Maternal Dietary (n-3) Fatty Acid Deficiency Alters Neurogenesis in the Embryonic Rat Brain[1,2]

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ABSTRACT Docosahexaenoic acid [22:6(n-3)] is enriched in brain membrane phospholipids and essential for brain function. Neurogenesis during embryonic and fetal development requires synthesis of large amounts of membrane phospholipid. We determined whether dietary (n-3) fatty acid deficiency during gestation alters neurogenesis in the embryonic rat brain. Female rats were fed diets with 1.3% energy [(n-3) control] or 0.02% energy [(n-3) deficient], from α-linolenic acid [18:3(n-3)], beginning 2 wk before gestation. Morphometric analyses were performed on embryonic day 19 to measure the mean thickness of the neuroepithelial proliferative zones corresponding to the cerebral cortex (ventricular and subventricular zones) and dentate gyrus (primary dentate neuroepithelium), and the thickness of the cortical plate and sectional area of the dentate gyrus. Phospholipids and fatty acids were determined by HPLC and GLC. Docosahexaenoic acid was 55–65% lower and (n-6) docosapentaenoic acid [22:5(n-6)] was 150–225% higher in brain phospholipids at embryonic day 19 in the (n-3) deficient ($n = 6$ litters) than in the control ($n = 5$ litters) group. The mean thickness of the cortical plate and mean sectional area of the primordial dentate gyrus were 26 and 48% lower, respectively, and the mean thicknesses of the cortical ventricular zone and the primary dentate neuroepithelium were 110 and 70% higher, respectively, in the (n-3) deficient than in the control embryonic day 19 embryos. These studies demonstrate that (n-3) fatty acid deficiency alters neurogenesis in the embryonic rat brain, which could be explained by delay or inhibition of normal development. J. Nutr. 136: 1570–1575, 2006.

KEY WORDS: • *(n-3) fatty acids* • *docosahexaenoic acid* • *neurogenesis* • *cerebral cortex* • *dentate gyrus*

Dietary deficiency or imbalance of key nutrients at critical stages of development can alter normal brain development, and have long-lasting and potentially irreversible effects on neural function, regardless of later restitution of an adequate diet (1,2). High amounts of the (n-3) fatty acid docosahexaenoic acid [DHA,[4] 22:6(n-3)] are accumulated in brain membrane phospholipids, particularly phosphatidylethanolamine (PE) and phosphatidylserine (PS), during fetal and neonatal development, paralleling membrane expansion in neurogenesis, dendritic aborization, and synaptogenesis (3,4). Since mammalian cells do not have the enzymes to form (n-3) fatty acids, all of the DHA in the brain must be derived from (n-3) fatty acids in the diet. Prior to birth, all of the DHA accumulated in the fetal brain must originate from (n-3) fatty acids in the maternal diet via placental transfer. DHA may be transferred preformed or may be synthesized in the fetal compartment from α-linolenic acid [ALA, 18:3(n-3)] derived from the mother. Many studies have shown that (n-3) fatty acid deprivation during development results in decreased DHA in brain membrane phospholipids, reduced performance in learning tasks, altered activity of membrane receptors and proteins, and altered metabolism of several neurotransmitters, including dopamine (3,5–8). Low DHA status is also associated with poorer development of visual acuity and lower indices of neural development in human infants (6,9–11). Of relevance to the developing brain, a doubling of membrane phospholipids is required in the S phase of mitosis for the creation of daughter cells (12,13), and decreased phospholipid turnover and synthesis has been reported in (n-3) fatty acid deficient animals (14–16). However, few studies have used morphometric and stereological approaches to assess potential effects of (n-3) fatty acid deficiency on neural cell growth in the developing brain. Relevant studies suggest that (n-3) fatty acid deficiency decreases the mean cell body size of neurons in the hippocampus, hypothalamus, and parietal cortex (17,18), decreases the complexity of dendritic arborizations on cortical neurons (19), and, in culture, DHA enhances neurite outgrowth of hippocampal neurons (20).

DHA is formed by desaturation and elongation of the dietary essential fatty acid ALA and is also present in the diet, but only in animal tissue lipids (3,6). Although ALA is relatively abundant in some oils, such as canola, soybean, flax seed, and walnut oils, many fats and oils including corn, safflower, sunflower, olive, coconut, palm, peanut, and hydrogenated oils

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[4] Abbreviations used: ALA, α-linolenic acid; BrdU, bromodeoxyuridine; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; E19, embryonic day 19, PC, phosphatidylcholine; PE phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine.

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and milk fats contain <1 g ALA/100 g of fatty acids (21). Furthermore, recent studies have suggested that pregnant women following Westernized diets consume less than the recommended intakes of (n-3) fatty acids (22,23), and that higher maternal DHA intakes during pregnancy and lactation increase measures of cognitive performance in infants (24–26).

Given the importance of DHA in brain gray matter phospholipids and the need for synthesis of new membrane components during neurogenesis, we sought to determine the effect of (n-3) fatty acid deficiency on neurogenesis in the embryonic rat brain. Because such studies are impossible in humans, we used a model of dietary (n-3) fatty acid deprivation in rats that we had previously found to reduce embryonic brain DHA accretion (27). We used 5-bromo-2′-deoxyuridine (BrdU), an analog of thymidine that crosses the blood barrier and enters the nucleotide pool to label mitotically active cells in the different zones of the cortex and hippocampus. Morphometric analyses were performed on embryonic day 19 (E19) to quantify the mean thickness of individual laminae within the developing telencephalic wall that corresponds to the cerebral cortex and dentate gyrus.

MATERIALS AND METHODS

Animals and diets. Long-Evans rats, 80–90 d of age (Charles River Laboratories) were housed in a temperature-controlled animal facility with a 12 h light-dark cycle (light, from 0700–1900), with free access to food and water. The rats were fed 1 of 2 semipurified diets that contained 16% energy from fat and differed only in the composition of the component fatty acids. One diet contained 1.2% energy from ALA from canola oil (control, $n = 6$) and the other diet contained 0.03% energy ALA from safflower oil ((n-3) deficient, $n = 6$). The diets also differed in (n-6) linoleic acid [18:2(n-6)] and oleic acid (18:1(n-9)) with 11.8 and 2.8% energy from 18:2(n-6) and 18:1(n-9) in the (n-3) deficient diet, and 3.1% energy from 18:2(n-6) and 2.4% energy from 18:1(n-9) in the control diet. This design, therefore, includes a high dietary (n-6) fatty acid intake and a high (n-6)/(n-3) ratio. The nutrient composition of the diets has been reported in detail (27). The diets were fed from 2 wk prior to mating, and then throughout gestation. Pregnancy was determined from the presence of a vaginal plug, the male was removed from the cage, and that day designated as E0 of gestation. Studies to assess the effects of in utero (n-3) fatty acid deprivation were conducted at E19 of gestation, at which time cortical neurogenesis is nearing completion in rats (28). All procedures involving animals were approved and carried out in accordance with the Animal Care Committee of the University of British Columbia and conformed to the guidelines of the Canadian Council on Animal Care guidelines.

Tissue preparation. On E18, dams ($n = 6$/diet) were given BrdU (50μg/g body weight in 0.1 mol/L PBS) by intraperitoneal injection, every 2 h for 6 h, beginning at 0900. Then, 24 h after the last injection of BrdU, the dams were anesthetized with 4% isoflurane, and the embryos were removed by hysterectomy and decapitated. The brains were dissected free of surrounding tissue, and then the brainstem and cerebellum were removed. Embryonic brain, pooled for 2 embryos/litter, was immediately frozen in liquid nitrogen, and then stored at −70°C for later lipid analysis. One embryonic brain/litter was taken from the center position of the uterine horn and fixed by immersion in 70% ethanol for immunohistochemical studies.

Lipid analyses. Total lipids were extracted, and individual phospholipids including PS, PE, phosphatidylcholine (PC), and phosphatidylinositol (PI) were separated by HPLC (29). Following HPLC separation, the column effluent was split to an evaporative light scattering detector for quantification of individual phospholipids and to a fraction collector for recovery (29). Phospholipid fatty acids were analyzed as their respective methyl esters by gas liquid chromatography (27,30). Protein was quantified by the method of Bradford (31).

Morphometric studies. For immunohistochemical studies, embryonic brains were embedded in paraffin and sectioned at 6 μm in the coronal plane. Individual sections were mounted on Colorfrost Plus glass slides (Fisher Scientific) and air dried. Consecutive sections were sampled through the cerebral hemispheres at the level of the anterior thalamus and 10 sections were labeled with BrdU. Briefly, sections were deparaffinized, rehydrated in descending grades of ethanol, and incubated in 2 mol/L HCl for 1 h. Sections were incubated with mouse monoclonal anti-BrdU antibody (1:75, BD Biosciences) for 1 h at room temperature and processed using a Vectastain Elite Kit for mouse IgG (Vector Laboratories). Slides were reacted for 3 min with 0.05% diaminobenzidine, 0.025% cobalt chloride, 0.02% nickel ammonium sulfate, and 0.01% hydrogen peroxide, then counterstained with 0.1% aqueous basic fuchsin. To delineate the boundary between the ventricular and subventricular zones of the proliferative neuroepithelium, sections were stained for the detection of Tbr2, a member of the T-box family of transcription factors that identifies intermediate progenitor cells in the subventricular zone (32). Briefly, sections were boiled in antigen unmasking solution (Vector Laboratories) for 10 min in a microwave oven, incubated with a rabbit polyclonal anti-Tbr2 antibody (1:800, provided by Dr. Robert Hevner, Seattle, WA) overnight at 4°C, processed using a Vectastain Elite Kit for rabbit IgG (Vector Laboratories), and then reacted and counterstained as described above. Slides incubated without the addition of primary antibodies served as negative controls.

Morphometric analyses were performed to measure the thickness of individual laminae within the embryonic telencephalic wall in regions corresponding to the primordial cerebral cortex and dentate gyrus. Histological sections were examined with an Olympus BH-2 compound microscope (40 × planapochromatic objective) interfaced to a Bioquant TCW98 image analysis system (R&M Biometrics) and visualized on a monitor at a magnification of 750×. For the primordial cerebral cortex, the lengths of the ventricular surface of the lateral ventricle and the pial surface were measured in μm. The areas of the ventricular zone, subventricular zone, intermediate zone, subplate layer, cortical plate, and marginal zone were measured bilaterally in μm^2. The mean laminar thickness was calculated by dividing the lamina area by the length of the ventricular surface (ventricular, subventricular, and intermediate zones) or the pial surface (subplate layer, cortical plate, and marginal zone). For the dentate gyrus, the length of the ventricular surface and the area of the primary dentate neuroepithelial layer were measured then used to calculate the mean thickness of the primary dentate epithelium. The surface areas of both the dentate gyrus and the hilus of the hippocampus were measured in μm^2.

Statistical analyses. Each litter was considered the experimental unit. Morphometric analysis and lipid analysis were completed on 6 litters from the (n-3) deficient group and 5 litters from the control group. Results are presented as means ± SEM. Significant differences between the control and (n-3) deficient groups were assessed using Student's t test. Differences resulting in a P-value < 0.05 were considered significant.

RESULTS

Embryonic brain lipids and fatty acids. Feeding the diets containing 1.2% (control) or 0.03% [(n-3) deficient] ALA did not affect maternal body weight gain. Control and deficient dams weighed 241 ± 29 g and 260 ± 19 g on E0, and 372 ± 66 g and 352 ± 36 g on E19, respectively. Weight gains were 130.9 ± 29.2 g in the controls and 94.4 ± 19.4 g, in the control (n-3) deficient group. Embryonic brain weights did not differ between the groups (0.12 ± 0.01 g in both) nor did the embryonic brain phospholipid concentrations differ (data not shown). However, the concentration of DHA was 55–65% lower in the embryonic brain PE, PS, PC, and PI in the (n-3) fatty acid deficient than in the control group (**Table 1**). This marked reduction in DHA was accompanied by significantly higher levels of 20:4(n-6) (arachidonic acid), 22:4(n-6), and, particularly, docosapentaenoic acid (DPA, 22:5(n-6)) levels in PC, PE, and PS in the fatty acid deficient than the control embryonic brain ($P < 0.001$). In PI, the increase in (n-6) fatty acids in response to (n-3) fatty

TABLE 1

Major (n-6) and (n-3) fatty acids in the brain of rat embryos at E19 fed the control or the (n-3) fatty acid deficiency diet[1]

	PE		PS		PC		PI	
Fatty acid	Control	(n-3) Deficient	Control	(n-3) Deficient	Control	(n-3) Deficient	Control	(n-3) Deficient
18:2(n-6)	0.33 ± 0.03	0.45 ± −0.03*	0.34 ± 0.06	0.37 ± 0.03	0.66 ± 0.05	0.92 ± 0.03**	0.28 ± 0.06	0.33 ± 0.04
20:4(n-6)	20.0 ± 0.47	22.4 ± 0.34*	8.42 ± 0.16	9.38 ± 0.22**	6.86 ± 0.16	8.20 ± 0.16***	45.8 ± 0.30	46.9 ± 0.50
22:4(n-6)	5.11 ± 0.28	7.22 ± 0.38**	8.65 ± 0.32	9.96 ± 0.31*	0.90 ± 0.05	1.12 ± 0.06*	1.41 ± 0.10	1.56 ± 0.11
22:5(n-6)	5.06 ± 0.17	15.57 ± 0.76*	6.54 ± 0.25	17.8 ± 0.90***	0.64 ± 0.02	2.07 ± 0.13***	0.70 ± 0.03	1.76 ± 0.15***
20:5(n-3)	0.10 ± 0.02	0.01 ± 0.01***	0.02 ± 0.01	<0.01	0.04 ± 0.01	<0.01*	0.08 ± 0.02	<0.01*
22:5(n-3)	0.49 ± 0.09	0.01 ± 0.00**	0.69 ± 0.12	0.03 ± 0.01**	0.07 ± 0.02	<0.01*	0.07 ± 0.03	<0.01
22:6(n-3)	21.7 ± 1.08	9.37 ± 1.07***	23.7 ± 0.88	10.4 ± 1.07***	2.78 ± 0.05	1.25 ± 0.09***	3.14 ± 0.06	1.14 ± 0.07***
Σ (n-6)	31.8 ± 0.81	46.0 ± 1.42***	24.6 ± 0.55	38.3 ± 1.21***	9.4 ± 0.28	12.8 ± 0.34***	48.8 ± 0.18	51.5 ± 0.21***
Σ (n-3)	22.3 ± 1.00	9.4 ± 1.08***	24.4 ± 0.80	10.4 ± 1.08***	2.89 ± 0.07	1.2 ± 0.09***	3.29 ± 0.08	1.15 ± 0.07***
Σ (n-6) + (n+3)	54.1 ± 0.46	55.4 ± 0.46	49.0 ± 0.86	48.7 ± 0.49	12.3 ± 0.33	14.0 ± 0.29**	52.1 ± 0.20	52.6 ± 0.22

[1] Values are means ± SEM, $n = 5$ (control), or $n = 6$ (deficient). Symbols indicate different from control group, $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

acid deficiency was compensated for by a significant increase in DPA, but not in 20:4(n-6) or 22:4(n-6) (Table 1). Over 70% of the reciprocal replacement of (n-6) for (n-3) fatty acids in the (n-3) fatty acid deficient brain compared with control embryonic brain was explained by an increase in DPA. Regardless of the (n-3) and (n-6) fatty acid content of the diet fed and the marked differences in individual (n-6) and (n-3) fatty acids in the E19 brain, the total (n-6) plus (n-3) fatty acids in PE, PS, and PI did not differ between the groups (Table 1). However, PC, which typically contains much lower (n-6) and (n-3) fatty acids than PE, PS, or PI, did not show this tight regulation of total (n-6) and (n-3) fatty acids; 18:2(n-6), 20:4(n-6), 22:4 (n-6), 22:5(n-6) and the total (n-6) fatty acids were all significantly higher in the brain PC of the E19 deficient group than in the control group.

Histological and morphometric analyses. The cerebral hemispheres of the (n-3) fatty acid deficient embryos were noticeably reduced compared with those of the control group, due largely to a decrease in the size of the cortical plate, primordial hippocampus, and dentate gyrus (**Fig. 1**). The thickness of the cortical plate (**Fig. 2** *A, B*) and the packing density of cells within the plate was decreased, and the thickness of the ventricular zone adjacent to the lateral ventricles was increased (Fig. 2 *C, D*), in the primordial cerebral cortex of the E19 (n-3) fatty acid deficient group compared with the control group. Similarly, we found a decrease in the size of the primordial dentate gyrus (**Fig. 3** *A, B*) and an increase in the thickness of the primary dentate

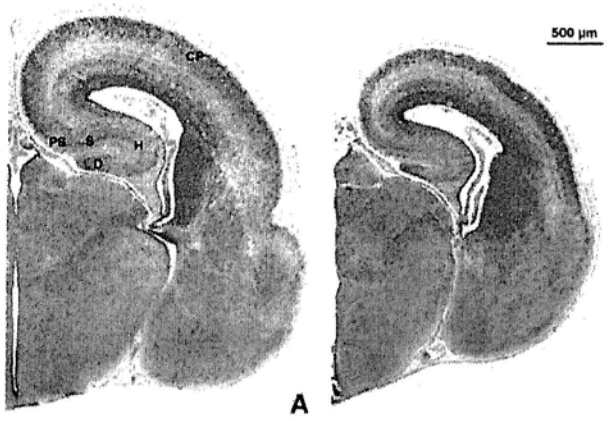

FIGURE 1 Developing telencephalic walls in brains of control (*A*) and (n-3) fatty acid (*B*) deficient rat embryos at E19. The photomicrographs show the overall decrease in the size of the brain in the (n-3) fatty acid deficient embryo, due largely to decreases in the size of the cortical plate (CP, primordial cerebral cortex), hippocampus (H), dentate gyrus (D), subiculum (S) and presubiculum (PS). Immunohistochemistry was for BrdU with a basic fuchsin counterstain. Calibration bar = 500 μm.

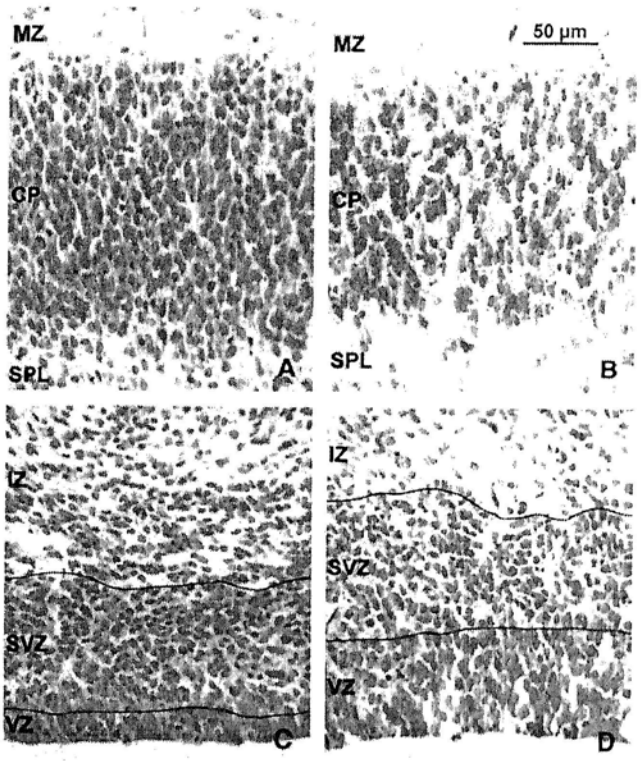

FIGURE 2 The marginal zone (MZ), cortical plate (CP), and subplate layer (SPL) (*A,B*) and proliferative neuroepithelium (*C,D*) of control (*A,C*) and (n-3) fatty acid deficient (*B,D*) rat embryos at E19. The photomicrographs show the reduced thickness of the cortical plate and decreased packing density of cells (*B* vs. *A*), and the higher thickness of the ventricular zone (VZ), with no difference in the thickness of the subventricular zone (SVZ), ventral to the intermediate zone (IZ) (*D vs. C*) in the (n-3) deficient embryonic brain. Sections stained with thionine for Nissl substance. The calibration bar of 50 μm applies to *A–D*.

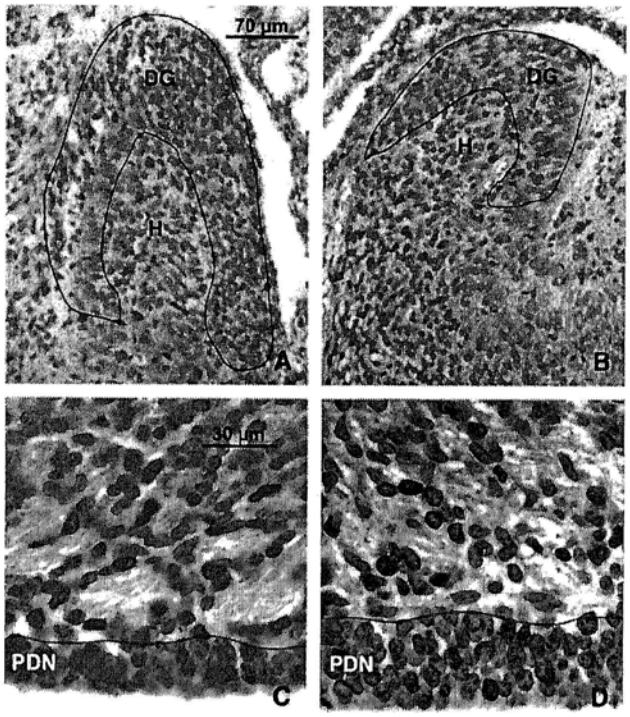

FIGURE 3 The primordial dentate gyrus (DG) and hilus (H) (*A,B*) and primary dentate neuroepithelium (PDN) (*C,D*) of control (*A,C*) and (n-3) fatty acid deficient (*B,C*) rat embryos at E19. The photomicrographs show the reduced area of the dentate gyrus and hilus in the (n-3) fatty acid deficient embryos (*B vs. A*) and the higher thickness of the primary dentate neuroepithelium (*C vs. D*). Immunohistochemistry for BrdU with a basic fuchsin counterstain. The calibration bar of 70 μm applies to *A* and *B*, and the calibration bar of 30 μm applies to *C* and *D*.

neuroepithelium adjacent to the lateral ventricles (Fig. 3 C, D) in the deficient group.

In the primordial cerebral cortex, the mean thickness of the cortical plate was 25% lower in the (n-3) fatty acid deficient embryos than in controls ($P = 0.017$), with no differences in the thickness of the marginal zone or subplate layer (Table 2). In the proliferative neuroepithelium, the thickness of the ventricular zone was 110% higher in the (n-3) fatty acid deficient group than in the control group ($P < 0.001$), with no differences in the thickness of the subventricular or intermediate zones. Because the boundary between the ventricular and subventricular zones is subtle on the histological sections stained with basic fuchsin, we repeated the measurements of thickness on sections stained with antibodies against Tbr2 (which selectively stain intermediate progenitor cells located predominantly in the subventricular zone) that had a sparse distribution in the intermediate and ventricular zones of the E19 embryo (Fig. 4). In this second morphometric analysis, the thickness of the ventricular zone was 27.5 ± 1.4 and 44.2 ± 2.8 μm in the control and (n-3) fatty acid deficient embryos, respectively, representing a 60% increase ($P < 0.001$). Again, the thickness of the subventricular zone did not differ between the groups: 75.7 ± 7.2 and 83.6 ± 7.2 μm in the control and (n-3) fatty acid deficient groups, respectively.

Similar to the findings for the ventricular zone, the thickness of the primary dentate neuroepithelium was 70% higher ($P = 0.003$) in the deficient compared with the control group (Table 2). In addition, the surface area of both the primordial dentate gyrus ($P = 0.002$) and the combined areas of the dentate gyrus and hilus of the hippocampus ($P = 0.023$) was 50% lower in the (n-3) fatty acid deficient compared with control embryos.

TABLE 2

Morphometric variables in the primordial cerebral cortex and dentate gyrus for (n-3) fatty acid deficient and control rat embryos at E19[1]

	Control	(n-3) Deficient
Primordial cerebral cortex		
Marginal zone, μm	52.3 ± 6.4	37.8 ± 2.9
Cortical plate, μm	192.1 ± 15.8	142.9 ± 8.1*
Subplate layer, μm	69.6 ± 9.4	64.7 ± 11.0
Intermediate zone, μm	232.6 ± 40.3	266.0 ± 22.9
Subventricular zone, μm	104.6 ± 3.0	109.9 ± 6.7
Ventricular zone, μm	26.3 ± 2.3	55.2 ± 5.1***
Primordial dentate gyrus		
Dentate gyrus,[2] μm^2	60,582 ± 4,038	31,236 ± 4,941**
Combined dentate gyrus and hilus,[2] μm^2	110,500 ± 14,938	54,712 ± 13,757*
Primary dentate neuroepithelium, μm	20.9 ± 2.2	35.4 ± 2.7**

[1] Values are means ± SEM, $n = 5$ (control), or $n = 6$ (deficient). Symbols indicate different from control group, $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

[2] Values represent the mean bilateral surface area for all histological sections in the sample.

DISCUSSION

In these studies, we tested whether dietary (n-3) fatty acid restriction, which reduces DHA and increases (n-6) fatty acids in the developing embryonic brain, alters neurogenesis. We found that restriction of dietary (n-3) fatty acids in dams fed an otherwise complete diet alters the morphology of telencephalic structures in the embryonic rat brain. Neurogenesis was studied on E19 when neurogenesis in the cerebral cortex of rats was nearing completion (28). The diets provided either 1.2 or 0.03% energy from (n-3) fatty acids as ALA, with 11.8 and 3.1% energy from linoleic acid (18:2(n-6)) in the control and deficient diets, respectively, and thus also differed in the amounts of (n-6) fatty acids and (n-6)/(n-3) fatty acid ratio. The percent energy from 18:2(n-6) used in our studies is within the range of the 18:2(n-6) in human diets. The diets were fed from 2 wk prior to gestation; yet this relatively short duration of

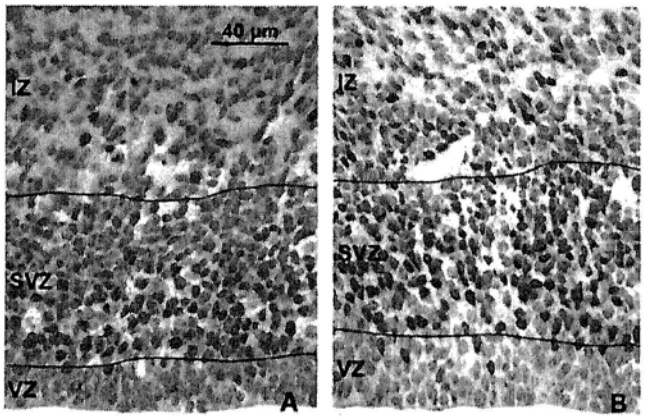

FIGURE 4 The ventricular zone (VZ), subventricular zone (SVZ) and intermediate zone (IZ) in control (*A*) and (n-3) fatty acid deficient embryos at E19 (*B*), as defined by immunoreactivity to Tbr2. Note the increased thickness of the ventricular zone in *B*, designated by the relative lack of Tbr2 immunoreactivity. Calibration bar = 40 μm.

maternal dietary deficiency resulted in a 50% decrease of DHA in the E19 embryonic brain. Consistent with previous studies (6,27,33–35), (n-3) fatty acid deficiency was accompanied by an increase in carbon chain 20 and 22 (n-6) fatty acids, particularly 22:5(n-6) in the developing brain. The changes in the embryonic brain fatty acids were accompanied by a 25% decrease in the thickness of the cortical plate and a 50% decrease in the area of the dentate gyrus and associated hilus of the hippocampal formation at E19. Similarly, the thickness of the ventricular zone (which contains progenitor cells giving rise to both intermediate progenitor cells and postmitotic neurons and glia destined for the cerebral cortex) was increased 110% and the primary dentate neuroepithelium (which contains progenitor cells giving rise to proliferative cells of the secondary dentate matrix and postmitotic neurons) was increased 70%. A similar pattern of increased thickness of proliferative zones and decreased size of target regions was observed in the hippocampus of the (n-3) fatty acid deficient compared with the control embryos. We interpret these morphological changes as consistent with the inhibition or delay of neurogenesis in the (n-3) fatty acid embryos commencing at earlier stages of development.

Neuronal proliferation in the neocortex normally extends from E14 to E20 in rats and is estimated to occur predominantly from 6 to 16 wk of gestation in humans (28). During normal development in rats, the duration of the mitotic cell cycle increases by almost 2-fold in the ventricular zone, due largely to an increase in the length of the G1-phase, whereas the length of the cell cycle in the subventricular zone and intermediate zones remains relatively constant (36–39). At the same time, the proportion of proliferative (i.e., mitotic) cells in the ventricular zone gradually decreases and the proportion of quiescent cells (i.e., cells exiting division) increases. Thus, the increased thickness of the ventricular zone and the primary dentate neuroepithelium in the E19 (n-3) fatty acid deficient embryos in our study is consistent with a delay or inhibition of neurogenesis, which could be explained by an increase in the length of the mitotic cycle or a later onset of neurogenesis in the cerebral cortex and dentate gyrus. We found no significant changes in the thickness of the subventricular and intermediate zones and no apparent difference in the density of cells within these laminae, due to (n-3) fatty acid deficiency. We used a second approach, employing staining with antibodies against Tbr2 to identify the boundaries between the ventricular and subventricular zone and repeated the measures of thickness in the different zones to verify the results of the morphometric assessments with basic fuchsin staining. Thus, using 2 approaches, we provide results that suggest a differential susceptibility of proliferative cells in the ventricular zone, as opposed to the subventricular and intermediate zones, to (n-3) fatty acid deficiency.

Neuron proliferation is estimated to occur from E15 to E20 in the rat hippocampus (40,41) and between 7 and 15 wk of gestation in the human hippocampus (28,42), thus being essentially complete before birth in both humans and rats. Neuron proliferation in the dentate gyrus, however, occurs in 2 stages and over a considerably longer period of development. Granule cells of the dentate gyrus originate mainly from postnatal day 20 to 30 in rats (40,41) and from 19 wk of gestation and continuing well into postnatal development in humans (28,42). Briefly, postmitotic cells from the proliferative primary dentate neuroepithelium migrate to form the secondary dentate matrix in the primordial hilus; proliferating cells in the secondary dentate matrix continue to undergo mitosis with the postmitotic neurons migrating into the granule cell layer of the dentate gyrus. Our results, showing an increased thickness of the primary dentate neuroepithelium and decreased size of the dentate gyrus and hilus in the (n-3) fatty acid deficient embryos, further illustrate the dependence of neurogenesis in telencephalic structures on an adequate supply of DHA.

Although our studies demonstrate that (n-3) fatty acids, and specifically the supply of DHA available to the developing brain, is important for neurogenesis, the cellular basis for our findings is unknown. It is also possible that the mechanisms involve both the decrease in DHA and disruption of normal (n-6) fatty acid metabolism. Several mechanisms can be suggested. Depletion of DHA from neural membranes is known to alter the activity of membrane-associated transporters and receptors, including G-protein coupled receptors, ion channel activities, to reduce phospholipid turnover and PS synthesis and to alter the metabolism of neurotransmitters such as dopamine, serotonin, and their receptors (3,5–8,14–16). Changes in dopamine metabolism have been reported in several studies with (n-3) fatty acid deficient animals and brain cortex dopamine is increased in E19 (n-3) fatty acid deficient rat embryos (7,8,27). Of relevance, dopamine has been shown to modulate cell cycle kinetics in the embryonic lateral ganglionic eminence, such that dopamine D1 receptor activation reduces the entry of progenitor cells from the G1- to S-phase of the cell cycle, while D2 receptor activation promotes G1- to S-phase entry (43). In addition, (n-3) fatty acids regulate the expression of multiple genes, which, in the brain, the hippocampus, and retinal explants, include genes involved in the control of synaptic plasticity and cytoskeleton and membrane assembly, as well as signal transduction and ion channel formation (44–47). Disruption of normal phospholipid synthesis and turnover, including PS, secondary to altered availability of (n-6) and (n-3) fatty acids, could also influence normal neurogenesis both through influencing the synthesis of new membrane components and through altered release of (n-6) and (n-3) fatty acid signal molecules. Of relevance, studies with rat retina photoreceptor cells have demonstrated that in vitro DHA enhances photoreceptor survival, possibly involving antiapoptotic effects (48).

In conclusion, we have demonstrated that neurogenesis in the embryonic brain is altered by (n-3) fatty acid deficiency. Deficiency at key stages of brain development can have lasting effects on neural function, regardless of later restitution of an adequate diet (1,2). Further studies are needed to address the mechanism, potential for recovery, and sensitive periods during development when (n-3) fatty acid restriction can impact normal neurogenesis. In this regard, recent studies have provided evidence that maternal intakes of DHA during pregnancy are associated with higher scores on tests of cognition in infants and preschool children (24–26), and a relation between in utero DHA deprivation and several neurologic birth defects has been proposed (49). Furthermore, we have demonstrated altered neurogenesis in the dentate gyrus of the hippocampus, which is 1 of 2 regions that continues to produce new neurons throughout adult life (50,51). The rate of neurogenesis has been linked to aging-related cognitive decline in hippocampal-dependent learning tasks, such as spatial memory tasks (50–53). In addition, DHA has also been linked to aging-related cognitive decline (54–56). We suggest a need for future studies to address the effects of dietary (n-3) fatty acids on normal neurogenesis, regardless of life stage.

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