n-3 Deficient and docosahexaenoic acid-enriched diets during critical periods of the developing prenatal rat brain

Mark Schiefermeier[1] and Ephraim Yavin[2]

Department of Neurobiology, Weizmann Institute of Science, 76100 Rehovot, Israel

Abstract **The last period of the intrauterine life in the rat (embryonic day 17 to 21, ED17–ED21) is demarcated by an increase in brain and body weight and active neuronogenesis. During this period, a rapid accumulation of DHA (22:6 n-3), unparalleled to other fatty acids, takes place. The details of DHA rapid acquisition in the fetal brain were investigated after imposing a diet deficient in n-3 fatty acids (FA) as of ED1 and subsequently examining the distribution of DHA in major brain phospholipid (PL) classes on ED20, having added on ED15 a triglyceride (TG) mixture enriched up to 43% with DHA. The n-3 deficiency maintained for 19 days resulted at ED20 in more than 30% reduction of DHA in PL, which was counterbalanced by an increase of docosapentaenoic acid (DPA, 22:5 n-6). No effect on body weight, nor major changes in PL composition or other FA in fetal brain PL were observed. Feeding dams a DHA-TG diet on ED15 induced an immediate increase of DHA in maternal liver PL, followed by a subsequent increase of DHA in fetal liver PL, as well as in fetal brain PL. Thus the content of fetal brain DHA in n-3 deficient embryos could be restored within 48 hours. Dietary manipulation of fetal tissues is a rapid phenomenon and can be used to enrich DHA at critical periods of development in utero.**—Schiefermeier, M., and E. Yavin. **n-3 Deficient and docosahexaenoic acid-enriched diets during critical periods of the developing prenatal rat brain.** ***J. Lipid Res.*** **2002.** 43: **124–131.**

Supplementary key words docosahexaenoic acid • n-3 deficiency • phospholipids • fetal brain • fetal liver • phosphatidylcholine • phosphatidylethanolamine • phosphatidylinositol • phosphatidylserine

PUFA are major structural components of the neuronal plasma membrane. Information on the prenatal acquisition and possible function of PUFA in brain ontogeny is very limited. During prenatal development, the PUFA and especially DHA (22:6n-3) and arachidonic acid (AA, 20:4n-6), the two major PUFA present in the nervous system, are delivered by maternal source via the utereoplacental circulation and may be synthesized from precursors by placenta and fetal tissues (1). The fetal brain is also able to convert the essential precursors α-linolenic (LNA, 18:3n-3) and linoleic (18:2n-6) acids into DHA and AA, respectively (2).

It is traditionally accepted that DHA accumulation in the developing rat brain occurs in the postnatal period, primarily during the three weeks before weaning (3). Until recently, accumulation of brain FA has scarcely been studied in the prenatal rat, despite the dramatic changes occurring virtually from day to day, particularly in terms of neurogenesis. The last period in the developing prenatal brain is demarcated in the rat by active neuronogenesis, a process that ontogenetically predates oligodendrogliogenesis (4–7). We have found that at this developmental stage the accretion rate of DHA is most profound compared to other FA (8, 9). Particularly within the last three days prior to birth, the accretion spurt of all FA is halted with the exception of DHA, which continues to rise dramatically.

LNA has been recently shown by ^{13}C-NMR to serve as a precursor for cholesterol and palmitate biosynthesis in the brain, suggesting a lesser availability of precursors for DHA production (10). Thus a deficiency in n-3 FA during prenatal development may have serious and irreversible consequences on further normal development (11–13). Feeding pregnant dams n-3 FA deprived diets resulted in a dramatic reduction of DHA content in the phospholipids (PL) of the fetal liver and brain, which was counterbalanced by an increase in the content of docosapentaenoic acid (DPA, 22:5n-6) (14). These changes were most pronounced in phosphatidylserine (PS) and phosphatidylethanolamine (PE), and were less notable in phosphatidylcholine (PC).

A rapid modulation of fetal DHA content, achieved by intra amniotic ethyl-DHA administration, permitted the investigation of DHA manipulation during different stages of intrauterine life under conditions of utero-placental insufficiency (15). Administration of ethyl-DHA via the intra amniotic route increased brain DHA content in normal animals and replenished the DHA content of n-3 deficient

Abbreviations: AA, arachidonic acid; ED, embryonic day; DPA docosapentaenoic acid; DTA, docosatetraenoic acid; FA, fatty acid; LNA, alpha-linolenic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, phospholipid; PS, phosphatidylserine.

[1] On leave from Dept. of Neurology, University of Vienna, Austria.

[2] To whom correspondence should be addressed.
e-mail: ephraim.yavin@weizmann.ac.il

embryos (14, 15). Although injected only once in utero, this procedure has invasive components due to surgical manipulation of the pregnant dam and penetration of the amniotic sac.

Prompted by this experimental drawback, we used nutritional means to manipulate the composition of the fetal brain and other tissues. To the best of our knowledge, very few studies have manipulated the DHA content by using, for example, n-3 deficient or DHA-enriched diets in animal models during the last period of gestation. On the contrary, in order to attain maximum deficiency of DHA, most investigators have used prolonged deficiency regimes, often spanning for more than one generation (11, 12, 16–20). The rationale for the present study stemmed from recent findings of the rapid accretion of DHA toward birth (14) and our working hypothesis that a partial DHA deficiency may be corrected by suitable dietary means. We report that *a*) indeed a dietary FA n-3 deficient status can be attained and practically expressed in all major PL classes of the developing brain in utero and *b*) that a diet enriched in triglycerides containing approximately 43% DHA can reverse the DHA-deficient status within two days. These findings should pave the way for dietary administration of DHA whenever n-3 deficiency conditions persist and maternal supplements are indicated.

MATERIALS AND METHODS

Animals and diets

Pregnant Wistar rats purchased from Harlan laboratories at the Weizmann Institute, (Rehovot, IL) one day after conception were subjected to a diet either sufficient or deficient in n-3 FA acids. On embryonic day (ED) 15, both the n-3 FA sufficient as well as the n-3 deficient groups were divided again. The n-3 FA sufficient group was either continued (control dams) or received a diet containing a DHA-enriched triglyceride (control/DHA-TG). The n-3 deficient group was also divided into two subgroups, one of which was supplemented with the DHA-TG (n-3 Def/DHA-TG) additive, while the other not. The diets were based on a semi purified fat-free basal mix of casein, DL-methionine, sucrose, corn starch, cellulose and a mineral mix as well as a vitamin mix (Custom Ward AIN76 modified diet from Harlan, Cleveland, OH) as reported previously (14). For lipid supplements, 5% (by weight) of local soybean oil (control diet), or 5% sunflower oil (n-3 deficient diet) were used. Enrichment with DHA-TG (2.5% by weight) was complemented with 2.5% soybean oil. The FA composition of soybean, sunflower oil, and DHA-TG oil supplement (kindly provided by Martek Biosciences, Columbia, MD) is given in **Table 1**.

Rats were housed at a maximum of three to a cage, and freshly prepared food was administered in specially designed jars (BioServe, Laurel, MD) every second day, ad libitum.

Lipid extraction and analytical procedures

Fetal and maternal tissues of all four diet groups were isolated on ED15, ED18.5 and ED20. In additional studies, tissue samples were taken from the control and n-3 deficient groups on ED1. Tissue samples were also taken from the n-3 Def./DHA-TG group 18 and 36 h after supplying the DHA-TG diet. Animals were sacrificed by cervical dislocation and an abdominal midline incision was performed. The uterine horns were exposed and the embryos were delivered alive, counted and weighed essentially as detailed elsewhere (14). Fetal brain and liver tissues as well as the maternal liver were isolated and immediately frozen in liquid nitrogen for further PL analysis. Frozen tissue was homogenized in a precooled mixture of hexane-isopropanol (3:2 by vol) containing 50 mg/l butylated hydroxytoluene (Sigma, St. Louis, MO) using a Branson tip sonifier in accordance with Hajra and Radin (21). After low speed centrifugation, the organic layer was separated and the aliquots subjected to thin layer chromatography on silica gel plates (Merck, Darmstadt, Germany) for further separation into the major PL classes using a solvent system consisting of chloroform-methanol-40% methylamine (130:70:30 v/v/v) containing 50 mg/l butylated hydroxytoluene as described previously (22). All solvents were of HPLC-grade. For the determination of lipid phosphorus, bands were visualized by exposure to iodine vapor. As for gas chromatography analysis, 0.2% dichlorofluorescein (Sigma) spray was used for identification. The individual PL bands visualized under UV were scraped off into glass tubes and FA methyl esters were prepared, separated on gas chromatography, and quantified as described previously (23). Lipid phosphorus was determined in accordance with Bartlett (24).

TABLE 1. Fatty acid composition of the experimental oils

Fatty Acid	Soybean Oil	Sunflower Oil	DHA-TG	Soybean/ DHA
C12:0	—	—	4.78	2.39
C14:0	—	—	15.36	7.68
C16:0	10.25	6.74	12.23	11.23
C18:0	4.04	3.91	0.80	2.06
C16:1	0.11	0.15	1.37	0.74
C18:1	23.16	25.92	19.55	21.35
C18:2n-6	55.41	60.28	0.81	28.11
C18:3n-3	6.69	0.26	<0.10	3.35
C20:4n-6	0.11	0.48	<0.10	<0.10
C22:4n-6	—	—	—	—
C22:5n-6	—	—	—	—
C20:5n-3	—	—	<0.10	<0.10
C22:5n-3	—	—	0.26	0.13
C22:6n-3	—	—	43.22	21.61

Values expressed as % FA. Only major FA are presented, thus they do not add up to 100%.

Statistical analysis

Statistical analysis was performed using the software package SPSS 8.0.0. The data of all experiments were either compared between the four different diet groups on ED20 or compared between ED15 and ED20 to determine the difference in developmental changes within each diet group. Statistical comparisons of mean values were based on embryos of five to 11 different mothers in each experiment. Differences of mean values between the different dietary treatments on ED20 were tested for homogeneity in variance with the Levene test, and further analyzed with a one factorial ANOVA subsequent multiple post hoc comparisons according to Tukey's honestly significant difference and assumed to be significant if $P < 0.05$. Mean differences of FA content on ED15 and ED20 within every dietary group were compared using the *t*-test and assumed to be significant if $P < 0.05$.

RESULTS

On ED20, one day prior to delivery, fetuses subjected to each of the four different groups of maternal diet manipulations initiated on the first day after conception and continued for 18 days showed no difference between each

TABLE 2. Fetal body weight and number of fetuses at various gestational periods following different diets

Embroynic Day (ED)	Control	Control DHA-TG	n-3 Def	n-3 Def DHA-TG
	Body weight (g) (average number of fetuses/number of dams tested)			
ED15	0.31 ± 0.02 (12/2)	nd	0.30 ± 0.02 (11/4)	nd
ED17	1.03 ± 0.02 (11/7)	nd	0.92 ± 0.03 (12/9)	nd
ED18.5	1.96 ± 0.12 (12/3)	2.09 ± 0.12 (12/3)	2.09 ± 0.15 (11/5)	2.03 ± 0.08 (11/3)
ED20	3.93 ± 0.08 (12/13)	3.85 ± 0.05 (11/4)	3.89 ± 0.07 (12/15)	3.90 ± 0.08 (11/4)

Data expressed as gram ± SEM ($2 < n > 15$). The average number of fetuses per number of dams measured is given in parenthesis. Note: There is no difference in the body weight or in the number of embryos between dams on the control diet, a diet enriched with DHA from ED15 to ED20 (Control/DHA-TG), the n-3 deficient diet (n-3 Def), or the n-3 deficient diet until ED15 followed by a DHA enriched diet until ED20 (n-3 Def/DHA-TG). nd, not determined.

other with respect to fetal body weight or number of fetuses per pregnant dam (**Table 2**).

There were no differences encountered in gross brain weight (data not shown). Fetal brain tissue extracts were analyzed by thin layer chromatography for the distribution of major PL species.

As shown in **Table 3**, the composition of major PL classes in the fetal brain after maternal n-3 deficiency remained practically the same as compared to the major PL classes in the fetal brains exposed to the control diet. Neither supplements of a diet enriched in DHA-TG showed compositional changes, although PE was slightly lower than the control diet. This is in marked contrast to previous studies using intra-amniotic ethyl-DHA supplements in which an increase in PS and PE content was noticed (15).

Fatty acid composition of fetal brain on ED20

A detailed analysis of the brain FA composition one day prior to birth is depicted in **Table 4**.

Little or no significant changes were observed in the content of the saturated FAs in PC, PE, and PS lipids after the various diets, or in the dimethylacetals (DMA) content of the PE species. Similarly, monounsaturated FA did not change markedly between the different diets in either one of the PLs measured. The sum of n-6 PUFA in PE and PS (28.2% and 22.1%, respectively) was far higher than that measured in the PC (6.9%) of the control fetal brain.

Changes in the diets resulted in distinct differences in the n-6 abundance in PE and PS at ED20. A 22% decrease was noticed for both PLs in animals treated for five days with DHA-TG compared to a 17–24% increase after an 18 day-long n-3 deficiency. Notably, the n-3 deficient animals treated for 5 days with DHA-TG showed values closer to the control animals treated with DHA-TG (23.5% and 18.6% for PE and PS, respectively). In particular, the different diets induced substantial changes in the content of DPA; e.g., the content of DPA in PE varied between 2.8% and 10.5%. Compared to changes in DPA levels, the differences of the contents of AA and docosatetraenoic acid (DTA, 22:4 n-6) between the four diet groups were minor.

Like the n-6 family, the n-3 family comprises mainly of DHA. The n-3 family is also the highest in the PE and PS species, accounting for 14.75 ± 0.2 and 15.04 ± 0.3 at ED20, respectively. As anticipated, diets deficient in n-3 substrates reduced the DHA content, while a DHA-TG supplement increased it. The highest contents of DHA in both PE and PS at ED20 were observed in the DHA-TG treated groups, in contrast to a reduction after 18 days of n-3 deficiency. Interestingly, the DHA-TG supplement given to the n-3 deficient group for five days restored the DHA content to nearly the levels of the DHA-TG supplemented control animals. This study indicates the remarkable capability of the developing fetal brain to overcome a dietary deficiency within a short period of time.

The effect of various diets on the course of PUFA accumulation in major PL during the critical period of brain neuronogenesis

The remarkable changes in the PUFA profile between ED15 and ED20 (the peak time of neuronal proliferation

TABLE 3. Fetal brain phospholipid content at ED20 after different diets

Phospholipid	Control	Control DHA-TG	n-3 Def	n-3 Def DHA-TG
		% of total lipid phosphorus		
Phosphatidylcholine	59 ± 2.0	61 ± 1.0	57 ± 2.0	59 ± 2.0
Phosphatidylethanolamine	27 ± 2.0	23 ± 1.0	27 ± 1.0	28 ± 2.0
Phosphatidylserine	8.7 ± 1.5	9.3 ± 0.4	8.8 ± 0.5	7.4 ± 0.7
Phosphatidylinositol	3.8 ± 0.7	4.6 ± 0.5	4.6 ± 0.4	3.3 ± 0.3
Sphingomyelin	1.7 ± 0.2	1.9 ± 0.2	2.2 ± 0.6	2.2 ± 0.4

Lipid extracts from fetal brains maintained on a control diet, a diet enriched with DHA from ED15 to ED20 (DHA-TG), a n-3 deficient diet (n-3 FA Def), and a n-3 deficient diet until ED15 followed by a DHA enriched diet until ED20 (n-3 FA Def/DHA-TG) were separated by TLC and lipid phosphorus in individual PL measured and % distribution calculated. Values expressed as percent of PL phosphorus are mean ± SEM (n = 7).

TABLE 4. Fatty acid composition of fetal brain phospholipids on ED20[a]

Fatty Acid	Control	Control DHA-TG	n-3 Def	n-3 Def DHA-TG
		Phosphatidylcholine (% FA)		
C14:0	2.91 ± 0.30	3.09 ± 0.13	3.34 ± 0.08	3.17 ± 0.09
C16:0	52.33 ± 0.66	51.04 ± 0.36	51.98 ± 0.55	51.15 ± 0.55
C18:0	5.85 ± 0.55	6.74 ± 0.15	6.55 ± 0.12	6.57 ± 0.19
C16:1	7.86 ± 0.24	8.08 ± 0.28	7.60 ± 0.12	7.83 ± 0.11
C18:1	21.21 ± 0.36***	22.06 ± 0.26*	20.43 ± 0.35**	22.09 ± 0.17*
C20:4n-6	5.48 ± 0.17*	4.77 ± 0.09**	5.63 ± 0.13*	4.92 ± 0.08**
C22:4n-6	0.72 ± 0.04*	0.54 ± 0.02**	0.76 ± 0.03*	0.55 ± 0.02**
C22:5n-6	0.70 ± 0.03*	0.30 ± 0.01**	1.31 ± 0.10***	0.38 ± 0.03**
C22:6n-3	1.49 ± 0.09*	2.01 ± 0.15**	1.02 ± 0.07***	1.97 ± 0.13**
		Phosphatidylethanolamine (% FA)		
C16-DMA	5.90 ± 0.49	6.29 ± 0.22	6.49 ± 0.27	6.19 ± 0.48
C18-DMA	4.32 ± 0.24	4.46 ± 0.16	4.38 ± 0.11	4.31 ± 0.28
C16:0	12.41 ± 0.25	12.93 ± 0.39	12.33 ± 0.17	13.02 ± 0.22
C18:0	20.50 ± 0.36	21.16 ± 0.36	19.93 ± 0.29	20.94 ± 0.35
C16:1	1.57 ± 0.09	1.63 ± 0.10	1.56 ± 0.08	1.71 ± 0.07
C18:1	10.98 ± 0.77	12.79 ± 0.43	11.52 ± 0.21	12.18 ± 0.46
C20:4n-6	16.75 ± 0.21*	14.81 ± 0.19**	17.06 ± 0.17*	15.45 ± 0.36**
C22:4n-6	5.37 ± 0.10*	4.29 ± 0.06**	5.36 ± 0.10*	4.38 ± 0.13**
C22:5n-6	6.09 ± 0.14*	2.83 ± 0.07**	10.52 ± 0.34***	3.71 ± 0.16**
C22:6n-3	14.75 ± 0.23*	17.36 ± 0.96**	9.61 ± 0.34***	16.86 ± 0.76**
		Phosphatidylserine (% FA)		
C16:0	6.97 ± 0.32	7.31 ± 0.39	7.48 ± 0.35	7.65 ± 0.52
C18:0	43.83 ± 0.55	45.64 ± 0.48	43.12 ± 0.32	45.41 ± 0.89
C16:1	1.30 ± 0.08	1.52 ± 0.10	1.42 ± 0.12	1.63 ± 0.18
C18:1	8.68 ± 0.84	8.41 ± 0.37	8.24 ± 0.30	8.06 ± 0.34
C20:4n-6	7.65 ± 0.08***	6.85 ± 0.15***	7.98 ± 0.15**	7.25 ± 0.38****
C22:4n-6	6.76 ± 0.20*	5.59 ± 0.18**	7.05 ± 0.16*	5.56 ± 0.22**
C22:5n-6	7.58 ± 0.15*	4.78 ± 0.35**	12.21 ± 0.44***	5.69 ± 0.31**
C22:6n-3	15.04 ± 0.30*	17.64 ± 0.61**	10.15 ± 0.44***	16.80 ± 1.17***

Lipid extracts from fetal brains maintained on a control diet, a diet enriched with DHA from ED15 to ED20 (DHA-TG), a n-3 deficient diet (n-3 FA Def), and a n-3 deficient diet until ED15 followed by a DHA enriched diet until ED20 (n-3 FA Def/DHA-TG) were separated by TLC and FA profile in each individual PL calculated. Values expressed as % FA ± SEM ($6 < n > 13$) of FA obtained from three major in fetal brain PL extracted from fetuses under the diets as detailed in Table 2.

[a] Only major FA are presented, thus they do not add up to 100%

*,**,***,**** Values in the same row with different asterisks are significantly different at $P < 0.05$.

to near birth) were studied in greater detail. **Table 5** illustrates the percent of change in the amount of DHA (fetal brain has few, if any, other n-3 intermediates), compared with the n-6 FA family, in the major PL classes in the fetal brain at ED15 and ED20.

The n-6 FA family (composed of AA, DTA, and DPA) is present in substantial amounts at this developmental stage and is subject to developmental alterations. During this critical 5 day period in brain development, the relative content of AA decreased in all PLs of the control fetuses. In contrast, the amount of DHA increased by approximately 8% in both PS and PE and less than 1% in PC species. This is in line with previous findings indicating an accretion of DHA during this developmental period, and a halt in the uptake of other PUFA (9, 14). As a result of a DHA-TG enriched diet, a further increase to approximately 11% in both PS and PE was noticed. The n-3 deficient diet, on the other hand, resulted in reduced amounts of esterified DHA to 4.81 ± 0.56 and 5.95 ± 0.47 in PE and PE species respectively, which constitutes, respectively, an over 40% and 30% reduction compared with the appropriate control PL. This reduction is striking given the fact that it is measured from a basal line of ED15. In agreement with other reports, the change in the DHA content in the n-3 deficient fetuses was counterbalanced by a marked increase in DPA (3.6 fold for the PE species and 2.4 fold for the PS species over the control animals). Table 5 also shows a most remarkable increase of DHA in PS and PE within 5 days of feeding the DHA-TG based diet to the dams that had been maintained on a n-3 deficient diet until ED15. Thus the relative increase in the DHA content of PE was 1.5 fold and 2.5 fold, for control and n-3 deficient animals, respectively. Similarly, the increase of DHA in PS was 1.5 fold for control animals and 2.1 fold for n-3 deficient animals. In general, there were lesser changes in AA and DTA. Conversely, restoration of DHA during the last five days of pregnancy was accompanied by a massive reduction of DPA. This matched decrease in the amount of n-6 species resulted in no net gains of the PUFA content (see Table 3), which notably accounted for about 80% of the FA species in PS and PE lipids in the developing brain. This remarkable increase, which took place over a period of 5 days, suggests the very effective corrective effect of the dietary supplementation.

TABLE 5. Change in polyunsaturated fatty acid composition of fetal brain phospholipids from ED15 to ED 20[a]

Fatty Acid	Control	Control DHA-TG	n-3 Def	n-3 Def DHA-TG
		Phosphatidylcholine (Δ% FA)		
C20:4n-6	−0.80 ± 0.29[b]	−1.51 ± 0.20[b]	−0.13 ± 0.29	−0.84 ± 0.27[b]
C22:4n-6	−0.07 ± 0.06	−0.25 ± 0.04[b]	0.20 ± 0.05[b]	−0.01 ± 0.05
C22:5n-6	0.13 ± 0.05[b]	−0.27 ± 0.02[b]	0.64 ± 0.14[b]	−0.30 ± 0.05[b]
C22:6n-3	0.82 ± 0.14[b]	1.34 ± 0.17[b]	0.66 ± 0.11[b]	1.62 ± 0.17[b]
		Phosphatidylethanolamine (Δ% FA)		
C20:4n-6	−2.32 ± 0.45[b]	−4.23 ± 0.45[b]	−2.90 ± 0.35[b]	−4.51 ± 0.55[b]
C22:4n-6	−0.62 ± 0.28[b]	−1.69 ± 0.38[b]	−1.21 ± 0.30[b]	−2.19 ± 0.29[b]
C22:5n-6	0.90 ± 0.28[b]	−2.36 ± 0.25[b]	3.25 ± 0.58[b]	−3.57 ± 0.37[b]
C22:6n-3	7.99 ± 0.41[b]	10.60 ± 1.04[b]	4.81 ± 0.56[b]	12.10 ± 1.02[b]
		Phosphatidylserine (Δ% FA)		
C20:4n-6	−0.77 ± 0.38	−1.56 ± 0.35[b]	−0.77 ± 0.51	−1.49 ± 0.74
C22:4n-6	1.67 ± 0.41[b]	−0.00 ± 0.37	1.18 ± 0.38[b]	−0.31 ± 0.42
C22:5n-6	2.11 ± 0.33[b]	−0.69 ± 0.53	5.04 ± 0.76[b]	−1.49 ± 0.60[b]
C22:6n-3	8.51 ± 0.64[b]	11.09 ± 1.07[b]	5.95 ± 0.47[b]	12.60 ± 1.23[b]

Lipid extracts from fetal brains maintained on a control diet, a diet enriched with DHA from ED15 to ED20 (DHA-TG), a n-3 deficient diet (n-3 FA Def), and a n-3 deficient diet until ED15 followed by a DHA enriched diet until ED20 (n-3 FA Def/DHA-TG) were separated by TLC and FA profile in each individual PL calculated. Values expressed as mean % FA difference (ED20-ED15) ± SEM ($4 < n > 13$) of FA obtained from three major in fetal brain PL extracted from fetuses under the diets as detailed in Table 2.

[a] Only changes in major PUFA are presented.

[b] The difference is significant ($P < 0.05$).

Time course of DHA uptake into PE in fetal brain and liver compared to maternal liver

The effective correction of the DHA levels in the n-3 deficient animals raised the question as to whether fetal brain tissue at this time in development is preferentially in demand of DHA or whether other fetal organs are equally in need. A partial answer to this question can be seen from the data in **Fig. 1**, where the daily acquisition of DHA in fetal brain and liver PE after different diet regimens is monitored.

In Fig. 1A, a relatively steep increase from 6.8 ± 0.3% to 14.8 ± 0.3% over 5 days is seen in animals under the control diet. A DHA-TG-enriched diet caused an even steeper increase in the amount of DHA; already by ED18.5 a DHA value of approximately 17% was evident whether the dams were n-3 deficient before or not. As expected, a slow down in the acquisition of DHA in the n-3 deficient diets was noticed. Given the fact that there was no further increase in DHA levels after ED18.5 and that there was no difference between the two DHA-TG animal groups, it is suggested that the DHA levels have reached a plateau at this stage of development. The reduced levels of DHA can be corrected by supplementing the maternal diet with DHA on ED15 as indicated previously. Acquisition of DHA is not restricted to the fetal brain. A similar time course of DHA acquisition was noticed in the fetal liver (Fig. 1B). Thus, it would appear that the fetal organs are subject to similar conditions when the maternal diet constituency is modified. Indeed the fetal liver is known to serve as a reservoir and also as a site of synthesis for PUFA and is likely to take an active part in the supply of PUFA to the brain (25, 26).

The changes in the DHA composition of maternal liver PL commencing from day 15 and up to day 20 of gestation, under various dietary conditions is depicted in Fig. 1C. Pregnant dams supplemented with DHA on the 15th day gestation showed a remarkable increase attaining near birth a value of about 25%. Compared to animals maintained on a control diet, liver PE content in n-3 deficient animals showed a nearly 45% reduction of DHA. Notably, a 31% reduction was already established by ED15. There were practically no differences in the DTA and AA contents of PE between control animals and n-3 deficient animals during the last five days of pregnancy. A massive increase of DPA from 5.3% ± 0.6 on ED15 to 13.6% ± 0.8 on ED20 was noticed in n-3 deficient animals, compared to 2.0% ± 0.2 and 5.8% ± 0.3 of the control animals. After DHA supplements were administered, the maternal liver in the n-3 deficient animals showed a substantial recovery, and attained within several days levels up to approximately 25%, whereas the content of DPA was reduced approximately 0.5%. Notably, the restitution of DHA content in the fetal liver predated that of the fetal brain. Taken together, it may be speculated from these findings that the DHA-DPA ratio in fetal organs is mainly driven and regulated by a compensatory mechanism that takes place initially in the maternal system.

DISCUSSION

n-3 Deficiency in utero

The last several days of intrauterine life in the fetal rat (ED15 to birth) are characterized by a rapid increase in brain and body weight. In parallel to that, particularly between ED17 and birth, we have recently demonstrated a rapid and selective outburst of DHA accumulation unmatched by other FA (9). For this report we manipulated time-selectively the course of the DHA accretion spurt by

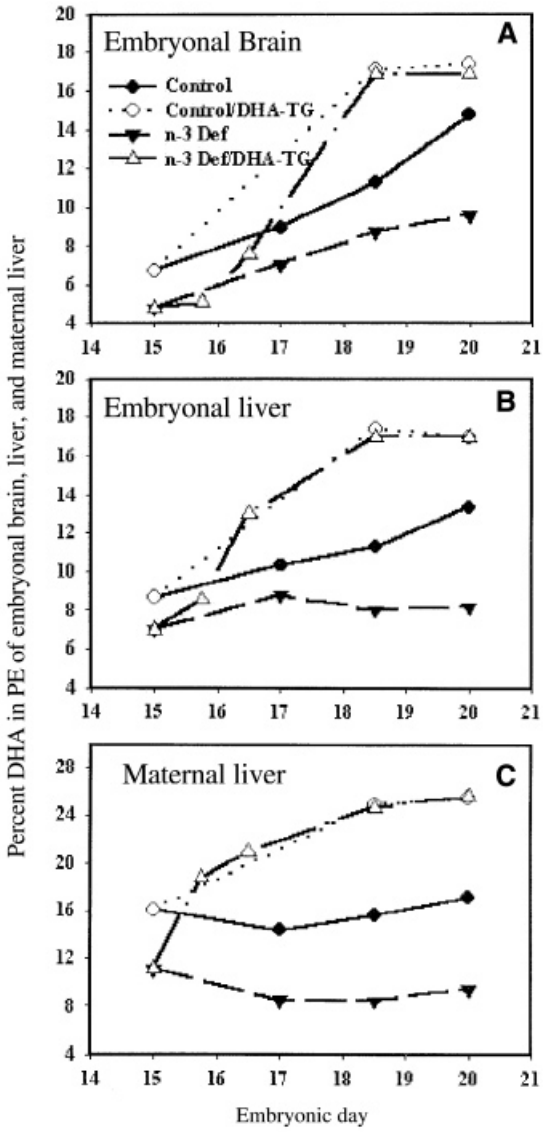

Fig. 1. Time course of DHA from ED15 to ED20 in fetal brain PE, fetal liver PE, and the maternal liver. Data at various time points are given as mean percent DHA ($3 < n > 12$) and spline functions of the fetal brain (A), fetal liver (B), and maternal liver (C). Dams received either a control diet, a diet n-3 FA deficient (n-3 Def), a control diet until ED15 followed by a DHA-TG diet until ED20 (Control/DHA-TG) or a n-3 deficient diet until ED15 followed by a DHA-TG diet until ED20 (n-3 Def/DHA-TG).

using a maternal diet deficient in n-3 FA and subsequently correcting the n-3 deficiency with direct dietary DHA supplements.

Over a period of two weeks, there were practically no changes in the relative percentage of PUFA in the n-3 deficient fetuses compared to the control fetuses. A detailed analysis of the PUFA composition in the various PLs however, revealed a significant decrease in DHA content, particularly in the PE and PS classes. The decrease in DHA (28.8−35.8%) was noticed in the n-3 deficient fetal brains after only two weeks. As indicated by the data, both PLs account for the bulk of DHA found in the fetal brain. Between ED15 and ED20, the decrease of DHA in PS and PE remained at similar levels (32.5% and 34.8%, respectively), suggesting that the dietary impact had reached a near plateau level. The fact that within two weeks a significant deficiency could be established in the fetal brain suggests a tight dependence of the fetus on maternal FA supplies.

The n-6/n-3 index in the fetal brain

In most studies using n-3 deficient diets, losses in DHA were always accompanied by an increase in the n-6 DPA content (14, 17, 27). The present study is in accordance with the notion of a compensatory mechanism involving the n-6 family, mostly DPA. As expected, the content of DHA in n-3 deficient animals was decreased whereas the content of DPA increased. DPA and DHA enrichment were inversely regulated, whereas AA showed no changes. Given the role of AA in many cellular regulatory mechanisms, maintaining a steady level may be important for the developing brain.

The consequences of DHA supplements

A major reason for initiating these studies was the notion that maternal diet manipulation is preferable from the point of view of non-invasiveness. The most sound observation stemming from this study, however, is that nearly normal DHA levels are established in n-3 deficient fetuses supplemented with DHA after a relatively short time period. The remarkable rise in DHA was already apparent after 48 h. After 5 days the supplements restored fetal brain DHA to the same level as the fetuses subject to the soybean (DHA control) diet (Table 5), and surpassed animals fed on regular commercial diets (data not shown). Thus, this study strongly indicates that an intrauterine n-3 deficiency may be manageable and the growing fetus can attain its PUFA levels for normal development.

A second important observation derived from this study is that, unlike intra-amniotic administration of Et-DHA, there were no changes in the PL composition resulting from the maternal diet. Although the source for DHA supplements is rather distinct, (Et-DHA vs. DHA-TG), it is possible that DHA derived via the maternal circulation is transported by a different route than that transported by the intra-amniotic injection and therefore each may act differently, possibly in non-lipid related metabolic functions.

Maternal essential PUFA status and its influence on fetal organs

A direct correlation between the maternal essential PUFA status and that of the brain of the fetus has been reported in many studies (8, 14, 17, 28). During pregnancy the maternal liver controls the flow of esterified DHA into the fetal maternal circulation (8). Furthermore, a concentration gradient of DHA between maternal and fetal organs during prenatal development has been shown (8). In the present study we show that the PUFA content in the maternal liver PL is extremely sensitive to dietary constraints during pregnancy so that DHA

and DPA are mutually interrelated. Taken together, a specific rapid adaptation of the maternal liver to the n-3 dietary transport in order to optimize for the requirements of PUFA of the fetus is highly indicated. Moreover, the dietary manipulation impacts PUFA composition in two major fetal organs (brain and liver) in a similar way, suggesting that organ specificity with respect to PUFA in the growing fetus is not yet defined. It also suggests that the liver may actively participate in the supply of PUFA for the brain.

Implications of DHA diets for the pathological fetus.

The accretion of DHA during fetal brain development has been widely studied. Nevertheless, there is still a big gap in our understanding the effects of n-3 PUFA on human brain development in utero. Elucidation of the precise role of DHA in the developing brain is of great significance in understanding pathological disorders in which chronic intrauterine stress leads to life-long physical and mental disabilities. Several major pediatric syndromes, including cerebral palsy (perinatal anoxic encephalopathies), mental retardation, epilepsy, and mild to moderate learning disabilities certainly have their etiologies, at least in part, in utero. A correlation between low levels of brain DHA and peroxisomal disorders with severe neurological symptoms (29), disturbed vision, changes in learning ability and altered behavior in n-3 deficiency (11–13, 19, 30, 31) have also been noted. Recent findings have established that intrauterine and early postnatal factors may influence adult neural development and cardiovascular function (32, 33) while supplements of LNA, an essential nutrient supplied in the early developmental period, can affect blood pressure later in life (34). The ability to intervene for limited periods in utero, by dietary means, the DHA content may pave the way for its administration whenever pathophysiological conditions indicate.

The technical assistance of N. Magid (MSc) and H. Guo-Yun is greatly appreciated. This work was supported in part by a grant from the Nationalbank, Austria (M.S.) and by the Gulton Foundation (E.Y.) New York. E.Y. is the incumbent of the Bee Wiggs Professorial Chair in Molecular Biology at the Weizmann Institute of Science.

Manuscript received 8 June 2001, in revised form 21 August 2001, and in re-revised form 28 September 2001.

REFERENCES

1. Crawford, M. A., A. G. Hassam, and G. Williams. 1976. Essential fatty acids and fetal brain growth. *Lancet.* **1:** 452–453.
2. Green, P., and E. Yavin. 1993. Elongation-desaturation of linolenic and linoleic acids in rat foetal brain in vivo. *J. Lipid Res.* **34:** 2099–2108.
3. Sinclair, A. J., and M. A. Crawford. 1972. The accumulation of arachidonate and docosahexaenoate in the developing brain. *J. Neurochem.* **19:** 1753–1758.
4. Frederiksen, K., and R. D. G. McKay. 1988. Proliferation and differentiation of rat neuroepithelial precursor cells in vivo. *J. Neurosci.* **8:** 1444–1151.
5. Bayer, S. A., K. V. Wills, L. C. Triarhou, and B. Ghetti. 1995. Time of neuron origin and gradients of neurogenesis in midbrain dopaminergic neurons in the mouse. *Exp. Brain Res.* **105:** 191–199.
6. Bayer, S. A., J. Altman, R. J. Russo, and X. Zhang. 1993. Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *Neurotoxicology.* **14:** 83–144.
7. Raedler, E., and A. Raedler. 1978. Autoradiographic study of early neurogenesis in rat neocortex. *Anat. Embryol. Berl.* **154:** 267–284.
8. Green, P., and E. Yavin. 1998. Mechanism of docosahexaenoic acid accretion in the fetal brain. *J. Neurosci. Res.* **52:** 129–136.
9. Green, P., S. Glozman, B. Kamensky, and E. Yavin. 1999. Developmental changes in rat brain membrane lipids and fatty acids: preferential accumulation of docosahexaenoic acid. *J. Lipid Res.* **40:** 960–966.
10. Cunnane, S. C., C. R. Menard, S. S. Likhodii, and M. A. Crawford. 1999. Carbon recycling into de novo lipogenesis is a major pathway in neonatal metabolism of linoleate and alpha-linolenate. *Prostaglandins Leukot. Essent. Fatty Acids.* **60:** 387–392.
11. Moriguchi, T., R. S. Greiner, and N. Salem, Jr. 2000. Behavioral deficits associated with dietary induction of decreased brain docosahexaenoic acid concentration. *J Neurochem.* **75:** 2563–2573.
12. Yamamoto, N., H. Hashimoto, Y. Takemoto, H. Okuyama, M. Nomura, R. Kitajima, T. Togashi, and Y. Tamai. 1988. Effect of dietary alpha-linolenate/linoleate balance on lipid compositions and learning ability of rats. II. Discrimination process, extinction process and glycolipid compositions. *J. Lipid. Res.* **29:** 1013–1021.
13. Enslen, M., H. Milon, and A. Malnoe. 1991. Effect of low intake of n-3 fatty acids during development on brain phospholipid fatty acid composition and exploratory behavior in rats. *Lipids.* **26:** 203–208.
14. Green, P., B. Kamensky, and E. Yavin. 1997. Replenishment of docosahexaenoic acid in n-3 fatty acid-deficient fetal rats by intraamniotic ethyl-docosahexaenoate administration. *J. Neurosci. Res.* **48:** 264–272.
15. Glozman, S., P. Green, and E. Yavin. 1998. Intraamniotic ethyl-docosahexaenoate administration protects fetal brain from ischemic stress. *J. Neurochem.* **70:** 2484–2491.
16. Favreliere, S., L. Barrier, G. Durand, S. Chalon, and C. Tallineau. 1998. Chronic dietary n-3 polyunsaturated fatty acids deficiency affects the fatty acid composition of plasmenylethanolamine and phosphatidylethanolamine differently in rat frontal cortex striatum and cerebellum. *Lipids.* **33:** 401–407.
17. Bourre, J. M., D. Pascal, G. Durand, M. Masson, O. Dumont, and M. Piciotti. 1984. Alternations in the fatty acid composition of rat brain cells (neurons, astrocytes and oligodendrocytes) and of subcellular fractions (myelin and synaptosomes) induced by a diet devoid of n-3 fatty acids. *J. Neurochem.* **43:** 342–348.
18. Bourre, J. M., O. Dumont, M. Piciotti, M. Clement, J. Chaudiere, M. Bonneil, G. Nalbone, H. Lafont, G. Pascal, and G. Durand. 1991. Essentiality of omega 3 fatty acids for brain structure and function. *World Rev. Nutr. Diet.* **66:** 103–117.
19. Salem, N., Jr., T. Moriguchi, R. S. Greiner, K. McBride, A. Ahmad, J. N. Catalan, and B. Slotnick. 2001. Alterations in brain function after loss of docosahexaenoate due to dietary restriction of n-3 fatty acids. *J. Mol. Neurosci.* **16:** 299–307.
20. Weisinger, H. S., A. J. Vingrys, and A. J. Sinclair. 1995. Dietary manipulation of long-chain polyunsaturated fatty acids in the retina and brain of guinea pigs. *Lipids.* **30:** 471–473.
21. Hajra, A., and N. S. Radin. 1978. Lipid extraction of tissue with low-toxicity solvent. *Anal. Biochem.* **90:** 420–426.
22. Yavin, E., and A. Zutra. 1977. Separation and analysis of 32P-labeled phospholipids by a simple and rapid two-directional thin layer chromatography procedure and its application to cultured neuroblastoma cells. *Anal. Biochem.* **54:** 1809–1811.
23. Green, P., and E. Yavin. 1995. Modulation of fetal rat brain and liver phospholipid content by intraamniotic ethyl-docosahexaenoate administration. *J. Neurochem.* **65:** 2555–2560.
24. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
25. Bazan, N. G., and B. L. Scott. 1990. Dietary omega-3 fatty acids and accumulation of docosahexaenoic acid in rod photoreceptor cells of the retina and at synapses. *Ups. J. Med. Sci. Suppl.* **48:** 97–107.

26. Scott, B. L., and N. G. Bazan. 1989. Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. *Proc. Natl. Acad. Sci. USA.* **86:** 2903–2907.
27. Galli, C., H. I. Trezeciak, and R. Paoletti. 1971. Effects of dietary fatty acids on the fatty acid composition of brain ethanolamine phosphoglyceride: reciprocal replacement of n-6 and n-3 polyunsaturated fatty acids. *Biochem. Biophys. Acta.* **248:** 449–454.
28. Neuringer, M., W. E. Connor, D. S. Lin, L. Barstad, and S. Luck. 1986. Biochemical and functional effects of prenatal and postnatal omega 3 fatty acid deficiency on retina and brain in rhesus monkeys. *Proc. Natl. Acad. Sci USA.* **83:** 4021–4025.
29. Martinez, M. 1992. Abnormal profiles of polyunsaturated fatty acids in the brain, liver, kidney and retina of patients with peroxisomal disorders. *Brain. Res.* **583:** 171–182.
30. Connor, W. E., M. Neuringer, and S. Reisbick. 1991. Essential fatty acids: the importance of (n-3) fatty acids in the retina and brain. *Nutr. Rev.* **50:** 21–29.
31. Wainwright, P. E. 1992. Do essential fatty acids play a role in brain and behavioural development? *Neurosci. Biobehav. Rev.* **16:** 193–205.
32. Meaney, M. J., J. Diorio, D. Francis, J. Widdowson, P. LaPlante, C. Caldji, S. Sharma, J. R. Seckl, and P. M. Plotsky. 1996. Early environmental regulation of forebrain glucocorticoid receptor gene expression: implications for adrenocortical responses to stress. *Dev. Neurosci.* **18:** 49–72.
33. Langley-Evans, S. C., A. G. Clamp, R. F. Grimble, and A. A. Jackson. 1996. Influence of dietary fats upon systolic blood pressure in the rat. *Int. J. Food Sci. Nutr.* **47:** 417–425.
34. Weisinger, H. S., J. A. Armitage, A. J. Sinclair, A. J. Vingrys, P. L. Burns, and R. S. Weisinger. 2001. Perinatal omega-3 fatty acid deficiency affects blood pressure later in life. *Nature Med.* **7:** 258–259.