concentration in breast milk, plus 30–50% more as a safety factor (Greene et al., 1992). Recently this approach has been questioned, especially for the "micropremie" (Greer, 2000). The data for estimating the vitamin K requirements of full-term infants have been reviewed in Raiten et al. (1998a). The two current domestic preterm infant formulas in the United States provide 8 and 12 μg vitamin K/100 kcal.

One study (Loughnan et al., 1996) described late-onset HDN in two preterm infants who had received IV vitamin K_1 in amounts expected to protect against the deficiency. The infants were born at GA 25 and 29 weeks, weighing 730 and 1630 g, respectively. The second infant was diagnosed with hepatitis. Profuse bleeding on days 84 and 74, respectively, was the result of the vitamin K deficiency. Before the onset of bleeding, the infants had received total IV doses of vitamin K_1 of 0.12 or 0.94 mg/kg, equivalent to doses of 0.4 and 3.3 mg in a full-term, 3500-g infant. The authors concluded that IV vitamin K_1 is less effective than IM administration and thus should not be used for long-term prophylaxis of HDN. Although these few data cannot be used to estimate a requirement for preterm infants, they do suggest that vitamin K_1 delivered by the IV route is less effective than that administered by the IM route.

Rossi et al. (1996) fed formula containing 3.4–6 μg of vitamin K_1/100 mL ($\sim$4.3–7.5 μg/100 kcal) to 50 or more preterm infants who received prophylactic vitamin K_1 at birth. They determined that prothrombin times were well within published reference values for neonates during the first 6 weeks of life (Rossi et al., 1996).

Toxicity. No vitamin K toxicity has been reported in the preterm infant (Greer, 2000). Early animal studies involving high doses of vitamin K found no toxicity, with the National Research Council (1987) concluding that vitamin K_1 exhibited "no effects when administered to animals in massive doses by any route." Thus, on the basis of the limited information available, no DRI-UL was set (Institute of Medicine, 2001d). In contrast, menadione administered to infants has been associated with hemolytic anemia and liver toxicity (Suttie, 1996), so menadione is not recommended as a therapeutic form of vitamin K. Olson (1992) indicated that there are no reports of toxicity of vitamin K_1 (phylloquinone) in humans at 500 times the 1989 recommended dietary allowance. In 1989, Olson (1989) proposed an upper limit of phylloquinone of 20 μg/100 kcal in an effort to prevent "excessive supplementation." The report *Assessment of Nutrient Requirements for Infant Formulas* (Raiten et al., 1998a) recommended a maximum for term infants of 25 μg/100 kcal on the basis of the 90th

percentile of Food and Drug Administration analyses of infant formulas.

Conclusions and recommendations

Preterm infants are thought to be more susceptible to vitamin K_1 deficiency than are full-term ones; however, this is not well documented or quantified in the literature. The AAP-CON (1998) recommended a prophylactic IM dose of 0.3–1.0 mg at birth, depending on BW. In infants requiring TPN and IV antibiotics, preterm infants had lower factor II activity as measured functionally and antigenically than full-term infants. Furthermore, the preterm infants required a second dose of prophylactic vitamin K_1 5–7 days after the initial dose because of decreased factor II activity (Goldschmidt et al., 1988). Similarly, factor II concentration was significantly lower in healthy preterm infants compared with term infants at 5 and 30 days postpartum (Andrew et al., 1988). Therefore, the vitamin K requirement may be greater in hospitalized preterm-LBW infants than term infants.

Prothrombin times were well within published reference values for preterm infants receiving formula containing 3.4–6 μg of vitamin K/100 mL ($\sim$4.3–7.5 μg/100 kcal) during the first 6 weeks of life (Rossi et al., 1996). Therefore, preterm formula containing $\sim$7.5 μg/100 kcal may support adequate clotting function in infants who received prophylactic vitamin K_1 within hours after birth.

Even after arbitrarily assuming a higher requirement and lower bioavailability, the 8 and 12 μg/100 kcal supplied by the two preterm infant formulas in the United States appear adequate to prevent a deficiency, when an initial prophylactic IM of vitamin K_1 is administered within hours of birth. Subclinical vitamin K deficiency might not be evident in infants if vitamin K status is assessed solely by coagulation factors. Further research to establish sensitive indices of vitamin K status in infants would make a major contribution in establishing the requirement for this essential vitamin.

A daily amount not to exceed 25 μg/100 kcal was recommended for full-term infants (Raiten et al., 1998a). This amount appears reasonable because there are no reports of toxic effects of vitamin K_1 even when massive doses were administered to animals or humans by different routes (Hawdon et al., 1992).

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of vitamin K_1 (phylloquinone) in preterm infant formula be 4 μg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum concentration of vitamin K_1 (phylloquinone) in preterm infant formulas be 25 μg/100 kcal.**

14. VITAMINS: WATER-SOLUBLE VITAMINS

VITAMIN C

Background

For specific information on the biochemistry, functions, and human nutriture of vitamin C, see Institute of Medicine (IOM) (2000b), Jacob (1998), Levine et al. (1996), Orazlesi and Lucchini (1993), Lucas (1993), and Goldsmith (1961). For reviews concerning recommendations and requirements for term infants, see Raiten et al. (1998a), Schanler (1997), Moran and Greene (1979), and Fomon (1993c). For general reviews related to the safety of high intakes of vitamin C, see Anderson (1975), Rivers (1987), dietary reference intakes

(DRIs) (Institute of Medicine, 2000b), and Levine et al. (1996).

Functions. In addition to the prevention of scurvy, vitamin C has numerous other functions and is a cofactor for at least eight enzyme systems, including two involved in tyrosine metabolism (Levine et al., 1996). The apparent in vivo antioxidant protection properties of vitamin C for adults were recently recognized when the estimated average requirement for vitamin C was established (Institute of Medicine, 2000b).

Data are accumulating that indicate lower antioxidant capacity in preterm infants than in term infants. Lower antioxidant capacity has been defined as low levels of one or more plasma components purported to be in vivo antioxidants (Becker, 1993; Gopinathan et al., 1994; Greenough et al., 1988; Gutcher et al., 1984; Hustead et al., 1984; Karmazsin et al., 1990; Miller et al., 1993; Rosenfeld et al., 1986; Shenai et al., 1981; Silvers et al., 1998; Sullivan & Newton, 1988). These proposed plasma antioxidants include vitamin A, vitamin E, antiproteinase, carotene, urate, ceruloplasmin, albumin, and thiol-containing amino acids. It is not established that all of these substances are true in vivo antioxidants, but vitamin C has been reported to be a more potent plasma antioxidant than the others (Frei et al., 1989).

Measurements of antioxidant potency are usually made using in vitro and not in vivo techniques, which are technically challenging. Sullivan and Newton (1988) reported a variable but significant ($P < 0.05$) positive association of birth weight (BW) with cord serum antioxidant activity, as measured by inhibition of thiobarbituric acid-reacting substances, in 25 infants of 830–3700 g. In contrast, another report indicated no significant difference between the plasma antioxidant activities, as measured by the total radical-trapping capacity of the antioxidants in plasma, of 18 preterm [mean gestational age (GA) of 32 weeks] and 20 term infants (Lindeman et al., 1989). This lack of difference may have been related to the relative immaturity of the infants (Miller et al., 1993). Bass et al. (1998) suggested that vitamin C administration may be a way to increase the antioxidant capabilities of preterm infants.

Review of the literature

Requirements. Three preterm-low birth weight (LBW) infants fed pooled, pasteurized human milk developed scurvy, diagnosed histologically postmortem (Ingalls, 1938). Samples of similarly treated milk contained, on average, 3 mg of vitamin C/L. Ingalls (1938) estimated that these infants received no more than 0.5–1.0 mg of vitamin C/d. Few data are available regarding the vitamin C requirements of either preterm or term infants (Fomon, 1993c; Greene & Smidt, 1993; Institute of Medicine, 2000b). Nevertheless, some early studies reported that preterm infants may have a higher requirement for vitamin C or be more vulnerable to vitamin C deficiency than term infants (Parks et al., 1935; Toverud, 1935; von Wiesener, 1966; Woolf & Edmunds, 1950). Reasons offered for the apparent higher requirements for preterm infants include these: protein levels of the preterm infant formula in the United States are higher than those in term formula (vitamin C is involved in protein metabolism); the rate of maternal transfer of vitamin C to the fetus is greatest during the latter half of the third trimester; healthy preterm infants experience a rapid catch-up growth exceeding that of term infants; and preterm infants are particularly vulnerable to free radical damage resulting from pulmonary oxygen toxicity, a major clinical problem (Wispe & Roberts, 1987).

Comparison of vitamin C content of preterm and term infant formulas. No reports have been published identifying vitamin

C deficiency among preterm infants fed preterm formula with a vitamin C content ranging from 8.5 to 37 mg/100 kcal (American Academy of Pediatrics. Committee on Nutrition, 1998). (The criteria for assessing deficiency were not given.) Preterm formulas presently available in the United States contain from 20 to 37 mg of vitamin C/100 kcal, compared with 9–12 mg/100 kcal for term infant formulas in the United States (American Academy of Pediatrics. Committee on Nutrition, 1998).

Vitamin C content of breast milk. Traditionally, breast milk has been used as the primary reference standard to assess nutritional requirements of term infants (Institute of Medicine, 2000b). The mean vitamin C concentration of human milk has been reported to vary from 34 to 90 mg/L within the first week after parturition [see IOM (2000b) for a summary of eight studies]. However, the IOM (2000b) has estimated the average vitamin C concentration of human milk to be 50 mg/L. McCormick (1989b) indicated that human milk provides 42 mg/L, and Raiten et al. (1998a) used the value of 40 mg/L (range: 30–100 mg/ L). More recently, the DRI (Institute of Medicine, 2000b) for vitamin C listed 40 mg/d (~6 mg/kg) as the adequate intake (AI) of term infants 0–6 months of age, on the basis of an average concentration in human milk of 50 mg/L. The European Society of Paediatric Gastroenterology and Nutrition Committee on Nutrition (ESPGAN-CON) (1987) stated that LBW infants fed fresh human milk may receive sufficient vitamin C to meet their requirements; nevertheless, ESPGAN-CON (1987) recommended supplementing 20 mg/d because of the wide variation in milk and possible destruction of vitamin C if heat treated. Lucas (1993) reported that once growth is established, unfortified preterm milk frequently fails to meet the preterm infant requirements for vitamin C and six other vitamins.

Blood vitamin C concentrations in preterm infants. Several studies have reported higher blood vitamin C concentrations at birth in preterm than in term infants, followed by a rapid decline (Gopinathan et al., 1994; Heinonen et al., 1986; Silvers et al., 1998). This decline can be ameliorated by administration of high amounts of vitamin C (Arad et al., 1982; Gopinathan et al., 1994; Heinonen et al., 1986; Silvers et al., 1998; Vobecky et al., 1976). However, the clinical significance of the high plasma levels and the rapid decline is not established. This decline also occurs in term infants, although not to the same extent. Thus, it appears that the decrease in vitamin C in preterm and full-term infants is a normal physiological phenomenon and does not indicate an inadequate status. However, Arad et al. (1982) and Heinonen et al. (1986; 1988) reported that plasma vitamin C concentrations were maintained above 0.6 mg/dL (reference normal) in preterm infants receiving supplements of vitamin C to 8 mg/d (6.7 mg/100 kcal if fed 120 kcal/d) and 10 mg/kg (8.3 mg/100 kcal if fed 120 kcal/d) compared with those fed pooled, pasteurized human milk or formula containing 0–0.54 mg/100 kcal.

Silvers et al. (1994) reported an association of poor outcome (early mortality) with high plasma vitamin C concentrations, but this finding could not be confirmed in a recent larger study by the same group (Silvers et al., 1998).

Vitamin C as a pro-oxidant. Böhles (1997) recently suggested a critical reevaluation of the metabolic effects of vitamin C, because high concentrations of the vitamin in the presence of free iron have pro-oxidant capabilities, supporting free radical formation (Fenton's reagent) (Frei et al., 1989). Several reports indicate that free iron may be more available in preterm than in term infants (Berger et al., 1990; Evans et al., 1992; Shaw, 1982; Sullivan, 1988; von Weippl, 1962). Vita-

min C at concentrations observed in preterm infants inhibited the ferroxidase activity of ceruloplasmin, assessed by in vitro tests (Powers H.J. et al., 1995). Cochrane (1965) suggested that scurvy in two infants whose mothers had ingested about 400 mg of vitamin C daily during pregnancy was due to a conditioning of the offspring to a higher requirement.

High vitamin C intakes to prevent or ameliorate tyrosinemia of prematurity. Levine et al. (1939) reported indications of a metabolic defect in preterm infants. They found five preterm infants who excreted hydroxphenyl compounds. The urinary levels of these compounds were markedly reduced by large doses (25–200 mg) of parenterally administered vitamin C. In an unpublished survey of 15,000 infants referred to by Avery et al. (1967), tyrosinemia and tyrosyluria were more prevalent in preterm infants (30%) than in term infants (10%), and diets high in protein were associated with an increased incidence (Dann, 1942; Levine et al., 1941; Woolf & Edmunds, 1950). In addition, smaller preterm infants were reported to have a higher incidence of tyrosinemia than larger ones (Light et al., 1966), 41% compared with 22%. In light of this, the Canadian Paediatric Society (CPS) (1976) stated that high daily vitamin C supplements, about 100 mg, should be given as a prophylaxis for tyrosinemia in the newborn, particularly the preterm infant. The mechanism whereby high intakes of vitamin C could lower tyrosinemia is an enhancement of the vitamin C-dependent hepatic p-hydroxyphenylpyruvic acid oxidase, which catabolized a metabolic product of tyrosine (American Academy of Pediatrics. Committee on Nutrition, 1993).

There are conflicting reports, especially in the earlier literature, concerning the need for higher vitamin C intakes to prevent or alleviate tyrosinemia of prematurity (Avery et al., 1967; Dann, 1942; Levine et al., 1939; 1941; Light et al., 1966; 1973; Mathews & Partington, 1964; Woolf & Edmunds, 1950). Levine et al. (1939) reported that high doses of parenteral vitamin C, up to 200 mg/single dose, in a total of 525 mg during a 4-day period, given to five premature infants fed dried cow milk without added vitamins C or B, resulted in a rapid decline in urinary tyrosine as measured by the Millon reaction.

Powers et al. (1994), using a ^{13}C-labeled tyrosine breath test, evaluated the metabolism of tyrosine in 25 preterm infants, whose GA was 25–35 weeks and BW was less than 1900 g, who received vitamin C supplements. Total daily intake of vitamin C was 8–100 mg/kg for a 5-day period. Daily vitamin C intakes of 20 mg/kg or more significantly increased the catabolism of tyrosine. However, Powers et al. (1994) indicated that because the increase was small, it was unlikely to be biologically significant. The relevance of this study to the clinical condition is questionable for three reasons: only 1 of the 25 infants studied exhibited high plasma levels of tyrosine, the plasma tyrosine concentrations at baseline were not normally distributed, and the infants assigned to different supplemental groups apparently had different plasma tyrosine levels at baseline.

Three provocative investigations reported intellectual deficits in children who had been born preterm and who had exhibited tyrosinemia (Mamunes et al., 1976; Menkes et al., 1972; Rice et al., 1989). However, others suggested that there was no clear evidence of a detrimental effect of the transient neonatal tyrosinemia of prematurity (Avery et al., 1967). Light et al. (1973) found no differences in motor or mental development skills in premature infants with high or low serum tyrosine concentrations. Likewise, Martin et al. (1974) failed to detect any abnormal intellectual, neurological, or developmental effects in 6-year-old children who had had transient tyrosinemia as infants.

Previous recommendations for term infants. The report *Assessment of Nutrient Requirements for Infant Formulas* recommended a minimum vitamin C content of 6 mg/100 kcal, based on the previous recommended dietary allowance (RDA) (Pohlandt & Mathers, 1989) of 30 mg for infants up to 6 months of age and an assumption that breast milk contains 40 mg/L of vitamin C, with a daily intake of 750 mL/d (Raiten et al., 1998a). The same report recommended a maximum formula content of vitamin C of 15 mg/100 kcal by extrapolation from an apparently safe intake of 1000 mg/d by a 70-kg adult. The most recent vitamin C recommendations do not include an RDA for term infants younger than 6 months but rather suggest an AI of 40 mg, or approximately 6 mg/kg; this suggestion was based on observed vitamin C intakes of infants fed 780 mL of human milk daily and an average vitamin C concentration of 50 mg/L was assumed (Institute of Medicine, 2000b). If this recommendation were appropriate for a 1000 g preterm-LBW infant, a formula with a vitamin C content of 5 mg/100 kcal would provide 6 mg/d, if the infant had an energy intake of 120 kcal/d.

The IOM (2000b) recently established a tolerable upper intake level (UL) of 2000 mg/d for adults based on the no observed adverse effect level (NOAEL), which was derived from the lowest observed adverse effect level (LOAEL) of 3000 mg/d. No DRI-UL was established for infants 0–6 months of age because of insufficient data.

Previous recommendations for preterm infants. Avery et al. (1967) recommended 75 mg of vitamin C daily as a reasonable allowance for most premature infants, because 60 mg was insufficient, in their studies, to prevent the tyrosinemic condition for those fed high protein (3.9 g /100 mL). A report from Germany also suggested a high daily level of vitamin C (75–120 mg) for preterm infants to prevent or alleviate tyrosinemia (Henze & Bremer, 1969). Ziegler et al. (1981), in a review of the literature, recommended 60 mg/d for preterm infants as adequate to prevent marked tyrosinemia when protein intakes were modest. ESPGAN-CON (1987) recommended at least 7 mg/100 kcal in the formula of preterm infants. If the formula provides less than 7 mg/100 kcal, a daily supplement of 20 mg was recommended. Greene and Smidt (1993), after reviewing the evidence in the literature, suggested an oral intake of 20 mg/100 kcal because of the likelihood of increased renal losses and increased protein metabolism in preterm infants. The CPS (1995) recommended 6–10 mg/(kg·d) [5–8.3 mg/100 kcal at 120 kcal/(kg·d)]; the volume of preterm mothers' milk necessary to meet these recommendations would be 120–200 mL/(kg·d). Because a typical daily intake volume is approximately 150 mL, some preterm infants would be unable to obtain the higher range recommended without supplementation. The current vitamin C recommendation of American Academy of Pediatrics Committee on Nutrition (AAP-CON) (1998) is 35 mg/100 kcal. The consensus recommendation is 15–20 mg/100 kcal (Tsang et al., 1993).

Levine et al. (1939) gave up to 200 mg as single parenteral dose to five preterm infants (GA not given). One infant was given 525 mg during a 4-day period without apparent adverse effects. Light et al. (1966) gave 70 preterm infants (BW of <2268 g) a daily supplement of 100 mg of vitamin C for 14 weeks to treat aminoacidemia of prematurity. Of these, 18 infants continued to receive the supplement for a total of 75 weeks. No adverse effects were noted.

More recently, Bass et al. (1998), in a randomized, placebo-controlled, double-blind study, gave 47 preterm infants (mean GA of 27 weeks, mean BW of 949 g) either saline or 100 mg of vitamin C arterially for the first 7 days of life. The study was for 30 days. Vitamin C had no significant effect on hemoglobin, hematocrit, red blood cell (RBC) morphology, bilirubin, number of blood transfusions, bronchopulmonary dysplasia, intraventricular hemorrhage, renal function, respiratory status, infections, or cranial ultrasonography findings. The immediate postbirth decline in plasma vitamin C was prevented, and there was no increased hemolysis. The investigators noted neither adverse nor beneficial effects on the indices measured.

Conclusions and recommendations

Minimum. Because human breast milk vitamin C content is markedly variable, the Expert Panel reasoned that basing the recommendation on breast milk alone was neither scientifically sound nor appropriate. The Expert Panel recommended a higher level than the 6 mg/100 kcal recommended for term infants (Raiten et al., 1998a) because the needs of preterm infants for growth and development are higher than those of term infants, and to prevent or ameliorate oxygen toxicity of prematurity. Total daily intakes of 20 mg/kg, or up to 100 mg/(kg·d) for five days (~17-83 mg/100 kcal) significantly increased the rate of tyrosine catabolism in preterm infants in a recent, although as yet not confirmed, study in which the in vivo ^{13}C-labeled tyrosine breath test was used (Powers et al., 1994). One current domestic formula contains 20 mg of vitamin C/100 kcal. The AAP-CON (1998) noted that there were no reports of vitamin C deficiency among preterm infants fed preterm formula containing at least 8.5 mg/100 kcal. Plasma vitamin C concentrations were maintained above 0.6 mg/dL (reference normal) in preterm infants fed 8.3 mg of vitamin C/100 kcal (Arad et al., 1982). However, no well-controlled, prospective, randomized controlled studies have assessed the ascorbic acid status of enterally fed preterm infants fed graded amounts of vitamin C.

Maximum. This recommendation was based on the amount of vitamin C currently provided in one domestic preterm infant formula. No apparent adverse effects of this level have been noted in the past several years that it has been fed to preterm infants. Therefore, the Expert Panel recommended a maximum concentration of 37 mg/100 kcal in preterm formula.

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of vitamin C in preterm infant formula be 8.3 mg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum concentration of vitamin C in preterm infant formula be 37 mg/100 kcal.**

FOLIC ACID

Background

Folate, a water-soluble vitamin, refers to a family of conjugated forms of pteroylglutamic acid with related biological activity. Folate is the generic term for the form occurring in food (i.e., breast milk); however, folic acid is the most stable form of the vitamin and is the specific form added to infant formula. The metabolically active form of folate is the tetrahydro vitamer, which functions as a coenzyme, serving as an acceptor of one-carbon units in amino acid and nucleotide metabolism (Ehrenkranz, 1993). As a result, inadequate folate can interfere with cellular differentiation, hematopoiesis, and growth and development, particularly of the nervous system. Folate interacts with vitamin B_{12}, and their common association with megaloblastic anemia is well established. For reviews of the assessment of folic acid and recommendations for term

infants, see Raiten et al. (1998a), IOM (1998), Schanler (1997), Fomon and McCormick (1993), Friel et al. (1996a), Ehrenkranz (1993), Orzalesi and Lucchini (1993), Picciano (1990), Schorah (1988), and Specker et al. (1992). Ek (1985) reviewed data relevant to the importance of adequate folate nutriture in embryonic and early fetal development, including the preterm infant. For reviews related to the safety of high intakes of folic acid, see DRIs (1998). For specific information on the biochemistry, function, and human nutriture of folate, see Bailey and Gregory (1999), Herbert (1996), IOM (Institute of Medicine, 1998), and Selhub and Rosenberg (1996).

There is active transport of folate from mother to fetus, with the increasing concentrations of folate in the fetal serum, RBCs, and liver as gestation proceeds (Loría et al., 1977; Shojania, 1984). Turnover of total body folate has been estimated at only 1% per day for adults (Greene et al., 1988; Schanler, 1989). However, stores of folate in preterm infants are limited and have been estimated to be sufficient for only several weeks (Ek, 1985; Greene et al., 1988; Moran & Greene, 1998; Schanler, 1989; Shojania, 1984). The free monoglutamate derivative is stored in the liver. Loria et al. (1977) reported that the total of liver folate levels for 6 small for gestational age (SGA) (<75% of median BW for GA) preterm infants of 30–32 weeks GA were 57 ± 34 μg (mean ± SD), compared with 179 ± 119 μg for 7 term SGA infants, 135 ± 50 μg for 6 appropriate for gestational age (AGA) preterm infants, and 234 ± 75 μg for 14 AGA (90–110% of median BW for GA) term infants of 37–41 weeks GA.

Review of the literature

Deficiency. Folate deficiency has been one of the most prevalent vitamin deficiencies reported for the neonate (Dallman, 1988; Greene et al., 1988; Moran & Greene, 1998; Schanler, 1989; Shojania, 1984), as measured by various hematological indices, including serum and RBC folate levels as well as blood smears (Dallman, 1988; Herbert, 1996; Schanler, 1989; Selhub & Rosenberg, 1996). The primary indicator of folate adequacy is RBC folate, because it reflects tissue stores and thus long-term status, whereas serum folate reflects more recent intake (Institute of Medicine, 1998).

Preterm infants are especially vulnerable to folate deficiency because of limited stores and rapid growth. In 1946, Zuelzer and Ogden (1946) described megaloblastic anemia in 25 infants, including five preterm ones, who responded to folic acid. In the 1950s and after, several studies indicated that preterm infants were at risk of megaloblastic anemia or other signs of folate deficiency (Burland et al., 1971; Gray & Butler, 1965; Kho & Odang, 1959; Vanier & Tyas, 1967; Zuelzer & Rutzky, 1953). Hoffbrand (1970) recommended that preterm infants weighing less than 1500 g at birth be given folic acid for a few months after birth as a prophylactic measure. Strelling et al. (1979) reported that folate deficiency was present in nearly 40% of 37 preterm infants (BW of <2001 g) in Plymouth, England. Based on their study, they concluded that folate deficiency was more prevalent in preterm infants than in term infants. Gastrointestinal problems and infections, common in preterm infants, can be contributing factors to folate deficiency (Kendall et al., 1974; Vanier & Tyas, 1967).

Burland et al. (1971), Kendall et al. (1974), and Stevens et al. (1979) investigated the effect of folic acid supplementation on the prevention of megaloblastic anemia in preterm infants. Although data from three studies cited by Specker et al. (1992) demonstrated that folic acid supplementation to LBW infants (<1800 or <2500 g) significantly increased RBC folate levels, the clinical relevance of these results was questioned. None of the infants in those investigations, whether receiving supplements or not, developed anemia. Specker et al. (1992) submitted the results of these three studies to statistical analysis and concluded that folic acid supplementation of preterm infants resulted in a weighted mean difference in hemoglobin of 1.0 g/dL at 6 months age.

Requirements. Few data are available relative to the folate needs of either preterm or term infants, and their requirements are unknown (Alpay et al., 1998; Fomon & McCormick, 1993). The preterm infant's reserves of folate are more limited than are those of term infants because of their shortened gestation. As a result, preterm infants may have a higher folate requirement or be more vulnerable to folate deficiency than term infants. Apparently in recognition of the increased need of preterm infants, the AAP-CON recommended that preterm infant formula contain folic acid at 33 μg/100 kcal (American Academy of Pediatrics. Committee on Nutrition, 1998), compared with the 4 μg/100 kcal that had been recommended for term infant formula (American Academy of Pediatrics. Committee on Nutrition, 1985b).

Shojania and Gross (1964) measured serum folate levels in preterm infants from birth to 7 months of age. Serum folate was elevated after birth, declined thereafter, and reached the lowest values 1–2 months postpartum in infants fed evaporated milk formula, estimated to contain 8–13 μg/100 kcal (Dallman, 1974). Some infants whose serum folate level was less than 5 ng/mL exhibited increased formiminoglutamic acid excretion, which improved after supplementation with folic acid (Shojania & Gross, 1964). The data suggest that preterm infants fed formula with 8–13 μg of folic acid/100 kcal have less than adequate folate status. Similarly, Burland et al. (1971) determined that some LBW infants fed evaporated milk formula containing 68 μg of folate/L had subnormal serum folate concentrations after 17, 28, and 90 days of life, and subnormal RBC folate levels were evident after 28 and 90 days of life. In one of the first estimations of the folate requirements for preterm infants, Dallman (1974) indicated that healthy infants weighing less than 2000 g needed a supplement of 50 μg/d when fed evaporated milk formula that supplied 8–13 μg/100 kcal. Pathak and Godwin (1972) reported decreases in serum folate levels to less than 4 ng/mL at an average of 40 days postpartum in 2 of 10 preterm infants fed evaporated milk formula containing 130 μg of folate/L. Strelling et al. (1979) reported that 15 μg of folic acid for 2 days, followed by two daily doses of 60 μg (all intramuscular) given to a 1560-g, folate-deficient preterm infant, of GA 32 weeks, failed to produce a hematological response. After completing a study of 14 folate-deficient preterm infants, they concluded that although hematological responses may occur with as little as 50 μg/d orally, this was unlikely to be enough to replenish the stores of overtly folate-deficient preterm infants.

Dallman (1988) estimated that infants require about 10 times more folate per unit of body weight than do adults, because of their higher protein requirements and lower storage reserves. After their previously cited statistical analysis of three studies in which daily 50 or 100 μg of folic acid supplementation was given to a total of 386 preterm infants weighing less than 2500 g, Specker et al. (1992) suggested that a total folate intake of 50 μg/d, including that in the diet, was probably sufficient. They further stated that stabilized, moderately preterm infants may quickly increase intakes of formula or milk sufficient to meet their folate requirements. However, they speculated that the folate needs of the smaller preterm infants might be greater.

Ek et al. and Ek (1984; 1985) came to similar conclusions from a long-term supplementation study involving 41 Norwegian preterm infants (mean GA of 32 weeks), randomized at 2

weeks of age and supplemented with 50 μg/d or no folic acid until 1 year old. The subjects were divided into two groups according to BW above or below 1750 g. One-half of each subgroup received the supplement. Prior to one month of age, the infants were fed human milk containing 16.9 μg of folate/L. Thirty-five breast-fed term infants considered to have adequate folate status, previously reported by Ek and Magnus (1979), were referred to as the control group. By one month of age, RBC folate concentration had decreased below that of term infants for the preterm-LBW infants of BW greater than 1750 g fed unsupplemented milk (2.5 μg/100 kcal, assuming the energy content of human milk was 670 kcal/L); whereas, red blood cell folate concentration was not significantly different from that of term infants for those preterm-LBW infants supplemented to a total intake of 28-36 μg/(kg·d) [22-27 μg/100 kcal, ~200 mL/(kg·d)]. The mean plasma folate concentration was not subnormal for any group at one month of age (Ek et al., 1984).

Friel et al. (1996a) reported an investigation involving the folate status of LBW infants fed a preterm infant formula containing folate at 37 μg/100 kcal. All infants received total parenteral nutrition (TPN) for an average of the first 9 days after birth, which is a confounding factor in this study. Blood samples were collected after weeks 2 and 3. Plasma folate and RBC levels were within normal ranges of those of breast-fed full-term infants (Smith et al., 1983; Smith et al., 1985). The authors suggested that 37 μg of folate/100 kcal was adequate for preterm-LBW infants in the first few weeks of life (Friel et al., 1996a).

Burland et al. (1973) fed a daily supplement of 50 μg of folic acid as either pteroylmonoglutamic acid or pteroylpolyglutamate hydrolase to preterm-LBW infants fed formula containing 49 μg/L. The total intake of folate was about 30 μg/kg (~25 μg/100 kcal) and about 28 μg/kg (~23 μg/100 kcal), respectively, assuming an energy intake of 120 kcal/(kg·d). By 14 days, these infants had significantly higher serum folate and RBC folate concentration than other preterm-LBW infants in the control group fed ~6 μg/100 kcal.

In part, the limited liver stores of folate in preterm infants (Loría et al., 1977) may be a result of poor maternal folate nutriture, because Scholl et al. (1996) reported that 832 women with a daily folate intake of less than 241 μg, studied prospectively, were at an approximately two-fold greater risk of having a preterm and LBW infant than were women with intakes greater than 241 μg. More recent similar data indicate a decreased incidence of LBW and SGA infants for folate-supplemented women (Rolschau et al., 1999). The study was double-blind and randomized one, involving 14,021 women who received folic acid supplements of 1.0 or 2.5 mg/d compared with control women receiving none.

Folic acid content in breast milk. ESPGAN-CON (1987) stated that human milk may not provide sufficient folate for LBW infants, particularly if the milk is heat-treated (Roberts et al., 1969). Lucas (1993) reported that unfortified preterm milk frequently fails to meet the preterm infant requirements for folate once growth is established. Lim et al. (1997), Brown et al. (1986a), and O'Connor et al. (1991) reported the mean folate concentration in term milk to be about 85 μg/L (12.7 μg/100 kcal, assuming an energy content of 670 kcal/L), which was the value used to estimate the AI for term infants 0–6 months of age.

Accurate analytic determinations of blood folate have suffered from methodological difficulties, lack of certified reference materials, and destruction during storage. Other variables include stage of lactation, time of day, whether foremilk or hindmilk is used, and differences among individuals

(O'Connor et al., 1997). The mean folate concentration of mature term human milk has been reported to vary from 20 to 141 μg/L, a 6.5-fold difference (O'Connor et al., 1991; O'Connor, 1994; O'Connor et al., 1997; Picciano, 1995; Schanler, 1997). The recent DRIs (Institute of Medicine, 1998) for folate have noted the wide difference in reported values and suggested that the older methods using a *Lactobacillus casei* bioassay may have underestimated the folate content; a modification of the method using enzymes to liberate available folate results in an increase in the analytic value (Lim et al., 1997). There is also the possibility of folate degradation during storage and preparation (Ek et al., 1984). A high bioavailability of breast milk folate was suggested by an in vitro study of folate uptake by isolated rat intestinal mucosal cells (Colman et al., 1981).

Current domestic preterm infant formulas available in the United States contain 37 or 35 μg of folic acid/100 kcal (Abbott Laboratories. Ross Products Division, 2001) (Mead Johnson Nutritionals, 2000).

Previous recommendations. The expert panel that assessed nutrient requirements for term infants recommended a minimum folic acid level of 11 μg/100 kcal in formula, based on a presumed breast milk content of 71 μg/L, derived from the mean of 80 μg/L in human milk minus 1 SD (Raiten et al., 1998a). The present DRIs (Institute of Medicine, 1998) for folate do not include an RDA for term infants 0–6 months of age but rather an AI of 65 μg/d. This AI is based on observed folate intakes of infants fed a mean of 780 mL of human milk with an average of 85 μg of folate/L; this is equivalent to a folate level of 12.7 μg/100 kcal, assuming a caloric value of 670 kcal for human milk. Thus, the recommendations for term infants by Raiten et al. (1998a) and the DRIs (Institute of Medicine, 1998) are almost identical.

For preterm infants, Ek et al. (1984) suggested a daily intake of 65 μg on the basis of original studies comparing biochemical indices and anthropometric indices of folate nutriture in preterm infants supplemented with folic acid and in term breast-fed infants. ESPGAN-CON (1987) recommended an intake of greater than 60 μg/100 kcal for preterm infants. Strelling et al. (1979) recommended an oral or intramuscular dose of 100–200 μg/d for folic acid-deficient preterm infants weighing less than 2000 g at birth. However, their recommendation was an effort to establish the minimum therapeutic dose to correct folate deficiency in preterm infants and was not intended as a requirement.

Dallman (1988) suggested that LBW infants fed human milk or formula be given 100 μg/d of folic acid as a supplement to provide an adequate intake of folate until they achieve an intake of 300 mL/d or a weight of 2000 g. Specker et al. (1992) recommended a total folate intake of 50 μg/d, on the basis of an analysis of three studies of the effect of folic acid supplementation on prevention of megaloblastic anemia.

Greene et al. (1992) suggested that the intake of preterm infants should be 56 μg/(kg·d). The CPS (1995) recommended 50 μg/(kg·d). The AAP-CON (1998) recommendation is 33 μg/(100 kcal. The consensus recommendation is 21–41 μg/100 kcal (Tsang et al., 1993). ESPGAN (1987) recommended a minimum level of greater than 60 μg/100 kcal.

Toxicity. Enlargement of the spleen and/or liver in LBW infants was reported after daily treatment with 60–5000 μg of folic acid intramuscularly or 50–2000 μg orally; the reason for this was not clear (Strelling et al., 1979). Because of lack of data on the adverse effects of high intakes of folic acid and infants' capacity to metabolize excess amounts, no NOAEL or LOAEL was developed for the recently published DRIs (Insti-

tute of Medicine, 1998); thus, no UL was established for folate intake for term infants 0–6 months of age. However, daily doses of 50–200 μg have been recommended for preterm infants (Fuller et al., 1992a; Strelling et al., 1979). Intakes of several hundred times the requirement are nontoxic for healthy adults (Raiten et al., 1998a). Ek (1985) refers in a review to unpublished data, by J. Ek and E. Magnus, for a group of preterm infants who received 155 μg of folate/d from at least 3 to 6 months. No side effects were reported, although the mean plasma and RBC folate concentrations were about double those of breast-fed term infants.

A study from the Medical Research Council of the United Kingdom (Fuller et al., 1992a) reported giving a daily supplement of 1000 μg to 83 preterm infants, starting between birth and 6 weeks of age, for periods varying from a few days to several months. No adverse effects were reported. However, this study noted that the plasma concentrations of the vitamin reached extremely high levels, with a median value (about 300 μg/L) more than 40 times the lower range of normal (Davis, 1986), after the second day of supplementation, before declining gradually. The metabolic consequences of such abnormally elevated plasma folate levels are unknown, although Fuller et al. (1992b) reported a significant inverse relationship between serum folate and zinc concentrations ($P < 0.0001$) in a study that included 60 preterm infants, 80% of them receiving 1000 μg of folic acid for up to 16 weeks after birth.

Conclusions and recommendations

Minimum. Infant formula containing 8–16 μg of folate/100 kcal (Dallman, 1974) failed to prevent a decline in serum concentrations of folate to less than 5 ng/mL [reference normal: 5–23 ng/mL (Burland et al., 1971)] in preterm-LBW infants after 1–2 months postpartum (Burland et al., 1971; Pathak & Godwin, 1972; Shojania & Gross, 1964). Unlike the minimum recommendation of 11 μg/100 kcal for term infant formula (Raiten et al., 1998a), the minimum recommendation for preterm formula is not based on breast milk folate concentration because of the wide range of literature values for breast milk, the inadequacy for optimal growth and development of some preterm infants, and the lower stores of the vitamin in preterm infants.

The recommendation for a minimum amount of folate in preterm infant formula was based on data related to the daily intake to support normal status as assessed by plasma and RBC folate concentrations (Ek et al., 1984; Ek, 1985; Friel et al., 1996a). Folic acid supplementation to about 29-36 μg/(kg·d) [~22-27 μg of folate/100 kcal; ~200 mL/(kg·d)] was apparently sufficient to support adequate plasma and RBC folate concentrations in preterm infants from 14 to 30 days of life (1984). In another study, the total intake of folate of about 35–37 μg/kg (~30-31 μg/100 kcal) resulted in significantly higher serum folate concentrations by 14 days in preterm-LBW infants compared to preterm-LBW infants not supplemented (~6 μg/100 kcal) (Burland et al., 1973). In addition, RBC and plasma folate levels were within normal ranges for preterm infants fed formula containing 37 μg of folate/100 kcal in the first few weeks of life (1996a). A range of 22–37 μg of folate/100 kcal was shown to support folate status based on biochemical indices, including plasma and RBC folate concentrations.

Maximum. Ek (1985) referred in a review to unpublished data, by J. Ek and E. Magnus, for a group of preterm infants who received folate at 155 μg/d from at least 3 to 6 months of age. No side effects were reported, although the mean plasma and RBC folate concentrations were about triple those of breast-fed term infants. Folic acid supplements of 1000 μg/d

that were administered to 48 preterm infants for up to the first 16 weeks of life depressed serum zinc concentrations (Fuller et al., 1992b). The median age when the 1000 μg/d oral folic aicd was introduced was 8.7 days. Therefore, the median BW of 1169 g of the entire group of 60 (12 subjects were not given the folic acid supplements of 1000 μg/d) was used to estimate the intake of 855 μg/kg, or approximately 700 μg/100 kcal, assuming an energy intake 120 kcal/kg.

No UL has been established for folic acid intake of preterm infants or term infants 0–6 months of age. Preterm infant formulas available in the United States contain 37 or 35 μg of folic acid/100 kcal (American Academy of Pediatrics. Committee on Nutrition, 1998). Ek et al. (1984) gave preterm infants supplements of 50 μg of folic acid/d for up to 1 year of life; they were fed formula containing 2.1 μg folic acid/100 kcal. The initial total intake of folate was estimated to average 53.2 μg/d (~ 44 μg folate/100 kcal), and this was apparently sufficient to support adequate blood folate parameters in preterm infants without reports of adverse effects.

Recommendations

Minimum. **The Expert Panel recommended that the minimum folic acid concentration of preterm infant formula be 30 μg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum folic acid concentration of preterm infant formula be 45 μg/100 kcal.**

VITAMIN B$_6$

Background

For specific information on the biochemistry, function, and human nutriture of vitamin B$_6$, see DRI (Institute of Medicine, 1998), Leklem (1996; 1998), McCormick and Chen (1999), and McCormick (1989a). A history of deficiency in infants was provided by Borschel (1995). For reviews concerning recommendations for vitamin B$_6$ intake of term infants, see Raiten et al. (1998a), DRIs (Institute of Medicine, 1998), Raiten (1995), Kirksey (1995), Borschel (1995), and Fomon and McCormick (1993). Vitamin B$_6$ status of the preterm infant from a clinical and metabolic perspective has been reviewed by Rassin and Raiten (1995). For general reviews related to the safety of high intakes of B$_6$, see IOM (1998), Leklem (1998), and Fomon and McCormick, (1993).

Review of the literature

Chemistry and function. The three naturally occurring forms of vitamin B$_6$ are pyridoxine, pyridoxamine, and pyridoxal. All three forms are water soluble, light sensitive, and heat labile in an alkaline medium but relatively stable in an acid medium. The form of vitamin B$_6$ used to fortify infant formula is pyridoxine hydrochloride, because it is the most heat stable of the vitamers (Fomon & McCormick, 1993).

Pyridoxal 5′-phosphate (PLP) and pyridoxamine 5′-phosphate are the active coenzyme forms of vitamer B$_6$; PLP is the biologically relevant form. Evidence suggests that PLP is involved in about 100 enzymatic reactions (Coburn, 1994; Sauberlich, 1985), of which transamination reactions account for 40%. In addition, vitamin B$_6$ is involved in decarboxylation of amino acids, conversion of tryptophan to niacin and to serotonin, neurotransmission (Ebadi et al., 1990), and synthesis of sphingomyelin and other important phospholipids (Fomon & McCormick, 1993). Vitamin B$_6$ is also involved in the transsulfuration of methionine to cysteine. A deficiency of this vitamin can result in homocysteinemia, a condition that is

associated with thrombovascular disease (Institute of Medicine, 1998).

Requirements. Only limited data are available regarding the vitamin B_6 requirements of either term or preterm infants. As a result, no RDAs have been established for these children (Institute of Medicine, 1998). Borschel (1995) provided a comprehensive review and historical perspective of infant requirements and how recommendations for vitamin B_6 intakes have been derived. Vitamin B_6 requirements of preterm infants are considered to be greater than those of term infants because they have lower stores, a result of interruption of fetal accumulation, and because of their higher protein requirements (American Academy of Pediatrics. Committee on Nutrition, 1998). Transport of vitamin B_6 to the fetus is most active during the last trimester, as assessed by an increase in the vitamin B_6 cord blood-to-maternal blood ratio (Schanler, 1988). However, Ramos et al. (1996) found no difference in the cord RBC concentrations of aspartate aminotransferase, considered to be a biomarker for nutritional status of vitamin B_6, among 23 AGA term infants, 19 SGA term infants, and 20 AGA preterm infants.

Two infants developed convulsive seizures when they were fed a proprietary milk-based formula providing about 85 μg of vitamin B_6/d for 1–4 months but did well with an evaporated milk formula providing 260 μg of vitamin B_6/d (Bessey et al., 1957). One of these infants also had biochemical evidence of vitamin B_6 deficiency. Others have reviewed similar cases of vitamin B_6 deprivation of infants fed formula containing 30–100 μg/L caused by destruction of the vitamin during processing (Rassin & Raiten, 1995). The data presented provide support that provision of vitamin B_6 at 15% below the DRI-AI of 100 μg of vitamin B_6/d (Institute of Medicine, 1998) may be overtly deficient.

McCullogh et al. (1990) reported that secretion of milk with vitamin B_6 concentrations less than 410 nmol/L (70 μg/L, if calculated as pyridoxine, or 10.4 μg/100 kcal) was associated with behavioral aberrations in two Egyptian mothers with low vitamin B_6 status and their infants at 3–6 months of age. Similarly, in a comprehensive review of infant requirements for vitamin B_6, Borschel (1995) concluded from studies of a vitamin B_6-depleted formula used in the 1950s that feeding infant formula containing vitamin B_6 at 62 μg/L, or 9.3 μg/100 kcal (4.13 μg/g protein) resulted in deficiency symptoms, whereas formula containing 96 μg/L, or 14.3 μg/100 kcal (5.56 μg/g protein) did not.

In the past, the vitamin B_6 requirement was often related to protein intake. For example, the AAP-CON (1976) recommended that infant formula have a minimum content of 15 μg of vitamin B_6/g of protein. Later, the AAP-CON (1998) reiterated that vitamin B_6 requirements are related to protein intakes but provided no details. The CFR (Food and Drug Administration, 1985) specified that the vitamin B_6 level in infant formula should be at least 35 μg/l00 kcal, plus 15 μg of vitamin B_6/g of protein in excess of 1.8 g/100 kcal. Moreover, the 1989 RDA (National Research Council. Food and Nutrition Board, 1989) recognized the association between vitamin B_6 requirements and protein intakes for infants 0–6 months of age.

Certain studies involving adolescents and elderly subjects, however, did not support the assumption that vitamin B_6 requirements depend on protein intakes (Pannemans et al., 1994). The IOM (1998) came to the same conclusion from a statistical analysis of four studies (Hansen & Diener, 1997; Kretsch M.J. et al., 1995; Ribaya-Mercado et al., 1991; Ward et al., 1998). The IOM (1998) also rejected linking the vitamin B_6 requirement to protein intake because this method

yielded unreasonably high values when the estimated average requirement and the RDA were being developed for children.

Breast milk vitamin B_6 content. According to the IOM (1998), there have been no reports of vitamin B_6 deficiency in exclusively breast-fed term infants in the United States or Canada since the publication by Bessey et al. (1957). However, Heiskanen et al. (1995) reported an association of low vitamin B_6 status with slowed growth in length of Finnish infants exclusively breast-fed for 6 months.

The vitamin B_6 content of term breast milk varies markedly, from 70 to 310 μg/L (10.4 to 46.3 μg/100 kcal) (Raiten et al., 1998a). Factors that affect the concentration in mature milk include nutritional status of the mother, stage of lactation, length of gestation, and use of drugs (Kang-Yoon et al., 1995). Information regarding the vitamin B_6 content of preterm mothers' milk is particularly limited, although one report indicated that the concentration is significantly lower in preterm milk than in term milk (Udipi et al., 1985).

In a comprehensive summary of 17 studies reporting the vitamin B_6 content of mature breast milk, Bates and Prentice (1994) calculated a mean content of 150 μg/L. Kirksey (1995) summarized the range of means of vitamin B_6 content of human milk, as published in 15 studies between 1942 and 1990, with the following as variables: stage of lactation, assay method, number of subjects, and whether supplementation with the vitamin was provided. In the six studies published from 1981 to 1990, the range of means was 482–2457 nmol/L (81–414 μg/L, calculated as pyridoxine). Other individual reports indicate means of 120 μg/L (17.9 μg/100 kcal assuming 670 kcal/L) (Andon et al., 1989) and 130 μg/L (19.4 μg/100 kcal assuming 670 kcal/L) (West & Kirksey, 1976). The IOM (1998) based the AI for infants 0–6 months of age on a mean breast milk vitamin B_6 level of 130 μg/L. Cow milk, the source of whey on which the most infant formulas are based, has markedly higher levels of vitamin B_6 (a mean of 554 μg/L) (Fomon & McCormick, 1993).

There is no agreement on whether preterm milk is adequate for vitamin B_6 nutriture of preterm infants. Lucas (1993) suggested that unfortified preterm milk frequently fails to meet the nutritional requirements for vitamin B_6 and six other vitamins for preterm infants, based on a review of the literature and research.

McCoy et al. (1985) fed preterm infant formula providing 200 μg/100 kcal to three preterm infants for 2–5 weeks. Porcelli et al. (1996) fed preterm infant formula providing 300 μg/kg to 57 preterm infants (BW of <1500 g) for the first month of life. No adverse effects related to vitamin B_6 administration were noted in these two studies (McCoy et al., 1985; Porcelli et al., 1996).

Previous recommendations. The recent DRI-AI for infants 0–6 months of age was 100 μg/d (~14 μg/kg) for vitamin B_6, based on an assumed mean concentration of vitamin B_6 for breast milk of 130 μg/L (Institute of Medicine, 1998). If this amount were appropriate for preterm infants, preterm formula would need to provide at least 11.7 μg/100 kcal. The RDA for children 1–3 years old, with an estimated mean weight of 12 kg, is 500 μg/d (42 μg/kg)(Institute of Medicine, 1998). Raiten et al. (1998a) recommended a minimum of 30 μg/100 kcal for term infant formula. The AAP-CON (1998) recommended a minimum intake for preterm infants of more than 35 μg/100 kcal; the consensus (Tsang et al., 1993) was 125–175 μg/100 kcal. The CPS (1995) recommended 15 μg/g of protein, and ESPGAN-CON (1987) recommended 35–250 μg/ 100 kcal as the enteral intake for premature infants. On the basis of a literature review of recommendations for infant formulas,

Schanler and Nichols (1985) suggested that LBW infants receive 250 μg/100 kcal. More recently, Green et al. (1992) suggested 150 μg/100 kcal for LBW infants (<1000g) on the basis of their own research and a literature review. Preterm infant formulas presently available in the United States contain 150 and 250 μg of vitamin B_6/100 kcal (Abbott Laboratories. Ross Products Division, 2001; Mead Johnson Nutritionals, 2000).

Toxicity. The neurotoxic syndrome associated with an excessive intake of vitamin B_6 has not been reported in infants (1993). Long-term daily consumption of 2–6 g of vitamin B_6 by adults has been reported to be toxic (Schaumburg et al., 1983). No subjects receiving daily doses below 2 g experienced toxic symptoms of sensory neuropathy. No UL for vitamin B_6 has been established for preterm or term infants 0–12 months of age (Institute of Medicine, 1998). However, a daily UL of 30 mg of vitamin B_6 as pyridoxine has been established for children 1–3 years of age (Institute of Medicine, 1998). Vitamin B_6 as pyridoxine (300–980 μg/kg) was added to TPN administered daily to preterm infants up to 14 days (Greene et al., 1991) and 28 days (Moore et al., 1986; Raiten et al., 1991). No classic signs of vitamin B_6 toxicity were reported (Greene et al., 1991; Moore et al., 1986; Raiten et al., 1991).

Conclusions and recommendations

Minimum. The Expert Panel was unable to find sufficient evidence on which to base a minimum vitamin B_6 concentration for preterm formula different from the minimum recommended for term formula, that is, 30 μg/100 kcal (Raiten et al., 1998a)

Maximum. The neurotoxic syndrome associated with an excessive intake of vitamin B_6 by adults has not been reported in infants (1993). In addition, no NOAEL and LOAEL for preterm infants have been established, so no UL has been identified. Two studies (McCoy et al., 1985; Porcelli et al., 1996) fed preterm infant formula providing 200–250 μg/100 kcal to preterm infants for 2–5 weeks. No known adverse effects of feeding up to 250 μg/100 kcal have been reported in the past several years. The recommendation of the Expert Panel for the maximum concentration of vitamin B_6 in preterm formula is 250 μg/100 kcal based on the amount currently provided in one domestic preterm infant formula (Abbott Laboratories. Ross Products Division, 2001).

Recommendations

Minimum. **The Expert Panel recommended that the minimum vitamin B_6 content of premature infant formula be 30 μg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum vitamin B_6 content of premature infant formula be 250 μg/100 kcal.**

RIBOFLAVIN

Background

For specific information on biochemistry, function, pharmacokinetics, biomarkers, and human nutriture of riboflavins, see Powers (1999), IOM (1998), McCormick (1998), Lakshmi (1998), Zempleni et al. (1996), and Fomon and McCormick (1993). For reviews concerning recommendations and requirements for term infants, see Raiten et al. (1998a) and IOM (1998). Riboflavin status of the preterm infant has been reviewed from a clinical and metabolic perspective by Specker (1992), Greene et al. (1992), Lucas (1993), and Greene and Smidt (1993). For general reviews related to the safety of high

intakes of riboflavin, see IOM (1998), Rivlin (1996), and Greene and Smidt (1993).

Review of the literature

Chemistry and function. Riboflavin is only slightly soluble in aqueous solutions. It is found in the diet bound to proteins, primarily as two phosphorylated forms, flavin mononucleotide and flavin adenine dinucleotide (FAD) (Bates, 1997; McCormick, 1998). Both of these function as coenzymes in numerous reduction-oxidation reactions, by acting as intermediates in the transfer of electrons in many metabolic pathways. Some of these pathways involve other vitamins, including vitamin B_{12} and vitamin B_6, and metals such as iron, molybdenum, and zinc. As a result, riboflavin is required for all cellular growth (Schanler & Nichols , 1985), and both coenzyme forms as well as free riboflavin are found intracellularly. FAD is the most common form found in the blood and tissues; it can be determined analytically by high-performance liquid chromatography methodology (Powers, 1999). The fluorescent properties of FAD are useful in its detection and quantitation in human biological samples, including plasma, RBCs, and urine. However, its light-absorbing property results in photodegradation, causing losses during preparation, administration, and storage, as well as in vivo during phototherapy for hyperbilirubinemia of the newborn.

Requirements and deficiency. Few data are available regarding the riboflavin requirements of either preterm or term infants. As a result, a DRI-AI but not an RDA has been established for term infants up to 6 months of age (Institute of Medicine, 1998). The DRI-AI was based on a mean riboflavin content of human milk of 350 μg/L, providing about 300 μg/d (Institute of Medicine, 1998). It has been assumed that riboflavin status of breast-fed infants of well-nourished mothers is adequate, although some reports suggest otherwise. For a review of riboflavin deficiency in term breast-fed infants, see Fomon and McCormick (1993).

Preterm infants are considered to be at higher risk for riboflavin deficiency, especially during the first few weeks of life. Their requirements may be greater than those of term infants because they have lower stores (because of the interruption of fetal accumulation), higher protein requirements, lower absorption, and a greater frequency of destruction by phototherapy for hyperbilirubinemia (American Academy of Pediatrics. Committee on Nutrition, 1998; Lucas & Bates, 1987).

In the first study designed to determine the minimum riboflavin requirement of term infants, Snyderman et al. (1949) conducted metabolic balances on three infants who were 14, 22, and 32 months of age, weighing 5.9, 8.6, and 9 kg respectively. Two of the infants were studied for 143 days. The investigators concluded that 400–500 μg (58 μg/kg or 48 μg/100 kcal) of riboflavin daily resulted in freedom from deficiency symptoms, normal urinary secretion, and maintenance of normal levels of riboflavin in the serum, RBCs, and white cells. Chronic respiratory infections, measles, and other conditions associated with negative nitrogen balance have been reported to reduce riboflavin status in physiologically stressed infants and children (Bamji et al., 1987; Greene & Smidt, 1993; Steier et al., 1976).

Rönnholm et al. (1986) studied 39 LBW infants in Finland who were randomized to be fed human milk with or without a daily supplement of 300 μg (0.8 μmol) of riboflavin for the first 12 weeks after birth. On average, unsupplemented infants received 66, 73, and 84 μg of riboflavin/kg at 2, 6, and 12 weeks respectively, compared with intakes of 317, 257, and 183 μg/kg in the infants receiving the supplement (Rönn-

holm, 1986). An elevated RBC glutathione reductase activation coefficient indicated a significantly higher incidence of riboflavin inadequacy in the group receiving unsupplemented breast milk. It was concluded that the intake of riboflavin by LBW infants from breast milk may be inadequate during the first few weeks of life. However, no overt clinical signs of deficiency appeared in the unsupplemented group.

A study by Lucas and Bates (1987) included 68 preterm infants (mean BW of 1335 g, mean GA of 30.5 weeks) born in two English hospitals. In the first hospital, 52 infants were fed either a preterm infant formula containing riboflavin at 677 μg/L (1.8 μmol/L) or human milk. All infants were given a commercial vitamin preparation providing an adult dose of 400 μg/d of riboflavin on or before day 7 of life. In the second hospital, 16 preterm infants were similarly treated except that the vitamin supplement was delayed until the end of the second week. The study was confounded by uncontrolled variables and by phototherapy to 60% of the infants during the first 2 weeks of life. The amount of riboflavin supplied by the milk was not stated. However, the results suggested that the riboflavin content of the breast milk alone was not adequate to ensure normal riboflavin status (as assessed by the RBC glutathione reductase activation coefficient), in well preterm infants. Later, Lucas (1993) reaffirmed from the literature and his own research that unfortified preterm human milk fails to meet the nutrient requirements of riboflavin for some preterm infants.

Breast milk riboflavin content. The riboflavin content of mature breast milk varies markedly, from 274 to 580 μg/L (41–87 μg/100 kcal) (Raiten et al., 1998a). The IOM (1998) estimated that human milk contained, on average, 350 μg of riboflavin/L. A calculated mean of 380 μg/L (57 μg/100 kcal) for breast milk was reported by Bates and Prentice (1994) for 10 studies in Western countries. Picciano (1995), however, suggested that the true mean riboflavin level in breast milk is 400–600 μg/L (60–90 μg/100 kcal) because of underestimation related to methodological problems.

Ford et al. (1983) reported that the riboflavin content in preterm milk (13 mothers) was 266 μg/L (range: 120–480 μg/L), whereas that in term milk (24 mothers) was 310 μg/L (range: 200–440 μg/L). Although no statistics were provided, the investigators stated that there was no significant difference between the two types of milk. They concluded that their data presented a strong case for supplementing term or preterm breast milk with B vitamins.

Previous recommendations

The Infant Formula Act of 1980 (Food and Drug Administration, 1985) mandated that infant formula contain at least 60 μg of riboflavin/100 kcal. The DRI-AI for infants 0–6 months of age is 0.3 mg/d (~0.04 mg/kg) (Institute of Medicine, 1998). If this were appropriate for preterm infants, formula would need to contain at least 33.3 μg/100 kcal. The report by Raiten et al. (1998a) recommended a minimum of 80 μg/100 kcal for term infant formula. The AAP-CON (1998) recommended a daily minimum intake for preterm infants of 60 μg/100 kcal; the consensus recommendation cited by the AAP-CON (1998) was 200–300 μg/100 kcal. The highest recommendation was 60–600 μg/100 kcal, suggested by ESPGAN-CON (1987). Greene et al. (1992) recommended 300 μg/100 kcal for all premature infants. The CPS (1995) recommended an intake of 360–460 μg/(kg·d) (300–383 μg/100 kcal) for stable, growing preterm infants. Preterm infant formulas available in the United States contain 300 or 620 μg/100 kcal (Abbott Laboratories. Ross Products Division, 2001; Mead Johnson Nutritionals, 2000).

Riboflavin toxicity has not been reported in infants (Raiten et al., 1998a; Schanler & Nichols, 1985), so it was not possible for the IOM (1998) to establish a UL. However, the IOM (1998) cautioned that there is a potential for adverse effects resulting from high intakes, such as those given to treat infants with hyperbilirubinemia. For a review of studies, in vivo and in vitro, involving large doses or daily intakes of riboflavin, see IOM (1998).

Levy et al. (1992) reported balance studies involving at least 11 LBW infants, with a mean BW of 1281 g and GA of 29.6 weeks, who were fed enteral preterm formula providing an average of about 660 μg of riboflavin/kg (~550 μg/100 kcal) during the second and third weeks of life. Friel et al. (1996b) fed LBW infants preterm formula containing 617 μg/100 kcal up to week 3 of life or longer. No adverse effects related to riboflavin were reported in these short-term studies.

Conclusions and recommendations

Minimum. The recent recommendation for term infant formula, principally based on breast milk content (Raiten et al., 1998a), was considered by the Expert Panel to be inadequate for preterm infants. Higher intakes were recommended for preterm infant formula because preterm infants have higher riboflavin requirements but lower reserves and more often receive phototherapy for hyperbilirubinemia. Also, the bioavailability of the riboflavin from the formula may be lower than from the breast milk.

On the basis of measures of the RBC glutathione reductase activation coefficient, Rönnholm et al. (1986; 1986) suggested that average riboflavin intakes in the range of 66–73 μg/kg (55–61 μg/100 kcal) from 2 to 6 weeks and 84 μg/kg (70 μg/100 kcal) at 12 weeks by preterm-LBW infants were inadequate compared with those receiving an average of 183–317 μg/kg. The Expert Panel found insufficient evidence on which to base a minimum riboflavin concentration for preterm formula different from the minimum recommended for term infant formula.

Maximum. No riboflavin NOAEL or LOAEL has been established for preterm infants, so no UL has been defined (Pohlandt & Mathers, 1989). ESPGAN-CON (1987) has recommended up to 600 μg/100 kcal in formula for preterm infants. Moreover, 620 μg/100 kcal has been fed enterally and is present at that level in one of the premature infant formulas available in the United States (Abbott Laboratories. Ross Products Division, 2001). There have been no reports of adverse effects or toxicity for preterm infants fed preterm formula containing about 620 μg/100 kcal (Friel et al., 1996b; Levy et al., 1992).

The plasma riboflavin concentration was significantly greater in preterm infants fed 750 μg of riboflavin/kg for 4 weeks compared with those fed 430 μg/kg (Porcelli et al., 1996). Porcelli et al. (1996) suggested that 750 μg of riboflavin/kg (~625 μg/100 kcal) was excessive for preterm formula and recommended lower daily riboflavin intake.

In one study (Moore et al., 1986), 18 preterm infants were intravenously administered 900 μg of riboflavin/d [equal to ~340–1140 μg/100 kcal if fed 120 kcal/(kg·d)] for up to 34 days without reported adverse effect. Measures of the riboflavin activity coefficient were consistently within the reference range (Moore et al., 1986). In another study (Baeckert et al., 1988), seven preterm infants (BW of 450–1360 g) receiving an average of 660 μg of riboflavin/kg [equal to ~550 μg/100 kcal if fed 120 kcal/(kg·d)] intravenously for 19–28 days had average RBC riboflavin concentrations that were consistently elevated from 1 to 4 weeks of life compared with values for adult women. The authors concluded that providing 660 μg of

riboflavin/(kg·d) in TPN was excessive. Although these doses of riboflavin were administered intravenously, on average, the total amount administered fell below the recommended maximum for enteral formula. Therefore, more research is needed to study the safety of feeding high amounts of riboflavin to preterm-LBW infants.

Recommendations

Minimum. **The Expert Panel recommended that the minimum concentration of riboflavin in preterm infant formula be 80 μg/100 kcal.**

Maximum. **The Expert Panel recommended that the maximum concentration of riboflavin in preterm infant formula be 620 μg/100 kcal.**

THIAMIN, NIACIN, VITAMIN B_{12}, PANTOTHENIC ACID, AND BIOTIN

The Expert Panel found that there is not enough evidence in the literature to support specific recommendations for these vitamins in preterm infant formulas. However, the Expert Panel found the evidence in support of standards for these vitamins for term infant formulas is significant and recommended extending those recommendations to preterm infant formulas with the exceptions that the maximum values be raised for thiamin, niacin, pantothenic acid and biotin (Table 14-1). These recommendations are based on the amounts of nutrients in one domestic preterm infant formula fed in the United States. There have been no reports of adverse effects or toxicity.

Thiamin. The IOM (1998) set the DRI-AI for thiamin at 200 μg/d ($\sim$ 30 μg/kg) for infants 0–6 months old. If this were appropriate for preterm infants, a minimum formula concentration of 25 μg of thiamin/100 kcal would be needed, assuming an energy intake of 120 kcal/(kg·d). One current domestic preterm formula has a thiamin content of 250 μg/100 kcal (Abbott Laboratories. Ross Products Division, 2001). There is no DRI-UL for infants 0–6 months of age. There are no known adverse effects caused by ingestion of thiamin in food and supplements. In one study (Moore et al., 1986), 18 preterm infants were intravenously administered 780 μg of thiamin/d up to 34 days without reported adverse effects.

Niacin. The IOM (1998) set the DRI-AI for niacin at 2000 μg of preformed niacin/d ($\sim$ 200 μg/kg) for infants 0–6 months old. If this were appropriate for preterm infants, a minimum formula concentration of 167 μg of niacin/100 kcal would be needed, assuming an energy intake of 120 kcal/(kg·d). There is no DRI-UL for infants 0–6 months of age. There are no known adverse effects caused by ingestion of niacin in food. Administration with meals of 30–50 mg of nicotinic acid/d (as 25 mg twice a day) is associated with

flushing, considered an adverse effect, in adults (Institute of Medicine, 1998). Domestic preterm formula contains niacin as niacinamide (Mead Johnson Nutritionals, 2000) (Abbott Laboratories. Ross Products Division, 2001). One current domestic preterm formula has a niacin content of 5000 μg/100 kcal (Abbott Laboratories. Ross Products Division, 2001). Further research is needed at graded levels of niacin intake with adequate monitoring of preterm-LBW infants for adverse effects. In one study (Moore et al., 1986), 18 preterm infants were intravenously administered 11 mg of niacin/d for up to 34 days without reported adverse effects. Blood levels of niacin were consistently maintained in the reference range (Moore et al., 1986).

Vitamin B_{12}. The IOM (1998) set the DRI-AI for vitamin B_{12} (cobalamin) at 0.4 μg/d ($\sim$ 0.05 μg/kg) for infants 0–6 months old. If this were appropriate for preterm infants, a minimum formula concentration of 0.04 μg of vitamin B_{12}/100 kcal would be needed, assuming an energy intake of 120 kcal/(kg·d). One current domestic preterm formula has a biotin content of 0.55 μg/100 kcal (Abbott Laboratories. Ross Products Division, 2001). The recommendation for the vitamin B_{12} content of term formula was 0.08 μg/100 kcal (Raiten et al., 1998a). There is no DRI-UL. There are no known adverse effects caused by ingestion of vitamin B_{12} in food and supplements in healthy individuals. In one study (Moore et al., 1986), 18 preterm infants were intravenously administered 0.7 μg of vitamin B_{12}/d for up to 34 days without reported adverse effects. Blood levels of vitamin B_{12} were consistently elevated above the reference range (Moore et al., 1986).

Pantothenic acid. The IOM (1998) set the DRI-AI for pantothenic acid at 1.7 mg/d ($\sim$ 0.2 mg/kg) for infants 0–6 months old. If this were appropriate for preterm infants, a minimum formula concentration of 0.17 mg of pantothenic acid/100 kcal would be needed, assuming an energy intake of 120 kcal/(kg·d). One current domestic preterm formula has a pantothenic acid content of 1900 μg/100 kcal (Abbott Laboratories. Ross Products Division, 2001). There is no DRI-UL. There are no known adverse effects caused by oral ingestion of pantothenic acid in food.

Biotin. The IOM (1998) set the DRI-AI for biotin at 5 μg/d ($\sim$ 0.7 μg/kg) for infants 0–6 months old. If this were appropriate for preterm infants, a minimum formula concentration of 0.6 μg of biotin/100 kcal would be needed, assuming an energy intake of 120 kcal/(kg·d). According to Schanler (1988), no deficiencies of biotin have been reported for enterally fed preterm infants. One current domestic preterm formula has a biotin content of 37 μg/100 kcal (Abbott Laboratories. Ross Products Division, 2001). There is no DRI-UL. There are no known adverse effects caused by ingestion of biotin in food and supplements. In one study (Moore et al., 1986), 18 preterm infants were intravenously administered 13 μg of biotin/d for up to 34 days without reported adverse effects. Blood levels of biotin were consistently elevated above the reference range (Moore et al., 1986). In one case of biotin deficiency during parenteral alimentation, a 12-month-old infant was given a biotin supplement of 1 mg/d in TPN for 7 days (Mock et al., 1981). Then the biotin dose was increased to 10 mg/d for 6 weeks, when it was reduced to 100 μg/d and continued for at least 1 month. Signs and symptoms of biotin deficiency in this child resolved within days for some indicators of deficiency and within weeks for others, and no adverse effects of supplementation were reported.

Recommendations

Recommendations for thiamin, niacin, vitamin B_{12}, pantothenic acid, and biotin are listed in Table 14-1.

TABLE 14-1

The minimum and maximum values for thiamin, niacin, vitamin B_{12}, pantothenic acid, and biotin in preterm infant formula recommended by the Expert Panel

Vitamin	Minimum (μg/100 kcal)	Maximum (μg/100 kcal)
Vitamin B_1, thiamin	30	250
Vitamin B_3, niacin	550	5000
Vitamin B_{12}, cobalamin	0.08	0.70
Pantothenic acid	300	1900
Biotin	1	37

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