Dietary Fish Oil Inhibits Δ6-Desaturase Activity *in vivo*

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ABSTRACT: Liver Δ6-desaturase activity was determined in mice which were made deficient in (i) n-6 and n-3 polyunsaturated fatty acids (PUFA), (ii) n-6 PUFA, or (iii) arachidonic acid (AA). Initially, the mice were subjected to two cycles of a fasting (1 d)/refeeding (2–3 d) protocol in which they were refed an essential fatty acid-deficient (EFAD) diet during the refeeding period. This 1-wk fasting/refeeding protocol, referred to as F/R EFAD, produced a rapid and substantial decline in tissue n-3 and n-6 PUFA and a corresponding increase in n-9 fatty acids, notably oleic acid and Mead acid (20:3n-9). Combined liver Δ6-desaturase/elongase/Δ5-desaturase activities *in vivo* were quantified by measuring the conversion of ^{14}C-linoleic acid (LA) to ^{14}C-AA in mouse liver. Although F/R EFAD caused, as expected, a substantial decline in liver AA and LA content, the conversion of ^{14}C-LA to ^{14}C-AA was the same in these mice as in chow-fed controls (approximately 33–34%). Subsequent refeeding of F/R EFAD mice with an EFAD diet, supplemented with corn oil, restored tissue n-6 PUFA levels without altering the conversion of ^{14}C-LA to ^{14}C-AA. In contrast, refeeding with an EFAD diet, supplemented with fish oil, inhibited ^{14}C-LA to ^{14}C-AA conversion by 78%. Significantly, inhibition of conversion of ^{14}C-LA to ^{14}C-AA was maintained in F/R EFAD mice that were subsequently fed an EFAD diet supplemented with a 1:1 mixture of fish oil/corn oil. This latter protocol yielded a unique liver fatty acid composition in which AA was selectively depleted, whereas LA and the n-3 PUFA were increased. The data suggest that dietary n-3 $C_{20–22}$ PUFA negatively regulate the *in vivo* synthesis of n-6 PUFA at the level of the Δ6-desaturase.
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KEY WORDS: Desaturase, essential fatty acid-deficient, fish oil, n-6/n-3 polyunsaturated fatty acids.

Arachidonic acid (AA; 20:4n-6) is derived from linoleic acid (LA; 18:2n-6) by sequential Δ6 desaturation, elongation, and Δ5 desaturation (Fig. 1) (1–5). A reduction in the content of AA in tissues or cells has been shown to be beneficial in several animal disease models (6–8). Because LA is an essential fatty acid, its availability in the diet cannot be drastically curtailed without the onset of side-effects associated with essential fatty acid deficiency (EFAD) (9–11). An alternative approach to achieve a reduction of AA involves a dietary-induced exchange of AA with other $C_{20–22}$ polyunsaturated fatty acids (PUFA), most notably the n-3 fatty acids that are abundant in marine fish oils (FO). A potential advantage of FO is that the eicosanoids synthesized from eicosapentaenoic acid (EPA) (e.g., PGE_3, TxA_3, LTB_5, or LTC_5) have reduced inflammatory properties (12,13). However, this dietary approach is slow in onset due to the recalcitrance of AA to be depleted from cellular lipids. An effective dietary approach to reduce tissue AA may thus involve exchange of AA by EPA/docosahexaenoic acid (DHA), while maintaining dietary LA to avoid EFAD and, at the same time, significantly reducing conversion of LA to AA. Previous *in vitro* studies that quantified desaturase activities in rat liver microsomes indicated that FO feeding reduced microsomal Δ6 desaturation of LA to γ-linolenic acid (GLA; 18:3n-6) (14–17). However, no data are available on whether Δ6-desaturase inhibition by FO occurs *in vivo*. The aims of this study were to develop a dietary protocol that yields a rapid and substantial exchange of n-6 for n-3 PUFA, and to determine whether such an exchange leads to inhibition of Δ6-desaturase activity *in vivo*.

EXPERIMENTAL PROCEDURES

Dietary protocols. Balb/C female mice at 4 wk of age were obtained from the Tel-Aviv University animal facility. In the first experimental protocol, one group of mice was fed the EFAD diet for 12 wk, while the other group of mice was maintained on the chow diet for 11 wk and then switched to either continuous feeding of the EFAD diet or to a protocol that involved 1 d of fasting, 2 d of refeeding, 1 d of fasting,

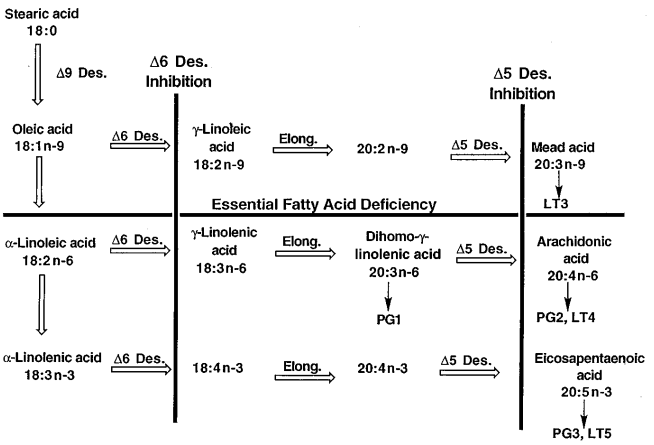

FIG. 1. Metabolic pathways of polyunsaturated fatty acids.

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and 3 d of refeeding. In the second experiment, 8-wk-old mice were subjected to one cycle of fasting/refeeding the EFAD diet, followed by two cycles of fasting/refeeding (F/R) the EFAD diet, supplemented with 5% (w/w) of either corn oil (CO), menhaden FO, or CO + menhaden FO (1:1, vol/vol) (CO + FO). A control group was not fasted and was fed the chow diet *ad libitum*.

The EFAD diet contained only 0.1% (w/w) fat, being comprised principally of saturated and monounsaturated fatty acids. The EFAD-supplemented diets were prepared by cutting the EFAD pellets into sizes of approximately 0.3 cm^3 and then mixing in either CO, FO, or CO + FO (5.0%, w/w) to yield a diet that contained a total of 5.1% (w/w) fat. The fatty acid composition of all diets is shown in Table 1. Fresh pellets were prepared weekly and kept at 4°C in sealed containers. Animals were provided with fresh pellets daily; uneaten pellets were discarded. At the end of the dietary treatment, the animals were anesthetized with 80:20 CO_2/O_2. Blood was withdrawn by retro-orbital bleeding into heparinized tubes, after which the mice were sacrificed by CO_2 inhalation. The livers were frozen on dry ice and stored at −70°C until lipid analysis was done.

Fatty acid composition analysis. Frozen liver tissue (100 mg) was homogenized in 0.6 mL of Dulbecco's phosphate-buffered saline with a Teflon–glass hand homogenizer. A portion of the homogenate (0.2 mL) was saponified, and fatty acid methyl esters (FAME) were obtained by direct transmethylation (18). The FAME were dissolved in hexane and analyzed by capillary gas chromatography (PAG capillary column; Supelco, Bellefonte, PA) in a Hewlett-Packard model 5790 gas chromatograph (Palo Alto, CA), equipped with a flame-ionization detector and an HP-3396 series II reporting detector/integrator. Heptadecanoic acid (10 μg) was added as an internal standard to the samples.

In vivo *assay of Δ6-desaturase activity.* An appropriate amount of [1-^{14}C]-LA (ethanolic solution) was evaporated to dryness under nitrogen and immediately dissolved in 18.2 mM Na_2CO_3 (10-fold molar excess) to a concentration of 100 μCi/mL. The animals were injected intraperitoneally with 0.1 mL (10 μCi) and sacrificed after 6 h by CO_2 inhalation. The livers were frozen on dry ice and then stored at −70°C. Total liver lipids were extracted (19) and then saponified in methanolic-KOH (2.5 N in methanol/water, 4:1). An aliquot ($1–2 \times 10^5$ dpm) of each sample was analyzed by argentation TLC on preparative $AgNO_3$-impregnated silica gel G plates developed with chloroform/methanol/acetic acid/water (90:8:1:0.8, by vol). Radioactive bands were visualized by autoradiography and identified by cochromatography with known ^{14}C-fatty acid standards. Radioactive substrate or product band(s) was then scraped into scintillation fluid and counted. Δ6-Desaturase activity was calculated as the sum of the percentage conversion of substrate (^{14}C-LA) to products (^{14}C-GLA, ^{14}C-DGLA, ^{14}C-AA).

Materials and reagents. Precoated preparative 1-mm silica gel G plates (Whatman, Clifton, NJ) were immersed for 15–20 s in a 10% $AgNO_3$ solution in water, air-dried for 2 d, and stored in light-tight boxes. The plates were activated for 1 h at 110°C prior to use. The fatty acids [1-^{14}C]-LA, [1-^{14}C]-AA, and [1-^{14}C]-dihomo-γ-linolenic acid (sp. act. approx. 55 mCi/mmole) were purchased from American Radiolabeled Chemicals (St. Louis, MO). Nonradioactive authentic fatty acids and FAME were purchased from Nu-Chek-Prep, Inc. (Elysian, MN). The EFAD diet (5803C, low essential fatty acid P.D.) was purchased from Purina Test Diets (Richmond, IN). The diet composition was 21% vitamin-free casein, 69% sucrose, 3% solka floc, 2% PMI vitamin mix, 5% PMI mineral mix #10, 0.15% DL-methionine, and 0.2% choline chloride. CO and menhaden FO were purchased from Sigma Chemical Co. (St. Louis, MO). All solvents and reagents were of analytical grade.

TABLE 1
Fatty Acid Composition and Fat Content of the Experimental Diets[a]

Fatty acid	EFAD (% of total)	EFAD + CO[b]	EFAD + FO[c]	EFAD + CO + FO (1:1)
16:0	40.6	14.0	2.2	5.1
16:1n-7	5.4	0.3	0.4	0.5
18:0	14.3	2.4	0.7	0.2
18:1n-9	36.3	24.1	5.5	14.2
18:2n-6	3.3	57.7	0.7	27.9
18:3n-3	n.d.	0.9	9.2	4.4
18:3n-6	n.d	n.d.	n.d.	n.d.
20:4n-6	n.d.	n.d.	1.9	1.0
20:5n-3	n.d.	n.d.	45.6	22.9
22:6n-3	n.d.	n.d.	22.6	11.4
Fat (% wt)	0.1	5.1	5.1	5.1

[a]n.d., Not detectable.
[b]CO, corn oil.
[c]FO, fish (menhaden) oil; EFAD, essential fatty acid-deficient.

RESULTS

Alterations in composition of liver fatty acids by fasting/refeeding. It has been previously demonstrated (20) that two–three cycles of a F/R protocol induced compositional changes in liver that were characteristic of EFAD. These changes included a reduction in n-6 PUFA and an increase in the n-9 (18:1 and 20:3) and n-7 (16:1) fatty acids. The potential of this F/R protocol to quickly modulate cellular acids was assessed in this study by comparing the F/R EFAD protocol with that of a nonfasting protocol in which the diet was simply switched from chow to EFAD for the same 1-wk period (designated acute EFAD). The F/R EFAD protocol was superior to the acute EFAD protocol in that depletion of LA, AA, and DHA was more severe (Fig. 2). As expected, depletion of these fatty acids was most pronounced after a chronic EFAD protocol in which weanlings were fed an EFAD diet for 12 wk.

Exchange of n-3 for n-6 fatty acids in mouse liver. The rapid and substantial reduction in liver LA and AA content after short fasting/refeeding suggested that PUFA added as a

supplement to an EFAD diet would quickly exchange with LA and AA, thereby reducing the otherwise compensatory synthesis of the n-9 fatty acids, oleic acid, and Mead acid. This hypothesis was tested with mice that were subjected to one cycle of F/R EFAD, followed by two cycles of F/R in which the EFAD diet was supplemented with CO, FO, or CO + FO. Compared to refeeding an EFAD diet alone, the EFAD + CO diet reduced the extent of essential fatty acid deficiency, as evidenced by the increased levels of LA and AA in liver lipids, and reduced the levels of palmitoleic acid (16:1n-7) and the n-9 fatty acids, oleic acid and, especially, Mead acid (Fig. 3). Refeeding an EFAD + FO diet also reduced the n-9 fatty acids but, in addition, caused the n-3 PUFA, EPA, and DHA to accumulate. Feeding on EFAD + FO diet also led to a selective deficiency in n-6 PUFA, similar to that seen with EFAD refeeding (Fig. 3). This deficiency was overcome by refeeding an EFAD + CO + FO diet. With this mixed oil diet, the n-3 PUFA profile was similar to that observed with EFAD + FO. On the other hand, the n-6 PUFA profile was distinct in that LA rebounded while the AA level remained depressed (Fig. 3).

Δ6-Desaturase activity in vivo. To test directly whether n-3 PUFA block the *in vivo* conversion of LA to AA, mice were subjected to one cycle of F/R EFAD, followed by two cycles of refeeding either an EFAD, EFAD + CO, EFAD + FO, or EFAD + CO + FO diet. ^{14}C-LA was then injected intraperitoneally to quantitate the overall conversion to ^{14}C-AA. Results showed that EFAD + FO reduced substantially the conversion of ^{14}C-LA to ^{14}C-AA (Table 2). The FO-mediated inhibition was also seen in mice that were refed an EFAD + CO + FO diet (Table 2), demonstrating that dietary FO inhibits the synthesis of AA, even when an ample dietary supply of precursor LA is present.

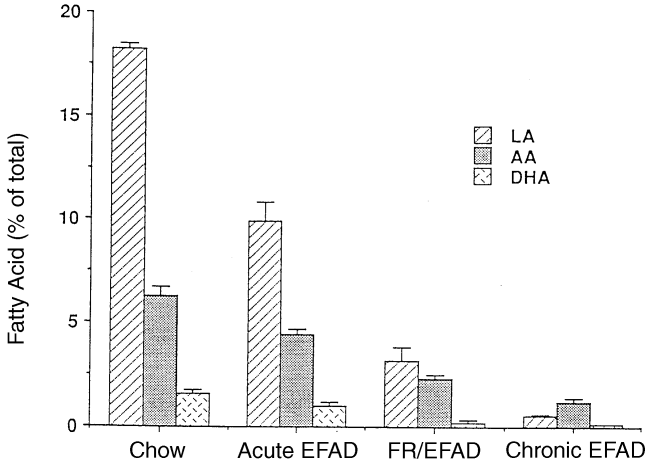

FIG. 2. Fatty acid composition of liver lipids from mice subjected to various dietary regimens. Mice were placed on one of the following dietary regimens: (i) Chow diet for 12 wk (CHOW). (ii) Chow diet for 11 wk and then an essential fatty acid-deficient (EFAD) diet for 1 wk (acute EFAD). (iii) Chow diet for 11 wk and then subjected to two cycles of fasting/refeeding an EFAD diet (F/R EFAD). (iv) EFAD diet for 12 wk (chronic EFAD). The content of individual fatty acids in liver tissue is expressed as percentage of total (mean ± SEM; n = 4/group). For each fatty acid (linoleic acid—solid bars; arachidonic acid—striped bars; docosahexaenoic acid (DHA)—stippled bars), the values in the various dietary regimens are significantly different [$P < 0.05$, analysis of variance (ANOVA)].

DISCUSSION

This study was undertaken to evaluate the effectiveness of the F/R dietary protocol as a means to substantially deplete AA in lipid pools. The data presented are the first *in vivo* demonstration that dietary supplementation with FO inhibits Δ6-desaturase activity, thus inhibiting the conversion of LA to AA. By employing fasting and refeeding with a FO + CO mixture, a selective AA-deficient state in liver lipids can be obtained while the level of LA is maintained. With this dietary manipulation, it should be possible to maintain a low level of AA, while still providing a sufficient level of LA in the diet to guard against undesired side-effects of an EFAD condition.

The results in this report showed that depletion of AA could be achieved by two mechanistic scenarios that may not be mutually exclusive. One possible mechanism is that n-3 PUFA may compete effectively with newly synthesized AA and reduce its acylation into liver lipids. Such competition between fatty acids has been documented previously in *in vitro* studies (21–24). A second possible mechanism is that one or more of the n-3 PUFA in FO inhibits the conversion of LA to AA. Work from several laboratories with liver microsomes from FO-fed rats indicated that Δ6 desaturation of ^{14}C-LA to ^{14}C-GLA was depressed (14,16,17). Initially, it was reported (15,25) that FO supplementation also inhibited liver microsomal Δ5 desaturation of ^{14}C-DGLA to ^{14}C-AA. Studies with rats that were fed diets with FO + evening primrose oil mixtures yielded data on tissue fatty acid composition that were consistent with such a possibility (26,27). However, additional studies found such effects to be minimal (16) or seen only in animals fed low levels of EPA and DHA (28).

TABLE 2
Conversion of ^{14}C-Linoleic Acid to ^{14}C-Arachidonic Acid in the Liver of Mice Subjected to Different Dietary Protocols[a]

Diet	Conversion (%)
F/R EFAD	34.8 ± 2.8
F/R EFAD + CO	33.1 ± 2.2
F/R EFAD + FO	7.4 ± 2.9
F/R EFAD + CO + FO	7.8 ± 3.6

[a]Mice were subjected to one cycle of fasting/refeeding (F/R) EFAD followed by two cycles of F/R EFAD, F/R EFAD + FO, F/R EFAD + CO, or F/R EFAD + CO + FO (see text for details). A control group was fed a chow diet for the duration of the dietary regimens (10 d). At the end of the feeding protocols, the mice were injected intraperitoneally with 10 μCi of ^{14}C-linoleic acid (LA) and sacrificed after 6 h. Liver lipids were extracted and saponified, and the fatty acids were separated by $AgNO_3$ thin-layer chromatography. The percentage conversion of ^{14}C-LA to ^{14}C-AA was quantitated by counting the radioactivity present in each respective ^{14}C-fatty acid band. Results are expressed as percentage conversion (mean ± SEM; $n = 4$/group). The values for the two fish oil-supplemented groups are significantly lower (analysis of variance, $P < 0.01$) than the corresponding EFAD and EFAD + CO groups. See Table 1 for other abbreviations.

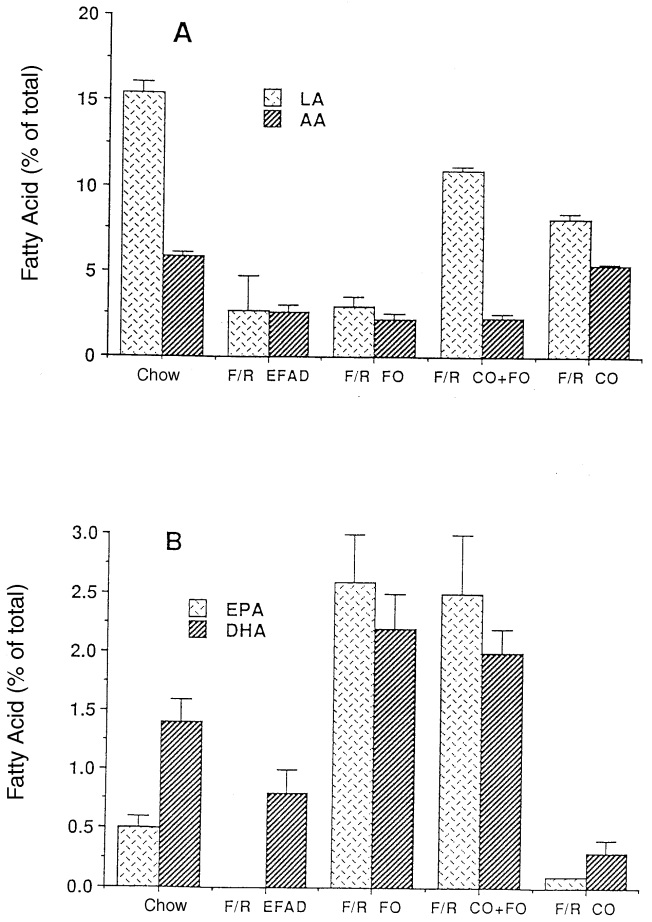

FIG. 3. Polyunsaturated fatty acid composition in liver lipids from mice subjected to fasting/refeeding (F/R) protocols with an EFAD diet supplemented with fish oil (FO) and/or corn oil (CO). Mice were subjected to one cycle of F/R and EFAD diet and then two cycles of F/R EFAD diet supplemented (5% w/w) with either FO (F/R EFAD), CO (F/R CO), a 1:1 CO/FO (F/R CO + FO), or no supplementation (F/R EFAD). A control group (CHOW) was fed the chow diet for the 10-d duration of the feeding protocols. The content of individual liver fatty acids is expressed as percentage of total (mean ± SEM; $n = 4$/group). Statistical significance was tested with ANOVA ($P < 0.05$). For linoleic acid (A, crosshatched bars), values in the different protocols are significantly different from each other, except for the F/R EFAD and the F/R FO groups. For arachidonic acid (A, striped bars), the CHOW and F/R CO groups had similar levels, which were different from the other three protocols. Eicosapentaenoic acid (EPA; 20:5 n-3) (B, crosshatched bars) and DHA (22:6n-3) (B, striped bars) levels were significantly different among the groups, except between the F/R FO and F/R CO + FO groups. See Figure 2 for other abbreviations.

In our studies, FO feeding did not lead to the accumulation of ^{14}C-DGLA (data not shown), suggesting that mouse Δ5 desaturase, as assayed *in vivo*, is not affected by FO feeding. It thus appears that in mammals the only control point and rate-limiting step in the synthesis of AA from its dietary precursor, LA, is the Δ6-desaturase. Elucidating the molecular mechanism for the FO-induced inhibition of Δ6-desaturase activity must await the purification, cloning, and reconstitution of Δ6-desaturase activity *in vitro*.

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