Effects of Parenteral Nutrition with High Doses of Linoleate on the Developing Human Liver and Brain

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The developmental changes in the fatty acid composition of ethanolamine phosphoglycerides (EPG) and choline phosphoglycerides (CPG) were studied in the liver and brain of 18 newborn infants with gestational ages ranging from 20 to 44 wk. A small group of five newborns receiving total parenteral nutrition (TPN) with high doses of linoleic acid (18:2ω6) was also studied and compared to controls of the same gestational age to look for effects on the developmental fatty acid patterns of liver and brain EPG and CPG. TPN with Intralipid 20% was given for 4–12 days, the total fat intake being 14.7–90 g (mean ± S.D. = 47.1 ± 29.8 g). The main developmental changes in the liver and brain of the control group were an increase in 22:6ω3 (docosahexaenoic acid) at the end of gestation and a linear decrease in 20:4ω6 (arachidonic acid) and 18:1ω9 (oleic acid) in EPG and CPG. A very good correlation in the percent values of these fatty acids in the brain and liver tissues was obtained. Very significant changes in the fatty acid composition of liver EPG and CPG could be found in the infants receiving TPN with Intralipid–mainly an increase in 18:2ω6, a decrease in the linoleate elongation/desaturation to longer members of the series and a decrease in the 22:6ω3 levels of liver EPG and CPG. In the brain, only an increase in the 18:2ω6 value of CPG, not accompanied by any increase in the longer ω6 fatty acids, could be detected. Possible adverse effects of high doses of 18:2ω6 on the tissue levels of long chain polyunsaturated fatty acids (PUFA), especially of 22:6ω3, are discussed.
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Neural tissues are by far the richest in polyunsaturated fatty acids (PUFA), and a remarkable constancy in the fatty acid composition of the brain, despite widely different dietary intakes of essential fatty acids (EFA) has been well documented (1–3). In contrast, the liver is much more readily influenced by differences in the diet (3,4). Dietary EFA are chain elongated and desaturated in the liver to form long chain PUFA, which are preferentially taken up by the brain, especially during development. A progressive increase in the fatty acid length and degree of unsaturation from maternal liver to placenta, fetal liver and fetal brain has been found (5). Direct incorporation of dietary long chain PUFA into the developing brain has also been demonstrated (6).

Besides being influenced by diet, the fatty acid composition of body organs changes with development. Special attention has been focused on developmental changes of the brain fatty acid patterns (7–11), but few data are available on the fatty acid composition of the mammalian liver during development (12,13). Clandinin et al. (14,15) have applied a quantitative approach to the study of total fatty acid accretion in the developing human liver and brain as a means of estimating neonatal dietary needs for ω6 fatty acids but, to our knowledge, a systematic study on the developmental fatty acid patterns of the main phosphoglycerides of human liver and brain is lacking.

For many years it has been general practice in pediatrics to supplement milk formulas with substantial amounts of linoleic acid (18:2ω6). Linoleate supplementation has even been extended to cases in which oral feeding is not possible, and several parenteral formulas highly enriched in 18:2ω6 have appeared. The content and proportion of α-linolenic acid (18:3ω3) have relatively been disregarded in most of these formulas. There is an increasing concern for the metabolic effects of such unphysiological EFA supplies, and some effects on the levels of arachidonic acid (20:4ω6) (16,17), long PUFA with 22 carbon atoms (17,18) and prostaglandin production (19) have been published. However, there is little information on the effects of total parenteral nutrition (TPN) with high doses of 18:2ω6 on the fatty acid composition of human tissues during development (19).

The aim of the present work was twofold: first, to correlate the fatty acid patterns of the main phosphoglycerides in the liver with those in the brain during normal human development and, second, to study the effects of a high supply of linoleic acid, a widely used formula (TPN with Intralipid) administered intravenously, on the developmental fatty acid patterns of human liver and brain. Some preliminary accounts of this work have appeared elsewhere (20,21).

MATERIALS AND METHODS

Developmental study. The developmental study comprised 18 newborn infants with gestational ages between 20 and 44 wk. Gestational ages were determined by maternal history and clinical examination; only those cases with body weights appropriate for gestational age according to Lubchenco's grid (22) were included. Those cases with any kind of neonatal problem that could affect cerebral integrity were excluded. The cause of death, occurring during the first 48 hr of life, was immaturity and/or acute respiratory disease.

Study on TPN. Five infants who received TPN and died during the neonatal period due to major surgical problems were studied. Four were full-term neonates (gestational ages between 37 and 43 wk) and the fifth had been born slightly premature (35 wk). Body weight was appropriate for gestational age in all cases. All infants had to be administered TPN because of multiple congenital defects requiring major surgery (two cases of esophagus atresia and tracheoesophageal fistula, one associated to duodenal obstruction; one case of myelomeningocele plus diaphragmatic hernia and arthrogryposis; one case of intestinal malrotation and duodenal stenosis; and one case of imperforate anus). TPN was infused immediately after operation, performed during the first 24 hr of life. Total fluid intake was 156 ml/kg/day, and total calories ranged from 85.5 to 102 kcal/kg/day. The intravenous infusion

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contained amino acids (1.5-2.5 g/kg/day), glucose (11.2-12.9 g/kg/day), electrolytes and minerals (Na, 2.2 meq/kg/day; K, 1.8 meq/kg/day; Cl, 2.0 meq/kg/day; Ca, 2.5 meq/kg/day; Mg, 0.5 meq/kg/day; P, 2 mmol/kg/day) and vitamins (A, 1000 IU; D, 100 IU; E, 0.5 mg; B_1, 5 mg; B_2, 1.4 mg; B_6, 1.5 mg; C, 50 mg; niacin, 10 mg; pantothenic acid, 2.5 mg). Fat was administered as Intralipid 20% (Vitrum AB, Stockholm, Sweden). A progressive amount of lipids was infused, increasing from 1.0 g to 4.0 g per kg per day in the course of 7 days. The infants died from various complications at 5-13 days after birth, having received TPN for a minimum of 4 days and a maximum of 12. This represented a total fat intake of 14.7-90 g (means ± S.D. = 47.1 ± 29.8 g). According to the analysis of our batch of Intralipid 20%, the total supply of 18:2ω6 ranged from 6.7 g to 41 g (21.5 ± 13.59 g) and that of 18:3ω3 from 1.2 g to 7.3 g (3.8 ± 2.41 g).

All the bodies were refrigerated at 4 C immediately after death. Autopsy, which was done within 12-36 hr of death, did not reveal any brain or liver anomalies. A cerebral hemisphere and the liver were removed, wrapped in double bags of aluminum foil and polyethylene, and stored frozen at −20 C until analysis.

Analytical procedures. All solvents and reagents were of highest purity, purchased from Merck (Darmstadt, Federal Republic of Germany). Just before lipid extraction, the complete hemisphere and the whole liver were homogenized in a Sorvall Omnimixer (Norwalk, Connecticut) to avoid regional differences. Brain and liver homogenates were diluted with an equal volume of water, and total lipids were extracted with 20 vol of chloroform/methanol (1:2, v/v) as specified elsewhere (23). Ethanolamine phosphoglycerides (EPG) and choline phosphoglycerides (CPG) were separated from aliquots of the lower phase by thin layer chromatography (TLC) in two different ways. In order to have a high fatty acid/impurities ratio, as much as 40 μg of lipid P were spotted on a precoated Silica Gel G plate (Chromatoplate, Merck) for the separation of EPG. For CPG, on the other hand, only 20-25 μg of lipid P were spotted on a 0.25-mm-thick Silica Gel G plate prepared manually in the laboratory, because this procedure was shown to give best separation between choline and serine phosphoglycerides. In both cases, the solvent system was chloroform/methanol/water (65:25:3.5, v/v/v). The individual phosphoglycerides were recovered from the plates by scraping off the spots, and the fatty acid methyl esters were obtained directly from the dried silica by cold methanolysis with 2 ml of 0.1 N sodium methoxide in dry methanol, according to Svennerholm (24). After neutralization with 1 N acetic acid, the fatty acid methyl esters were extracted three times with 2 ml of very pure petroleum ether (40-60 C), washed twice with water and dried with anhydrous sodium sulfate. After evaporation with N_2, the fatty acid methyl esters were dissolved in a small volume of hexane and injected into the gas chromatograph.

A Perkin Elmer 900 gas chromatograph was used, equipped with flame ionization detectors (FID) and a 10-ft, 1/8 inch OD, stainless steel column, packed with 15% ethylene glycol succinate (LAC 4R 886) on Chromosorb W 100-120 mesh, acid-washed and dimethyldichlorosilane-treated. The carrier gas was N_2, and the column was operated on a temperature program from 160 C to 195 C, at a rate of 3 C/min. A relatively high volume (10 μl) was injected so that the descending effect of the solvent tail compensated for the column bleeding, and a good base line was obtained despite the use of a single column. The peak areas were measured with an Autolab electronic computer-integrator, system IV model, and the results were expressed as area percentages, which were essentially equivalent to weight percentages within the range of fatty acids studied. The statistical analyses were effected with an electronic programmer (Olivetti, 203 model). For the study on normal development, regression analysis was used, either linear or quadratic depending on the developmental trend. All cases were analyzed individually, with gestational ages plotted on the X axis against corresponding fatty acid values on the Y axis (for correlation between liver and brain, the fatty acid value for the liver was plotted on the X axis against the corresponding value for the brain of the same child on the Y axis, as shown in Figs. 1-3). The levels of significance of the regression coefficients are indicated in Results. On

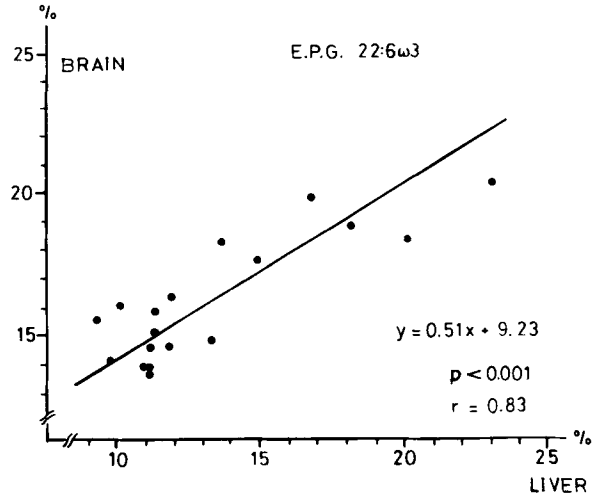

FIG. 1. Brain against liver percent values of docosahexaenoic acid in ethanolamine phosphoglycerides of a group of newborn infants with gestational ages from 20 to 44 wk.

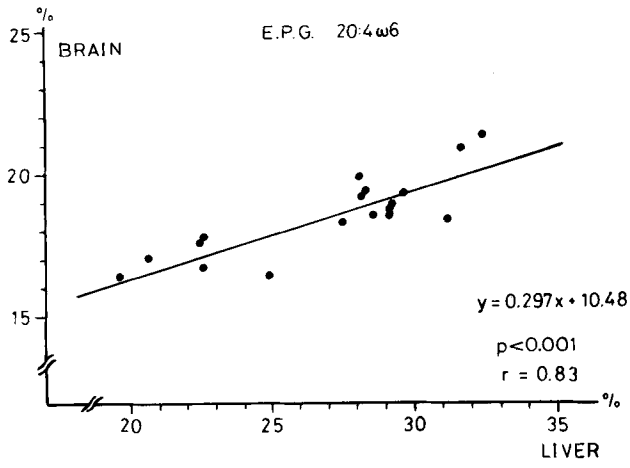

FIG. 2. Brain against liver percent values of arachidonic acid in ethanolamine phosphoglycerides of a group of newborn infants with gestational ages from 20 to 44 wk.

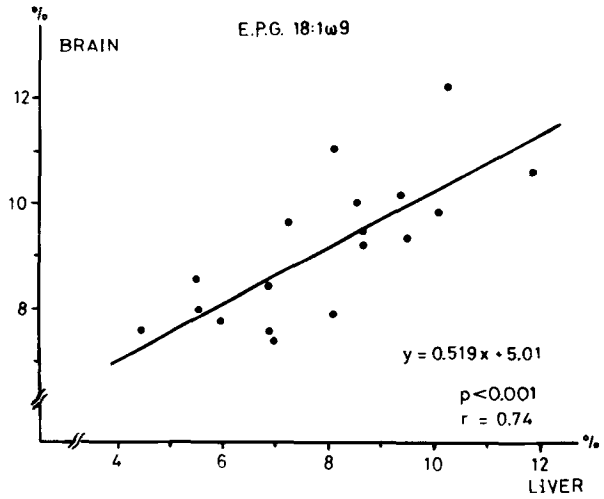

FIG. 3. Brain against liver percent values of oleic acid in ethanolamine phosphoglycerides of a group of newborn infants with gestational ages from 20 to 44 wk.

the other hand, for the study on the effects of TPN on tissue fatty acid composition, all cases were distributed into three age groups (see Results and Tables 1 and 2). This allowed us to compare the cases receiving TPN to controls of the same age. The differences observed were validated by means of Student's t-tests for independent variables, and the levels of significance are given in the tables.

RESULTS

Normal development. Under "controls," Tables 1 and 2 summarize the developmental changes in the fatty acid composition of the main phosphoglycerides of the human liver and brain during the second half of gestation. For the sake of simplicity, Tables 1 and 2 show all the cases distributed into three age groups: a very immature group, ranging from 20–25 wk of gestational age; an immature group in a developmental stage just before the beginning of the "chemical spurt" (23), 28–31 wk; and a full-term group, with gestational ages from 37 wk onward. However, for statistical analysis, all control data were considered individually (by means of regression lines) and not by groups, so that the developmental changes could find a more real statistical expression.

Confirming previous data (10,11), the main developmental changes in the brain were an increase in 22:6ω3 (docosahexaenoic acid) at the end of gestation and a quite linear decrease in 18:1ω9 (oleic acid) and 20:4ω6 in EPG. The elongation/desaturation of arachidonic acid to its products 22:4ω6 (docosatetraenoic or adrenic acid) and 22:5ω6 (docosapentaenoic acid), expressed by the index (22:4ω6 + 22:5ω6)/20:4ω6, increased linearly with gestational age in brain EPG, and the ratio 22:4ω6/22:5ω6 increased with maturation in EPG and CPG as shown before (10). All these changes were very significant statistically ($p < 0.001$).

Very similar changes could be observed in the liver, with 22:6ω3 increasing markedly at the end of gestation in both phosphoglycerides. A linear decrease in 18:1ω9

TABLE 1

Fatty Acid Composition of the Main Phosphoglycerides of the Human Liver—Effect of Development and of Total Parenteral Nutrition (TPN)

	Ethanolamine phosphoglycerides				Choline phosphoglycerides			
	Controls			TPN	Controls			TPN
	20–25 wk (n = 6)	28–31 wk (n = 6)	>36 wk (n = 6)	>36 wk (n = 5)	20–25 wk (n = 6)	28–31 wk (n = 6)	>36 wk (n = 6)	>36 wk (n = 5)
16:0	16.3 ±2.08	16.2 ±1.05	19.3 ±2.94	16.0 ±2.08[a]	35.4 ±2.33	33.7 ±1.06	36.5 ±1.34	32.1 ±1.56[d]
16:1ω7	0.8 ±0.22	0.7 ±0.16	0.8 ±0.22	0.5 ±0.20[b]	4.1 ±0.76	3.3 ±0.38	3.6 ±1.07	2.2 ±0.61[b]
18:0	22.5 ±1.62	24.9 ±1.42	23.6 ±2.55	25.2 ±2.08	11.2 ±0.39	12.3 ±0.79	11.4 ±1.52	11.8 ±1.34
18:1ω9	9.4 ±2.00	8.2 ±0.95	6.2 ±1.31	8.4 ±0.77[c]	21.0 ±2.48	20.4 ±2.62	17.2 ±2.66	15.4 ±0.81
18:2ω6	3.0 ±0.88	4.4 ±0.86	4.9 ±1.64	15.9 ±3.58[f]	5.7 ±1.61	8.7 ±1.28	9.2 ±2.62	24.8 ±2.86[f]
20:3ω9	1.0 ±0.51	0.8 ±0.17	0.2 ±0.15	0.2 ±0.08	0.8 ±0.43	0.6 ±0.12	0.2 ±0.10	tr. —
20:3ω6	1.6 ±0.27	1.8 ±0.39	1.5 ±0.21	0.7 ±0.21[f]	2.1 ±0.39	2.3 ±0.35	2.0 ±0.57	0.9 ±0.33[d]
20:4ω6	29.7 ±1.89	29.1 ±1.19	21.9 ±2.13	21.6 ±3.89	14.7 ±2.67	14.2 ±2.23	13.1 ±1.91	8.8 ±1.93[d]
22:4ω6	2.2 ±1.20	1.8 ±0.74	1.7 ±0.76	1.2 ±0.19	0.3 ±0.08	0.3 ±0.01	0.3 ±0.04	0.2 ±0.05[b]
22:5ω6	1.4 ±0.75	1.0 ±0.22	1.2 ±0.50	0.7 ±0.20[a]	0.4 ±0.13	0.3 ±0.04	0.3 ±0.12	0.2 ±0.07[b]
22:5ω3	0.3 ±0.11	0.3 ±0.06	0.6 ±0.21	0.5 ±0.13	0.1 ±0.04	0.1 ±0.03	0.3 ±0.07	0.2 ±0.05
22:6ω3	11.5 ±1.05	10.6 ±0.96	17.7 ±3.50	9.1 ±2.57[e]	3.3 ±0.61	3.3 ±0.58	5.4 ±1.66	2.7 ±0.69[d]
ω3/ω6	0.31±0.04	0.29±0.03	0.60±0.16	0.25±0.10[e]	0.20±0.02	0.20±0.03	0.23±0.06	0.08±0.03[f]
22:4ω6/22:5ω6	1.60±0.47	1.78±0.46	1.44±0.43	1.74±0.50	0.94±0.18	1.05±0.17	0.87±0.30	1.25±0.37[a]
18:2ω6/18:1ω9	0.35±0.19	0.54±0.10	0.78±0.18	1.88±0.33[f]	0.28±0.10	0.43±0.09	0.54±0.14	1.62±0.26[f]
Elongation/desaturation 18:2ω6*	12.6 ±4.15	7.97±1.51	5.96±1.93	1.59±0.47[f]	3.22±0.65	2.01±0.34	1.79±0.51	0.41±0.11[f]
Elongation/desaturation 20:4ω6**	0.12±0.07	0.09±0.04	0.13±0.06	0.09±0.02	0.05±0.02	0.04±0.01	0.04±0.01	0.04±0.01

All values represent wt % of each fatty acid (mean values, for the number of cases indicated, *n*) ± S.D. The statistically significant changes for the children receiving TPN are signaled by letters according to the following levels of significance (based on Student's t-test with 9 degrees of freedom): *a*, <0.1; *b*, <0.05; *c*, <0.02; *d*, <0.01; *e*, <0.002; *f*, <0.001.

*(20:3ω6 + 20:4ω6 + 22:4ω6 + 22:5ω6)/18:2ω6; **, (22:4ω6 + 22:5ω6)/20:4ω6.

TABLE 2

Fatty Acid Composition of the Main Phosphoglycerides of the Human Forebrain—Effect of Development and of Total Parenteral Nutrition (TPN)

	Ethanolamine phosphoglycerides				Choline phosphoglycerides			
	Controls			TPN	Controls			TPN
	20–25 wk (n = 6)	28–31 wk (n = 6)	>36 wk (n = 6)	>36 wk (n = 5)	20–25 wk (n = 6)	28–31 wk (n = 6)	>36 wk (n = 6)	>36 wk (n = 5)
16:0	7.8 ±0.56	7.6 ±0.66	6.2 ±0.36	5.8±0.56	50.3±0.66	50.6 ±0.71	50.4 ±0.80	49.9 ±0.45
16:1ω7	0.6 ±0.18	0.8 ±0.09	0.5 ±0.12	0.6 ±0.11	7.4 ±0.41	6.7 ±0.50	6.3 ±0.61	6.5 ±0.55
18:0	25.6 ±0.68	27.5 ±1.69	26.7 ±1.49	26.7 ±1.07	7.5 ±0.48	7.9 ±0.50	9.1 ±0.87	8.6 ±0.54
18:1ω9	10.0 ±1.44	9.4 ±1.18	8.0 ±0.41	7.4 ±0.66	23.0 ±0.36	22.6 ±0.88	22.4 ±0.61	21.8 ±0.50
18:2ω6	0.5 ±0.10	0.6 ±0.15	0.5 ±0.12	0.6 ±0.23	0.7 ±0.19	0.8 ±0.18	0.7 ±0.16	1.6 ±0.41*f*
20:3ω9	1.4 ±0.43	1.0 ±0.20	0.8 ±0.31	0.7 ±0.10	0.2 ±0.07	0.2 ±0.07	0.2 ±0.05	0.1 ±0.03
20:3ω6	0.6 ±0.12	0.9 ±0.18	1.1 ±0.17	1.1 ±0.23	0.3 ±0.18	0.5 ±0.06	0.8 ±0.13	0.6 ±0.18
20:4ω6	20.0 ±0.90	18.5 ±0.21	17.0 ±0.58	17.7 ±0.93	4.5 ±0.45	4.7 ±0.32	5.3 ±0.48	5.4 ±0.15
22:4ω6	11.6 ±0.80	13.1 ±1.06	14.8 ±1.02	14.7 ±0.86	0.6 ±0.04	0.7 ±0.04	0.8 ±0.09	0.9 ±0.24
22:5ω6	5.8 ±0.40	6.0 ±0.58	4.6 ±0.85	5.3 ±1.10	0.3 ±0.03	0.3 ±0.04	0.2 ±0.11	0.3 ±0.09
22:5ω3	0.3 ±0.05	0.3 ±0.05	0.5 ±0.21	0.6 ±0.24	tr. —	tr. —	tr. —	tr. —
22:6ω3	15.3 ±0.58	13.8 ±1.09	18.9 ±1.06	18.5 ±1.81	1.1 ±0.11	1.0 ±0.15	1.2 ±0.19	1.4 ±0.23
ω3/ω6	0.41±0.02	0.36±0.03	0.50±0.06	0.49±0.06	0.17±0.02	0.17±0.02	0.18±0.02	0.16±0.03
22:4ω6/22:5ω6	2.01±0.15	2.20±0.11	2.78±0.49	2.84±0.52	2.00±0.13	2.14±0.20	3.51±1.09	2.70±0.45*a*
18:2ω6/18:1ω9	0.05±0.01	0.06±0.01	0.06±0.02	0.08±0.03	0.03±0.01	0.04±0.01	0.03±0.01	0.08±0.02*f*
Elongation/desaturation 18:2ω6*	86.3 ±16.5	73.3 ±19.7	86.9 ±19.2	78.5 ±32.7	8.34±1.56	7.82±1.38	10.8 ±2.21	4.77±1.40
Elongation/desaturation 20:4ω6**	0.88±0.05	1.04±0.09	1.09±0.10	1.13±0.06	0.21±0.03	0.23±0.02	0.20±0.03	0.23±0.05

All values represent wt % of each fatty acid (mean values, for the number of cases indicated, *n*) ± S.D. The statistically significant changes for the children receiving TPN are signaled by letters according to the following levels of significance (based on Student's t-test with 9 degrees of freedom): *a*, <0.1; *f*, <0.001.

*, (20:3ω6 + 20:4ω6 + 22:4ω6 + 22:5ω6)/18:2ω6; **, (22:4ω6 + 22:5ω6)/20:4ω6.

and 20:4ω6 was also found in EPG and CPG, significant at the 0.001 level. The 22:4ω6/22:5ω6 ratio, on the other hand, did not show significant variations with age, these fatty acids being quantitatively less important in the liver than in the brain due to a poorer elongation/desaturation of arachidonate [see the index (22:4ω6 + 22:5ω6)/20:4ω6 in the tables]. As for the parent ω6 fatty acid, 18:2ω6, it is present only in very low amounts in brain because of its very active elongation and desaturation to the higher members of the family. In liver, on the other hand, it is an important constituent of phosphoglycerides, and it increased slowly with maturation in EPG ($p < 0.05$) and CPG ($p < 0.01$), at the same time as its elongation/desaturation slowed down in both phosphoglycerides ($p < 0.001$). As a consequence of these maturational changes, the ratio 18:2ω6/18:1ω9 increased linearly and very significantly ($p < 0.001$) in liver EPG and CPG.

The striking similarity of some fatty acid profiles in the liver and the brain was emphasized by plotting the brain values against the corresponding liver values. This resulted in a very significant ($p < 0.001$) positive correlation between the brain and liver values of 22:6ω3 (Fig. 1), 20:4ω6 (Fig. 2) and 18:1ω9 (Fig. 3). Although only a minor constituent, 20:3ω9 was also studied for its significance in relation to nutrition and showed a parallel decrease with maturation in EPG of liver ($p < 0.001$) and brain ($p < 0.01$) and in liver CPG ($p < 0.001$).

Influence of TPN on fatty acid patterns of liver and brain. The fatty acid composition of liver and brain EPG and CPG in a group of full-term infants receiving TPN with Intralipid is shown in Tables 1 and 2 beside the normal patterns corresponding to controls of the same age, so that the effects of TPN can be easily detected. It can be seen that there was a threefold increase in linoleic acid in the two phosphoglycerides of liver and a twofold increase in brain CPG, all very significant statistically (a t-test was applied between the two full-term groups, control and TPN-treated; see tables for levels of significance). This increase in 18:2ω6, however, was not accompanied by any parallel increase in the long members of the family. On the contrary, 20:3ω6 (dihomo-γ-linolenic acid) decreased very significantly in liver EPG and CPG, and 20:4ω6 decreased markedly in liver CPG (although not in EPG). The longer ω6 PUFA, 22:4ω6 and 22:5ω6, showed a tendency to decrease in liver EPG. It can be deduced, therefore, that the elongation/desaturation of 18:2ω6 was significantly decreased in both phosphoglycerides of the liver, leading mainly to reductions in 20:4ω6 in CPG and in the 22 carbon atom members in EPG.

The ratio of 18:2ω6 to 18:1ω9, which increased slowly with maturation in the liver of the normal child, was much more augmented in the liver phosphoglycerides of infants receiving TPN. Palmitic (16:0) and palmitoleic (16:1ω7) acids were slightly reduced in the liver of the TPN-treated group, more significantly in CPG than in EPG, but 18:0 (stearic acid) did not show any significant variation, and 18:1ω9 slightly increased in liver EPG and showed a non-significant tendency to decrease in liver CPG.

A most interesting finding was the very significant decrease in the 22:6ω3 level of the liver, which was reduced to about half the normal value at this age in liver EPG and CPG. As a consequence, the ω3/ω6 ratio was also greatly reduced in the liver of children receiving TPN.

It is interesting to note that the only change in the brain of the parenterally nourished infants was the above-mentioned increase in 18:2ω6 in brain CPG. A consequence of this was a very significant reduction of the 18:2ω6 elongation/desaturation and an increase in the 18:2ω6/18:1ω9 ratio in brain CPG. The rest of brain CPG fatty acids, however, were totally within normal limits, and no variation at all could be found in brain EPG.

DISCUSSION

The developmental changes in the fatty acid composition of the main phosphoglycerides in the human brain confirm our previous data on a larger number of cases (10,11), i.e., there is a significant increase in the 22:6ω3 level of the brain EPG and CPG after 32 wk of gestational age and a linear decrease in the 20:4ω6 and 18:1ω9 values during the second half of gestation, mainly in brain EPG.

The present data show an excellent correlation between the main developmental fatty acid changes in the brain and the liver during early human development. In other words, docosahexaenoate increases in the human liver also in a parabolic manner, whereas arachidonate and oleate decrease in a linear way in the two main phosphoglycerides, mainly in EPG. During this crucial stage of brain development the percentage of 22:6ω3 in the liver EPG is almost as high, or even a little higher toward the end of gestation, as that in the brain EPG, indicating that during this period elongation and desaturation of ω3 fatty acids in the human liver is very active and capable of providing the high levels of long ω3 PUFA required by the developing brain.

Upon comparison of fatty acid patterns in the infants receiving TPN with controls of the same age, the decrease in the docosahexaenoate level in both liver phosphoglycerides is striking. Such a decrease in 22:6ω3 has not even been described in rats receiving high doses of 18:2ω6 intravenously (17), in which a similar reduction was noted in liver CPG, but not in liver EPG.

A significant decrease in liver 20:4ω6 has been found in rats (16,17) and humans (19) receiving TPN with high doses of 18:2ω6. In our study, this decrease was only significant in liver CPG, the levels of arachidonate in liver EPG being totally within normal limits for the age. On the other hand, we found a very important increase in 18:2ω6 in both phosphoglycerides of the liver and even in brain CPG, which has not been described in the experimental animal; as a consequence, the ratio of 20:4ω6 to 18:2ω6 (or, as we prefer, the elongation/desaturation of linoleate expressed by the index [20:3ω6 + 20:4ω6 + 22:4ω6 + 22:5ω6]/18:2ω6) was greatly reduced, even much more so than in the rat receiving a large supply of parenteral 18:2ω6 (16).

For many years, studies on EFA have focused mainly on the linoleate (ω6) family. On the other hand, the essentiality of α-linolenic acid (18:3ω3) has been questioned (25), because no signs of ω3 deficiency could be discovered in laboratory animals subjected to ω3-deficient diets. However, from a quantitative point of view, the main long chain PUFA of the ω3 series, docosahexaenoic acid (22:6ω3), is a very important component of neuronal and retinal membranes. Although its functions are still not fully understood, 22:6ω3 seems to be an important factor in membrane fluidity of neurons and outer segments of the retina, probably enhancing the various movements of rhodopsin (26). Docosahexaenoate may even play a role in neurotransmission, as indicated by its preferential accumulation in synaptic membranes (27). Furthermore, another member of the ω3 family, eicosapentaenoic acid (EPA, 20:5ω3), plays an important role in platelet aggregation (28–31), and the supply of ω3 fatty acids is now recommended in the prevention of thromboembolic disorders (32–35).

Recently, clinical evidence of ω3 deficiency, caused by a poor supply of α-linolenic acid, has been presented in the human (36) and in the monkey (37). The present results suggest that a relative deficiency of ω3 fatty acids can also be produced by a different mechanism. It is very possible that the exclusive concern for the ω6 fatty acids and the very high doses of linoleate used in many milk formulas and most forms of TPN has caused the natural preference of the Δ6-desaturase system by the ω3 family (38,39) to be overcome and displaced towards the predominant ω6 fatty acids. Thus, even if a ω3 fatty acid deficiency is spontaneously very rare, if possible at all, because of the abundance of these fatty acids in nature, their small minimum requirements and the enzyme preferences for the linolenate family, our results show that it is possible to artificially produce a relative deficiency in ω3 fatty acids by supplying a great excess of ω6 fatty acids, as has also been shown in the rat brain and retina (40) and liver (17). It seems advised, therefore, to warn clinicians, mainly pediatricians, against the use of excessive amounts of linoleic acid in the diet, especially during early development.

Our cases received TPN only for a short period (4–12 days), and this was enough to produce important alterations in the fatty acid composition of the main liver phospholipids and even a significant increase in 18:2ω6 in brain CPG. The brain's resistance to changing its fatty acid composition despite dietary manipulation is well known (1,2,41,42), and we could not find any alteration in the brain long chain PUFA in our cases receiving TPN. However, it is possible that a more prolonged diet with large doses of 18:2ω6 could affect these fatty acids, as has been shown in the experimental animal (40). In any case, a diet capable of reducing the level of liver EPG and CPG docosahexaenoate to half its normal value during a critical developmental stage should certainly be proscribed. In agreement with very recent data in the rat (17), our results indicate that a correct ratio of linoleate to linolenate is not the only relevant factor in devising an EFA diet, especially when the nutrient has to be administered intravenously. An excess of 18:2ω6, even if a theoretically correct ratio of 18:2ω6 to 18:3ω3 is maintained, should be avoided if the balance of long chain PUFA is to be kept unaltered, an aim particularly important during brain development.

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