

Docosahexaenoic and Arachidonic Acid Prevent a Decrease in Dopaminergic and Serotonergic Neurotransmitters in Frontal Cortex Caused by a Linoleic and α -Linolenic Acid Deficient Diet in Formula-fed Piglets¹

Sylvia de la Presa Owens and Sheila M. Innis²

Department of Paediatrics, University of British Columbia, Vancouver, Canada V5Z 4H4

ABSTRACT This study examined the effects of diets deficient (D) in linoleic [18:2(n-6)] and linolenic acid [18:3(n-3)] at 0.8 and 0.05% energy, respectively, or adequate (C) in 18:2(n-6) and 18:3(n-3) at 8.3 and 0.8% energy, respectively, without (–) or with (+) 0.2% energy arachidonic [20:4(n-6)] and 0.16% energy docosahexaenoic [22:6(n-3)] acid in piglets fed from birth to 18 d. Frontal cortex dopaminergic and serotonergic neurotransmitters and phospholipid fatty acids were measured. Piglets fed the D– diet had significantly lower frontal cortex dopamine, 3,4-dihydroxyphenylacetic (DOPAC), homovanillic acid (HVA), serotonin and 5-hydroxyindoleacetic acid (5-HIAA) concentrations than did piglets fed the C– diets. Frontal cortex dopamine, norepinephrine, DOPAC, HVA, serotonin and 5-HIAA were higher in piglets fed the D+ compared to those fed the D– diet ($P < 0.05$) and not different between piglets fed the D+ and those fed the C– diets or the C– and C+ diets. Piglets fed the D– diet had lower frontal cortex phosphatidylcholine (PC) and phosphatidylinositol (PI) 20:4(n-6) and PC and phosphatidylethanolamine (PE) 22:6(n-3) than did piglets fed the C– diet ($P < 0.05$). Piglets fed the D+ diet had higher frontal cortex PC and PI 20:4(n-6) and PC, PE, PS and PI 22:6(n-3) than did piglets fed the D– diet. These studies show that dietary essential fatty acid deficiency fed for 18 d from birth affects frontal cortex neurotransmitters in rapidly growing piglets and that these changes are specifically due to 20:4(n-6) and/or 22:6(n-3). *J. Nutr.* 129: 2088–2093, 1999.

KEY WORDS: • essential fatty acids • docosahexaenoic acid • arachidonic acid • dopaminergic • serotonergic • piglets

Brain lipids contain high proportions of the polyunsaturated fatty acids (PUFA)³ arachidonic acid [20:4(n-6)] and docosahexaenoic acid DHA [22:6(n-3)] (Sastry 1985). Arachidonic acid [20:4(n-6)] and 22:6(n-3) are derived from the dietary essential fatty acid (EFA) linoleic acid [18:2(n-6)] and α -linolenic acid [18:3(n-3)], respectively, by sequential desaturation and elongation (Sprecher et al. 1995), and by dietary intake. Brain phospholipids contain high proportions of 20:4(n-6) and 22:6(n-3), but only small amounts of the precursors 18:2(n-6) and 18:3(n-3) (Sastry 1985). Dietary deficiency of (n-6) and (n-3) fatty acids results in reduced 20:4(n-6) and 22:6(n-3), respectively, in the brain and influences various aspects of neural function at the behavioral level, including changes on behavioral tests of learning, memory and habituation (Bourre et al. 1989, Enslen et al. 1991, Frances et al. 1996, Lamptey and Walker 1976, Wainwright

and Ward 1997, Yamamoto et al. 1988). The biochemical explanation for the effects of dietary (n-6) and (n-3) fatty acids on brain functions are not known, although changes in some central nervous system (CNS) enzyme activities (Bourre et al. 1989) and in the levels of monoaminergic neurotransmitters (Delion et al. 1994 and 1996) were reported in rats after prolonged feeding with (n-3) fatty acid-deficient diets. Changes in monoamine metabolism are of interest because the dopaminergic and serotonergic systems are involved in the regulation of some behaviors, memory and learning processes (Brozosky et al. 1979, McEntee and Cook 1991, Simon et al. 1980). Previous studies concerning the effects of dietary (n-6) and (n-3) fatty acid deficiency on behavior and brain fatty acids used animals fed deficient diets through gestation and early development, and often for one or more generations (Bourre et al. 1989, Enslen et al. 1991, Frances et al. 1996, Lamptey and Walker 1976, Reisbick et al. 1990, Wainwright and Ward 1997, Yamamoto et al. 1988).

Despite substantial evidence of the importance of 20:4(n-6) and 22:6(n-3) in normal brain function, little is known about the pathways by which the brain normally acquires (n-6) and (n-3) fatty acids. Recent studies, however, have identified fatty acid-binding and -transport proteins in the developing brain (Utsunomiya et al. 1997, Xu et al. 1996). The brain takes up

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² To whom correspondence should be addressed.

³ Abbreviations used: C–, adequate without 20:4(n-6) and 22:6(n-3); C+, adequate with 20:4(n-6) and 22:6(n-3); D–, deficient without 20:4(n-6) and 22:6(n-3); D+, deficient with 20:4(n-6) and 22:6(n-3); DOPAC, 3,4-dihydroxyphenylacetic; EFA, essential fatty acid; HVA, homovanillic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, Phosphatidylserine; PUFA, polyunsaturated fatty acids.

18:2(n-6) and 18:3(n-3), and recent studies have shown the brain is able to synthesize 20:4(n-6) and 22:6(n-3) from the 18 carbon chain precursors (Dhopeswarkar et al. 1971a and 1971b; Edmond et al. 1998, Moore et al. 1990 and 1991). However, the importance of uptake of 20:4(n-6) and 22:6(n-3) from plasma rather than desaturation-elongation of 18:2(n-6) and 18:3(n-3) as a source of 20:4(n-6) and 22:6(n-3) for the growing brain is not yet clear. Numerous studies showed that blood lipid levels of 20:4(n-6), 22:6(n-3) and 22:6(n-3) are lower in infants fed formula with 18:2(n-6) and 18:3(n-3), but not 20:4(n-6), than are those in breast fed infants (Innis et al. 1994b, Makrides et al. 1995, Putnam et al. 1982). Thus, it is important to understand the relative importance of dietary 18:2(n-6) and 18:3(n-3), compared to 20:4(n-6) and 22:6(n-3), on the composition of brain fatty acids and neurotransmitter metabolism. The purpose of this study was to determine the importance of dietary 18:2(n-6) and 18:3(n-3), and their longer-chain derivatives 20:4(n-6) and 22:6(n-3), respectively, on frontal cortex levels of dopaminergic and serotonergic neurotransmitters in neonatal piglets. Piglets were used for these studies because the perinatal patterns of piglet brain growth and pig milk lipids resemble those of humans and because pigs can be bottle-fed from birth (Innis 1992). The latter allows complete control of the diet fatty acid composition in the early neonatal period.

MATERIALS AND METHODS

Animals and diets. Newborn male piglets weighing >1 kg at birth and <12 h old (Kintail Meats, Langley, British Columbia) were assigned to be fed one of four formula diets ($n = 6/\text{group}$). The composition of the liquid formulas was based on the macro- and micro-nutrient composition of pig milk and differed only in the composition of the fat. Two of the formulas were deficient (D) in 18:2(n-6) and 18:3(n-3), with about 0.8% energy 18:2(n-6) and 0.05% energy 18:3(n-3) [18:2(n-6)+18:3(n-3) deficient]; the other two formulas had adequate (C) 18:2(n-6) and 18:3(n-3), representing 8.3 and 0.8% energy, respectively, [18:2(n-6)-18:3(n-3) adequate] (Table 1). One of each of the 18:2(n-6) + 18:3(n-3) deficient and 18:2(n-6)+18:3(n-3) adequate formulas had 0.2% energy (0.4g/100 g total fatty acids) 20:4(n-6) and 0.16% energy 22:6(n-3) (D+, C+, respectively). The other two formulas had no other added oil sources (D-, C-). The 20:4(n-6) and 22:6(n-3) were from single-cell triglycerides and were included in the formula during preparation by Nestlé Research Center (Lausanne, Switzerland). The formulas contained 57.9 g fat and 4.143 MJ/L, with a macronutrient and micro-nutrient composition similar to that used previously (Innis and Dyer 1997). Littermates were not assigned to the same diet. The formula-fed piglets were bottle-fed by hand to 18 d of age (Innis and Dyer 1997). The procedures involving the piglets were approved by the Animal Care Committee of the University of British Columbia and conformed to the guidelines of the Canadian Council on Animal Care.

Sample collection. The piglets were anesthetized (Ketaset® 37.5: 3.75 mg/kg MTC Pharmaceuticals, Cambridge, Canada; Bayvet Division, Chenango, Etobicoke, Canada, respectively, by intramuscular injection) at 18 d of age, 3–4 h after the last feeding (Innis and Dyer 1997). Blood samples were drawn by intracardiac puncture, and the animals were killed by intracardiac injection of 200 mg pentobarbital/kg. The brain was rapidly removed, weighed and the frontal cortex dissected and frozen in liquid nitrogen. The remaining cerebrum tissue was homogenized (5 mL/g, 0.32 mol sucrose/L, 15 mmol Tris HCl/L with 1 mmol EDTA/L, 1 mmol MgCl/L and 1.5 mmol glutathione/L, pH 7.4) then frozen in liquid nitrogen. All of the samples were stored at -80°C until analysis.

Frontal cortex and total cerebrum lipid analyses. Frontal cortex and total cerebrum lipids were extracted (Folch et al. 1957), then phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS) were separated on thin layer chromatography plates (Whatman PK6F Silica Gel 60 A)

TABLE 1

Major fatty acid components of formulas varying in 18:2(n-6) and 18:3(n-3) without or with 20:4(n-6) and 22:6(n-3)¹

Fatty acid	Formula			
	D-	D+	C-	C+
	g/100g fatty acids			
8:0	8.0	7.4	17.2	14.9
10:0	6.7	6.5	13.5	13.0
12:0	44.2	42.9	1.0	0.3
14:0	17.1	16.8	0.8	0.6
16:0	9.5	9.5	11.3	10.9
18:0	0.4	3.5	3.2	3.3
16:1	0.1	0.1	0.1	0.2
18:1	8.1	9.3	33.3	35.1
18:2(n-6)	1.6	1.9	15.6	16.4
18:3(n-6)	—	0.1	—	0.1
20:4(n-6)	—	0.4	—	0.4
18:3(n-3)	0.1	0.1	1.5	1.6
22:6(n-3)	—	0.3	—	0.3

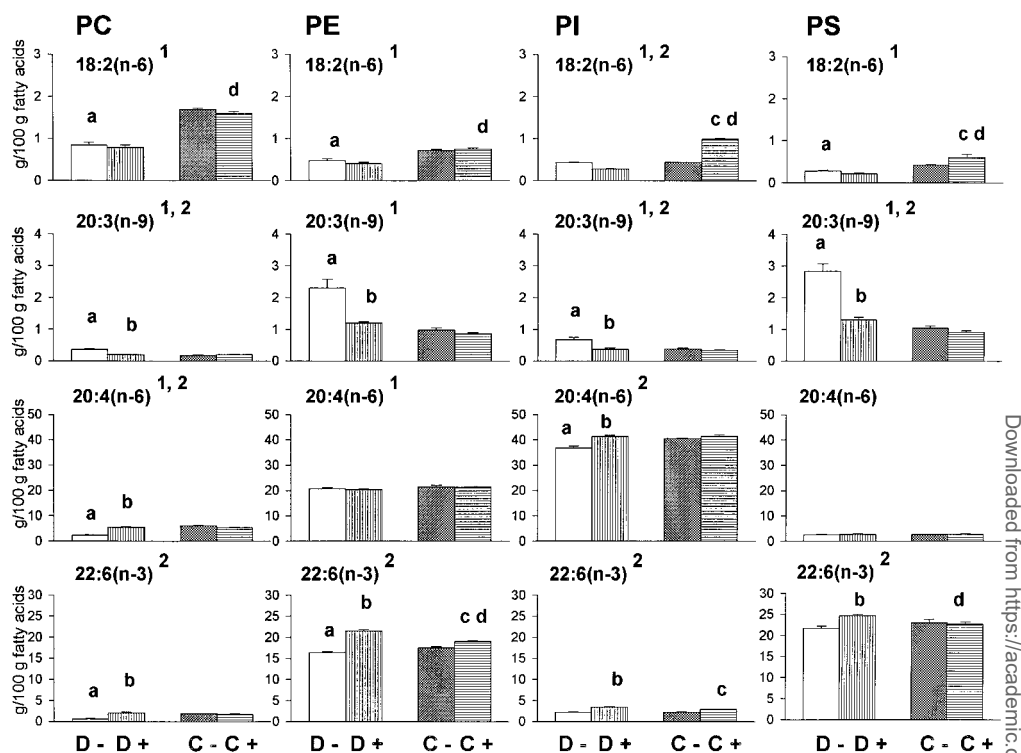
¹ The formulas contained 57.9 g of fat and 4.143 MJ/L, with the same macro- and micro-nutrient composition as in Innis and Dyer (1997) and were deficient (D) or adequate (C) in 18:2(n-6) and 18:3(n-3) without (-) or with (+) 20:4(n-6) and 22:6(n-3) from single cell triglycerides.

by using methyl acetate/n-propanol/chloroform/methanol/KCl 0.25% (25:25:28:10:7 v/v/v/v/v) followed by methyl acetate/n-propanol/chloroform/methanol/KCl 0.25% (25:25:25:10:9 v/v/v/v/v) in the same dimension. The separated phospholipids were recovered, the fatty acid components converted to their respective methyl esters, separated, identified and quantified by gas liquid chromatography (Innis et al. 1994a). Total cerebrum cholesterol was analyzed by using enzymatic reagents (Diagnostic Chemical Limited, Charlottetown, Prince Edward Island, Canada), and phospholipid was analyzed according to Chen et al. (1956).

Total cerebrum CNPase activity, DNA and protein. The activity of brain 2', 3' cyclic nucleotide 3'-phosphohydrolase (CNPase), which increases in parallel with myelination (Norton and Cammer 1985) was determined according to Prohaska et al. (1973). The brain DNA concentration was estimated fluorometrically with DyNA Quant 200 (Hoefer, San Francisco, CA), with calf thymus DNA as the standard. Protein was determined according to Lowry et al. (1951).

Frontal cortex monoamines. Frontal cortex (200 mg) was homogenized (Sonic Materials, Danbury, CT) in 760 μL of perchloric acid 0.1 mol/L, with 40 μL of 3,4-dihydroxybenzylamine (3 mg/L) as an internal standard. The resulting homogenate was then centrifuged at 172,400 $\times g$, for 1 h at 4°C in a L7-55 ultracentrifuge equipped with 50.2 Ti type rotor (Beckman Instruments, Palo Alto, CA.). The supernatant was then transferred to a low volume insert vial (Waters Div. of Millipore, Milford, MA). The frontal cortex monoamine concentrations were then determined by HPLC, using a Waters Alliance 2690 separation module equipped with a refrigerated autosampler (Waters, Mississauga, Ontario, Canada) with electrochemical detection (EG&G Princeton Applied Res, Princeton, NJ, electrochemical detector model 400) with a glass carbon electrode cell block and reference electrode 3M NaCl/Sat AgCl filling solution. The analytical column was a Symmetry C18, 2.1 \times 150 mm, coupled to a guard column Sentry Symmetry C18, 3.9 \times 20 mm (Waters, Milford, MA). The mobile phase consisted of 6 g sodium acetate/L, 10 mg EDTA/L, 125 mg octyl sulfate sodium salt/L, 27 mL glacial acetic acid/L, and 20 mL HPLC grade methanol/L. The mobile phase was filtered and degassed by using a solvent filtration apparatus with GV 0.22 μm (pore size) Millipore filters. The separation was performed under isocratic conditions with a column temperature of 32°C and a flow rate of 0.3 mL/min, allowing for the separation of dopa-

FIGURE 1 Levels of 18:2(n-6), 20:3(n-9), 20:4(n-6) and 22:6(n-3) in frontal cortex phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS) of piglets fed formula deficient in 18:2(n-6) and 18:3(n-3) without (D-) or with (D+) 20:4(n-6) and 22:6(n-3), or adequate in 18:2(n-6) and 18:3(n-3) without (C-) or with (C+) 20:4(n-6) and 22:6(n-3). Values shown are means \pm SEM, $n = 6$ /group. 1: Main effect of 18:2(n-6)-18:3(n-3) deficient compared to adequate (D/C). 2: Main effect of addition compared to no addition of 20:4(n-6) and 22:6(n-3) (-/+). a: Different from value for 18:2(n-6)-18:3(n-3) adequate. b: Different from value for 18:2(n-6)-18:3(n-3) deficient. c: Different from value for 18:2(n-6)-18:3(n-3) adequate. d: Different from value for 18:2(n-6)-18:3(n-3) deficient with 20:4(n-6) and 22:6(n-3). $P < 0.05$. Significant interactions between the addition of 20:4(n-6) and 22:6(n-3) and the amount of 18:2(n-6) and 18:3(n-3) in the formula are described in the text.



mine, serotonin, norepinephrine, and their major metabolites, 3,4-dihydroxy-phenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindolacetic acid (5-HIAA), as well as the precursors tyrosine and tryptophan. The working electrode potential was maintained at 775 mV and 5 nA for the range current.

Statistical analyses. Results were compared between the groups by using two-way ANOVA, with the level of 18:2(n-6)-18:3(n-3) (D or C, respectively) and no addition or addition of 20:4(n-6) and 22:6(n-3) (- or +, respectively) as the main effects. The homogeneity of the variances was analyzed by the Levene test and was found to be not significantly different. Formal tests for significant difference were made by using Fisher's least significant difference and were performed only for ANOVA results with $P < 0.05$. The results given are means \pm SEM, $n = 6$ /group.

RESULTS

Brain frontal cortex fatty acid composition. There were no significant differences in the brain weight among piglets fed the different diets (46.8 ± 0.5 , 48.3 ± 1.3 , 45.6 ± 1.4 , 46.8 ± 1.5 g, for piglets in the D-, D+, C-, C+ groups, respectively). Similarly, there were no significant differences in the brain protein, DNA, cholesterol or phospholipid concentrations, or CNPase activity among piglets fed the different formulas (data not shown).

The frontal cortex of the piglets fed the D- formula had a significantly lower percentage of 18:2(n-6), 20:4(n-6), and 22:6(n-3) and a higher percentage of 20:3(n-9) in PC; lower 18:2(n-6) and 20:4(n-6) and higher 20:3(n-9) in PE; lower 18:2(n-6) and 20:4(n-6) and higher 20:3(n-9) in PI; and lower 18:2(n-6) and higher 20:3(n-9) in PS than did piglets fed the C- formula (Fig. 1). The effects of including 20:4(n-6) and 22:6(n-3) in the formula depended on the formula content of the precursors 18:2(n-6) and 18:3(n-3) ($P < 0.05$). The frontal cortex of piglets fed the D+ formula had a significantly higher percent 22:6(n-3) in PC, PE, PI, and PS, and higher 20:4(n-6) in PC and PI, and lower 20:3(n-9) in PC, PE, PI, and PS than in that of piglets fed the D- formula. In contrast,

the frontal cortex phospholipid fatty acid composition of piglets fed the C+ formula was not different from that of piglets fed the C- formula, except for a significantly higher percent of 22:6(n-3) in PE and PI and higher 18:2(n-6) in PI and PS. The frontal cortex of piglets fed the D+ formula had a significantly higher percentage of 22:6(n-3) in PE and PS and lower 18:2(n-6) in PS than that in piglets fed the C+. The percentages of 20:3(n-9) in frontal cortex PC, PE, PI and PS were not different between piglets fed the C- or C+ diets.

Frontal cortex endogenous monoamine. Piglets fed the formulas with 20:4(n-6) and 22:6(n-3) had significantly higher frontal cortex dopamine, HVA and norepinephrine, as well as tryptophan and serotonin concentrations than did piglets fed the formulas without 20:4(n-6) and 22:6(n-3) (Fig. 2). Piglets fed the D formulas deficient in 18:2(n-6)-18:3(n-3) had significantly lower dopamine and serotonin concentrations than did piglets fed the C formulas with adequate 18:2(n-6) and 18:3(n-3). Piglets fed the D- formula had a significantly lower concentration of dopamine and its degradation metabolites, DOPAC and HVA, and lower serotonin and its degradation metabolite, 5-HIAA, in the frontal cortex than did piglets fed the C- or C+ formula. Piglets fed the D+ formula had significantly higher frontal cortex dopamine, norepinephrine, DOPAC, HVA, serotonin and 5-HIAA concentrations than did piglets fed the C- ($P < 0.05$). Thus, the concentrations of all frontal cortex monoamines and metabolites in piglets fed D+ formula were not different from those of piglets fed the C- or C+ adequate formulas. The inclusion of 20:4(n-6) and 22:6(n-3) in the 18:2(n-6)-18:3(n-3) adequate formula had no significant effect on any of the frontal cortex monoamines or metabolites measured (C+ compared to C-).

DISCUSSION

These studies are the first to show that feeding a formula deficient in (n-6) and (n-3) fatty acids, with $< 1\%$ energy

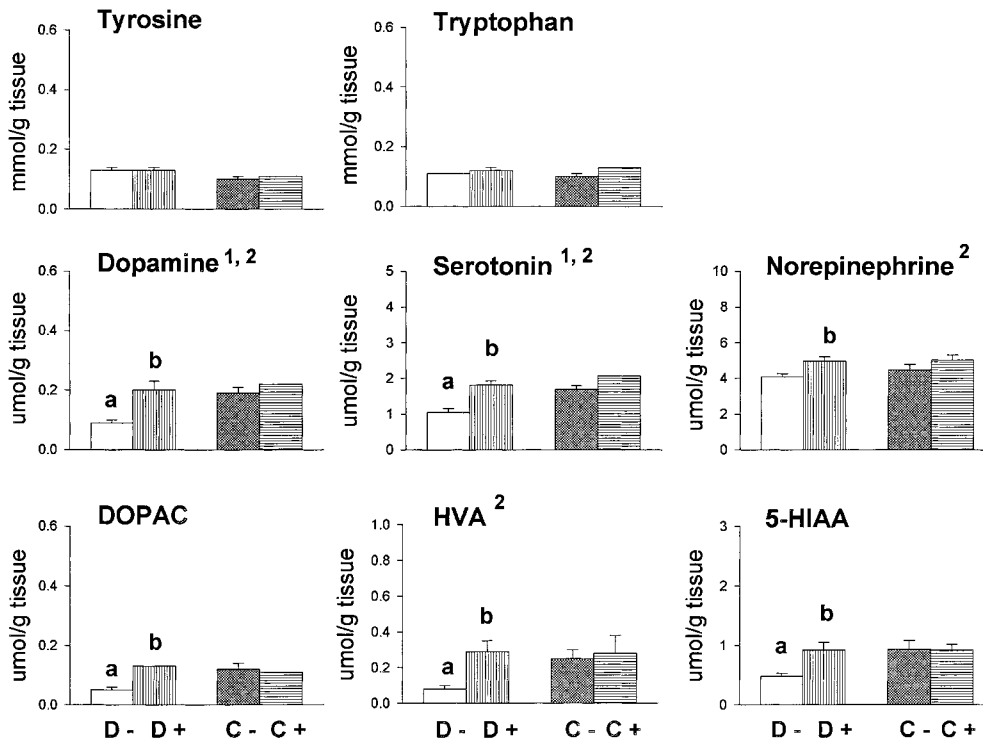


FIGURE 2 Levels of monoamines, precursors and metabolites in frontal cortex of piglets fed formula deficient in 18:2(n-6) and 18:3(n-3) without (D-) or with (D+) 20:4(n-6) and 22:6(n-3) or adequate in 18:2(n-6) and 18:3(n-3) without (C-) or with (C+) 20:4(n-6) and 22:6(n-3). Values shown are means + SEM, $n = 6$ /group. 1: Main effect of 18:2(n-6)-18:3(n-3) deficient compared to adequate (D/C). 2: Main effect of addition compared to no addition of 20:4(n-6) and 22:6(n-3) (-/+). a: Different from value for 18:2(n-6)-18:3(n-3) adequate. b: Different from value for 18:2(n-6)-18:3(n-3) deficient. c: Different from value for 18:2(n-6)-18:3(n-3) adequate. $P < 0.05$. No significant interactions between the amount of 18:2(n-6) and 18:3(n-3) and the addition of 20:4(n-6) and 22:6(n-3) were found.

18:2(n-6) and $< 0.05\%$ energy 18:3(n-3), for only 18 d from birth, results in significant alteration of developing frontal cortex concentrations of dopamine and serotonin and the major metabolites DOPAC, HVA and 5-HIAA. The formula-induced changes in dopamine and serotonin in piglets fed the (n-6) and (n-3) fatty acid deficient formula were accompanied by reduced frontal cortex 20:4(n-6) and 22:6(n-3). The studies here are also the first to show that providing a small amount of 20:4(n-6) and 22:6(n-3), representing about 0.2 and 0.16% dietary energy, respectively, prevents the effect of a 18:2(n-6)-18:3(n-3) deficient formula on frontal cortex dopamine and serotonin and their metabolites, DOPAC, HVA and 5-HIAA. These findings suggest that 20:4(n-6) and 22:6(n-3), not the precursors 18:2(n-6) and 18:3(n-3), are important determinants of at least some aspects of brain neurotransmitter metabolism, and that dietary and plasma 20:4(n-6) and 22:6(n-3) are available to the brain. However, piglets fed the 18:2(n-6)-18:3(n-3) adequate formula, with about 8.3% energy 18:2(n-6) and 0.8% energy 18:3(n-3), had frontal cortex dopamine, norepinephrine, serotonin, DOPAC, HVA and 5-HIAA concentrations not different from that of piglets fed the 18:2(n-6)-18:3(n-3) deficient formula with 20:4(n-6) and 22:6(n-3). No significant effects of including 20:4(n-6) and 22:6(n-3) in the 18:2(n-6)-18:3(n-3) adequate formula on frontal cortex monoaminergic neurotransmitters were found. The results to show dietary 20:4(n-6) and 22:6(n-3) prevent the decrease in frontal cortex 20:4(n-6) and 22:6(n-3) in piglets fed a 18:2(n-6)-18:3(n-3) deficient formula favors a hypothesis that the brain is able to use preformed 20:4(n-6) and 22:6(n-3) for plasma that is used in membrane lipid synthesis. Recent studies have identified fatty acid-transport and lipid-binding proteins in the brain that have preference for the longer chain PUFA (Utsunomiya et al. 1997, Xu et al. 1996). Alternatively, the provision of a dietary 20:4(n-6) and 22:6(n-3) could spare the very limited dietary supply of 18:2(n-6) and 18:3(n-3) for the brain.

The studies here cannot address the relative importance of

plasma 18:2(n-6)-18:3(n-3) compared with 20:4(n-6) and 22:6(n-3) for brain lipid synthesis under conditions of adequate 18:2(n-6)-18:3(n-3) intake. However, piglets fed the formula with about 8.3% energy 18:2(n-6) and 0.8% energy 18:3(n-3) had levels of 20:4(n-6) in their frontal cortex phospholipids that were not different from that of piglets fed the same (adequate) formula or the deficient formula with 20:4(n-6) and 22:6(n-3). This finding is consistent with previous studies that suggested that when provided with adequate 18:2(n-6) and 18:3(n-3), the newborn piglet is able to make sufficient 20:4(n-6) and 22:6(n-3), either in the liver or in the brain itself, to meet the needs of the developing brain (Arbuckle et al. 1992 and 1994). Others have shown the brain is able to form 20:4(n-6) and 22:6(n-3) from the respective 18:2(n-6) and 18:3(n-3) precursors (Clandinin et al. 1985, Moore et al. 1990 and 1991). The percentage of 22:6(n-3) was ~20 and 36% higher in frontal cortex PE and PI in piglets fed the D+ and the D- formulas, respectively, and ~9 and 24% higher in PE and PI of piglets fed the C+ and C- formulas, respectively. Previous studies have shown that the addition of 22:6(n-3) from fish oil to formula results in a dose-dependant increase in brain and brain synaptic plasma membrane PE 22:6(n-3), although to a much smaller extent than in the plasma or liver (Arbuckle et al. 1991 and 1992). Diets high in 22:6(n-3) were also shown to increase brain and retina 22:6(n-3) in rodents and nonhuman primates (Lin et al. 1990, Wainwright et al. 1997, Weisinger et al. 1996, Yeh et al. 1998). This may suggest that brain uptake and regulation of 20:4(n-6) and 22:6(n-3) differs; however, dose-response studies of brain lipids after high intakes of 20:4(n-6) do not seem to have been reported.

Behavioral changes in tests of learning and memory were noted in several, but not all, studies with rodents fed diets very low in 18:2(n-6) and 18:3(n-3) or adequate in 18:2(n-6) but deficient in 18:3(n-3) (Bourre et al. 1989, Enslin et al. 1991, Frances et al. 1996, Lamprey and Walker, 1976, Wainwright and Ward, 1997, Yamamoto et al. 1988). Whether or not

changes in neurotransmitter metabolism can explain the behavioral changes associated with (n-6) and/or (n-3) fatty acid deficiency is not yet clear. Studies with second generation rats fed a 18:3(n-3) deficient diet reported lower frontal cortex dopamine, but not serotonin, concentrations; reduced D2 receptor binding; and higher 5-HT₂ receptor density (Delion et al. 1994 and 1996). Subsequent studies with second generation rats fed a 18:3(n-3) deficient diet found increased levels of dopamine metabolites, without modification of basal dopamine levels, and reduced release of dopamine during tyramine-stimulated, but not basal conditions, of microdialysis (Zimmer et al. 1998). Because dopamine stored in synaptic vesicles is recruited to maintain requirements, and the lower dopamine caused by the 18:3(n-3) deficiency was not accompanied by changes in monoamine oxidase activity (Delion et al. 1994), it was suggested that 18:3(n-3) deficiency decreases the storage pool of dopamine (Zimmer et al. 1998). Yoshida et al. (1997), on the other hand, based on evidence of low synaptic vesicle densities after a learning task in rats fed a (n-3) fatty acid deficient diet (safflower oil) compared to a high (n-3) fatty acid diet (perilla oil), suggested changes in the turnover rate of synaptic vesicles. Our studies with neonatal piglets showed that feeding a formula similar in nutrient composition to milk (except for fatty acids) and low in both 18:2(n-6) and 18:3(n-3) results in lower frontal cortex concentrations of dopamine and in the mitochondrial monoamine oxidase products DOPAC and HVA, as well as serotonin and its product, 5-HIAA, after only 18 d feeding from birth. The decrease in both dopamine and serotonin and in the metabolites DOPAC, HVA and 5-HIAA in our studies with piglets suggests the possibility of decreased synthesis, which will need to be evaluated through measures of neurotransmitter synthesis and turnover. Possibly, the effects of combined 18:2(n-6) and 18:3(n-3) deficiency are more severe, or 20:4(n-6) may have specific or additional effects from (n-3) fatty acids on frontal cortex dopamine and serotonin synthesis and metabolism. It is also possible that the effects of (n-6) and (n-3) fatty acids on brain function may differ between piglets and rats. However, an important aspect of our studies with piglets is that the dietary deficiency was imposed in the early neonatal period that is associated with exclusive milk feeding by feeding formulas with a defined fat composition. This avoids potential transfer of (n-6) and (n-3) fatty acids from maternal stores via milk to the developing young.

The explanation for the changes in frontal cortex dopamine and serotonin, as well as the metabolites DOPAC, HVA and 5-HIAA, with dietary (n-6) and (n-3) fatty acid deprivation is not known. The (n-6) and/or (n-3) fatty acid composition of neural membranes could alter membrane properties or the function of membrane associated transport systems, receptors or enzymes, or possibly lead to structural changes in developing synapses. In this regard several studies showed that dietary (n-3) fatty acid deficiency results in reduced 22:6(n-3) in synaptosomal membranes (Arbuckle and Innis 1992, Bourre et al. 1989, Foote et al. 1990, Hrboticky et al. 1989, Youyou et al. 1986). Wainwright et al. (1999) also recently reported evidence of altered dendritic morphology in the brains of 16-wk-old mice fed an EFA deficient diet through development. The diet-induced changes in 20:4(n-6) and 22:6(n-3) in the frontal cortex phospholipids of piglets fed the 18:2(n-6) and 18:3(n-3) deficient diet in the studies presented here were relatively modest when compared to the marked differences in frontal cortex monoaminergic neurotransmitters. For example, the frontal cortex PI had 35.8 ± 0.7 and $40.1 \pm 0.5\%$ 20:4(n-6), and PE had 16.0 ± 0.3 and $21.2 \pm 0.4\%$ 22:6(n-3), whereas the frontal cortex concentration of dopamine was

0.09 ± 0.0 $\mu\text{mol/g}$ and 1.04 ± 0.1 $\mu\text{mol/g}$ and serotonin was 0.2 ± 0.0 $\mu\text{mol/g}$ and 1.81 ± 0.1 $\mu\text{mol/g}$, for piglets fed the D- and D+ formulas, respectively. Furthermore, the effects of the formula fatty acid composition on frontal norepinephrine were much smaller (4.09 ± 0.16 , 4.97 ± 0.25 $\mu\text{g/g}$ for piglets fed the D- and D+ formulas, respectively) than for dopamine or serotonin. Whether the effects of (n-6) and (n-3) fatty acids are mediated by some mechanism other than a change in the membrane phospholipid fatty acid composition, for example, involving specific signaling systems, which may have developmental, neurotransmitter and/or species specific relevance, and if neurotransmitters other than those measured here are affected may be worth considering.

The 18:2(n-6)-18:3(n-3) deficient formula fed in our studies had a high content of 8:0–14:0, representing about 77% fatty acids, whereas 8:0–14:0 represented only about 20% fatty acids in the 18:2(n-6)-18:3(n-3) adequate formula. Thus, the possibility that the changes in frontal cortex monoamine metabolism were due to saturated rather than polyunsaturated fatty acids needs to be considered. The addition of 20:4(n-6) and 22:6(n-3) to the 18:2(n-6)-18:3(n-3) deficient formula, however, increased dopamine, norepinephrine, serotonin, DOPAC, HVA and 5-HIAA in the frontal cortex. From this it is reasonable to believe that the changes in dopamine and serotonin were due to the absence of the (n-6) and (n-3) fatty acids, 20:4(n-6) and 22:6(n-3), and not to the high saturated fat content of the formula.

In summary, these studies showed that feeding an EFA deficient diet to the neonatal piglet for as little as 18 d from birth significantly decreased frontal cortex 20:4(n-6) and 22:6(n-3) and decreased the frontal cortex concentration of dopaminergic and serotonergic neurotransmitters. The absence of decreased frontal cortex dopaminergic and serotonergic neurotransmitters in piglets fed small amounts of 20:4(n-6) and 22:6(n-3), representing about 0.2% and 0.15% dietary energy, respectively, provide clear evidence of the role for 20:4(n-6) and/or 22:6(n-3) in normal brain function. The explanation for the effects of dietary (n-6) and (n-3) fatty acids on frontal cortex monoaminergic neurotransmitters, the relevance to early parenteral nutrition support without lipid, or to human diets containing oils very low in (n-6) and (n-3) fatty acids, is not yet known.

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