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International Zinc Nutrition Consultative Group (IZiNCG) Technical Document #1

Assessment of the Risk of Zinc Deficiency in Populations and Options for Its Control

Christine Hotz and Kenneth H. Brown, guest editors

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List of abbreviations

ANOVA	Analysis of variance
CV	Coefficient of variation
EAR	Estimated average requirement
EZP	Exchangeable zinc pool
F	Female
FAO	Food and Agriculture Organization
FBS	Food Balance Sheets
FFQ	Food frequency questionnaire
FNB	Food and Nutrition Board (United States of America)
GRAS	Generally Regarded as Safe
IAEA	International Atomic Energy Association
IOM	Institute of Medicine (United States of America)
IP-5, IP-6	Inositol penta-phosphate, inositol hexa-phosphate
IZiNCG	International Zinc Nutrition Consultative Group
LOAEL	Lowest Observed Adverse Effect Level
M	Male
MW	Molecular weight
NHANES	National Health and Nutrition Examination Survey (United States of America)
NIST	National Institute of Standards and Technology (United States of America)
NOAEL	No Observed Adverse Effect Level
PPM	Parts per million
RDA	Recommended dietary allowance
RT-PCR	Reverse transcriptase-polymerase chain reaction
SD	Standard deviation
SOD	Superoxide dismutase
SRM	Standard reference material
US	United States of America
WHO	World Health Organization

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The Micronutrient Initiative (MI) is a not-for-profit organization specializing in addressing vitamin and mineral deficiencies. MI is governed by an international Board of Directors. MI supports and promotes food fortification and supplementation programs in Asia, Africa, and Latin America and provides technical and operational support in those countries where vitamin and mineral deficiencies are most prevalent. MI carries out its work in partnership with other international agencies, governments, and industry. MI is based in Ottawa, Canada, and maintains regional offices in New Delhi, India, and Johannesburg, South Africa.

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Preface

In recent years, a considerable number of well-designed intervention trials have been completed in many parts of the world—including both lower-income and industrialized countries—to assess the impact of zinc supplementation in populations thought to have an elevated risk of zinc deficiency. These studies have confirmed the critical importance of adequate zinc nutriture to support child growth, reduce the risk of common infections, prevent adverse outcomes of pregnancy, and improve other aspects of human health and function. Because of the likely widespread occurrence of zinc deficiency, especially in low-income groups, and the important health consequences of this condition, efforts are needed to define more precisely the risk of zinc deficiency in vulnerable populations and to develop programs to control this condition where necessary.

The present document was prepared by the Steering Committee (SC) of the newly established International Zinc Nutrition Consultative Group (IZiNCG) and several other experts in zinc nutrition invited by IZiNCG to assist in its preparation. The SC was appointed by the United Nations University's Food and Nutrition Program for Human and Social Development (UNU/FNP) and the International Union of Nutritional Sciences (IUNS). The document was reviewed by 10 independent experts selected by the UNU/FNP and the IUNS. The IZiNCG's response to the reviews was assessed by two additional reviewers appointed by the UNU/FNP and IUNS. Therefore, the present publication reflects the input from experts both within and outside the IZiNCG SC.

This document's primary objective is to provide a summary of current knowledge on zinc as it pertains to public health issues, primarily in low-income countries. It presents a comprehensive background review of information on zinc metabolism, zinc requirements, risk factors for zinc deficiency, methods of assessing population zinc status, and available options for developing intervention programs to control zinc deficiency. The document is not intended to replace current reference values set by other international or national agencies with normative and/or policy roles, but to assess the scientific support of current reference values and to make recommendations for their reevaluation as appropriate. The implication of these considerations to available options for developing intervention programs to control zinc deficiency is also a key focus of this report.

Because this information has not been summarized previously in a single text, we have intentionally presented the material in some detail. An abbreviated companion document will be made available subsequently to facilitate access to the key points that need to be considered prior to designing programmatic interventions. The present document should be useful to nutrition researchers concerned with health-related aspects of zinc nutrition and to other health professionals who are planning nutrition and/or health surveys and public health intervention programs.

Introduction

During the first half of the 20th century, researchers discovered that zinc is essential for the normal growth and survival of higher plants, poultry, rodents, and swine [1]. Despite these observations, many nutritionists doubted that zinc deficiency occurred in humans because of the element's ubiquitous distribution in the environment and the lack of obvious clinical signs of deficiency in presumably high-risk human populations. Nevertheless, evidence of human zinc deficiency began to emerge during the 1960s, when cases of zinc-responsive dwarfism and delayed sexual maturation were first reported among Egyptian adolescents [1]. Since then, clinical studies of children with acrodermatitis enteropathica—an inborn error of zinc metabolism that results in poor zinc absorption and, consequently, in severe, secondary zinc deficiency—have ascertained the critical role of zinc in physical growth of humans and normal functioning of the gastrointestinal tract and immune system [2].

Since these early observations in people with

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acrodermatitis enteropathica, a number of well-designed intervention trials have been completed in a broad range of populations throughout the world. Results of these trials have confirmed that zinc supplementation increases growth among stunted children [3] and reduces the prevalence of common childhood infections, such as diarrhea and pneumonia, in populations at risk [4]. Moreover, data from a recent study in northern India indicated that daily zinc supplementation among full-term, small-for-gestational-age infants significantly reduced mortality by 68% [5]. Data are also accumulating to suggest that zinc deficiency may be related to adverse outcomes of pregnancy [6] and compromised neurobehavioral function in children [7]. These findings argue strongly for the need to define further the extent of human zinc deficiency worldwide and to initiate public health intervention programs to control this problem in at-risk populations.

Regrettably, there are no simple, quantitative, biochemical or functional markers of zinc status currently available that are sufficiently sensitive to identify mild to moderate zinc deficiency in individuals. The absence of such sensitive biomarkers of individual zinc status has, to some extent, undermined efforts to quantify the global prevalence of zinc deficiency, and the resulting lack of information has hampered the development of relevant intervention programs. Nevertheless, experts in zinc nutrition have presented several cogent arguments to suggest that zinc deficiency may, in fact, be very common in many lower-income countries [8–10]. For example, foods that are particularly rich sources of absorbable zinc are inaccessible to many of the world's poorer populations. Animal products, such as shellfish and red meat, which contain substantial amounts of zinc in readily absorbable form, are not consumed extensively due to their high cost, limited supply, and, in some cases, religious or cultural practices. Whole-grain cereals and legumes, which are more widely available than animal-source foods, also contain reasonably high amounts of zinc, but the zinc contained in these grains is absorbed less efficiently because uptake by the intestine is inhibited by other components of these foods. Thus, many people—particularly those in lower-income settings—have limited access to diets that meet their theoretical requirements for zinc.

The notion that zinc deficiency may be widespread in lower-income populations is further supported by the results of zinc supplementation trials completed in a broad range of countries. Provision of supplemental zinc during these intervention trials has led to improved growth among underweight or stunted children, thus demonstrating that their habitual zinc intakes were inadequate to meet physiologic requirements [3]. Nearly one-third of preschool children in lower-income countries have stunted growth [11], and the foregoing results indicate that a considerable proportion of this growth failure is likely attributable to zinc deficiency.

It is well established that iron deficiency is extremely common; anemia, due largely to iron deficiency, affects between one-third and one-half of the preschool children and women of reproductive age in lower income countries [12]. Because absorbable forms of iron and zinc are found in many of the same foods, these high rates of iron deficiency provide further suggestive evidence of the probable widespread occurrence of zinc deficiency.

Considering the likely common occurrence of zinc deficiency and the critical roles of adequate zinc nutriture in supporting normal growth and development, preventing morbidity from common infections, and possibly reducing child mortality, health planners are strongly advised to implement appropriate measures to evaluate the zinc status of their target populations and to use this information in considering whether programmatic interventions are indicated. To assist with the development of these activities, the present document provides the following: (1) background information on zinc metabolism and new estimates of the physiologic and dietary requirements for zinc; (2) recommendations for approaches that can be used to assess a population's risk of zinc deficiency; and (3) a review of the range of programmatic options that are available to enhance zinc nutriture in populations at risk of zinc deficiency.

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Chapter 1

Overview of Zinc Nutrition

1.1 Biological functions of zinc

Zinc is ubiquitously present throughout all biologic systems and has abundant and varied functions within these systems. These characteristics owe to its unusually versatile physicochemical properties. For example, zinc is able to assume a number of coordination numbers and geometries, which make it steriochemically adaptable to the functional needs of various ligands [1]. Moreover, under physiologic conditions, zinc is not subject to oxido-reductive reactions, so it is relatively non-toxic. These properties make zinc an ideal element to participate in catalytic, structural, and cellular regulatory functions [2].

More than 100 specific enzymes require zinc for their catalytic function [2]. If zinc is removed from the catalytic site, activity is lost; replacement of zinc restores activity. Unlike any other metal, examples of zinc-requiring enzymes are found in all six enzyme-classes (oxidoreductases, transferases, hydrolases, lysases, isomerases, and ligases) and include RNA polymerase, alcohol dehydrogenase, carbonic anhydrase, and alkaline phosphatase [1]. Zinc may provide activity to these enzymes by serving as an electron acceptor. In its structural role, zinc facilitates the folding of proteins into three-dimensional configurations, thus enabling their biologic activity. This folding often involves chelation of zinc with the amino acids cysteine and histidine and the formation of finger-like motifs, referred to as 'zinc fingers.' Metal transcription factor 1, retinoic acid receptor, and enzymes (such as copper-zinc superoxide dismutase) are examples of proteins that require zinc in a structural role. Processes regulated by zinc include expression of the metallothionein gene, apoptosis (or programmed cell death) and synaptic signaling.

In summary, zinc is the most ubiquitous of all trace elements involved in human metabolism. Zinc participates in all major biochemical pathways and plays multiple roles in the perpetuation of genetic material, including transcription of DNA, translation of RNA, and ultimately cellular division.

1.2 Tissue zinc distribution and reserves

The zinc content of the adult human body ranges from 1.5 to 2.5 g, with higher average contents in men than in women. Zinc is present in all organs, tissues, fluids, and secretions in the body. However, most zinc is located in the fat-free mass, with about 30 mg zinc/kg tissue, almost all of which (> 95%) is intracellular. Due to the bulk of skeletal muscle and bone in the body, zinc in these tissues accounts for the majority (83%) of whole body zinc [3]. The concentration and total zinc content of various tissues, and the proportion contributed to total body zinc, are shown in table 1.1.

When total body zinc content is reduced during depletion, the loss of zinc is not uniform across all tissues. Skeletal muscle, skin, and heart zinc are maintained, while zinc levels decline in bone, liver, testes, and plasma [4]. It is not known what signals certain tissues to continue to release zinc during depletion while others retain zinc.

There are no conventional tissue reserves of zinc that can be released or sequestered quickly in response to variations in dietary supply. Nevertheless, it has been proposed that bone may serve as a passive reserve because some zinc may become available during normal turnover of osseous tissue. Thus, although the release of zinc from bone does not increase during deficiency [5], less of the zinc that is released during normal remodeling of bone may be re-deposited in the skeleton when the dietary supply is very low [6]. This passive reserve of zinc may be even more important in growing individuals, as bone turnover is more active. For example, young rats fed zinc-deficient diets for 24 days had nearly 50% less bone zinc content than did rats in a control group [7]. Interestingly, chicks fed higher zinc-containing diets during a baseline study period accumulated more skeletal zinc and were more resistant to growth failure during a subsequent period of very low zinc intake than were comparison animals fed a diet that was marginally adequate for zinc during the baseline period [8]. This suggests that intermittent zinc supplementation may be able to reduce the risk of

TABLE 1.1. Zinc content of major organs and tissues in an adult (70 kg) man[a]

Tissue	Zinc concentration (mg/kg wet weight)	Total zinc content (mg)	Proportion of total body zinc (%)
Skeletal muscle	50	1,400	63
Skeleton			
Bone	90	450	20
Marrow	20	60	3
Cartilage	34	30	1
Periarticular tissue	11	11	< 1
Liver	40	72	3
Lung	40	40	2
Skin	15	39	2
Whole blood	6	33	1
Kidney	50	15	1
Brain	10	14	1
Teeth	250	11.5	1
Hair	200	4	< 1
Spleen	20	3.6	< 1
Lymph nodes	14	3.5	< 1
Gastrointestinal tract	15	1.8	< 1
Prostate	100	1.6	< 1
Other organs/tissues	Variable	50	2
Total		2,240	100

a. adapted from Iyengar [3]

symptomatic zinc deficiency among people with poor dietary intakes.

1.3 Zinc metabolism

Zinc is released from food as free ions during digestion. These liberated ions may then bind to endogenously secreted ligands or to exogenous material in the intestinal lumen before their transcellular uptake in the distal duodenum and proximal jejunum [2]. Zinc transport into the enterocyte demonstrates saturable kinetics, suggesting involvement of a specific carrier mechanism. With high intakes, zinc is also absorbed through a passive, paracellular route. Other specific transporters, such as zinc transporter protein–1 (ZnTP-1) may facilitate passage of zinc across the basolateral membrane of the enterocyte into the portal circulation [9].

The portal system carries absorbed zinc directly to the liver, where it is taken up rapidly and released into the systemic circulation for delivery to other tissues [2]. About 70% of zinc in circulation is bound to albumin, and any conditions that alter serum albumin concentration have a secondary effect on serum zinc levels. For example, serum zinc concentration declines in concert with serum albumin during pregnancy, due to expansion in plasma volume. Serum zinc concentration also falls with the hypoalbuminemia that accompanies aging and protein-energy malnutrition. The concentration of circulating zinc is also altered by conditions that affect its uptake by tissues. For example, infections, acute trauma, and other stresses that induce increased secretion of cortisol and cytokines (such as interleukin 6) also augment tissue zinc uptake and thereby reduce serum zinc concentrations. During fasting, serum zinc concentrations rise due to release by muscle during catabolism; following meals, serum zinc levels decline progressively in association with hormonal changes and tissue uptake of circulating nutrients induced by fuel metabolism. Although serum zinc represents only 0.1% of the whole body zinc, the circulating zinc turns over rapidly (~ 150 times per day) to meet tissue needs. Notably, during the course of 24 hours, the equivalent of approximately one-fourth to one-third (~ 450 mg) of total body zinc exchanges between the bloodstream and other tissues [5].

Loss of zinc through the gastrointestinal tract accounts for approximately half of all zinc eliminated from the body. Considerable amounts of zinc (~3–5 mg) are secreted into the intestine from the pancreas following each meal, and biliary and intestinal secretions also contain sizeable amounts of zinc [10]. The total endogenous gastrointestinal zinc secretion may well exceed the amount consumed in the diet. However, much of the zinc that is secreted into the intestine is subsequently reabsorbed, and this process serves as an important point of regulation of zinc balance. Other routes of zinc excretion include the urine, which accounts for approximately 15% of total zinc losses, and epithelial cell desquamation, sweat, semen, hair, and menstrual blood, which together account for approximately 17% of total zinc losses [11]. The fecal loss of endogenous zinc from the body is less than 1 mg/d when a virtually zinc-free diet is consumed by healthy individuals studied under experimental conditions [10]. Under these conditions, urinary zinc losses decline by about 95%, largely due to the effects of glucagon and renal zinc transporters [2, 10].

In general, the amount of endogenous zinc excreted in the feces goes up as the total absorbed zinc increases; fecal excretion of endogenous zinc declines when either dietary zinc intake is reduced or zinc needs are increased due to growth or lactation [12–14]. When dietary zinc is decreased, the individual goes into negative zinc balance for a period of time before zinc balance is re-established at the lower level of intake [5]. This transient negative zinc balance results in a small loss of zinc from the exchangeable zinc pool; the amount lost depends on the length of time required to achieve zinc balance. This small loss of exchangeable zinc could have a subtle effect on zinc function, for example a reduction in immune function. However, functional consequences are generally not apparent until the capacity of these

adaptive mechanisms is exceeded.

1.4 Importance of zinc for human health

Given the diverse array of biologic functions of zinc, it is not surprising that multiple physiologic and metabolic functions, such as physical growth, immuno-competence, reproductive function, and neuro-behavioral development are all affected by zinc status. When the supply of dietary zinc is insufficient to support these functions, biochemical abnormalities and clinical signs may develop. Evidence regarding the effects of zinc status on physiologic function has been derived from three types of studies in human subjects: (1) evaluations of individuals with acrodermatitis enteropathica; (2) studies of the association between markers of zinc status and specific functions; and (3) clinical or community-based intervention trials. In some cases, studies in experimental animals also provide insights into the functional consequences of zinc deficiency.

Acrodermatitis enteropathica is a rare autosomal recessive genetic disorder that results in zinc malabsorption [15]. The classification of clinical signs associated with this disease has provided much insight into the functional outcomes of zinc deficiency, and therefore the physiologic roles of zinc. The clinical manifestations of acrodermatitis enteropathica and the frequency with which they have been observed are listed in table 1.2 [16]. Impairments of the dermal, gastrointestinal, neurologic, and immunologic systems predominate. Iatrogenic causes of zinc deficiency—such as prolonged administration of total parenteral nutrition with inadequate zinc content [17], long-term penicillamine therapy for Wilson's disease, which results in chelation of circulating zinc [18], and chlorthiazide administration [19], which increases urinary losses of zinc—have produced similar clinical signs as those described for acrodermatitis enteropathica.

TABLE 1.2. Clinical manifestations of acrodermatitis enteropathica[a]

Symptom	Frequency (%)
Dermatitis	84
Intermittent diarrhea	54
Alopecia	48
Growth retardation (stunting)	46
Weight loss (wasting)	43
Mood changes	39
Birth defects	31
Recurrent infections	30
Nail deformation	25
Miscarriage	23
Blepharoconjunctivitis	22
Death	20
Anorexia/hypogeusia	15
Photophobia	14
Pale skin	8
Neurological defects	3

a. adapted from Van Wouwe [16]

The severity and manifestations of frank zinc deficiency may vary at different ages [20]. In infants up to 2 months of age, diarrhea is a prominent symptom. Early zinc deficiency leads to cognitive function impairment, behavioral problems, mood changes, memory impairment, problems with spatial learning, and neuronal atrophy (optic and cerebellar) [21]. Skin problems become more frequent and gastrointestinal problems, anorexia, and mood changes less frequent as the child grows older [16]. Alopecia (hair loss), growth retardation, blepharoconjuctivitis (inflammation of eyelids and conjuctiva), and recurrent infections are common findings in school-aged children. Chronic non-healing leg ulcers and recurrent infections occur among the elderly [22].

Of the aforementioned types of study designs, placebo-controlled intervention trials provide the strongest inferences regarding the functional importance of zinc nutriture and the expected impact of interventions designed to enhance zinc status. When measurable functional changes occur in response to supplemental zinc in adequately controlled trials, preexisting zinc deficiency can be inferred, and the specific functions that are responsive to zinc can be identified. Unlike the situation with acrodermatitis enteropathica described above, where the myriad of clinical signs are likely to reflect severe deficiency, functional impairments identified in community-based trials may be more representative of mild or moderate deficiency. The range of functional impairments reported from these trials is described in the following sections.

It is important to recognize that the results of these studies may be affected by concurrent deficiency of other nutrients. Among the set of other nutrients that are commonly consumed in inadequate quantities, several also have demonstrated effects on growth [23], immune function [24], or neurologic or behavioral function [25]. While several of the zinc supplementation trials cited in this report have included other nutrients in the supplement formulation (e.g., iron plus folate, multi-micronutrients), zinc was the only factor differing between control and treatment groups in the results reported herein. In cases where zinc was given alone and compared with a placebo, the effect of zinc treatment may have been reduced or absent, either because zinc deficiency was not prevalent or because other concurrent nutrient deficiencies limited the impact of zinc [26, 27]. Considerations for the inclusion of zinc with other nutrition programs are discussed later in section 3.5 of chapter 3.

Immune function and risk of infection

The role of zinc in immune function has been reviewed in detail elsewhere [28, 29]. Zinc affects both nonspecific and specific immune function at a variety of levels. At least some effects of zinc on immune function are mediated via release of glucocorticoids, decreased thymulin activity, and possibly antioxidant properties. In terms of nonspecific immunity, zinc affects the integrity of epithelial barrier, and function of neutrophils, natural killer cells, monocytes, and macrophages. With regard to specific immunity, lymphopoenia and declined lymphocyte function occur, as do alterations in the balance of T helper cell populations (TH1 and TH2) and cytokine production.

Although most knowledge of the effects of zinc on immune function has been derived from experimental animals or in vitro models, several studies have shown that perturbations of zinc status can affect immune competence in adult human subjects [30–37]. For example, elderly subjects in higher-income countries who received supplemental zinc demonstrated improvements in delayed cutaneous hypersensitivity [34, 36], the number of circulating T cells, and serum IgG antibody response to tetanus toxoid [36]. In other studies of experimentally induced mild zinc deficiency among adults, reduced serum thymulin and IL-2 activity, and reductions in specific subpopulations of lymphocytes occurred during zinc depletion, and these returned to normal levels following zinc repletion [30, 37]. The specific links between zinc-related aspects of immune function and the incidence and severity of different infections are not well understood. Nevertheless, it can be assumed that the reported changes in immune function are clinically important because decreased rates of infection have been observed following zinc supplementation in population-based studies, as described below.

Diarrhea

Several studies have demonstrated reductions in the incidence and duration of acute and persistent diarrhea in zinc-supplemented children compared with their placebo-treated counterparts [38–41]. In two cases [42, 43], beneficial effects of supplemental zinc on the incidence of diarrhea extended beyond the actual period of zinc administration. Recently, a pooled analysis of randomized, controlled trials of zinc supplementation performed in nine lower-income countries in Latin America and the Caribbean, south and southeast Asia, and the western Pacific, indicated that supplemental zinc led to an 18% reduction in the incidence of diarrhea and a 25% reduction in diarrheal prevalence [44]. Notably, this analysis did not find differences in the effect of zinc by age, baseline serum zinc status, presence of wasting, or sex, suggesting that the benefits of zinc supplementation are likely to occur in all subgroups of children living in areas where there is an elevated risk of zinc deficiency (see section 2.2 in chapter 2). Since the publication of the pooled analysis just cited, results from two additional zinc supplementation trials have been reported from Africa [45, 46]. Both of these trials demonstrated significant reductions in the incidence or number of days with diarrhea, thus confirming that the preventative effect of zinc on diarrheal infection is consistent across a wide range of geographic regions. It is noteworthy that the impact of supplemental zinc on reducing diarrheal morbidity is comparable to that observed in programs to improve water quality, water availability, and excreta disposal [47].

Respiratory infections

Reductions in the incidence of acute lower respiratory infections in response to zinc supplementation have also been documented [48, 49]. The recent pooled analysis of trials conducted in India, Jamaica, Peru, and Vietnam indicated an overall 41% reduction in the incidence of pneumonia among zinc-supplemented children [44].

Malaria

Randomized, placebo-controlled studies in Gambia [50] and Papua New Guinea [51] suggest that zinc may play a role in morbidity reduction related to *Plasmodium falciparum* infections. The trial conducted in Gambia demonstrated a 32% reduction in clinic visits due to *P. falciparum* infections among those given 70 mg zinc twice weekly for 18 months. Similarly, the trial in Papua New Guinea showed a 38% reduction in clinic visits attributable to *P. falciparum* parasitemia among pre-school children provided with 10 mg zinc daily [51]. In the latter study, zinc supplementation resulted in an even greater reduction (69%) in clinic-based malarial episodes with high densities of *P. falciparum* parasites in the blood (i.e., > 100,000/μl). On the other hand, one recent trial conducted in Burkina Faso did not find any reduction in episodes of falciparum malaria among children who received daily supplementation with 10 mg zinc for 6 months [45]. However, malaria episodes were identified using daily household visits in this study. The contrast in results may occur because zinc has an ameliorating effect on the severity of falciparum-related morbidity, possibly resulting in fewer clinic visits, but not necessarily fewer infections.

Mortality

Only limited information is available concerning the impact of zinc supplementation on child mortality, although several large-scale studies are currently in progress. In one study of full-term, small-for-gestational-age infants in north India, daily supplementation

of zinc (1–5 mg/day, from 15–30 days of age, followed by 5 mg/day, from 30 days of age and continued until reaching 269 days of age) significantly reduced mortality by 67% compared with a control group that did not receive zinc supplements [52]. Another, smaller trial of older children in Burkina Faso also found that mortality from all causes was reduced by more than 50% among those who received zinc supplements, although this difference was not statistically significant [45].

Growth and development

Given the multiple roles of zinc in DNA replication, RNA transcription, endocrine function and metabolic pathways, it is not surprising that the state of zinc nutriture affects growth and development. Although the primary mechanism(s) whereby zinc influences growth is uncertain, there is a large body of literature indicating that zinc depletion limits growth and development, as summarized in the following paragraphs.

Low-birthweight infants

Low-birthweight infants (i.e., those < 2500 g at birth) may be especially vulnerable to zinc deficiency. Two studies of low-birthweight infants have been undertaken in lower-income countries, and in both cases weight gain increased among those who received the zinc supplement [43, 53]. Responses in linear growth have been less consistent. Castillo-Duran et al. [53] reported increased growth among low-birthweight Chilean infants, but this was not seen in the study in Brazil [43], possibly because the zinc supplement (5 mg zinc/day) was given for only a short period (i.e., 8 weeks) in the latter study. In a study of very low-birthweight, premature infants in Canada [54], a significant increase in linear growth was reported, but only in the zinc-supplemented female infants.

Severely malnourished infants and children

Some of the earliest studies of zinc supplementation in severely malnourished children were carried out in Jamaica, where zinc supplementation (1.6–9.8 mg/kg body weight/day, for 2 weeks, starting between the 4th and 12th week after hospital admission) was found to increase weight gain and lean tissue synthesis compared to unsupplemented children [55, 56]. Subsequent trials in Bangladesh [57, 58] have likewise found greater weight gain among severely malnourished inpatients who received supplemental zinc (10 mg/kg body weight/day up to a maximum of 50 mg/day, for 3 weeks) during the course of nutritional rehabilitation. However, one group of investigators [59] who provided either 1.5 or 6 mg zinc/kg body weight/day for 15 or 30 days starting immediately after hospital admission, found that severely malnourished inpatients who received the higher dose of supplemental zinc had increased mortality compared with similar patients who received 1.5 mg zinc/kg body weight/day, suggesting that excessive zinc supplementation may increase the risk of severe complications.

There have been fewer reports of a positive effect of zinc supplementation on children s linear growth during recovery from severe malnutrition, perhaps because most of the available studies were too brief to detect significant changes in linear growth, which generally occurs only after recovery of weight deficits in these patients [60].

Infants and children

The effects of zinc supplementation on children's growth were examined in a recently completed meta-analysis of 33 randomized intervention trials that were conducted in pre-pubertal children [61]. Zinc supplementation produced highly significant positive responses in linear growth and weight gain (mean effect sizes of 0.30–0.35 SD units), but no effect on weight-for-height indices. Growth responses were greater in children with low initial weight-for-age or height-for-age Z-scores. Thus, the beneficial effect of zinc on children's growth may be limited to populations with evidence of pre-existing growth failure. The magnitude of the zinc-induced growth impact tended to be greater in studies that enrolled younger children, but these age-related differences were not statistically significant, possibly because of the limited number of studies available for analysis.

In some studies, zinc supplementation had a greater impact in males than in females [62–64], but this finding was not consistent in all of the trials that identified a significant impact of zinc supplementation. Males have a higher percentage of total body weight comprised of muscle, which in turn contains a higher content of zinc than fat. Additionally, the growth rate of males is generally higher than females, so their zinc requirements are probably greater.

Adolescents

The first cases of human zinc deficiency described in the 1960s were reported in male adolescents from the Middle East [65, 66]. In this group, zinc deficiency was characterized by delayed sexual development, short stature, anemia, enlargement of the liver and spleen, and abnormalities in skeletal maturation. Zinc supplementation resulted in significantly increased height, weight, bone development, and sexual maturation [26, 67]. Decreased sperm counts and testosterone levels were also observed during experimental zinc depletion among adolescent males [68]. Since these early studies, very few zinc supplementation trials have been performed in this age group.

In a study of Chilean adolescents with idiopathic short stature, zinc supplementation (10 mg zinc/day as zinc sulfate) for 12 months significantly increased height-for-age Z-scores in boys, but not in girls, com-

pared with their unsupplemented counterparts [69].

Maternal health and pregnancy outcome

Zinc supplementation trials during pregnancy have been reviewed extensively by Tamura and Goldenberg [70], Caulfield et al. [71], and King [72]. Adverse outcomes associated with zinc status in at least some of these trials include intrauterine growth retardation, low-birthweight, poor fetal neurobehavioral development, and increased neonatal morbidity. Adverse maternal outcomes include preterm delivery and pregnancy-induced hypertension. Other possible consequences of maternal zinc deficiency on pregnancy outcome have been suggested from clinical observations of women with acrodermatitis enteropathica and cross-sectional studies of maternal zinc status. Notable associative outcomes not observed in controlled trials include fetal congenital anomalies, intra- or postpartum hemorrhage, and prolonged labor.

Figure 1.1 summarizes the consequences of maternal zinc deficiency, as derived from the controlled trials (shaded), and other possible consequences determined from observational studies, with indication of possible relationships among the various outcomes.

Most of the zinc supplementation trials have measured only a subset of the possible outcomes described above. Of the 13 published, randomized, placebo-controlled zinc supplementation trials identified for this report, only four have been conducted in lower-income countries: South Africa [73], India [74], Peru [75], and Bangladesh [76]. Six of the 13 trials reported no effect on pregnancy outcomes measured. Two studies found significantly improved fetal growth, as measured by birth weight, one of which was carried out in the US among African American women with below average plasma zinc concentrations [77], and the other among poor urban Indian women [74]. Significant reductions in preterm deliveries were reported in three of the zinc supplementation trials [74, 77, 78], although only in women of normal body weight in the study of Cherry et al. [78]. Two studies observed reductions in the incidence of maternal complications [79, 80], although only one of these studies statistically analyzed the outcome data [79]; this study of adult Hispanic Californian women showed a significant reduction in pregnancy-induced hypertension. However, a similar study conducted among Hispanic adolescents in California did not show any effect of zinc supplementation on blood pressure [81].

Fetal and infant health have also been assessed as pregnancy outcome variables. In the Peruvian trial, inclusion of zinc with iron and folate in maternal

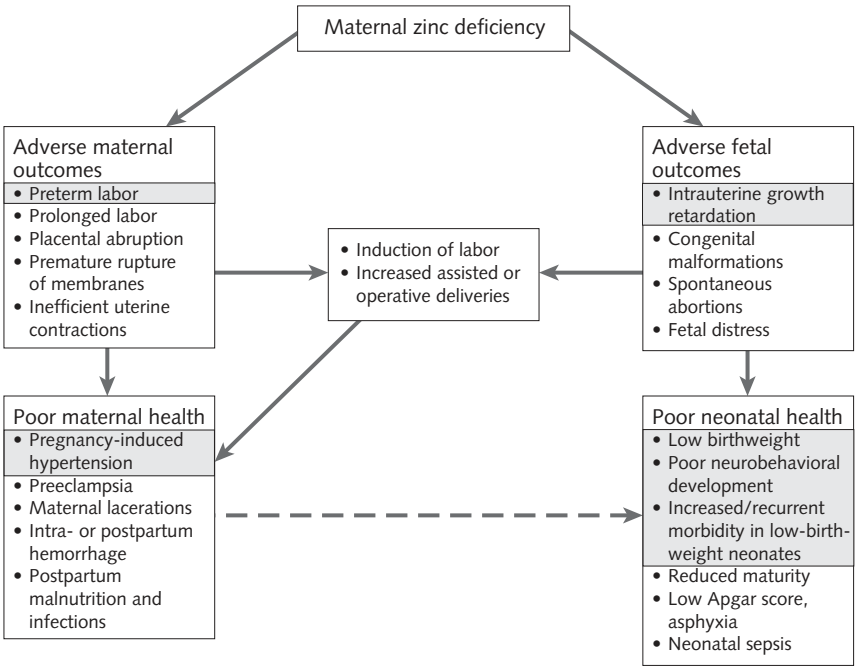

FIG. 1.1. Possible consequences of maternal zinc deficiency on birth outcomes and maternal and perinatal health. Outcomes observed in randomized, controlled zinc supplementation trials are shaded, indicating greater confidence in their association with zinc deficiency. Unshaded outcomes are those derived from observational studies of human maternal zinc status and pregnancy outcome, and their association with zinc deficiency can be considered only tentative.

supplements resulted in greater fetal heart rates and fetal movements, indicators of fetal neurobehavioral development, compared with those receiving only iron and folate [82]. Although no effects on birth weight were observed in the Bangladeshi trial, a follow-up study found that the risks of acute diarrhea, dysentery, and impetigo at 6 months were lower among infants whose mothers had received zinc supplements during pregnancy [83]. No effects on mental development or behavior, as assessed by the Bayley and modified Wolke's scales, respectively, were identified during later follow-up on the same infants at 13 months of age [84].

In summary, the results of zinc supplementation trials during pregnancy have been inconsistent. Several reasons may account for these discrepancies, including small sample sizes, varying degrees of underlying zinc deficiency, differing levels and periods of supplementation and measures of pregnancy outcome, and failure to account for confounding factors, including concurrent nutrient deficiencies. Clearly, more double-blind, placebo-controlled trials among pregnant women are needed in those lower-income countries where there is an elevated risk of zinc deficiency and where poor fetal growth is prevalent. Further, it is important to look beyond poor fetal growth alone as a possible outcome of maternal zinc deficiency, and to consider that suboptimal maternal zinc status may also manifest during postnatal development as poor infant growth and increased risk of infant morbidity and mortality.

Degenerative changes among the elderly

Several of the degenerative changes associated with aging may be due in part to zinc deficiency. These include a decline in immunocompetence [85], a decrease in taste acuity (hypoguesia) [86–88], delayed wound healing [89, 90], certain limitations of neurologic and psychologic function [91, 92], and deterioration of glucose tolerance [93, 94].

Neurobehavioral function

Some studies provide evidence that zinc deficiency contributes to compromised neuro-behavioral function among infants and children. One study among very low-birthweight infants showed improved developmental scores among those receiving supplemental zinc in formula [54]. In a zinc supplementation trial among infants in rural Guatemala, attainment of motor milestones was not affected, but activity patterns were improved with zinc supplementation [95]. A study among one-year-old Indian children also indicated that supplemental zinc plus vitamins resulted in higher activity levels than when vitamins alone were given [96]. School-aged children in China demonstrated improved neuropsychologic test performance with supplemental zinc, with the greatest improvements observed when other micronutrients were also given [27].

Zinc and appetite

Zinc deficiency has also been associated with reductions in appetite and may thereby contribute to deficiencies of other nutrients. Decreased food intake is observed early during the course of zinc depletion in animal models [97], and anorexia is a symptom of clinical zinc deficiency in humans [16]. Zinc-responsive anorexia has been demonstrated in population studies as well. A controlled zinc supplementation trial among low-income US children with evidence of mild zinc deficiency resulted in increased dietary intakes (137% energy intake of control group) after one-year of zinc supplement use (~ 4.2 mg zinc/day) [98]. A significant reduction in reported anorexia was also observed following zinc supplementation among stunted Ethiopian children [46]. However, because the high rates of morbidity were also responsive to zinc in the latter study, it is not possible to determine to what extent anorexia resulted primarily from zinc deficiency or was associated with morbidity. The mechanisms that link zinc status to appetite control are not well understood, and it is unclear whether appetite reduction precedes growth retardation or vice versa [99]. Nonetheless, the effects of zinc status on growth and appetite may be integrally related and both outcomes would likely be corrected simultaneously through improved zinc intakes.

1.5 Human zinc requirements

Since the mid 1990s, the World Health Organization/Food and Agriculture Organization/International Atomic Energy Association (WHO/FAO/IAEA) and the Food and Nutrition Board (FNB) of the US Institute of Medicine (IOM) have convened expert committees to develop estimates of human zinc requirements and to propose the corresponding dietary intakes that are needed to satisfy these requirements [11, 100, 101]. For most age and physiologic groups, the committees used a factorial method to estimate the average physiologic zinc requirement, which is defined as the amount of zinc that must be absorbed to offset the amount of endogenous zinc lost from both intestinal and non-intestinal sites. Non-intestinal sources of zinc loss include the urine, "surface losses" (desquamated skin, hair, nails, sweat), and, in adolescents and adults, semen or menstrual flow. In growing children and pregnant women, the amount of zinc retained in newly accrued tissue is also factored into total physiologic needs, and in lactating women the zinc transferred in breastmilk is added to the requirements.

When applying this conceptual framework, the committees considered the amounts of zinc lost from non-intestinal sites to be fixed, because these losses are generally constant across a wide range of zinc intakes [11]. On the other hand, intestinal excretion of endogenous zinc varies considerably in relation to the amount of absorbed zinc. Thus, the figure used for intestinal loss of endogenous zinc is appropriately estimated as the level that occurs when total absorbed zinc is just adequate to meet the theoretical physiologic needs. In the following paragraphs, we will describe in greater detail the conceptual frameworks and specific sources of data used by the various expert committees to estimate the physiologic requirements for absorbed zinc in different age and physiologic groups. In section 1.6, we will then discuss several issues concerning the absorption of dietary zinc and provide estimates of dietary zinc requirements and recommended dietary intakes.

1.5.1 Adult men

As indicated above, the physiologic requirement for zinc can be defined as the amount of zinc that must be absorbed to counterbalance the sum of endogenous zinc lost through all routes of excretion plus the amount of zinc retained in newly accrued tissue. The FNB/IOM estimated mean urinary zinc excretion by adult males to be 0.63 mg/day, based on the amounts reported from 17 previously published studies of individuals whose zinc intakes (4 to 25 mg/day) were within the range at which urinary concentrations are not influenced by zinc intake [11]. The corresponding figure published by WHO (0.3 mg/day) was based on just two studies [102, 103], which were conducted in men who had very low zinc intakes (0.8 to 3.6 mg/day); the observed amounts of urinary zinc were then inflated by 40% to account for the degree of reduction in urinary zinc excretion that occurred in response to the low intakes that were provided in those same studies. The authors of the present document conclude that the information derived by the FNB/IOM committee is more reliable because that report did the following: (1) reviewed a larger number of studies; (2) included only studies in which zinc intakes fell within the range in which urinary excretion is constant and which is likely to include the true physiologic requirement; and (3) provided more extensive documentation of the analytic process.

The FNB/IOM report considered just one study of integumental and sweat losses of zinc [104], which was carried out in 11 adult males whose mean zinc losses of 0.54 mg/day did not change in response to different levels of dietary zinc intakes ranging from 1.4 to 10.3 mg/day during periods of 28–35 days. Hence, this single figure was applied for surface losses of endogenous zinc. The WHO reports referred to a single earlier study of eight adult male volunteers [102] in whom surface losses of zinc declined from 0.49 mg/day when they consumed a diet containing 8.3 mg zinc per day to 0.28 mg/day when they consumed only 3.6 mg zinc per day. The IZiNCG SC concluded that although surface losses of zinc may decline with very restricted zinc intakes, it is preferable to estimate endogenous losses through this route when intakes are sufficient to meet physiologic needs. Thus, the IZiNCG SC decided that until more information becomes available it would be appropriate to use the study results applied by the FNB/IOM committee. However, as it is desirable to adjust zinc losses by this route for body size, as discussed in further detail below, the IZiNCG SC applied the figure per kg body weight (i.e., 6.5 μg/kg) as derived from Johnson et al [104]. Thus for a 65-kg adult man, the amount of zinc lost via the integument is 0.42 mg/day.

Unlike the WHO committees, which did not include an estimate of zinc loss in semen, the FNB/IOM committee considered information provided in two papers [104, 105] on the zinc concentration of semen and ejaculate volume of the same 11 volunteers for whom surface losses were reported above. The men's semen zinc concentrations (0.11 mg/ml) did not change with restricted dietary zinc intakes, and the ejaculate volume decreased significantly only at the lowest level of zinc intake (1.4 mg/day). Thus, the FNB/IOM committee decided to use a single figure of 0.10 mg zinc loss per day in semen, considering a mean ejaculate volume of 2.8 ml and a mean number of 2.45 ejaculations per week. The IZiNCG committee agrees with the general approach used by FNB/IOM, although more information is needed from a greater range of individuals, particularly on the mean daily volume of semen. Pending the availability of additional information, IZiNCG accepts the figure of 0.10 mg/day for average zinc loss in semen.

To estimate the intestinal loss of endogenous zinc, the WHO committees used the results from one study that reported a total fecal zinc excretion of 0.5 mg/day in six young adult males who received 0.28 mg zinc per day for 4–9 weeks [106]. This level of fecal zinc output was felt to represent the minimal amount that might be excreted after adaptation to a severely restricted diet. The WHO committee then inflated the figure for fecal losses by 40%, although the basis for this adjustment was not well substantiated in the WHO reports. Thus, the derivation of the figure of 0.8 mg/day used by WHO to indicate endogenous fecal zinc losses in adult men not adapted to low intakes of zinc is questionable.

The FNB/IOM committee considered a larger number of studies for their analyses and applied a somewhat different conceptual approach to estimate intestinal losses of endogenous zinc. In particular, the FNB/IOM committee identified 10 studies from 7 published articles that measured total absorbed zinc and intestinal excretion of endogenous zinc

using radio- or stable-isotope techniques, where the absorbed zinc was estimated from a whole day's dietary intake. Only studies that were conducted in North American or European men 19–50 years of age were accepted for inclusion in the analysis. After examining this information, the FNB/IOM committee concluded that, "excretion of endogenous zinc via the intestine is a major variable in the maintenance of zinc homeostasis and is strongly correlated with absorbed zinc." Therefore, to estimate the physiologic requirement for absorbed zinc, they decided that it would be necessary to consider the amount of intestinal losses of endogenous zinc that would occur when the absorbed zinc is just sufficient to offset the sum of all sources of endogenous zinc lost from both intestinal and non-intestinal sites.

Using this analytic approach, the FNB/IOM committee concluded that 2.57 mg/day of endogenous zinc would be excreted in feces when the amount of absorbed zinc is equivalent to the total losses of endogenous zinc from all sources, and the physiologic requirement for absorbed zinc in adult men is therefore 3.84 mg/day (i.e., with endogenous losses 0.63 mg/day from urine, 0.54 mg/day from integument and sweat, 0.10 mg/day from semen, and 2.57 mg/day in feces, it would be necessary to replace a total of 3.84 mg/day of endogenous losses of zinc). The estimate of the physiologic requirement for absorbed zinc derived from these data is illustrated graphically in figure 1.2, which is excerpted from the FNB/IOM report. The ten data points that the FNB/IOM committee used for these analyses are summarized in table 1.3 [13, 14, 103, 107–115]. As shown in the table, the studies employed diets that had a fairly low range of phytate:zinc ratios, including several that were formula diets prepared from purified ingredients.

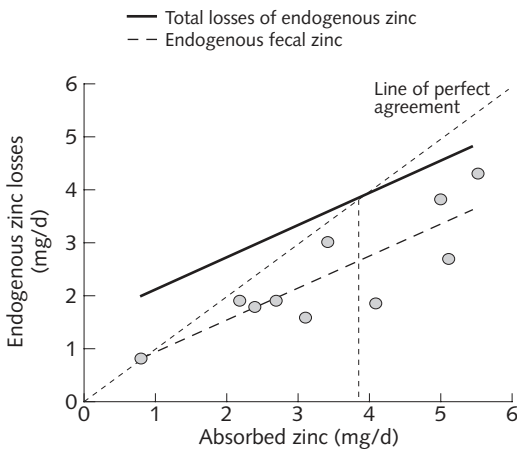

FIG. 1.2. Graphical representation of the conceptual model used by FNB/IOM [11] to estimate intestinal endogenous losses of zinc and total endogenous losses of zinc, when the amount of absorbed zinc is sufficient to offset all losses. The 10 data points represent mean data from 7 published studies of zinc absorption and intestinal losses of endogenous zinc in adult American or Western European men (19–50 years of age). The regression line (- - -) of the data points represents the relationship between total absorbed zinc and intestinal losses of endogenous zinc. The parallel line above (▬) represents the total endogenous losses of zinc after adding the static losses through urine, integument, and semen. The line of perfect agreement (-----) indicates where total endogenous losses would be equal to the amount of absorbed zinc. The vertical line (¦) is derived from the point where the total endogenous losses of zinc crosses with the line of perfect agreement, thus representing the physiologic requirement for absorbed zinc for North American adult men (i.e., 3.84 mg/day).

The IZiNCG SC concluded that the conceptual approach used by the FNB/IOM committee to estimate intestinal excretion of endogenous zinc is more appropriate than that used by WHO. However, the IZiNCG SC felt that, for the development of internationally relevant estimates of zinc requirements, it is appropriate and desirable to use the same methodologic types of studies as the FNB/IOM, but to expand the database used in this analysis to include all available studies of apparently healthy men and women, regardless of their age and nationality. Further, the IZiNCG SC initially restricted its analysis to those studies that used mixed diets prepared from common foods (i.e., studies in which supplemental zinc salts or exogenous phytate were added to the diet were excluded). Studies that manipulated several nutrients or isolated food components simultaneously were also excluded. Using these criteria, nine new studies were identified. For this new set of studies, the relationship between total absorbed zinc and fecal endogenous losses was examined by linear regression analysis, weighting by the sample size of the respective studies.

The relationship between total absorbed zinc and fecal losses of endogenous zinc was then explored to determine whether this relationship was inherently different between the new set of studies added and those used previously by the FNB/IOM. Notably, there were no significant differences in the slopes or intercepts of the respective best-fit lines, as shown in figure 1.3. Further, when all studies that employed diets prepared with common foods were then compared with those that employed semi-purified, formula diets, there were no significant differences in the relationship between absorbed zinc and endogenous fecal losses of zinc. Moreover, there were no significant differences in this relationship when studies conducted in men or women were compared. It may thus be interpreted that, once absorbed, the relationship between the amount of absorbed zinc and the amount of endogenous zinc excreted via the intestine is not dependent on the source of zinc ingested (i.e., zinc salts vs dietary zinc).

Because of the limited number of available studies in

TABLE 1.3. Summary of studies used by the FNB/IOM committee [11], the IZiNCG SC, or both committees, to estimate the relationship between total absorbed zinc and intestinal losses of endogenous zinc.

Source of data	Diet description	*n*	Total zinc intake (mg/day)	Phytate: zinc molar ratio	Intestinal losses of endogenous zinc (mg/day)	Total absorbed zinc (mg/day)
FNB/IOM only						
Lee et al. [13]	Soy protein-based, 6 months	8	4.1	21	1.8	2.4
Taylor et al. [102]	Semi-purified formula	5	5.6	0	1.9	2.2
Taylor et al. [102]	Semi-purified formula	5	0.9	0	0.8	0.8
Turnlund et al. [106]	Purified formula diet, young men	6	15.0	0	3.8	5.0
Turnlund et al. [107]	Semi-purified formula	4	15.0	0	2.7	5.1
FNB/IOM and IZiNCG						
Lee et al. [13]	Hospital	8	12.6	*	4.3	5.5
Jackson et al. [108]	Mixed	1	7.2	*	3.0	3.4
Hunt et al. [109]	Mixed	14	14.0	5	1.6	3.1
Wada et al. [110]	Mixed	6	16.5	4	1.9	4.1
Wada et al. [110]	Mixed	6	5.5	12	1.9	2.7
IZiNCG only						
Knudsen et al. [111]	High-fiber, mixed	8	10.7	6	2.6	3.1
Hunt et al. [112]	Lacto-ovo-vegetarian	21	9.1	18	0.8	2.4
Hunt et al. [112]	Mixed	21	11.1	5	1.4	3.7
Hunt et al. [113]	Low meat content	14	6.7	15	0.4	2.0
Hunt et al. [113]	High meat content	14	13.0	8	0.9	3.6
Sian et al. [14]	Plant-based	10	5.2	11	1.3	1.6
Sian et al. [14]	Mixed	10	8.1	10	2.3	2.8
Hunt et al. [109]	Mixed	14	7.8	5	2.0	2.3
Lowe et al. [114]	Mixed	6	7.0	8	2.0	2.0

* = Not available.

each of the foregoing subsets analyzed by the FNB/IOM and IZiNCG committees, respectively, the IZiNCG SC decided that the most reliable estimate of the relationship between total absorbed zinc and endogenous fecal zinc should be based on the full set of available information from all 19 studies (including the ten studies in the original FNB/IOM analysis and the nine additional studies identified by the IZiNCG SC). Figure 1.4 shows the relationship between total absorbed zinc and fecal endogenous zinc for the combined data set. Based on this combined set of information, 1.54 mg/day of endogenous zinc would be excreted in feces when the amount of absorbed zinc is equivalent to the total losses of endogenous zinc from all sources, and the physiologic requirement for absorbed zinc in adult men is therefore 2.69 mg/day. That is, with endogenous losses of 0.63 mg/day from urine, 0.42 mg/day from integument and sweat, 0.10 mg/day from semen, and 1.54 mg/day in feces, it would be necessary to replace a total of 2.69 mg/day of endogenous losses of zinc. Estimates for the various sources of endogenous losses of zinc for 65-kg adult men derived by the IZiNCG SC are summarized in table 1.4. For the purpose of comparison, the estimates derived by the WHO and FNB/IOM committees are also shown.

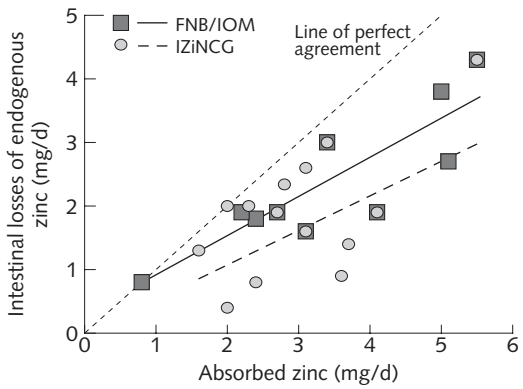

FIG. 1.3. Comparison of regression lines of total absorbed zinc and intestinal losses of endogenous zinc used by the FNB/IOM committee [11] and the IZiNCG SC for the estimation of physiologic zinc requirements.

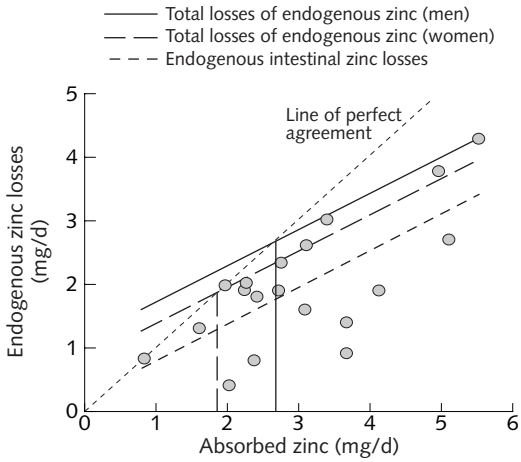

FIG. 1.4. Graphical representation of the model used by the IZiNCG SC in the present report to estimate intestinal endogenous losses of zinc and total endogenous losses of zinc, when the amount of absorbed zinc is sufficient to offset all losses. The 19 data points represent mean data from 12 published studies of zinc absorption and intestinal losses of endogenous zinc in adult men and women (19–50 years of age). The regression line (- - -) of the data points represents the relationship between total absorbed zinc and intestinal losses of endogenous zinc. The parallel lines above (—— men, – – women) represent the total endogenous losses of zinc after adding the static losses through urine, integument, and semen. The line of perfect agreement (-----) indicates where total endogenous losses would be equal to the amount of absorbed zinc. The vertical lines (| men, ¦ women) are derived from the point where the total endogenous losses of zinc crosses with the line of perfect agreement, thus representing the physiologic requirement for absorbed zinc for 65 kg adult men (i.e., 2.69 mg/day) and 55 kg adult women (i.e., 1.86 mg/day).

One final consideration for the derivation of physiologic zinc requirement estimates that are internationally applicable is that of reference body weights. The FNB/IOM Dietary Reference Intake committee applied reference body weights that are suitable for North American populations. However, the IZiNCG SC felt that the reference body weights adopted by the WHO, and based on the NCHS/CDC 1977 growth reference data, are more suitable for the present report. Based on the available data for body weight of subjects from 17 of the 19 studies used in the analysis of endogenous losses of intestinal zinc, the average body weights of the adult subjects (men, 71 kg; women, 61 kg) were intermediate to the reference body weights used by FNB/IOM (men, 75 kg; women, 65 kg) and those used by WHO (men, 65 kg; women, 55 kg). The IZiNCG SC thus felt it was unnecessary to correct intestinal losses according to different body weights among adult men. Similarly, it is unlikely that urinary losses of zinc would vary predictably according to different body weights among adults of different sizes. On the other hand, as integumental zinc losses may be more directly associated with body surface area and hence body size, it was felt appropriate to apply these losses to both adult men and women according to reference body weight.

TABLE 1.4. Estimated physiological requirements for absorbed zinc in adult men and women, as developed by expert committees of the WHO [99, 100], the US FNB/IOM [11], and as reviewed by IZiNCG

Endogenous zinc losses (mg/day) in adult men and women, by source of loss	Source of estimated physiological requirements		
	WHO	IOM	Revisions suggested by IZiNCG
Men			
Reference body weight (kg)	65	75	65
Urinary excretion	0.30	0.63	0.63
Integument	0.30	0.54	0.42
Semen	—	0.10	0.10
Total non-intestinal endogenous losses	0.60	1.27	1.15
Intestinal excretion of endogenous zinc	0.80	2.57	1.54
Total endogenous losses	1.40	3.84	2.69
Women			
Reference body weight (kg)	55	65	55
Urinary excretion	0.30	0.44	0.44
Integument	0.20	0.46	0.36
Menstrual blood	—	0.10	0
Total non-intestinal endogenous losses	0.50	1.00	0.80
Intestinal excretion of endogenous zinc	0.50	2.30	1.06
Total endogenous losses	1.00	3.30	1.86
Additional requirements for pregnancy (first, second, third trimesters)	0.1, 0.3, 0.7	0.16, 0.39, 0.63	0.70[a]
Additional requirements for lactation (0–3 months, 3–6 months, > 6 months)	1.4, 0.8, 0.5	1.35[b]	1.0[b]

a. A single estimate for additional zinc requirements is applied throughout pregnancy.
b. A single estimate for additional zinc requirements is applied throughout lactation.

1.5.2 Adult women

The foregoing conceptual issues concerning zinc

requirements generally apply to women as well as men. However, for several reasons, the specific figures used for endogenous zinc losses differ by sex, as described in the following paragraphs. The FNB/IOM committee examined the results of 10 published studies to calculate a mean urinary zinc excretion of 0.44 mg/day for adult females. The WHO committee relied on the results of just one study of women who were receiving very restricted zinc intakes, and then inflated the results by 40%, as described previously for men. For the same reasons described above, the IZiNCG SC has more confidence in the figure proposed by the FNB/IOM committee for urinary losses of zinc in women.

Each of the former expert committees estimated women's surface losses of zinc by adjusting for differences in body surface area to extrapolate from the data available from men. Because the IZiNCG SC preferred the original estimates for adult males proposed by the FNB/IOM committee, these same figures (adjusted for body size) were adopted for adult females (i.e., 0.0065 mg zinc/kg body weight/day × 55 kg = 0.36 mg zinc/day). There is little information on endogenous zinc losses in menstrual fluid. In one study [116], the average excretion of menstrual fluid during a single period was 60 g, and the mean zinc content was approximately 2.8 μg/g menstrual fluid or 154 μg during each menstrual period. This resulted in an average daily zinc loss of about 5 μg/day, considering the average cycle length of the women in the study. The WHO committees did not account for menstrual zinc losses, but the FNB/IOM committee estimated average menstrual losses to be 0.1 mg/day. However, this estimate was based on erroneous interpretation of data from the aforementioned study [116]. Because loss of zinc by this route is negligible, the IZiNCG SC concluded that it can be ignored in estimates of zinc requirements.

As discussed above, the IZiNCG SC prefers the conceptual approach used by the FNB/IOM committee to estimate fecal losses of endogenous zinc. Using this approach, the amount of intestinal losses of endogenous zinc that would occur in adult women is 1.06 mg/day when the amount of absorbed zinc is just sufficient to offset the sum of all sources of endogenous zinc loss (1.86 mg/day) from both intestinal and non-intestinal sites, assuming a reference body weight of 55 kg, as shown in figure 1.4. The endogenous losses of zinc by intestinal and non-intestinal routes for a 55-kg adult woman are summarized in table 1.4.

1.5.3 Children

Infants 0-6 months

Very little empirical information is available on zinc homeostasis, and, therefore, little information is available on physiologic requirements for absorbed zinc in infants less than 6 months of age. Moreover, there is some evidence that young infants may be able to acquire a portion of their zinc needs by mobilizing hepatic reserves accumulated during gestation [117], thereby possibly modifying their need for absorbed zinc from the diet. Studies of exclusively breastfed, healthy, term infants in the United States found no differences in the growth patterns of those who received zinc supplements or placebo from 4–6 months of age, suggesting that their zinc intake from breastmilk, along with any additional zinc contributed from pre-existing stores, was adequate to maintain normal growth [118]. On the other hand, European investigators found that infants 4–9 months of age of immigrant populations had increased rates of growth when supplemented with zinc (5 mg/day for three months) [119]. However, these latter infants were not exclusively breastfed, so it is conceivable that foods with lower zinc density were displacing breastmilk and/or these foods interfered with zinc absorption from breastmilk. Although more information is needed, it appears that zinc transfer from breastmilk is adequate for full-term, normal-birthweight, exclusively breastfed infants until about 6 months of age.

The FNB/IOM committee did not attempt to estimate physiologic requirements of zinc for young infants. Instead, the committee described presumably adequate intakes (AIs), based on the content of zinc in breastmilk at different ages and the average amount of milk consumed. It is important to note that because the zinc concentration of human milk declines sharply during the first few months post-partum, the total zinc transferred through milk falls from about 2.5 mg/day at one month to approximately 0.8 mg/day at six months [11]. Based on average figures for zinc transfer in breastmilk from 0–5 months post-partum, the FNB/IOM proposed 2.0 mg/day as the AI for infants in this age range. By contrast, the WHO committees developed estimates of physiologic zinc requirements of young infants by extrapolating from data for adults in relation to metabolic rate and then adding the amount of zinc incorporated into newly deposited tissue. Using this approach, the WHO committees suggested that the requirement for absorbed zinc from 0–5 months of age ranges from 0.7–1.3 mg/day, depending on age and sex. This compares with the estimate for absorbed zinc of ~ 0.7 mg/day developed by Krebs and Hambidge [120]. Considering the available information, IZiNCG concludes that breastmilk is a sufficient source of zinc for exclusively breastfed, normal-birthweight term infants until about 6 months of age. Non-exclusively breastfed infants need to absorb approximately 1.3 mg/day during the first three months of life and 0.7 mg/day during months 3–5.

Even less information is available on the zinc requirements of infants with low birth weight due to prematurity and/or intra-uterine growth retardation. Low-birthweight infants may have greater needs

for absorbed zinc than normal-birthweight infants because of limited hepatic reserves at birth and higher rates of postnatal growth. Notably, several researchers have found that low-birthweight infants had increased growth rates following zinc supplementation, which ranged from 2 to 5 mg/day of supplemental zinc in the available studies [43, 53, 54]. More research is needed to establish the physiologic requirements for zinc among low-birthweight infants.

Children 6 months to 18 years

The FNB/IOM used a factorial method to estimate physiologic zinc requirements of older infants and children. Losses of endogenous zinc from non-intestinal sites (i.e., urinary and surface losses) were estimated to be 0.014 mg/kg/day on the basis of extrapolations from adults per unit body weight. Fecal excretion of endogenous zinc was estimated to be 0.050 mg/kg/day for infants 6–11 months of age, based on empirical data obtained from breastfed infants, and 0.034 mg/kg/day for older children, as extrapolated from adult data. To these figures for endogenous losses were added the amount of zinc required for growth, which is estimated to be 0.020 mg/g of tissue gained. The figures for endogenous losses and zinc content of accrued tissue were then multiplied, respectively, by the reference body weight and the expected rate of weight gain at different ages. For male adolescents 14–18 years of age, an additional 0.1 mg/day was included in the estimated physiologic requirements to account for losses in semen. The WHO committees estimated physiologic zinc requirements throughout childhood by extrapolating from the data used to estimate endogenous losses in adults.

For the sake of consistency with the information discussed above for adults, the IZiNCG SC prefers to follow the factorial approach used by the FNB/IOM, but to base intestinal losses of endogenous zinc on the estimates derived by IZiNCG and to use the NCHS/CDC/WHO reference body weights, as summarized in table 1.5. Total endogenous zinc losses are calculated as 0.064 mg/kg/day for infants 6–11 months of age and 0.034 mg/kg/day for children one year of age and older (i.e., urinary losses 0.0075 mg/kg/day, surface losses 0.0065 mg/kg/day, and intestinal losses of 0.05 mg/kg/day for infants 6–11 months or 0.02 mg/kg/day for children 1 year and older). For example, children 6–11 months of age who have a reference body weight of 9 kg and expected weight gain of 13 g/day, need 0.576 mg/day (i.e., 9 kg × 0.064 mg/kg) to replace endogenous losses and 0.260 mg/day (13 g/day × 0.020 mg/g) for tissue accrual, resulting in a total physiologic requirement of 0.836 mg/day. The same procedure was used in children 1–3, 4–8, 9–13, and 14–18 years of age, using their respective reference body weights and rates of weight gain (table 1.5).

1.5.4 Pregnancy

Accrual of zinc in newly synthesized fetal and maternal

TABLE 1.5. Estimated physiologic requirements for absorbed zinc during childhood by age group and sex, and during pregnancy and lactation, as developed by expert committees of the WHO [100, 101], the US FNB/IOM [11], and as reviewed by IZiNCG

WHO			FNB/IOM			Revisions suggested by IZiNCG		
Age, sex	Reference weight (kg)	Physiologic requirement (mg/day)	Age, sex	Reference weight (kg)	Physiologic requirement (mg/day)	Age, sex	Reference weight (kg)	Physiologic requirement (mg/day)
6–12 mo	9	0.84	7–12 mo	9	0.84	6–11 mo	9	0.84
1–3 yrs	12	0.83	1–3 yrs	13	0.74	1–3 yrs	12	0.53
3–6 yrs	17	0.97	4–8 yrs	22	1.20	4–8 yrs	21	0.83
6–10 yrs	25	1.12						
10–12 yrs, M	35	1.40	9–13 yrs	40	2.12	9–13 yrs	38	1.53
10–12 yrs, F	37	1.26						
12–15 yrs, M	48	1.82						
12–15 yrs, F	48	1.55						
15–18 yrs, M	64	1.97	14–18 yrs, M	64	3.37	14–18 yrs, M	64	2.52
15–18 yrs, F	55	1.54	14–18 yrs, F	57	3.02	14–18 yrs, F	56	1.98
Pregnancy	—	2.27	Pregnancy (1st, 2nd, 3rd trimester)	—	4.12, 4.42, 5.02	Pregnancy	—	2.68
Lactation	—	2.89	Lactation (0–3, 3–6, 6–12 mo)	—	4.92, 3.82, 4.52	Lactation	—	2.98

tissue during pregnancy imposes an additional physiologic requirement for zinc. Both the FNB/IOM and WHO committees based these requirement estimates on data derived from Swanson and King [121]. The FNB/IOM estimated these additional zinc needs as 0.16 mg/day during the first trimester of pregnancy, 0.39 mg/day during the second trimester, and 0.63 mg/day during the third trimester; and WHO provided similar estimates of the respective amounts needed in each trimester, as follows: 0.1 mg/day during the first trimester, 0.3 mg/day during the second, and 0.7 mg/day during the third. To provide a single figure for the amount of additional zinc that needs to be absorbed during pregnancy, IZiNCG proposes using the figure of 0.7 mg/day, which covers the amount needed in the third trimester. It should be recognized, however, that this single figure overestimates the average requirements for absorbed zinc in the first and second trimesters. This additional amount of zinc needed during pregnancy should be added to the usual age-specific physiologic requirements for absorbed zinc of adolescent or adult women.

1.5.5 Lactation

The amount of zinc transferred from mother to infant in breastmilk must be added to lactating women's physiologic requirements for absorbed zinc. This amount is calculated by multiplying the average volume of milk transferred to the infant by the zinc concentration of human milk at different post-partum periods. To complete these calculations, the FNB/IOM committee applied milk volumes that were measured in US women during the first year post-partum (0.78 L/day). The FNB/IOM also summarized the results of 12 studies to provide age-specific information on the zinc concentration of human milk (2.75 mg/L at 4 weeks, 2.0 mg/L at 8 weeks, 1.5 mg/L at 12 weeks, and 1.2 mg/L at 24 weeks). Using these two sets of information, the FNB/IOM committee produced a single estimate of 1.35 mg/day for the average additional amount of absorbed zinc needed to support lactation, after discounting an assumed 1 mg/day of endogenous zinc that may become available during the first month post-partum because of involution of reproductive tissue. The WHO committees used data from just 3 of the 12 studies cited by FNB/IOM to estimate the zinc content of human milk (2.5 mg/L at 1 month, 0.9 mg/L at 3 months, and 0.7 mg/L at 4 months). WHO estimated that an additional 1.4 mg zinc/day is needed from 0–3 months post-partum, 0.8 mg/day from 3–6 months, and 0.5 mg/day thereafter.

Because women from developing countries typically breast feed for longer periods of time than do US women, and because breastmilk volume changes with infant age, the IZiNCG SC felt it would be desirable to derive estimates of zinc transfer in breastmilk using data on milk output from women in developing countries. Because the zinc concentration of human milk seems to be affected minimally, if at all, by maternal zinc status, it seems reasonable to use the more extensive set of data on milk zinc concentrations that was summarized by the FNB/IOM. Table 1.6 shows the mean amount of milk consumed by infants in developing countries at different ages, as published in a recent review [122]. These figures were multiplied by the mean zinc concentration of human milk for the same postpartum periods as reported by FNB/IOM [11] to estimate the total amount of zinc excreted in breastmilk. As indicated in table 1.6, the additional zinc needs imposed by lactation are considerable, especially during the early months of breastfeeding. On average, about 1 mg of additional zinc must be absorbed during lactation. Although more than this amount might be needed during the initial months, it is likely that this is partially offset by zinc released during involution of reproductive tissue. Thus, the figure of 1 mg/day seems to be a reasonable estimate of the additional amount of absorbed zinc needed throughout lactation. This additional amount should be added to the usual age-specific physiologic requirements for absorbed zinc of adolescent or adult women.

1.6 Dietary sources of zinc and suggested revisions of Recommended Daily Intakes

To translate physiologic requirements for absorbed zinc into recommendations for daily dietary zinc intakes, it is necessary to take into account the proportion of zinc in the diet that is absorbed by the intestine. In this section, we present the following: (1) a review of the dietary factors that affect zinc absorption; (2) estimates of zinc absorption from different diets; and (3) the derivation of dietary requirements, which incorporate information on the physiologic requirements for absorbed zinc (as described in section 1.5) and the estimated average zinc absorption.

TABLE 1.6. Amount of zinc transferred from mother to child in human milk, by infant age

Age range (months)	Milk volume (ml/day)[a]	Zinc concentration (mg/100 ml)[b]	Zinc amount (mg/day)
0–2	714	0.230	1.64
3–5	784	0.135	1.06
6–8	776	0.120	0.93
9–11	616	0.120	0.74
12–23	549	0.120	0.66

a. Data from Brown et al. [122]
b. Data from FNB/IOM [11]

1.6.1 Dietary sources of zinc and factors affecting the proportion of zinc available for absorption

Zinc occurs in a wide variety of food sources, but is found in highest concentrations in animal-source foods, particularly in the organs and/or flesh of beef, pork, poultry, fish and shellfish, and with lesser amounts in eggs and dairy products. Zinc content is relatively high in nuts, seeds, legumes, and whole-grain cereals, and is lower in tubers, refined cereals, fruits, and vegetables. Average ranges of zinc content (mg/100 g fresh weight) and zinc density (mg/100 kcal) in a variety of food sources are summarized in table 1.7, using information provided in the International MiniList (WorldFood Dietary Assessment System, 2.0, University of California, Berkeley; www.fao.org/infoods/software/worldfood.html).

As a result of physico-chemical interactions, dietary factors can alter the proportion of zinc that is available for absorption in the intestine by as much as 10-fold. Most of the available information on the effect of specific dietary factors on zinc absorption has been derived from studies measuring absorption from single test meals. However, it is questionable whether zinc absorption determined from single meal studies reflects the true proportion of zinc that would be absorbed from meals over the course of a whole day, as discussed below. Nonetheless, the large amount of data from these single meal studies is useful to identify the factors that do affect zinc absorption and to indicate their relative impact. Based on these single meal studies, the dietary components that demonstrate a substantial impact on the absorption of zinc are phytate and dietary calcium, which inhibit zinc absorption, and protein, which enhances absorption [123]. The total zinc content of a meal also influences the absorption of zinc; specifically, the percent absorption decreases with increasing intake of zinc [124], although the absolute amount of zinc absorbed increases.

Myo-inositol hexaphosphate (phytic acid) consists of a ring of six phosphate ester groups. Phytate is the magnesium, calcium, or potassium salt of phytic acid; the term "phytate" is used generically in this document to refer to the phytic acid molecule, as well as its salt forms. Phytate is a phosphorus storage molecule with a high natural content in seeds, including cereal grains, nuts, and legumes, and a lower content in other plant foods, such as fruits, leaves, and other vegetables. In legumes, phytate is uniformly distributed and associated with protein, whereas in cereal grains it is generally concentrated in the bran; in maize, the majority of phytate exists in the germ. Phytate is a strong chelator of minerals, including zinc. Because phytate cannot be digested or absorbed in the human intestinal tract, minerals bound to phytate also pass through the intestine unabsorbed. The inhibitory effect of phytate on zinc absorption appears to follow a dose-dependent response, and the phytate:zinc molar ratio of the diet has been used to estimate the proportion of absorbable zinc. The phytate:zinc molar ratio of foods or diets is calculated as follows:

$$\frac{\text{mg phytate}/660}{\text{mg zinc}/65.4},$$

TABLE 1.7. Zinc content, zinc density, phytate content, and phytate-to-zinc molar ratios of commonly consumed foods; data derived from the International MiniList[a]

	Zinc content		Phytate content	
Food groups	mg/100 g	mg/100 kcal	mg/100 g	Phytate:zinc molar ratio
Liver, kidney (beef, poultry)	4.2–6.1	2.7–3.8	0	0
Meat (beef, pork)	2.9–4.7	1.1–2.8	0	0
Poultry (chicken, duck, etc.)	1.8–3.0	0.6–1.4	0	0
Seafood (fish, etc.)	0.5–5.2	0.3–1.7	0	0
Eggs (chicken, duck)	1.1–1.4	0.7–0.8	0	0
Dairy (milk, cheese)	0.4–3.1	0.3–1.0	0	0
Seeds, nuts (sesame, pumpkin, almond, etc.)	2.9–7.8	0.5–1.4	1,760–4,710	22–88
Beans, lentils (soy, kidney bean, chickpea, etc.)	1.0–2.0	0.9–1.2	110–617	19–56
Whole-grain cereal (wheat, maize, brown rice, etc.)	0.5–3.2	0.4–0.9	211–618	22–53
Refined cereal grains (white flour, white rice, etc.)	0.4–0.8	0.2–0.4	30–439	16–54
Bread (white flour, yeast)	0.9	0.3	30	3
Fermented cassava root	0.7	0.2	70	10
Tubers	0.3–0.5	0.2–0.5	93–131	26–31
Vegetables	0.1–0.8	0.3–3.5	0–116	0–42
Fruits	0–0.2	0–0.6	0–63	0–31

a. WorldFood Dietary Assessment Program, 2.0, University of California, Berkeley

where 660 = the molecular weight of phytate, and
65.4 = the molecular weight of zinc.

The phytate content, and the phytate:zinc molar ratio of some commonly consumed foods are shown in table 1.7. In general, seeds, nuts, legumes, and unrefined cereal grains have the highest phytate:zinc molar ratios, which range from 22–88, while other plant foods have phytate:zinc molar ratios in the range of 0–42. Animal source foods do not contain phytate and therefore have a phytate:zinc molar ratio equivalent to zero.

Calcium also has an inhibitory effect on zinc absorption, although this may only occur when phytate is also present in the diet [123]. The inhibitory effect of calcium may result from the formation of insoluble calcium-zinc-phytate complexes in the intestinal tract. Both the total amount and type of protein in the diet influence zinc absorption. Increasing protein content results in a greater percent absorption of dietary zinc [124]. Animal protein, such as from meat and eggs, including whey protein, appear to have further enhancing effects on zinc absorption, although casein may be inhibitory [123].

1.6.2 Revised estimates of dietary requirements and recommended intakes for zinc

Two committees charged with developing dietary reference values, the FAO/WHO/IAEA Expert Consultation [100, 101] and the US Food and Nutrition Board/Institute of Medicine Standing Committee on the Evaluation of Dietary Reference Intakes [11], have estimated the percent absorption of dietary zinc. Both committees used a similar conceptual approach to develop these estimates, although the types of studies used in their analyses differ markedly. Each committee extracted data from studies of zinc absorption and plotted the mean amount of absorbed zinc against the total zinc ingested from the meal or diet being tested. A regression equation was then derived from the data and used to determine the amount of total zinc that would need to be ingested such that the amount of absorbed zinc would be equivalent to the physiologic requirement. This amount of total zinc ingested represents the daily "estimated average requirement" from the diet, or the EAR. The physiologic requirement for absorbed zinc divided by the associated total zinc intake (× 100%) represents the "critical" average zinc absorption, i.e., the percent of dietary zinc that is absorbed when the level of intake is just adequate to satisfy the physiologic requirement.

The IZiNCG SC reviewed the methods used by the WHO and FNB/IOM committees to estimate zinc absorption, taking into consideration the methodology used to measure absorption, the types of diets and subjects from which data were derived, as well as the models used to summarize these data.

Two general types of study designs have been used most commonly to estimate dietary zinc absorption: single-meal studies and total-diet studies. Single-meal studies are those that measure absorption from a single test meal, whereas total-diet studies measure zinc absorption from multiple meals consumed over 1 or more days. Zinc absorption data derived from total-diet studies have two main advantages. First, these studies label meals with either radioisotopes or stable-isotopes of zinc and, using fecal monitoring techniques, are able to estimate true zinc absorption for each individual by correcting for the simultaneous intestinal losses of endogenous zinc that occur during digestion. On the contrary, most single-meal studies have used radioisotope tracers and whole-body counting to measure zinc retention. This method can only estimate true absorption of zinc by applying an average correction factor for intestinal losses of endogenous zinc, derived from a separate study. Second, there is also evidence from studies of iron absorption that the percent iron absorption measured from a single test meal differs significantly from that measured from a total diet of similar composition as the single test meal [125, 126]. One of these studies [125] suggested that the enhancing and inhibiting effects of dietary factors (e.g., ascorbic acid, phytate) on iron absorption are exaggerated when measured from single test meals, whereas another suggested that iron absorption is much higher when measured from a breakfast meal than when the same test meal is consumed at later times during the day. It is possible that the same situation holds for zinc, although this has not been studied directly. For these reasons, the IZiNCG SC considered the total-diet study methodology as the reference method for measuring zinc absorption from the diet, particularly where the goal is to calculate dietary zinc requirements.

In determining its zinc absorption estimates, the WHO committee used data from a combination of single-meal studies and total-diet studies, although the specific studies used were not referenced [100]. Given the availability of zinc absorption studies at the time these estimates were made, it is likely that most of the data used were derived from single-meal studies. The WHO committee divided the available data into three categories according to the phytate:zinc molar ratio of the test meal or diet; where ratios of < 5, 5–15, and > 15 were considered to represent diets of relatively high, moderate, and low absorption levels, respectively. The process described above was applied to each of the three sets of data to derive zinc absorption estimates. A description of the diet types for each of the three categories and the associated absorption estimates are summarized in table 1.8. Because the specific studies used in these estimates were not reported, it is not possible to describe the specific meals or diets studied, nor the sex or geographic origin of the participants.

The FNB/IOM committee selected 10 data points

TABLE 1.8. Estimates of dietary zinc absorption, as developed by WHO [100, 101], FNB/IOM [11], and IZiNCG, and summaries of the data used to derive them

	WHO			IOM	IZiNCG	
Diet types represented	Highly refined[a]	Mixed/ refined vegetarian[b]	Unrefined[c]	Mixed, n = 5 Semi-purified, n = 4 EDTA-washed soy protein, n = 1	Mixed, n = 11 Refined vegetarian, n = 3	Unrefined, cereal-based, n = 1
Study type	Single meal & total diet			Total diet	Total diet	
Subjects	NA[d]	NA	NA	Men 19–50 yrs	Men & women 20+ yrs	
Phytate:zinc molar ratio	< 5	5–15	> 15	NA	4–18	> 18
Zinc absorption[e]	50%	30%	15%	41%	26% men 34% women	18% men 25% women

a. Refined diets low in cereal fiber, and where animal foods provide the principal source of protein. Includes semi-purified formula diets.
b. Mixed diets, and lacto-ovo-vegetarian diets that are not based on unrefined cereal grains or high extraction rate (> 90%) flours.
c. Cereal-based diets, with > 50% of energy intake from unrefined cereal grains or legumes and negligible intake of animal protein.
d. NA = not available
e. These figures represent the "critical" level of zinc absorption, or that which occurs when zinc intakes are just sufficient to meet physiologic requirements for absorbed zinc.

from 7 published studies of zinc absorption, using only total-diet studies, likely for the reasons described above [11]. The studies used by the FNB/IOM committee included only those of North American or Western European adult male subjects (19–50 years) and the diet types represented both mixed diets and semi-purified formula diets; all data points were considered in a single diet category, regardless of the composition of the diet. The same regression line relating zinc intake and total absorbed zinc that was derived from the studies of men was used to derive a zinc absorption estimate for women, based on their physiologic requirement for absorbed zinc. The studies included in the analysis and the absorption estimates derived are summarized in table 1.8.

For the reasons described above, the IZiNCG SC concurred with the FNB/IOM that total diet studies of zinc absorption provide the most valid estimates of dietary zinc absorption. Further, the IZiNCG SC felt it was important to consider differences in zinc absorption based on diet type and dietary content of known enhancers and inhibitors of zinc absorption, as done by the WHO, because—on the basis of their higher content of inhibitors of zinc absorption [127]—diets consumed habitually by a large proportion of the global population would be expected to have a lower fractional absorption of zinc than the diets considered by the FNB/IOM committee to establish their estimates. As a further step to ensure the appropriateness of the absorption estimates for internationally representative diet types, the IZiNCG SC rejected absorption studies that included semi-purified diets or other diets that included exogenous sources of zinc in the form of zinc salts. These types of diets do not represent typical diets consumed by populations, and the zinc absorption is expected to be higher from liquid formulas than from solid food matrices [128], and possibly higher from soluble zinc salts added exogenously than from an equivalent amount of zinc endogenous to the food. It also appeared unnecessary to exclude zinc absorption data derived from studies of women because the same regression curve is used to derive the zinc absorption estimate for both men and women. Finally, as the absorption estimates are intended for international use, geographic restriction on the origin of studies is unnecessary. Therefore, the selection criteria for the present IZiNCG analysis included the following: (1) radio- or stable-isotope studies that estimated true zinc absorption from total diets by correcting for intestinal losses of endogenous zinc; (2) studies of typical mixed, refined vegetarian, or unrefined, cereal-based diets, but not those that used semi-purified formula diets or diets with exogenous zinc salts added; and (3) studies with male or female adults, with no geographic limitations.

Initially, 17 data points from 11 published articles meeting the above criteria were identified. Information on the content of zinc, phytate, protein, and calcium of the study diets was derived either from the published article, by estimation from the published food composition of the study diet using a dietary assessment program, and/or from unpublished information obtained from the authors. Zinc and phytate contents of the study diets were available for 15 studies, and, calcium and protein contents were also available for 12 of these studies. The 15 data points, derived from 9 separate published articles, for which at least zinc and phytate contents were available were used in the final analyses [14, 110–115, 129, 130]. The diet types represented by these studies are summarized in table 1.8.

Data for these four dietary factors were log trans-

formed and a logit regression model was used to describe their relationship with the percentage of zinc intake that was absorbed. The logit model was used because, unlike the logarithmic transformation, it constrains predicted zinc absorption to between 0 and 100%. Neither protein nor calcium added significant predictive power, so the final model ($r^2 = 0.413$, $p < 0.001$) included only zinc and the phytate:zinc molar ratio, both of which were highly significant predictors of percent zinc absorption. Therefore, it appears to be valid to continue to use the phytate:zinc molar ratio to define diet types, with respect to zinc absorption. The prediction equation for proportion of absorbed zinc given as a fraction, using the dietary phytate:zinc molar ratio and zinc content derived from this model is*:

$$\text{Logit (fraction of absorbed zinc)} = 1.1365 - 0.6129 \times \ln(\text{mg zinc}) - 0.3164 \times \ln\left(\text{phytate:zinc molar ratio}\right)$$

and

$$\text{Fraction of absorbed zinc} = \frac{\exp(\text{logit(fraction of absorbed zinc)})}{1 + \exp(\text{logit(fraction of absorbed zinc)})}$$

The range of phytate:zinc molar ratios was divided into two categories: (1) 4–18, which represents mixed or refined vegetarian diets, and (2) 18–30, representing unrefined, cereal-based diets (table 1.8). Using the prediction equation above, the phytate:zinc molar ratio was set at the midpoint of the range for the first diet category (i.e., 11) and then at the midpoint for the second diet category (i.e., 24), and the percent absorption of zinc associated with each level of total zinc intake between 4.2 and 16.5 mg (i.e., the range of values for zinc in the diets of the studies included in the analysis) was used to calculate the associated amounts of absorbed zinc. Curves were then generated showing the relationship between total zinc intake and absorbed zinc for the two diet categories (figure 1.5). Using these curves and the physiologic requirement for absorbed zinc of adult men (2.69 mg zinc/day) and women (1.86 mg zinc/day), as described in section 1.5, the amount of total zinc intake needed to meet this requirement was determined for each diet type, as shown in figure 1.5; this amount represents the dietary requirement. The percent zinc absorption represented

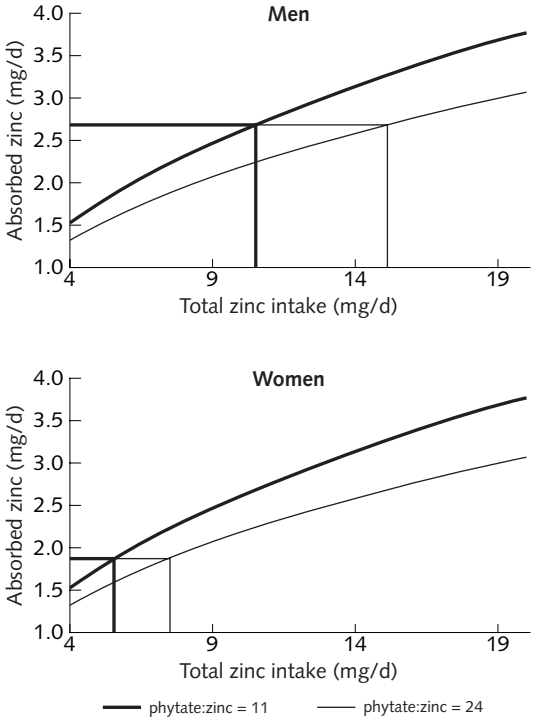

FIG. 1.5. Derivation of the estimated average requirement for men and women and the critical level of zinc absorption for mixed/refined vegetarian diets (P:Z = 11)and unrefined cereal-based diets (P:Z = 24), using the association between total zinc intake and absorbed zinc for each diet type and the physiologic requirement. Top panel is for men. Bottom panel is for women.

at this level of intake is referred to as the "critical" level of absorption. For example, the critical absorption level of an adult man consuming a mixed diet is calculated as 1.86 mg zinc/day (physiologic requirement) ÷ 10.4 mg zinc/day (dietary requirement) × 100% = 26%. Following this example, the calculated critical levels of zinc absorption were 26% for men and 34% for women for mixed/refined vegetarian diets, and 18% for men and 25% for women for unrefined, cereal-based diets.**

These estimates of zinc absorption should be considered as tentative until further data are available from a wider range of diet types, particularly from unrefined diets with a high phytate:zinc molar ratio (i.e., > 18), for which we identified only one data point from total diet studies of zinc absorption in adults. Notably, the estimate for zinc absorption from mixed or refined

* This prediction equation may be used to estimate fractional zinc absorption from adult diets. However, because the equation is dependent on total zinc intake it may not provide accurate estimates of fractional absorption from children's diets given that their dietary requirements and usual intakes are lower than for adults.

** The figures for zinc absorption which correspond to the amount of ingested zinc needed to meet the physiologic requirements of adult men and women with the higher reference body weights assumed by the FNB/IOM committee (i.e., men 75 kg and women 65 kg) are 24% for men and 31% for women consuming mixed/refined vegetarian diets and 16% for men and 22% for women consuming unrefined, cereal-based diets.

vegetarian diets in the present report is not as high as the estimate made by the FNB/IOM committee (41%) for North American diets or the upper estimate made by the WHO committee (50%) for highly refined diets, likely due to the IZiNCG SC's exclusion of data from semi-purified formula diets.

The IZiNCG SC felt that there was no justification at this time for assuming different levels of zinc absorption for different age groups, and therefore the mean of the absorption figures for adult men and women from each diet type were applied to children 1–18 years of age (i.e., 31% absorption from mixed/refined vegetarian diets and 23% from unrefined, cereal-based diets). The FNB/IOM committee applied lower figures for the critical absorption level for children than they used for adults. However, these data for children were based on results from only two studies of zinc absorption from single meals, and the average absorption from these two studies was 30%, similar to the absorption level that the IZiNCG SC derived for adults consuming mixed or refined vegetarian diets. The WHO committee did not propose different levels of zinc absorption for pregnant or lactating women. The FNB/IOM committee concluded that zinc absorption is not increased significantly during pregnancy. This conclusion was based on the results of one study in which zinc absorption was measured in women prior to conception and at 24–26 weeks and 34–36 weeks of gestation [12], with average zinc intakes of 15 mg/day throughout the study. In that study, the slight increase from 15% absorption at preconception to 19% absorption during pregnancy was statistically insignificant. Although these results do not exclude the possibility that zinc absorption is increased during pregnancy in women with lower zinc intakes, the IZiNCG SC concurs with the FNB/IOM committee that there is insufficient evidence to suggest a higher level of zinc absorption for pregnancy. Therefore, the IZiNCG SC also felt it was reasonable to apply the same zinc absorption estimates for pregnant as well as non-pregnant women, for each diet type. On the other hand, it does appear that zinc absorption increases significantly during lactation [12, 131–133]. In the study by Fung et al. [12], for example, absolute zinc absorption was increased by 10% among healthy, North American, lactating women who ingested 15 mg zinc per day from a combination of diet and supplements, compared to a non-pregnant, non-lactating, control group. The FNB/IOM committee suggested a level of absorption for lactating women that was 10% greater than that determined for pregnant women above (i.e., 27% + 10%), on the basis of the study by Fung et al. [12]. The study by Moser-Veillon et al. [131] reported a mean zinc intake of 8.0 mg/day by lactating women with 15% higher zinc absorption than measured in non-lactating women, and the study by Sian et al. [133] reported a mean zinc intake of 7.6 mg/day by lactating women, and a 19% greater absolute zinc absorption than determined in a separate study of non-lactating women. As there is insufficient information to determine whether these lactating women were meeting their zinc requirements, it is preferable to assume the figure of 10% increased absorption, as determined by Fung et al. [12]. Therefore, the IZiNCG SC estimated zinc absorption during lactation as 44% (≥ 19 years of age) and 40% (14–18 years of age) for those consuming mixed or refined vegetarian diets and 35% (≥ 19 years of age) and 32% (14–18 years of age) for those consuming unrefined, cereal-based diets.

Recommended derivation of the estimated average requirements

The estimates for absorption can now be applied to the physiologic requirements for absorbed zinc to derive EARs and Recommended Daily Allowances (RDA) for dietary intakes of zinc. The derivation of these Dietary Reference Intakes and their uses are described in the following paragraphs.

Different types of dietary reference intakes are derived depending on whether they are being used to assess the intakes of individuals or populations. Methods for calculating these reference intake values and their uses have been described previously by the FNB/IOM Dietary Reference Intake Committees [11], and the same terminology and methods are applied here. The EAR and the RDA for zinc developed by the IZiNCG SC for the purpose of international application are presented below. The upper limits for zinc intakes will be discussed in section 1.7.

The EAR represents the mean dietary requirement, or the dietary intake level at which 50% of individu-

TABLE 1.9. Revised estimated average requirement (EAR) for zinc by life stage and diet type, as suggested by IZiNCG

Age	Sex	Reference body weight (kg)	Revisions suggested by IZiNCG for EAR for zinc (mg/d)	
			Mixed or refined vegetarian diets	Unrefined, cereal-based diets
6–11 mo	M + F	9	3	4
1–3 yr	M + F	12	2	2
4–8 yr	M + F	21	3	4
9–13 yr	M + F	38	5	7
14–18 yr	M	64	8	11
14–18 yr	F	56	7	9
Pregnancy	F	—	9	12
Lactation	F	—	8	9
≥ 19 yr	M	65	10	15
≥ 19 yr	F	55	6	7
Pregnancy	F	—	8	10
Lactation	F	—	7	8

als would meet their physiologic requirement. The EAR is thus derived by dividing the mean physiologic requirement for absorbed zinc by the estimated average absorption of zinc. For example, the EAR for adult women (55 kg) consuming unrefined, cereal-based diets would be calculated as: 1.86 mg absorbed zinc/day ÷ 0.25 = 7.4 mg zinc/day, and rounded to 7 mg/day. The EAR for all age, sex, and life stage groups is given in table 1.9, for both mixed/refined vegetarian diets and for unrefined, cereal-based diets.

For consideration of breastfeeding infants 6–12 months of age, the FNB/IOM committee assumed that 50% of zinc in breastmilk is available for absorption [134] and that the average breastmilk consumption is 0.76 L/day. The amount of zinc required from complementary foods was then determined by difference. The EAR for breastfed children was calculated as the amount of zinc acquired from breastmilk plus the amount of zinc required from complementary foods, assuming 30% zinc absorption from the complementary diet. The WHO committee assumed that the absorption of zinc from breastmilk was 80%, although this estimate was not based on direct measures of absorption, and estimates of zinc intake from breastmilk in exclusively breastfed infants were derived from a single study of infants 1–3 months of age [135]. The IZiNCG committee used a similar approach as the FNB/IOM committee and also assumed 50% absorption of zinc from breastmilk. However, different estimates for average breastmilk consumption and milk zinc concentrations were used in each age group, as described in section 1.5 (table 1.6). Using these figures, the calculated total zinc requirements are somewhat lower than those that are derived when it is assumed that all dietary zinc is derived from complementary foods. The IZiNCG SC felt that it is unnecessary to provide two different sets of EARs for breastfed or non-breastfed children. Therefore, the slightly higher EARs, calculated assuming that all dietary zinc is derived from non-breastmilk sources, is provided in table 1.9.

The EAR has two primary uses, both of which apply to assessing the adequacy of dietary zinc intake by populations. First, the EAR can be used to evaluate the risk of inadequate intakes by a population by determining the proportion of the population whose intakes fall below the EAR. Second, the EAR may also be used for setting a *recommended mean intake for a population* (considering the observed variation in intakes of the population), such that only a small proportion of the population's intakes fall below the EAR. The application of the EAR in assessment of adequacy of zinc intakes by populations will be discussed in further detail in section 2.3.1, which describes methods to estimate the risk of inadequate zinc intakes.

Recommended derivation of the recommended daily allowances

It is not possible to know the true nutrient requirements of a particular individual, as these vary among individuals. However, when the normal variation of a physiologic nutrient requirement is known, the recommended intake level of that nutrient can be set at two standard deviations (SD) above the EAR. When calculated as such, almost all individuals (97.5%) whose intakes meet or exceed the recommended amount for any given nutrient will theoretically meet their physiologic requirement. This recommended intake level used for the purpose of individual assessments is commonly referred to as the RDA. The FNB/IOM committee has set RDAs for zinc. As for all nutrients for which adequate information on requirement distributions does not exist, zinc requirements were assumed to vary by ± 10% (i.e., the coefficient of variation (CV) of the requirement distribution is 10%) and the RDA was thus set as 120% (mean + 2 SD) of the EAR. The 1996 WHO report did not attempt to estimate the variability in zinc requirements. However, the more recent 2002 report assumed that the CV for zinc requirements was 25%, although the rationale for this assumption can be challenged. This estimate was based on two components. First of all, it was assumed that variation in the physiologic requirement for zinc is similar to that for protein (i.e., 12.5%) because both are related to tissue turnover and growth. An additional 12.5% was then added to account for variation in zinc absorption, resulting in an assumed total variability of ±25%, and the RDA was set at 150% of the EAR. Nevertheless it could be argued that the variation in protein digestibility is incorporated in the figure for variability in protein requirements, which was used as the basis for the estimate of the variability in zinc requirements, so it may not be justifiable to increase the estimate further to account for variation in zinc absorption. For this reason, the IZiNCG SC concluded that an estimate of ±12.5% for the variability in the zinc requirement might be more appropriate, and that is the figure that is adopted for the present report. The assumptions of each respective committee regarding the inter-individual variation in zinc requirements for women are the same as those for men.

The RDA for dietary zinc intakes was thus calculated as the EAR plus two times the CV (2 × 12.5), and is equivalent to 125% of the EAR. The RDAs for dietary zinc intakes derived by the IZiNCG SC are presented in table 1.10 for each sex and life-stage group. It is noteworthy that there is a negligible difference in the resultant RDAs when the CV of the physiologic requirement is assumed to be 10% vs. 12.5%.

TABLE 1.10. Revised recommended dietary allowances (RDAs) for zinc, by life stage and diet type, as suggested by IZiNCG

Age	Sex	Reference body weight (kg)	Revisions suggested by IZiNCG for RDA for zinc (mg/d)	
			Mixed or refined vegetarian diets	Unrefined, cereal-based diets
6–11 mo	M + F	9	4	5
1–3 yr	M + F	12	3	3
4–8 yr	M + F	21	4	5
9–13 yr	M + F	38	6	9
14–18 yr	M	64	10	14
14–18 yr	F	56	9	11
Pregnancy	F	—	11	15
Lactation	F	—	10	11
≥ 19 yr	M	65	13	19
≥ 19 yr	F	55	8	9
Pregnancy	F	—	10	13
Lactation	F	—	9	10

1.7 Zinc toxicity

Individuals may be exposed to high intakes of zinc, either through supplemental zinc or by contact with environmental zinc. Overt toxicity symptoms, such as nausea, vomiting, epigastric pain, diarrhea, lethargy and fatigue, may occur with acute, high zinc intakes [136]. Approximately 225–450 mg zinc is known to produce immediate vomiting in adults. Short-term exposure to very high levels of contaminant zinc (> 300 ppm) from the improper storage of food or beverages in galvanized vessels has caused acute gastroenteritis [136]. After receiving 150 mg zinc/day for six weeks, 26/47 human subjects reported gastrointestinal disturbances (abdominal cramps, nausea, and vomiting) [138].

Chronic overdosage of zinc, in the range of 100–300 mg zinc/day for adults, may induce copper deficiency [139] and alterations in the immune response and serum lipoprotein levels [140]. Some of these disturbances may also occur at lower doses (i.e., 50 mg zinc/day), although the data are conflicting and require confirmation [141–144]. Doses of 25–35 mg zinc/day in adults do not appear to pose a health hazard [145]. Intakes as low as 50 mg supplemental zinc/day affected copper metabolism, as measured by a decrease in erythrocyte copper-zinc SOD activity [144, 146]. However, the clinical significance of the depressed erythrocyte SOD activity is unknown. This same level of intake also resulted in a decline in serum ferritin concentration, which did not occur when 50 mg of iron was included with the daily zinc supplement [144]. Doses between 50 and 160 mg/day lowered the levels of high-density lipoprotein cholesterol in some, but not all studies [11, 147]. Among female subjects in one study who received 100 mg zinc/day, there was a significant reduction in high-density lipoprotein cholesterol levels after four weeks [142]. However, these levels returned to normal after eight weeks, suggesting that this effect may only be transient. A single case of a 13-month old child who received 16 mg zinc/day for six months and 24 mg zinc/day for one month was associated with copper deficiency attributed to excessive zinc intake [148].

The WHO/FAO/IAEA Expert Consultation derived upper limits for zinc intakes [100, 101]. These were based on the observation that 60 mg of supplemental zinc/day resulted in adverse interactions with other nutrients, although the source of these data was not provided, and it was considered that intakes should not exceed this amount. After accounting for a 25% possible variation in population intakes, the upper limit for males was set at 45 mg/day. Due to the limited availability of studies that looked at possible adverse effects of supplemental zinc, the upper limit for adult males was extrapolated to other age and sex groups based on differences in metabolic rates. These upper limits are shown in table 1.11. Although the case report of the 1-year-old child cited above was not included in the derivation of these upper limits, the 16 mg/day intake, after accounting for a 25% possible variation in intakes, is consistent with the 13 mg/day upper limit set by WHO [100].

The FNB/IOM committee also established upper tolerable limits for zinc [11]. This committee also based the upper limits on the studies that measured the effect of supplemental zinc intakes on measures of copper status, including erythrocyte superoxide dismutase (SOD) activity or the concentration of copper or ceruloplasmin in serum, but estimated the upper tolerable limits based on the Lowest Observed Adverse Effect Level (LOAEL) and the No Observed Adverse Effect Level (NOAEL). For adults, a LOAEL of 60 mg zinc/day (50 mg zinc/day from the supplement and an estimated 10 mg/day from the diet) was derived from the results of the study by Yadrick et al [144], as described above, and were supported by the data from Fischer et al. [146]. To take into account intra-individual variation in this response, an uncertainty factor of 1.5 was assumed, and this factor was used to extrapolate the LOAEL (60 mg zinc/day) to the NOAEL (40 mg zinc/day) for both male and female adults. For children, the upper limit was based on the results of just one available study [149]. This study measured serum copper and cholesterol concentrations in newborn infants receiving a formula that provided 5.8 mg zinc/L, compared with a formula with 1.8 mg zinc/L. No changes in measures of copper status were found in either group after 6 months. Based on an estimated consumption of 0.78 L formula/day, the formula with

TABLE 1.11. Upper limits or no observed adverse effects levels (NOAEL) for zinc intake by life stage, as developed by expert committees of the WHO [100, 101], the US FNB/IOM [11], and as reviewed by IZiNCG

WHO		FNB/IOM		Revisions suggested by IZiNCG	
Age/sex	Upper limit (mg/d)	Age/sex	Upper limit (mg/d)	Age/sex	No observed adverse effect level (mg/d)
0–6 mo	—	0–6 mo	4	0–5 mo	—
7–12 mo	13	7–12 mo	5	6–11 mo	6
1–3 yr	23	1–3 yr	7	1–3 yr	8
3–6 yr	23	4–8 yr	12	4–8 yr	14
6–10 yr	28				
10–12 yr, M	34	9–13 yr	23	9–13 yr	26
10–12 yr, F	32				
12–15 yr, M	40				
12–15 yr, F	36				
15–18 yr, M	48	14–18 yr, M	34	14–18 yr, M	44
15–18 yr, F	38	14–18 yr, F	34	14–18 yr, F	39
18–60+ yr, M	45	≥ 19 yr, M	40	≥ 19 yr, M	40[a]
18–60+ yr, F	35	≥ 19 yr, F	40	≥ 19 yr, F	40[a]

a. Represent upper limits.

5.8 mg zinc/L was estimated to provide an average intake of 4.5 mg zinc/day, and this figure was used as the NOAEL for infants 0–6 months of age. This was rounded down to 4 mg zinc/day for the upper limit; an uncertainty factor of 1 was applied. This upper limit was then extrapolated to older children based on differences in reference body weights (section 1.5). The upper limits derived by the FNB/IOM committee are presented in table 1.11.

The IZiNCG SC concurs with the upper limit of 40 mg zinc/day set for adults by the FNB/IOM, as derived from the LOAEL of 60 mg zinc/day; no further data on the effects of supplemental zinc on copper status in adults were found since the publication of the FNB/IOM report. Unfortunately, there is a lack of adequate data to better define the upper limits for children. By definition, the level of zinc intake described by the NOAEL does not exclude the possibility that chronic intakes of higher levels of zinc would also not cause an adverse effect. It is appears likely that the upper limits proposed by the FNB/IOM for young children (< 3 years of age) may be inappropriately low. This presents concern for the development of interventions to improve zinc intakes among this age group, because the margin between the RDA for zinc and the upper limit is rather narrow (only 0–5 mg zinc/day, depending on age and diet type). Further, it is apparent that a large proportion of US children have usual zinc intakes greater than the IOM/FNB upper limits. For example, the median intake of zinc by presumably healthy US infants 2–11 months of age from the diet is 5.5 mg/day (with or without consideration of supplement use) (NHANES III [150], whereas the upper limit for zinc for children in this age range set by FNB/IOM was 5 mg/day. The median zinc intake by children 1–3 years of age in the United States is 6.3 mg/day from the diet alone, and 6.4 mg/day when zinc supplements are also included, and the FNB/IOM upper limit for zinc for this age group is 7 mg/day. Although the proportion of children with intakes above the upper limit was not reported, it is likely that about half of children 2–11 months of age exceeded the upper limit of 5 mg/day and many children 1–3 years old would have exceeded the upper limit of 7 mg zinc/day. Given the unlikelihood that the described toxic effects of excessive zinc intakes occur in such a large proportion of children from this relatively healthy, US population, the degree of confidence in the upper limit is relatively low. Therefore, the IZiNCG SC is unsatisfied with the upper limits presented by the FNB/IOM for children, as these may have important implications on recommendations for the design of intervention strategies to improve zinc status among young children, particularly where supplements are used (section 3.1.2). For the reasons described above, IZiNCG will report only a NOAEL for children to indicate that insufficient data exists to set an upper limit with confidence.

Results from two community-based zinc supplementation studies in children have become available recently, and the IZiNCG SC felt it was important to take these into consideration in the calculation of the NOAEL. One study was conducted in India among children 6–30 months of age [151]. For a period of

4 months, children between 6 and 12 months of age received 10 mg zinc/day and those 1–2.5 years of age received 20 mg zinc/day. Plasma copper concentration was reported to be lower in the group that received supplemental zinc compared to the placebo group. The results for plasma copper were not presented according to age group, however, so it cannot be distinguished as to what dosage level and which age group was associated with changes in plasma copper concentration. The second study was conducted in Indonesia among children 6 months of age [152], who were provided with either 10 mg zinc or a placebo for 6 months. In this study, plasma copper concentration did not differ between the zinc supplemented group and the control group at the end of the supplementation period. The results from the Indonesian study may thus be used to set the NOAEL and derive upper limits for infants and older children. Considering unconsumed portions of the supplement monitored in the Indonesian study, the estimated intake of zinc from the supplement was 8.2 mg/day. However, as information was not available on the usual dietary zinc intake by the infants in the Indonesian study, the total zinc intake applied to the NOAEL will be somewhat underestimated. On the other hand, it was noted that the zinc supplement was provided apart from meals, therefore possibly avoiding direct interference between the supplemental zinc and the absorption of dietary copper or endogenous copper secreted in the intestine postprandially. Given that some further interference of the supplement with copper absorption may occur if supplements are consumed with a meal, an uncertainty factor of 1.5 was applied. Based on the average zinc intake from the supplement of 8.2 mg/day, and the mean body weight between baseline and the end of the study of 7.9 kg, the zinc intake was equivalent to 1.0 mg/kg body weight/day, or 0.7 mg/kg/d when considering the uncertainty factor of 1.5. This figure is then applied to the reference body weights for children to derive the NOAEL (table 1.11).

Reporting of the NOAELs for children does not preclude studies of possible adverse effects of higher intakes of zinc, with the caveat that appropriate monitoring is included. Indeed, further prospective studies of the possible adverse effects of varying levels of supplemental zinc are urgently required to improve the derivation of upper limits for total zinc intakes, particularly among children. Several issues, however, must be considered and controlled for in the design of such studies to facilitate the interpretation of results, including: (1) the proportion of zinc acquired from the diet versus zinc derived from pharmacologic supplements; (2) the comparative effects of zinc on copper status when supplemental zinc is taken with copper-containing meals, or between meals; (3) if supplemental zinc is taken with meals, the estimated bioavailability of zinc based on the phytate:zinc molar ratio of the usual diet of the subjects involved; and (4) the baseline copper status and copper intake of individuals and the possible influence of infections on biochemical indicators of copper status.

1.8 Causes of zinc deficiency and groups at high risk

Development of zinc deficiency can be attributed to at least five general causes occurring either in isolation or in combination. These include inadequate intake, increased requirements, malabsorption, increased losses, and impaired utilization [153]. The conditions or circumstances underlying these mechanisms are described below.

Inadequate dietary intake of absorbable zinc is likely to be the primary cause of zinc deficiency in most situations. This may result from a combination of low total dietary intake, heavy reliance on foods with a low zinc content and/or with zinc that is poorly absorbable. Several estimates of dietary zinc intakes indicate that inadequacy of intakes is widespread, occurring across a wide variety of geographical areas and dietary patterns [154, 155]. Low intakes of absorbable zinc are further exacerbated by physiologic or pathological conditions that lead to greater requirements for zinc (per kg body weight). The physiologic and pathologic conditions associated with elevated zinc requirements place individuals in these subgroups at an increased risk of zinc deficiency; these subgroups are described in further detail below.

Malabsorption of zinc may result from a number of different conditions. For example, acrodermatitis enteropathica is a rare genetic defect that specifically affects zinc absorption (section 1.4). Certain disease states, such as malabsorption syndromes and inflammatory diseases of the bowel, may result in poor absorption and/or losses of zinc from the body. Hence, these conditions may precipitate secondary zinc deficiency states, particularly in the presence of marginal dietary zinc intakes [156]. Certain drugs, such as phenytoin and tetracycline, are also noted to reduce the absorption of zinc [157]. Several studies suggest that zinc absorption is antagonized by pharmacologic doses of iron which would result from competitive interaction between these elements [12, 158–160].

Impaired utilization of zinc may occur as a result of administration of certain drugs (e.g., ethambutol, halogenated 8-hydroxyquinolines, penacillamine) that chelate zinc systemically and make it less available for use by tissues [156]. Presence of infection in general results in sequestration of zinc in the liver [161], and decreased circulating levels of zinc, which will reduce the availability of zinc to other tissues. In response to infection-induced secretion of cytokines, such as interleukin-1 and tumor necrosis factor–alpha,

by monocytes and activated macrophages, there is increased hepatic synthesis of metallothionein (MT), an intracellular metal-binding protein [162], and subsequently increased hepatic uptake of zinc and reduction in serum zinc concentration. It is not known whether these alterations in zinc metabolism may benefit the host by making more zinc available for particular processes in the liver or by reducing zinc availability in the peripheral blood.

Certain disease states or conditions that result in increased losses of endogenous zinc from the body include chronic renal disease, trauma, prolonged bed rest, and other conditions associated with bone or muscle atrophy. As the secretion and re-absorption of endogenous zinc in the intestine are key mechanisms in maintaining zinc homeostasis, conditions that perturb intestinal function or the integrity of the intestinal mucosa may have profound effects on the body's ability to maintain zinc status. For example, endogenous zinc losses are increased in infants with cystic fibrosis [163], and fecal zinc excretion is elevated during acute diarrhea [164]. However, it is unclear to what extent the increased fecal zinc represents unabsorbed dietary zinc or zinc of endogenous origin. Because diarrheal disease is a common infection in many lower-income countries, the possible effects of diarrhea on endogenous zinc depletion merit further study and quantification. Not only does zinc deficiency appear to augment the susceptibility to, and severity of, childhood diarrhea, but increased losses of endogenous zinc that occur during diarrhea may further deplete body zinc and propagate a cycle of diarrhea and further zinc depletion. Two studies of zinc homeostasis in Malawian children have demonstrated unusually high intestinal losses of endogenous zinc, even among apparently healthy children [165, 166]. While the causes of these increased losses were not identified, it was speculated that this may be attributed in part to poor intestinal health and may parallel the occurrence of poor intestinal permeability, which is consistently observed in lower-income populations [167], including infants [168]. Further measures of intestinal losses of endogenous zinc among populations living in areas with high exposure to environmental pathogens are needed to determine whether this phenomenon is widespread and to what extent it may contribute to zinc deficiency.

Population subgroups at increased risk of zinc deficiency

Population subgroups with particularly high risks of zinc deficiency can be identified on the basis of their age and physiologic status or the presence of particular pathologic conditions, as described in the following sections.

Infants and young children

Theoretical estimates of zinc requirements suggest that exclusively breastfed infants of mothers with adequate zinc nutriture can satisfy their zinc requirements for the first 5–6 months of life [120]. This is well supported by experimental evidence [169–172]. However, after approximately six months of age, it is unlikely that breastmilk alone can supply sufficient zinc to meet infants' needs [120, 173]. Therefore, if the introduction of complementary foods to breastfed infants is delayed until after six months of age, or if the complementary foods introduced contain inadequate amounts of absorbable zinc, infants will be at increased risk of zinc deficiency. In many lower-income countries, cereals or starchy roots or tubers are used as the basis for complementary foods and these foods often have a low content of total or absorbable zinc. Thus, the complementary diet fails to meet the estimated needs for zinc [174].

Conversely, the premature introduction of other food sources will reduce net zinc absorption if these foods displace breastmilk, have a lower concentration of absorbable zinc than breastmilk, and/or contain substances like phytate, which may interfere with absorption of zinc from breastmilk [175]. Notably, one zinc supplementation trial of non-exclusively breastfed infants of African immigrants to France found that those infants who received supplemental zinc for 3 months beginning at 4–9 months of age had increased weight gain and linear growth, possibly because the foods that had been introduced in addition to breastmilk had an adverse effect on total zinc intake and/or absorption [119]. This combined set of results suggests that premature introduction of complementary foods may impose an increased risk of poor zinc status in early infancy.

Adolescents

Physiologic requirements for zinc peak during adolescence at the time of the pubertal growth spurt, which generally occurs in girls between 10–15 years, and in boys between 12–15 years. Even when the growth spurt has ceased, adolescents may require additional zinc to replete tissue zinc pools depleted during puberty [176].

Pregnant and lactating women

Increased nutritional demands during pregnancy and lactation predispose women to developing zinc deficiency. These demands are greater for lactation than for pregnancy, although physiologic adjustments in zinc absorption help to meet the needs for lactation [72]. Smoking and alcohol abuse during pregnancy may also reduce the amount of zinc available for fetal development by compromising blood flow, and therefore transfer of zinc, to the placenta. As noted above, several studies have indicated that

iron supplements reduce the absorption of zinc [12, 158–160]. All of these studies were conducted in pregnant and/or lactating women and included either prophylactic or therapeutic doses of iron ranging from 60 to > 200 mg/day. Where dietary intakes of zinc are low, supplemental iron, in dosages as low as 60 mg/day, may prevent women from meeting their increased needs for zinc during pregnancy and lactation [160].

Elderly

Dietary surveys indicate that zinc intakes in the elderly, even in higher-income countries, are often inadequate [177]. Zinc deficiency among the elderly has been reported in various countries, and senior citizens living in nursing homes appear to be at increased risk [178]. A number of factors may contribute to the risk of poor zinc nutrition among the elderly, including reductions in total food intake due to reduced mobility, decreased energy needs, and possibly depression, and low intakes of zinc-rich foods, such as meat, poultry, or fish due to poverty or physical disabilities (e.g., swallowing and dental problems). Low zinc intakes may be compounded if efficiency of zinc absorption decreases with age, as has been suggested by some [179– 181], but not all [182], investigators.

Low-birthweight infants

Low-birthweight infants have a reduced size at birth, and hence a smaller content of hepatic zinc metallothionein, which reportedly acts as a zinc reserve in young infants [117]. For low-birthweight infants born prematurely, their body zinc content at birth will be further compromised because more than two-thirds of the zinc is transferred during the last trimester of pregnancy [183]. Moreover, preterm infants may have reduced absorption because of their immature gastrointestinal tract. These impairments result in elevated zinc requirements during the neonatal period, although specific requirements for these infants have not been established.

Malnourished infants and children

The dietary requirements for zinc in malnourished children are estimated to be between 2 and 4 mg/kg/day, depending on the volume of food intake and rate of growth [184]. These zinc requirements are markedly higher than those estimated for healthy children (e.g., 0.17 mg zinc/kg/day for children 1–3 years of age; table 1.9), presumably due to prior zinc depletion, the need for zinc for tissue synthesis, problems of malabsorption due to changes in the intestinal tract, and possibly increased losses due to diarrhea.

1.9 Summary

Due to the multiple biologic functions of zinc and its ubiquitous distribution in human tissues, there is a broad range of physiologic signs of zinc deficiency, which may vary depending on the affected individuals' sex and stage of the lifecycle. The functional effects of zinc deficiency have been determined primarily through community-based zinc supplementation trials and clinical studies of individuals with acrodermatitis enteropathica, children with severe malnutrition, and the elderly. The adverse consequences of zinc deficiency include the following: (1) impaired immunocompetence and increased prevalence and incidence of childhood infections, such as diarrhea and pneumonia, which may result in increased rates of mortality; (2) impaired growth and development of infants, children and adolescents; and (3) impaired maternal health and pregnancy outcomes. These complications of zinc deficiency may be better defined when the specific biochemical mechanisms that link zinc status to these outcomes are elucidated.

Although other factors may contribute to the development of zinc deficiency, inadequate dietary intake of absorbable zinc is likely to be the most common cause. The adequacy of zinc intake is affected by the presence of dietary factors that inhibit zinc absorption, primarily the phytate:zinc molar ratio. Diets based largely on unprocessed cereals or tubers and negligible amounts of animal source foods increase the dietary requirements for zinc, and therefore heighten the challenge of acquiring an adequate amount of zinc from the diet. It is also recognized that zinc deficiency in many populations may be attributable to underlying social and economic problems, such as poverty, poor quality food supply, lack of nutrition education, and elevated exposure to pathogens because of poor environmental sanitation and/or personal hygiene. The identification of high-risk groups within populations on the basis of socio-demographic variables is covered in section 2.5.

Groups at increased risk of zinc deficiency include those with high requirements for zinc and those for whom other factors make it difficult to acquire diets with adequate zinc content. These high-risk groups include pre-term infants, small-for-gestational-age term infants, young children after the period of exclusive breastfeeding, children presenting with and recovering from malnutrition, adolescents, pregnant and lactating women, and the elderly. Based on the large body of evidence for positive effects of supplemental zinc on multiple outcomes of concern to public health, it is evident that similar benefits would be realized in programs designed to improve zinc intakes among those at high risk for zinc deficiency. Identification of nutritional zinc deficiency and its specific causes has therefore become a growing concern for public health planners. Methods to estimate the risk of zinc deficiency in populations and strategies for improving zinc status are considered in the following chapters.

There are still a number of zinc-related issues for which additional research is needed: (1) the full range of functional consequences of zinc deficiency, (2) zinc requirements and safe upper limits of zinc intake, and (3) zinc absorption from mixed diets. New information on the functional consequences of zinc deficiency would be useful to motivate greater interest in the likely benefits of zinc intervention programs and to define the full range of conditions for which such interventions might be helpful. Additional research on zinc requirements is needed to provide relevant information for different population subgroups, as defined by age, sex, and physiologic status. For example, more information is needed on the quantitative losses of endogenous zinc from different sites to define physiologic requirements more precisely for these subgroups. In addition, research is needed on exogenous factors, such as infections or pre-existing malnutrition, that might modify these estimates of physiologic requirements. Studies are also needed to define more precisely excessive levels of zinc intake. Finally, additional studies are needed on zinc absorption from a broad range of mixed diets with varying levels of factors that are known or believed to modify zinc absorption (e.g., levels of zinc, phytate, protein from different sources, and calcium and other minerals), and on the effects of commonly occurring diseases, such as tropical enteropathy, acute and persistent diarrhea, and intestinal helminthic infections, on zinc absorption. A more detailed discussion of these research priorities is presented in chapter 4.

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Chapter 2

Assessment of the Risk of Zinc Deficiency in Populations

2.1 Objectives of assessment

Assessing the nutritional status of a population is critical in developing nutrition intervention programs that enhance human health and well-being. The results of nutritional assessment efforts are necessary both to determine the presence and magnitude of particular nutrition problems and, when indicated by the results, to elicit public interest and garner resources for action. Assessment data are used to determine the level of risk of deficiency in the general population and thereby indicate the probable consequences for human health and productivity. Information derived from assessments is also used to identify specific segments of the population at elevated risk so that interventions may be targeted to those in greatest need, or to determine whether population-wide interventions are indicated. Assessments can also be used to monitor changes in nutritional conditions over time, thereby permitting decisions on the effectiveness of intervention programs and need for their continuation.

The following paragraphs review some general aspects of nutritional assessment. Available methods for the specific assessment of zinc status in individuals and populations are described in the subsequent sections.

Identifying high-risk groups

A major objective of nutritional assessment efforts is to identify high-risk groups who might be targeted by nutrition (and possibly other health) intervention programs. Targeting enables limited resources to be used most efficiently to reach those in need and to protect those who do not require the program from any possible adverse effects of the intervention. Risk groups can be defined in terms of almost any easily identifiable descriptor, which might include the following:

» Physiologic status, as defined by age group, phase of reproductive cycle (e.g., pregnant or lactating), or presence of illness (e.g., presence of persistent diarrhea, HIV infection);
» Political or geographic region of inhabitance (e.g., regions, districts, urban vs. rural, coastal vs. inland);
» Socioeconomic status (e.g., level of maternal education, income, employment, or access to health, water and sanitation services).

Information on the population's zinc status, or overall risk of zinc deficiency, should be disaggregated according to some of these possible risk factors, as appropriate, such that sub-populations at elevated risk may be identified. Physiologic factors associated with an increased risk of zinc deficiency are described in section 1.8 in chapter 1. As with most other nutrient deficiencies, the groups that appear to be most commonly affected by zinc deficiency are infants and young children, adolescents, women during pregnancy, and the elderly. Socioeconomic factors that may also be used to identify at-risk populations or sub-populations are described in section 2.5.

Applications for program evaluation

Nutritional assessments should be repeated periodically to determine changes in the population's status over time. Ideally, the same techniques that are used to examine the nutritional condition at baseline should be used consistently during follow up to facilitate interpretation of any differences that are encountered. Other indicators of environmental or economic conditions should be included in the assessment to determine whether changes in nutritional status likely occurred due to elimination of the underlying ecologic or socioeconomic causes of the problem or due to success of the program itself. Specific methods for the monitoring and evaluation of programs and possible indicators are provided in section 3.6 in chapter 3.

Importance of assessing both nutrition deficiency and excess

Nutritional abnormalities can be defined in terms of deficiency, excess, or imbalance of particular nutrients

or foods. Although nutrition interventions should be designed appropriately to provide the most favorable ratio of benefits to risks, even under the best of circumstances these programs may not be entirely free from possible adverse consequences. Thus, it is prudent to include an assessment of possible undesirable outcomes of these interventions as well as their positive impacts. In the case of zinc, excessive intake may result in abnormalities of copper, iron, and/or lipoprotein metabolism. It may not be feasible to include biochemical indices of copper status and lipoprotein profiles in large surveys. However, it is advisable to include these assessments in efficacy trials of zinc interventions programs to identify any possible adverse effects in the population of interest. Information on evaluating the risk of excessive zinc intake is given in section 2.6.

Individual versus population assessment

Nutritional assessment may focus on individuals or populations. A population is defined as any group of individuals who share a common trait, often nationality. Whereas assessment of individuals leads to case-specific treatment or counseling, assessment of populations is used to plan and evaluate population-based interventions. Thus, it is not critical for the population assessment techniques to provide certainty with regard to any particular individual's true status. This is an important distinction with regard to assessing zinc status, because while available techniques may misclassify some individuals, they may be appropriate for detecting populations at high risk of deficiency.

Assessment of zinc status in individuals will find application most often in clinical settings among those seeking medical attention for a health condition. In the context of lower-income countries, it is likely that diagnosis of isolated zinc deficiency will be rare, but rather will be found in association with a variety of health conditions for which primary treatment is being sought. For example, children presenting with severe malnutrition, diarrheal infections, or respiratory illnesses may be zinc depleted, in which case usual treatment strategies should ensure correction of the zinc deficiency state.

Population assessment is applied to a sample of the population of interest. This sample may be chosen in a number of different ways, but the sampling technique must select representative members of the whole population. Ideally, the assessment procedures used in population surveys should be simple, low-cost, and rapid, and any necessary equipment must be easily transportable. The primary goal of population-level assessment of zinc status is to characterize the degree of risk of deficiency in the population and the urgency with which the situation needs to be addressed, if at all.

Available methods for assessing risk of zinc deficiency in populations

As with other nutrients, a number of general techniques can be used to estimate the risk of zinc deficiency in individuals or in populations. These are categorized as the following:

1. The presence or prevalence of clinical outcomes of zinc deficiency (e.g., stunting, diarrhea), or other ecologic factors associated with risk of zinc deficiency or risk of inadequate zinc intakes;
2. Assessment of the adequacy of dietary zinc intakes in relation to theoretical requirements for absorbed zinc;
3. Biochemical measures of zinc concentration, activity of zinc-dependent enzymes, or other zinc-responsive biocomponents in biologic fluids or tissues, assessed in comparison to reference values or established cutoffs;
4. Measurement of functional responses following the intake of adequate supplemental zinc.

The selection of which risk indicators to use will depend on the specific objectives of the assessment and available resources. When applied, these indicators should be assessed in a representative sample of the target population. Ideally, several of these measures should be considered in combination, or at different stages of the assessment. The following sections provide details of the range of assessment methods currently available and their application and interpretation. The focus of these sections is the assessment of the risk of zinc deficiency in *populations*; applicability of these methods for assessments in individuals will be mentioned where appropriate. The assessment methods will be classified into two categories: (1) those that provide suggestive evidence of the risk of zinc deficiency based on existing data collected for other purposes (section 2.2); and (2) those that are applied specifically to estimate the risk of zinc deficiency in a population (section 2.3).

2.2 Suggestive evidence for the risk of zinc deficiency in populations

Certain health or ecologic conditions may be associated with, although not necessarily specific to, zinc deficiency. Nevertheless, these conditions may provide useful suggestive evidence that a population is at risk of zinc deficiency. For example, stunting (low height-for-age) among preschool children is a common clinical manifestation of zinc deficiency. Although other nutritional or environmental factors can also cause stunting, an elevated prevalence of this condition may be used as suggestive evidence of zinc deficiency in a population. Another kind of suggestive information is provided by national food balance sheets, which can

be used to assess the adequacy of zinc in national food supplies and to estimate the risk of inadequate zinc intake at the national level. Finally, the prevalence of iron-deficiency anemia is another type of suggestive information on the risk of zinc deficiency. Although iron deficiency does not cause zinc deficiency, both the distribution of iron and zinc in the food supply, and the dietary components that modify their absorption, are similar, suggesting a comparable risk for deficiency. Therefore rates of iron-deficiency anemia may be used as suggestive evidence of the risk of zinc deficiency.

A notable advantage of these proposed supporting data is that they may be compiled from existing sources of information and therefore be used as preliminary evidence for the likely presence of zinc deficiency in a given population. It must be recognized that these data are limited in that they cannot provide reliable estimates of the true proportion of the population at risk of zinc deficiency. In some cases, for example, the data may be available only at the national level or they may be representative of a selected sub-population group, such as children under five years of age. *Nonetheless, because these data are readily available for most countries, they can be used immediately by decision makers as a first step to assess the expected risk of zinc deficiency in the population and the degree of urgency with which to conduct more specific population assessments*. The rationale for use of these data and their application in assessing the risk of zinc deficiency in a population are described below.

2.2.1 Rates of stunting

It has been well established, both by studies in experimental animals and by human intervention trials, that zinc deficiency is growth-limiting. In a recent meta-analysis, the results of more than thirty community-based intervention trials completed in different parts of the world were examined to determine the overall magnitude of growth responses to zinc supplementation [1]. Notably, the responses to zinc supplementation were significantly greater in those studies that enrolled subjects with pre-existing nutritional stunting or underweight, defined respectively as height-for-age or weight-for-age Z-scores < -2 in relation to international reference data. By contrast, there were no significant effects of zinc supplementation in those studies that enrolled mostly children who were non-stunted and/or had adequate weight-for-age. These results indicate that children with low height-for-age or weight-for-age are likely to be zinc deficient, and they further suggest that the national prevalence of stunting or underweight among children under 5 years of age can be used as indirect indicators of a population's risk of zinc deficiency.

The aforementioned meta-analysis found no effect of zinc supplementation on weight-for-height indices, suggesting that zinc mostly affects linear growth. Thus, the rate of stunting, or low height-for-age, is probably the best anthropometric indicator of risk of zinc deficiency. The WHO considers national stunting rates of ≥ 20% to be a level of public health concern [2]. The same cutoff can be applied to indicate when there may be a substantial risk of zinc deficiency, in which case further assessment of zinc status should be considered. Data on the prevalence of stunting are collected routinely in many countries and are compiled in the WHO Global Data Base on Child Growth and Malnutrition [3]. Updated information is available on the Internet (http://www.who.int/nutgrowthdb/). Thus, countries can use this existing information to assess the likelihood that zinc deficiency is a local problem. The following map, reproduced from the WHO data (figure 2.1), indicates those countries where the prevalence of childhood stunting exceeds 20%, and specific data can be found in appendix 1.

2.2.2 Adequacy of zinc in the national food supply

Studies of dietary intake can be used to estimate the risk of inadequate zinc intake in a population. However, at present, information on zinc consumption has been obtained from representative samples of the national populations of very few lower-income countries. In lieu of available information on dietary zinc intake, a simple alternative method, based on the zinc content of the national food supply in relation to the population's theoretical zinc needs, can be applied to estimate the risk of inadequate zinc intake. The proposed method is described in this section.

Each year the Food and Agriculture Organization (FAO) of the United Nations publishes national food balance sheets (FBS), which currently provide data on the amounts of 95 food commodities available for human consumption in 176 countries. Despite the inherent weaknesses of this type of aggregated, national-level information, the FBS do provide reasonably accurate, frequently updated information that, with appropriate interpretation and considerable caution, can be used as an ecological indicator of the risk of inadequate zinc intake in the population. The general approach for estimating the amount of absorbable zinc in national food supplies and judging its adequacy has been described in detail elsewhere [4]. This earlier analysis has been updated for the present document to include more recent information from FBS for the period 1992–2000 and to account for the current IZiNCG revised estimates of the average physiologic requirements for absorbed zinc and the level of zinc absorption expected from different types of diets.

For these analyses, the amounts of each of the food commodities reported in the national FBS were multiplied by their zinc and phytate contents to determine the amounts of these food components that are avail-

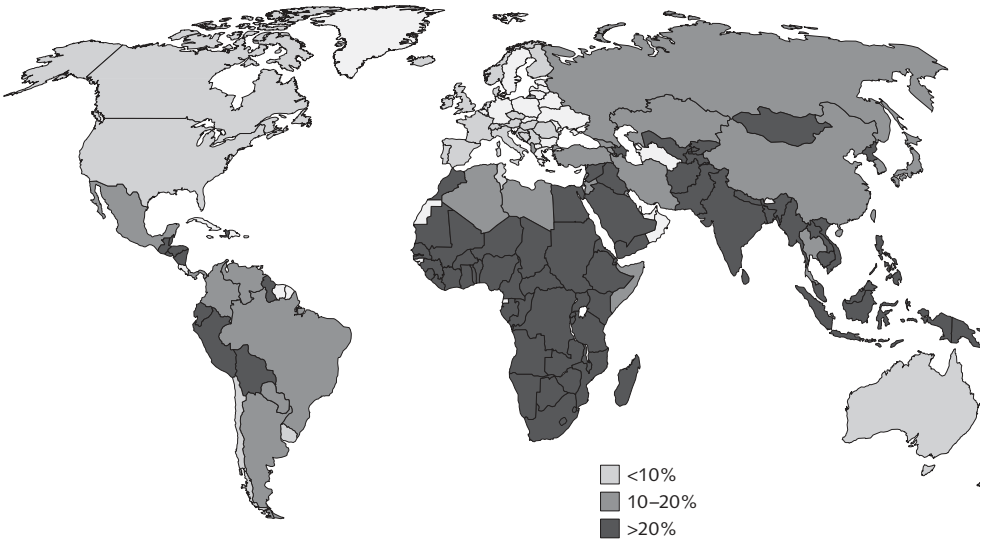

FIG. 2.1. Estimated prevalence of stunting (< –2 Z-score) among children under 5 years of age, by nation; adapted from the WHO Global Data Base on Child Growth and Malnutrition [3]

able for human consumption in each country. The amount of zinc that might be absorbed from these foods was then calculated, by using the amounts of zinc and phytate to estimate the country-specific mean fractional absorption of zinc, using the equation presented in section 1.6. The estimated mean daily per capita amount of absorbable zinc in the food supply was then compared with the mean physiologic requirement for absorbed zinc for the population (table 1.9), after weighting these theoretical requirements for the population's age and sex distribution. Finally, the proportion of the population at risk of inadequate zinc intake was estimated by calculating the percent of individuals whose intake of absorbable zinc is likely to provide less than their physiologic requirement, assuming the following: (1) that the mean intake of absorbable zinc in each country is the same as the mean daily per capita absorbable zinc content of the food supply; (2) that the habitual intake of absorbable zinc is normally distributed; and (3) that there is a 25% inter-individual coefficient of variation in intake [5]. This method is akin to the EAR cut-point method for estimating the adequacy of nutrient intakes in a population, as described in the recent FNB/IOM publication on dietary assessment [6]. The assumptions necessary to assure the validity of this method to estimate inadequate intakes in a population are described in further detail in section 2.3.1.

It is important to recognize that the accuracy of this approach is undermined to some extent by the lack of well-founded, quantitative information on the food processing techniques employed in different countries. For example, the extent of milling and fermentation of cereal staples markedly changes their zinc and phytate contents, and hence the estimates of the absorbable zinc contents of these foods. Thus, several additional sets of assumptions had to be applied for the present analyses. In particular, with regard to wheat, the following were assumed: (1) that 90% of the wheat in the regions of North Africa and the eastern Mediterranean and South Asia is consumed as whole wheat and 10% is consumed as 75%-extraction white flour; (2) that 10% of the wheat in Latin America and the Caribbean and in sub-Saharan Africa is consumed as whole wheat and the rest is consumed as white flour; and (3) that in all other countries 1% of the wheat is consumed as whole wheat and the remainder as white flour. In all cases, it was assumed that whole-grain wheat is not fermented and 58.5% of the white flour is fermented with yeast, as is the practice in the United States [7]. In the case of maize, the following were assumed: (1) in Central America all available maize is processed into tortillas; (2) in West Africa all available maize is fermented; and (3) in all other countries maize is consumed as unfermented, unrefined maize. Finally, it was assumed that all rice is consumed as unfermented, milled, white rice and all other cereals are consumed as unfermented, whole-grain products. These assumptions are based on just a limited number of consultations with experts on national food supplies, so if more accurate information on food processing is available at the country level, the information presented herein should be revised accordingly. In a paper to be published separately, we examine in greater detail how modifying the assumptions regarding food processing techniques might affect the estimated percent of individuals at risk of inadequate zinc intake. Briefly, these estimates are affected to only a modest degree in all regions except the eastern Mediterranean, where the predicted percent of the population at risk of inadequate zinc

intake might range from 7% to 15%, and South Asia, where the predicted values might range from 24% to 34%, depending on the assumptions that are applied.

Table 2.1 displays regional data on the mean daily per capita availability of the following items in the food supply of 176 countries: total energy (kcal/day), percent of energy from animal-source foods, total zinc (mg/day), zinc density (mg/1000 kcal), percent of zinc from animal source foods, total phytate (mg/day), phytate: zinc molar ratio, estimated fractional absorption of zinc (percent of the mean daily per capita amount of available zinc), estimated absorbable zinc (i.e., the mean daily per capita total available zinc multiplied by the estimated fractional absorption of zinc, in mg/day), absorbable zinc as a percentage of the weighted daily mean population zinc requirement, and the estimated percent of the population at risk of inadequate zinc intake. The regions are ranked in descending order by the amount of absorbable zinc in the national food supplies.

The mean daily per capita amount of total zinc in the national food supply is about 15 mg/day in North Africa and the eastern Mediterranean, where nearly 50% of the food energy is derived from wheat (presumably, mostly whole-wheat) products, 11–12 mg/day in the countries of Europe, North America, China, and the western Pacific, and about 9–10 mg/day in the countries of Latin America and the Caribbean, South and Southeast Asia, and sub-Saharan Africa. The total zinc content of national food supplies is strongly associated with total energy content and percent of energy provided by animal source foods (data not shown). Notably, in the wealthier countries more than half the zinc is provided by animal source foods compared with just 15–25% in the poorer ones, leading to sizeable differences in the phytate:zinc molar ratios among regions and approximately 50% differences in the estimated amount of zinc that is likely to be absorbed from the available foods (table 2.1). Using the assumptions described above, it appears that fewer than 10% of the population of the Western Europe, North American, and North African/eastern Mediterranean regions is at risk of inadequate zinc intake compared with more than 25% of the population in Latin America and the Caribbean, South and Southeast Asia, and sub-Saharan Africa. Additional country-specific information on the absorbable zinc content of national food supplies is available in appendix 1.

Several concerns must be highlighted with regard to the interpretation of this information. First of all, the data base that was used to estimate the zinc and phytate contents of national food supplies contains information on a fairly limited number of foods, so in some cases information had to be imputed for the full range of commodities reported in the FBS. Secondly, national FBS provide information on food availability, not food consumption, so the actual within-country distributions of food intake, and hence zinc consumption, are uncertain. The assumption of 25% inter-individual variation in zinc intake that was applied is based on a single national nutrition survey conducted in the United Kingdom [8] and may vary across countries and age groups. Finally, the estimates of zinc absorption do not account for these inter-individual differences in intake, and they rely on additional assumptions regarding the types of food processing that are employed in different countries. For all these reasons, the estimates of the absolute percent of individuals at risk of inadequate zinc intake must be interpreted with a great deal of caution. Nevertheless, the rank order of risk level in different countries and regions should be reasonably reliable, so these results can be used to identify those settings where there is a greater likelihood of inadequate zinc intakes.

2.2.3 Rates of anemia

Although anemia may result from a variety of factors, about half of all cases are believed to be attributable to iron deficiency [9]. Iron and zinc have a similar distribution in the food supply, and some of the same food components similarly affect the absorption of both minerals. The richest sources of iron and zinc are meat and other animal flesh foods, and both are found in moderate concentrations in cereal grains, which have much lower proportions available for absorption. As the nutritional causes of iron deficiency and zinc deficiency are similar, high rates of iron-deficiency anemia may be used as suggestive evidence of the risk of zinc deficiency.

There are several data sets that demonstrate a positive correlation between anemia and indicators of the risk of zinc deficiency. For example, the prevalence of anemia is inversely correlated with the amount of absorbable zinc in national food supplies ($r = -0.47$, $p < 0.01$; Wuehler et al, unpublished data). In a study of New Zealand women ($n = 238$), serum zinc concentrations were modestly but significantly positively correlated with hemoglobin ($r = 0.182$; $p = 0.002$), and serum ferritin ($r = 0.10$; $p = 0.05$) among those not using oral contraceptive agents.* Significant correlations between serum zinc and hemoglobin ($r = 0.291$; $p < 0.05$) have also been noted in Italian adults [10]. In a study of pregnant Filipino women, hemoglobin and serum zinc were significantly correlated at 24 weeks ($r = 0.22$; $p < 0.001$), but not at 36 weeks of gestation [11]. Results from the recent National Nutrition Survey in Mexico [12] also demonstrated a significant correlation between hemoglobin and serum zinc concentration among women ($r = 0.221$; $p < 0.001$), and school-aged children ($r = 0.090$; $p < 0.05$), although not among preschool children ($r = -0.025$; $p > 0.05$). A lack of

* RS Gibson, personal communication.

TABLE 2.1. Daily mean amounts of selected nutrients and food components in the national food supplies of 176 countries, by region (mean ± SD)[a,b] (See text for a detailed description of the analytic approach)

Variable	W. Europe	USA & Canada	E. Europe	N. Africa & E. Medit.	China, (+Hong Kong)	W. Pacific	Latin America & Carib.	South Asia	Southeast Asia	Sub-Saharan Africa	Total
Number of countries	20	2	27	17	1	12	35	6	10	46	176
Population (millions)	457	305	413	342	1,256	223	498	1,297	504	581	5875
Energy (kcals)	3,411 ± 129	3,602 ± 166	2,916 ± 277	2,796 ± 455	2,918	2,806 ± 267	2,781 ± 317	2,381 ± 106	2,626 ± 260	2,212 ± 411	2,755 ± 443
% of energy from animal sources	28.4 ± 8.0	27.9 ± 0.3	23.7 ± 4.4	9.9 ± 4.2	16.5	19.6 ± 7.4	18.2 ± 4.9	8.2 ± 3.5	8.4 ± 4.6	6.6 ± 5.5	14.9 ± 8.4
Zinc (mg)	12.4 ± 1.4	12.5 ± 0.5	10.6 ± 1.6	15.4 ± 2.2	12.4	11.3 ± 0.9	10.3 ± 2.0	10.8 ± 1.3	9.2 ± 0.9	9.4 ± 2.2	11.3 ± 2.1
Zinc per 1000 kcals (mg)	3.6 ± 0.4	3.5 ± 0.0	3.6 ± 0.3	5.5 ± 0.4	4.3	4.0 ± 0.3	3.7 ± 0.4	4.5 ± 0.4	3.5 ± 0.1	4.3 ± 0.8	4.1 ± 0.6
% of zinc from animal sources	56.2 ± 12.2	60.8 ± 1.6	49.6 ± 6.0	15.0 ± 6.8	37.2	39.4 ± 14.5	41.9 ± 11.5	10.9 ± 3.3	21.2 ± 11.2	15.1 ± 10.2	30.6 ± 18.4
Phytate (mg)	1,282 ± 271	1,338 ± 51	1,215 ± 292	3,877 ± 706	2,056	1,852 ± 482	2,030 ± 794	2,827 ± 265	2,231 ± 618	2,469 ± 668	2,221 ± 797
Phytate:zinc ratio	10.6 ± 3.4	10.6 ± 0.0	11.4 ± 2.5	24.9 ± 2.2	16.4	16.6 ± 5.2	19.8 ± 6.7	26.1 ± 1.3	23.8 ± 4.9	26.1 ± 4.0	19.8 ± 6.7
Fractional absorption, IZiNCG	0.24 ± 0.01	0.24 ± 0.00	0.26 ± 0.02	0.18 ± 0.01	0.22	0.23 ± 0.01	0.23 ± 0.03	0.21 ± 0.01	0.23 ± 0.02	0.22 ± 0.03	0.22 ± 0.02
Absorbable zinc, IZiNCG (mg)	3.0 ± 0.4	3.0 ± 0.1	2.7 ± 0.3	2.7 ± 0.2	2.7	2.6 ± 0.4	2.4 ± 0.4	2.2 ± 0.2	2.1 ± 0.1	2.1 ± 0.3	2.5 ± 0.4
% IZiNCG estimate of mean physiological requirement for absorbed zinc (adj. for phytate)	148 ± 14.6	149 ± 3.5	137 ± 13.3	151 ± 10.2	137	128 ± 18.2	126 ± 20.2	120 ± 10.5	113 ± 6.5	121 ± 15.2	131 ± 16.6
Estimated % of population at risk of inadequate zinc intake	10.9 ± 5.2	9.5 ± 1.3	16.2 ± 10.5	9.3 ± 3.6	14.1	22.1 ± 8.2	24.8 ± 12.0	26.7 ± 9.4	33.1 ± 5.9	28.2 ± 15.0	20.5 ± 11.4

a. Data on food availability obtained from FAO National Food Balance Sheets (http://apps.fao.org/page/collections?subset=nutrition). Demographic data obtained from United Nations' Women's Indicators and Statistics Database (Wistat CD-ROM version 3, 1994).

b. SDs represent the dispersion of data for different countries within the same region. China is considered as a separate region, hence no SD could be calculated.

relationship between serum zinc and hemoglobin has been reported in some populations, such as pregnant Malawian women [13] and young Vietnamese children [14], and this may in part be attributed to the effect of confounding factors, such as the presence of concurrent infections, including malaria, on the biochemical indices. Thus, it must be recognized that the occurrence of anemia does not necessarily indicate the presence of zinc deficiency.

Anemia rates are often measured in large-scale population health surveys, so information is available for many countries, primarily for high-risk groups, such as women of childbearing age and young children. Where the prevalence of iron-deficiency anemia in any age group is considered to be high, further assessments of zinc deficiency are warranted. Current guidelines suggest that a prevalence of anemia > 40% is indicative of a severe public health problem requiring urgent corrective action [15] (http://www.who.int/nut/documents/ida_assessment_prevention_control.pdf). A global database of prevalence rates of anemia and iron deficiency is currently maintained by The Micronutrient Initiative (http://www.mn-net.org/idastat/). The United Nations Women's Indicators and Statistics Database (Wistat) provides data on anemia rates among pregnant and non-pregnant women in more than 200 countries and is available on compact disc (United Nations publications, New York/Geneva).

2.2.4 Composite index of the national risk of zinc deficiency, based on stunting rates and the adequacy of zinc in the national food supply

Two of the foregoing pieces of information that are suggestive of a population's risk of zinc deficiency—namely, the percent of preschool children who are stunted (low height-for-age) and the percent of individuals at risk of inadequate zinc intake (based on data obtained from national Food Balance Sheets)—use information that is already widely available and routinely published by the UN agencies. Therefore, these sources of information allow for immediate estimation of the risk of zinc deficiency in many countries. However, for the reasons stated above, neither of these pieces of information can provide a true estimate of the risk of zinc deficiency in a particular population. In an attempt to derive stronger inferences than might be possible from either one of these sources of information alone, the IZiNCG SC explored the possibility of combining both sets of information to construct a composite index of the national risk of zinc deficiency.

Preliminary analyses indicated that national-level data concerning the percent of preschool children who are stunted and the percent of individuals at risk of inadequate zinc intake are significantly correlated ($r = 0.60$, $p < 0.0001$), although there is considerable variability about the best-fit line (figure 2.2). Because of this variability and the fact that these indicators only provide suggestive information on the risk of zinc deficiency, countries were then classified according to the combined set of information. As discussed previously (section 2.2.1), the WHO considers a rate of stunting ≥ 20% as indicative of a public health problem. With regard to the FBS information, a cutoff of 25% estimated prevalence of inadequate zinc intake was applied because of the multiple sources of uncertainty in using these data to estimate the risk of population zinc deficiency. Using the two sets of cutoffs, it is possible to identify a set of countries with a relatively high risk of zinc deficiency according to both indicators (figure 2.3). Likewise, the two sets of data can be combined to identify countries where the rate of stunting is less than 10% and the percent of individuals at risk of inadequate zinc intake is less than 15%, in which cases the risk of zinc deficiency is likely to be low. Finally, countries with intermediate rates of either stunting or prevalence of risk of inadequate intake can be considered to have an intermediate risk of zinc deficiency. According to this combined indicator, as shown in figure 2.3, selected countries in South and Southeast Asia, Southern Africa, Central America, and the Andean region appear to have the highest risk of zinc deficiency, and many other countries in these same regions are classified as having a moderate risk of zinc deficiency. Country-specific information on the available suggestive evidence of zinc deficiency and the combined indicator of risk is provided in appendix 1. Notably, approximately one third of the world's population live in countries identified as having a high risk of zinc deficiency and one half live in countries found to have a moderate risk of zinc deficiency.

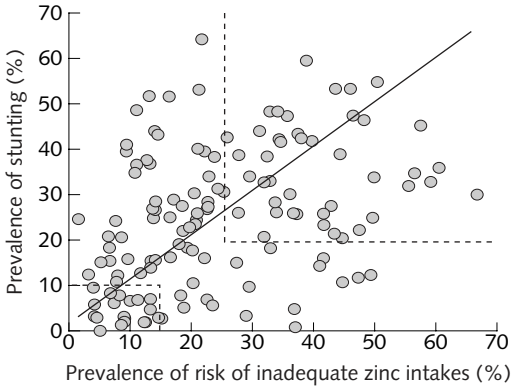

FIG. 2.2. Relationship between two sets of suggestive information concerning national risk of zinc deficiency: the prevalence of stunting (low height-for-age) in preschool children and the percentage of the population at risk of inadequate zinc intake (based on national food balance sheet data) ($r = 0.61$, $p < 0.001$).

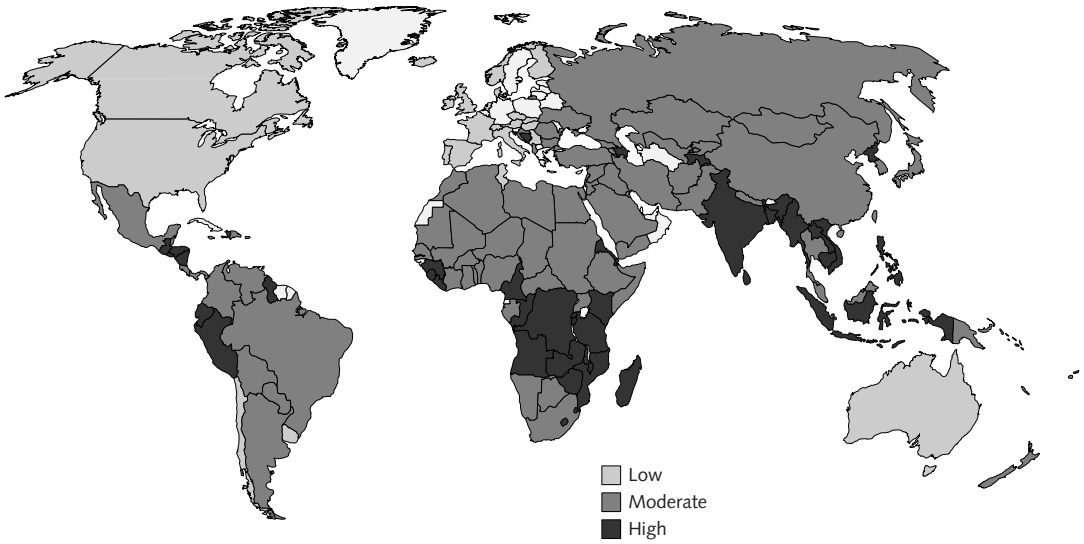

FIG 2.3. National risk of zinc deficiency based on combined information regarding the prevalence of childhood growth-stunting and the percent of individuals at risk of inadequate zinc intake

2.3 Methods to estimate the risk of zinc deficiency in populations

As indicated by the suggestive evidence described in section 2.2, zinc deficiency is expected to be widespread. However, to move forward with the development and evaluation of programs to improve population zinc status, it is necessary to derive more precise estimates of the magnitude of risk of zinc deficiency using more direct measures of a population's zinc status. Currently, national prevalence estimates of zinc deficiency based on direct measures are lacking for most countries. The methods for assessing the risk of population zinc deficiency that will be discussed in this section include assessment of dietary intakes of zinc, biochemical indicators of zinc status, and functional response to zinc supplementation. This section suggests methods for the direct measurement of zinc status, and possible approaches to the interpretation of results; application of standardized methods will assist in the comparison of information on indicators of zinc status of different populations.

2.3.1 Assessment of dietary zinc intakes

Because inadequate dietary intake of zinc is the most likely cause of zinc deficiency, dietary assessment is an important component in evaluating the risk of zinc deficiency. Information on the adequacy of dietary zinc intakes should be interpreted together with data derived from other assessment methods, such as biochemical assessment (section 2.3.2), to facilitate interpretation of the risk of zinc deficiency in the population. Standard dietary assessment methods can be applied to evaluate the adequacy of zinc intakes in populations, and to support results of biochemical assessments. Further, information derived from dietary surveys is useful for determining the specific dietary causes of inadequate zinc intake and therefore to help identify appropriate food-based approaches to intervention.

It is unlikely that it will be feasible to develop large-scale population-based dietary surveys to assess intakes of a single nutrient in most countries. However, where national nutrition surveys are planned, assessment of zinc intakes should be included, particularly where suggestive evidence indicates an elevated risk of zinc deficiency in the population (section 2.2). Likewise, when such dietary surveys have already been completed, it should be possible to re-analyze the information on food intake to assess the adequacy of zinc intake. This section provides information that will help guide the choice of appropriate assessment methods and includes considerations for the design of dietary surveys and analysis of data where zinc is to be incorporated in the assessment.

Determining the objectives of the dietary survey

Before selecting specific dietary assessment methods and developing a survey design, the objectives of the dietary assessment study must be clearly defined. There are two possible approaches to collecting dietary survey data. The preferred approach is to estimate the prevalence of inadequate intakes based on the distribution of usual intakes in the population. In this case, the distribution of observed dietary intakes in a group must be corrected for intra-individual (day-to-day) variation in intake. The assessment must therefore cover at least two, preferably non-consecutive, days of dietary intakes for each individual, or for an appro-

priate sub-sample of individuals in the survey (i.e., at least 30–40 individuals; [6]). In the case where it is possible to estimate usual intakes for all individuals in the sample, the number of days of dietary data for each individual required to derive this estimate can be calculated using the information in box 2.1. A less desirable approach is to characterize mean intakes of a group, in which case dietary information for a one-day period is collected for each person in the survey sample. This method is limited because only the mean intake can be estimated with certainty; the true distribution of usual intakes by the population is not known because variability due to intra-individual variation in intakes is not measured and therefore cannot be removed subsequently from the intake distribution. In both cases, each day of the week should be equally represented in the final sample.

The choice of survey approach will depend on the

BOX 2.1. Assessing the adequacy of individuals' intakes: an example

Source of data	n	Mean zinc intake (mg/day)	Intra-individual variance (%)	Inter-individual variance (%)	Variance ratio
Malawian women:					
2 × 24 hr recall	60	6.2	34	21	2.6
2 × 1-day weighed record	60	6.8	44	23	3.7
Ecuadorian women: 4 × 24 hr recalls	13	6.3	37	18	4.4
Ecuadorian men: 4 × 24 hr recalls	15	6.9	58	0	–

The ratio of the intra- to inter-subject variance, or variance ratio, can then be calculated. A ratio of 1.0 indicates that the intra-individual and inter-individual variances are equal, whereas a ratio > 1.0 indicates that the intra-individual variance is greater than the inter-individual variance [16]. In some cases, all or most of the intake variance is associated with intra-individual variation in intakes, despite the consumption of relatively monotonous diets.

After calculating the intra-individual variation using analysis of variance, the intra-individual coefficient of variation (CV_{intra}), can be determined as:

$$(\sqrt{\text{variance of intra-individual intake}} \div \text{mean intake}) \times 100\%$$

The CV_{intra} can then be used in the following equation to estimate the number of days required per subject to estimate an individual's zinc intake to within 20% of their true mean 95% of the time [17]:

$$n = (Z_{\alpha} CV_{intra}/D_0)^2$$

where:
- n = the number of days needed per subject
- $\boldsymbol{Z_{\alpha}}$ = the normal deviate for the percentage of times the measured value should be within a specified limit (i.e., 1.96 in the example below)
- $\boldsymbol{CV_{intra}}$ = the intra-individual coefficient of variation
- $\boldsymbol{D_0}$ = the specified limit, as a percentage of long-term true intake (i.e., 20% in example given below modified from Willett [18]).

Example: To calculate the number of days needed to estimate a Malawian woman's zinc intake using 24 hr recalls to within 20% of the true mean, 95% of the time:

$$\boldsymbol{Z_{\alpha}} = 1.96 \quad \text{and} \quad \boldsymbol{CV_{intra}} = 34\%$$

then:

$$n = (1.96 \times 34\%/20\%)^2 = 11 \text{ days}$$

The mean dietary zinc intake data derived for the determined number of days can then be compared to the RDA appropriate for the sex, life stage, and usual diet type of the individual (table 1.10). Usual intakes well below the corresponding RDA indicate a risk that the individual's zinc requirements may not be met. Further assessment of clinical and biochemical status of the individual would be required to determine if zinc deficiency exists.

objectives of the survey, the expected application of the survey data, and the availability of resources. A survey designed to estimate usual intakes for individuals in the population will provide more accurate information on the distribution of usual dietary zinc intakes in the population and allow a greater capacity to do the following: (1) estimate the proportion of individuals in a population with inadequate intakes; (2) design specific, food-based zinc intervention programs (e.g., determining an adequate and safe level of zinc fortification for a specific food vehicle); and (3) evaluate the effectiveness of programs to improve the zinc status and health of the target group(s) in relation to changes in usual zinc intakes. In the case that usual intakes for each individual in the sample can be estimated from multiple days of dietary data, it will also be possible to assess correlations between zinc intakes and other indicators of zinc status. However, the survey design required to meet this objective will also require more resources (e.g., time, staffing, budget) and more intensive data analysis procedures.

Dietary surveys that only allow the mean population intake to be assessed may be carried out where resources for conducting surveys are limited. Mean population intake data can be used to identify foods that are the primary contributors of specific nutrients in the population and to identify possible food vehicles for use in fortification programs. However, such data cannot be used reliably to determine the prevalence of inadequate intakes in the population because data on the distribution of usual zinc intakes are not available. Instead, only a crude estimate of the risk of inadequate intakes in the population can be made. The choice of survey design will also have implications for data analysis, and this is discussed in further detail below.

Implementation of the dietary assessment protocol

There are four stages in the implementation of a dietary assessment protocol designed to evaluate the adequacy of zinc intakes in individuals or populations: (1) measurement of food intakes; (2) calculation of the nutrient and anti-nutrition contents of the foods eaten; (3) estimation of the proportion of dietary zinc available for absorption; and (4) comparison of the mean usual intake of absorbable zinc for the population, or the distribution of usual zinc intakes within the population, to appropriate requirement estimates to assess the adequacy of intakes.

Measurement of usual food intakes

Several quantitative methods for assessing usual dietary intakes of individuals exist: weighed food records, recalls, and semi-quantitative food frequency questionnaires [19, 20]. Of these, food records and recalls are designed to measure the quantity of each food consumed over a one-day period. By contrast, a food frequency questionnaire (FFQ) obtains retrospective information on the pattern of food consumption during a longer time period, and sometimes on the usual intakes of certain nutrients.

Weighed food records completed by trained research assistants in households have been used to collect reliable quantitative data on dietary intakes, including zinc, in lower-income countries [21]. Methods for collecting weighed food records are described in detail elsewhere [22]. Although this method is more time consuming and costly, has a higher respondent burden than other methods, and may increase the likelihood that respondents change their dietary intakes during the recording period, weighed food records are the most accurate method of determining actual intakes during the recording period.

Dietary recalls can be used for estimating zinc intakes among non-literate populations, provided that portion sizes of the staple can be recalled accurately [23, 24]. Proper training of field workers in recall interview techniques can minimize bias and non-response rates [25]. As well, several strategies can be used to reduce memory lapses and facilitate portion size estimates, including training respondents in the use of food picture charts, bowls, plates and utensils familiar to the locale, and samples of actual cooked or raw foods that are commonly consumed [24]. Recalls are suitable for areas where diets are not very diverse and are predominantly plant based, as is the case in many lower-income countries. Although some accuracy is compromised by their use, recalls are easier, faster, and less expensive than weighed food records and are less invasive, so that compliance is enhanced, and the tendency to alter food intake is reduced.

An interactive 24-hour recall method has been specially designed for measuring usual intakes of total and absorbable zinc in lower-income countries. The feasibility and the relative and concurrent validity of this method were tested in rural Malawi, in sub-Saharan Africa [13, 24]. Intakes of available zinc calculated from three interactive 24-hour recalls and indices of absorbable dietary zinc were significantly associated with hair zinc concentrations, confirming that this assessment method can provide valid estimates of the amount of zinc available for absorption at the individual level. Further validation of this method to estimate usual intakes of absorbable zinc by individuals in other populations would be useful. Details of this interactive 24-hour recall method for determining intakes and adequacy of absorbable and total zinc (and iron) are given in Gibson and Ferguson [26].

Semi-quantitative FFQs have not yet been validated for the estimation of usual zinc intakes by individuals. Unlike nutrients such as vitamin A and calcium, which are concentrated in a relatively small number of foods or specified food groups, zinc occurs in a wide range of plant-based food items as well as animal source foods

and therefore may be quantified less accurately using this method. Although FFQs may prove to be suitable for determining mean population intakes, more research is required on the validity of this technique for estimating usual intakes of absorbable zinc for individuals before it can be applied with confidence.

Calculating total zinc intakes and estimating dietary zinc absorption

Once the daily food intake has been measured, total zinc intakes can be calculated by multiplying the amount (g) of each food consumed by its zinc content (mg zinc/100 g). It is preferable to use local food composition data for calculating zinc intakes, when available, because the zinc content of locally grown plant based foods can vary according to soil conditions, agronomic practices, and local food processing and preparation techniques [27]. However, when local food composition tables are not available, data from regional or global tables can be used. Factors influencing the proportion of dietary zinc that is absorbed in the gut and the importance of considering these factors in assessing the adequacy of dietary zinc intakes are discussed in section 1.6 (chapter 1). Two different levels of zinc absorption were suggested to represent the estimated usual absorption of zinc based on diet type and the phytate: zinc molar ratio: (1) mixed diets or refined vegetarian diets were those with a phytate:zinc molar ratio ranging from 5 to 18, and having an estimated average zinc absorption of 27% (adult men), 35% (adult women), or 31% (children); (2) unrefined, cereal-based diets were those with a phytate:zinc molar ratio > 18, and having an estimated average zinc absorption of 19% (adult men), 26% (adult women), or 23% (children). As the EAR (and RDA) for zinc are dependent on the assumed level of zinc absorption, two different levels of EARs and RDAs are presented in section 1.6 for the two different levels of zinc absorption. It is thus useful to calculate total phytate intake, in addition to total zinc intake, from the dietary intake data, such that the phytate:zinc molar ratio can be calculated, as described in section 1.6. The diet type and phytate:zinc molar ratio can thus be used to select the most appropriate EAR for use in assessing the adequacy of zinc intakes by populations. Likewise, the appropriate RDA can be selected for assessing adequacy of an individual's intakes, as discussed in box 2.1.

At present, the amount of data available on the zinc content of foods is not as extensive as for some other nutrients, but is increasing. Also, very few local food composition tables contain values for the phytate content of local plant-based staple foods, which will make it difficult in many cases to quantify total dietary phytate intakes. Regional or national data centers of the International Network of Food Data Systems (INFOODS) may be contacted for information on the availability of data on the zinc and phytate content of local foods. Data on the zinc content of foods are available from the US Department of Agriculture (USDA) food composition database, which may be downloaded from the Internet. Data on the phytate content of US foods are available on the University of Minnesota Nutrition Coordinating Center Nutrient Database; these data are updated regularly and the software system including the food composition database can be purchased from the Center. The phytate and zinc content of foods in several lower-income countries (Egypt, India, Indonesia, Kenya, Mexico, Senegal) derived from the International Minilist are available through the WorldFood Dietary Assessment System, 2.0. Although this database is not being updated, the dietary assessment software program can be downloaded free of charge from the INFOODS website (http://www.fao.org/infoods/software_worldfood_en.stm). Contact information for each of these resources is provided in appendix 2. Where adequate data on the phytate or zinc contents of foods are not available, it is preferable to determine these contents by direct analysis of locally acquired foods. When resources are not available to complete such laboratory analyses, the phytate content of foods may be extrapolated from the average phytate contents of common foods or food categories. A table of the phytate content of various foods is also provided in appendix 2.

The most commonly used method for analysis of zinc content of foods is flame atomic absorption spectrophotometry (AAS). Preparation of samples for analysis includes dry ashing to remove organic material followed by dilution with acid. Detailed methods can be found in Horwitz [28] and Aurand et al. [29]. Standard reference materials (SRM) suitable for food composition analysis are available from the National Institute of Standards and Technology (NIST; Gaithersburg, Maryland, USA) and Analytical Quality Control Services (Seibersdorf, Austria).

The adequacy of zinc intakes by individuals (as opposed to populations, or groups of individuals) can be estimated by comparing the usual intakes of the individual to the corresponding Recommended Dietary Allowance (RDA). The derivation of the RDAs for zinc is described in section 1.6, and the RDAs by sex and life-stage group, and by usual diet type, are summarized in table 1.10. To estimate the adequacy of zinc intakes by individuals it is first necessary to determine their usual intake of zinc, for which multiple days of dietary intake data are required.

To calculate the number of days required to assess usual zinc intakes of individuals, data on the intra-individual (within-subject) variation in zinc intakes are required. This may be derived from previously, or prospectively, collected data from a similar population group (i.e., similar age, gender, and socio-cultural group) for which more than one day of food intake data was collected for each individual [19]. To date,

very few estimates are available on the intra- and inter-individual variance for zinc intakes. Available data from lower-income countries are shown in box 2.1 (adapted from Gibson and Ferguson [30]).

The analysis of phytate content of foods on a wider scale is hindered by the present lack of a universally accepted laboratory method and certified reference material. Nonetheless, several methods exist and continue to be developed. The analytic method employed for phytic acid should preferably use high performance liquid chromatography (HPLC). This method is preferred because it can separately identify and quantify both the higher (hexa- and penta-inositol phosphates) and lower inositol phosphates [31]. Only the higher inositol phosphate forms (IP-6 and IP-5) compromise zinc absorption [32]. Use of the HPLC method is especially important for certain prepared foods that have undergone soaking, germination, and/or fermentation, because some enzymatic and non-enzymatic hydrolysis of hexa- and penta-inositol phosphates to lower inositol phosphates may occur [33].

Assessing the adequacy of zinc intakes

To evaluate the adequacy of dietary zinc in populations, intakes must be compared with an appropriate set of dietary reference values, taking into account the estimated percent absorption of dietary zinc. Although several different sets of recommended dietary intakes for zinc exist, comparison to a single set of recommendations is desirable to facilitate cross-comparison of dietary adequacy among populations. Currently available dietary recommendations for zinc intakes are described in section 1.6. The IZiNCG SC reviewed available information and presented a revised set of dietary recommendations for zinc (EARs, table 1.9); these recommendations take into account variation in intake requirements due to differences in estimated percent zinc absorption based on diet type, and are appropriate for international use. These recommendations can thus be used to assess the adequacy of dietary zinc intakes for different sex and life-stage groups. Information on the assessment of dietary zinc intakes for individuals and use of the RDA (table 1.10) is presented in box 2.1.

When the survey design allowed for the estimation of day-to-day (intra-individual) variation in intakes, the full probability approach may be used to estimate the risk of inadequate intakes in the population. It is beyond the scope of this document to describe the details of this method; for in-depth information on the theories and methods the reader is referred to the publications of the National Research Council [16] and the Food and Nutrition Board, Institute of Medicine [6]. To use this method, it is necessary that the distribution of the requirements is known and is symmetrical about the mean, and that the physiologic requirements for the nutrient are independent of its intake. The coefficient of variation of zinc requirements has been estimated to be 12.5%, as described in section 1.6. In the case of zinc, independence of requirements and intakes can be assumed.*

The observed intake data must be adjusted to remove variability introduced by intra-individual variation. This can be done using specialized software programs (e.g., C-SIDE, Iowa State University, Department of Statistics and Statistical Laboratory, Ames, Iowa, USA), or other statistical software with appropriate programming; detailed information describing this statistical methodology can be found in the report of the National Research Council [16, 34, 35]. As distributions of dietary intake data are typically skewed, the data are mathematically transformed prior to the adjustment for intra-individual variation, and can be reverse transformed following adjustment. The corrected distribution of intakes is then compared to the distribution of the EAR, and the probability of individual intakes falling below the EAR is computed. When the overall probability of inadequate intake is ≥ 25%, it is considered that there is an elevated risk of zinc deficiency in the population.

As an alternative to the probability approach, a more simplified method to estimate the prevalence of inadequate intakes may be used, which is referred to as the EAR cut-point method. Theoretical aspects and application of this methodology have been described by Beaton [36] and the FNB/IOM [6]. Briefly, the requirements for use of this method are the same as those indicated for the probability approach, but include an additional requirement that the variability in intakes among individuals in a population is greater than the variability in requirements of individuals. The latter assumption is likely to be valid in most cases, as the CV of the distribution of population zinc intakes has been assumed to be 25% [5, 37] based on data from a survey in the United Kingdom [8], which greatly exceeds the assumed distribution of zinc requirements (i.e., 12.5%; section 1.6). Once a distribution for zinc

* It is apparent from figure 1.4 that absorbed zinc intakes above the point where intake equals endogenous losses of zinc results in increased endogenous losses of zinc via the intestine, and therefore it is unlikely that increased intakes result from increased requirements. Although zinc requirements may become somewhat dependent on absorbed zinc intakes in the range of intakes just at or below the physiologic requirement for zinc as a result of homeostatic adaptations that reduce intestinal losses of endogenous zinc, the overall correlation between zinc intakes and zinc requirements across a range of dietary zinc intakes in a population is expected to be low (e.g., < 0.25–0.30), in which case any bias introduced by the dependency effect is likely to be minimal [6]. Nonetheless, it should be recognized that where a large proportion of individuals in a population have intakes near to the EAR, it is possible that either the probability approach or the EAR cut-point method will overestimate the proportion of individuals with inadequate intakes.

intakes with intra-individual variation removed is derived, as described above for the probability approach, the prevalence of inadequate intakes can simply be estimated by determining the proportion of individuals with intakes below the EAR. The accuracy of this method can approach that of the probability approach, particularly when the actual prevalence of the inadequate intakes is not very high or very low. Where ≥ 25% of individuals in the population have intakes less than the EAR, it may be considered that there is an elevated risk of zinc deficiency in the population. A hypothetical example of the estimation of the proportion of individuals with inadequate zinc intakes in a population of adult women is depicted in figure 2.4.

When usual zinc intakes of each individual in the sample have been determined, the mean intake data for each individual can be used and no further correction of the distribution is required. To estimate the prevalence of inadequate intakes, it is simply necessary to determine the proportion of individuals with usual intakes below the EAR.

For surveys that collected data for only a single day's intake by each individual, the true intra-individual variation and distribution of usual zinc intakes in that population are not known. In this case, it may be assumed that the CV of usual intakes by the population is equivalent to 25% [5, 37], as noted above. Assuming this distribution, the EAR cut-point method may be used to crudely estimate the proportion of individuals with inadequate intakes, as described above. The proportion of individuals in the population with intakes below the EAR can be determined using a cumulative distribution function, such as CDF.NORM in SPSS (SPSS, Inc., Chicago, IL, USA), where the SD of intakes is assumed to be 25% of the mean. However, caution must be used in the interpretation of these data because the true variability in usual population intakes is not known. The assumption of a 25% CV for population intakes would not be valid, for example, if the distribution of the observed (uncorrected) intakes had a CV of less than 25%. Where the true variability of usual intakes by a population greatly exceeds 25%, the prevalence of inadequate intakes will be underestimated.

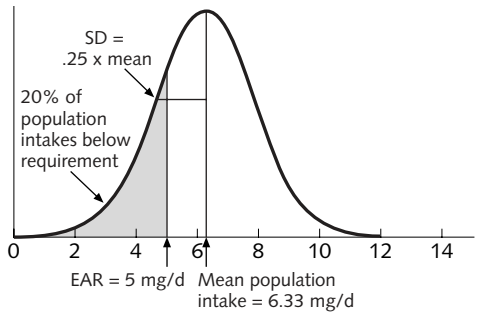

FIG. 2.4. Hypothetical, graphical representation of the estimation of the proportion of adult women with dietary zinc intakes below the estimated average requirement (EAR) for zinc from a typical mixed diet, assuming a mean intake of 6.3 mg/day and CV of the corrected distribution of usual intakes of 25%. Shaded area represents the percentage of the population (20%) with inadequate intakes.

Considerations for designing dietary assessments

A sample size estimate for a dietary survey can be made based on the anticipated prevalence of inadequate zinc intakes, and the desired precision of the estimate of inadequate intakes. The anticipated proportion of an individual's zinc intakes falling below the EAR may be derived from pre-existing dietary data, or from an indirect assessment of the adequacy of zinc in the food supply (section 2.2.2). The desired precision of the estimate will be represented by the width of the 95% confidence interval. In general, for a given confidence interval width, the required sample size will be higher where greater proportions of an individual's intakes are expected to fall below the requirement. However, where high proportions of inadequate intakes are anticipated (e.g., > 30%), wider confidence intervals may be acceptable, thus minimizing the required sample size. Sample sizes based on half-width 95% confidence intervals of 0.02–0.06, expressed as the proportion of an individual's intakes (i.e., the ability to determine the mean proportion within 0.02–0.06 on either side of the mean, with a 95% level of confidence), are given in table 2.2. In situations where it is intended to quantify the change in dietary zinc intakes from surveys repeated at intervals, narrower confidence intervals may be desirable.

Ultimately, the sample size for a large-scale dietary survey will often take into account the sample size

TABLE 2.2. Estimated sample sizes for dietary surveys assessing usual zinc intakes of individuals within a population by anticipated proportion of individuals with inadequate intakes and width of 95% confidence interval

Estimated proportion[a]	Confidence interval (half-width)				
	± 0.02	± 0.03	± 0.04	± 0.05	± 0.06
0.05	457	203	115	73	51
0.10	865	385	217	139	97
0.15	1,225	545	307	196	137
0.20	1,537	683	385	246	171
0.25	1,801	801	451	289	201
0.30	2,017	897	505	323	225
0.40	2,305	1,025	577	369	257
0.50	2,401	1,068	601	385	267

a. Anticipated proportion of individuals with zinc intakes below the estimated average requirement (EAR)

needed to assess adequacy of dietary intakes for other nutrients, or other survey variables, and hence the final survey sample size may be dictated by those requirements. When designing population surveys, the sample size estimates should be applied to the different population strata, as appropriate. For example, samples may be selected to represent populations in different regions or districts or in urban versus rural areas. When collecting multiple days of intake data for individuals, non-adjacent days representative of the range of days to be studied should be selected to enhance statistical information. In studies of rural areas in lower-income countries, market days as well as weekend and weekdays should be proportionately included because the foods consumed can vary between market and non-market days [24].

Summary

The procedures for carrying out dietary surveys to estimate the adequacy of zinc intakes by populations are summarized in figure 2.5. The objective of the survey, and other factors such as the availability of resources for survey implementation will determine the survey design used. Weighed records or 24-hour recalls can be used to measure food intakes. Where the objective of the survey is only to estimate the adequacy of group mean zinc intakes, a single day of dietary intake data is collected. However, where the objective is to determine the proportion of individuals in the population with inadequate intakes of absorbable zinc, then at least two, non-consecutive days of dietary intake data are required for all individuals (or at least 30–40 individuals) in the survey sample. The usual diet type (e.g., mixed diet, refined vegetarian diet, or unrefined, cereal-based diet) and the phytate:zinc molar ratio of the diet, can be used to estimate an appropriate level of assumed zinc absorption, and thus to select the most appropriate set of EARs for determining the adequacy of zinc intakes by populations (section 1.6).

If the survey was designed to measure intra-individual variation in usual zinc intakes, the distribution of zinc intakes should be adjusted to remove this variation before estimating adequacy of zinc intakes. Using this corrected distribution, either the probability approach may be used to estimate the risk of individuals' intakes falling below the EAR, or the EAR cut-point method may be used to determine the proportion of individuals with intakes below the EAR. If the survey was not designed to estimate and correct for intra-individual variation in zinc intakes, the distribution of zinc intakes may be assumed to have a CV of 25%; the proportion of the population with intakes below the corresponding EAR can be crudely estimated using this assumption. Nonetheless, caution should be used in the interpretation of these data. For either study design, when ≥ 25% of individuals' intakes fall below the EAR, the risk of zinc deficiency should be considered substantial. Nonetheless, to assist with the interpretation of the risk of zinc deficiency, it is recommended to combine estimates of adequacy of zinc intakes with data from biochemical assessments of zinc status (section 2.3.2).

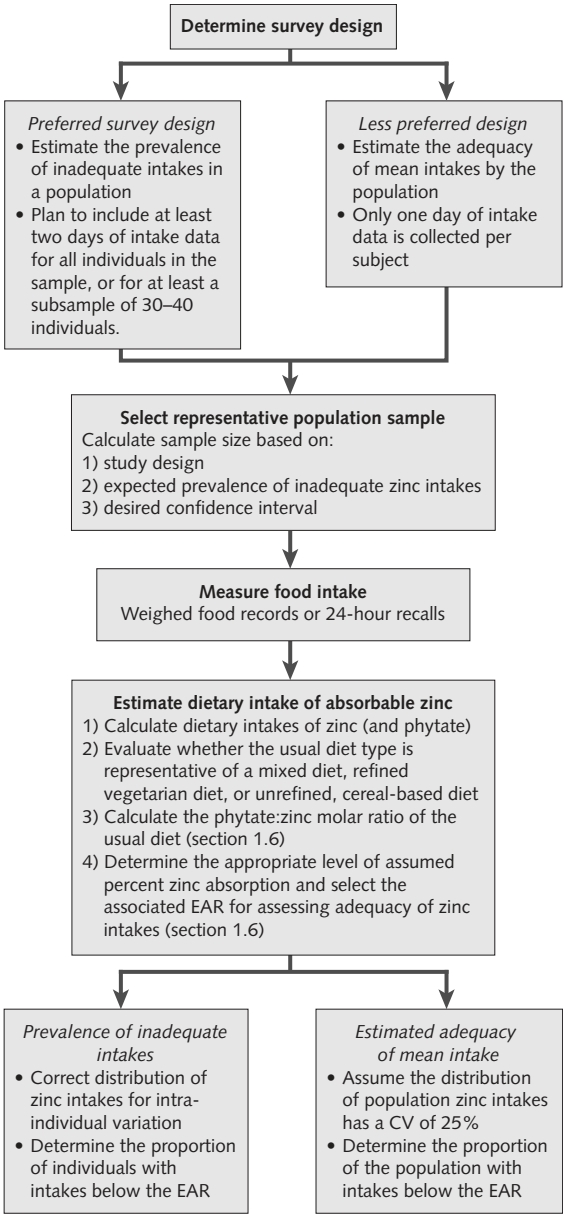

FIG. 2.5. Flowchart summarizing steps in assessing the dietary adequacy of zinc intakes in populations

2.3.2 Serum zinc concentration

Serum and plasma zinc concentrations are the most widely used biochemical markers of zinc status. Strictly

speaking, zinc concentrations measured in serum or plasma are not entirely comparable, largely as a consequence of the different collection and separation procedures used. Nevertheless, for the sake of simplicity, the following general discussions on circulating zinc concentrations will refer to serum zinc for both types of specimens. Although circulating zinc concentrations may have limitations in validity and reliability regarding the identification of mild to moderate zinc deficiency in individuals, several lines of evidence suggest that this index is useful in assessing zinc status at the population level [1, 38, 39]. In particular, the mean serum zinc concentration of groups of individuals responds as expected to dietary modifications and in association with functional outcomes following zinc supplementation. For example, in the United Kingdom National Diet and Nutrition Survey, there was a significant positive correlation between dietary zinc intakes, assessed by seven-day weighed food records, and fasting serum zinc concentration for girls aged 4 to 18 years, although not for boys [8]. In a study of New Zealand women not using oral contraceptives, there were significant negative correlations between serum zinc concentrations and intakes of dietary phytate and the phytate:zinc molar ratio [40]. An inverse correlation between serum zinc and dietary phytate: zinc molar ratios has also been reported in adolescent Canadian women consuming lacto-ovo vegetarian, semi-vegetarian, and omnivorous diets [41]. Further, in two experimentally controlled studies in which healthy adults were first fed omnivorous diets and then switched to vegetarian diets, measurable declines in serum zinc values on the vegetarian diets were observed [42, 43].

In an earlier meta-analysis of the effect of zinc supplementation on children's growth, which included studies of severely malnourished inpatients, the initial mean serum zinc concentration was inversely associated with the magnitude of the growth response to zinc supplementation [44]. However, this relationship was not statistically significant in an updated version of the meta-analysis that excluded severely malnourished children [1]. Low serum zinc concentration was found to be predictive of increased risk of diarrheal morbidity among Indian children [45]. In the aforementioned meta-analysis of the effect of zinc supplementation on children's growth, data on mean serum zinc concentration before and after the intervention were available for 15 studies [1]. In all but one study there was a positive response to zinc supplementation. The overall effect of zinc supplementation on serum zinc concentration was large (0.82 SD) and highly significant ($p < 0.001$). Thus, the population mean serum zinc concentration is a useful indicator of successful delivery and absorption of zinc supplements in children.

The following sections describe: (1) factors affecting the interpretation of serum zinc levels; (2) reference data for serum zinc concentration and the derivation of lower cutoffs for estimating the risk of zinc deficiency in populations; (3) technical considerations for the collection, preparation, storage, and analysis of serum samples for determination of zinc concentration; (4) quality control issues; and (5) considerations for survey design.

Factors affecting the interpretation of serum zinc concentration

Serum zinc concentrations fluctuate by as much as 20% during a 24-hour period [46], largely due to the effects of food ingestion. Following a meal, there is an immediate initial increase, after which the concentration declines progressively for the next 4 hours and then rises until food is eaten again. During an overnight fast, the concentration of serum zinc increases slightly, so the highest levels of the day are generally seen in the morning [47, 48]. However, diurnal variations in serum zinc concentration among fasted individuals have also been observed, whereby serum zinc decreased from morning to mid-afternoon and then began to rise again to morning levels [49].

Low serum zinc concentrations can occur in the presence of several conditions, representing a normal physiologic response and not necessarily indicative of low zinc status. Serum zinc concentrations are reduced during acute infections and inflammation, which is likely due to the redistribution of zinc from the plasma to the liver [50]; cytokines released during the acute phase response activate hepatic metallothionein synthesis [51], a metal-binding protein which appears to alter the hepatic uptake of zinc [52]. Elevated concentrations of C-reactive protein or other markers of the acute phase response can be used to indicate the presence of infection and should be considered in the interpretation of results. Stress and myocardial infarction also reduce serum zinc levels [53]. Because zinc is transported in plasma bound to albumin, diseases, such as cirrhosis and protein-energy malnutrition, that produce hypoalbuminemia result in lower serum zinc concentrations [54]. Hemodilution, as observed during pregnancy, oral contraceptive use, and other hormonal treatments, also results in a lower serum zinc concentration [55, 56]. On the other hand, conditions resulting in intrinsic or extrinsic hemolysis of blood cells can result in extremely high serum zinc levels because the concentration of intracellular zinc is considerably greater than in serum.

Reference data for serum zinc concentration and derivation of lower cut-offs

The largest population-based survey to include analysis of serum zinc concentrations in a presumably healthy,

non-malnourished, population was the United States National Health and Nutrition Examination Survey II (NHANES II: 1976–1980). NHANES II provides data for serum zinc concentrations in a representative sample of persons aged 3–74 years. Details of the NHANES II survey methodology, other data collected, and laboratory procedures have been previously reported [57, 58]. For both men and women, serum zinc values were lower in childhood, peaked during adolescence and young adulthood, and declined with age thereafter. From adolescence onwards, men had higher serum zinc values than did women, with the greatest differences occurring among adults aged 20–40 years. In the analysis of data originally reported by NHANES [58], serum zinc concentrations were described separately for three sets of samples collected: (1) in the morning from subjects in a fasted state ('AM Fasting'); (2) in the morning from subjects who were not asked to fast ('AM Other'); or (3) in the afternoon or evening ('PM'). As noted above, fasting state and diurnal variation are known to affect serum zinc concentrations. For each of the three sets of samples grouped by fasting state and time of day, the mean –2 SD was considered to represent the cut off level below which zinc deficiency is likely, and these values were reported as follows: AM Fasting, < 70 μg/dl (< 10.7 μmol/L); AM Other, < 65 μg/dl (< 9.9 μmol/L); and PM, 60 μg/dl (< 9.2 μmol/L). However, these proposed cutoffs did not take into account the known effects of age and sex on serum zinc concentrations, as reported in previous studies [59, 60]. Therefore, NHANES II data from a total of 14,770 individuals were reanalyzed for the present report to account for differences in reference values based on age and sex, as well as fasting status and time of day of sample collection. The results of this analysis are summarized here, and will be reported in detail elsewhere.

After eliminating data for 1307 subjects (13,463 remaining) for whom adequate information was not available, each of the four major variables (age, sex, time of day of blood sample collection [AM, PM, or Evening], and fasting status [≥ 8 hours fasted vs. fasting status unspecified or fasted < 8 hours]) were found to have significant main effects on serum zinc concentration ($p < 0.0001$; ANOVA). It is noteworthy that AM fasting samples were only collected from subjects 20 years and older. Data for an additional 1604 subjects were then excluded due to presence of conditions having a significant effect on serum zinc concentration, but where this effect may be independent of the subject's zinc status. These conditions were: low serum albumin (< 3.5 g/dl; $p < 0.01$); high white blood cell count (> 11.5 x 10^9/L; $p < 0.001$); currently pregnant ($p < 0.0001$) or lactating (females 14–42 yrs. only; $p < 0.05$); current use of oral contraceptives (females ≥ 13 years; $p < 0.01$), hormones (≥ 17 years; $p < 0.01$), or steroids (≥ 14 years; $p = 0.059$); and current diarrhea (≤ 10 years; $p < 0.05$).

Data for the remaining 11,857 subjects were then used to develop smoothed curves for the 2.5th percentile of AM Fasting, AM Other, and PM collected samples for each sex. Age groups were formed in 5-year intervals, from 0–4 years up to 70–74 years, although data were only available for children 3–4 years of age in the first age group. These curves were then assessed for the need to establish different cutoff points based on sex, time of day of sampling and fasting status. Differences equivalent to 4.3 μg/dl serum zinc were considered to be meaningful; this allows for a margin of analytic error equivalent to a 5% coefficient of variation. Based on the latter criterion, separate reference curves for the 2.5th percentile were developed for each sex and, within each sex, for AM Fasting, AM Other, and PM (PM and Evening combined) sample collections (figure 2.6). Data for Evening samples were included with the PM samples as the differences between these curves in any age group were small. Within each of these curves, age

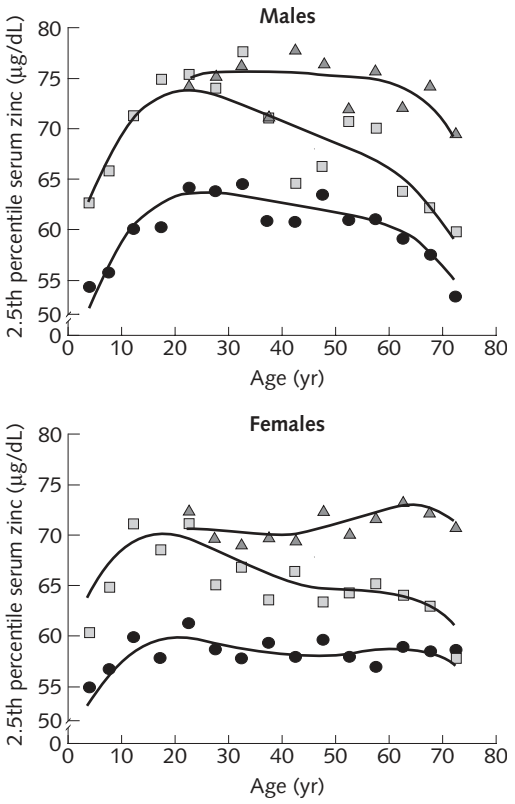

FIG. 2.6. 2.5th percentile of serum zinc concentration for males and females aged 3–74 years, by age and collection time/fasting status from NHANES II. Symbols represent the mid-point for five-year age intervals. Curves were fitted using a 4th order polynomial function for age in years. Data for each time of day and fasting status group are shown as: AM fasted ≥ 8 h (▲); AM non-fasted (□); PM/Evening non-fasted (●).

groupings with a difference of > 4.3 μg/dl were identified. The geometric means ± CV for these groups are given in table 2.3. The 2.5th percentile data for these groupings were then assessed for the need to establish different lower cutoffs by age groups. Appropriate suggested cutoffs for assessing serum zinc status are summarized in table 2.4.

The lower cutoffs for boys and girls aged 3–9 years were merged, as the differences between sexes were negligible. Although the 2.5th percentiles for serum zinc among males > 65 years are lower than for younger adult males, it would be prudent to apply the same cutoff established for males < 65 years, given the possibility that the decline in serum zinc concentration among males > 65 years is attributable to declining nutritional status [61].

Collection of morning fasting samples has previously been proposed as a standardized approach. However, it is recognized that in large population-based surveys this may be logistically very difficult and undesirable to implement, particularly where infants and young children are included. Further, there may be no apparent advantage to requesting fasting samples in a population survey in terms of reducing variability of serum zinc concentration due to meal effects, as the CVs for samples taken in presumably non-fasted samples are similar to those from fasting samples. Therefore, it is simply recommended that the time of blood collection and the fasting status (where fasting is considered to be > 8 hours since the last meal) of all subjects be recorded and serum zinc concentration for those individuals be

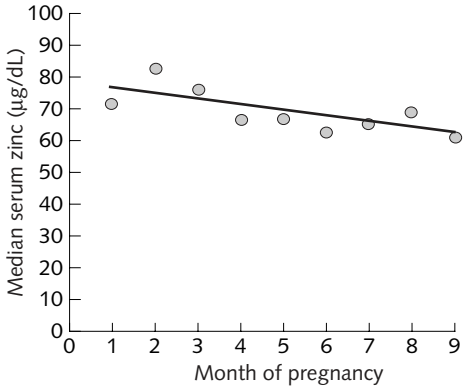

FIG. 2.7. Median serum zinc concentration by month of pregnancy derived from NHANES II (1976–1980)

TABLE 2.3. Geometric mean ± CV for serum zinc concentration by sex, time of collection/fasting status, and age

		Serum zinc concentration, μg/dl (μmol/L)[a] geometric mean ± CV		
Male	Age group (yr)	3–9	10–64	65+
	AM Fasting	na[b]	98 (15.0) ± 14%[c]	92 (14.1) ± 13%
	AM Other	85 (13.0) ± 14%	94 (14.4) ± 15%	82 (12.5) ± 15%
	PM	77 (11.8) ± 17%	82 (12.5) ± 15%	76 (11.6) ± 16%
Female	Age group (yr)	3–9	10–70+	
	AM Fasting	na[b]	90 (13.8) ± 13%[c]	
	AM Other	86 (13.2) ± 15%	86 (13.2) ± 14%	
	PM	75 (11.5) ± 15%	78 (11.9) ± 14%	

a. Conversion factor: μmol/L = μg/dl ÷ 6.54
b. na = not available
c. Based on data from subjects 20 years and older only

TABLE 2.4. Suggested lower cutoffs (2.5th percentile) for the assessment of serum zinc concentration in population studies, derived from NHANES II data

	Serum zinc concentration, μg/dl (μmol/L)[a]			
Age group	< 10 yr	≥ 10 yr		
		Females		
	Children	Non-pregnant	Pregnant[b]	Males
AM Fasting[c]	na[d]	70 (10.7)	1st trimester: 56 (8.6) 2nd/3rd trimester: 50 (7.6)	74 (11.3)
AM Other	65 (9.9)	66 (10.1)		70 (10.7)
PM	57 (8.7)	59 (9.0)		61 (9.3)

a. Conversion factor: μmol/L = μg/dl ÷ 6.54
b. Lower cutoffs given control for time of day/fasting status
c. Based on data from subjects 20 years and older only
d. na = not available

compared to the appropriate cutoff values, as given in table 2.4.

As noted above, serum zinc concentration is normally much lower in women during pregnancy. A regression line for the median serum zinc concentration by month of pregnancy from the NHANES II data indicates a distinct trend for declining serum zinc concentration throughout pregnancy (figure 2.7). However, the survey sample size for pregnant women is limited (n = 61) and, as a result, it is not possible to establish with reliability the 2.5th percentile from this distribution for each time of day/fasting group in each trimester of pregnancy. ANOVA of serum zinc indicated that the 2.5th percentile for the first trimester was 56 μg/dl. The 2.5th percentile for the second and third trimesters did not differ significantly from each other and the pooled value was 50 μg/dl.

It was not possible to derive a reliable estimate of the 2.5th percentile for lactating women due to the limited amount of data for this group (n = 23). Nonetheless, the mean serum zinc concentration of lactating women is not as low as during pregnancy. Until further reference data are available for this subgroup, it may be prudent to compare serum zinc concentrations to the lower cutoffs derived for non-pregnant women.

Given the relatively large number of women in the survey who were using oral contraceptive agents, 2.5th percentiles were also estimated for women up to 44 years of age in this sub-group. Women of childbearing age are a high-risk group for nutrient deficiencies and therefore are often over-sampled in surveys. Thus, there may be a large number of women included in surveys who are using oral contraceptives. The 2.5th percentiles for serum zinc for this group were 65, 61, 57, and 53 μg/dl for AM Fasting, AM Other, PM, and Evening samples, respectively. However, as the hormonal composition of the oral contraceptives used by women in NHANES II is not known, the applicability of the 2.5th percentile to users of different types of oral contraceptives is uncertain. Until further reference data on the effects of a variety of oral contraceptives on serum zinc concentration are available, these tentative lower cutoffs should be used with caution.

Unfortunately, the NHANES II survey did not provide reference data for children less than 3 years of age. Nevertheless, a few smaller studies have collected serum zinc data with the intent of establishing pediatric reference values for younger children [60, 62]. However, only the study of healthy Australian preschoolers by Karr and colleagues disaggregated the data for children less than 3 years of age. In this latter study, the 2.5th percentiles for serum zinc concentration reported were 59 and 52 μg/dl for children 9–23 months (n = 132) and 24–35 months (n = 109) of age, respectively, although the time of day of sampling, fasting state, or other possible confounders apparently were not considered for data collection or analysis. Nonetheless, the 2.5th percentiles for the children 9–35 months of age were similar to those reported for children 3–5 years of age (52 μg/dl, n = 226) [62] and were intermediate to those found in the NHANES II survey for AM and PM collected samples among children (table 2.4). Therefore, until appropriate reference data are available for children less than 3 years of age, it appears reasonable to apply the same lower cutoffs presented for the 0–5 years age group, as derived from the NHANES II data.

In surveys that include an assessment of serum zinc concentration, information on possible confounding variables (i.e., current infection, pregnant or lactating, current use of oral contraceptives or other hormones or steroids) should be noted. It would be preferable to avoid sampling of subjects while they have current infections to minimize the confounding effects of infection. Data for women in their first or second/third trimester of pregnancy may be compared to the suggested cutoffs in table 2.4. Serum zinc data for lactating women and users of oral contraceptives, or other hormones/steroids should be interpreted with caution.

Technical considerations for collecting and analyzing serum zinc

The collection and preparation of biologic materials for zinc analysis should be performed in a controlled environment to ensure accurate assessment. Contamination of samples with adventitious sources of zinc can produce erroneously high results and several precautions should be taken to avoid this. Blood collection, separation, and preparation techniques can also affect zinc concentrations, and therefore standardized methods are also recommended in this section.

Contamination

Contamination of blood samples with adventitious sources of zinc will lead to false and inconsistent results during analysis of zinc concentration. Every surface with which blood comes into contact during collection, processing, and analysis, as well as dust or smoke in the air, are potential sources of contamination. Contaminant sources of zinc can also be introduced by the technician handling the blood, through sweat, fingernails or saliva (via sneezing or coughing), and transportation of dust particles. Specific measures to avoid contamination are described below.

Airborne particulate matter is a substantial source of zinc contamination. Therefore, sample preparation and analysis should be performed in an adequately controlled environment. The optimal laboratory environment for trace element analyses is a filtered air environment such as a laminar-flow class-100 clean room. However, less rigorous methods of providing a clean environment and minimizing environmental

sources of contamination have been found to be acceptable. Use of laminar flow boxes or hoods during sample processing is recommended, together with other practices for minimizing trace element contamination in laboratories, such as described by the National Bureau of Standards [63]. More recent information can be found in Iyengar [64].

All equipment used in blood collection, processing, and storage must be rendered trace element-free prior to use. Common sources of zinc contamination during blood sampling include improperly washed lab ware; rubber, which may be used for plunger tips in syringes or stoppers for blood collection tubes; lubricants used in blood collection tubes; anti-coagulants used for the separation of plasma; pipettes, pipette tips and storage containers; water, preservatives, and reagents [65– 67]. Stainless steel needles are acceptable for zinc analyses. Siliconized needles, or polypropylene or Teflon catheters, are also acceptable options. Use of syringes with rubber tip plungers is not advised as the rubber is a source of contaminant zinc. For population-based surveys, trace element-free evacuated blood collection tubes that utilize siliconized, rather than rubber, stoppers are suggested. Polyethylene serum separators with polyethylene stoppers and olefin-oligomer have been recommended [64]. In general, disposable lab ware (e.g., storage containers, pipette tips) made of polyethylene or polypropylene is recommended. For the selection of appropriate equipment, manufacturers should be consulted as to which products are considered to be trace element-free.

Regardless of whether manufacturers specify equipment to be trace element-free, samples from each shipment should be pre-screened before use. A recommended screening procedure is to expose the equipment for 24 hours to solutions of standard reference materials for blood, serum, or plasma, with certified zinc content [64]. Analysis of these solutions for zinc should produce values within about 5% of the certified value to confirm that equipment is trace element-free. Disposable lab ware that contains detectable zinc, and all other lab ware used for the analyses, should be decontaminated; a suggested procedure is to immerse equipment for 24 hours in a chelating solution such as a 10–20% solution of ultrapure, concentrated hydrochloric or nitric acid, 1% disodium ethylene-diaminetetraacetate (EDTA), or Isoclean [68], followed by a thorough rinsing (3–4 times) with distilled, deionized water. Acid cleaning is preferred for gross contamination of glassware [69]. Suggested equipment and procedures for minimizing contamination are summarized in table 2.5.

TABLE 2.5. Practices to eliminate adventitious zinc contamination in serum zinc analysis

- » Disposable polyethylene gloves, free of talc or other coatings, worn by those handling blood samples
- » Samples processed in laminar flow clean rooms, laminar flow hoods, or otherwise clean, dust and smoke-free laboratory
- » Stainless steel needles
- » Anti-coagulants (if separating plasma) that are pre-screened for zinc
- » Trace element-free polyethylene evacuated tubes, stoppers, and serum separators (should be pre-screened for zinc prior to use)
- » Pre-screened polyethylene processing and storage vials
- » All equipment (except pre-screened disposable equipment) decontaminated by washing procedures (soaked for 24 hours in ultrapure 10–20% HCl or HNO_3 solution and rinsed 3–4 times in distilled, deionized water)
- » All materials and equipment stored covered or sealed to avoid dust

Sample collection

Variation in serum zinc results may be caused by changes in intravascular pressure at the time of the blood draw, which varies with stress levels, position, and venous occlusion through use of a tourniquet. Intravascular pressure causes the outward movement of fluid into interstitial space, therefore increasing the concentration of serum proteins and zinc. To minimize this source of variation, it is recommended that the subject is generally free of stress, is in a seated position, and that the subject's arm is occluded with a tourniquet for a standardized length of time (i.e., about one minute). Typically blood is drawn from the antecubital vein. The subject's skin should first be cleaned, preferably with alcohol-soaked gauze pads, at the site of venipuncture to remove contaminant zinc from the skin surface.

Sample preparation: serum versus plasma

Either serum or plasma can be used for the analysis of circulating zinc concentrations. Notably, when blood samples were collected simultaneously from the same individuals and separated as either serum or plasma, the zinc concentrations were greater in serum than plasma [70, 71]. Differences between plasma and serum appear to be partly dependent on the time between collection and separation. Plasma is commonly separated shortly after collection, but for serum, adequate time is needed to allow samples to clot prior to separation. During this clotting time, zinc may be released from platelets. Both plasma and serum samples showed a linear increase of 6% in zinc concentration over the first 2 hours, after which only the plasma levels increased [70]. However, this increase in serum or plasma zinc concentration with time before separation is avoidable by storing the samples under refrigeration or on ice prior to separation [72].

Anti-coagulants, such as heparin or EDTA, required for the separation of plasma are potential sources of zinc contamination [73], and each batch must be tested

prior to use. Further, heparin has been shown to bind zinc selectively [74], and some anticoagulants (e.g., oxalate and EDTA) efficiently chelate metallic ions so that plasma values will be falsely low. Citrate can alter osmotic pressure and therefore causes changes in intracellular water content and changes in the apparent concentration of zinc in plasma following separation. To facilitate comparison among results from different studies or surveys, it is recommended that a single anticoagulant be chosen as the standard for use in plasma zinc analysis, and zinc-free heparin is suggested as the anticoagulant of choice.

The choice between using serum or plasma samples to measure zinc concentration in population surveys may ultimately be determined according to the preference of the analytic laboratory. If plasma is preferred, heparin is recommended as an anti-coagulant as noted above, but it should first be screened to ensure it does not contain contaminant levels of zinc. Some analysts prefer serum to plasma, as precipitates that form in plasma samples can be problematic due to clogging of the aspirator in atomic absorption spectrometry. Using serum also avoids the possibility of contamination by anti-coagulants, where this is a concern. A standardized clotting time of 30–40 minutes is recommended, with samples kept under refrigeration or on ice during this time. It is also noteworthy that serum samples were used to derive the reference data for circulating zinc concentration in NHANES II, as described above. Therefore, analysis of serum zinc in future surveys may be most appropriate for comparison to the available reference data.

During sample separation, collection tubes and centrifuge tubes should be closed with trace element-free stoppers, particularly during centrifugation where metal particles may be liberated. Centrifugation procedures should be adequate (e.g., 2000–3000 × *g* for 10–15 minutes) to remove all blood cells, as these contain higher concentrations of zinc and effectively serve as a source of contamination [64]. Samples that are obviously hemolyzed (i.e., red in color) should be discarded, as the zinc released from erythrocytes into the serum/plasma will produce falsely high results.

Sample storage

It is generally recommended that refrigeration (4° C) of plasma or serum samples is acceptable for short-term storage (i.e., 2–3 weeks) prior to analysis. For longer storage periods, samples should be kept frozen at –25° C or lower. In general, zinc will be stable in frozen samples for prolonged periods. However, long-term storage of samples before analysis can cause dehydration of the sample, especially if "frost-free" freezers are used. Dehydration increases the concentration of the analyte and the use of ice cubes in heat-sealed plastic bags along with the samples can be used to prevent dehydration [75]. It is also advised to minimize the air space in sample tubes.

Analytic techniques

A number of different analytic methods can be employed for the measurement of zinc concentrations in serum samples. Flame atomic absorption spectrometry (FAAS) is most widely used. Others include graphite furnace atomic absorption spectrometry (GFAAS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), Instrumental Neutron Activation Analysis (NAA), X-Ray Spectrometry (Proton-induced emission; PIXE), and Anodic Stripping Voltammetry (ASV). The suitability of different analytic methods for the analysis of serum zinc has been reviewed [5]; FAAS, ICP-AES, NAA, and PIXE have the capacity to produce high precision results (CV ~ 1%), whereas GFAAS has a lower precision (CV ~ 10%). FAAS is considered to be the most simple and practical technique that is suitable for use in lower-income country settings and capable of producing accurate results with proper staff training and application of quality control techniques. Ultimately, the choice of analytic technique will be dependent on the other potential uses for the equipment, taking into consideration the elemental analytes of interest, detection limits, and the sample matrix. It is beyond the scope of this document to review each of these techniques; more detailed information on these analytic methods and sample preparation procedures appropriate for each (e.g., sample dilution and/or digestion) can be found in Herber and Stoeppler [76] and Iyengar [64].

The majority of current FAAS methods are direct techniques in which the sample is diluted with deionized water [77], aqueous acid solution (e.g., 0.1 M HCl), organic alcohols (e.g., n-butanol or n-propanol), or with a signal enhancing mixture [64, 78, 79]. The main purpose of dilution is to reduce the solids content, and hence viscosity, of the plasma/serum sample to equal that of the standard solutions. Differences in viscosity affect the rate of aspiration of samples and hence will affect the FAAS readings. Reducing the solids content of the samples will also prevent blockage of the burner. Approaches that have been suggested to avoid analytic error due to differential viscosity between samples and standards are to use 5% aqueous glycerol solution as the solvent for the standards [80] or, preferably, to use 6% aqueous butanol or 10% aqueous propanol as the sample diluent in a 5-fold dilution [64, 78]. Dilutions of between 5- and 10-fold are suggested to minimize viscosity differences; as high as 20-fold have been suggested but they may cause added problems of weakened signals and decreased precision due to pipetting errors.

Quality control issues

In addition to applying adequate technical precautions, quality control procedures are required for accurate assessments of serum zinc concentration. The quality of results is highly dependent on the skill of the analyst and the laboratory practices employed to monitor accuracy and precision.

Two types of quality control procedures should be used. The first is a primary reference material, or a Standard Reference Material, with a certified, analyzed mean (± SD) zinc content. These standards should be used to validate the accuracy of analytic methodology and to assure the accuracy of results. Serum-based reference materials should be chosen (table 2.6) for consistency of the matrix. Accuracy can also be monitored through participation in external proficiency testing programs. Secondary reference materials, or carefully prepared 'in-house' or 'bench' controls such as a bulk sample of pooled serum should be used with every run to monitor precision. These can be prepared to cover the low, normal, and elevated levels in the normal range of serum zinc concentrations. This control measure is suitable for monitoring day-to-day variation, short-term "noise", and long-term "drift" or fluctuations of the instrument reading in the absence of a signal. Inherent in the use of quality control measures is the establishment of standards or tolerance limits for analyzed results. Precision is determined through the repeated analysis of reference materials, both within runs and between runs, and calculating the CV. A CV of 5% should be attainable for zinc using atomic absorption spectroscopy. Suggested quality assurance procedures and resources are summarized in table 2.6.

Due to the multiple methods available for sampling and analyzing serum zinc, it will be important to employ a standard method to improve the comparability of results within and among laboratories. Recommended standardized procedures for the collection, preparation, and analysis of serum zinc samples are summarized in table 2.7.

TABLE 2.6. Quality assurance procedures and resources for the analysis of serum zinc concentration

- » Certified standard reference material suitable for serum zinc analysis:
  - Bovine serum, SRM 1598; National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA
  - Animal blood, IAEA-A-13; International Atomic Energy Association (IAEA), Seibersdorf, Austria
- » In-house or bench reference materials, such as pooled serum sample:
  - Pooled sample should be analyzed against a certified standard reference material to establish its zinc content
  - Samples should be prepared at the low, mid, and high range of normal serum zinc concentrations
  - Pooled sample should be prepared in a large enough quantity to provide a single standard over a useful duration of time (e.g., two to three years for use in a monitoring program or on-going survey)
- » Participation in an external proficiency testing program:
  - College of American Pathologists Quality Assurance Service, New York, USA

Considerations for survey design

Sample size determination

The sample size required to detect the prevalence of low serum zinc concentrations with a reasonable degree of precision is dependent to some extent on the anticipated prevalence of zinc deficiency. When the prevalence of zinc deficiency is anticipated to be low (< 5%), a narrow confidence interval is desirable to define prevalence more precisely. In this case, a large sample size is needed. Where a higher prevalence is expected, a wider confidence interval for the distribution of results is acceptable, and therefore a smaller sample size can be selected. Based on the distributions of serum zinc concentrations determined in the NHANES II survey, the confidence intervals and suggested survey sample sizes for expected low, moderate, and high prevalences of low serum zinc concentration are given in table 2.8. The range of sample size given for each anticipated level of prevalence reflects the range in percentage points of the confidence interval suggested.

TABLE 2.7. Summary of suggested procedures for serum zinc analysis

- » Employ appropriate practices throughout the procedure to avoid adventitious trace element contamination (table 2.5).

Sample collection and preparation*:*

- » The subject should be seated
- » Clean subject's skin with alcohol at site of the antecubital vein
- » Restrict occlusion of subject's arm with tourniquet to about 1 minute
- » Draw blood using stainless steel needle, and collect into trace element-free evacuated blood collection tubes *without* anticoagulant for processing serum
- » Place blood sample in refrigerator or on ice and allow to clot 30–40 minutes
- » Centrifuge blood sample at 2000–3000 × g for 10–15 minutes
- » Discard any obviously hemolyzed samples
- » Store samples frozen unless they are to be analyzed immediately

Sample analysis:

- » Dilute sample 5- to 10-fold in solvents such as 6% aqueous butanol or 10% aqueous propanol
- » Read sample zinc concentration using Flame Atomic Absorption Spectrometry with appropriate standard dilutions, in-house quality controls, and standard reference materials (table 2.6)

TABLE 2.8. Suggested confidence intervals and estimated sample sizes for surveys of serum zinc concentration

Prevalence of low serum zinc concentration	Confidence interval (percentage points)	Sample size
Low (< 5%)	1–2	1,825–457
Moderate (5%–10%)	2–3	865–385
Moderately high (10%–20%)	3–4	683–307
High (> 20%)	4–5	505–289

Data analysis and interpretation

To determine the proportion of the population with low serum zinc status, it is first necessary to group data according to whether the blood drawn represented (1) morning fasting samples; (2) morning, non-fasting samples; or (3) afternoon samples. Based on these groupings, the result should be compared to the appropriate cutoff for age, sex, and physiologic status (table 2.4) and classified as either low or normal.

Information on expected prevalences of low serum zinc concentration in at-risk populations is somewhat limited. A recent National Nutrition Survey in Mexico indicated that 25% of preschool and school-aged children, and 30% of women had low serum zinc concentrations (defined as < 65 μg/dl in that survey) [12]. In rural areas, these prevalences reached 40% among children and 34% among women. Some community-based zinc supplementation trials from various populations have reported prevalences of low serum zinc. Examples include a study of 6–24 month old Vietnamese infants (n = 163) where the prevalence of low serum zinc (defined as < 70 μg/dl) was ~ 34% at baseline and dropped to < 10% after 3 months of supplementation with a concurrent increase in height-for-age Z-score among initially stunted children [14]. A group of Indian children 6–35 months of age (n = 609) had a prevalence of ~ 36% low plasma zinc (defined as < 60 μg/dl) concentration at baseline [80]. In the group receiving zinc supplements for four months, the prevalence was reduced to 11.6% with a concurrent reduction in the incidence of acute lower respiratory infection. A group of peri-urban 6- to 8-year-old Guatemalan school children (n = 155) had a prevalence of low plasma zinc concentration (defined as < 70 μg/dl) of 12.3% among boys and 1.5% among girls [81]. Following 3 months of supplementation, plasma zinc concentrations increased and the children demonstrated some body composition changes, without improvements in growth or other functional tests of zinc deficiency [82].

Although the above studies are not entirely comparable because of different sampling techniques and use of different cutoff points, they can be used to set tentative guidelines for taking action based on prevalences of low serum zinc concentrations. In populations with a prevalence of low serum zinc concentrations less than 10%, zinc deficiency would not be considered a public health concern warranting national level programs. A prevalence of between 10% and 20% for low serum zinc values warrants further assessment of results, as the slightly elevated prevalence suggests that some segments of the population may be at high risk of zinc deficiency. Programs targeted to high-risk groups may be necessary. Where the prevalence of low serum zinc values exceeds 20%, national level programs may be considered following further assessment to identify groups at elevated risk.

Prevalence rates should be examined according to possible risk factors to identify high-risk groups. Demographic variables to assess can include age groups, sex, location (e.g., region, district, urban/rural), as well as indicators of socioeconomic status (section 2.5). Appropriate statistical procedures, such as logistic regression or log-linear models, then may be used to identify associative risk factors for low zinc status for purposes of research or for development of more intensive, targeted public health programs.

Summary

Serum zinc concentration is the most widely used biochemical indicator of zinc status. Reference values and cutoffs suitable for most individuals have been suggested, taking into account age, sex, fasting status, and time of day of sample collection. Adequate information on all four of the latter variables is necessary so that results can be compared to the appropriate lower cutoff value. Several physiologic factors, such as low serum albumin, elevated white blood cell counts, pregnancy, lactation, and use of oral contraceptives or other hormones, can affect serum zinc levels and must be considered in the interpretation of results. Although these statistically derived lower cutoffs are suitable for estimating the risk of zinc deficiency in populations, further population-based studies are needed to validate the lower cutoffs against functional indices of zinc deficiency.

Either serum or plasma can be used to measure circulating zinc concentration. Proper measures to avoid contamination of samples with adventitious sources of zinc are essential to produce meaningful results. The use of serum samples avoids the need for anti-coagulants during blood collection, which can be a source of contamination. For separation of serum, a recommended, standardized procedure is to allow serum samples to clot, under refrigeration, for 30–40 minutes. Rigorous quality control measures are also required to produce accurate and precise results.

For population surveys, adequate sample sizes are needed to determine the prevalence of low serum zinc concentrations with a desired level of precision.

A prevalence of between 10% and 20% for low serum zinc suggests that some segments of the population may be at high risk of zinc deficiency. Where the prevalence of low serum zinc values exceeds 20%, national level programs may be considered. The assessment of serum zinc concentrations should be combined with other zinc status assessment methods, particularly with dietary zinc intake data, to strengthen the estimates of risk of zinc deficiency.

2.3.3 Hair zinc concentration

The zinc content of the hair shaft reflects the quantity of zinc that was available to the hair follicle during an earlier time interval, so hair zinc concentration has been proposed as a useful index of longer-term zinc status. During infancy and early childhood, hair zinc concentration declines from high neonatal values, reaching a minimum at about 2–3 years [83, 84]. These trends in hair zinc concentrations may arise from a gradual depletion of tissue zinc pools induced by rapid growth. During pre-pubertal years, hair zinc concentrations tend to increase from the lower levels of infancy and early childhood to reach a normal adult value of about 150 μg/g (2.3 μmol/g) [85]. Hair zinc concentrations differ markedly according to sex [86–88]. Boys have consistently lower hair zinc concentrations than girls of the same age, even when food consumption patterns and energy and nutrient intakes are comparable. Such sex differences in hair zinc concentrations may arise because of higher zinc requirements for boys or because of changes in growth hormone and testosterone concentrations [89]. Seasonal differences in hair zinc concentrations have also been described in a population in Canada [90]. Hair zinc values tend to be lower in the spring/summer. This may result from seasonal changes in linear growth velocity, such that rapid periods of growth lead to a gradual depletion of tissue zinc pools.

Significant relationships between hair zinc concentrations and functional outcomes of zinc status, such as impairments in linear and ponderal growth, taste acuity and appetite, as well as selected dietary zinc indices have frequently been observed [81, 84, 91–96]. The clinical signs of mild zinc deficiency have been associated with hair zinc values in infants and young children of less than 70 μg/g (1.07 μmol/g) in the spring or summer [84, 87] and less than 110 μg/g (1.68 μmol/g) in the winter [90]. Some studies have reported associations between hair zinc concentration and dietary zinc indices. For example, in adolescent Canadian women consuming omnivorous and vegetarian diets, high phytate intakes ($r = -0.194$; $p = 0.03$) and high phytate:zinc molar ratios ($r = -0.215$; $p = 0.01$) were negatively correlated with hair zinc concentration [97]. Similar negative correlations between hair zinc and phytate:zinc molar ratios have been observed in Canadian [98] and Malawian preschool children [99]. Pregnant women in rural Malawi consuming diets with phytate:zinc molar ratios > 17 (the median) had lower hair zinc concentrations than those consuming diets with a phytate:zinc molar ratio < 17 [13].

The application of hair zinc concentrations as an indicator of zinc status has several advantages, and several limitations. Unlike serum zinc, concentrations of zinc in hair are more stable and not affected by diurnal variation, prolonged fasting, meal consumption, and acute infection. Collection of hair samples is less invasive than drawing blood, and may be more appropriate in some populations where collection of blood is not culturally acceptable, particularly from young children. Unlike blood, hair samples do not need to be processed in the field and refrigeration is not required.

The limitations of using hair zinc concentration as an index of zinc status include the limited availability of reference data, and some problems in the interpretation of results. The cutoffs currently used for infants and children may not apply to adolescents or adults. Controversy surrounds the interpretation of apparently normal or even high hair zinc concentrations in malnourished children with linear growth retardation. This has been attributed to a reduced rate of hair growth arising from a limited supply of zinc to the hair follicle [100–102]. An alternative explanation may be that the growth failure in these malnourished children was not induced by zinc deficiency *per se* but rather from environmental factors such as parasitic infections, morbidity, and/or deficiencies of other growth limiting nutrients [95]. As a result of the reduction in growth rate, requirements for zinc are also reduced, resulting in apparently normal or even high hair zinc concentrations. Therefore, although normal or high hair zinc concentrations do not necessarily indicate normal zinc status, low hair zinc concentrations would suggest that zinc status is not optimal.

Standardized procedures for collecting, washing, and analyzing hair samples are essential in all studies. Samples should be collected from close to the occipital portion of the scalp with stainless steel scissors, and only the proximal 1.0–1.5 cm of the hair strands retained for analysis. Care must be taken to remove all sources of adventitious contamination (e.g., nits and lice) prior to washing the hair samples according to a standard procedure. A non-ionic detergent (e.g., Actinox) with [102] or without acetone [103] can be used. Hair samples can be analyzed by instrumental neutron activation (INA) [83] or flame atomic absorption spectrophotometry [103]. Accuracy of the analytic methods can be assessed using a certified reference material for human hair (e.g., Community Bureau of Reference, Certified Reference Material #397; Institute for Reference Materials and Measurements, Retieseweg., B-2440 Geel, Belgium). Variations in

hair zinc concentrations due to age, sex, season, hair color, hair beauty treatments, growth rate, severity of malnutrition, and rate of hair growth have been described. The effects of these possible confounding factors must be considered in the interpretation of results.

Summary

Due to the lack of established cutoffs for most age groups and the uncertainties in interpreting results among malnourished children, the usefulness of hair zinc analysis in population zinc status assessment is presently limited. However, hair zinc concentrations may be useful as an alternative to serum zinc determinations when collection of blood samples from young children is not permitted. In this case, the proportion of young children with values less than 70 μg/g (1.07 μmol/g) can be used for samples collected in the spring/summer and the proportion less than 110 μg/g (1.68 μmol/g) can be used for those collected in the winter. A high proportion of infants or young children with hair zinc concentrations falling below these cutoffs may be used to support a diagnosis of zinc deficiency where it is suspected. Hair zinc concentrations may be useful for tracking trends in zinc status over time within a population. Further research would be useful to validate lower cutoffs for the assessment of zinc status in different age groups and seasons.

2.3.4 Other biochemical indicators of zinc status

Other biochemical indicators of zinc status described in this section are those that are still being explored and developed, and that merit further study of their usefulness as indicators of zinc status. These methods are presented for informational purposes only, and are not currently recommended for use in population assessments.

Enzymes and circulating proteins

Several zinc-dependent enzymes have been shown to be affected by zinc intake or zinc status in experimental animal models and human populations. These include plasma or serum alkaline phosphatase, 5'-nucleotidase, ribonuclease, lactic dehydrogenase, delta-aminolevulinic acid dehydrogenase and extracellular superoxide dismutase (EC-SOD). Results have been highly variable between studies and none of these has been shown consistently to reflect zinc status. Another common problem is specificity; several of these enzymes (e.g., alkaline phosphatase) are also affected by nutrients other than zinc. Metallothionein is a metal storage protein that is present in serum at a low concentration; the circulating concentration of metallothionein appears to correlate with zinc intake. However, similar to several of the enzymes mentioned previously and to serum zinc, metaollothionein may be affected by other factors, such as infection and stress, although this has not been confirmed by direct studies. Because of these limitations and the relative difficulty of performing these assays outside the research laboratory, it is presently unlikely that they will be useful for assessment of zinc status at the population level.

Cells

Cells are more likely to reflect long-term zinc status than the rapidly turning over plasma pool. Various enzymes and binding proteins affected by zinc have been measured in cells, such as erythrocytes, leukocytes and platelets. Although results have been encouraging for some of these, they have received very little clinical use and there are no established reference values. Another limitation is the difficulty in separating the cells under field conditions and, for leukocytes and platelets, the large volume of blood required, which may lead to decreased participation rates and elimination of infants and children from studies.

Molecular techniques

Modern molecular techniques are being used increasingly to measure mRNA for proteins whose expression is regulated by metal ions, such as zinc. Usually, quantitative reverse-transcriptase polymerase chain reaction assays (RT-PCR) are used, but these require specialized equipment and procedures that are not yet widely available. Metallothionein mRNA in monocytes and erythrocytes has been shown to be affected by zinc intake [104, 105], but only a few studies have been performed and our knowledge regarding other factors, such as other metal ions, infection and stress, that may affect its expression is very limited.

Differential mRNA display and gene microarrays are likely to be helpful in identifying genes that are specifically altered by zinc status. However, it is not yet known whether this new technology will ultimately prove suitable for assessment of zinc status of individuals or populations.

Kinetic markers: pool sizes and turnover rates

Kinetic studies of zinc metabolism provide a powerful means of summarizing the integrated whole-body response to changes in zinc status. Kinetic parameters, estimated from a compartmental model, relate shifts in zinc absorption, excretion, cellular zinc fluxes, and the size of body pools with changes in zinc intake. Because the compartmental model integrates all of the adjustments in homeostasis, subtle changes in zinc status may be detected when the response of a single measurement is not evident. Several compartmental

models of zinc metabolism have been developed and used to study kinetic responses to severe zinc depletion in men [106, 107], moderate changes in zinc intakes [108], and zinc excess [109]. Parameters that seem to reflect changes in zinc status include the size of the exchangeable zinc pool(s), plasma fractional turnover rates, and total plasma zinc flux.

Exchangeable zinc pools (EZPs)

The zinc that is available for maintaining zinc-dependent functions is thought to be mobilized from small, rapidly exchanging zinc pools found primarily in the plasma and liver [108, 109]. The size of this pool can be estimated from the tracer-tracee disappearance curves using kinetic modeling software. The total exchangeable mass varies with the length of time over which the decay curves are measured. For example, if tracer disappearance is followed for 3 hours, the EZP mass is approximately 18 mg in healthy men; if the tracer disappearance is followed for 192 hours, or eight days, it is approximately 150 mg or about 10% of the whole-body zinc pool. A decline in one or more of the EZPs could be associated with a reduction in the zinc available for zinc-dependent functions, especially among rapidly turning over proteins [110]. If so, then EZP mass would provide a good indication of tissue zinc status.

EZP mass has been measured in individuals freely selecting their diets [108], in men fed zinc-depleted diets [106, 111], and in populations with chronically low zinc intakes. Among individuals freely selecting their zinc intake, EZP mass varied directly with dietary zinc, both in individuals [108] and populations [112]. Also, experimental acute, severe zinc depletion induced in adults by feeding a diet providing 0.23 mg zinc/day for five weeks [106] lowered total EZP by 36%. Plasma zinc concentrations declined 65% in that study suggesting that plasma zinc is more sensitive to severe zinc depletion than is EZP mass. When dietary zinc was reduced to a marginal level (4.6 mg/day) in a group of healthy men, EZP mass did not change [111]. Thus total EZP mass does not appear to be a good indicator of modest short-term changes in zinc intake. However, longer-term low intakes or acute zinc depletion causing a reduction in whole-body zinc content appears to cause a concomitant reduction in EZP.

EZP mass is correlated with fat-free mass in adults. This is expected since zinc is an integral part of the protein mass in lean tissue. Possibly, EZP would be more meaningful if it is expressed in terms of fat-free mass. Body size and body composition of individuals should be considered when the EZP values of individuals are compared.

Serum zinc turnover rates

The small amounts of zinc in the serum turn over rapidly in the body. To meet tissue needs, the rate of turnover is expected to increase when total body zinc is reduced, such as during zinc deficiency. Thus, serum zinc turnover rates may serve as an indicator of zinc status. With acute, severe zinc depletion in a group of healthy men, fractional plasma turnover increased from ~ 150 to ~ 200 times/day, but the total zinc flux declined from ~ 475 to ~ 230 mg due to a marked decline in plasma zinc concentrations [106]. This decline in the amount of zinc available to the tissues was associated with the onset of the clinical symptoms of zinc depletion in these men. One study reported that the fractional plasma zinc turnover was reduced in young women with low serum ferritin levels [113]. It was assumed that women with low iron status also had a low zinc status, and that this faster fractional zinc turnover reflected the poor zinc status. No other indicators of zinc status were reported, however. Fractional plasma zinc turnover rates were found to be 50% greater in zinc-deficient Egyptian subjects than in normal controls [114]. The use of fractional plasma turnover rates or total plasma zinc flux as a biomarker of zinc status needs further validation in humans.

In summary, kinetic measures of EZP and plasma zinc flux seem to reflect chronic zinc intakes of individuals and populations. These kinetic parameters may be useful in defining the status of individuals and populations that have marginal intakes leading to a loss of whole-body zinc. As these techniques are costly and intensive, however, they will not find use in population assessments. Rather, findings from small clinical-based studies using these techniques will contribute to our understanding of zinc requirements, zinc homeostasis, and the dietary and physiologic conditions that influence zinc status. Moreover, these techniques may ultimately prove useful to validate novel simpler techniques to assess zinc status as they become available.

2.4 Functional indicators: response to supplementation

Because of uncertainties in the interpretation of the foregoing techniques to assess zinc status, many recent studies have relied on the identification of a functional response to zinc supplementation as the basis for diagnosing preexisting zinc deficiency in the supplemented individuals (section 1.4). Use of a functional response to indicate deficiency requires randomly administering either zinc or placebo to members of the target population and comparing the responses in the two groups of subjects. Ideally, the subjects are selected to be a representative sample of a larger population, so inferences can be drawn for the population as a whole. Functional responses that have been used previously include physical growth, immune function and rates of specific infections, physical activity and performance on psychometric tests, and hormonal responses,

among others. The major disadvantage of using these functional responses to zinc supplementation as indicators of zinc deficiency is the long delay that is often required for the response to be detectable and the consequent high cost of this diagnostic technique. Appropriate efforts to ensure that the supplements are actually consumed contribute further to the cost of these assessments. Considerations for supplementation programs are covered in section 3.1.

2.5 Socioeconomic status indicators to identify high-risk groups

Social and economic factors are important underlying determinants of childhood morbidity, mortality and malnutrition, including micronutrient malnutrition. In the absence of clinical, biochemical, or dietary evidence of zinc deficiency, the general level of deprivation, as assessed through socioeconomic indicators, can be useful to inform on a population's potential vulnerability to zinc deficiency. These socioeconomic indicators can also be used to select priority areas or sub-populations for targeting interventions. Because of their unknown sensitivity and specificity, however, socioeconomic indicators should not be used for the monitoring and evaluation of interventions to address zinc or other nutrient deficiencies [115]. A few potentially useful socioeconomic indicators are suggested below.

Maternal education

Maternal education has been shown consistently to be critically important for child health, nutrition and survival [116, 117]. Although the precise mechanisms by which maternal education affects child outcomes are not fully understood, evidence from various countries indicates that childcare and feeding practices are key pathways [118–120]. Thus, lower maternal education is likely to lead to inadequate child feeding, hygiene and health-seeking behaviors, which in turn are likely to be associated with increased risk of zinc deficiency among children. Maternal education is also known to be highly correlated with socioeconomic status and household food security. Because maternal schooling is easier to measure and is less prone to recall bias than income or expenditure data, it may be a more useful overall proxy for poverty.

Indicators of maternal schooling could include the following:

» Rates of illiteracy among women;
» Mean number of years of schooling;
» Percentage of women having completed primary, secondary, or higher levels of schooling.

A cutoff point of 50% illiteracy among women 15–44 years of age has been suggested to define greater vulnerability to vitamin A deficiency, along with information on food availability and dietary patterns [115]. This cutoff point may also be useful to predict vulnerability to zinc deficiency, although it is important to recognize the arbitrary nature of this definition. In situations where the only information available is education of the head of household, it can also be used as an overall population level proxy for education because maternal education has been shown to correlate with the education level of the head of household.

Income

The association between poverty and child nutrition has long been recognized, and anthropometric indices of children under 5 years of age are often used as an indicator of socioeconomic development. Because poor populations often rely heavily on monotonous plant-based diets low in animal products and high in phytate content, poverty is bound to be associated with poor zinc status. Reliable income data are not widely available because they are difficult, time-consuming and expensive to collect. This is particularly true among populations relying on agriculture as their main means of subsistence, which constitutes a large proportion of the poor in lower-income countries. Similarly, collecting income data on individuals in urban populations who may have up to three different occupations, or who are self-employed or working in informal or even illegal activities, poses similar challenges in the assessment of income. Food expenditure data are often used as a proxy for income, but these data are also time-consuming and expensive to collect and are subject to recall bias. Economists have not yet agreed on a simple, reliable, proxy measure for income, but some commonly used alternatives are listed below:

» Number of household assets (e.g., furniture, television, electrical appliances, vehicles, etc.);
» Value of household assets (estimated value of assets possessed by the household);
» Type and quality of dwelling (e.g., whether dwelling is a room, apartment or house; whether the house is considered formal or informal; construction material of the walls, roof, floor; availability of water and sanitary services). (Because these variables are highly correlated, they are sometimes combined into an index using factor analysis or other data reduction approaches.);
» Availability of electricity (more useful at the community level, as the majority of households in a community are likely to have electricity if it is available);
» Access to land (for rural areas: land ownership, size of land, size of cultivated land).

Employment

In urban areas, where populations are highly dependent

on cash income, employment can be a useful indicator of standards of living. It may also be possible to rank different types of employment according to a salary scale. For example, unskilled laborers usually have lower wages than skilled laborers, and factory workers have lower salaries than office or bank employees. Thus, type of employment (sometimes referred to as 'functional groups') may be used as a crude indicator of socioeconomic status. Other potential indicators at the household level include the ratio of adults generating income per capita, and the gender of the head of household. Women-headed households are commonly found in urban areas and constitute a particularly vulnerable group because these women must assume complete responsibility for their households' livelihood and food security, and they often receive lower wages than do men for the same employment.

In rural areas, type of employment can be useful to predict income, but access to land is likely to be more important in agrarian societies. A potentially useful classification is subsistence versus cash-cropping agriculture to reflect differences in income. Similarly, the specific type of agricultural production that households are engaged in is relevant for the zinc supply and producers could usefully be classified into those who produce animal products (e.g., cattle-rearing, small animal husbandry, aquaculture) and those who do not.

Access to health, water, and sanitation services

Populations with poor access to health, water and sanitation are at increased risk of infectious diseases, which increases the risk of zinc deficiency. More remote and deprived populations often have poorer access to these services. Data on the percentage of the population with access to health, water and sanitation services are usually available at the national level and are often disaggregated at the provincial and district level and by urban and rural areas. The types of indicators typically available include the proportion of the population with access to the following:

» an adequate and safe water supply
» sanitary services
» health services

2.6 Indicators of the risk of excess zinc intake

The clinical symptoms of overt zinc toxicity and the biochemical changes that may represent subclinical zinc toxicity were described in detail in section 1.7 (zinc toxicity). Presently used indicators for excess zinc intakes include biochemical indicators of copper status, such as serum or plasma copper concentration and superoxide dismutase activity in erythrocytes [5, 37, 121]. Any statistically significant, negative change in biochemical indicators of copper status has been interpreted to be indicative of zinc toxicity. However, the physiologic significance of small changes in these indicators has not been defined. Changes in copper status indices to identify a toxic effect of supplemental zinc should be evaluated with caution in populations where infections are common; supplemental zinc has well-documented effects on reducing the prevalence of certain infections (section 1.4), and a decline in copper status indicators may reflect the shift from elevated circulating copper concentrations that are normally observed during infections, back down to concentrations normally observed in a non-infectious state [122]. In such cases, it would be preferable to interpret changes in copper status in relation to the proportion of cases that fall outside the normal reference range or to restrict the analysis to uninfected individuals. Further research is required in this area.

Although supplemental zinc (50 mg zinc/day for 10 weeks) has been shown to decrease iron stores of healthy, adult subjects, as measured by serum ferritin [123], the use of iron status as a reliable and specific indicator of excess zinc intake may be problematic in many settings. This in large part would be due to the likely, frequent co-occurrence of zinc and iron deficiencies, and the subsequent suitability of including both minerals in the same supplement formulation, thus making it difficult to attribute changes in iron status to the intake of supplemental zinc. Nonetheless, whether zinc is given alone or together with iron, it is recommended that iron status be monitored to assure that no negative impact on iron status results.

Declines in the serum concentration of high density lipoproteins have also been observed to occur during zinc supplementation [124, 125] and this biochemical alteration may also reflect zinc toxicity. The mechanisms for, and possible pathological significance of these changes in lipoprotein metabolism are not well defined.

2.7 Summary

Several factors must be considered before deciding whether to initiate an intervention program to reduce the rate of zinc deficiency and what type of program(s) might be appropriate. The first, and most obvious, question is whether zinc deficiency does, in fact, occur in the population with a frequency or degree of severity that should be considered a public health problem. Unfortunately, because of the lack of easily interpretable indicators of zinc status, it is still difficult to classify countries definitively with regard to the prevalence of zinc deficiency. Nevertheless, as an interim measure, it is possible to assess the likelihood that zinc deficiency is an important public health problem by reviewing existing information on the

adequacy of zinc in the national food supply, the rate of stunting in preschool children, and other possibly contributing ecological factors. The suggested steps in conducting this assessment are summarized in the following paragraphs and the recommended responses to this information are shown in figure 2.8.

As described in section 2.2.4, when ≥ 25% of the population is at risk of inadequate zinc intake, based on the amount of absorbable zinc available in the national food supply, the population may be considered to have an elevated risk of zinc deficiency. Also, because childhood stunting seems to be zinc responsive, a prevalence of stunting ≥ 20% may indicate an increased likelihood of zinc deficiency. Thus, if a country has ≥ 25% of the population at risk of inadequate zinc intakes based on data from the national food supply or from direct assessments of dietary intakes, and ≥ 20% stunting among preschool children, the country should be considered at high risk of zinc deficiency (figures 2.2 and 2.3). By contrast, if the country has < 15% of the population at risk of inadequate zinc intakes, and < 10% prevalence of stunting, it is unlikely that zinc deficiency is a major public health problem. Those countries with either moderate rates of stunting (≥ 10%, < 20%) or moderately low prevalence of inadequate zinc intakes (≥ 15%, < 25%) are considered to have an intermediate level of risk of zinc deficiency. Further evidence to support determination of the level of risk in a population can be derived from indirect indicators of zinc status, such as rates of iron-deficiency anemia, or predominant diet types, as described in section 2.2.

The following initial assessments are recommended:

» If a country is decidedly at low risk of zinc deficiency, no further population-level intervention is likely to be necessary, although evaluation and treatment of selected high-risk individuals may still be indicated in clinical settings. Segments of the population who are at elevated risk for zinc deficiency may have to be identified using more detailed regional and local data on indicators of zinc status; isolated groups at elevated risk may be defined by their stage of lifecycle, place of inhabitance (e.g., state, district, urban/rural), socioeconomic status, or other factors generally associated with disparity in nutritional status.

» When a country has an intermediate risk of zinc deficiency, further population assessment is appropriate. In this case, measurement of dietary zinc intake and/or serum zinc concentrations in a representative sample of the population is recommended. This will allow a more complete assessment of risk, and further information can be derived to identify segments of the population at highest risk and to guide the choice of appropriate intervention strategies.

» When a country has a high risk of zinc deficiency, either further assessment is indicated or planning for programmatic intervention may be initiated. If a decision is made to initiate an intervention program, further objective assessment is still desirable, so that baseline information will be available for future comparison.

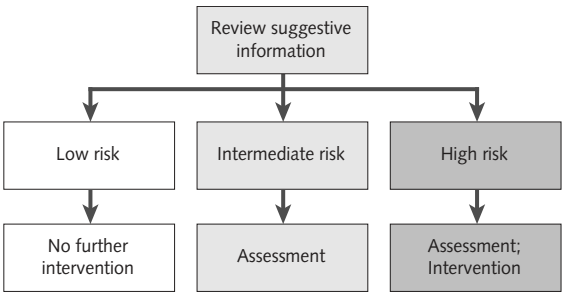

FIG. 2.8. Steps in assessment of population zinc status

A summary of possible indicators for assessing a population's risk of zinc deficiency and the suggested criteria for determining the magnitude of risk are presented in table 2.9. The range of possible indicators described relies on either suggestive information on the population's risk of zinc deficiency or on more objective measures of the population's zinc status. Using preexisting suggestive information on the national risk of zinc deficiency (namely, rates of childhood stunting and absorbable zinc contents of national food supplies) individual countries have been classified as having low, medium, or high risk of zinc deficiency, as shown in appendix 1.

There is an urgent need to develop better methods to assess the zinc status of individuals and populations and to evaluate the relationships of these biomarkers of zinc status to known functional consequences of zinc deficiency and excess. Pending the availability of such biomarkers, the risk of population zinc deficiency can be inferred from ecologic evidence, such as the absorbable zinc content of the food supply, rates of stunting, dietary zinc intake and possibly rates of anemia and other diseases. Research is needed to validate these proposed approaches to classify countries according to suggestive evidence of their risk of zinc deficiency against other markers of zinc status. With regard to dietary assessment, information is needed on the zinc and phytate contents of local foods; and simplified dietary methods, such as food frequency questionnaires or other techniques, should be developed and evaluated with regard to their ability to predict the risk of zinc deficiency. Although reference data are available for assessing the adequacy of serum zinc concentrations in relation to most age groups, sex, time of day and fasting status, additional information is still needed on appropriate cutoffs for children less than 3 years of age, elderly people, and pregnant and lactating women.

TABLE 2.9. Summary of indicators for the assessment of zinc status in a population

Indicator category	Measurement variable	Variable unit criteria	Recommended cutoff to identify an elevated risk of zinc deficiency
Suggestive evidence (existing health/ecologic information)	Rates of stunting among children < 5 years	Length-for-age or height-for-age Z-score < –2	> 20%
	Absorbable zinc content in the food supply, based on national Food Balance Sheets	Estimated percent of population with access to absorbable zinc in food supply below the weighted mean physiologic requirement	> 25%
Dietary	Adequacy of population zinc intakes based on dietary surveys	Probability approach: Probability of zinc intakes below the EAR (table 1.9)	> 25% of population at risk of inadequate intakes
		EAR cut-point method: Proportion of individuals with intakes below the EAR (table 1.9)	> 25% of population with inadequate intakes
Biochemical	Plasma/serum zinc concentration	Prevalence of low concentrations compared to appropriate age, sex, fasting status, and time of day cut-off (table 2.4)	> 20% below cut-off
Response to zinc supplementation	Change in weight-for-age or height-for-age Z-score among a representative sample of children	Effect size compared to appropriate control group	> 0.2 SD units; $p < .05$

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Chapter 3

Developing Zinc Intervention Programs

Once it is decided that programmatic intervention is needed, several factors must be considered for the development of suitable action. First, the evidence for zinc deficiency and its public health implications should be used to motivate the public and private sectors to develop interventions and to promote public acceptance of these actions. Based on information derived from population assessments, the distribution of zinc deficiency in the population will determine whether there is a need to reach the population as a whole and therefore establish national level programs, or whether the problem of zinc deficiency is isolated to specific high-risk groups, in which case targeted interventions may be more appropriate and efficient (section 2.1). The level of risk of zinc deficiency in the population will determine the urgency with which the situation needs to be addressed, and hence will influence the choice of intervention strategy, or combination of strategies. The choice of intervention strategies will also be influenced by the in-country resources available to develop and maintain the infrastructure and/or technology necessary to deliver and sustain the intervention. The three major categories of nutrition-focused zinc intervention strategies—supplementation, fortification, and dietary diversification/modification—are discussed in the following sections.

It is clear from the results of numerous zinc supplementation trials that a wide range of health benefits can be realized by increasing the intake of zinc where intakes were previously insufficient (section 1.4). These results argue strongly for the development of programs to improve zinc nutriture in high-risk populations. However, because it is unlikely that zinc deficiency will occur in isolation of other nutritional deficiencies and health problems, programs to address zinc deficiency should be incorporated into more comprehensive new or existing health and nutrition programs when possible. Opportunities for linking zinc interventions with existing programs are discussed in section 3.5.

3.1 Supplementation

3.1.1 General issues of supplementation programs

Supplementation refers to the provision of additional nutrients, usually in the form of some chemical (or pharmaceutical) compound, rather than in food. Supplementation programs are particularly useful for targeting vulnerable population subgroups whose nutritional status needs to be improved within a relatively short time period. For this reason, such programs are often viewed as short-term strategies. In many cases, however, supplementation programs may be the only effective strategy to reach specific target groups, such as vulnerable populations who may not be reached by fortification programs due to lack of access to processed foods, or young children and pregnant women, whose requirements for zinc may not be met, even with fortification or dietary diversification/modification programs. Even in higher-income countries, there is currently no alternative approach for pregnant women than to recommend use of iron and folate supplements. In such cases, supplementation programs need to be pursued over extended periods of time, or indefinitely. It is thus possible that supplementation will continue to be the approach of choice for some subgroups of the population and for some selected micronutrients, even in the long-term. On the other hand, for other, less vulnerable populations, targeted supplementation programs may need to be maintained only until the benefits of other longer-term approaches, such as fortification and dietary diversification/modification, begin to accrue.

For supplementation programs to be successful, a health system or other delivery channel must be able to provide a consistent supply, distribution, and delivery of the supplement to the targeted groups, and to encourage their use. All too often, supplementation programs have failed because of the absence of commitment at the national and community levels, lack

of supplement supply, poor coverage, poorly designed communication messages, and poor compliance. Increased burden to already overloaded health care delivery systems is also a contributing factor [1]. Poor compliance is a key limiting factor for the success of iron-supplementation programs, and has often been linked with the onset of side effects. However, recently it has also been attributed to several important behavioral barriers that may apply equally when zinc or any other supplements or medications are recommended for long periods, such as throughout pregnancy. Examples include concerns about long-term medications being harmful to the baby or resulting in a bigger baby, thus imposing difficulties during delivery [2]. A review of the World Bank's experience with supplementation programs suggests that key elements of success include social marketing efforts to raise awareness and create a demand, effective targeting of vulnerable population subgroups, increased outreach, and improved quality of services [3]. Further qualitative research is required to understand more fully the complex reasons for poor compliance in supplementation programs. Development of more innovative delivery systems and research to identify dosage schedules and chemical forms of supplements that lessen any unwanted side effects would also be useful.

Most of the existing experience with zinc supplementation is derived from research trials; we are not aware of any attempts to deliver zinc supplements in ongoing, large-scale programs. Issues that must be considered in the development of supplementation programs include the following: (1) the physical and chemical forms of the zinc compound; (2) the dosage level and frequency of administration; (3) the possible inclusion of other micronutrients in the supplement; (4) the administration of supplements with or without foods; (5) the packaging and distribution system; and (6) any possible risk of toxicity. Specific recommendations for zinc supplementation programs are discussed in section 3.1.2.

3.1.2 Choosing a supplement type, dosage, and method of administration

Chemical form of the supplement

There are several different chemical forms of zinc that can be used in supplements. Characteristics of available chemical forms of supplements are summarized in table 3.1 [4]. The costs of some zinc compounds ($US/kg zinc) are compared in table 3.3, in section 3.2, where fortification is discussed.

The choice of a particular chemical form should be based on its solubility in water, intragastric solubility, taste, cost, side effects and safety [5]. Water-soluble compounds are preferable because they are absorbed more efficiently. A number of studies have been conducted to assess the absorption of different chemical forms of supplemental zinc (zinc acetate, aminoate, ascorbate, citrate, gluconate, histidine, methionine, oxide, picolinate and sulfate), although results have been variable and sometimes conflicting in terms of their relative absorption [6–9]. In general, water-soluble compounds, such as zinc acetate, zinc gluconate, and zinc sulfate, are considered to be more readily absorbable than compounds with limited solubility at neutral pH. Some studies suggest that zinc oxide is poorly absorbed because its low solubility at the basic pH of the small intestine may prevent it from dissociating in the gastro-intestinal tract [8–10]. However, this may only present a problem when gastric acidity is reduced, as may occur in malnourished children. In a study of pregnant adolescents receiving prenatal supplements containing iron and elemental zinc as oxide or sulfate, plasma zinc levels of those

TABLE 3.1. Characteristics of zinc compounds available for supplementation (adapted from [4])

Compound	Color	Taste	Odor	Solubility in water (20°C)
Zinc acetate	White/slightly efflorescent	Astringent	Slight odor of acetic acid	Soluble
Zinc carbonate	White	Astringent	Odorless	Insoluble
Zinc chloride	White	Astringent	Odorless	Soluble
Zinc citrate	White		Odorless	Slightly soluble
Zinc gluconate	White		Odorless	Soluble
Zinc lactate	White		Odorless	Slightly soluble
Zinc methionine	White	Slightly sour and bitter	Vanilla odor	Soluble
Zinc oxide	White, gray, yellowish white	Bitter, astringent	Odorless	Insoluble
Zinc stearate	White		Faint	Insoluble
Zinc sulfate anhydrous	Colorless		Odorless	Soluble
Zinc sulfate heptahydrate	Colorless	Astringent	Odorless	Soluble

receiving the supplemental zinc oxide (25 mg zinc) remained at levels comparable to those of the unsupplemented women; only those receiving the zinc sulfate supplement (20 mg zinc) had increased plasma zinc levels [10]. Zinc methionine and zinc histidine have also been suggested [7, 11] because the amino acid ligands facilitate zinc absorption. However, the possible benefit of improved zinc absorption from these compounds may not justify their higher costs. More research needs to be carried out to compare the relative absorption, cost, and acceptability of various zinc compounds for use as supplements.

Physical form of the supplement

For infants and small children, zinc supplements have often been given in the form of a flavored syrup. Chewable tablets containing micronutrients with and without zinc (as amino acid chelate) have been used for school children [12]. Newer formulations provide tablets that are either chewable or dispersible in liquids. Recently, zinc has also been included in a mixture of micronutrients provided as a high-fat spread to be consumed alone or added to certain component(s) of the existing diet [13]. Another approach is the use of single-dose sachets of dry micronutrients (sprinkles), or crushable tablets that are added to food at the time of serving.

The optimal physical form of the supplement depends on the age of the target group, cultural preferences, and the possible desirability or need to include additional nutrients in the supplement. Young children will need to receive a liquid preparation or one that can either be made into a liquid in the household or added directly to foods, such as the sprinkles referred to above. In contrast, dry supplements (tablets, capsules or powders) are less expensive, more stable and permit inclusion of a broader range of nutrients. Recent trials with high-fat, micronutrient-fortified spreads suggest that these may provide another option for supplementation programs, although more experience is needed to assess their acceptability, efficacy, and impact on consumption of the usual household diet.

Considerations for providing zinc supplements with other supplemental nutrients or meals

Zinc supplements can be given alone or as an additional component of multi-nutrient supplements, such as prenatal iron and folate preparations. These supplements can be provided either with or between meals. In general, when minerals are consumed in the fasting or post-absorptive state, absorption is substantially greater because dietary components, particularly phytate, do not interfere with absorption [14].

When formulating multi-nutrient supplements, it is recommended that salts that are readily absorbed should be selected to avoid antagonistic interactions between zinc and other minerals. Interactions between zinc and calcium [15], zinc and iron [16], and zinc and copper [17, 18] have been described. When multi-micronutrient supplements are consumed with food, the presence of ligands in food appears to minimize the inhibitory effect of non-heme iron on zinc absorption [19–21] and vice versa [18, 22, 23]. Nevertheless, total zinc absorption is likely to be greater when the supplements are given apart from meals because of the inhibitory effect of many foods on zinc absorption. Interestingly, in one study Sandstrom et al. [19] gave iron and zinc supplements in a water solution with and without added histidine. Iron inhibited zinc absorption when the solution contained a high (25:1) iron:zinc ratio, but not when histidine, a ligand known to assist the absorption of zinc, was added to the solution.

Very few of the available zinc supplementation trials have provided details on whether the supplements were given with or without food. Therefore, it is difficult to assess whether the meals may have affected the efficacy of the interventions. In many of the studies of children, zinc supplements were given under the supervision of teachers, health care workers, or field staff, so it may be assumed that they were given without food. However, direct comparisons are needed to determine the implications of nutrient/food interactions on zinc absorption, and to assess the efficacy of various zinc dosage levels according to method of administration.

Frequency of administration

Zinc supplements probably should be given frequently, as most of the zinc in the human body exists in non-labile pools (e.g., muscle and bone) and is not readily released in response to zinc deprivation [24]. Nevertheless, some evidence suggests that providing zinc supplements less often than once daily may be efficacious. A supplementation trial among Gambian infants, for example, provided 70 mg zinc as zinc sulfate twice weekly for 1.25 years [25]. A significant improvement in arm circumference and a reduction in malarial incidence were observed. No significant effects were observed on the biochemical indices of zinc status or linear growth, but it is not certain whether zinc was the first growth-limiting nutrient in these infants. A supplementation trial among Vietnamese infants compared the efficacy of daily (5 mg) versus weekly (17 mg) zinc supplements [26] in a multi-micronutrient formulation containing vitamin A and iron. A comparable positive impact of both dosing regimens on linear growth among initially stunted children and a comparable improvement in serum zinc concentration were observed. Thus, it is conceivable that the functional impact of temporary improvement of zinc status lasts longer than the period during which the rapidly exchangeable pool of zinc is expanded. The only study to counter these findings is a study in rats, which showed that daily rather than intermittent doses of zinc were required to produce a growth response

that fully compensated for a previously deficient zinc intake [27]. Given the level of uncertainty and paucity of studies making direct comparisons of efficacy between daily and weekly supplements, daily provision (5–7 days/week) of zinc supplements is recommended at this time. Further studies are needed to compare the efficacy of different dosing regimens with zinc alone, or zinc in combination with other nutrients.

Recommended daily dosage levels

The appropriate dose of supplemental zinc for the prevention of zinc deficiency in different age groups and clinical conditions has not been studied systematically. Therefore, tentative recommendations have been derived based on the RDA for zinc (section 1.6) and with consideration of the dosages used in published clinical trials of zinc supplementation in various age groups. The suggested daily dosages of supplemental zinc are summarized by life stage group in table 3.2. These have been planned to avoid the possibility of chronic overdosage, as described in section 1.7.

The recommended doses for children 7 months to 3 years (5 mg/day), and for those greater than 3 years (10 mg/day), were derived by considering the RDAs (section 1.6), the NOAELs or upper limits (section 1.7), and dosage levels used in zinc supplementation trials. A meta-analysis of randomized, controlled zinc supplementation trials measuring effects of supplemental zinc on growth among children 6 months to 10 years of age showed an overall positive growth response to zinc among growth-retarded children with dosage levels that ranged from 1–20 mg/day [28]. There was no apparent association between dosage level and magnitude of the growth response, therefore suggesting that the lower zinc dosage levels, which approximate the recommended daily intakes, may be equally efficacious as higher doses in preventing growth retardation due to zinc deficiency. For young children recovering from severe malnutrition (weight-for-height Z-score < –3), a higher dose is recommended (10 mg/day) to cover the increased requirements for catch-up growth. Assuming that tissue accretion is approximately 30 g/day and 20 μg zinc is required per g of accrued tissue, the total physiologic zinc requirements are approximately doubled. As a result, the recommended daily dosage level for zinc was doubled for young children recovering from malnutrition. For pregnant women, doses of 15–30 mg elemental zinc per day (generally as zinc sulfate) have been used most frequently (table 3.2).

As noted in the foregoing sections, available evidence suggests that the absorption of supplemental zinc is much lower (approximately half) when given with foods or supplemental iron than when consumed in their absence. These recommendations may be modified upward if the supplements are designed to be administered with foods, particularly foods with high levels of inhibitors of zinc absorption, such as phytate (section 1.6). However, a specific recommendation for higher dosage levels when zinc supplements are to be given with food or iron containing supplements is pending, as direct comparisons of the efficacy of different zinc dosage levels in relation to the method of supplementation are currently lacking. There is presently very little information on the prevalence of copper deficiency in lower-income country populations. However, in populations or high-risk groups where copper

TABLE 3.2. Daily dosages of supplemental zinc by lifestage suggested by IZiNCG

Age/sex	Range (median) of zinc doses (mg/day) used in controlled trials	Number of trials represented	RDA suggested by IZiNCG (mg zinc/d)[a]	No Observed Adverse Effect Level suggested by IZiNCG (mg zinc/d)	Dose of zinc supplements recommended by IZiNCG (mg/day)
7–11 mo	5–20 (10)	9	3/5	6	5
1–3 yr	5–20 (10)	13	2/3	8	5
4–8 yr	3–10 (10)	7	3/5	14	10
9–13 yr	15–18 (17)	3	6/9	26	10
14–18 yr, M	—	—	10/14	44	10
14–18 yr, F	—	—	8/11	39	10
Pregnancy	20–30 (25)	2	11/15	39	20
Lactation	—	—	9/12	39	20
≥ 19 yr, M	—	—	13/19	40[b]	20
≥ 19 yr, F	—	—	7/9	40[b]	20
Pregnancy	9–45 (23)	11	9/13	40[b]	20
Lactation	15	1	8/10	40[b]	20
Severe malnutrition (children < 4 yr)	5–50 (40)	3	—	—	10

a. RDAs for mixed/refined vegetarian, or unrefined, cereal-based diets, respectively
b. Represent upper limits for zinc intakes

deficiency is suspected, possible adverse effects of zinc supplementation on copper status may be avoided by including copper in the supplement. Molar ratios of zinc:copper in the supplements should be ~ 10:1, up to a maximum of 1 mg/day of copper.

3.1.3 Zinc supplementation as adjunctive therapy for diarrhea

Results of several randomized clinical trials have shown consistently that zinc supplementation reduces the duration and severity of diarrhea in children [29]. Moreover, results of one metabolic study indicate that there is excessive fecal loss of zinc during diarrhea [30]. For both reasons, it seems worthwhile to include zinc supplements in the treatment regimen of children with diarrhea, particularly in settings where there is an elevated risk of zinc deficiency in the population. A group of experts in the management of childhood diarrhea who participated in a recently convened meeting on this topic concluded that, "There is now enough evidence demonstrating the efficacy of zinc supplementation on the clinical course of diarrhea, with regard to the severity and duration of the episode" [31]. They further recommended that zinc supplementation should be provided at a dose of about two times the age-specific RDA per day for 14 days, both to reduce the severity and duration of the episode and to replenish excessive zinc losses. As noted in section 1.8, it is possible that diarrhea or other conditions that affect intestinal health increase intestinal losses of endogenous zinc, thus increasing zinc requirements. Therefore, it is also conceivable that other programs to prevent or treat diarrhea may reduce the risk of zinc deficiency by decreasing excessive losses of zinc via the intestine.

3.1.4 Cost of including zinc in ongoing supplementation programs

Given the similar requirements for frequent administration of supplemental iron for prevention of iron deficiency, and possibly other micronutrients, it would be most feasible to include zinc in programs already delivering daily or weekly nutrient supplements. The only additional costs in delivery of supplements with zinc included are the cost of the zinc compound, additional costs of quality control during supplement manufacturing, and additional costs of measuring program impact in terms of improved zinc status. Based on an average cost of zinc as zinc sulfate of US$25.7 per kg (table 3.3), the additional cost of zinc would range from US$0.05–0.19 per person per year. Previously estimated costs of an iron supplementation program were in the range of US$3.17–5.30 per person per year [3]. While the cost of programs to provide daily supplements needs to be updated, it demonstrates that the additional cost of including zinc in an existing program is minimal. If program monitoring is to be included, the cost-model for wheat flour fortification programs (table 3.4) can be used to derive an estimate. This hypothetical model program was designed to reach 1,290,000 preschool children and include a sample of 1,500 children in the evaluation activities (three surveys over 5 years). This amounts to an additional US$6,000 per year for biochemical analysis of zinc status. Therefore, the additional costs for monitoring zinc status in such a population would amount to less than US$0.01 per person per year.

TABLE 3.3. Cost of zinc compounds (US$) in 2001

Compound	Cost per kg compound	Cost per kg zinc
Zinc acetate	10.2	28.6
Zinc carbonate	16.0	30.7
Zinc chloride[a]	32.5	67.8
Zinc citrate	8.0	23.4
Zinc gluconate[a]	20.9	145.6
Zinc methionine	25.4	83.4
Zinc oxide[a]	4.5	5.6
Zinc stearate[a]	4.9	47.4
Zinc sulfate[a]	10.4	25.7

a. Listed by the US Food and Drug Administration as "generally regarded as safe" (GRAS)

3.2 Fortification

3.2.1 General issues of fortification programs

Food fortification is defined as the addition of nutrients to commonly eaten foods, beverages or condiments at levels higher than those found in the original food, with the goal of improving the quality of the diet. In higher-income countries, fortification has played a major role in increasing the dietary intake of those micronutrients for which deficiencies are common and of public health concern; the contribution of fortification programs to the virtual elimination of micronutrient deficiencies in these countries is widely acknowledged [32].

In lower-income countries, fortification is increasingly recognized as an effective strategy to improve the micronutrient status of the population. Relative to other approaches, fortification is thought to be the most cost-effective means of overcoming micronutrient malnutrition [3]. Programs are designed such that success does not require changes in the dietary habits of the population, nor any personal contact with recipients. Public education is still required, however, to create a demand for the fortified products. Once a suitable fortification program is developed and established, it can be easily sustainable. Fortification programs represent long-term strategies that may effectively prevent

the development of nutrient deficiencies among their recipients, although fortification alone may not be adequate to treat existing deficiencies.

Where the micronutrient deficiency is widely distributed in the population, universal or national level fortification of centrally processed foods is an appropriate strategy. An example of a country with a nationwide zinc fortification program is Mexico, where zinc and other micronutrients are added to wheat and lime-processed corn flours that are used in preparing bread and tortilla, the two principal staples in the country. In the case where large segments of the population at risk do not have ready access to centrally processed foods, fortification may also be implemented at the community level. With the latter strategy however, quality assurance and control are more difficult to achieve.

Targeted fortification programs can be developed to increase the intake of zinc or other nutrients by specific segments of the population who are at elevated risk of zinc deficiency, such as infants, young children, or pregnant and lactating women. In this case, special-purpose foods, such as infant cereals, other processed infant foods, or foods distributed in school lunch programs, can be fortified and distributed or made available in the regular marketplace. There are several examples of the addition of zinc to foods in targeted fortification programs. In higher-income countries and in some lower-income countries infant formulas, infant cereals and ready-to-eat breakfast cereals are currently fortified with zinc. Several Latin American countries, including Guatemala, Peru, Colombia, and Mexico have used or are currently using centrally processed complementary foods for children that are fortified with zinc and other micronutrients [33]. Mexico also has developed a fortified, milk-based, beverage mix targeted toward pregnant and lactating women [33].

The government, food industry, research community, and consumer groups all play key roles in developing successful fortification programs; cooperation among these groups is extremely important for programmatic success and should be sought at an early stage of program development. A committee comprised of representatives of these groups should be created for planning, designing, promoting and regulating the fortification program. The government generally plays a vital role as the initiator, coordinator, and monitoring agency. The scientific community should be involved in determining the prevalence of zinc deficiency, the absorption and sensory acceptability of the chosen zinc salt, and the efficacy and effectiveness of the zinc-fortification program. If results from scientific studies indicate that zinc fortification is an efficacious and effective strategy to reduce zinc deficiency, the government should create legislation to implement an intervention program. The food industry can help researchers in defining feasible, affordable fortification strategies, in the identification of appropriate food vehicles and fortificants, in the definition, development and implementation of quality assurance systems and in educational efforts to reach target populations. Consumer groups are able to represent any users' concerns regarding the suitability of the fortified products.

Technical issues of specific relevance to the inclusion of zinc in food fortification programs are discussed in section 3.2.2 below. Considerations for the costs of including zinc in existing fortification programs are discussed in section 3.2.3.

3.2.2 Technical considerations for zinc fortification programs

Selection of the food vehicle(s)

Ideally, a sizable proportion of the target population should consume a proposed food vehicle in relatively constant amounts so that the fortification will result in a predictable and fairly stable level of intake of the added nutrient. The food should be able to be processed in units large enough to permit controlled fortification, should not have any objectionable changes in taste, color or appearance after fortification, should retain appropriate levels of the added nutrients after further processing or cooking and should not be consumed in amounts that present a risk of consumption of toxic levels of the fortificant in any segment of the population [32]. Information derived from dietary surveys used in the initial assessment of a population's risk for zinc deficiency can be used to identify appropriate food vehicles and usual amounts of foods consumed by different segments of the population or target group. Food vehicles that are candidates for universal fortification include staples such as rice, wheat, and maize, or condiments, like salt, that are consumed by a large proportion of the population.

Selection of the form of zinc fortificant

There are several zinc compounds that are available for fortification (table 3.1). Of these, zinc chloride, zinc gluconate, zinc oxide, zinc stearate, and zinc sulfate are listed by the US Food and Drug Administration as generally recognized as safe (GRAS). There is no consensus as to which of the GRAS compounds is most appropriate for fortification programs, especially when gastric acid production is reduced, as may occur more frequently in lower-income countries. Zinc salts vary in their solubility in water and range from very soluble (zinc acetate, zinc chloride, zinc gluconate, and zinc sulfate), to slightly soluble (zinc citrate and zinc lactate), to insoluble in water (zinc carbonate, zinc oxide, and zinc stearate). Water-soluble compounds are generally better absorbed than less soluble or insoluble compounds. As mentioned above, the chemical form of zinc chosen for fortification must not alter the organoleptic characteristics of the final product. Finally, the zinc compounds mentioned above vary

in their cost, which should also be taken into account during the selection (table 3.3). Zinc sulfate and zinc oxide are the GRAS salts that are least expensive and most commonly used by the food industry. Of these, zinc sulfate theoretically should provide more reliable absorption because of its greater solubility, although it is more expensive than zinc oxide. Despite these theoretical concerns about zinc solubility, two recent studies found no difference in zinc absorption from wheat products fortified with either zinc oxide or zinc sulfate [34, 35].

Determining the level of zinc fortificant

The proper level of zinc fortification is that which would increase the intake of zinc by the targeted individuals, without imposing a risk of excessive intake on the rest of the population. The IZiNCG SC recommends an intake of no more than 40 mg of zinc per day by adults. To determine the appropriate level of fortification, it is necessary to measure or estimate the amount of the food vehicle being consumed by different segments of the population. If, for example, adult men and women consume a mean of 100 g of cereal flour/day and pre-school children consume 50 g/day, fortification of the flour with 60 mg zinc per kg of flour would provide 40%, 67%, and 100% of the respective RDAs, assuming that the diet is based mainly on unrefined cereals (table 1.10). To reach daily zinc intakes that are considered excessive, adults would have to consume approximately 667 g/day of cereal flour fortified at this level and children would have to consume approximately 267 g/day of the fortified flour, both of which seem unlikely. Recently, participants of a conference on zinc in human health concluded that the appropriate levels of zinc fortification of cereal staples generally should be between 30 and 70 mg of zinc per kg of flour [11].

Consumer acceptability of zinc-fortified foods

Sensory trials are necessary to determine whether the chosen zinc compound and level of fortification alter the organoleptic qualities of the fortified product and to assess consumer acceptance. For example, Saldamli et al. [36] found that fortifying wheat flour with 300 mg of zinc as zinc acetate per 100 g of flour did not affect the rheologic or baking properties of the wheat dough and that the sensory properties of the breads were acceptable. Sensory trials can also be used to compare organoleptic qualities and consumer acceptance of products fortified with different forms of zinc and at different levels of zinc fortification. For example, the sensory acceptability of wheat products made from wheat flour fortified per kg of flour with 30 mg of iron as ferrous sulfate alone, or both iron and either 60 or 100 mg of zinc as either zinc sulfate or zinc oxide were assessed [37]. The authors concluded that zinc-fortified bread and noodles were well accepted, regardless of the chemical form of zinc, even at 100 mg of zinc/kg of flour, although noodles fortified with iron and zinc oxide were slightly less acceptable than those fortified with iron and zinc sulfate, especially at the higher levels of zinc fortification.

Determining the absorption of zinc from fortified foods

Some potential food vehicles may have high amounts of inhibitors of zinc absorption, such as phytate, which can affect the absorption of zinc fortificants added to these foods. Since experience with zinc fortification is still limited, it is worthwhile to conduct absorption studies, using either radioisotopes or stable isotopes of zinc, to quantify the absorption of different fortificants used in candidate vehicles before final selections of fortificants and vehicles are made. Available techniques to measure zinc absorption are summarized in appendix 3.

Monitoring and evaluation issues

Once the fortification program is in place, the effectiveness of the program to reduce zinc deficiency in the target group must be monitored and evaluated. A system should be created to monitor changes in population zinc status, using the same indicators described in chapter 2. The quality of the fortified product also must be monitored on a regular basis, both at the level of the production site and at the point of purchase, to ensure that it contains an appropriate amount of the fortificant. General information on monitoring programs can be found in section 3.6. Published guidelines set for monitoring and evaluation of iron fortification programs [38] may be consulted for useful information that is also relevant for monitoring zinc fortification programs.

3.2.3 Cost of including zinc in ongoing fortification programs

Estimating costs is an important step in planning a food fortification program. Such estimates must include both industry costs (e.g., capital investment and recurrent costs, such as the purchase of fortificant) and public sector costs (e.g., quality control, monitoring and evaluation).

Zinc fortification is unlikely to occur independently of other micronutrient fortification programs. Wheat flour is the most widely fortified staple food product, and iron is the most frequently added nutrient in large-scale fortification programs. The cost of a national program to fortify wheat flour with iron has been estimated [32, 38] and this detailed estimation is used here as a model to determine the additional costs of adding zinc to an existing program.

The cost of establishing a national wheat flour fortification program will vary according to factors such as the number and size of mills, existing quality

TABLE 3.4. Estimated hypothetical costs (US$) of wheat flour fortification with iron and zinc[a]

	Annual cost of iron fortification	Additional annual cost of including zinc	Total annual costs
A. Industry Costs			
1. Capital investment	820	0	820
2. Recurrent Costs			
Equipment (maintenance, depreciation)	600	0	600
Ferrous sulfate fortificant[b]	57,090	—	57,090
Zinc sulfate fortificant[c]	—	102,600	102,600
Quality control	7,920	2,880[d]	10,800
Total industry costs	66,430	105,480	171,910
Cost per MT fortified wheat flour	0.66	1.05	1.72
B. State Costs			
1. Capital investment and maintenance	2,625	0	2,625
2. Mill inspection and monitoring (salaries & transportation)	3,500	0	3,500
Laboratory analysis and reports (including technician salaries)	1,000	96[e]	1,096
Quality assurance and monitoring training	1,500	500[f]	2,000
3. Program monitoring (dietary intake; travel, per diem, analysis, reports)	1,400	0	1,400
4. Evaluation			
Travel, per diems, and collection of biological samples	3,000	0	3,000
Laboratory analysis and reports (including technician salaries)	5,000	3,600[g]	8,600
Total State costs	18,025	4,196	22,221
Total program costs	84,455	109, 676	194,131
Cost per MT fortified wheat flour	0.84	1.10	1.94

a. Adapted from [38]
b. Cost of ferrous sulfate (US$8.65/kg elemental iron, including additional 33% for shipping) added to 100,000 MT wheat flour at 66 ppm
c. Cost of zinc sulfate (US$34.20/kg elemental zinc, including additional 33% for shipping) added to 100,000 MT wheat flour at 30 ppm
d. Additional costs of zinc analysis: 10 samples fortificant premix (5 samples per lot @ 2 lots per year) @ $4 per sample = $20; 2 analyzed samples per day, 360 d per year @ $4 per sample = $2,880 annual (semi-quantitative analysis of iron from samples taken every 2 hours should serve to ensure presence of zinc once zinc content of premix is analyzed)
e. Additional cost of analyzing fortified flour samples collected at market; one sample per month analyzed in duplicate, 12 months/year @ $4 per sample = $96
f. An additional 50% of the cost of quality assurance and monitoring training was included for zinc assessment.
g. Program evaluation for serum zinc analysis based on a sample of 1,500 preschool children @ $4 per sample = $6,000 per assessment; assessments conducted three times in a 5-year period (baseline, 12–15 months, and 5 yr post-program initiation) = $18,000/5-year period or $3,600 annually

assurance facilities, functional regulatory and food inspections, and the quantity of micronutrients being added [38]. The estimated annual cost of a program that fortifies 100,000 metric tons (MT) flour per year with 66 ppm (parts per million) elemental iron in the form of ferrous sulfate at one mill using a continuous fortification system is US$84,455, considering amortization of the capital investment over 10 years. In this example, the cost of the iron fortificant is 68% of the total program cost. The additional cost of including zinc in this program will be the cost of adding zinc fortificant in the iron premix, quality control in production, and monitoring and evaluation. Current prices of zinc fortificants in the international market are given in table 3.3. The cost for a particular industry in a specific country should include the product cost plus shipping to the country, importation taxes, and the cost of transportation within the country.

Although most costs can be shared with the cost for quality control, monitoring and evaluation of the iron fortification program, some additional cost should be added to account for the laboratory analysis for both quality control determination and for zinc determination in biological samples during program monitoring and evaluation. The estimated additional costs, US$4,196 per year, are detailed in table 3.4.

Table 3.5 provides a summary of the estimated additional annual costs of including zinc in a wheat flour iron-fortification program using either zinc oxide or zinc sulfate as the fortificant forms. Each fortificant type was included at two different levels, 30 and 70 ppm zinc, representing the lower and upper levels of the recommended range for addition of zinc fortificant. The price of mixing the zinc and iron is assumed to

TABLE 3.5. Estimated additional program cost of adding zinc to iron-fortified wheat flour

Zinc source	Cost of zinc fortificant (US$/kg zinc)	Plus 33% shipping costs	Fortification level (ppm)	Additional cost per MT of flour (US$)	Additional cost for 100,000 MT of flour	Total additional cost (including quality control and evaluation)[a]	% of cost of iron fortification program[b]
Zinc oxide	5.6	7.4	30	0.22	22,200	29,276	35
			70	0.52	51,800	58,876	70
Zinc sulfate	25.7	34.2	30	1.03	102,600	109,676	130
			70	2.39	239,400	246,476	292

a. The additional cost of quality control and evaluation of zinc fortification was estimated at US$7,076/year
b. The total cost of iron fortification of 100,000 MT of wheat flour with ferrous sulfate at 66 ppm, including quality control, monitoring and evaluation was estimated at US$84,455 (adapted from [38])

be negligible and therefore the increase in the cost of the zinc/iron premix is due only to the cost of the zinc fortificant. In the example of adding zinc sulfate to provide 30 ppm zinc, the fortificant cost was estimated at US$25.70 per kg zinc (table 3.3) plus an additional 33% for shipping, giving US$34.20 per kg additional cost to the plant. The additional cost per MT flour (US$34.20/kg × 30 kg zinc/1000 MT ×1 MT/1000 kg) is US$1.03 per MT or US$102,600 for the 100,000 MT. After accounting for quality control analyses at the industry level, and costs for equipment and monitoring and evaluation (US$4,196), the total program cost increases from US$84,455 to US$194,131. It is clear that the majority of additional program costs are due to the cost of the zinc fortificant; therefore reduction in zinc fortificant prices that may occur with increased demand would contribute substantially to limiting the additional costs of including zinc in an existing fortification program.

3.3 Dietary diversification/modification

3.3.1 General issues of dietary diversification/modification programs

Strategies to diversify or modify the diet aim to enhance the access to, and utilization of, foods with a high content of absorbable zinc throughout the year. These strategies can involve changes in food production practices, food selection patterns, and traditional household methods for preparing and processing indigenous foods. Dietary diversification/modification represents a sustainable, economically feasible, and culturally acceptable approach that may be used to improve the adequacy of dietary intakes of several micronutrients simultaneously with limited risk of antagonistic interactions.

Dietary diversification/modification strategies encompass a wide variety of approaches, but all are generally regarded as long-term strategies in terms of development, implementation, and potential for impact. They are often described as a sustainable approach because the process empowers individuals and households to take ultimate responsibility over the quality of their diet through self-production or acquisition of nutrient-rich foods and informed consumption choices [39]. Once the expected behavior changes are achieved, it is also expected that inputs will be minimal as the practices become self-perpetuating through the natural mechanisms of information sharing. Because impacts are likely to be achieved only in the long term, however, these strategies should be implemented jointly with other shorter-term approaches, such as supplementation and fortification, as required, to address the needs of specific target groups.

As discussed in section 1.6, diets in lower-income countries are often based predominantly on cereals and legumes or starchy roots and tubers, while consumption of foods with a high content of readily absorbable zinc, such as meat, poultry, and fish, is often limited because of economic, cultural and/or religious constraints. Dietary strategies described herein are directed toward improving intakes of absorbable zinc, some of which aim to increase the total intake of zinc, whereas others aim to enhance zinc absorption by altering the levels of food components that modify zinc absorption. Some strategies can be implemented at the level of agricultural production, whereas others are directed toward community or household level application.

The conditions for success of dietary diversification/modification strategies will vary depending on the specific approach used. For example, new agricultural strategies must not compromise crop yield, they must not involve additional costs for farmers, and they should not significantly alter the organoleptic and overall nutritional quality of the products. Dietary interventions involving changes in production, processing or consumption patterns must be practical, culturally acceptable, economically feasible, and sustainable for the target group. At the household level, they must not increase the cost or result in increased time and workload required by the caregiver, or require substantial changes in the types and quantity of foods consumed. Specific strategies that may be directed to improving

total or absorbable zinc intakes are summarized in the sections below.

3.3.2 Agricultural strategies to increase total and/or absorbable zinc content in staple foods

Several strategies can be used to increase the zinc content of plant-based staples. These include the use of zinc fertilizers and plant breeding and genetic modification techniques. Although these methods appear promising, more research is needed to evaluate their economic, environmental, and health effects before these strategies can be recommended for implementation. These techniques, referred to as "field fortification," are described below.

Zinc fertilizers

When applied to zinc-deficient soil, zinc-containing fertilizers can increase the zinc content of cereal grains, and this technique is used extensively to enhance yields under these conditions. For example, in Turkey the zinc concentrations in wheat grains (7–12 mg/kg) are well below the accepted critical levels of zinc for adequate plant nutrition, and application of zinc to the soil at a rate of 22 kg of zinc per hectare raised the mean concentration of zinc in the plant from 8 to 13 mg/kg [40].

Plant breeding

Plant breeding can produce new cereal varieties that have higher grain zinc concentrations than pre-existing wild strains and that better tolerate zinc-deficient soils. These zinc-efficient genotypes are also more disease resistant and have improved seedling vigor, enhanced germination, and a higher grain yield [41]. Hence, their use will not decrease crop productivity or increase costs to farmers.

The identification of crop varieties that naturally contain high levels of specific micronutrients has been aided by the germplasm screening approach. Research has focused on five main crops (rice, wheat, maize, beans, and cassava) and on three micronutrients (iron, zinc, and β-carotene). All crops show significant genotypic variation in mineral content of up to twice that of common cultivars. In one study of rice grains, for example, zinc concentration averaged 24.5 ppm with a range of 13.5 to 41.6 ppm [42]. The range of genotypic differences in zinc (and iron) concentration measured in maize was around 50% of the mean value.

Plant breeding has also been used to develop mutants of corn, barley, and rice with more than 50% reduction in levels of phytate phosphorus in the kernels [43]. A 78% increase in average zinc absorption was reported in a study of five adults when fed a corn-based polenta diet in which the phytate content of the corn was reduced by 55–63% of the parent, wild-type variety [44]. Iron absorption from a low-phytate maize mutant was also improved by 50% (i.e., from 5.5% to 8.2% of intake) compared with the wild-type strain maize [45].

The amino acids methionine and cysteine form soluble ligands with zinc, and thus enhance its absorption [46]. Maize has shown some genetic variability in content of these amino acids, the highest being about 50% above the lowest levels [42, 47]. Only a small increase in the concentration of these amino acids in the diet may be needed to enhance the absorption of zinc (or iron), and therefore it is unlikely to be a constraint for plant functions [47]. At this time, there is little information about the agronomic advantages or disadvantages to increasing the concentration of sulfur-containing amino acids in staple foods. The efficacy of this modification in improving total zinc absorption in humans should be quantified in comparison with the other strategies mentioned above.

Genetic modification

Genetic engineering has recently been employed to produce rice grains containing an increased content of iron as well as a significant amount of β-carotene in the endosperm [48]. With additional research, this technique could be applied to enhance the zinc content of rice and other cereal grains.

Genetic modifications can also be used to incorporate phytase enzymes (myo-inositol hexaphosphate phosphohydrolases) into staple crops. This would dramatically decrease their phytic acid (myo-inositol hexa phosphate:IP6) content; phytase enzyme hydrolyzes phosphate groups from the inositol ring to yield intermediate myo-inositol phosphates (bi-, tri-, tetra-, and penta-phosphates; [49]). As noted in section 2.3.1, myo-inositol phosphates with less than five phosphate groups (i.e., IP-4 to IP-1) do not inhibit zinc absorption [50].

Genetic modification can also be used to increase the level of promoters of zinc absorption in plant-based staples. A gene that codes for a sulfur-rich metallothionein-like protein has recently been introduced into rice *(Oryza sativa)* to increase iron absorption [51]; zinc absorption would be expected to improve simultaneously.

3.3.3 Strategies to increase production and/or intake of zinc-rich foods

To increase the zinc content of diets, small-livestock husbandry, aquaculture, and production of other indigenous zinc-rich foods can be promoted, where feasible. Education and behavior change strategies can also be used, either alone or in combination with production activities, to promote greater intake of zinc-rich foods.

Small livestock production

Production of a variety of small livestock (e.g., guinea pigs, poultry, rabbits, and small ruminants) can be promoted to increase the availability of these zinc-rich foods within a community or household. Education is key to the success of these interventions, and efforts must be made to ensure that the livestock produced is not entirely sold for cash. Some of the food that is produced should be reserved for household consumption and targeted to those household members at higher risk of inadequate zinc intake. Regrettably, evidence from Bangladesh and Ethiopia suggests that the increases in household income achieved through increased livestock production do not necessarily translate into improved dietary quality among producer households [52, 53]. Nutrition education and behavior change interventions seem to be essential in achieving nutritional impact because the increases in income may be invested in basic necessities or consumer goods other than food [39]. It is also important to ensure that small livestock are not regarded by producer households as ceremonial foods (e.g., guinea pigs in highland Ecuador) that are only consumed on special occasions. A limitation of this type of intervention is that animal product intake is sometimes constrained by cultural or religious factors that prohibit their inclusion in the diet.

Aquaculture

Aquaculture may be a more suitable strategy in countries where economic, religious, and/or cultural factors prevent the consumption of meat and poultry. Inclusion of fish (whole) can increase the content and density of zinc and other nutrients (fat, iodine, iron, selenium, niacin, and riboflavin) in the diet. Use of whole dried fish is more desirable as it contains more zinc than fish flesh without bones [54, 55] and also does not require refrigeration. Fish flour or meal prepared from small, whole dried fish, including the bones, can be used to enrich cereal-based porridges for infant and child feeding. A new farmer-focused, systems approach has been carried out successfully in Bangladesh and Malawi whereby aquaculture was incorporated into existing farming systems with the minimum of investment [56]; further exploration of these methods is recommended. As was described for small livestock production, promotion of aquaculture must also include educational efforts to ensure that increased production translates into greater intakes by vulnerable groups.

Indigenous zinc-rich foods

Agronomic and genetic improvements have led to the development of higher-yielding genotypes of some indigenous wild plants (e.g., wild fruits, nuts, seeds, leaves), as well as varieties that are resistant to drought or heat stress, tolerant of poor soils, and easily cultivated and accepted by local rural communities [57]. In some regions (e.g., Korea, Vietnam, Sahel in Western Africa, Zambia), an inventory of certain edible species has been compiled and some analyses of minerals undertaken [58]. Sago grubs, which are consumed in Papua New Guinea, and locusts, which are consumed in Malawi during certain seasons, are rich sources of zinc [59]. More information is required on the content of zinc and zinc absorption modifiers in local indigenous foods to identify those that might be suitable sources of absorbable zinc.

Processed snacks

The absorbable zinc content of processed food products such as chips or noodles can be enhanced by incorporating zinc-rich food items, such as dried fish, fish liver, and other organ meats. In Thailand, for example, beef or chicken livers are used to enrich a popular, locally produced snack food prepared from a 2:1 mixture of sago flour and tapioca flour and processed by steaming to enhance vitamin A retention [60].

3.3.4 Household food processing methods to increase absorbable zinc in the diet

At the household level, reduction in the phytate content of the diet can be achieved in two ways: (1) by inducing activity of plant-associated phytase (myo-inositol hexaphosphate phosphohydrolases; EC 3.1.3.26) and the enzymatic hydrolysis of phytic acid (myo-inositol hexaphosphate) through germination, fermentation, and soaking; and (2) via diffusion of water-soluble phytate through soaking.

Germination

Most cereal grains and legumes contain some endogenous phytase, but the activity varies among species and varieties. Endogenous phytase activity is high in rye and wheat, but very low in maize and sorghum, and is negligible in dry or dormant seeds. Germination increases the activity of endogenous phytases in cereals and legumes as a result of *de novo* synthesis or activation of the enzyme, although the extent of this increase also depends on the species and variety [61]. After 2 to 3 days of germination, the hexa-inositol phosphate content of cereals is reduced by 13–53% in cereals and 23–53% in legumes [62, 63]. Soaking also activates endogenous cereal phytases, and the level of activity varies with temperature and pH [62].

Flours prepared from germinated grains can be added to ungerminated flours to promote phytate hydrolysis during food processing. For example, addition of 10% germinated maize flour to maize dough decreased the phytate content of *kenkey* (a traditional maize dough in Ghana) by 56% [64]; even greater phytate reductions can be achieved if these doughs or flour slurries are incubated at the optimal temperature, pH, and time to maximize phytase activity. A similar process can

be applied to lower the phytate content of porridges used in infant and young child feeding. In this case, use of germinated flour has an added advantage in that denser porridges can be prepared (e.g., with 20–28% dry matter and a comparably higher zinc density), as the high amylase content of the germinated flour decreases water-binding capacity of the starch and lowers the viscosity of the porridge to that of a porridge with lower dry matter content (e.g., 7–10% dry matter). The result is a porridge with higher energy and nutrient density and a lower phytate:zinc molar ratio.

Care must be taken when using amylase-rich foods to ensure that the porridges are decontaminated by heating prior to use. Germination may increase the concentration of *Enterobacteriaceae*, fungi, *bacillus* species, etc., including potentially pathogenic and toxinogenic species [65]. If germinated cereal grains are not thoroughly dried before being milled into flour, they may become contaminated with aflatoxin, which is produced by *Aspergillus flavus*, *A. parasiticus*, and *A. nomius* when storage conditions are poor (i.e., 88–95% relative humidity; 25–30° C) [58].

Fermentation

Fermentation can induce phytate hydrolysis via the action of microbial phytases (EC.3.1.3.8), which can originate either from the microflora on the surface of cereals and legumes or from a starter culture inoculate [66]. The levels of phytate reduction reportedly achieved by fermenting cereal-based flour slurries or porridges are variable, but reductions of about 50% appear to be achievable [67]. A number of factors within the lactic acid fermenting system probably influence microbial phytase activity, and hence the level of phytate reduction achieved, including the types of fermenting organisms present, pH, incubation temperature, and ratio of solids to water [68]. High tannin content in cereals (e.g., bullrush millet, red sorghum) also appears to inhibit phytase activity [69].

In the future, commercial phytase enzymes prepared from *Aspergillus* o*xyzae*, A. *niger*, *or A. fumigatus* may be available for human use. Only the phytase prepared from *A. fumigatus* is heat stable, and hence can be incorporated into the staple food prior to cooking. The high cost of these enzymes is likely to preclude their use in many lower-income countries at the present time.

Soaking

Soaking can reduce the phytate content of certain cereals (e.g., maize and rice) and most legumes because their phytate is stored in a relatively water-soluble form, such as sodium and potassium phytate, and hence can be removed by diffusion. Levels of water-soluble phytate in legumes and cereals vary widely, ranging from only 10% in defatted sesame meal to as much as 70–97% in California small white beans, red kidney beans, corn germ, and soy flakes [70, 71]. Removal of water-soluble phytate can be achieved more effectively by soaking legume flours rather than whole legumes.* Reductions in IP5 + IP6 content ranging from 51–57% have been achieved, when maize flour is soaked and the excess water is removed by decanting [67]. In Malawi, a method of soaking pounded maize was found to be well accepted, and a nearly 50% reduction in phytate content of maize flours was achieved by the rural participants [72]. Soaking may also remove other antinutrients such as saponins and polyphenols. The potential loss of water-soluble nutrients following this procedure needs to be quantified.

Zinc absorption from cereal-based foods by humans has been improved by employing some of these processing methods to reduce phytate content, such as the fermentation of bread [73], and the germination and soaking of oats [74]. Animal studies have shown greater femoral zinc in rats fed diets containing fermented soybean meal than those fed regular soybean meal, due to the increase of zinc solubility in the small intestine [75]. Increases in *in vitro* levels of soluble iron have also been reported after fermenting porridges prepared from white sorghum and maize with a starter culture, with and without the addition of commercially prepared wheat phytase enzyme [69].

To date, the efficacy of these interventions to improve human zinc status has not been extensively tested. One community-based intervention study using a quasi-experimental design employed a range of dietary strategies to increase the content of micronutrients (including zinc) and to enhance the absorption of zinc among a group of Malawian children with maize-based diets and a high prevalence of stunting. The strategies were implemented using formative research and included promotion of the consumption of animal source foods, notably whole, dried, soft-boned fish, and reduction of the phytate content of the maize-based porridges. The latter was accomplished primarily by soaking maize flour prior to cooking, and also by encouraging the fermentation of porridges and the addition of germinated cereal flour during the preparation of the porridges [62]. After 12 months, which included a six month intervention period, Z-scores for mid-upper-arm circumference and arm muscle area ($p < 0.001$), although not weight or height Z-scores, were greater in the experimental group compared with the control group. After controlling for baseline variables, the incidence of common infections was lower in intervention children compared to controls, although with no change in malaria or hair zinc status [76]. These results corresponded to dietary changes in the treatment group, which resulted in diets that supplied significantly more animal source foods, especially soft-boned fish, and less phytate ($p < 0.01$), and in higher median intakes of absorbable zinc, and a reduction in the

* L. Perlas, personal communication.

prevalence of inadequate zinc intakes ($p < 0.01$) [77].

3.3.5 Programmatic experience with dietary modification/diversification strategies to increase micronutrient intake and status

During the past few decades, home gardening and nutrition education interventions using social marketing techniques have been popular food-based strategies, especially for the control of vitamin A deficiency [39, 78, 79]. More recently, a few innovative programs have been designed to address multiple micronutrients, including iron and vitamin C in addition to vitamin A (see Ruel and Levin [39]). None of the programs aiming at increasing the production and/or intake of micronutrient-rich foods, however, has specifically addressed zinc as a target nutrient, even those focusing on multiple micronutrient deficiencies. This opportunity should be pursued in the future. Interventions to increase production/intake of animal sources of iron, such as small livestock production and aquaculture, could also provide an opportunity for adding zinc as a target nutrient without any additional costs.

Plant breeding strategies are also suitable approaches for addressing iron and zinc deficiencies simultaneously because positive correlations between zinc and iron concentrations (and other trace minerals) have been consistently found when screening for genetic variability [80]. Again, adding zinc as a target nutrient could be achieved at no additional cost.

Programmatic experience with the promotion of home processing techniques to increase absorbable zinc in the diet is surprisingly limited, considering the large amount of literature showing their effectiveness in reducing phytate and in increasing zinc absorption in clinical studies. Several small-scale studies have documented that amylase-rich foods improve the nutrient density of the diet, and the potential usefulness of these approaches for increasing the micronutrient concentration of complementary foods for young infants has been shown [81–83]. There is, however, little evidence of the efficacy or effectiveness of such approaches for the control of zinc or other micronutrient deficiencies, with the exception of the Malawian study discussed above. Research in this area is urgently needed to determine the potential of these approaches to contribute to the alleviation of zinc deficiency among vulnerable groups. There is no information available on the cost or cost-effectiveness of dietary modification/diversification strategies.

3.4 Formative research for program planning

Formative or consultative research is an approach that has been developed to design effective programs to improve infant and young child feeding [84, 85]. The approach has also been used in diarrheal disease control programs [86, 87], in the design of vitamin A interventions [88], including a successful social marketing program to promote intake of vitamin A–rich foods in Thailand [89], and in a dietary intervention to enhance micronutrient adequacy of rural Malawian diets [62]. The formative research methodology is based on the premise that community nutrition programs will be more effective in modifying child feeding practices and in improving child nutrition if communities are directly involved in their design and formulation. Program planners need to understand the behaviors, practices, culture and constraints faced by the population targeted by their program [84]. The methodology involves the following steps:

1. Define the key problems and practices.
2. Identify simple and effective actions within the household.
3. Test the recommended practices in the homes to determine which ones are most practical and culturally acceptable.
4. Develop an effective strategy for the promotion of the selected practices among the targeted population.

Formative research is largely based on qualitative methods adapted from anthropology, market research, and nutrition education, which may be complemented by some semi-quantitative or quantitative methods as appropriate. The ultimate objective is to understand "what people say, believe, do, and want to do" [84, page 1], and to use this knowledge for improved program planning and effectiveness.

Although experience in the use of formative research for the design of micronutrient programs is rather limited, recent efforts by Helen Keller International to adopt this methodology for vitamin A programs should provide useful guidelines for its use in the design of zinc interventions [88]. The approach could be particularly useful to design food-based interventions, in particular the strategies aimed at increasing the content or the absorption of zinc in the diet through changes in household purchasing, preparation, and processing techniques, or intra-household allocation of resources. Approaches previously developed for use with other micronutrients could be adapted to explore available, culturally acceptable, and affordable food sources of zinc at the community level. For example, a detailed protocol was previously developed to assess natural food sources of vitamin A at the local level, using focused ethnographic studies [90, 91]. Other interventions requiring a behavior change component, such as promoting the use of a fortified product, or even motivating the population to comply with a supplementation program, could also benefit from using a formative research approach for program design.

3.5 Linking zinc interventions with other nutrition and health programs

To reach the specific target groups with a particular intervention, such as zinc supplements or educational messages to promote dietary modification, an appropriate delivery mechanism must be identified. For example, infants might be reached through well-baby clinics or growth monitoring programs if these have suitably high population coverage. Educational messages for pregnant and lactating women might be delivered through community-based women's groups, antenatal clinics, or religious organizations. Formative research is generally needed to select the optimal delivery mechanism. Obviously, if a successful micronutrient program is already in progress, it might be most prudent to link zinc activities with the existing program. A listing of existing health and nutrition programs and opportunities for the inclusion of specific types of zinc interventions is summarized in table 3.6.

The number of asterisks (*) in each cell of this table reflects the potential of a particular program to serve as a vehicle or distribution mechanism for three broad types of interventions to control zinc deficiency. One asterisk (*) represents a possible opportunity, but one that is not likely to be the most effective. Two asterisks (**) represent an opportunity that has limited usefulness because the frequency of exposure to the intervention is too limited or irregular. An example of this is immunization. Although the immunization schedule has the advantage of starting very soon after birth, thus allowing an early first contact of the infant with the health system, the immunization schedule does not allow for providing a supply of zinc supplements monthly. The same is true for other interventions that bring mothers to the health center infrequently, such as vitamin A supplementation, which is repeated only once every 6 months. For zinc supplementation, monthly contacts with the health services, such as to receive a monthly supply of supplements, would be ideal, although bi-monthly provision of zinc supplements could be similarly effective and even more efficient by reducing the burden on health centers imposed by monthly delivery. Three asterisks (***) are given to interventions that theoretically bring mothers to the health center on a monthly basis, but high compliance to the monthly schedule would need to be achieved for the intervention to be an optimal approach for zinc supplementation. Four asterisks (****) are given to interventions that are prime opportunities for the particular zinc intervention considered. Examples include iron supplementation programs for women, which could easily add a zinc supplementation component. Similarly, existing food fortification programs could add zinc as one of the nutrients included in the fortification process. The cost implications of adding zinc to supplements or food fortification programs have been discussed in sections 3.1.4 and 3.2.3, respectively.

Education interventions to promote increased zinc intake or the use of home processing techniques to increase zinc absorption can be included in any nutrition or health education program curriculum. It is particularly relevant in the context of programs promoting exclusive breastfeeding and optimal complementary feeding practices for young infants. In situations where effective prenatal care programs are in place and bring mothers in frequent contact with the health system, increased zinc intake could also be promoted among mothers and their families.

Finally, agricultural programs combining increased production of animal products and education to promote greater intake by vulnerable groups are the ideal mechanism to target multiple micronutrients, including zinc, at little additional cost.

3.6 Evaluation of zinc interventions: surveillance and monitoring

Monitoring and evaluation are essential components of intervention programs and thus should be integrated into the overall program strategy development. Monitoring and evaluation are key to ensuring that programs are implemented as planned, that they are reaching their target population in a cost-effective manner, and that they are having the expected impact.

Evaluative research has been defined as the systematic collection of information on the design, implementation, and effect of projects on targeted populations [92]. Monitoring is usually referred to as the component of evaluative research that addresses the implementation aspects of a program, whereas evaluation is concerned with the program's effects or impacts. Because their objectives are different, monitoring and evaluation require different methodologic approaches, although many of the general concepts related to the selection of designs and indicators apply to both.

Monitoring implies the continuous collection and review of information on project implementation activities, coverage and use, which can be used to re-design or re-orient the program and to strengthen its implementation and the quality of service delivery in an ongoing fashion. A monitoring system can be used to assess service provision (availability, accessibility, and quality), utilization, coverage, and cost [93]. More specifically, monitoring can help assess the following [94]:

» delivery of the service or intervention (whether or not it is delivered)
» timeliness of service delivery
» quality of service delivery
» coverage of the intervention (the degree to which targeted individuals and communities are reached)

» appropriateness of the intervention and the level of use of the services by the targeted populations
» costs of implementation
» overall effectiveness of implementation of the different activities (whether the actual implementation follows the implementation plan)

Evaluation seeks to determine the extent to which the project goals and objectives have been achieved and whether the intervention is having the expected impact on the targeted population. Clearly, in order to achieve impact, program performance must be achieved, and this should be ensured through an effective monitoring system.

TABLE 3.6. Opportunities for linking interventions to control zinc deficiency with existing maternal and child health and nutrition programs

Maternal and child health and nutrition programs	Opportunities for linking with interventions to control zinc deficiency		
	Supplementation	Fortification	Dietary diversification/ modification
Child health and nutrition (prevention)			
Malaria control (bed nets, education, prophylaxis drugs)	contacts with health services may be too infrequent and irregular*		combined education programs*
Immunization	may allow early exposure to health services during first few months of life**		
Growth monitoring/well-baby clinic	good vehicle if once/ month***		education as part of growth monitoring***
Education and behavior change interventions			
Promotion of exclusive breastfeeding	may allow early exposure to health services**		promotion of exclusive breastfeeding is an excellent food-based strategy for children 0–6 months****
Education on complementary feeding practices	timely because of increased needs of infants from 6 months of age**	excellent for promoting the use of processed, fortified complementary foods, if available locally****	excellent for the promotion of home preparation and processing techniques to increase zinc content and absorption****
Hygiene, prevention of illnesses	may be too infrequent or irregular*		education regarding fermentation (improved zinc absorption/diarrhea prevention)*
Control and treatment of infectious diseases	may be too infrequent or irregular for prophylaxis* opportunity to include zinc in treatment regime***		
Deworming	may be too infrequent or irregular; possible to provide shorter term supply (e.g., 1–2 months)*		
Vitamin A capsules distribution	too infrequent: every 6 months*		
Iron supplementation	excellent: zinc can be included in supplement****		
Food distribution programs	good opportunity if once per month***	excellent opportunity to fortify with zinc if food donations are fortified locally****	

continued

TABLE 3.6. Opportunities for linking interventions to control zinc deficiency with existing maternal and child health and nutrition programs *(continued)*

Maternal and child health and nutrition programs	Opportunities for linking with interventions to control zinc deficiency		
	Supplementation	Fortification	Dietary diversification/ modification
Child health (curative)			
Treatment of ARI	opportunity to treat severe zinc deficiency*		
Treatment of diarrhea	treatment should include zinc***		
Treatment of severe PEM	opportunity to treat severe zinc deficiency***		
Maternal health, nutrition and care			
Prenatal care	timely, but may be infrequent**		if contacts are frequent enough, can be a good vehicle for education**
Prevention and control of iron-deficiency anemia (supplementation)	supplementation programs can include zinc****		
Family planning, reproductive health	may be too irregular and infrequent*		
Vitamin A supplementation	too infrequent: every 6 months; possible to provide shorter term supply (e.g., 1-2 months)*		
School girls/adolescent girls nutrition and health programs	depends on the nature of the program*		
School feeding programs (food for education)	good vehicle for supplementation****	can be a good vehicle for distribution of fortified foods****	can increase zinc intake, depending on foods provided and menu****
Other			
Food fortification (of staples)		zinc can be included****	
AIDS prevention	depending on frequency of exposure*		
Agriculture programs (e.g., promotion of animal products [fish ponds, small livestock]) combined with education			opportunity to promote home production and intake of animal products****

*Selecting evaluation designs and indicators**

Although the distinction between monitoring and evaluation is useful from a programmatic point of view, this section will use the general term "evaluation" because the principles described apply to both monitoring and evaluation designs.

Two main questions must be asked when selecting an evaluation design: What is the purpose of the evaluation? And what is the level of precision needed by the users of the information?

Purpose of the evaluation and appropriate indicators

The specific purpose of the evaluation, or what the evaluation intends to measure, is the first consideration in the choice of an evaluation design. For example, results of evaluations can be used to make decisions about whether or not to continue a program, expand it, modify and/or strengthen it, or discontinue it.

Habicht, Victora, and Vaughan [95] classify evalua-

* This section draws extensively on the work of Habicht and collaborators [93, 95].

tion objectives into four categories: provision, utilization, coverage, and impact. Relative to the distinction made previously between monitoring and evaluation, the first three objectives would refer to monitoring and the fourth one to impact evaluation. A fifth objective could be added to this list: evaluation of the cost of the program. Examples of indicators to address these five objectives for the three main types of interventions to address zinc deficiency (supplementation, fortification, dietary modification) are presented in table 3.7.

TABLE 3.7. Examples of indicators that can be used for different evaluation purposes and different types of interventions for the control of zinc deficiency

	Types of interventions for the control of zinc deficiency		
Objective of evaluation	Supplementation	Fortification	Dietary modification (Education, home processing and production interventions)
Provision and availability of services	Number of programs or facilities offering supplements Number of supplements available for target population	Number of food products fortified Number of markets with fortified products available	Number of education sessions provided Number of inputs distributed to promote small animal husbandry or fish ponds
Accessibility of services	% of the population living at reasonable distance from distribution point	% of the population with access to markets where fortified products are available	% of the population who could be reached by education or distribution of inputs
Quality of services	% staff trained in intervention (importance of zinc, vulnerable groups, supplementation dosage, schedule)	Quality control of product: level of fortification, stability during storage	Quality of the education, communication, behavior change: Number of staff trained, duration of training, knowledge of staff Duration and intensity of education sessions Quality of inputs provided, amount and quality of education provided with production intervention
Utilization of services by targeted population	Number of individuals coming to receive supplements	Number of families who purchase fortified product in sufficient amounts Number of individuals who receive fortified product with regular frequency	Number of families who have heard messages or attended education sessions Number of families who have received and used production inputs
Coverage of the targeted population	% at-risk individuals who take supplements with recommended frequency	% of at-risk population consuming sufficient amounts and right frequency of fortified product	% of at-risk population who have received education and other inputs
Impact on the targeted population	Assessment of changes in biochemical, clinical, functional indicators of zinc deficiency in targeted individuals	Amounts and frequency of intake of fortified product by targeted individuals Changes in biochemical, clinical, functional indicators of zinc deficiency in targeted population	% of targeted population with increased knowledge % of targeted population who adopted recommended practices or production activity Changes in amount of food (animals, fish) produced and/or consumed Changes in amount and frequency of intake of sources of bioavailable zinc Changes in biochemical, clinical, functional indicators of zinc deficiency

For all three types of programs, the indicators used to assess the provision of services refer to operational issues such as whether the program is providing the inputs and services in a timely fashion to the right population and with adequate quality. To assess for coverage, the indicators should reflect what percentage of the targeted population (based on need and vulnerability) actually receives the intervention as planned, and for service utilization, appropriate indicators include the percentage of the population who actually use the inputs provided.

Impact indicators, in the case of zinc interventions, can include final outcomes such as biochemical, clinical, and functional signs of zinc deficiency, or intermediary outcomes such as changes in knowledge and attitudes, changes in dietary intakes of zinc or zinc-rich foods, or adoption of recommended home processing techniques or animal or fish production activities (table 3.7).

Level of precision needed for evaluation

Following the approach described by Habicht, Victora, and Vaughan [95], the next question to be answered in selecting an evaluation design is, what is the level of precision required for decision makers to have sufficient information about the performance and/or impact of their program? Or, more specifically, how confident do program planners and decision makers need to be that the effects observed are due to the intervention, or what is the level of inferences required? The three levels of inferences defined by Habicht [93] are adequacy, plausibility, and probability. Taking zinc interventions as an example, an adequacy evaluation would refer to assessing the prevalence of zinc deficiency relative to some pre-determined criteria. For example, it may be that the overall goal of a country is to reduce the prevalence of zinc deficiency among children to 10% or less (or any cut-off point used to define a public health problem). Thus, adequacy would be achieved if the evaluation showed that the prevalence of zinc deficiency at the time of the evaluation was lower than the 10% pre-established cut-off point. Adequacy evaluations are usually the simplest and least costly types of evaluations because they do not require randomization or the use of a control group (table 3.8). They do, however, require the same level of scientific rigor as other types of evaluation.

Plausibility and probability, on the other hand, require more resources and a more sophisticated design because they have to demonstrate with some certainty that the achievements in reducing the prevalence of zinc deficiency are related to the intervention being evaluated. For an evaluation to be plausible, it must be able to demonstrate that the zinc intervention program seemed to have an effect above and beyond other external influences that may also have affected the prevalence of zinc deficiency (such as, for example, increased income and resulting improvements in

TABLE 3.8. Types of intervention designs recommended to achieve various levels of inferences (focusing on impact evaluations)[a]

Type of inferences	Purpose of the evaluation	Types of designs
Adequacy	Determine whether the current prevalence of zinc deficiency is adequate (according to some pre-determined criteria, which could be the level considered as representing a public health problem, if there was one).	Cross-sectional survey of the prevalence of zinc deficiency at a certain point in time, and comparison of prevalence findings with pre-established criteria of adequacy
Plausibility	Determine whether it is plausible to conclude that the zinc intervention contributed to current prevalences of zinc deficiency or to the observed changes in the prevalence of zinc deficiency.	Quasi-experimental and other designs that can be used: » Cross-sectional survey with treatment and comparable control group » Longitudinal study with before and after measurements (looking at changes in treatment group only) » Longitudinal-control study, with both a comparison group and before and after measurements » Case-control study with measurement of cases, compared to matched comparable controls
Probability	Determine, with a pre-established level of probability, that the impact on zinc prevalence is due to the intervention	Double-blind, randomized experimental design: » Randomization of the intervention » Before and after measurements » Intervention and control group » Both subjects and researchers are blind to the treatment during the intervention and the analysis

a. Based on Habicht [93].

dietary quality that were not related to the specific zinc intervention). This is achieved by effectively controlling for these potential confounding factors and biases through a careful selection of evaluation design and appropriate multivariate data analysis methodologies. Quasi-experimental or case-control designs are the designs of choice for achieving plausibility (table 3.8).

Finally, probability evaluations are the types of evaluations that provide the highest level of confidence that the intervention caused the outcome, or in the case of zinc, that the zinc intervention is responsible for the reduction in the prevalence of zinc deficiency. This is usually achieved by using a randomized, controlled, double-blind experiment. The probability design is based on the premise that there is only a small known probability (usually < 0.05) that the observed difference in the prevalence of zinc deficiency between treatment and control communities (or individuals) is due to other factors, such as confounding factors or biases, or to chance alone. Probability designs are the reference standard of efficacy* research; they require randomized, double-blind, placebo-controlled designs, which are the only types of designs that can be used to establish causality.

Table 3.8 summarizes the types of intervention designs that can be applied to achieve these different levels of inferences for impact evaluations of zinc interventions. For more details and for examples of designs to address other evaluation objectives (provision, utilization, and coverage), see Habicht, Victora, and Vaughan [95].

Additional discussions on the choice of evaluation designs and indicators and on the design and implementation of monitoring and evaluation systems can be found in several published documents on monitoring and evaluation [92, 94] and in the vitamin A literature [96, 97].

3.7 Summary

Three possible direct approaches may be taken to improve zinc intakes in whole populations or particular subgroups that are at risk of zinc deficiency: supplementation, fortification, and dietary modification/diversification. Given the small amount of programmatic experience to date in the control of zinc deficiency, research efforts need to be directed toward the study of the efficacy, effectiveness, cost-effectiveness, and acceptability of different strategic approaches. A considerable amount of research is still required to produce the necessary scientific evidence for designing effective interventions.

Although there is much experience with zinc supplementation in small-scale, controlled research trials, there is as yet very limited experience in ongoing, large-scale supplementation programs that include zinc. Nevertheless, a series of specific recommendations have been proposed for zinc supplementation programs, based on currently available information. For example, research suggests that soluble forms of zinc salts (e.g., zinc acetate, zinc sulfate, zinc gluconate) should be used in the supplement formulation, that the supplements should be administered between meals to maximize absorption, and that the supplements should be offered daily. Suggested daily dosage levels for supplemental zinc have been derived (table 3.2), considering the IZiNCG RDAs, the NOAELs or upper limits for zinc intake, and dosage levels used in controlled trials of zinc supplementation that are considered to be effective and have no apparent adverse clinical effects. In areas or subgroups that may be at risk of low copper status, it is recommended to include copper in the zinc supplement (zinc:copper molar ratio 10:1, to a maximum of 1 mg/day copper), so as to minimize the possible risk of altered copper metabolism. Supplemental zinc is also recommended as an adjunct therapy during the treatment of diarrhea in children, where the recommended daily dosage is equivalent to two times the age-specific RDA, for 14 days. Direct studies are needed to better define the optimal doses (amount, frequency, and duration) of zinc supplements for different age and physiologic status groups, and the implications on dosage levels of modifying the method of administration, such as giving the supplement with or between meals and combining zinc with other micronutrients in the supplement. The effects of different chemical and physical forms of supplements on zinc absorption and the cost, shelf life, and acceptability of the supplement also require evaluation. Cultural and behavioral factors that influence adherence to the proposed dosage schedules should also be assessed, and studies are needed of the effectiveness and efficiency of different distribution systems.

Experience with the fortification of foods with zinc is, at present, largely represented by the fortification of infant formulas, infant cereals, and ready-to-eat breakfast cereals. Several Latin American countries are using or have used centrally processed complementary foods fortified with multiple micronutrients, including zinc. Mexico currently has a national, voluntary fortification program for wheat and lime-processed corn flours (the efficacy of this program in preventing zinc deficiency in Mexico has not yet been evaluated). It is recommended that the selected food vehicle for zinc fortification be one that is widely consumed in stable and predictable amounts, and that it is processed on a reasonably large scale to permit adequate quality control. Further, its organoleptic properties should not be affected by the

* An efficacy trial is one that applies an intervention under controlled conditions to determine the magnitude of effect that can be achieved under the best possible circumstances [93].

addition of zinc fortificants and it should be able to retain appropriate levels of the zinc fortificant during processing, storage, and preparation. Examples of candidate zinc fortification vehicles include rice, wheat, and maize or condiments such as salt or fish sauce. Selection of the fortificant form of zinc should take into consideration its acceptance for use (e.g., listed as GRAS by the US Food and Drug Administration), its solubility, lack of effect on organoleptic properties of the food vehicle, and cost. Both zinc oxide and zinc sulfate are relatively inexpensive and have been used as zinc fortificants. Zinc oxide may be less well absorbed in humans than zinc sulfate because it is not water-soluble, although current evidence suggests that zinc absorption from these two fortificants does not differ appreciably. Nevertheless, this needs to be confirmed in additional human studies in populations with high rates of enteropathy and/or hypochlorhydria. Although the appropriate level of zinc fortificant to be added to a food vehicle should be assessed in each specific case, a suggested range for fortification of cereal staples is 30–70 mg zinc per kg of flour. Further information is required on the absorption of zinc, sensory acceptability, shelf life, and final product cost, when different chemical forms of zinc are added to different food vehicles. The latter studies should also consider these outcomes when zinc is included with other micronutrient fortificants to take possible interactions into account. Studies of both the efficacy and effectiveness of fortified food products, including fortified complementary foods, in improving zinc status are needed.

There are several possible strategies for increasing the intake of total zinc and/or absorption of zinc from the diet. These include the following: (1) agricultural strategies to increase the total zinc content, or decrease the content of phytate, of staple food crops using zinc fertilizers, plant breeding, or genetic modification techniques; (2) community or home-based strategies to increase the production and/or intake of zinc-rich foods, such as through promotion of small-livestock production, aquaculture, indigenous zinc-rich foods or processed snacks; and (3) household food processing methods to increase the amount of absorbable zinc in the diet. Examples of household food processing techniques include germination, fermentation, or soaking procedures to reduce the phytate content of cereals or legumes. Experience with these strategies in intervention studies or small-scale programs is still limited. All possible dietary diversification/modification strategies need to be evaluated in terms of their efficacy, acceptability, effectiveness, and cost-effectiveness. In addition, agricultural methods to increase the zinc content of foods and/or improve zinc absorption from foods need to be evaluated in terms of their possible economic and environmental impact.

All of the program options for improving zinc intakes would benefit from the use of formative research, because all methods require some degree of behavior change. Qualitative methods already developed for use in other health and nutrition programs may be adapted and applied to improve the effectiveness of zinc interventions.

Given the likelihood of the coexistence of several micronutrient deficiencies, intervention programs to address zinc deficiency should be linked to programs that address other problem micronutrients to make more efficient use of resources. Intervention programs that address other health issues may also be used as opportunities to include the delivery of zinc-related interventions. Some of the many possibilities, as summarized in table 3.6, include incorporation of nutrition education to improve intakes of dietary zinc in child health and nutrition prevention programs (e.g., growth-monitoring clinics, programs to promote exclusive breastfeeding, improved complementary feeding practices, or improved hygiene practices); inclusion of supplemental zinc in diarrhea treatment programs; inclusion of supplemental zinc where other micronutrient or food supplements are distributed; and inclusion of zinc in fortification programs with other micronutrients.

As with all program interventions, monitoring and evaluation activities should be included in zinc intervention programs. Several indicators can be incorporated into these activities for all types of program strategies. Examples of possible indicators include evaluation of the provision of services, the utilization of services, the coverage of the program in the target population, and its impact on population zinc status or other functional outcomes (table 3.7).

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Chapter 4

Research Needs

While compiling the present document, we identified several knowledge gaps. The research needed to bridge these gaps can be grouped according to the following issues: zinc and function; zinc requirements and toxicity; zinc absorption; assessment of zinc status; and zinc intervention programs. Each of these sets of research needs is described briefly below.

4.1 Zinc and function

Additional information on the functional consequences of zinc deficiency is needed, both to support advocacy for zinc intervention programs and to define the full range of conditions for which zinc interventions might be desirable. Moreover, some of these functional outcomes may provide useful indicators of zinc status and response to intervention programs. Specific functional domains that require additional research are zinc and infection, zinc and reproductive health, and zinc and neurobehavioral development.

In the area of zinc and infection, further information is needed on the mechanisms of the protective effects of zinc against infection. Also, the effects of zinc on specific etiologies of infection, including malaria and other parasitic diseases, tuberculosis, and HIV, should be studied. Finally, additional information is needed on the role of zinc in reducing the risk of mortality.

Further information is required to define the role of zinc in reproductive health and the consequences of zinc deficiency during pregnancy, including fetal development, delivery complications, postpartum maternal health, and infant health. Clinical trials that take into consideration the possible risk factors for poor zinc status during pregnancy (e.g., initial serum zinc concentration, pre-supplementation nutrition status, maternal health, and reproductive history) may help to better define those outcomes that are associated with zinc deficiency.

Little is known about the magnitude of effects of zinc deficiency on neurobehavioral development. Studies in a variety of populations and age groups are needed to define the range and magnitude of these effects.

4.2 Zinc requirements and toxicity

Many questions remain regarding zinc requirements in different subgroups, as defined by age, sex, and physiologic status. Information is needed on the presence and size of zinc stores at birth (in term, pre-term, and small-for-gestational-age babies) and whether these contribute to zinc homeostasis of the newborn and young infant. More information is also needed on quantitative losses of endogenous zinc from different sites, including integument, semen, and menstrual fluid, in individuals with adequate zinc status and different stages of depletion. Empirical data are needed on total endogenous zinc losses (and hence physiologic requirements) in infants (including low-birthweight infants) and children, so that these do not need to be derived by extrapolation from data on adults. Investigation is also needed on the possible impact of common conditions that affect the integrity of the intestinal tract, such as tropical enteropathy or intestinal parasitemia, on the control of endogenous losses of zinc via the intestine. Finally, information is needed on zinc requirements for optimal compensatory growth of patients recovering from severe malnutrition and/or infectious disease.

Relatively little empirical data are available regarding risk assessment of zinc toxicity. Information is needed to define intakes at which there are no observable adverse effects (NOAEL) and levels of intake at which these effects first occur (LOAEL) in different population groups. Adverse effects may include interference with maintenance of nutrition adequacy with regard to other nutrients.

4.3 Zinc absorption

Additional studies are needed on zinc absorption from a broad range of mixed diets with varying levels of factors known to modify zinc absorption (e.g., levels of zinc, phytate, protein from different sources, and calcium and other minerals). Information is needed with particular urgency for diets with high phytate: zinc ratios. Studies are also needed on the effects on

zinc absorption of commonly occurring diseases, such as tropical enteropathy, acute and persistent diarrhea, and intestinal helminthic infections. Studies that measure zinc absorption from a total day's diet and that estimate the true absorption of zinc for individuals by correcting for intestinal losses of endogenous zinc are recommended.

4.4 Assessment of zinc status

A critical area for future investigation is the development of better methods to assess the zinc status of individuals and populations. Identification of easily obtainable, low-cost biomarkers of individual zinc status and their relationship to functional outcomes of zinc deficiency and excess is an area of high priority. Pending the development of such biomarkers, the risk of population zinc deficiency can be inferred from ecologic evidence, such as the absorbable zinc content of the food supply, rates of stunting, dietary zinc intake, and possibly rates of anemia and other diseases. Research is needed to validate these indicators against other markers of zinc status and to develop more information on appropriate cutoffs that are associated with widely recognized public health problems.

With regard to dietary assessment, information is needed on the zinc and phytate contents of local foods with the goal of incorporating this information into food composition databases. Moreover, simplified dietary methods, such as food frequency questionnaires or other techniques, should be developed and evaluated with regard to their ability to predict the risk of zinc deficiency.

Additional information is needed on appropriate reference values and cutoffs of serum zinc concentration of healthy individuals, especially for children less than 3 years of age, elderly individuals, and pregnant and lactating women.

4.5 Zinc intervention programs

There is relatively little programmatic experience to date in the control of zinc deficiency. Thus, information is needed on the efficacy and effectiveness of different strategic approaches, as well as their cost-effectiveness and acceptability. Specific research needs are described, as follows, for each of the different programmatic approaches that have been proposed.

4.5.1 Supplementation

Research is needed on the optimal doses (amount, frequency, and duration) of zinc supplements for different groups, as defined by age and physiologic status. The extent to which these dosage recommendations should be modified according to the method of administration (e.g., with or after meals) and whether the supplements are provided as zinc alone or combined with other micronutrients should also be determined. The efficacy of weekly versus daily supplementation and of short-course treatment with supplemental zinc, also deserve study. Evaluation is needed of the effects of different chemical forms (i.e., the particular zinc salt or organic ligand) and physical forms (liquid, tablet, sprinkles, spreads, etc.) of supplements on zinc absorption and the cost, shelf-life, and acceptability of the supplement. Cultural and behavioral factors that influence adherence to the proposed dosage schedules should also be assessed, and studies are needed on the effectiveness and efficiency of different distribution systems.

4.5.2 Fortification

The absorption of zinc from a variety of chemical forms of zinc fortificant compounds in different food vehicles requires further study. Sensory trials are also needed in relation to the chemical form and amounts of zinc fortificants added to different food vehicles. The effects of zinc fortification on final product cost, shelf-life, and acceptability should also be determined. Studies of both the efficacy and the effectiveness of fortified food products, including fortified complementary foods, to improve zinc status are needed. Interactions of zinc with other nutrients should be assessed, in cases where these nutrients may be included in a mixture of multiple fortificants.

4.5.3 Dietary diversification/modification

Studies are needed to plan and evaluate different approaches for enhancing dietary zinc intake and absorption, including food production and processing and nutrition education. Agricultural methods to increase the zinc content of foods and/or improve zinc absorption from foods need to be evaluated, not only in terms of their efficacy but also in terms of their possible economic and environmental impact. Possible approaches include the use of zinc-containing fertilizers, plant-breeding strategies to select for high-zinc strains, and genetic modification to alter the content of inhibitors and enhancers of zinc absorption. Studies are also needed on household processing techniques that could be applied to improve zinc absorption. Finally, the feasibility and nutrition impact of incorporating non-traditional zinc-rich foods into the diet (e.g., animal-source foods, insects, and wild plants) should be evaluated.

Appendix 1

Estimated Risk of Zinc Deficiency by Country

This table presents country-specific information on the per capita amounts of selected nutrients and food components (including absorbable zinc content) of national food supplies. Information is included on the individual and combined indicators of risk of zinc deficiency: prevalence of stunting (height- or length-for-age < –2 SD) of children under 5 years of age, and the percent of the population at risk for inadequate zinc intake, based on data derived from national food supplies. See section 2.2 for details on the derivation of these data, the definition of risk categories, and the limitations in their interpretation.

Region/Country	Population (thousands)	Energy (kcal/d)	Zinc (mg/d)	Phytate (mg/d)	Phytate: zinc molar ratio	% of energy from animal-source foods	Zinc density (mg/1000 kcal)	Estimated zinc absorption (%)	Absorbable zinc (mg/d)	Absorbable zinc as % of IZiNCG EAR	Estimated % of population at risk of inadequate intake, IZiNCG	Prevalence of stunting	Risk category
Western Europe													
Austria	8,140	3,607	13.0	1,239	9.4	34.4	3.6	24.1	3.1	152.6	8.4		Low
Belgium-Luxembourg	10,563	3,618	11.3	985	8.6	32.0	3.1	26.3	3.0	144.6	10.9		
Denmark	5,270	3,354	13.3	1,420	10.6	37.5	4.0	23.2	3.1	149.8	9.2		Low
Finland	5,154	3,101	13.5	1,033	7.6	38.5	4.4	25.0	3.4	165.5	5.7		Low
France	58,683	3,551	14.8	1,086	7.3	37.9	4.2	24.2	3.6	176.2	4.2	5.8	Low
Germany	82,133	3,379	12.2	1,410	11.5	31.2	3.6	23.7	2.9	140.3	12.5		
Greece	10,600	3,604	12.7	1,364	10.7	22.6	3.5	23.7	3.0	144.8	10.8		
Iceland	276	3,138	16.2	1,205	7.4	41.3	5.2	23.1	3.7	187.7	3.1		Low
Ireland	3,681	3,527	14.3	1,264	8.8	31.4	4.1	23.5	3.4	168.6	5.2		Low
Israel	5,984	3,463	12.2	1,842	14.9	19.5	3.5	22.2	2.7	141.0	12.2		
Italy	57,369	3,546	12.5	1,107	8.8	26.1	3.5	25.0	3.1	150.0	9.1	2.7	Low
Malta	384	3,383	12.8	1,610	12.4	26.6	3.8	22.7	2.9	145.4	10.6		
Netherlands	15,678	3,254	13.6	1,304	9.5	34.5	4.2	23.6	3.2	157.0	7.3		Low
Norway	4,419	3,297	12.9	1,218	9.3	34.0	3.9	24.3	3.1	154.2	8.0		Low
Portugal	9,869	3,555	13.0	1,464	11.1	26.9	3.7	23.2	3.0	147.4	9.9		Low
Spain	39,628	3,305	12.5	1,117	8.9	27.1	3.8	24.9	3.1	150.4	9.0	3.1	Low
Sweden	8,875	3,089	10.9	918	8.4	33.7	3.5	26.9	2.9	143.3	11.3		
Switzerland	7,299	3,293	12.6	1,009	7.9	34.7	3.8	25.5	3.2	155.8	7.6		Low
Turkey	64,479	3,423	9.9	1,817	18.2	11.4	2.9	23.4	2.3	123.7	22.2	16.0	Medium
United Kingdom	58,649	3,255	12.1	1,049	8.6	31.6	3.7	25.5	3.1	151.9	8.6	1.3	Low
USA/Canada													
Canada	30,563	3,105	11.1	1,185	10.6	28.7	3.6	25.3	2.8	138.5	13.3	4.7	Low
USA	274,028	3,658	12.7	1,355	10.6	27.8	3.5	23.7	3.0	150.1	9.1	2.0	Low
Eastern Europe													
Albania	3,119	2,788	9.9	1,036	10.4	25.5	3.5	26.7	2.6	138.2	13.4	15.4	Medium
Armenia	3,536	1,981	6.4	826	12.8	16.3	3.2	30.9	2.0	100.4	49.4	12.3	Medium
Azerbaijan	7,669	2,222	6.9	1,020	14.7	15.6	3.1	29.0	2.0	101.6	47.5	22.2	High
Belarus	10,315	3,140	15.1	1,771	11.6	29.7	4.8	21.3	3.2	164.9	5.8		
Bosnia and Herzegovina	3,675	2,699	11.3	2,752	24.1	13.2	4.2	20.5	2.3	114.7	30.4		

continued

Region/Country	Population (thousands)	Energy (kcal/d)	Zinc (mg/d)	Phytate (mg/d)	Phytate: zinc molar ratio	% of energy from animal-source foods	Zinc density (mg/1000 kcal)	Estimated fractional absorption	Absorbable zinc (mg/d)	Absorbable zinc as % of IZiNCG EAR	Estimated % of population at risk of inadequate intake, IZiNCG	Prevalence of stunting	Risk category
Eastern Europe (cont'd)													
Bulgaria	8,336	2,787	9.9	1,056	10.6	24.4	3.5	26.6	2.6	128.7	18.6		Medium
Croatia	4,481	2,502	7.9	1,078	13.5	20.6	3.2	27.8	2.2	109.0	37.0	0.8	Medium
Czech Republic	10,282	3,157	10.6	967	9.0	27.1	3.4	26.7	2.8	140.8	12.3	1.9	Low
Estonia	1,429	2,898	13.8	1,827	13.1	30.9	4.8	21.6	3.0	152.7	8.4		
Georgia	5,059	2,289	8.1	1,782	21.8	14.3	3.5	24.6	2.0	101.7	47.3	11.7	Medium
Hungary	10,116	3,388	9.9	838	8.4	32.2	2.9	28.1	2.8	135.6	14.7	2.9	Low
Kazakhstan	16,319	3,009	11.6	1,170	10.0	21.9	3.8	25.1	2.9	148.3	9.6	15.8	Medium
Kyrgyzstan	4,643	2,547	9.5	823	8.6	21.6	3.7	28.5	2.7	137.4	13.8	24.8	Medium
Latvia	2,424	2,930	12.1	1,469	12.0	30.1	4.1	23.5	2.8	145.1	10.7		
Lithuania	3,694	2,983	13.0	1,568	12.0	26.2	4.3	22.8	3.0	150.9	8.9		
Macedonia, The Fmr Yug Rep	1,999	2,707	9.5	1,656	17.2	18.9	3.5	24.1	2.3	113.7	31.5		
Moldova, Republic of	4,378	2,840	10.2	2,262	22.0	16.3	3.6	22.0	2.2	114.4	30.8		
Poland	38,718	3,342	12.5	1,371	10.9	27.4	3.7	23.8	3.0	148.7	9.5		
Romania	22,474	3,216	11.1	1,663	14.8	21.7	3.5	23.3	2.6	129.2	18.3	7.8	Medium
Russian Federation	147,434	2,896	10.8	1,102	10.1	24.6	3.7	25.9	2.8	142.3	11.7	12.7	Medium
Slovakia	5,377	3,012	10.6	1,244	11.6	27.0	3.5	25.2	2.7	132.4	16.4		Medium
Slovenia	1,993	2,988	12.7	1,762	13.7	30.1	4.3	22.3	2.8	140.3	12.5		
Tajikistan	6,015	2,075	5.5	811	14.5	9.2	2.7	31.9	1.8	90.2	66.8	30.0	High
Turkmenistan	4,309	2,618	8.2	874	10.5	18.1	3.1	28.9	2.4	121.2	24.2		Medium
Ukraine	50,861	2,953	10.3	1,247	12.0	21.6	3.5	25.4	2.6	133.4	15.8		Medium
Uzbekistan	23,574	2,528	8.4	941	11.1	17.5	3.3	28.3	2.4	121.0	24.4	31.3	Medium
Yugoslavia, Fed Rep of	10,635	2,909	11.6	1,192	10.2	34.9	4.0	25.0	2.9	143.5	11.3	6.8	Low
Eastern Mediterranean													
Afghanistan	21,354	1,737	12.0	3,143	26.0	9.2	6.9	19.5	2.3	132.4	16.4	51.6	Medium
Algeria	30,081	2,952	17.2	4,240	24.4	10.0	5.8	16.5	2.8	160.2	6.6	18.3	Medium
Cyprus	771	3,207	17.7	2,946	16.5	28.2	5.5	18.1	3.2	162.4	6.2		
Egypt	65,978	3,279	17.4	4,830	27.5	7.0	5.3	15.9	2.8	151.8	8.6	20.6	Medium
Iran, Islamic Rep of	65,758	2,938	16.0	3,984	24.6	9.1	5.4	17.1	2.7	159.9	6.7	15.4	Medium
Iraq	21,800	2,196	11.1	2,919	26.2	4.2	5.0	20.3	2.2	128.8	18.5	27.5	Medium
Jordan	6,304	2,718	14.5	3,349	22.9	12.1	5.3	18.3	2.7	153.1	8.3	7.8	Low
Kuwait	1,811	2,987	17.1	2,828	16.3	23.6	5.7	18.4	3.2	176.4	4.2	3.2	Low

Lebanon	3,191	3,165	16.5	3,754	22.5	13.0	5.2	17.3	2.9	154.2	8.0	12.2	Medium
Libyan Arab Jamahiriya	5,339	3,271	17.2	3,874	22.4	11.7	5.2	17.0	2.9	168.2	5.2	15.1	Medium
Morocco	27,377	2,971	17.3	4,553	26.0	7.0	5.8	16.2	2.8	155.6	7.6	24.2	Medium
Saudi Arabia	20,181	2,787	13.8	2,786	20.0	14.7	4.9	19.5	2.7	149.0	9.4	41.0	Medium
Sudan	28,292	2,312	13.1	3,159	23.9	19.1	5.7	19.1	2.5	144.8	10.8	34.8	Medium
Syrian Arab Republic	15,333	2,958	15.3	3,594	23.3	13.0	5.2	17.8	2.7	161.1	6.5	20.8	Medium
Tunisia	9,335	3,270	18.6	4,525	24.1	9.2	5.7	16.0	3.0	159.5	6.8	8.3	Low
United Arab Emirates	2,353	3,122	16.7	2,394	14.2	25.0	5.3	19.3	3.2	156.4	7.5		
Yemen	16,887	2,039	11.7	3,157	26.7	6.4	5.7	19.6	2.3	138.9	13.1	51.7	Medium
China													
China	1,262,817	2,918	12.4	2,056	16.4	16.5	4.3	21.5	2.7	136.7	14.1	15.6	Medium
Western Pacific													
Australia	18,520	3,145	13.3	928	6.9	32.9	4.2	25.7	3.4	169.1	5.1	0.0	Low
Fiji Islands	796	2,773	10.1	1,369	13.4	20.2	3.6	24.9	2.5	134.6	15.2	2.7	Medium
French Polynesia	227	2,826	12.2	1,344	10.9	28.6	4.3	24.0	2.9	157.3	7.3		
Japan	126,281	2,793	11.0	1,754	15.8	21.2	3.9	23.0	2.5	122.0	23.5	5.6	Medium
Kiribati	81	2,826	9.1	1,519	16.5	12.3	3.2	24.9	2.3	111.7	33.7	28.3	High
Korea, Dem People's Rep	23,348	2,204	9.8	2,834	28.8	5.9	4.4	21.0	2.1	107.6	38.8	59.5	High
Korea, Republic of	46,109	3,031	11.9	2,157	18.0	13.8	3.9	21.5	2.6	127.6	19.4	18.3	Medium
Mongolia	2,579	1,930	12.7	453	3.5	45.3	6.6	30.6	3.9	216.7	1.6	24.6	Medium
New Caledonia	206	2,746	10.7	1,077	9.9	25.8	3.9	26.0	2.8	137.7	13.7		
New Zealand	3,796	3,248	14.1	1,096	7.7	34.7	4.3	24.4	3.4	172.9	4.6	2.9	Low
Solomon Islands	417	2,191	7.9	1,223	15.3	9.9	3.6	27.0	2.1	123.0	22.8	27.3	Medium
Vanuatu	182	2,573	11.2	1,576	14.0	16.5	4.3	23.6	2.6	129.7	18.0	19.1	Medium
Latin America													
Antigua and Barbuda	67	2,334	9.5	766	8.0	34.6	4.1	28.9	2.8	147.1	10.0	6.6	Low
Argentina	36,123	3,157	14.0	890	6.3	31.2	4.4	25.7	3.6	186.2	3.2	12.4	Medium
Bahamas	296	2,481	11.2	1,139	10.1	31.5	4.5	25.5	2.8	146.0	10.4		
Barbados	268	3,071	11.6	1,474	12.6	24.7	3.8	23.7	2.8	138.4	13.3	7.0	Low
Belize	230	2,829	9.5	1,612	16.8	23.4	3.4	24.3	2.3	123.7	22.2	39.5	Medium
Bermuda	64	3,034	14.7	1,450	9.8	27.7	4.8	22.6	3.3	177.2	4.1		
Bolivia	7,957	2,173	8.8	1,556	17.4	16.7	4.1	24.9	2.2	123.2	22.6	26.8	Medium
Brazil	165,851	2,890	10.5	1,931	18.2	19.2	3.6	22.7	2.4	126.2	20.3	10.5	Medium
Chile	14,824	2,783	10.5	1,203	11.4	21.5	3.8	25.5	2.7	140.3	12.5	1.9	Low
Colombia	40,803	2,541	9.0	1,613	17.7	16.7	3.5	24.6	2.2	117.6	27.4	15.0	Medium
Costa Rica	3,841	2,749	8.6	1,534	17.7	17.5	3.1	25.2	2.2	116.1	29.0	3.3	Medium

continued

Region/Country	Population (thousands)	Energy (kcal/d)	Zinc (mg/d)	Phytate (mg/d)	Phytate: zinc molar ratio	% of energy from animal-source foods	Zinc density (mg/1000 kcal)	Estimated fractional absorption	Absorbable zinc (mg/d)	Absorbable zinc as % of IZiNCG EAR	Estimated % of population at risk of inadequate intake, IZiNCG	Prevalence of stunting	Risk category
Latin America (cont'd)													
Cuba	11,116	2,395	7.1	1,132	15.7	14.0	3.0	28.1	2.0	100.5	49.3		
Dominica	71	2,964	11.8	1,204	10.1	23.0	4.0	24.8	2.9	156.7	7.4	6.1	Low
Dominican Republic	8,232	2,295	6.7	1,107	16.5	14.7	2.9	28.7	1.9	103.4	44.7	10.7	Medium
Ecuador	12,175	2,644	8.0	1,323	16.4	16.1	3.0	26.5	2.1	115.5	29.6	34.0	High
El Salvador	6,032	2,498	8.9	3,132	34.7	11.4	3.6	20.9	1.9	105.5	41.7	23.3	High
Grenada	93	2,709	9.9	1,292	12.9	23.3	3.7	25.4	2.5	134.5	15.2		Medium
Guatemala	10,801	2,274	8.0	2,950	36.3	8.6	3.5	21.8	1.8	101.1	48.3	46.4	High
Guyana	850	2,531	8.3	1,445	17.2	14.1	3.3	25.7	2.1	113.3	31.9	20.7	High
Haiti	7,952	1,901	6.8	1,870	27.3	6.1	3.6	25.3	1.7	96.6	55.6	31.9	High
Honduras	6,147	2,369	7.9	2,466	30.7	13.9	3.4	22.8	1.8	103.7	44.3	38.9	High
Jamaica	2,538	2,622	8.7	1,131	12.8	15.0	3.3	26.9	2.3	123.2	22.6	6.9	Medium
Mexico	95,831	3,146	12.2	3,413	27.7	16.9	3.9	19.0	2.3	126.4	20.2	17.7	Medium
Netherlands Antilles	213	2,507	10.8	1,106	10.2	32.1	4.3	25.8	2.8	148.8	9.5		
Nicaragua	4,807	2,185	7.5	2,696	35.4	8.1	3.4	22.6	1.7	100.2	49.7	24.9	High
Panama	2,767	2,385	8.1	1,407	17.1	22.3	3.4	26.0	2.1	112.4	33.0	18.2	Medium
Paraguay	5,222	2,519	11.0	1,926	17.3	23.7	4.4	22.5	2.5	138.4	13.4	13.9	Medium
Peru	24,797	2,391	7.6	1,547	20.2	13.9	3.2	25.8	2.0	105.6	41.6	25.8	High
Saint Kitts and Nevis	39	2,598	10.0	1,015	10.1	25.4	3.8	26.8	2.7	142.8	11.5		
Saint Lucia	150	2,797	11.1	1,020	9.1	27.0	4.0	26.2	2.9	155.0	7.8	10.8	Medium
Saint Vincent/ Grenadines	112	2,499	9.4	1,423	15.1	18.6	3.7	25.1	2.4	125.7	20.7	23.5	Medium
Suriname	414	2,623	8.1	1,316	16.1	13.6	3.1	26.4	2.1	114.9	30.2		
Trinidad and Tobago	1,283	2,642	7.5	1,250	16.5	15.8	2.8	27.2	2.0	109.2	36.9	4.8	Medium
Uruguay	3,289	2,803	14.7	1,150	7.7	34.6	5.2	23.9	3.5	177.9	4.0	9.5	Low
Venezuela	23,242	2,366	7.6	1,488	19.4	15.5	3.2	26.0	2.0	106.0	41.0	14.3	Medium
South Asia													
Bangladesh	124,774	2,061	7.4	2,064	27.7	3.1	3.6	24.2	1.8	99.7	50.4	54.8	High
India	982,223	2,419	10.9	2,906	26.3	7.5	4.5	20.4	2.2	119.3	25.9	42.6	High
Maldives	271	2,533	11.0	2,166	19.5	22.4	4.3	21.9	2.4	137.6	13.7	26.9	Medium
Nepal	22,847	2,317	11.1	3,146	28.2	6.8	4.8	19.9	2.2	124.9	21.3	53.1	Medium

Pakistan	148,166	2,423	13.0	2,974	22.7	17.1	5.3	19.4	2.5	144.0	11.1	36.6	Medium
Sri Lanka	18,455	2,278	8.6	2,221	25.4	6.1	3.8	23.0	2.0	103.4	44.7	20.4	High
Southeast Asia													
Brunei Darussalam	315	2,750	12.3	2,094	16.8	20.5	4.5	21.5	2.6	139.6	12.8		
Cambodia	10,716	1,945	7.1	1,722	24.0	8.0	3.7	25.5	1.8	104.2	43.6	53.3	High
Indonesia	206,338	2,866	10.0	2,859	28.4	4.4	3.5	20.9	2.1	111.2	34.4	42.2	High
Laos	5,163	2,168	7.9	2,031	25.6	6.6	3.6	24.0	1.9	110.1	35.7	47.3	High
Malaysia	21,410	2,895	10.3	1,534	14.7	20.2	3.6	24.1	2.5	136.7	14.1	26.6	Medium
Myanmar	44,497	2,764	9.3	2,612	27.9	3.9	3.4	21.7	2.0	111.0	34.6	41.6	High
Papua New Guinea	4,600	2,192	9.0	1,036	11.4	11.2	4.1	27.3	2.4	135.9	14.6	43.2	Medium
Philippines	72,944	2,300	7.8	1,344	17.1	14.0	3.4	26.5	2.1	113.3	31.9	32.7	High
Thailand	60,300	2,407	8.1	1,610	19.7	12.7	3.4	25.2	2.0	105.6	41.6	16.0	Medium
Viet Nam	77,562	2,461	9.2	2,008	21.6	9.5	3.7	23.2	2.1	117.3	27.8	38.7	High
Sub-Saharan Africa													
Angola	12,092	1,830	6.5	1,616	24.6	8.3	3.6	26.4	1.7	102.6	46.0	53.3	High
Benin	5,781	2,489	10.8	2,826	26.0	4.0	4.3	20.6	2.2	132.1	16.5	25.0	Medium
Botswana	1,570	2,270	10.2	2,244	21.8	18.7	4.5	22.1	2.3	131.1	17.1	28.9	Medium
Burkina Faso	11,305	2,333	13.4	4,149	30.7	4.6	5.7	17.7	2.4	138.6	13.3	36.8	Medium
Burundi	6,457	1,690	7.6	2,668	34.6	2.7	4.5	22.6	1.7	102.3	46.5	47.4	High
Cameroon	14,305	2,192	9.0	2,339	25.6	5.8	4.1	22.5	2.0	117.3	27.7	26.0	High
Cape Verde	408	3,186	10.8	2,548	23.3	14.6	3.4	21.1	2.3	132.0	16.6	16.2	Medium
Central African Republic	3,485	1,901	8.8	1,920	21.5	9.8	4.7	23.7	2.1	123.0	22.7	28.4	Medium
Chad	7,270	1,978	11.2	3,462	30.6	7.3	5.7	19.4	2.2	125.1	21.1	40.1	Medium
Comoros	658	1,815	6.0	1,348	22.4	5.9	3.3	28.1	1.7	100.1	49.9	33.8	High
Congo, Dem Republic of	49,139	1,770	5.7	1,421	24.7	2.9	3.2	28.0	1.6	95.5	57.5	45.2	High
Congo, Republic of	2,785	2,084	6.2	1,163	18.7	7.4	3.0	28.8	1.8	104.7	42.9	27.5	High
Côte d'Ivoire	14,292	2,523	8.8	1,914	21.5	4.0	3.5	23.7	2.1	125.5	20.8	24.4	Medium
Djibouti	623	2,046	6.2	955	15.4	11.7	3.0	30.1	1.9	108.8	37.3	25.7	High
Eritrea	3,577	1,654	8.2	2,223	26.8	5.8	5.0	23.2	1.9	112.9	32.4	38.4	High
Ethiopia	59,649	1,770	9.9	2,747	27.3	5.9	5.6	21.1	2.1	124.3	21.7	64.2	Medium
Gabon	1,167	2,511	9.0	1,345	14.7	14.5	3.6	25.6	2.3	128.7	18.6	22.0	Medium
Gambia	1,229	2,279	8.1	2,200	26.9	5.3	3.6	23.4	1.9	109.7	36.1	30.1	High
Ghana	19,162	2,493	9.0	1,828	20.1	4.3	3.6	23.9	2.1	125.2	21.0	25.9	Medium
Guinea	7,337	2,228	7.3	1,592	21.7	3.1	3.3	25.9	1.9	111.5	33.9	26.1	High
Guinea-Bissau	1,161	2,400	8.9	2,163	24.2	7.0	3.7	23.0	2.0	116.1	29.0		
Kenya	29,008	1,932	8.1	2,195	26.7	12.6	4.2	23.4	1.9	112.5	32.9	33.0	High

continued

Region/Country	Population (thousands)	Energy (kcal/d)	Zinc (mg/d)	Phytate (mg/d)	Phytate: zinc molar ratio	% of energy from animal-source foods	Zinc density (mg/1000 kcal)	Estimated fractional absorption	Absorbable zinc (mg/d)	Absorbable zinc as % of IZiNCG EAR	Estimated % of population at risk of inadequate intake, IZiNCG	Prevalence of stunting	Risk category
Sub-Saharan Africa (cont'd)													
Lesotho	2,062	2,282	10.2	3,500	33.8	5.0	4.5	19.7	2.0	114.0	31.2	44.0	High
Liberia	2,666	2,112	5.4	1,162	21.2	3.3	2.6	29.6	1.6	94.5	59.2	32.8	High
Madagascar	15,057	2,018	7.4	1,554	20.8	10.4	3.7	25.9	1.9	112.5	32.9	48.3	High
Malawi	10,346	2,030	8.9	3,370	37.3	2.7	4.4	20.6	1.8	111.4	34.2	48.3	High
Mali	10,694	2,314	12.6	3,284	25.7	9.1	5.5	19.1	2.4	144.0	11.1	48.6	Medium
Mauritania	2,529	2,654	10.0	1,793	17.7	17.1	3.8	23.4	2.3	137.0	14.0	44.0	Medium
Mauritius	1,141	2,935	9.1	1,535	16.6	14.0	3.1	24.8	2.3	115.6	29.5	9.7	Medium
Mozambique	18,880	1,797	6.2	1,861	29.6	2.8	3.5	25.8	1.6	93.7	60.5	35.9	High
Namibia	1,660	2,572	11.7	2,886	24.5	9.2	4.5	20.1	2.3	136.6	14.2	28.5	Medium
Niger	10,078	2,000	13.6	3,651	26.5	5.8	6.8	18.2	2.5	149.1	9.4	39.5	Medium
Nigeria	106,409	2,780	12.0	3,035	25.1	3.2	4.3	19.7	2.4	139.8	12.8	37.6	Medium
Rwanda	6,604	2,073	7.5	2,329	30.7	2.9	3.6	23.5	1.8	106.9	39.8	41.8	High
Sao Tome and Principe	141	2,246	7.0	1,489	21.0	4.4	3.1	26.5	1.9	109.3	36.7	25.9	High
Senegal	9,003	2,267	9.1	2,184	23.9	9.1	4.0	22.8	2.1	119.9	25.3	30.6	High
Seychelles	76	2,375	8.3	1,303	15.6	19.4	3.5	26.3	2.2	128.4	18.8	5.1	Medium
Sierra Leone	4,568	2,021	6.2	1,703	27.1	3.4	3.1	26.3	1.6	96.1	56.5	34.7	High
Somalia	9,237	1,586	7.9	1,092	13.6	40.3	5.0	27.7	2.2	131.2	17.1		Medium
South Africa	39,357	2,862	11.2	2,710	23.9	13.5	3.9	20.6	2.3	127.0	19.7	22.8	Medium
Swaziland	952	2,517	10.3	2,503	24.0	12.8	4.1	21.4	2.2	125.9	20.5	30.3	Medium
Tanzania, United Rep of	32,102	1,915	7.9	2,392	30.1	6.7	4.1	23.0	1.8	108.7	37.5	43.4	High
Togo	4,397	2,307	9.8	2,657	26.9	3.5	4.2	21.4	2.1	122.8	22.9	34.0	Medium
Uganda	20,554	2,279	9.4	2,661	28.2	6.6	4.1	21.6	2.0	121.7	23.8	38.3	Medium
Zambia	8,781	1,918	8.3	2,874	34.3	5.1	4.3	21.8	1.8	108.3	38.0	42.4	High
Zimbabwe	11,377	2,033	8.3	2,953	35.2	7.1	4.1	21.6	1.8	104.3	43.4	21.4	High

Appendix 2

Resources for Food Composition Data for Zinc and Phytate, and Phytate Content of Selected Foods

INFOODS (International Network of Food Data Systems) Secretariat
c/o FAO
ESNA
Viale delle Terme di Caracalla
00100 Rome
Italy
Telephone: +39 06 570 53728
FAX: +39 06 570 54593
http://www.fao.org/infoods/index_en.stm

International Minilist/WorldFood Dietary Assessment System, 2.0.
(University of California, Berkeley; Berkeley, CA)
The software program including food composition databases can be downloaded at no cost from the INFOODS website: http://www.fao.org/infoods/software_worldfood_en.stm

US Department of Agriculture (USDA). Nutrient database for standard reference. Release 14. Washington, DC: United States Department of Agriculture, 2001.
Nutrient Data Laboratory
Agricultural Research Service
Beltsville Human Nutrition Research Center
10300 Baltimore Avenue
Building 005, Room 107, BARC-West
Beltsville, MD 20705-2350
Telephone: 301-504-0630
FAX: 301-504-0632
http://www.nal.usda.gov:80/fnic/foodcomp/

Phytate content of foods (adapted from the International Minilist [WorldFood Dietary Assessment Program, 2.0; University of California, Berkeley, USA])

Food group	Description	Phytate content (mg/100 g)
Cereals and grains	Whole-grain cereals (barley, maize, millet, sorghum)	800
	Refined cereals (extracted flours, rolled oats)	197
	Bran, maize	263
	Bran, wheat	3,011
	Bread, whole-wheat	845
	Bread, white, wheat	30
	Bread, unleavened	200
	Rice, brown	262
	Rice, white	126
	Tortilla, maize	480
Seeds, nuts, and legumes	Beans, peas, lentils	358
	Seeds (lotus, pumpkin, sesame)	3,465
	Nuts (almonds, peanuts, walnuts)	1,760
	Soybean and products (tempeh, tofu)	374
Starchy roots and tubers	Cassava, potatoes, yams	54

continued

Phytate content of foods (adapted from the International Minilist [WorldFood Dietary Assessment Program, 2.0; University of California, Berkeley, USA]) *(continued)*

Food group	Description	Phytate content (mg/100 g)
Vegetables	Broccoli, cabbage, carrots, eggplant, lettuce, mushrooms, onions, squash, sweet corn, tomatoes, turnip	0
	Green beans, green peas	60
	Green leaves	42
	Pepper (capsicum), chiles	35
	Seaweed, kelp	97
Fruits	Berries, citrus, melons, stonefruit	0
	Apple	63
	Coconut	324
	Mango	20
Meats	Beef, pork, other game, poultry, organ meat	0
Fish and seafood	Fish, shellfish	0
Insects	Grubs, locusts	0
Dairy and eggs	Milk, cheese, yogurt	0
	Eggs	0

Appendix 3

Techniques for Measuring Zinc Absorption

The specific features of zinc metabolism—a high endogenous intestinal excretion, a rapid turnover of zinc in plasma, and a constant urinary excretion over a wide range of dietary intakes—limit the possible range of methods that can be used to measure zinc absorption. The conventional chemical balance technique, where the *apparent absorption* is calculated as the difference between dietary zinc intake and fecal zinc content, can at best give information about the overall balance of body zinc. However, long periods (> 30 days) of constant zinc intake are needed to achieve steady state conditions and gain reliable information [1]. To measure *true absorption* of zinc, endogenous sources of excreted zinc must be separated from unabsorbed dietary zinc, and for this determination, isotope techniques are necessary. Suitable radio- and stable-zinc isotopes are available and have been used extensively to study zinc absorption from single meals and, to a limited extent, from total diets. These techniques require advanced analytic equipment and skills and are mainly suited for research laboratories and studies of small groups of subjects. Further research in this area is required to better quantify the effects of physiologic and dietary conditions that affect the efficiency of zinc absorption, particularly from total diets. These techniques will also be useful in assessing the potential efficacy of different zinc compounds for use in food fortification as well as zinc supplements.

Whole-body counting

The use of the gamma-emitting radioisotope ^{65}Zn with a physical half-life of 243.6 days and determination of absorption from measurements of the whole-body retention of the isotope is regarded as the reference method for zinc absorption. Test meals or total diets are extrinsically labeled before intake and retention is measured in a whole-body counter at a time when unabsorbed isotope has been excreted from the body (minimum seven days) [2]. Endogenous excretion of absorbed isotope from the time of intake to the first retention measurement is corrected for by measuring the excretion of an intravenous dose in the same subject, or using the average rate of excretion determined in a group with similar characteristics. The whole-body counting technique has high precision and is simple for the participating subjects, but the required equipment is available in only a limited number of centers.

Fecal monitoring

Measurement of appearance of zinc isotopes (stable or radioactive) in fecal samples is at present the only alternative method that has been validated against the whole-body counting technique [3]. When ^{65}Zn is used, fecal samples can be measured directly in large-volume gamma-counters without further pretreatment. Intake of radio-opaque markers followed by x-ray of the fecal samples or of a non-absorbed marker (e.g., ^{51}Cr) can be used to relate excretion to period of intake and thereby limit the number of fecal samples, which also means less influence of endogenous zinc excretion. This approach could be a relatively cheap and simple field technique.

Three stable isotopes of zinc are of low enough natural abundance to be used in a similar way as tracers. These are ^{67}Zn, ^{68}Zn, and ^{70}Zn, with natural abundance rates of 4.1%, 18.8%, and 0.6%, respectively. Isotopic ratios can be determined using mass spectrometric techniques. Single or dual stable-isotope techniques with fecal monitoring have been applied to study zinc absorption. Endogenous excretion of zinc is corrected for by extrapolating a linear fit of rate of excretion after the unabsorbed, orally administered isotope has been excreted or by simultaneous intravenous injection of a second isotope. This correction is necessary as relatively long fecal collection periods (10–12 days) are required. Sample pretreatment prior to analysis is laborious and contributes to variation in results and therefore larger study groups are needed compared to the whole-body

counting technique [3]. Due to the need for advanced analytic equipment, application of this method is limited to research laboratories.

Urinary monitoring

Total urinary excretion is relatively constant and not related to intake within the range of typical dietary consumption. Thus, this method cannot be used to evaluate dietary zinc absorption. However, urinary ^{65}Zn excretion during 48 hours after intake of a labeled meal does appear to be correlated to zinc absorption determined by the whole-body counting technique.* Urinary radioisotope content can be measured in a similar way as fecal samples in large-volume gamma counters and could also be a relatively inexpensive field technique. A dual-isotope technique with simultaneous oral and intravenous administration of different stable isotopes of zinc and determination of isotope ratios in urine during the following 48 hours has been used to study zinc absorption from single meals [4]. The technique is based on the non-proven assumption that absorbed zinc is cleared from plasma in the same way as intravenously injected zinc. Relatively large oral doses are necessary when a low absorption is expected, as could be the case for diets in many lower-income countries, to achieve a detectable level of enrichment. It has not been conclusively demonstrated that an intravenous infusion of zinc does not affect systemic zinc metabolism. Nonetheless, as only a spot urine sample is required, this method is simple for the participating subjects, and the number of samples to be analyzed is limited. Thus, if the validity of this approach can be documented, it could be a feasible method for field studies. Its application is, however, limited to single-meal studies and it would consequently be most valuable in populations with a monotonous food intake.

[8] M. Hansen, personal communication.

In vitro methods and models

For mechanistic studies of zinc absorption and evaluation of the effect of individual food components on zinc uptake, cell models (e.g., Caco-2 cells) may be useful. This method is less suited for studies of complex diets and it is unable to give quantitative information about absorption. Qualitative information about zinc availability may also be obtained from animal studies. A rat pup model has been demonstrated to be able to rank zinc absorption from infant formula in the same order as results from human studies using the whole-body counting technique [5], while adult rats were less suitable for this purpose.

An *in vitro* model simulating intestinal absorption conditions originally developed for iron [6] has also been applied to zinc [7]. After pepsin digestion at low pH, > 50% of zinc in cereal-based meals is released and dialyzable (MW cut off 6000–8000) while further trypsin digestion at pH 8 reduces the dialyzable fraction. A comparison with *in vivo* measurements of absorption showed a good correlation at pH 8 but not at the lower pH [7]. It is possible that with further development and validation this method could be used to give qualitative information about zinc availability for the screening of different possible zinc intervention strategies. Its ability to compare different foods or diets and to give information that can be used to judge the adequacy of a total diet is probably limited.

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Appendix 4

List of Contributors

Contributing authors

Zulfiqar Bhutta, MD, PhD, currently holds the Husein Lalji Dewraj Professorship of Paediatrics at the Aga Khan University and is also the director of neonatal services at the affiliated University Medical Center. Dr. Bhutta graduated from the Khyber Medical College (University of Peshawar) and received training in pediatrics at several leading hospitals of Pakistan and the United Kingdom, with subspecialty training in neonatal pediatrics and a PhD in nutrition from the Karolinska Institute, Stockholm. He is a fellow of the Royal College of Physicians of Edinburgh as well as the Royal College of Paediatrics and Child Health (UK). Dr. Bhutta is the president-elect of the Commonwealth Association of Paediatric Gastroenterology and Nutrition and a member of the Global Advisory Group on Health Research for the World Health Organization and the International Zinc Nutrition Consultative Group. He is also a member of the Child Health and Nutrition Board of the Global Forum for Health Research. Dr. Bhutta is the Chair of the Health Sciences projects committee of the Biotechnology Commission of Pakistan and an Advisor to the Pakistan Medical Research Council. He also serves on several international editorial boards of medical journals including the *British Medical Journal*, *Maternal and Child Nutrition*, *Transactions of Royal Society of Tropical Medical & Hygiene*, and *Current Pediatrics*. Dr. Bhutta has wide-ranging research interests including community-based perinatal care, interactions between nutrition and infection, micronutrient malnutrition and public health nutrition interventions. He has written two books, 25 book chapters, and more than 150 indexed publications and was recently awarded the Tamgha-i-Imtiaz by the President of Pakistan for contributions towards education and research.

Kenneth H. Brown, MD, is Professor of Nutrition and Director of the Program in International Nutrition at the University of California, Davis, where he has been a member of the faculty since 1989. Dr. Brown completed medical school at the University of Pennsylvania and specialty training in Pediatrics at the Boston Children's Hospital Medical Center. His research focuses on the nutritional problems of infants and young children in lower income countries, with special emphasis on the diagnosis and control of micronutrient deficiencies, infant and child feeding (breast feeding and complementary feeding), and interactions between nutrition and infection. Dr. Brown has participated in expert committees of the World Health Organization, the Pan American Health Organization, UNICEF, and the US National Academy of Science; and he has served as assistant editor or member of the editorial board of several journals, including the *American Journal of Clinical Nutrition*, the *European Journal of Clinical Nutrition*, and *the Journal of Health, Population, and Nutrition*. He is a former president of the Society of International Nutrition Research and Councilor of the American Society of Clinical Nutrition. He has helped to organize multiple international conferences and symposia on zinc and health and has served as the chairman of the IZiNCG Steering Committee since its inception. Dr. Brown is a recipient of the International Award for Modern Nutrition, the Kellogg International Nutrition Research Prize of the Society for International Nutrition Research, and the E.V. McCollum Award of the American Society for Clinical Nutrition.

Rosalind S. Gibson, PhD, is a Professor of Human Nutrition at the University of Otago, New Zealand, where she has held a personal chair since 1996. From 1979 to 1995, Dr. Gibson held academic appointments in the Division of Applied Human Nutrition at the University of Guelph, Ontario, Canada. She received a BSc degree (nutrition) from Queen Elizabeth College, University of London, a MS degree (public health nutrition) from the University of California, and a PhD degree (nutrition) from the University of London. She has served as a consultant for the World Health Organization and the International Atomic Energy Authority, and been a member of several national committees in Canada and New Zealand, including

the New Zealand National Food Advisory Committee. Her research has focused on the etiology and functional health consequences of zinc deficiency and, to a lesser extent, iron deficiency, in high-risk population groups. Much of her recent research has involved the development of food-based strategies to enhance the content and bioavailability of micronutrients, especially zinc, in the diets of infants and children in Malawi, and most recently Thailand. She is the author of a standard reference text and laboratory manual on nutritional assessment, published by Oxford University Press in New York, and the author or co-author with her graduate students of more than 130 refereed scientific papers. In 2002, Dr. Gibson was elected to Fellowship of the Royal Society of New Zealand.

Christine Hotz, PhD, currently works as an investigator and professor at the Instituto Nacional de Salud Pública (Mexico). She received her bachelor and master of science degrees (nutrition) from the University of Manitoba (Canada). She conducted her master's research in the nutrition research laboratories of Health Canada, and worked briefly in the nutrition programs and promotions unit of Health Canada before starting a doctoral program at the University of Otago (New Zealand). During her doctoral research, she worked with communities in rural Malawi, designing and evaluating methods to improve complementary feeding practices, with an emphasis on improved zinc nutrition. Dr. Hotz served as executive officer of IZiNCG during its first 2 years after inception and continues to offer technical assistance to the group. Her research activities include the study of zinc and iron absorption from cereal-based diets using isotopic techniques, methods to improve iron and zinc absorption, methods to evaluate zinc status, and interactions between intake and status of zinc, iron, and copper. Dr. Hotz is a member of the American Society of Nutritional Sciences (U.S.) and the Society for International Nutrition Research (U.S.).

Janet C. King, PhD, RD, is a scientist at Children's Hospital Oakland Research Institute and Professor emerita of Internal Medicine and Nutrition at the University of California, Davis. A member of the Institute of Medicine, she is recognized internationally for her research in zinc metabolism and in maternal nutrition. Dr. King has published more than 200 papers and abstracts and has trained more than 50 graduate students and post-doctoral fellows. She pioneered the use of stable isotopes of iron, copper, and zinc to study mineral metabolism in humans. This technique opened up a new approach for studying dietary mineral requirements of pregnant and lactating women, infants, and children, and the technique is used widely around the world. Dr. King also conducts research on energy requirements during pregnancy. She showed that maternal fat stores at conception dictate changes in energy metabolism during gestation. This finding led to the development of different weight gain standards for underweight, normal weight, and overweight women by an Institute of Medicine (IOM) Committee chaired by Dr. King. She is currently studying the role of maternal diet on body weight and metabolic adjustments during pregnancy. Dr. King also has a strong interest in the translation of research findings into nutrition policies and practice. Therefore, she is working to establish a Center for the Prevention of Obesity in Children at the Oakland Children's Hospital and Research Center. She also was recently appointed to the 2005 Dietary Guidelines Advisory Committee, one of the major sources of nutrition policy in the United States. Dr. King holds a PhD in nutrition from the University of California, Berkeley, where she also has a faculty appointment.

Bo Lönnerdal, PhD, is a Professor of Nutrition and Internal Medicine and a member of the Program in International Nutrition at the University of California (UC), Davis. He received his PhD in biochemistry at the University of Uppsala, Sweden, and has been at UC, Davis since 1978. His research background includes studies of lactation, the composition of breastmilk and the transfer of nutrients from the lactating mother to the breast-fed infant, the biochemistry of breast milk components and the function of breast milk proteins. He has also studied the absorption of trace elements, such as zinc, iron, copper and manganese, in experimental animals and humans and how various dietary factors affect their absorption. Current research interests include mechanisms of trace element absorption and transport, which are studied in cells, experimental animals and humans, and micronutrient interactions, both antagonisms and synergisms. He is a member of the American Society for Nutritional Sciences (ASNS), American Society for Clinical Nutrition (ASCN), Society for International Nutrition Research (SINR), the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), and the International Society for Research on Human Milk and Lactation (ISRHML). He has been awarded the Borden Award, the International Prize in Modern Nutrition, the Macy-Gyorgy Award and an Honorary Doctorate in Medicine at the University of Uppsala, Sweden.

Daniel Lopez de Romaña Forga, MS, is a researcher at the Instituto de Investigación Nutricional in Lima and a professor at the public nutrition master's degree program at the National Agrarian University La Molina in Lima. He has a MS in nutrition from the University of California, Davis and is a doctoral student in the Program in International Nutrition at the same institution. He is a member of the American Society of Nutritional Sciences and the American Society of Clinical Nutri-

tion. His research interests are the causes, treatment and prevention of micronutrient deficiencies in developing countries, with emphasis in iron and zinc.

Janet M. Peerson, MS, has been a senior statistician and statistical programmer for the Program in International Nutrition at the University of California (UC), Davis since 1989. She received her bachelor's and master's degrees in agricultural economics from UC Davis, and has completed doctoral-level coursework in statistics. Ms. Peerson has contributed to more than 40 published articles in the field of nutrition and has acted as a consultant to the UC Davis nutrition department and its associated programs. She is a member of the American Statistical Association.

Juan A. Rivera, PhD, is the founding Director of the Center for Research in Nutrition and Health at the National Institute of Public Health and Professor of Nutrition in the School of Public Health in Mexico. Dr. Rivera earned both his master's degree and doctorate degree in international nutrition from Cornell University, with a minor in epidemiology. Dr. Rivera's research interests include the epidemiology of nutritional stunting, the short- and long-term effects of supplementary feeding during early childhood in malnourished children, the effects of zinc and other micronutrient deficiencies on growth and health, the study of malnutrition in Mexico, and the design and evaluation of programs to improve nutrition status of children. A leader in nutrition research in Mexico and Latin America, he is a former director of Nutrition and Health at the Nutrition Institute of Central America and Panama (INCAP). More recently, he coordinated a national nutrition survey in Mexico, is a principal investigator of the Global Forum Coalition for the Latin America Region, and serves on several national and international committees. He has been a member of the PAHO Advisory Committee on Nutrition since 1995 and has recently been appointed to the board of the International Union of Nutritional Sciences, as member of the Global Alliance for Improved Nutrition (GAIN) Board, and to the National Academy of Medicine in Mexico. Dr. Rivera is also an adjunct professor at Cornell University and the Rollins School of Public Health at Emory University. He has published more than 100 scientific articles, book chapters, and books and is currently a member of the Latin American Nutrition Society, the American Society for Nutritional Sciences, and the Society for International Nutrition Research.

Marie T. Ruel, PhD, is a research fellow at the International Food Policy Research Institute (IFPRI) in Washington, DC. At IFPRI, Dr. Ruel is currently developing a new multi-country research program on "Diet Quality, Diet Changes and Health of the Poor" to analyze the impacts of food policies on diet quality and nutrition, with concerns ranging from micronutrient deficiencies to problems of over-nutrition. Between 1996 and 2002, she led a research program to analyze the food security and nutrition implications of rapid urbanization in developing countries. In the area of micronutrient deficiencies, Dr. Ruel's research focus has been on developing and evaluating effective food-based strategies, with a special emphasis on the monitoring and evaluation of such interventions. Since 2002, she has been involved in the preparation of a WHO Technical Document to Develop Guidelines on Food Fortification. She has also recently been appointed to the newly formed WHO International Micronutrient Advisory Group of Experts (IMAGE). Dr. Ruel earned a PhD in international nutrition from Cornell University. Before joining IFPRI in 1996, she was head of the Nutrition and Health Division of the Institute of Nutrition of Central America and Panama/Pan American Health Organization (INCAP/PAHO) in Guatemala, where she worked for 6 years. While at INCAP/PAHO, she conducted epidemiological research in maternal and child health and nutrition, in breastfeeding and complementary feeding, and in child growth and micronutrient deficiencies with a special emphasis on zinc and vitamin A deficiencies and the impact of supplementation on child morbidity and growth.

Brittmarie Sandström, PhD, was professor in the research department of human nutrition at the Royal Veterinary and Agricultural University in Copenhagen, Denmark until the time of her death (October 22, 2002). Dr. Sandström received a BSc in home economics from the University of Umeå and a BSc in nutrition from the Institute for Nutrition Research, University of Oslo, Norway. Dr. Sandström trained as a dietician at the Department of Clinical Nutrition, University of Gothenburg, Sweden, where she later received her PhD in nutrition. During her distinguished career, Dr. Sandström developed and validated several methodologies related to mineral absorption including a radioisotope method using whole-body counting and a rat-pup model for measuring zinc absorption, and a radioisotope method for measuring manganese absorption. She was a pioneer in the study of zinc absorption from infant milks and formulas, the effects of phytate on zinc absorption, and the mutual, competitive inhibition of iron and zinc on their absorption. She also contributed to the study of dietary factors affecting iron absorption. Her later research endeavors expanded to include preventive nutrition and contemporary public health issues, encompassing studies of fat quality on risk indicators of cardiovascular disease, the effect of copper in the prevention of cardiovascular disease, and the interactions of diet and osteoporosis. Dr. Sandström's expertise in mineral and trace element requirements

had many practical applications, contributing to the establishment of zinc requirements for infants, and later to the recommended dietary intakes for zinc published by the WHO, the latter representing a landmark in our understanding of human dietary zinc requirements. Dr. Sandström participated in executive committees and councils of the WHO, the European Union, the International Life Science Institute (ILSI, Europe) and the Danish Nutrition Council. She had a close affiliation with the Swedish National Food Administration and was part of the Swedish Expert Group for Food and Physical Exercise and Health 1987 and was a key member of a Committee under the Nordic Council of Ministers, with the mandate to establish *Nordic Nutrition Recommendations* (*NNR*), which she chaired from 1992 to 1996. Her research inspired others and will remain of considerable significance in the field of human nutrition for many years to come.

Emorn Wasantwisut (Udomkesmalee), PhD, is the Director of the Institute of Nutrition, Mahidol University. Dr. Wasantwisut holds a BSc in biochemistry from Chulalongkorn University, Thailand (1977), a MSc in nutrition from Brigham Young University, Utah, USA (1980), and a PhD in nutritional biochemistry and metabolism from Massachusetts Institute of Technology, Massachusetts, USA (1985). Her postdoctoral training was at the vitamin and mineral nutrition laboratory, Beltsville Human Nutrition Research Center, US Department of Agriculture, Beltsville, Maryland, USA (1987). Currently she also is the chairwoman of the Thai RDA Sub-Committee on Trace Elements and a consultant to the Ministry of Public Health/Thailand vitamin A program. She is the chairwoman of the Asian Task Force for Capacity Strengthening in Nutrition (under the United Nations Standing Committee on Nutrition–Working Group on Capacity Development) and is on the Global Steering Committee of the Ellison Medical Foundation–International Nutrition Foundation Fellowship Program and the Steering Committee of the International Zinc Consultative Group (IZiNCG). Dr. Wasantwisut is a member of the ILSI/South East Asia Micronutrient Task Force, a vitamin A correspondent for the *Sight and Life Newsletter*, and curator for the *International Journal of Vitamin and Nutrition Research*. Her research interests include vitamin A assessment, nutrient bioavailability and metabolism as well as nutrient interactions, particularly of vitamin A and zinc, and iron and zinc, and nutrients and immune function.

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