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Maternal dietary 22 : 6*n*-3 is more effective than 18 : 3*n*-3 in increasing the 22 : 6*n*-3 content in phospholipids of glial cells from neonatal rat brain

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One of the debates in infant nutrition concerns whether dietary 18 : 3*n*-3 (linolenic acid) can provide for the accretion of 22 : 6*n*-3 (docosahexaenoic acid, DHA) in neonatal tissues. The objective of the present study was to determine whether low or high 18 : 3*n*-3 *v.* preformed 22 : 6*n*-3 in the maternal diet enabled a similar 22 : 6*n*-3 content in the phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS) of glial cells from whole brain (cerebrum and cerebellum) of 2-week-old rat pups. At parturition, the dams were fed semi-purified diets containing either increasing amounts of 18 : 3*n*-3 (18 : 2*n*-6 to 18 : 3*n*-3 fatty acid ratio of 7·8 : 1, 4·4 : 1 or 1 : 1), preformed DHA, or preformed 20 : 4*n*-6 (arachidonic acid) + DHA. During the first 2 weeks of life, the rat pups from the respective dams received only their dam's milk. The fatty acid composition of the pups' stomach contents (dam's milk) and phospholipids from glial cells were quantified. The 20 : 4*n*-6 and 22 : 6*n*-3 content in the stomach from rat pups at 2 weeks of age reflected the fatty acid composition of the dam's diet. The 20 : 4*n*-6 content of PE and PS in the glial cells was unaffected by maternal diet treatments. Preformed 22 : 6*n*-3 in the maternal diet increased the 22 : 6*n*-3 content of glial cell PE and PS compared with maternal diets providing an 18 : 2*n*-6 to 18 : 3 *n*-3 fatty acid ratio of 7·8 : 1, 4·4 : 1 or 1 : 1 ($P<0·0001$). There was no significant difference in the 20 : 4*n*-6 and 22 : 6*n*-3 content of glial cell PC and PI among maternal diet treatments. It was concluded that maternal dietary 22 : 6*n*-3 is more effective than low or high levels of maternal dietary 18 : 3*n*-3 at increasing the 22 : 6*n*-3 content in PE and PS of glial cells from the whole brain of rat pups at 2 weeks of age. The findings from the present study have important implications for human infants fed infant formulas that are devoid of 22 : 6*n*-3.

Linolenic acid: Docosahexaenoic acid: Glial cells: Rats

Arachidonic acid (AA; 20 : 4*n*-6) and 22 : 6*n*-3 are the most abundant PUFA in the phospholipids of the central nervous system (O'Brien & Sampson, 1965; Sun & Horrocks, 1970) and can be synthesised in animal tissues by the desaturation and elongation of their dietary precursors, 18 : 2*n*-6 and 18 : 3*n*-3 (Dhopeshwarkar & Subramanian, 1976; Bourre *et al.* 1990). AA and 22 : 6*n*-3 are found in high concentrations in synaptic plasma membranes (Cotman *et al.* 1969; Breckenridge *et al.* 1972; Sun & Sun, 1974; Foot *et al.* 1982) and in photoreceptor cells (Anderson *et al.* 1974). AA plays an important role as a precursor of biologically active molecules such as prostanoids, leukotrienes and other lipoxygenase products (reviewed by Kinsella *et al.* 1990). AA and 22 : 6*n*-3 are involved in providing an optimum microenvironment within the phospholipid bilayer that influences important membrane functions such as ion or solute transport, receptor activity and adenylate cyclase activity (reviewed by Stubbs & Smith, 1984 and Sastry, 1985; Litman & Mitchell, 1996; Huster *et al.* 1998; Mitchell & Litman, 1998; Jump, 2002).

20 : 4*n*-6 and 22 : 6*n*-3 accumulate rapidly in the brain during the fetal and early postnatal periods, depending on the species concerned. In rats, 22 : 6*n*-3 accumulates during the embryonic period and first 3 postnatal weeks of life (Kishimoto *et al.* 1965; Sinclair & Crawford, 1972; Green & Yavin, 1998). In man, the accretion of 20 : 4*n*-6 and 22 : 6*n*-3 takes place during the last trimester and first 6–10 months after birth (Clandinin *et al.* 1980*a*,*b*). The rapid accumulation of 20 : 4*n*-6 and 22 : 6*n*-3 in rat and human brain suggests that these fatty acids may be essential for neural and visual development (Okuyama *et al.* 1997; Carlson & Neuringer, 1999; Moriguchi *et al.* 2000). This issue is of particular importance to preterm infants, who miss the period of peak accumulation of 20 : 4*n*-6 and 22 : 6*n*-3 in the brain during the last trimester of pregnancy (Clandinin *et al.* 1980*a*,*b*; Innis, 1991).

The manipulation of brain 22 : 6*n*-3 content by 22 : 6*n*-3 deprivation has been attempted to determine some functions of 22 : 6*n*-3 in the central nervous system. In this regard, rats fed diets deficient in 18 : 3*n*-3 but with adequate 18 : 2*n*-6 had lower levels of 22 : 6*n*-3 in brain and retina compared with rats fed 18 : 3*n*-3 (Bourre *et al.* 1989) and had delayed electrophysiological responses in the retina (Wheeler *et al.* 1975; Bourre *et al.* 1989) together with poorer performance in behavioural tests of

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; LNA, linolenic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine.

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learning, memory and habituation (Lamptey & Walker, 1976; Tinoco, 1982; Yamamoto *et al.* 1987; Bourre *et al.* 1989; Enslen *et al.* 1991; Frances *et al.* 1996; Wainwright *et al.* 1998; Carrie *et al.* 1999, 2000; Moriguchi *et al.* 2000). The poorer performance in behaviour tests with rats fed *n*-3-deficient compared with adequate diets may be due to alterations in the dopaminergic (Delion *et al.* 1994; Chalon *et al.* 1998; Innis & LaPresa Owens, 2001; Zimmer *et al.* 2002), serotoninergic (Delion *et al.* 1994; Kodas *et al.* 2004) or cholinergic (Aid *et al.* 2003) systems. The activity of Na^+,K^+-ATPase has also been shown to be lower in rats fed diets deficient compared with adequate in 18 : 3*n*-3 (Bourre *et al.* 1989; Gerbi *et al.* 1994; Tsutsumi *et al.* 1995; Bowen & Clandinin, 2002).

Evidence from human infant nutrition studies suggests that feeding formulas with low *n*-3 PUFA leads to suboptimal cognitive and visual functions (Willatts & Forsyth, 1998, 2000; Carlson & Neuringer, 1999; Jacobson, 1999; Birch *et al.* 2000); however, the exact mechanisms by which alterations in the membrane content of *n*-3 PUFA affect human brain functions remains to be fully elucidated. Nevertheless, these studies reinforce the essential nature of 22 : 6*n*-3 in rat and human brain. The importance of 22 : 6*n*-3 in brain has given rise to the question of whether 18 : 3*n*-3 is sufficient to enable the synthesis of adequate amounts of 22 : 6*n*-3 for optimal brain growth and development.

Astrocytes (glial cells) are essential for neurotransmission and neuroprotection because they maintain the composition of the extracellular milieu, respond to neuromediators and supply energetic substrates to neurones (reviewed by Morgane *et al.* 1993). Studies with isolated brain cells have provided evidence that astrocytes and cerebroendothelial cells, but not neuronal cells, can synthesise 20 : 4*n*-6 and 22 : 6*n*-3 from 18 : 2*n*-6 and 18 : 3*n*-3, respectively (Moore *et al.* 1991; Moore 1993, 2001; Bernoud *et al.* 1998; Willard *et al.* 2001, 2002). Further work has also shown that neuronal cells take up 22 : 6*n*-3 released by astrocytes and cerebroendothelial cells, and incorporate 22 : 6*n*-3 into neural plasma membranes (Moore, 1993; Delton-Vandenbroucke *et al.* 1997). It is not, however, known whether the neuronal cell requirement for 20 : 4*n*-6 and 22 : 6*n*-3 *in vivo* is derived from synthesis from precursors by astrocytes and cerebroendothelial cells or from the uptake of preformed 20 : 4*n*-6 and 22 : 6*n*-3 supplied by the mother. Furthermore, these observations on the cellular partitioning of 22 : 6*n*-3 synthesis in astrocytes and cerebroendothelial cells are based primarily on the rat and are largely undetermined for the human brain.

In weanling rats, increasing maternal dietary 18 : 3*n*-3 by decreasing the 18 : 2*n*-6 to 18 : 3*n*-3 fatty acid ratio from 7·3 : 1 to 4 : 1 increased the 22 : 6*n*-3 content only in neuronal cell phosphatidylethanolamine (PE) from the cerebellum during the first 2 weeks of life (Jumpsen *et al.* 1997*a*,*b*). Other phospholipids from the frontal or hippocampal regions were not significantly affected (Jumpsen *et al.* 1997*a*,*b*). Similarly, it has been shown that increasing maternal dietary 18 : 3*n*-3 by lowering the 18 : 2*n*-6 to 18 : 3*n*-3 fatty acid ratio does not significantly increase the 22 : 6*n*-3 content in the phosphatidylcholine (PC), PE and phosphatidylserine (PS) of neuronal cells from whole brain of 2-week-old rat pups (Bowen *et al.* 1999). Taken together, these studies suggest that astrocytes and cerebroendothelial cells may not synthesise enough 22 : 6*n*-3 from 18 : 3*n*-3 to provide for maximal levels of 22 : 6*n*-3 in the plasma membrane phospholipids of neuronal cells in the brain.

It is not known whether maternal dietary 18 : 3*n*-3 compared with 22 : 6*n*-3 can significantly increase the 22 : 6*n*-3 content of glial cell phospholipids from whole brain (cerebrum and cerebellum) of rats. Previous studies have not examined high levels of maternal dietary 18 : 3*n*-3 intake or the 22 : 6*n*-3 content of isolated glial cells from whole brain. Therefore, the present study used neonatal rat brains at 2 weeks of age, before the consumption of solid food, to test the hypothesis that maternal dietary 22 : 6*n*-3 but not 18 : 3*n*-3 will significantly increase the 22 : 6*n*-3 content of glial cell phospholipids of whole brain.

Materials and methods

Animals and diets

All animal procedures were approved by the University of Alberta Animal Ethics Committee. Sprague-Dawley rats were obtained from the University of Alberta vivarium. During breeding, three females and one male were housed together for a 2 week mating period. Females were then moved to individual cages in a room maintained at 21°C with a 12 h light and 12 h dark cycle. Water and food were supplied *ad libitum.* Laboratory rodent diet 5001 (PMI Feeds, Inc., St. Louis, MO, USA) was fed to the rats when not receiving experimental diets. Dams were switched to the experimental diet on the day of parturition. All the litters were culled to twelve rat pups following parturition. Rat pups received only maternal milk. Pups were killed at 2 weeks of age. One entire litter of rat pups fed the same diet was sexed and weighed prior to decapitation. Excised brains (cerebrum and cerebellum) were placed in ice-cold 0·32 mol/l sucrose. Six brains from the same sex were pooled per sample. The stomach contents of three rats from each litter were also removed and analysed for fatty acid composition to reflect the composition of maternal milk. Three litters per diet treatment were used.

The basal diet fed to the dams contained 200 g total fat/kg diet (Table 1). The fat level used in this study reflects a typical North American diet in which approximately 40 % of total energy derives from fat, and is closer to the high content of energy from fat in human or rat milk. The different fat blends in the basal diet were made by mixing vegetable and/or single-cell oils, and each diet was designed to provide approximately 17 g/100 g total fatty acids as 18 : 2*n*-6 (7·6 % energy 18 : 2*n*-6) so that only the effects of *n*-3, and not those of *n*-6 fatty acids, on the glial cell phospholipids of 2-week-old rat pups were studied. Diet fats were formulated to approximate the fatty acid composition of an existing infant formula providing an 18 : 2*n*-6 to 18 : 3*n*-3 fatty acid ratio of 7·8 : 1 (control; 1·0 % energy 18 : 3*n*-3) (Jumpsen *et al.* 1997*a*,*b*). This fat blend served as the control fat treatment. The diet with an 18 : 2*n*-6 to 18 : 3*n*-3 fatty acid ratio of 4·4 : 1 (medium linolenic acid (LNA); 1·7 % energy 18 : 3*n*-3) and 1 : 1 (high LNA; 7·8 % energy 18 : 3*n*-3) was obtained by the addition of flaxseed oil to the control diet. The 22 : 6*n*-3 (docosahexaenoic acid (DHA); 0·3 % energy 22 : 6*n*-3) diet was achieved by the addition of 0·6 g/100 g total fatty acids of 22 : 6*n*-3 to the control diet. The 20 : 4*n*-6 (AA; 0·5 % energy 20 : 4*n*-6) + 22 : 6*n*-3 diet was achieved by the addition of 1 g/100 g total fatty acids of 20 : 4*n*-6 and 0·6 g/100 g total fatty acids of 22 : 6*n*-3 to the control diet. The AA (ARASCO) and DHA (DHASCO) were provided as single-cell triacylglycerols, with 20 : 4*n*-6 from the fungus *Mortierella*

Table 1. Fatty acid composition of experimental diets fed to lactating dams (g/100 g total fatty acids)

Dietary fat	Control*	Medium LNA†	High LNA†	DHA‡	AA + DHA§
12 : 0	8·4	9·4	6·0	7·5	7·7
14 : 0	5·2	5·5	3·6	5·3	5·3
16 : 0	14·0	13·9	11·4	14·6	14·5
18 : 0	7·4	6·9	6·4	7·3	7·4
18 : 1*n*-9 + *n*-7	39·9	37·4	33·8	38·6	39·4
18 : 2*n*-6	17·2	16·6	17·6	16·2	16·5
18 : 3*n*-3	2·2	3·8	17·5	1·8	1·9
20 : 4*n*-6	ND	ND	ND	ND	1·0
20 : 5*n*-3	ND	ND	ND	ND	ND
22 : 6*n*-3	ND	ND	ND	0·6	0·6
Σ Sat‖	35·0	35·7	27·4	34·7	34·9
Σ Mono¶	39·9	37·4	33·8	38·6	39·4
Σ *n*-6**	17·2	16·6	17·6	16·2	17·5
Σ *n*-3††	2·2	3·8	17·5	2·4	2·5
18 : 2*n*-6 : 18 : 3*n*-3	7·8	4·4	1·0	9·0	8·7

AA, arachidonic acid; DHA, docosahexaenoic acid; LNA, linolenic acid; ND, not detected.
* Control diet approximates the fatty acid composition used in SMA® infant formula.
† Medium- and high-LNA diets were obtained by the addition of flaxseed oil to the control diet.
‡ DHA was obtained by the addition of 0·6 g/100 g total fatty acids as 22 : 6*n*-3 triacylglycerols to the control diet.
§ AA + DHA diet was obtained by addition of 1 g/100 g total fatty acids as 20 : 4*n*-6 and 0·6 g/100 g total fatty acids as 22 : 6*n*-3 triacylglycerols to the control diet.
‖ Sum of saturated fatty acids.
¶ Sum of monounsaturated fatty acids.
** Sum of *n*-6 fatty acids.
†† Sum of *n*-3 fatty acids.

alpina and 22 : 6*n*-3 from the algae *Crypthecodinium cohnii* (Martek Biosciences, Columbia, MD, USA).

The amount of 20 : 4*n*-6 (1 g/100 g total fatty acids) and 22 : 6*n*-3 (0·6 g/100 g total fatty acids) used in the present study has been shown by Clandinin *et al.* (1989) to reflect the 20 : 4*n*-6 and 22 : 6*n*-3 content of human milk, and studies with preterm infants have shown that supplementation with 22 : 6*n*-3 without the addition of 20 : 4*n*-6 can reduce blood lipid 20 : 4*n*-6 and growth (Carlson *et al.* 1992, 1996). All maternal diets, with the exception of the high-LNA diet, contained similar total saturated, monounsaturated, *n*-6 and *n*-3 fatty acids. The increase in the level of 18 : 3*n*-3 in the high-LNA maternal diet was concurrent with a decrease in the amount of saturated and MUFA, particularly, 16 : 0 and 18 : 1*n*-9 + *n*-7, respectively.

These diets were nutritionally adequate, providing for all known essential nutrient requirements (ingredients and concentration (g/kg diet), respectively): fat 200; starch, 200; casein, 270; glucose, 207·65; non-nutritive fibre, 50; vitamin mix, 10; mineral mix, 50·85; L-methionine, 2·5; choline 2·75; inositol, 6·25. The AOAC vitamin mix (Teklad Test Diets, Madison, WI, USA) provided the following per kilogram of complete diet: 6 mg vitamin A; 50 μg vitamin D; 100 mg vitamin E; 5 mg menadione; 5 mg thiamine HCl; 8 mg riboflavin; 40 mg pyridoxine HCl; 40 mg niacin; 40 mg pantothenic acid; 2000 mg choline, 100 mg myoinositol; 100 mg *p*-aminobenzoic acid; 0·4 mg biotin; 2 mg folic acid; 30 mg vitamin B_{12}. Bernhart Tomarelli mineral mix (General Biochemicals, Chargin Falls, OH, USA) was modified to provide 77·5 mg Mn^{2+} and 0·06 mg Se^{2+} per kilogram of complete diet.

To minimise any changes in sample composition due to fatty acid oxidation, diets were sealed under N_2 and stored in a freezer at −30 °C in darkness. Each day, the required amount of diet was mixed thoroughly and placed in individual feed cups.

Isolation of glial cells from whole brain

Glial cells were isolated from whole brain (cerebrum and cerebellum) according to the method described by Sellinger & Azcurra (1974). Briefly, pooled brains were placed in beakers containing 7·5 % (w/v) polyvinylpyrrolidone and 10 mmol/l $CaCl_2$ at pH 4·7 and 25 °C. Brain tissue was minced and poured into a 20 ml plastic syringe, fitted with a reusable filter unit (Swinnex disc holder, 25 mm; Billerica, MA, USA). The samples were pressed, three times each, through a series of combined nylon mesh filters. The final filtrate volume was adjusted and then layered on a two-step sucrose gradient of 1·0 mol/l and 1·75 mol/l. Gradients were centrifuged in a Beckman SW-28 rotor (Beckman Instruments, Palo Alto, CA, USA) at 41 000 **g** for 30 min at 4 °C.

Glial cells were obtained at the interface of 1·0 mol/l and 1·75 mol/l sucrose. Aliquots of glial cell samples were stained with methylene blue and examined for purity under a light microscope (1600 ×; Carl Zeiss, oberkochen, Germany). Gel electrophoresis and immunoblotting were performed to ensure the purity of the glial cell fraction prepared by these procedures (Jumpsen *et al.*, 1997*a*,*b*). Proteins isolated from glial cells were compared by gel electrophoresis and immunoblotting with glial fibrillary acid protein standard, an astrocyte-specific marker, and neurofilament standard, a neuronal cell specific marker (Jumpsen *et al.* 1997*a*,*b*; Ubl & Reiser, 1997).

Lipid extraction and fatty acid analysis

The glial cell lipid was extracted by a modified Folch method (Folch *et al.* 1957). Individual phospholipids were separated by TLC (Touchstone *et al.* 1980) and identified by comparison with authentic phospholipid standards after visualisation with 0·1 g/100 ml aniline naphthalene sulphonic acid in water. Fatty acid methyl esters from the individual phospholipids of glial cells were prepared following the method of Morrison and Smith (1964). Fatty acid methyl esters were separated by automated GLC (Varian model 6000 GLC equipped with a Vista 654 data system and a Vista 8000 autosampler; Varian Instruments, Georgetown, ON, USA) using a bonded fused silica BP20 capillary column (25 mm × 0·25 mm inside diameter) and quantified using a flame ionisation detector (Bowen *et al.* 1999; Bowen & Clandinin, 2000). These conditions are capable of separating methyl esters of saturated, *cis*-monounsaturated and *cis*-PUFA from 14 to 24 carbons in chain length. The quantitation and identification of peaks was based on relative retention times compared with known standards (PUFA 1 and 2, bacterial methyl ester mix-14; Supelco Canada, Mississauga, ON, Canada; Bowen & Clandinin, 2000).

Statistical analysis

The effect of maternal diet treatment on the fatty acid composition of glial cell PE, PS, PC and phosphatidylinositol (PI) was assessed by a one-way ANOVA procedure using the SAS package, version 6.11 (SAS Institute Inc., 1988). Significant differences between maternal diet treatments were determined by a *post hoc* Fisher's least significant difference test at a significance level of $P < 0·05$ after a significant ANOVA (Steel & Torrie, 1960). Values are expressed as means and standard deviation for *n* 6.

Results

Whole-body and brain weights

Whole-body and brain weights were not significantly different for male and female rat pups (data not shown); hence, statistical analyses to test the subsequent effects of diet treatments were combined for both sexes. The whole-body and brain weights, and brain to body weight ratio (data not shown), were not significantly different among the five maternal diet treatments, indicating that whole body and tissue growth in the 2-week-old rat pups was no different between maternal diets with or without 22 : 6*n*-3. Mean final body weights were 35·8 (SD 2·4) g, 35·9 (SD 2·4) g, 35·6 (SD 3·1) g, 36·1 (SD 1·2) g and 36·1 (SD 1·2) g for control, medium-LNA, high-LNA, DHA and AA + DHA maternal diet treatments, respectively. Mean final brain weights were 1·21 (SD 0·2) g, 1·22 (SD 0·2) g, 1·21 (SD 0·2) g, 1·22 (SD 0·2) g and 1·23 (SD 0·2) g for control, medium-LNA, high-LNA, DHA and AA + DHA maternal diet treatments, respectively. Differences in fertility were not observed between the maternal dietary treatments.

Purity of glial cell preparations from whole brain

Glial cell preparations contained only minor cross contamination (<5 %) from cell membrane fragments and microvessels, as determined by microscopic examination. The glial cell samples were all positive for glial fibrillary acid protein and negative for neurofilament protein (data not shown). The presence of glial fibrillary acid protein in glial cell samples from rat brain was previously verified by gel electrophoresis and immunoblotting (Jumpsen *et al.* 1997*a*,*b*). These results indicate that the cell preparations were primarily glial cells.

Fatty acid composition of stomach contents

The rat pup stomach contents at day 14 of life contained no particulates indicative of diet consumption and therefore reflected the composition of their dams' milk. The fatty acid composition of the stomach contents of the rat pups was analysed. These analyses have been shown to be similar to dams' milk composition (Nouvelot *et al.* 1983; Yonekubo *et al.* 1993; Lien *et al.* 1994; Jumpsen *et al.* 1997*a*,*b*; Bowen *et al.* 1999; Moriguchi *et al.* 2000). The major fatty acids in the stomach contents of rat pups fed maternal diets providing an 18 : 2*n*-6 to 18 : 3*n*-3 fatty acid ratio of 7·8 : 1, 4·4 : 1 or 1 : 1, DHA or AA + DHA at 2 weeks of age were 12 : 0, 14 : 0, 16 : 0, 18 : 1*n*-9 + *n*-7 and 18 : 2*n*-6 (Table 2). The 22 : 6*n*-3 content was significantly increased in the stomach of rat pups fed the DHA and AA + DHA maternal diets compared with the maternal diets providing an 18 : 2*n*-6 to 18 : 3*n*-3 fatty acid ratio of 7·8 : 1, 4·4 : 1 and 1 : 1 at 2 weeks of age (Table 2), indicating that the maternal dietary fat fed in the present experiment produced similar changes in the fat composition of the dams' milk.

Glial cells phospholipid fatty acid composition

Consideration of the maternal diet-related changes in the present study will be given to the 20 : 4*n*-6 and 22 : 6*n*-3 content of glial cell phospholipids of rat pups at 2 weeks of age.

Phosphatidylethanolamine. The analysis of the fatty acid composition of glial cells from whole brain of 2-week-old rat pups fed the different maternal diets is shown in Table 3. The predominant fatty acids in PE were 16 : 0, 18 : 0, 20 : 4*n*-6 and 22 : 6*n*-3 (Table 3). There was no significant difference in the 20 : 4*n*-6 content of glial cell PE among maternal diet treatments (Table 3). The 22 : 6*n*-3 content of glial cell PE was significantly increased with rats fed the DHA and AA + DHA maternal diet but not the diets providing a 18 : 2*n*-6 to 18 : 3*n*-3 fatty acid ratio of 7·8 : 1, 4·4 : 1 and 1 : 1 ($P<0·0001$; Table 3).

Phosphatidylserine. The fatty acid composition of glial cell PS from whole brain of 2-week-old rat pups fed the different maternal diets is shown in Table 4. The major fatty acids of glial cell PS were 16 : 0, 18 : 0, 18 : 1*n*-9 + *n*-7, 20 : 4*n*-6, 22 : 4*n*-6 and 22 : 6*n*-3 (Table 4). The 20 : 4*n*-6 content of PS from glial cells was not significantly different among the maternal diet treatments (Table 4). Feeding rat pups the DHA and AA + DHA maternal diet significantly increased the 22 : 6*n*-3 content of glial cell PS compared with the maternal diets providing an 18 : 2*n*-6 to 18 : 3 *n*-3 fatty acid ratio of 7·8 : 1, 4·4 : 1 and 1 : 1 ($P<0·0001$; Table 4).

Phosphatidylcholine. The predominant fatty acids observed in PC were 16 : 0, 18 : 0 and 18 : 1*n*-9 + *n*-7 (43–49 %, 9–16 % and 20–22 % of the total fatty acids, respectively; Table 5).

Table 2. The content of fatty acids in the stomach of rat pups at 2 weeks of age (g/100 g total fatty acids)*

(Values are mean and SD with *n* 9 for each maternal diet)

	Control		Medium LNA		High LNA		DHA		AA + DHA	
Diet fat	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10 : 0	7·2[a]	0·8	8·9[a]	1·9	6·9[a]	2·2	6·7[a]	0·7	6·2[a]	0·8
12 : 0	15·1[a]	0·4	16·0[a]	2·3	10·8[c]	0·6	13·8[b]	0·2	12·2[b]	0·1
14 : 0	15·3[a]	0·5	9·8[b]	0·5	7·2[c]	0·3	9·3 [b]	0·1	9·2[b]	0·1
16 : 0	17·7[a]	0·7	13·9[bc]	1·4	12·9[c]	0·4	14·9[b]	0·2	15·9[b]	0·2
18 : 0	3·4[c]	0·1	4·0[b]	0·2	4·8[a]	0·2	4·6[a]	0·1	4·8[a]	0·1
18 : 1*n*-9 + *n*-7	24·7[b]	0·7	28·0[b]	1·5	26·7[b]	1·0	31·5[a]	0·8	29·2[a]	0·6
18 : 2*n*-6	12·5[b]	0·1	10·4[c]	0·1	13·7[a]	0·1	13·8[a]	0·2	13·1[b]	0·1
20 : 4*n*-6	0·5[b]	0·0	0·5[b]	0·1	0·5[b]	0·1	0·5[b]	0·0	1·1[a]	0·1
18 : 3*n*-3	1·6[c]	0·0	2·1[b]	0·1	8·8[a]	0·1	1·5[c]	0·0	1·6[c]	0·0
22 : 6*n*-3	0·1[b]	0·0	0·1[b]	0·0	0·2[b]	0·0	0·6[a]	0·0	0·7[a]	0·0
18 : 2*n*-6 to 18 : 3*n*-3	7·8		5·0		1·6		9·2		8·2	

AA, arachidonic acid; DHA, docosahexaenoic acid; LNA, linolenic acid.

[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$; Fisher's least significant difference test).

* For details of fatty acid composition of experimental diets, see Table 1 and p. 602.

Table 3. Effect of low to high maternal dietary 18 : 3*n*-3 compared with feeding 22 : 6*n*-3 on the fatty acid composition of glial cell phosphatidylethanolamine (g/100 g total fatty acids)*

(Values are mean and SD with *n* 6 for each maternal diet)

	Control		Medium LNA		High LNA		DHA		AA + DHA	
Diet fat	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14 : 0	1·5^{a}	0·2	1·5^{a}	0·2	1·6^{a}	0·3	0·9^{a}	0·1	1·0^{a}	0·2
16 : 0	12·6^{b}	0·2	13·7^{a}	0·7	11·9^{b}	0·3	8·8^{c}	0·2	9·9^{c}	0·5
18 : 0	21·8^{a}	0·5	22·6^{a}	0·6	21·4^{a}	0·6	21·5^{a}	0·1	21·4^{a}	0·3
18 : 1*n*-9 + *n*-7	7·8^{b}	0·3	9·5^{a}	0·3	8·2^{b}	0·2	7·1^{c}	0·1	7·3^{b}	0·1
18 : 2*n*-6	0·4^{b}	0·1	1·0^{a}	0·1	0·8^{a}	0·0	0·5^{b}	0·0	0·4^{b}	0·0
20 : 4*n*-6	18·7^{a}	0·7	18·8^{a}	0·4	19·3^{a}	0·3	18·7^{a}	0·2	19·1^{a}	0·6
22 : 4*n*-6	6·8^{a}	0·4	5·2^{b}	0·4	6·6^{b}	0·1	7·4^{a}	0·4	6·5^{b}	0·1
22 : 5*n*-6	1·9^{a}	0·2	1·4^{a}	0·1	1·8^{a}	0·0	1·5^{a}	0·0	1·8^{a}	0·1
18 : 3*n*-3	0·1^{a}	0·0	0·0^{a}	0·0	0·0^{a}	0·0	0·1^{a}	0·0	0·0^{a}	0·0
20 : 5*n*-3	0·0^{b}	0·0	0·0^{b}	0·0	0·3^{a}	0·0	0·0^{b}	0·0	0·3^{a}	0·2
22 : 5*n*-3	0·2^{b}	0·1	0·5^{a}	0·1	0·7^{a}	0·1	0·2^{b}	0·1	0·4^{a}	0·2
22 : 6*n*-3	24·1^{b}	0·3	23·2^{b}	0·6	24·3^{b}	0·4	30·8^{a}	0·4	28·6^{a}	0·4

AA, arachidonic acid; DHA, docosahexaenoic acid; LNA, linolenic acid.

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$; Fisher's least significant difference test).

* For details of fatty acid composition of experimental diets, see Table 1 and p. 602.

The 20 : 4*n*-6 and 22 : 6*n*-3 content of PC from glial cells was not significantly different among the maternal diet treatments of 2-week-old rat pups (Table 5).

Phosphatidylinositol. PI represents approximately 4 % of the total brain phospholipids (Green & Yavin, 1996*a*). The PI from glial cells contained a large amount of saturated fatty acids, particularly 16 : 0 and 18 : 0 (9–15 % and 27–33 %, respectively; Table 6). 20 : 4*n*-6 (28–34 %) and 22 : 6*n*-3 (7·5–8·3 %) present in PI were the predominant *n*-6 and *n*-3 fatty acids, respectively (Table 6). Similarly to PC, the 20 : 4*n*-6 and 22 : 6*n*-3 content of glial cell PI was not significantly different among maternal diet treatments of 2-week-old rat pups (Table 6).

Discussion

Two-week-old rat pups were used in the present study because, at this age, very active brain growth occurs with a rapid accretion of PUFA, especially 22 : 6*n*-3, for brain membrane synthesis (Sinclair & Crawford, 1972; Dobbing & Sands, 1979). Therefore, the demand for 22 : 6*n*-3 in 2-week-old rat pups for postnatal brain growth and development is high and must be provided by the maternal diet.

The present results establish the hypothesis that maternal dietary 22 : 6*n*-3 but not an increased intake of 18 : 3*n*-3 will significantly increase the 22 : 6*n*-3 content of glial cell PS and PE from whole brain (cerebrum and cerebellum) in 2-week-old rat pups. These observations extend previous information (Jumpsen *et al.* 1997*a*,*b*) by establishing that maternal dietary 22 : 6*n*-3 is more effective at increasing the 22 : 6*n*-3 content of whole brain glial cell membrane phospholipid in 2-week-old rat pups than is feeding low or high levels of maternal 18 : 3*n*-3. The results from the present study are in accordance with other studies showing that rat brain uses 22 : 6*n*-3 instead of 18 : 3*n*-3 to maintain the 22 : 6*n*-3 content of membrane phospholipids (Sinclair, 1975; Anderson & Connor, 1988; Woods *et al.* 1996; Edmond *et al.* 1998). The present findings also confirm *in vitro* studies using radiolabelled 18 : 3*n*-3 and

Table 4. Effect of low to high maternal dietary 18 : 3*n*-3 compared to feeding 22 : 6*n*-3 on the fatty acid composition of glial cell phosphatidylserine (g/100 g total fatty acids)*

(Values are mean and SD with *n* 6 for each maternal diet)

	Control		Medium LNA		High LNA		DHA		AA +DHA	
Diet fat	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14 : 0	0·5^{a}	0·2	0·4^{a}	0·1	0·3^{a}	0·1	0·4^{a}	0·1	0·3^{a}	0·1
16 : 0	14·8^{a}	0·1	13·1^{a}	0·4	11·0^{a}	0·8	9·2^{b}	0·8	6·0^{c}	0·2
18 : 0	36·2^{a}	0·2	37·4^{a}	0·9	35·9^{a}	1·1	39·0^{a}	2·6	35·8^{a}	0·3
18 : 1*n*-9 + *n*-7	8·3^{b}	0·2	11·1^{a}	0·1	10·6^{a}	0·4	6·2^{c}	1·4	7·4^{b}	0·2
18 : 2*n*-6	0·4^{b}	0·0	1·1^{a}	0·2	0·9^{a}	0·1	0·4^{b}	0·0	0·3^{b}	0·0
20 : 4*n*-6	6·3^{a}	0·2	5·8^{a}	0·2	5·8^{a}	0·1	5·6^{a}	0·1	6·3^{a}	0·1
22 : 4*n*-6	4·8^{b}	0·4	3·7^{c}	0·2	4·3^{b}	0·2	5·2^{b}	0·1	6·6^{a}	0·1
22 : 5*n*-6	2·3^{b}	0·3	1·8^{b}	0·1	1·9^{b}	0·1	1·9^{b}	0·1	2·9^{a}	0·1
18 : 3*n*-3	0·0^{a}	0·0	0·2^{a}	0·1	0·1^{a}	0·1	0·0^{a}	0·0	0·0^{a}	0·0
20 : 5*n*-3	0·1^{a}	0·1	0·5^{a}	0·2	0·9^{a}	0·4	0·0^{a}	0·0	0·4^{a}	0·1
22 : 5*n*-3	0·4^{b}	0·1	0·4^{b}	0·0	0·6^{a}	0·0	0·4^{b}	0·0	0·4^{b}	0·0
22 : 6*n*-3	23·1^{b}	0·6	21·4^{b}	0·8	22·6^{b}	0·7	29·1^{a}	1·7	31·0^{a}	0·3

AA, arachidonic acid; DHA, docosahexaenoic acid; LNA, linolenic acid.

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$; Fisher's least significant difference test).

* For details of fatty acid composition of experimental diets, see Table 1 and p. 602.

Table 5. Effect of low to high maternal dietary 18 : 3*n*-3 compared to feeding 22 : 6*n*-3 on the fatty acid composition of glial cell phosphatidylcholine (g/100 g total fatty acids)*

(Values are mean and SD with *n* 6 for each maternal diet)

	Control		Medium LNA		High LNA		DHA		AA + DHA	
Diet fat	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14 : 0	1·7[a]	0·0	0·6[b]	0·7	0·8[b]	0·0	1·6[a]	0·0	1·5[a]	0·1
16 : 0	48·5[a]	0·3	43·4[b]	5·9	48·6[a]	0·3	45·2[b]	0·3	43·5[b]	0·5
18 : 0	8·9[b]	0·0	15·9[a]	5·2	13·9[a]	0·3	11·8[b]	1·1	10·1[b]	0·2
18 : 1*n*-9 + *n*-7	20·4[a]	0·0	19·7[a]	1·0	20·7[a]	0·2	21·1[a]	0·2	20·8[a]	0·0
18 : 2*n*-6	1·2[b]	0·0	2·3[a]	1·2	1·4[b]	0·0	1·1[b]	0·1	1·0[b]	0·0
20 : 4*n*-6	7·7[a]	0·1	6·2[a]	1·9	6·8[a]	0·3	5·1[a]	0·7	7·4[a]	0·3
22 : 4*n*-6	0·6[a]	0·1	0·3[a]	0·4	0·6[a]	0·1	0·6[a]	0·1	0·8[a]	0·1
22 : 5*n*-6	0·1[a]	0·0	0·0[a]	0·0	0·1[a]	0·0	0·1[a]	0·0	0·2[a]	0·0
18 : 3*n*-3	0·0[a]	0·0	0·0[a]	0·0	0·0[a]	0·0	0·0[a]	0·0	0·0[a]	0·0
20 : 5*n*-3	0·0[a]	0·0	0·7[a]	1·4	0·0[a]	0·0	0·0[a]	0·0	0·1[a]	0·1
22 : 5*n*-3	0·1[b]	0·0	0·7[a]	0·4	0·1[b]	0·0	0·1[b]	0·0	0·1[b]	0·0
22 : 6*n*-3	3·3[a]	0·1	3·1[a]	0·9	3·1[a]	0·3	3·1[a]	0·3	3·3[a]	0·2

AA, arachidonic acid; DHA, docosahexaenoic acid; LNA, linolenic acid.

[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$; Fisher's least significant difference test).

* For details of fatty acid composition of experimental diets, see Table 1 and p. 602.

22 : 6*n*-3 that astrocytes' incorporation of preformed 22 : 6*n*-3 far exceeds the efficiency with which 18 : 3*n*-3 can be converted to 22 : 6*n*-3 for membrane lipids (De la Presa Owens & Innis, 1999; Willard *et al.* 2001; Innis & Dyer, 2002; Champeil-Potokar *et al.* 2004). In fact, the limiting step in the metabolism of 18 : 3*n*-3 to 22 : 6*n*-3 in astrocytes has been shown to be at the level of elongation to the 24-carbon chain metabolites (Innis & Dyer, 2002). Other animal and human studies have also shown that preformed 22 : 6*n*-3 is more efficient in supporting organ 22 : 6*n*-3 composition than 18 : 3*n*-3 (Salem *et al.* 1996; Sauerwald *et al.* 1996; Abedin *et al.* 1999; Uauy *et al.* 2000). The reason that preformed 22 : 6*n*-3 is more bioavailable than 18 : 3*n*-3 for membrane lipids is because it is a poor substrate for peroxisomal and mitochondrial β-oxidation pathways and it is preferentially acylated into membrane phospholipids (Sinclair, 1975; Leyton *et al.* 1987; Anderson & Connor, 1988; Madsen *et al.* 1998).

Increasing the maternal dietary 18 : 3*n*-3 content eight-fold (Table 1) did not significantly increase the 22 : 6*n*-3 content in PE, PS, PC and PI from glial cells compared with feeding maternal diets containing 22 : 6*n*-3. The lack of significant increase in 22 : 6*n*-3 in rat pups fed the high-LNA maternal diet may be explained by δ-6 desaturase, the rate-limiting enzyme in the synthesis of 22 : 6*n*-3, not being fully active at 2 weeks of age (Bernhart & Sprecher, 1975). Rodent studies have demonstrated that liver (Nouvelot *et al.* 1986), astrocytes (Moore *et al.* 1990, 1991), choroid plexus (Bourre *et al.* 1997) and microvessel endothelial cells (Delton-Vandenbroucke *et al.* 1997) have δ-6 desaturase activity and that 18 : 3*n*-3 is converted to 22 : 6*n*-3 in these cell types (Ravel *et al.* 1985; Sanders & Rana, 1987; Bourre *et al.* 1990). Whether rat pups at 2 weeks of age are able to convert sufficient amounts of 18 : 3*n*-3 into 22 : 6*n*-3 to provide for the maximal incorporation of 22 : 6*n*-3 into glial cell

Table 6. Effect of low to high maternal dietary 18 : 3*n*-3 compared to feeding 22 : 6*n*-3 on the fatty acid composition of glial cell phosphatidylinositol (g/100 g total fatty acids)*

(Values are mean and SD with *n* 6 for each maternal diet)

	Control		Medium LNA		High LNA		DHA		AA + DHA	
Diet fat	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14 : 0	0·6[a]	0·0	0·3[a]	0·0	0·7[a]	0·1	0·6[a]	0·1	0·7[a]	0·1
16 : 0	8·9[b]	1·2	11·3[b]	0·5	15·3[a]	0·4	9·0[b]	0·4	10·6[b]	0·5
18 : 0	33·1[a]	0·5	33·4[a]	0·5	29·6[b]	0·2	27·8[c]	0·4	26·6[c]	1·4
18 : 1*n*-9 + *n*-7	7·6[a]	0·4	8·0[a]	1·8	8·8[a]	0·2	6·8[a]	0·2	8·7[a]	0·5
18 : 2*n*-6	0·6[a]	0·0	0·7[a]	0·1	0·7[a]	0·2	0·6[a]	0·1	0·4[a]	0·0
20 : 4*n*-6	33·6[a]	0·9	29·1[a]	2·2	28·3[a]	1·1	32·9[a]	0·9	31·7[a]	0·9
22 : 4*n*-6	1·3[a]	0·3	2·6[a]	0·7	2·0[a]	0·5	1·7[a]	0·1	2·2[a]	0·2
22 : 5*n*-6	0·5[b]	0·1	1·0[a]	0·1	1·0[a]	0·1	0·4[b]	0·1	0·6[b]	0·1
18 : 3*n*-3	0·1[a]	0·0	0·2[a]	0·0	0·1[a]	0·0	0·2[a]	0·1	0·0[a]	0·0
20 : 5*n*-3	0·2[a]	0·0	0·4[a]	0·1	0·3[a]	0·1	0·8[a]	0·4	0·2[a]	0·0
22 : 5*n*-3	0·2[b]	0·0	0·2[b]	0·0	0·4[a]	0·0	0·1[b]	0·0	0·1[b]	0·0
22 : 6*n*-3	7·5[a]	0·5	7·5[a]	0·2	7·8[a]	0·8	8·2[a]	0·8	8·3[a]	0·9

AA, arachidonic acid; DHA, docosahexaenoic acid; LNA, linolenic acid.

[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$; Fisher's least significant difference test).

* For details of fatty acid composition of experimental diets, see Table 1 and p. 602.

membrane phospholipid synthesis is not known. The findings from the present study with glial cells are in agreement with those of our previous study showing that low to high maternal 18 : 3*n*-3 diets do not significantly increase the 22 : 6*n*-3 content of neuronal cell membrane phospholipids (Bowen *et al.* 1999).

It is also conceivable that the lack of a significant increase in 22 : 6*n*-3 content of glial cell phospholipids in 2-week-old rat pups when fed low to high maternal 18 : 3*n*-3 may be the result of competitive δ-6 desaturase inhibition. An excess of 18 : 3*n*-3 from flaxseed oil might provide for the synthesis of some 20 : 5*n*-3 in the initial phase of the synthetic pathway to 22 : 6*n*-3, but the excess of 18 : 3*n*-3 might have suppressed the final δ-6 desaturase step needed to produce 24 : 6*n*-3 from 24 : 5*n*-3, with subsequent retroconversion to 22 : 6*n*-3 in the peroxisomes (Voss *et al.* 1991; Sprecher *et al.* 1999; Sprecher, 2000; Ferdinandusse *et al.* 2001). However, whether the δ-6 desaturase responsible for the desaturation of 18 : 3*n*-3 and 24 : 5*n*-3 is the same or a different enzyme is not completely understood (Sprecher *et al.* 1999).

In 2-week-old rat pups, the metabolic fate of feeding a high maternal 18 : 3*n*-3 diet is a deposition of 18 : 3*n*-3 in the skin, including subcutaneous fat and adipose tissue (Bowen & Clandinin, 2000; Fu & Sinclair, 2000; Bazinet *et al.* 2003*a*,*b*), β-oxidation to acetyl-CoA, which is recycled into cholesterol and non-essential fatty acids, or further metabolism to CO_2 (Cunnane *et al.* 1994, 1999; Sheaff-Greiner *et al.* 1996; Menard *et al.* 1998; DeLany *et al.* 2000; Bazinet *et al.* 2003*a*,*b*).

The 20 : 4*n*-6 content of glial cell PE, PS, PC and PI was not significantly different between rat pups fed the different maternal diet treatments at 2 weeks of age, and these findings corroborate those reported for guinea-pig brain (Kurvinen *et al.* 2000), rat brain microsomes (Garcia *et al.* 1998) and rat pineal gland (Zhang *et al.* 1998). The reason for the non-significant difference in the 20 : 4*n*-6 content of individual phospholipids of rats fed the different maternal diet treatments may be explained by the 20 : 4*n*-6 present in the stomach contents (see Table 2). It is possible that the level of the 20 : 4*n*-6 (>0·5 % of the total fatty acids) present in the stomach contents of rat pups may be sufficient to prevent a significant decrease in the 20 : 4*n*-6 content of PE, PS, PC and PI of glial cells from whole brain of 2-week old rat pups.

In early postnatal rat brain, PE and PS constitute approximately 30 % and 6 % of the total phospholipids, respectively (Green & Yavin, 1995, 1996*a*,*b*, 1998). Both PE and PS in brain are particularly enriched in 22 : 6*n*-3 (Breckenridge *et al.* 1972; Salem *et al.* 1980; Sastry, 1985; Martinez, 1989; Salem *et al.* 2001) and contain most of the 22 : 6*n*-3 (approximately 92 %) esterified into the total brain phospholipids by the first week of postnatal life (Green & Yavin, 1995, 1996*a*,*b*). Therefore, any changes in whole-brain glial cell 22 : 6*n*-3 content caused by the maternal dietary fat treatments used in this study should be detected in PE and PS.

The functional implications of an increase in the 22 : 6*n*-3 content of PE and PS of the glial cells of rat pups fed maternal diets with 22 : 6*n*-3 on 2-week-old rat pups is not known. Investigations of functional changes associated with an increase in the 22 : 6*n*-3 content of PE and PS from glial cells would be of great interest since modification of the PUFA content of cell membranes has a large impact on membrane properties and the functioning of a variety of membrane-associated proteins such as transporters, enzymes, and receptors (reviewed by Spector & Yorek, 1985 and Clandinin, 1997; Huster *et al.* 1998; Mitchell & Litman, 1998). Microarray analysis has recently shown that a diet enriched in 22 : 6*n*-3 can induce changes in brain and hippocampal expression of 100 and 23 genes, respectively (Barcelo-Coblijn *et al.* 2003; Puskas *et al.* 2003; Kitajka *et al.* 2004).

PS is involved in variety of cell functions (Salem & Niebylski, 1995) such as signal transduction, via its activation of several protein kinase C isoforms (Bell & Burns, 1991) or Raf-1 kinase, to cell membranes (Ghosh *et al.* 1996), the modulation of synaptosomal benzodiazepine receptors (Levi deStein *et al.* 1989) and increases in synaptic efficiency (Borghese *et al.* 1993). The 22 : 6*n*-3 content in PS has been shown to modulate signal transduction pathways by increasing the PS content in neuronal membranes, which in turn promotes the activation of Raf-1 and PI-3 kinase pathways (Garcia *et al.* 1998; Hamilton *et al.* 2000; Kim *et al.* 2000; Akbar & Kim, 2002; Murthy *et al.* 2002). The Raf-1 and PI-3 kinase pathways are involved in inducing neurite growth in PC12 and H19-7 hippocampal cell lines (Wood *et al.* 1993; Kuo *et al.* 1996; Kobayashi *et al.* 1997; Kita *et al.* 1998; Calderon & Kim, 2004). Both PE and PS synthesis have also been shown to be stimulated by 22 : 6*n*-3 in differentiated PC12 cell lines (Ikemoto *et al.* 1999). Hence, as neurite growth requires newly synthesised membrane components, this mechanism may play an important role in promoting neurite growth (Ikemoto *et al.* 1999). These studies therefore support the idea that the maternal diet-induced alterations in glial cell 22 : 6*n*-3 content observed in both PE and PS in the present study may have a physiological impact.

In conclusion, the findings from this study demonstrate that maternal dietary 22 : 6*n*-3 is more effective in increasing the 22 : 6*n*-3 content of PE and PS in glial cells from whole brain in 2-week-old rat pups than maternal dietary 18 : 3*n*-3. In both 2-week-old rat pups and 6-month-old human infants, early gliogenesis and macroneurogenesis have been completed, whereas microneurogenesis, late gliogenesis and myelination are continuing in both species during this period (reviewed by Morgane *et al.* 1993). Rat pups and human infants have a similar metabolic pathway for the synthesis of 20 : 4*n*-6 and 22 : 6*n*-3 via the desaturation and elongation of precursors. Rodents have a markedly higher desaturase activity than human infants (Cunnane *et al.* 1984; Horrobin *et al.* 1984). It is thus reasonable to speculate that infants may produce relatively less 22 : 6*n*-3 in glial cell phospholipids compared with 22 : 6*n*-3 production by rat pups fed the same level of 18 : 3*n*-3.

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