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Fish oil inhibits Δ6 desaturase activity in vivo: Utility in a dietary paradigm to obtain mice depleted of arachidonic acid

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In mice that were alternately fasted and then refed an essential fatty acid-deficient (EFAD) diet, there was a rapid and substantial decline in tissue n-3 and n-6 polyunsaturated fatty acids (PUFAs) and a corresponding increase in n-9 fatty acids. Combined in vivo activities of Δ6 + Δ5 desaturases were quantified directly by measuring the conversion of ^{14}C-linoleic acid (intraperitoneal injection) to ^{14}C-arachidonic acid in liver lipids. Δ5 desaturase activity was quantified by measuring the conversion of ^{14}C-dihomo-γ-linolenic acid (intraperitoneal injection) to ^{14}C-arachidonic acid in liver lipids. The combined Δ6 + Δ5 desaturase activities in EFAD mice was very similar to that in chow-fed control mice (35% vs. 33% conversion of ^{14}C-linoleic acid to ^{14}C-arachidonic acid, respectively). Subsequent refeeding of EFAD mice with an EFAD diet supplemented with corn oil restored tissue n-6 PUFA levels, but did not alter Δ6 + Δ5 desaturase activities (33%). In contrast, subsequent refeeding of EFAD mice with a fish oil-supplemented diet markedly inhibited Δ6 + Δ5 desaturase activities (7%). Fatty acid analysis of the livers from the fish oil-fed mice showed that there was a depletion of the n-6 PUFAs, linoleic acid, and arachidonic acid, and an increase in the n-3 PUFAs, eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3). The inhibition of Δ6 + Δ5 desaturase activities was also maintained in EFAD mice fed a 1:1 mixture of fish oil:corn oil. As a consequence, a unique fatty acid composition in liver and plasma was obtained in which arachidonic acid was selectively depleted, whereas linoleic acid and n-3 PUFAs were increased. Δ5 desaturase activity was not affected by any of the fasting/refeeding paradigms. The data demonstrate that dietary n-3 PUFAs negatively regulate the in vivo synthesis of n-6 PUFAs at the level of the Δ6 desaturase. The inhibition of Δ6 desaturase activity by n-3 PUFAs provides a basis for a unique dietary route to selectively reduce tissue arachidonic acid, while providing sufficient linoleic acid, an essential fatty acid, to support normal cellular metabolism. This dietary paradigm may be effective in attenuating diseases characterized by excessive production of arachidonic acid-derived eicosanoids. (J. Nutr. Biochem. 8:558–565, 1997) © *Elsevier Science Inc. 1997*

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Introduction

A reduction in the content of arachidonic acid (AA; 20:4 n-6) in tissues or cells has been shown to be beneficial in several animal disease models.[1–3] A popular approach to achieve a reduction of AA involves a dietary-induced exchange of AA with other C_{20-22} polyunsaturated fatty acids (PUFAs), most notably the n-3 fatty acids, eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3), both of which are abundant in marine fish oils. The potential advantage of fish oil is three-fold:[2] 1) EPA will partially displace AA from eicosanoid precursor pools, thus reducing the availability of AA for eicosanoid synthesis; 2) EPA will compete directly with remaining AA for oxygenation by the eicosanoid-synthesizing enzymes (cyclooxygenases and lipoxygenases); and 3) eicosanoids

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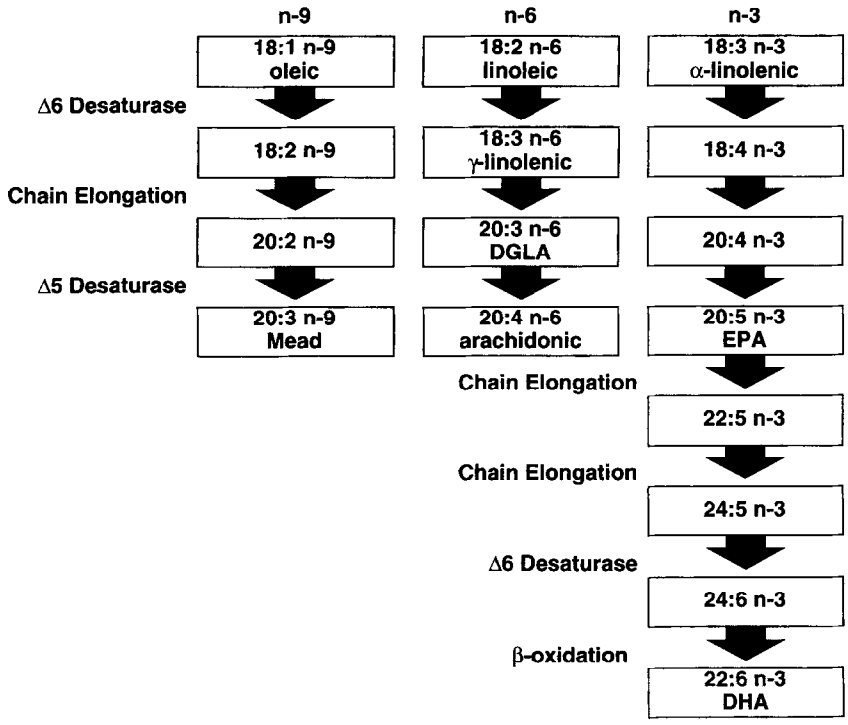

Figure 1 Desaturation and elongation pathways of n-9, n-6, and n-3 fatty acids.

synthesized from EPA (e.g., PGE_3, TxA_3, LTB_5, or LTC_5) have reduced inflammatory properties.[4,5]

AA is derived from linoleic acid (LA; 18:2 n-6) by sequential Δ6 desaturation, elongation, and Δ5 desaturation (*Figure 1*).[6–10] Because LA is an essential fatty acid, its availability in the diet cannot be drastically curtailed without the onset of untoward side-effects associated with essential fatty acid deficiency (EFAD).[11–13] The goal of a dietary approach to limit inflammation may thus involve substantial exchange of AA by EPA/DHA, whereas maintaining dietary LA and, at the same time, significantly reducing conversion of LA to AA via the Δ6 desaturase/elongase/Δ5 desaturase system (*Figure 1*). Previous in vitro studies that quantified desaturase activities in rat liver microsomes indicated that fish oil feeding reduced microsomal Δ6 desaturation of LA to γ-linolenic acid (GLA; 18:3 n-6) and possibly Δ5 desaturation of dihomo-γ-linolenic acid (DGLA; 20:3 n-6) to AA.[14–18] At present, no data are available as to whether Δ6 and/or Δ5 desaturase inhibition by fish oil occurs in vivo. The aims of this study were to: 1) develop a dietary paradigm that allows for a rapid and substantial exchange of n-6 for n-3 PUFAs in mice; 2) determine whether such an exchange leads to inhibition of Δ6 and/or Δ5 desaturase activity in vivo; and 3) assess possible in vivo regulation of Δ5 desaturase activity by dietary PUFAs.

Methods and materials

Materials and reagents

Precoated preparative silica gel G plates (60 Å, 1 mm thick) were purchased from Whatman (Clifton, NJ, USA). The plates were immersed for 15 to 20 sec. in a 10% $AgNO_3$ solution in water, after which they were drained and then air-dried for a minimum of 2 days. For storage, the plates were wrapped in foil and stored in light-tight boxes. The plates were activated for 1 hr at 110°C before use. The fatty acids [1-^{14}C]-linoleic acid, [1-^{14}C]-arachidonic acid, and [1-^{14}C]-dihomo-γ-linoleic acid (specific activity approximately 55 mCi/mmol) were purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). Authentic fatty acids and fatty acid methyl esters were purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA). Corn oil (CO), borage oil (BO), and menhaden fish oil (FO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tri-γ-linolenyl triglyceride (γ-LN-TG) was purchased from Nu-Chek Prep, Inc. All solvents and reagents were of analytical grade.

Diet formulation

The essential fatty acid-deficient (EFAD) diet (5803C, low essential fatty acid PD) was purchased from Purina Test Diets (Richmond, IN, USA). The diet composition was 21% vitamin-free casein, 69% sucrose, 3% solka floc, 2% PMI vitamin mix, 5% PMI mineral mix No. 10, 0.15% DL-methionine, and 0.2% choline chloride. The EFAD diet contained only 0.1% (w/w) as fat, being comprised principally of saturated and mono-unsaturated fatty acids (*Table 1*). 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 (5.0%, w/w), FO (5.0%, w/w), CO + FO (1:1, 2.5% each, w/w), or BO (5.0%, w/w) to yield a diet containing a total of 5.1% (w/w) as fat. Additionally, γ-LN-TG was added to the EFAD pellets (2%, w/w) to yield a diet containing a total of 2.1% (w/w) as fat. Fresh pellets were prepared weekly, divided into daily amounts, and kept at 4°C in sealed containers. The fatty acid composition of the EFAD and EFAD-supplemented diets is shown in *Table 1*. Following is the fatty acid composition of the standard chow diet: 16:0 (23%), 16:1 n-7 (3%), 18:0 (7%), 18:1 n-9 (24%), 18:2 n-6 (34%), 18:3 n-3 (3%), 20:4 n-6 (0.5%), 20:5 n-3 (1%), and 22:6 n-3 (1%).

Table 1 Fatty acid composition and fat content of the experimental diets

Fatty acid (% of total)	EFAD	EFAD + [a]CO	EFAD + [b]FO	EFAD + CO + FO (1:1)	EFAD + [c]BO	EFAD + [d]γ-LN-TG
16:0	40.6	14.0	2.2	5.1	13.2	n.d.
16:1 n-7	5.4	0.3	0.4	0.5	0.2	n.d.
18:0	14.3	2.4	0.7	0.2	5.6	n.d.
18:1 n-9	36.3	24.1	5.5	14.2	19.2	0.5
18:2 n-6	3.3	57.7	0.7	27.9	39.5	1.1
18:3 n-3	n.d.	0.9	9.2	4.4	n.d.	n.d.
18:3 n-6	n.d.	n.d.	n.d.	n.d.	21.9	98.4
20:4 n-6	n.d.	n.d.	1.9	1.0	n.d.	n.d.
20:5 n-3	n.d.	n.d.	45.6	22.9	n.d.	n.d.
22:6 n-3	n.d.	n.d.	22.6	11.4	n.d.	n.d.
Fat (% wt.)	0.1	5.1	5.1	5.1	5.1	2.1

[a]CO, corn oil.
[b]FO, fish (menhaden) oil.
[c]BO, borage oil.
[d]γ-LN-TG, tri-γ-linolenyl triglyceride.
n.d., not detectable.

Animals and dietary paradigms

Balb/C female mice were purchased from the Tel-Aviv University animal facility. Mice were subjected to a protocol involving one day of fasting followed by 2 or 3 days of refeeding. This paradigm is defined as one cycle of fasting/refeeding. The mice were used in three experimental paradigms involving varied numbers of fasting/refeeding cycles, in which the refeeding was done with either an EFAD diet or an EFAD diet supplemented with CO, FO, BO, tri-γ-linolenyl triglyceride, or a combination of CO + FO. The diets were provided ad libitum during refeeding. Water was provided ad libitum throughout. A control group was not fasted and was fed the chow diet ad libitum. Except for the mice on the prolonged (3 months) EFAD diet which gained less weight, all groups consumed approximately equal amounts of food and showed no differences in weight gain during the seven 10-day periods of dietary manipulations.

Animals were kept four to a cage and provided 20.0 ± 0.2 g fresh pellets/day. Uneaten pellets were discarded. At the end of the dietary treatment, the animals were anesthetized with 80/20 CO_2/O_2. Blood (0.3–0.4 mL) was withdrawn by retro-orbital bleeding into heparinized tubes and plasma was subsequently prepared. The mice were then killed by CO_2 inhalation and decapitated. Residual blood was drained and the livers were removed quickly and frozen on dry ice. Livers and plasma were stored at −70°C until sample preparation.

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 liver homogenate (0.2 mL) or plasma (0.2 mL) was treated by a single-step extraction/saponification/methylation to generate fatty acid methyl esters (FAMEs).[19] The FAME mixture was dissolved in hexane and analyzed by capillary gas chromatography using a Hewlett-Packard model 5790 gas chromatograph equipped with a PAG capillary column (Supelco), 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 at the start of saponification.

In vivo assay of Δ6 and Δ5 desaturase activities

An appropriate amount of [$^{1\text{-}14}$C]-LA or [$^{1\text{-}14}$C]-DGLA (ethanolic solution) was evaporated to dryness under nitrogen and immediately dissolved in 18.2 mM Na_2CO_3 (10-fold molar excess) to a specific activity of 100 μCi/mL. The mice (n = 4/group) were injected intraperitoneally with 0.1 mL (10 μCi) of ^{14}C-fatty acid and after 6 hr (^{14}C-LA injection) or 2 hr (^{14}C-DGLA injection) they were killed by CO_2 inhalation. The livers were removed quickly, frozen on dry ice, and then stored at −70°C. Total liver lipids were extracted[20] and then saponified in methanolic-KOH (2.5 N in methanol:water, 4:1). Approximately 92% to 95% of the radioactivity in the crude liver extract was recovered in the chloroform phase. Portions of each chloroform extract (10^5–2×10^5 dpm) were analyzed by argentation TLC using preparative $AgNO_3$-impregnated silica gel G plates. The solvent system was chloroform:methanol:acetic acid:water (90:8:1:0.8). Radioactive bands were visualized by autoradiography and identified by co-chromatography with known ^{14}C-fatty acid standards. Silica gel containing the appropriate radioactive substrate or products band(s) was then scraped into scintillation fluid and ^{14}C activity was counted in a scintillation counter. Δ6 desaturase activity was calculated as the percent conversion of substrate (^{14}C-LA) to products (^{14}C-GLA + ^{14}C-DGLA + ^{14}C-AA). Δ5 desaturase activity was calculated as the percent conversion of substrate (^{14}C-DGLA) to product (^{14}C-AA).

Results

Alterations in composition of liver fatty acids by fasting/refeeding

Lefkowith[21] demonstrated previously that two to three cycles of a fasting/refeeding (F/R) paradigm induced compositional changes in liver that were characteristic of EFAD. These changes included a reduction in n-6 PUFAs and an increase in the n-7 (16:1) and n-9 (18:1 and 20:3) fatty acids. Similarly, Chen and Cunane[22] showed that a F/R paradigm in pregnant rats lead to a selective depletion of whole-body n-6 and n-3 PUFAs. The potential of this F/R paradigm to quickly manipulate tissue fatty acids was confirmed in this study by comparing the F/R EFAD

Table 2 Fatty acid composition of livers from mice subjected to various dietary paradigms

Fatty Acid (% of total)	Chow	Acute EFAD	F/R EFAD	Chronic EFAD
16:0	31.0 ± 0.9	28.1 ± 1.5	26.4 ± 1.0	23.1 ± 1.2
16:1 n-7	3.1 ± 0.5	6.7 ± 0.4	10.7 ± 2.0	11.9 ± 2.3
18:0	12.5 ± 0.1	9.8 ± 0.3	7.3 ± 0.2	7.0 ± 0.6
18:1 n-9	18.2 ± 0.3[a]	28.5 ± 1.1[b]	39.9 ± 0.7[c]	43.3 ± 1.8[c]
18:2 n-6	16.8 ± 0.6[a]	9.9 ± 0.9[b]	3.2 ± 0.6[c]	0.5 ± 0.1[d]
20:3 n-9	0.1 ± 0[a]	1.0 ± 0.1[b]	1.6 ± 0.1[c]	4.5 ± 1.3[d]
20:3 n-6	0.3 ± 0.1	0.2 ± 0	0.1 ± 0.1	n.d.
20:4 n-6	6.3 ± 0.4[a]	4.4 ± 0.3[b]	2.3 ± 0.2[c]	1.2 ± 0.2[d]
20:5 n-3	0.1 ± 0	n.d.	n.d.	n.d.
22:6 n-3	1.6 ± 0.2[a]	1.0 ± 0.2[b]	0.2 ± 0.1[c]	0.1 ± 0[c]

Four week-old mice were manipulated dietarily by one of the following regimens: 1) Fed a chow diet for 12 weeks (designated "Chow"); 2) fed a chow diet for 11 weeks and then fed an EFAD diet continuously for 1 week (designated "Acute EFAD"); 3) fed a chow diet for 11 weeks and then subjected to two cycles of fasting/refeeding an EFAD diet (designated "F/R EFAD"); 4) fed an EFAD diet for 12 weeks (designated "Chronic EFAD"). Results are expressed as relative % of total fatty acids (mean ± SEM; n = 4/group). For each individual fatty acid, values indicated with a different subscript are significantly different ($P < 0.05$, ANOVA).
n.d., not detectable

paradigm with that of a nonfasting paradigm in which the diet of adult mice was simply switched from chow to EFAD for the same time interval (referred to as acute EFAD). The F/R EFAD paradigm was clearly superior to the acute EFAD paradigm in that depletion of LA, AA, and DHA was more severe (*Table 2*). However, depletion of LA, AA, and DHA was most severe in chronic EFAD mice (*Table 2*) in which weanlings were fed an EFAD diet for 12 weeks.

Exchange of n-6 for n-3 PUFAs in mouse liver and plasma

The rapid and substantial reduction in liver LA and AA by the fasting/refeeding paradigm (*Table 2*) suggested that PUFAs added as a supplement to an EFAD diet could efficiently exchange with LA and AA without enhancing 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 with refeeding an EFAD diet alone, refeeding an EFAD + CO diet reduced the extent of essential fatty acid deficiency as evidenced by the increased levels of LA and AA in liver and plasma lipids and reductions in palmitoleic acid (16:1 n-9) and the n-9 fatty acids, oleic acid and, especially, Mead acid (*Table 3*). Even so, the level of LA was only approximately half of that in livers of mice maintained on the chow diet (*Table 3*A, B). The capacity of the F/R EFAD to stimulate depletion of n-6 PUFAs is clearly seen when comparing animals merely switched to EFAD + FO without previous F/R to animals that were F/R EFAD and then fed EFAD + FO. In the former groups, only a modest decline in relative LA and AA content and a concomitant increase in EPA and DHA was observed. In contrast, the latter group exhibited a marked decline in LA and AA in liver (81% and 63%, respectively) and plasma (93% and 48%, respectively), whereas EPA and DHA increased significantly in both liver (5-fold and 1.6-fold, respectively) and plasma (17-fold and 2.7-fold, respectively) (*Table 3*A, B). The major depletion in LA was overcome by refeeding an EFAD + CO + FO diet (*Table 3*A). With this mixed oil diet, the n-3 PUFA profile was similar to that obtained after refeeding with only FO. On the other hand, the n-6 PUFA profile was distinct, in that LA rebounded to the same level as in mice refed EFAD + CO, whereas AA remained depleted (*Table 3*A, B).

The total fatty acid content in plasma was similar for all of the groups, except the EFAD + FO group where it was 5% to 7% lower. The changes in fatty acid composition of plasma (*Table 3*B) from mice subjected to the different dietary paradigms were similar to those seen in liver (*Table 3*A).

In vivo Δ6 and Δ5 desaturase activities

After the injection of ^{14}C-LA, the overall recovery of radioactivity from the liver was maximal by 2 hr (8.5–12.5 × 10^5 dpm/liver) and decreased somewhat by 6 hr (6.5–9.5 × 10^5 dpm/liver) and substantially by 22 hr (2.1–4.9 × 10^5 dpm/liver). The rate of conversion of ^{14}C-LA to ^{14}C-AA was nearly maximal by 6 hr (33% with chow-fed mice). Hence, the 6-hr time point was chosen for killing mice injected with ^{14}C-LA. The recovery of injected ^{14}C-DGLA was 11.0–23.5 × 10^5 dpm/liver after 2 hr and 7.3–16.5 × 10^5 dpm/liver after 6 hr. The conversion of injected ^{14}C-DGLA to ^{14}C-AA was considerably faster than that of ^{14}C-LA to ^{14}C-AA, plateauing by 2 hr. Therefore, the 2-hr time point was chosen for killing mice injected with ^{14}C-DGLA.

To test directly whether dietary n-3 PUFAs inhibit the conversion of LA to AA in vivo, mice were subjected to one cycle of fasting/refeeding an EFAD diet, followed by two cycles of fasting/refeeding either an EFAD, EFAD + CO, EFAD + FO, or EFAD + CO + FO diet. ^{14}C-LA was then injected intraperitoneally to quantify the combined Δ6 desaturase/elongase/Δ5 desaturase activities directly in liver. The results showed that whereas EFAD diet did not change the Δ6 + Δ5 desaturase activities (35% for EFAD vs. 33% for EFAD + CO) (Figure 2A), changing to a FO

Table 3 Fatty acid composition of livers (A) or plasma (B) from mice subjected to fasting/refeeding regimens with an EFAD diet supplemented with corn oil and/or fish oil.

A. Liver fatty acid composition						
Fatty Acid (% of total)	Chow	FO	F/R EFAD	F/R CO	F/R FO	F/R CO + FO
16:0	46.9 ± 1.7	39.4 ± 2.1	34.4 ± 1.1	40.6 ± 1.2	36.1 ± 1.3	33.7 ± 1.2
16:1 n-7	6.1 ± 0.7	9.8 ± 0.5	14.5 ± 0.2	13.8 ± 0.5	13.5 ± 0.7	9.3 ± 0.4
18:0	10.6 ± 0.3	8.9 ± 0.6	5.4 ± 0.3	6.5 ± 0.1	4.5 ± 0.3	8.4 ± 0.7
18:1 n-9	10.7 ± 0.6^{a}	15.7 ± 0.6^{b}	31.1 ± 2.0^{c}	22.1 ± 0.5^{d}	23.4 ± 2.0^{d}	22.2 ± 1.7^{d}
18:2 n-6	15.4 ± 0.7^{a}	10.6 ± 0.9^{b}	2.7 ± 2.0^{c}	8.1 ± 0.3^{d}	2.9 ± 0.6^{c}	10.9 ± 0.3^{b}
20:3 n-9	0.1 ± 0^{a}	0.2 ± 0.0^{b}	1.7 ± 0.3^{c}	0.5 ± 0.1^{d}	0.6 ± 0.2^{d}	0.5 ± 0.1^{d}
20:3 n-6	0.4 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.4 ± 0.1
20:4 n-6	5.9 ± 0.2^{a}	4.8 ± 0.5^{b}	2.6 ± 0.4^{c}	5.4 ± 0.1^{d}	2.2 ± 0.3^{c}	2.3 ± 0.2^{c}
20:5 n-3	0.5 ± 0.1^{a}	1.5 ± 0.2^{b}	n.d.	0.1 ± 0^{c}	2.6 ± 0.4^{d}	2.5 ± 0.5^{d}
22:6 n-3	1.4 ± 0.2^{a}	1.9 ± 0.2^{b}	0.8 ± 0.2^{c}	0.3 ± 0.1^{d}	2.2 ± 0.3^{b}	2.0 ± 0.2^{b}
B. Plasma fatty acid composition						
16:0	27.7 ± 1.1	26.9 ± 1.4	22.6 ± 0.9	23.6 ± 1.2	28.7 ± 2.0	26.3 ± 2.2
16:1 n-7	1.8 ± 0.3	2.2 ± 0.3	3.5 ± 0.2	1.8 ± 0.1	2.9 ± 0.3	2.4 ± 0.1
18:0	14.0 ± 0.9	12.5 ± 1.1	7.4 ± 0.4	13.3 ± 0.8	11.4 ± 0.7	13.4 ± 0.8
18:1 n-9	18.2 ± 1.6^{a}	16.8 ± 1.0^{b}	38.0 ± 0.5^{c}	18.6 ± 1.3^{a}	19.6 ± 1.6^{a}	17.5 ± 1.6^{ab}
18:2 n-6	20.7 ± 0.6^{a}	7.1 ± 0.9^{b}	1.3 ± 0.2^{c}	16.1 ± 0.9^{d}	1.4 ± 0.2^{c}	14.8 ± 0.6^{d}
20:3 n-9	0.4 ± 0.1^{a}	0.2 ± 0.1^{a}	11.3 ± 0.8^{b}	0.7 ± 0.1^{c}	0.3 ± 0.0^{a}	0.2 ± 0.1^{a}
20:3 n-6	1.2 ± 0.2	0.5 ± 0.1	0.5 ± 0.0	1.3 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
20:4 n-6	7.3 ± 0.9^{a}	5.5 ± 0.4^{b}	2.9 ± 0.4^{c}	12.6 ± 0.5^{d}	3.8 ± 0.3^{e}	3.9 ± 0.2^{e}
20:5 n-3	0.6 ± 0.1^{a}	5.3 ± 0.6^{b}	n.d.	n.d.	10.6 ± 0.5^{c}	6.1 ± 0.3^{b}
22:6 n-3	5.1 ± 1.0^{a}	8.2 ± 1.0^{b}	1.4 ± 0.2^{c}	2.2 ± 0.2^{d}	14.1 ± 1.3^{e}	8.8 ± 0.5^{b}

Ten week-old mice were subjected to one cycle of 1 day fasting/3 days refeeding an EFAD diet followed by two cycles of 1 day fasting/2 days refeeding an EFAD diet that was supplemented as follows: 1) no supplementation (designated "F/R EFAD"); 2) EFAD + corn oil supplementation (designated "F/R CO"); 3) EFAD + fish oil supplementation (designated "F/R FO"); and 4) EFAD ± corn oil:fish oil (1:1) supplementation (designated "F/R CO + FO"). A control group of mice was fed a chow diet, while an additional group of mice was fed an EFAD + FO diet for the duration of the dietary regimens (10 days). Results are expressed as relative % of total fatty acids (mean ± SEM; n = 4/group). For each individual fatty acid, values indicated with a different subscript are significantly different ($P < 0.05$, ANOVA).
n.d., not detectable.

diet reduced substantially the conversion of ^{14}C-LA to ^{14}C-AA (7.5%)(*Figure* 2A). Of further significance, FO-mediated inhibition of the conversion of ^{14}C-LA to ^{14}C-AA was also present in mice refed an EFAD + CO + FO diet (8.0%) (Figure 2A), demonstrating the capacity of FO to inhibit the synthesis of AA in the presence of an ample supply of LA precursor. Fish oil refeeding did not lead to the accumulation ^{14}C-DGLA (*Figure* 2B), nor to enhanced accumulation of nonradioactive DGLA in liver or plasma lipids (*Table* 3A, B). Also, in vivo activity of liver Δ5 desaturase was not affected (*Figure 3*). These results demonstrate that the Δ5 desaturase is not the key enzymatic step affected by FO refeeding.

The regulation of Δ5 desaturase activity by either its precursors, GLA or DGLA, or its product, AA, was evaluated in vivo. In mice subjected to one round of F/R EFAD followed by two rounds of F/R EFAD + BO, there was a substantial repletion of DGLA and AA in liver tissue (*Table 4*). These results indicated indirectly that DGLA or AA did not inhibit Δ5 desaturase activity in vivo. After three cycles of F/R EFAD or one cycle of F/R EFAD followed by two cycles of F/R EFAD + BO, liver Δ5 desaturase activity was measured directly in vivo by injection of ^{14}C-DGLA. Liver Δ5 desaturase activity was unaffected by the different dietary paradigms (*Figure 3*), supporting the contention that GLA, DGLA, or AA does not affect Δ5 desaturase activity in vivo.

It was possible that fatty acids in borage oil other than GLA might mask a modulatory effect mediated by GLA/DGLA on Δ5 desaturase activity. To rule out this possibility, the EFAD diet was supplemented with pure γ-LN-TG. As expected, the levels of GLA, DGLA, and AA increased, even to higher levels than obtained in chow-fed mice (*Table 4*). However, Δ5 desaturase activity remained unchanged (*Figure 3*); it was not upregulated when AA was partially depleted in liver (F/R EFAD), nor was it downregulated when excess AA accumulated in liver (F/R EFAD+γ-linolenyl triglyceride).

Discussion

The aims of the present study were to 1) evaluate dietary manipulation as a means to substantially exchange AA with n-3 PUFAs; 2) determine whether dietary fish oil inhibits Δ6 and/or Δ5 desaturase activities in vivo; and 3) whether desaturase inhibition could be used to selectively deplete AA. Using a paradigm of short fasting followed by refeeding a fat-free diet and then a FO diet, a rapid and substantial exchange of AA for EPA in mouse liver and plasma lipids was obtained. By fasting and then refeeding a CO + FO mixture, a selective AA-deficient state can be obtained; the level of AA is kept depressed, whereas simultaneously, the level of LA is maintained. Using this paradigm, it should be possible to maintain a low level of AA, while still providing

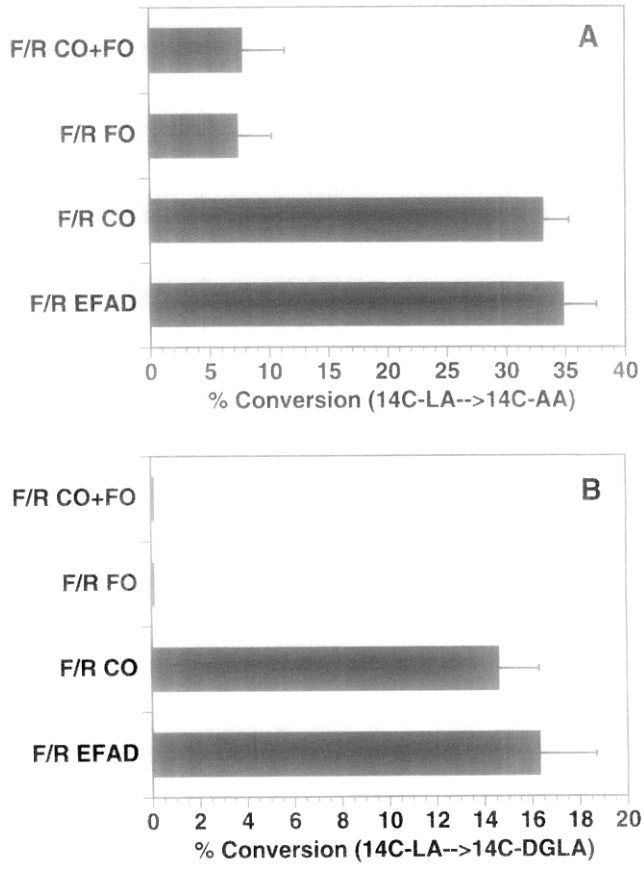

Figure 2 Conversion of ^{14}C-linoleic acid to ^{14}C-arachidonic acid (A) or ^{14}C-linoleic acid to ^{14}C-dihomo-γ-linolenic acid (B) in mouse liver. Mice were subjected to one cycle of fasting/refeeding an EFAD diet followed by two cycles of F/R an EFAD diet that was supplemented as follows: 1) no supplementation (designated "F/R EFAD"); 2) EFAD + corn oil supplementation (designated "F/R CO"); 3) EFAD + fish oil supplementation (designated "F/R FO"); and 4) EFAD + corn oil:fish oil (1:1) (designated F/R CO + FO). A control group of mice was fed a chow diet for the duration of the dietary regimens (10 days). At the end of the fasting/refeeding regimens, the mice were injected intraperitoneally with 10 μCi of ^{14}C-LA. After 6 hr, the animals were killed and their livers were removed. Lipids were extracted from liver tissue and saponified. Liberated fatty acids were transmethylated and resolved by $AgNO_3$-TLC. The % conversion of ^{14}C-LA to ^{14}C-DGLA or ^{14}C-AA was quantified by counting the radioactivity present in each respective ^{14}C-fatty acid band. See "Methods and Materials" for details. Results are expressed as relative % conversion (mean ± SEM; n = 4/group). The two fish oil-supplemented groups in (A) and (B), EFAD + FO and EFAD + CO + FO, had values significantly lower than the corresponding EFAD and EFAD + CO groups (ANOVA, $P < 0.01$).

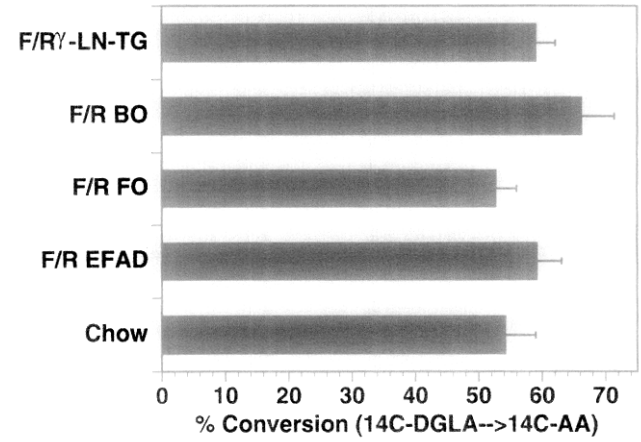

Figure 3 Conversion of ^{14}C-dihomo-γ-linolenic acid to ^{14}C-arachidonic acid in mouse liver. Mice were subjected to one cycle of fasting/refeeding an EFAD diet followed by two cycles of F/R an EFAD diet that was supplemented as follows: 1) no supplementation (designated "F/R EFAD"); 2) EFAD + fish oil supplementation (designated "F/R FO"); 3) EFAD + borage oil supplementation (designated "F/R BO"); and 4) EFAD + tri-γ-linolenyl triglyceride (designated F/R γ-LN-TG). A control group of mice was fed a chow diet for the duration of the dietary regimens (10 days). At the end of the fasting/refeeding regimens, the mice were injected intraperitoneally with 10 μCi of ^{14}C-DGLA to measure Δ5 desaturase activity. After 2 hr, the animals were killed and their livers were removed. Lipids were extracted from liver tissue and saponified. Liberated fatty acids were transmethylated and resolved by $AgNO_3$-TLC. Results are expressed as relative % conversion (mean ± SEM; n = 4/group). The difference between the two dietary groups is not statistically different ($P < 0.01$, Student's t test).

a sufficient level of LA in the diet to ward off at least some of the untoward side effects of EFAD.[11] Indeed, the applicability of a fasting/FO refeeding paradigm in humans to produce a relatively rapid exchange of EPA for AA in plasma lipids has recently been described by us.[23]

The results in this report showed that partial depletion of AA was achieved by two mechanistic scenarios that may not be mutually exclusive: 1) n-3 PUFAs may compete effectively with newly synthesized AA and prevent its acylation into liver lipids. Whereas such competition between fatty acids has been documented previously,[22,24–27] the total lack of AA replenishment into liver or plasma lipids argues against this as the sole explanation; and 2) one or more of the n-3 PUFAs in FO inhibits the conversion of LA to AA. This is the more plausible explanation. The inhibitory effect of FO is likely to be exerted on either the Δ6 desaturase or the Δ5 desaturase or both. Work from several laboratories using microsomes from FO-fed rats indicated that desaturation of ^{14}C-LA to ^{14}C-GLA by liver microsomal Δ6 desaturase activity was depressed.[14,16,17] Also, Garg et al.[15] showed that FO supplementation inhibited desaturation of ^{14}C-DGLA to ^{14}C-AA by liver microsomal Δ5 desaturase activity. However, other studies found such effects to be marginal[16] or seen only in animals fed low levels of EPA and DHA.[28] Our results are the first demonstration that dietary supplementation with FO inhibits Δ6 desaturase activity in vivo, significantly reducing the conversion of LA to AA. Two lines of evidence from our in vivo studies suggest that the inhibitory effect of FO on conversion of ^{14}C-LA to ^{14}C-AA was attributable mainly, if not solely, to inhibition of Δ6 desaturase activity. Firstly, FO feeding did not lead to the accumulation of ^{14}C-DGLA (*Figure 2*B), indicating that the Δ5 desaturase was not the key enzymatic step affected. In agreement with this result, the level of nonradioactive DGLA in liver and plasma lipids did not increase and, instead, a small decrease in the level of DGLA occurred (*Table 3*A, B). Second, in vivo activity of liver Δ5 desaturase was not affected by the fasting/refeeding regimen using a FO-supplemented diet (*Figure 3*).

The effects of GLA and DGLA on Δ6 desaturase activity were not investigated because either fatty acid is readily converted to AA, irrespective of any effect on Δ6 desaturase activity. Numerous human studies have shown that the

Table 4 Fatty acid composition of livers from mice subjected to fasting/refeeding regimens with an EFAD diet supplemented with borage oil or tri-γ-linolenyl triglyceride

Fatty acid (% of total)	Chow	F/R EFAD	F/R EFAD + BO	F/R EFAD + γ-LN-TG
16:0	31.5 ± 1.3	25.6 ± 1.1	29.1 ± 1.3	31.6 ± 0.8
16:1 n-7	2.9 ± 0.4	11.1 ± 1.4	9.9 ± 1.6	8.1 ± 0.5
18:0	12.1 ± 0.3	7.7 ± 0.5	10.1 ± 0.3	12.0 ± 0.8
18:1 n-9	18.0 ± 0.3	38.2 ± 1.7	28.1 ± 1.0	22.1 ± 1.3
18:2 n-6	17.8 ± 1.4[a]	3.2 ± 0.4[b]	7.2 ± 0.1[c]	3.8 ± 0.5[b]
18:3 n-6	0.1 ± 0.0[a]	n.d.	1.8 ± 0.3[b]	3.8 ± 0.3[c]
20:3 n-9	0.2 ± 0.0[a]	1.5 ± 0.2[b]	0.4 ± 0.1[c]	0.7 ± 0.1[d]
20:3 n-6	0.6 ± 0.2[a]	0.1 ± 0.0[b]	1.4 ± 0.1[c]	2.7 ± 0.1[d]
20:4 n-6	6.2 ± 0.4[a]	2.5 ± 0.3[b]	5.9 ± 0.4[a]	8.9 ± 0.4[c]
22:6 n-3	1.7 ± 0.1[a]	0.2 ± 0.1[b]	0.5 ± 0.1[c]	1.9 ± 0.1[a]

Ten week-old mice were subjected to one cycle of 1 day fasting/3 days refeeding an EFAD diet followed by two cycles of F/R an EFAD diet that was supplemented as follows: 1) no supplementation (designated "F/R EFAD"); 2) EFAD + borage oil supplementation (designated "F/R BO"); and 3) EFAD + tri-γ-linolenyl triglyceride (designated F/R γ-LN-TG). A control group of mice was fed a chow diet for the duration of the dietary regimens (10 days). Results are expressed as relative % of total fatty acids (mean ± SEM; n = 4/group). For each individual fatty acid, values indicated with a different subscript are significantly different ($P < 0.05$, ANOVA).
n.d., not detectable

maximum conversion of dietary LA to AA is achieved at a relatively low level of dietary LA, whereas bypassing the Δ6 desaturase by providing supplemental GLA or DGLA caused plasma AA to increase markedly.[28] In this study, no correlation was found between the level of AA in liver or plasma lipids and Δ5 desaturase activity in vivo. It thus appears that in mice the only control point and rate-limiting step in the synthesis of AA from its dietary precursor, LA, is the Δ6 desaturase.

The molecular mechanism of inhibition of Δ6 desaturase activity by PUFAs is unknown. Recent studies provided evidence for transcriptional regulation by PUFAs of selective lipogenic and glycolytic enzymes, including fatty acid synthase,[29,30] acetyl CoA carboxylase,[29,30] stearoyl-CoA desaturase,[31,32] and pyruvate kinase.[33] Investigation of the molecular mechanism responsible for the negative effect of FO on liver Δ6 desaturase activity awaits the purification, cloning, and reconstitution of Δ6 desaturase activity in vitro.

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References

1 Wan, J.M.-F., Haw, M.P., and Blackburn, G.L. (1989). Symposium on the interaction between nutrition and inflammation. Nutrition, immune function, and inflammation: An overview. *Proc. Nutr. Soc.* **48,** 315–335

2 Kinsella, J.E., Lokesh, B., Broughton, S., and Whelan, J. (1990). Dietary polyunsaturated fatty acids and eicosanoids: Potential effects on the modulation of inflammatory and immune cells: An overview. *Nutrition* **6,** 24–44

3 Kinsella, J.E. and Lokesh, B. (1990). Dietary lipids, eicosanoids, and the immune system. *Crit. Care Med.* **18,** S94–S113

4 Reingold, D.-Felsen and Needleman, P. (1980). Eicosapentaenoic acid and the triene prostaglandins: Pharmacology and therapeutic potential. *Trends Pharmacol. Sci.* **1,** 359–361

5 Galli, C., Marangoni, F., and Galella, G. (1993). Modulation of lipid-derived mediators by polyunsaturated fatty acids. *Prostaglandins Leukotrienes Essent. Fatty Acids* **48,** 51–55

6 Jeffcoat, R. (1980). The biosynthesis of unsaturated fatty acids and its control in mammalian liver. In *Essays Biochem* (P.N. Campbell and G.D. Greville, eds.), vol. 15, pp. 1–36, Academic Press, London UK

7 Holloway, P. (1983). Fatty acid desaturation. In *The Enzymes* (P.D. Boyer, ed), vol. 16, pp. 63–83, Academic Press, NY USA

8 Sprecher, H. (1983). The mechanisms of fatty acid chain elongation and desaturation in animals. In *High and Low Erucic Acid Rapeseed Oils* (J.K.G. Kramer, F.D. Sauer, and W.J. Pigden, eds.), pp. 385–411, Academic Press, Canada

9 Brenner, R.R. (1987). Biosynthesis and interconversion of the essential fatty acids. In *CRC Handbook of Eicosanoids: Prostaglandins and Related Lipids. Vol I. Chemical and Biochemical Aspects. Part A.* (A.L. Willis, ed.), pp. 99–117, CRC Press, Inc., Palo Alto, CA USA

10 Brenner, R.R. (1989). Factors influencing fatty acid chain elongation and desaturation. In *The Role of Fats in Human Nutrition, 2nd Ed.* (A.J. Vergroesen and M. Crawford, eds.), pp. 45–79, Academic Press, London UK

11 Holman, R.T. (1968). Essential fatty acid deficiency. In *Progress in the Chemistry of Fats and Other Lipids* (R.T. Holman, ed.), vol. 9, pp. 279–348, Pergamon Press, London UK

12 Lefkowith, J.B., Evers, A.S., Elliott, W.J., and Needleman, P. (1986). Essential fatty acid deficiency: A new look at an old problem. *Prostaglandins Leukotrienes Med.* **23,** 123–127

13 Lefkowith, J.B., Sprecher, H., and Needleman, P. (1986). The role and manipulation of eicosanoids in essential fatty acid deficiency. *Prog. Lipid. Res.* **25,** 111–117

14 Garg, L.M., Sebokova, E., Thomson, A.B.R., and Clandinin, M.T. (1988). Delta-6 desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or ω-3 fatty acids. *Biochem. J.* **249,** 351–356

15 Garg, L.M., Thomson, A.B.R., and Clandinin, M.T. (1988). Effect of dietary cholesterol and/or ω-3 fatty acids on lipid composition and delta-5 desturase activity of rat liver microsomes. *J. Nutr.* **118,** 661–668

16 Ulmann, L., Blond, J.P., Poisson, J.P., and Bezard, J. (1994). Incorporation of delta-6 and delta-5 desaturation of fatty acids in liver microsomal lipid classes of obese Zucker rats fed n-6 or n-3 fatty acids. *Biochim. Biophys. Acta* **1214,** 73–78

17 Dinh, L., Bourre, J.M., Dumont, O., and Durand, G. (1995). Comparison of recovery of previously depressed hepatic delta-6 desaturase activity in adult and old rats. *Ann. Nutr. Metab.* **39,** 117–123

18 Dang, A.Q., Kemp, K., Faas, F.H., and Carter, W.J. (1989). Effects of dietary fats on fatty acid composition and delta-5 desaturase in normal and diabetic rats. *Lipids* **24,** 882–889

19 Garces, R. and Mancha, M. (1993). One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. *Anal. Biochem.* **211,** 139–143

20 Bligh, E.G. and Dyer, W. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37,** 911–917

21 Lefkowith, J.B. (1990). Accelerated essential fatty acid deficiency by delta-9 desaturase induction: Dissociation between the effects of liver and other tissues. *Biochim. Biophys. Acta.* **1044,** 13–19

22 Chen, Z. and Cunnane, S.C. (1993). Refeeding after fasting increases apparent oxidation of N-3 and N-6 fatty acids in pregnant rats. *Metabolism* **42,** 1206–1211

23 Yosefy, C., Viskoper, R.J., Varon, D., Ilan, Z., Pilpel, D., Lugassy, G., Schneider, R., Savyon, N., Adan, Y., and Raz, A. (1996). Repeated fasting and refeeding with 20:5 n-3, eicosapentaenoic acid (EPA): A novel approach for rapid fatty acid exchange and its effect on blood pressure, plasma lipids and hemostasis. *J. Human Hypertension* **10,** S135–S139

24 Lokesh, B.R. and Kinsella, J.E. (1994). Effect of n-3 polyunsaturated fatty acids on the reacylation of arachidonic acid in peritoneal macrophages. *Prostaglandins Leukotrienes Essent. Fatty Acids* **51,** 235–239

25 Banerjee, N. and Rosenthal, M.D. (1985). High affinity incorporation of 20-carbon polyunsaturated fatty acids by human skin fibroblasts. *Biochim. Biophys. Acta* **835,** 533–541

26 Fernandez, B., Solis-Herruzo, J.A., and Balsmide, J. (1992). Mouse peritoneal macrophages contain an acylating system specific for twenty-carbon polyunsaturated fatty acids. A study in intact cells. *Eicosanoids* **5,** 115–120

27 Manku, M.S., Morse-Fisher, N., and Horrobin, D.F. (1988). Changes in human plasma essential fatty acid level as a result of administration of linoleic and gamma-linolenic acid. Europ. *J. Clin. Nutr.* **42,** 55–60

28 Gronn, M., Christensen, E., Hagve, T.A., and Christophersen, B.O. (1992). Effect of dietary purified eicosapentaenoic acid [20:5 (n-3)] and docosahexaenoic acid [22:6 (n-3)] on fatty acid desaturation and oxidation in isolated rat liver cells. *Biochim. Biophys. Acta* **1125,** 35–43

29 Clarke, S.D. and Jump, D.B. (1994). Dietary polyunsaturated fatty acid regulation of gene transcription. *Annu. Rev. Nutr.* **14,** 83–98

30 Jump, J.B., Thelen, A., Clarke, S.D., and Liimatta, M. (1994). Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. *J. Lipid Res.* **35,** 1076–1084

31 Landschultz, K.T., Jump, D.B., MacDougald, O.A., and Lane M.D. (1994). Transcriptional control of the stearoyl-CoA desaturase-1 gene by polyunsaturated fatty acids. *Biochem. Biophys. Res. Comm.* **200,** 763–768

32 Ntambi, J.M. (1995). The regulation of stearoyl-CoA desaturase (SCD). *Prog. Lipid Res.* **34,** 139–150

33 Liimatta, M., Towle, H.C., Clarke, S.D., and Jump, D.B. (1994). Dietary polyunsaturated fatty acids interfere with the insulin/glucose activation of L-type pyruvate kinase gene transcription. *Mol. Endocrinol.* **8,** 1147–1153