Docosapentaenoic Acid Does Not Completely Replace DHA in n-3 FA-Deficient Rats During Early Development

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ABSTRACT: The reciprocal replacement of DHA by docosapentaenoic acid (DPAn-6) was studied in rats that consumed an n-3 FA-deficient or n-3 FA-adequate diet. Dams were fed the two experimental diets from weaning and throughout pregnancy and lactation. Their pups were then fed the respective diets after weaning. Cortex FA analysis was performed at various times (0, 5, 10, 20, 50, and 91 d) after birth to determine whether DPAn-6 completely replaced DHA in the n-3-deficient group. Cortical DHA levels were significantly lower (average 86%) in the n-3-deficient rats. DPAn-6 increased significantly in the n-3-deficient rats starting with a 6.5-fold increase at day 0 up to a 54-fold increase at day 91 compared with the n-3-adequate group. However, this significant increase did not completely replace the loss of DHA at postnatal days 5, 10, and 20 in which there was still an 11.5, 10.3, and 8.0% deficit in the sum of DHA and DPAn-6, respectively, in the n-3-deficient group. Once docosatetraenoic (DTA) and arachidonic acids (AA) were included in the sum (DHA + DPAn-6 + DTA + AA), the levels between the two groups were similar. These results suggest that not only DPAn-6 but also other n-6 FA, including DTA and AA, replace DHA in n-3-deficient rats. The lack of total 22-carbon (22C) FA in the brain during the rapid membrane biogenesis that occurs during early development could be a factor in the nervous system functional deficits associated with n-3 FA deficiency.

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DHA (22:6n-3), a long-chain PUFA (LC-PUFA) of the n-3 family, is highly concentrated and retained in the central nervous system when α-linolenic acid (LNA; 18:3n-3) and/or DHA is present in the diet (1,2). A severe depletion of DHA in the brain, induced by an n-3 FA-deficient (n-3 Def) diet, is associated with abnormal visual development and learning-related deficits in rodents (3–10).

When rats consume an n-3 Def diet for several generations, tissue DHA levels decrease and docosapentaenoic acid (DPAn-6; 22:5n-6) levels increase (11,12) due to the competition between the n-3 and n-6 families for elongation and desaturation enzymes and the change in the available substrates (13–16). High levels of brain DPAn-6, a member of the n-6 FA family, are present only in the DHA-deficient tissue, presumably to maintain an optimal amount of these highly unsaturated FA in the brain. Other polyunsaturated n-6 FA such as docosatetraenoic acid (DTA; 22:4n-6) and arachidonic acid (AA; 20:4n-6) also increase in response to an n-3 FA deficiency. Many studies describe a complete reciprocal replacement of DHA with DPA-n-6 (11,12,17) such that there are no significant differences between an n-3 Def brain and an n-3 FA-adequate (n-3 Adq) brain in terms of total DHA plus DPAn-6. It is thus intriguing that deficits in central nervous system functions are detectable in n-3 Def animals because DPAn-6 has an identical structure to DHA except for the absence of a double bond in the n-3 position. This suggests that even if DHA is completely replaced by DPAn-6, DHA is the critical FA necessary for optimal function. However, in large part, only adult central nervous system DHA and DPAn-6 levels have been discussed (11,12) in the reports in which reciprocal replacement was claimed. This raises the question whether DPAn-6 completely replaces DHA at critical early developmental periods. If not, it is possible that the decrease in total 22-carbon (22C) FA or total PUFA during early brain development may account, at least in part, for the abnormal functional outcomes in n-3 Def rodents. Green *et al.* (18) determined the accumulation of DHA in the rat brain from embryonic day 12 to postnatal day 16, where the dams were consuming only an n-3-Adq diet. Otherwise, few papers describe the fatty acyl composition during brain development, much less the reciprocal replacement of DHA with DPAn-6 during development.

The purpose of this study was to determine whether the DHA in cortex, an area with a high neuronal content, is completely reciprocally replaced by DPAn-6 at various developmental stages including days 0, 5, 10, 20, 50, and 91 in n-3 Def rats compared with n-3 Adq rats. A further question was: If DHA is not completely replaced by DPA, what other FA are increased in the n-3 Def rat cortex?

MATERIALS AND METHODS

Animals and study design. This study was performed under a protocol approved by the NIAAA, NIH Animal Care and Use

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Abbreviations: AA, arachidonic acid; 22C, 22-carbon; DPAn-6, docosapentaenoic acid; DTA, docosatetraenoic acid; LC-PUFA, long-chain PUFA; LNA, α-linolenic acid; n-3 Def, n-3 FA-deficient; n-3 Adq, n-3 FA-adequate.

Committee. Two cohorts of Long Evans (Charles River, Portage, MI) female rats arrived at our animal facility when they were 21 d old. They were immediately matched for weight and randomized into one of two dietary groups. All rats were maintained in our animal facility under conventional conditions with controlled temperature (23 ± 1°C) and illumination (12 h; 0700–1900 h). Food and water were consumed on an *ad libitum* basis. At ~11 wk of age, the female rats (F1 generation) were mated with chow-fed (NIH 31) males. The male offspring (F2 generation) of these females were used for this study. Only males were used in this study to minimize possible effects of hormonal factors.

Different stages of brain development were of interest in this study; thus, days 0 and 5 were selected to represent the newborn stage. For brain growth spurt stages (19), days 10 and 20 were selected, and days 50 and 91 were selected to represent the mature, adult brain. Our goal was to obtain six male rats at each time point. Because of our stringent requirement that each pup in each group be from a separate litter, $n = 6$ was obtained in all groups with the exception of the day 5-adequate and day 20-deficient groups, which each contained five pups, the day 0-deficient and day 50-adequate groups, which each contained four pups and the day 0-adequate group, which contained three pups. Rat pups were killed by decapitation. The whole brains were removed and weighed; the cortex was dissected out and frozen at –80°C before lipid extraction and FA analysis.

Experimental diets. The AIN-93 diet was the base for the two custom-made experimental diets (Dyets, Bethlehem, PA). To achieve the lowest level of n-3 FA possible in this diet, several ingredient substitutions were made. Vitamin-free casein was used instead of unprocessed casein because the latter contains n-3 FA. Much of the cornstarch, which also contains n-3 FA, was replaced with maltose-dextrin and other carbohydrates (Table 1). Both diets contained 10 wt% fat, and the only difference between the two diets was the addition of a small amount of flaxseed (source of 18:3n-3) and algal oils (source of DHA; Martek Biosciences, Columbia, MD) to the n-3 Adq diet to supply n-3 FA. The n-3 Def diet contained hydrogenated coconut oil in place of the flaxseed and algal oils. Table 1 shows the complete FA profile of each experimental diet.

FA analysis. The cortex samples were thawed and lipids were extracted using the method of Folch *et al.* (20). Subsequent to lipid extraction, a small aliquot of each total lipid extract was used for FA analysis. Transmethylation was performed with BF_3-methanol by a modification of the method of Morrison and Smith (21) as described by Salem *et al.* (22). Methyl esters were then quantified on a gas chromatograph (Hewlett-Packard, model 5890/series II, Palo Alto, CA) equipped with an FID and fused-silica capillary column (DB-FFAP; 30 m × 0.25 mm × 0.25 μm; J&W, Folsom, CA). The carrier gas (hydrogen) linear velocity was 50 cm/s, and injector and detector temperatures were set to 250°C with the oven temperature program as follows: 130–175°C at 4°C/min, 175–210°C at 1°/min, and then to 245°C at 30°C/min with a final hold for 15 min at 245°C. The tissue FA were identified by comparison with the retention times of a standard mixture of FA (462; Nu-Chek-Prep, Elysian, MN), and the concentrations of each FA were determined by reference to an internal standard (ethyl ester 22:3n-3; 100–500 μg depending on tissue size), which was added to the sample before lipid extraction.

TABLE 1
Composition of the Diets

Ingredient	(g/100 g diet)	
Casein, vitamin free	20	
Carbohydrate[a]	60	
Cellulose	5	
Salt and mineral mix	3.5	
Vitamin mix	1	
L-Cystine	0.3	
Choline bitartrate	0.25	
Fat	10	
Fat sources	n-3 adequate	n-3 deficient
Hydrogenated coconut oil	7.43	8.1
Safflower oil	1.77	1.9
Flaxseed oil	0.5	—
DHASCO[b]	0.3	—
FA[c]		
Total saturated	73.0	80.0
Total monounsaturated	4.6	3.5
18:2n-6	15.2	15.5
Σn-6	15.2	15.5
18:3n-3	3.0	0.05
22:6n-3	1.5	ND[d]
Σn-3	4.5	0.05
18:2n-6/18:3n-3	5.0	310.0
n-6/n-3	3.4	310.0

[a]The carbohydrate was composed of 20, 15, 15, and 10 g/100 g diet of dextrose, cornstarch, maltose-dextrin, and sucrose, respectively.
[b]DHASCO: 46% DHA (from Martek Bioscience, Columbia, MD).
[c]Presented as the percentage of total FA weight.
[d]ND, not detected.

Statistical analysis. Data were analyzed using Statistica (StatSoft, Tulsa, OK). The differences in FA concentrations between the two experimental groups at each time point were analyzed using the Student's *t*-test. Differences were considered significant when $P \leq 0.05$.

RESULTS

There were no differences in body, brain, or liver weights between the n-3 Adq and n-3 Def rats at day 0, 5, 10, 20, or 50. There were also no differences in brain or liver weights at day 91. However, at day 91, there was a significant difference in body weight between the two groups. Body weights at day 91 were 589 ± 42 and 511 ± 34 g (mean ± SD, $P < 0.01$, $n = 6$) for the n-3 Adq and n-3 Def groups, respectively.

Cortex total FA concentrations were not different between the groups at any time point. Cortex DHA levels were significantly lower ($P < 0.000001$) in the n-3 Def group compared with the n-3 Adq group at all time points (Fig. 1). The most significant decrease in cortex DHA in the n-3 Def group was seen at

day 10 in which DHA was 88% lower ($P < 0.000001$) compared with the n-3 Adq group. The average loss of cortex DHA over all time points was 86% in the n-3 Def group. As expected, cortex DPAn-6 levels were significantly higher ($P < 0.000002$) in the n-3 Def rats compared with the n-3 Adq rats at all time points. At day 0, DPAn-6 was 6.5-fold higher ($P < 0.000001$) in the n-3 Def rats. The increase in DPAn-6 in the n-3 Def rats was 7.6, 12.2, 22.8, 36.7, and 53.8-fold compared with the n-3 Adq rats on days 5, 10, 20, 50, and 91, respectively. This increase over time was due mainly to the decrease in DPAn-6 in the n-3 Adq group as the rats aged, rather than an increase in DPAn-6 in the n-3 Def group. In other words, the DPAn-6 as a percentage of FA decreased slightly in the n-3 Adq rats as they matured, and the DPAn-6 decreased slightly or stayed constant in the n-3 Def rats as they matured.

Previous work indicated a "reciprocal replacement" of DHA with DPAn-6 in the brains of adult rats given a diet low in n-3 fats (11,12). Therefore, the sum of the DHA and DPA-n-6 in the n-3 Def rat brain would be expected to be similar to that in the n-3 Adq rat brain. Cortex DHA plus DPAn-6 levels in the n-3 Adq and n-3 Def rats are shown in Figure 2. On days 5, 10, and 20, the DHA plus DPAn-6 levels were significantly lower in the n-3 Def rats compared with the n-3 Adq rats, indicating that DPAn-6 did not completely replace DHA in the n-3 Def rats. There was 11.5 ($P < 0.005$), 10.3 ($P < 0.0001$), and 8.0% ($P < 0.01$) less DHA plus DPAn-6 on days 5, 10, and 20 in the n-3 Def rats, respectively.

Cortex DTA levels were significantly higher ($P < 0.05$) at all time points except day 0 in the n-3 Def rats. When the DTA levels were summed together with DHA and DPAn-6, the total 22C was still significantly lower at days 5 and 10 ($P < 0.05$) in the n-3 Def rats (Fig. 3). There were no significant differences between groups on any other days analyzed.

AA levels were significantly higher ($P < 0.02$) at most time points (all except day 5) in the n-3 Def group. Summed cortex DHA, DPAn-6, DTA and AA levels in the two groups are shown in Figure 4. When the levels of these four FA are considered, the PUFA content of the n-3 Def cortex was equal to or even greater (at later time points) than that of the n-3 Adq cortex, indicating that AA completes the DHA replacement by the C22 n-6 polyunsaturates during early development.

There were no differences in the cortex levels of the saturated FA, 16:0 and 18:0, between the two groups; however, there was a significantly higher percentage ($P < 0.03$) of 18:1n-9 in the n-3 Adq group at days 5, 10, 50, and 91 (data not shown).

The complete fatty acyl composition at the 10-d time point is presented in Table 2. These data illustrate all of the features presented above. There were highly significant losses in DHA and

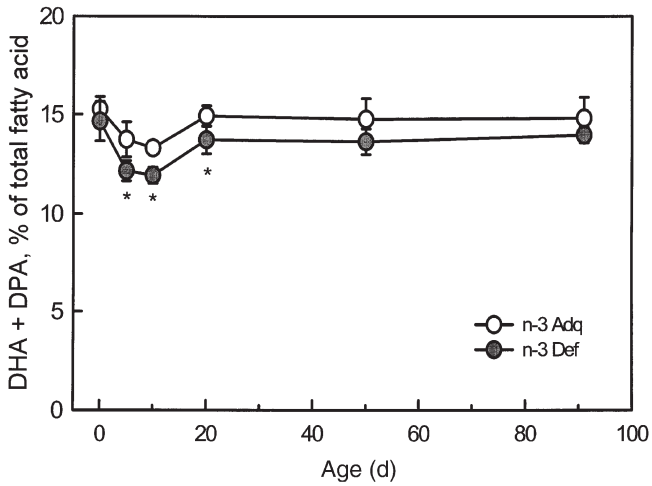

FIG. 2. Effects of an n-3 FA deficiency on DHA + docosapentaenoic acid (DPAn-6) levels in rat cortex over 91 d. For each time point, the sum of DHA + DPAn-6 was calculated for each rat and then averaged with the sums from the other rats at that time point. *Significantly different from the n-3 Adq group ($P < 0.05$). For abbreviations see Figure 1.

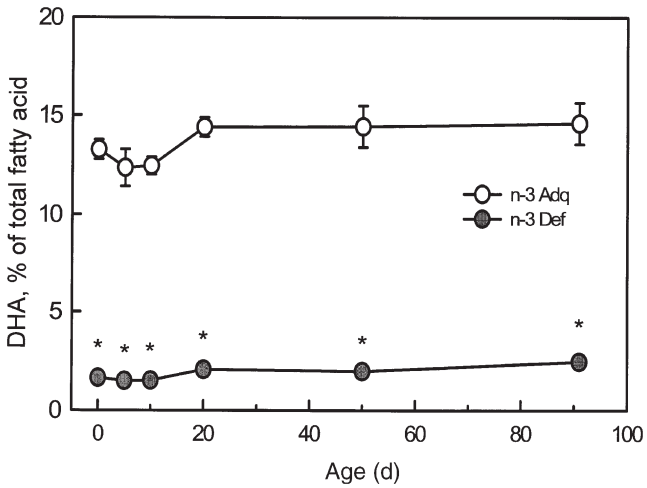

FIG. 1. Effects of an n-3 FA deficiency on the DHA levels in rat cortex over 91 d. DHA levels are presented as the mean percentage of total FA ± SEM. DHA levels at each time point were significantly different between the two groups ($P < 0.05$). n-3 Adq, n-3 FA-adequate; n-3 Def, n-3 FA-deficient.

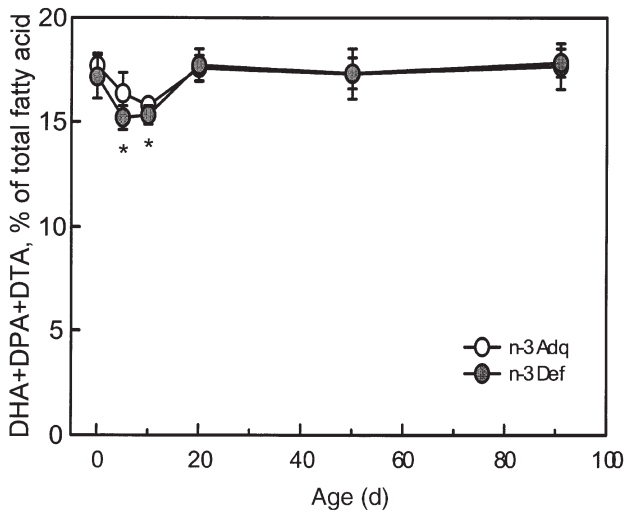

FIG. 3. Effects of an n-3 FA deficiency on DHA + docosapentaenoic acid (DPAn-6) + docosatetraenoic acid (DTA) levels in rat cortex over 91 d. For each time point, the sum of DHA + DPAn-6 + DTA was calculated for each rat and then averaged with the sums from the other rats at that time point. *Significantly different from the n-3 Adq group ($P < 0.05$). For abbreviations see Figure 1.

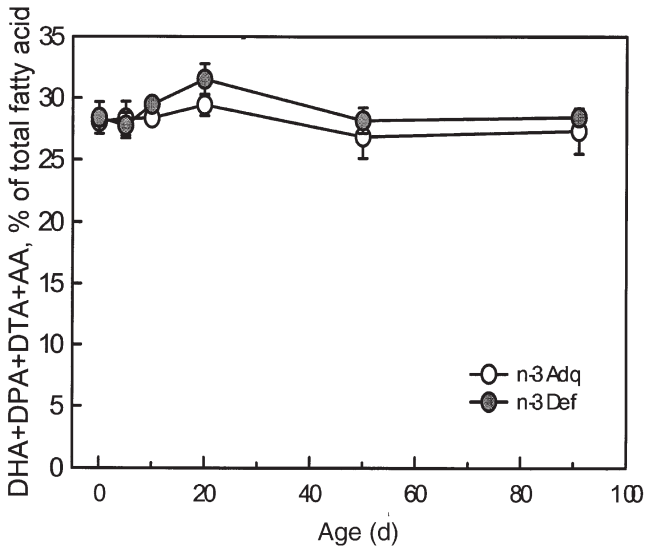

FIG. 4. Effects of an n-3 FA deficiency on DHA + docosapentaenoic acid (DPAn-6) + docosatetraenoic acid (DTA) + arachidonic acid (AA) levels in rat cortex over 91 d. For each time point, the sum of DHA + DPAn-6 + DTA + AA was calculated for each rat and then averaged with the sums from the other rats at that time point. No significant differences were found for this variable. For abbreviations see Figure 1.

22:5n-3 and increases in AA, DTA, and DPAn-6 in the n-3 Def group compared with the n-3 Adq group. It was interesting that the shorter-chain and less unsaturated 18- and 20-C n-6 polyunsaturates actually decreased in the n-3 Def group. There was also a slight decrease in 18:1n-9 with an accompanying increase in 18:1n-7 in the n-3 Def group.

TABLE 2
Rat Cortex Fatty Acyl Composition in the Second Generation of Rats Consuming an n-3-Adequate (n-3 Adq) or n-3-Deficient (n-3 Def) Diet at 10 days of Age[a]

FA	n-3 Def ($n = 6$)	n-3 Adq ($n = 6$)
Nonessential		
14:0	1.8 ± 0.05	1.8 ± 0.11
16:0	29.3 ± 0.41	29.4 ± 0.18
18:0	13.9 ± 0.14	14.2 ± 0.18
20:0	0.05 ± 0.03	0.05 ± 0.001
16:1n-7	1.7 ± 0.01*	1.6 ± 0.02
18:1n-9	8.9 ± 0.10**	9.3 ± 0.04
18:1n-7	2.4 ± 0.06	2.3 ± 0.02
20:1n-9	0.13 ± 0.04	0.17 ± 0.03
24:1n-9	1.4 ± 0.29	0.79 ± 0.19
n-6 Polyunsaturates		
18:2n-6	0.84 ± 0.02***	1.13 ± 0.03
18:3n-6	0.09 ± 0.026	0.12 ± 0.040
20:2n-6	0.13 ± 0.003***	0.18 ± 0.007
20:3n-6	0.31 ± 0.017***	0.61 ± 0.025
20:4n-6	14.1 ± 0.10***	12.6 ± 0.13
22:4n-6	3.38 ± 0.03***	2.47 ± 0.07
22:5n-6	10.4 ± 0.16***	0.9 ± 0.06
n-3 Polyunsaturates		
22:5n-3	0.04 ± 0.002***	0.28 ± 0.18
22:6n-3	1.5 ± 0.03***	12.4 ± 0.13
Total FA (mg/g wet weight)	18.2 ± 0.58	17.8 ± 0.45

[a]Rats were 10 d of age at the time of killing. Data are expressed as wt% of total FA. *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$ vs. n-3 Adq group (Student's *t*-test).

DISCUSSION

The n-3 Def diet was successful in producing rats with low brain DHA. Despite the extreme differences in n-3 FA between the groups, the body and organ weights did not differ except for body weight on day 91. Because brain weights were not different between groups at any time point and body weights were not different at other time points, this single body weight difference at day 91 is not expected to affect the results or the interpretation significantly.

In the adult rats (days 50 and 91), depleted DHA was reciprocally replaced by DPAn-6, as reported in previous investigations (11,12). However, DPAn-6 did not completely reciprocally replace DHA in rat cortex total lipids during early development (days 5, 10, and 20). Levels of LC-PUFA were "restored" in the n-3 Def rat cortex at the early time points only when increases in DTA and AA, the other dominant n-6 FA in cortex, were also considered.

Although it was reported previously that DPAn-6 can replace DHA in adult nervous system tissues, the present results indicate that DPAn-6 is not completely replacing DHA during early developmental periods. Availability of adequate LC-PUFA supplies may be most critical during these early days because DPAn-6 cannot replace DHA in terms of the function of these tissues (23–25). For example, when rhodopsin is reconstituted in liposomes composed of DPAn-6 phospholipids, phosphodiesterase activity after light activation is significantly less than that in membranes composed of DHA-phospholipids (26).

Clearly, in this study, n-6 metabolism did not proceed at a rate such that it was capable of synthesizing enough 22C long-chain n-6 FA to completely replace the depleted DHA at days 5, 10, and 20. That this occurred only at these initial time points is no doubt related to the explosive brain growth during this time period. Eventually, n-6 FA elongation/desaturation can provide adequate DPAn-6 to replace all of the lost DHA. Only after DTA and AA, a 20C FA, were considered was the LC-PUFA level fully replete in the n-3 Def brain. This raises the question of what would happen in terms of functional outcomes if 22C n-6 FA were adequate during early development. Other questions raised include whether any 22C unsaturated FA is adequate to support the functional requirements of neural cells during this time or whether DHA is specifically required.

The critical experiments required to answer the aforementioned questions include a study in which DPAn-6 is fed to pregnant and lactating n-3 FA Def dams. The aim would be to maintain brain levels of 22C FA throughout all critical periods of development, thus providing replacement of depleted DHA with preformed DPAn-6 rather than relying on *in vivo* n-6 metabolism. Subsequently, functional outcomes would have to be assessed to determine whether supplying adequate 22C FA to the brain during early development prevents the previously described effects of an n-3 FA deficiency. The persistence of losses in nervous system function in that case would strongly support the conjecture that DHA is specifically required for optimal function.

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