**Influence of Dietary Arachidonic Acid on Metabolism in vivo of 8cis,11cis,14-Eicosaatrienoic Acid in Humans**


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**ABSTRACT:** This study investigated the influence of dietary arachidonic acid (20:4n-6) on Δ5 desaturation and incorporation of deuterium-labeled 8cis,11cis,14-eicosaatrienoic acid (20:3n-6) into human plasma lipids. Adult male subjects (n = 4) were fed diets containing either 1.7 g/d (HI20:4 diet) or 0.21 g/d (L020:4 diet) of arachidonic acid for 50 d and then dosed with a mixture containing ethyl esters of 20:3n-6[d4] and 18:1n-9[d2]. A series of blood samples was sequentially drawn over a 72-h period, and methyl esters of plasma total lipid, triacylglycerol, phospholipid, and cholesteryl ester were analyzed by gas chromatography–mass spectrometry. Based on the concentration of 20:3n-6[d4] in total plasma lipid, the estimated conversion of 20:3n-6[d4] to 20:4n-6[d4] was 17.7 ± 0.79% (HI20:4 diet) and 2.13 ± 1.44% (LO20:4 diet). The concentrations of 20:4n-6[d4] in total plasma lipids from subjects fed the HI20:4 and LO20:4 diets were 2.10 ± 0.6 and 0.29 ± 0.2 μmole/mL plasma/mmol of 20:3n-6[d4] fed/kg of body weight. These data indicate that conversion of 20:3n-6[d4] to 20:4n-6[d4] was stimulated 7-8-fold by the HI20:4 diet. Phospholipid acyltransferase was 2.5-fold more selective for 20:3n-6[d4] than 18:1n-9[d2], and lecithin:cholesterol acyltransferase was 2-fold more selective for 18:1n-9[d2] than 20:3n-6[d4]. These differences in selectivity were not significantly influenced by diet. Absorption of ethyl 20:3n-6[d4] was about 33% less than ethyl 18:1n-9[d2]. The sum of the n-6 retroconversion products from 20:3n-6[d4] in total plasma lipids was about 2% of the total deuterated fatty acids. Neither absorption nor retroconversion appears to be influenced by diet.

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The ratio of 20:3n-6 to 20:4n-6 in tissues is involved in the regulation of the balance between the series-1 and series-2 eicosanoids which effects their physiological impact. Because dihomo-γ-linolenic acid (20:3n-6) and arachidonic acid (20:4n-6) are substrates for the synthesis of these eicosanoids, the possibility has been suggested that diets rich in 20:4n-6 decrease the ratio of 20:3n-6 to 20:4n-6 in tissues and may have a negative effect on a variety of chronic diseases such as heart disease, arthritis, asthma, and hypertension (1–3). Conversely, supplementation of diets with the precursor of 20:3n-6, γ-linolenic acid (18:3n-6), has been reported to have beneficial health effects (1–4). For these reasons, a better understanding of the influence of dietary 20:4n-6 on 20:3n-6 metabolism has potential nutritional and health importance.

A number of studies have used isotope-labeled 18:3n-6 and 20:5n-6 to investigate the metabolism of 20:3n-6 in animal models and *in vitro* (1,5). The metabolism of 20:3n-6 in human subjects has been investigated in studies from two groups (6,7), and there is no information available for the effect of dietary 20:4n-6 on 20:3n-6 metabolism in man. Of the two human studies that have investigated the metabolism of 20:3n-6, one study investigated the effect of single and multiple oral doses of unlabeled 20:3n-6 on the ratio of 20:3n-6 to 20:4n-6 in plasma, platelet, and red cell lipids of three volunteers. The other study used deuterium-labeled 20:3n-6 to follow incorporation and desaturation in four normal and four diabetic subjects. In neither study was the fatty acid composition of the subjects' diets controlled. Results from both studies provide evidence that in humans the conversion of 20:3n-6 to 20:4n-6 by Δ5 desaturation is low. In contrast, Δ5-desaturase activity in rats and mice is much higher than in humans (5,6).

This metabolism study in adult male subjects was part of a dietary arachidonic acid study that investigated the effect of dietary 20:4n-6 on platelet function, eicosanoid production, lipoprotein concentrations, immune status, and the fatty acid composition of plasma, red cell, and adipose lipids. The questions addressed by this report are whether the addition of 20:4n-6 to a human diet alters the desaturation of 20:3n-6 to 20:4n-6, the absorption and incorporation of 20:3n-6 into plasma lipid classes relative to 18:1n-9, or the retroconversion of 20:3n-6 to 18:3n-6 and 18:2n-6.

**EXPERIMENTAL PROCEDURES**

*Deuterated fatty acids.* Deuterium-labeled oleic acid (9cis-18:1-9,10-d2) and dihomo-γ-linolenic acid (8,11,14-20:3-17,17,18,18-d4) were synthesized, purified, and converted to their ethyl esters by previously described methods (8–10). The isotopic purity for 9cis-18:1-9,10-d2 was 79.5% d2, 18.4%...
The subjects were fed these diets for 50 d prior to being fed experimental meals. Subjects were provided a no-fat breakfast at 8:00 a.m. to avoid dilution of the labeled fatty acid with exogenous fatty acids. A low-fat (ca. 15% fat calories) lunch was provided at 12:00, and the usual diets were fed at 5:00 p.m. and on subsequent days. The amount of deuterated fatty acid ethyl esters in the mixture fed to each subject is given in Table 2. The \( \mu \) mole data for the deuterated fatty acid esters fed have been adjusted by multiplying the weighted amounts by their percent isotopic and chemical purity.

**Sample collection.** Blood samples (ca. 14 mL each) were collected by venipuncture at 0, 4, 6, 8, 12, 24, 48, and 72 h for analysis of plasma lipid class fatty acids. Additional blood samples (ca. 11 mL) were collected at 2, 4, 6, and 8 h for analysis of the chylomicron total lipid. Standard preparative ultracentrifuge methods (13) were used to separate the chylomicron fraction. An aliquot of the chylomicron samples was analyzed by electrophoresis to confirm the purity of the chylomicron fractions (14).

### TABLE 1

<table>
<thead>
<tr>
<th>Fatty Acid Composition of Diets*</th>
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<tbody>
<tr>
<td>Fatty acid</td>
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<tr>
<td>-----------</td>
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<tr>
<td>Saturates</td>
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<tr>
<td>20:4n-6</td>
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<tr>
<td>18:1n-9</td>
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<tr>
<td>18:2n-6</td>
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<tr>
<td>18:3n-3</td>
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<tr>
<td>20:0</td>
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<tr>
<td>Total</td>
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*Diets: 2800 total calories; 27.1-27.8 energy percentage (% total fat, 15 % protein, and 57 % carbohydrate.

### TABLE 2

<table>
<thead>
<tr>
<th>Deuterium-Labeled Fatty Esters in Mixtures Fed</th>
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<tr>
<td>Subject code</td>
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<tr>
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</tr>
<tr>
<td>HG3</td>
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<td>HG9</td>
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<tr>
<td>HG8</td>
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<td>HG10</td>
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*Data include corrections for isotopic and chemical purity.
Analysis of plasma lipid fatty acids. Plasma total lipids (TL) were extracted with 2:1 chloroform/methanol (15). Preparative TLC was used to isolate TG, cholesteryl ester (CE), and phospholipid (PL) from plasma lipids (16,17). Known weights of triheptadecanoin, cholesteryl heptadecanoate, and dioleododecanoyl-sn-phosphatidylethanolamine (Applied Science, State College, PA) were added as internal standards to the TL extract. The 17:0 internal standard data were used to determine the concentrations (μmol/mL) of each deuterated and nondeuterated fatty acid in the plasma lipid classes. Methyl esters of the isolated lipid classes were prepared by heating the samples with a 5% HCl–methanol solution (10). Quantitative analysis of the deuterium-labeled fatty acids incorporated into plasma and lipoprotein lipids was by GC–MS analysis of their methyl esters (18).

Fatty acid percentages and concentrations for both labeled and unlabeled fatty acids were obtained by analysis of their methyl esters with a Hewlett-Packard model 5988A quadrupole mass spectrometer (Palo Alto, CA) operated in a positive chemical ionization mode with isobutane as the ionization reagent. The GC–mass spectrometer was equipped with a Supelcowax 10 fused-silica column (30 m × 0.25 mm; Supelco Inc., Bellefonte, PA) and was temperature programmed from 165 to 265°C at 5°C/min with a 20-min final hold. The GC–MS methodology utilized selected ion monitoring of the appropriate ion masses for the fatty acid in each GC peak. The areas for each of the ion masses monitored were obtained by integration of the peaks. The specific operating conditions and computer-assisted storage and processing of the MS data have been described previously (18,19).

Determination of the weight data for both the isotopelabeled and nonlabeled fatty methyl ester derivatives of the plasma lipid samples was based on the known weight of 17:0 added as an internal standard prior to conversion of the lipid classes to their methyl esters. Response factors were determined by analysis of standard mixtures containing weighed amounts of pure fatty methyl esters purchased from Nu-Chek-Prep Inc. (Elysian, MN) and Applied Science. The accuracy of the GC–MS data was determined by adding known weights of 9c-18:1n-9[d2], 20:3n-6[d4], and 20:4n-6[d4] to TG, PL, and CE samples isolated from plasma samples drawn from subjects that were not fed the deuterated fatty ethyl ester mixture. The weight for each of the deuterated fatty esters added was equal to about 0.57% of the total unlabeled fatty acids in the samples. Standard deviations were based on three replicate analyses. The range for the standard deviations for the 18:1n-9[d2], 20:3n-6[d4], and 20:4n-6[d4] in the TG, PL, and CE spiked samples was 0.005 to 0.008%.

The quantitation of the unlabeled methyl ester data obtained by GC–MS analysis was confirmed by analysis of a subset of samples using a Varian model 3400 gas chromatograph (Varian, Walnut Creek, CA) equipped with a 100 m × 0.25 mm SP2560 fused-silica capillary column (Supelco) and a flame-ionization detector. Operating conditions were: split ratio, 1:100; linear velocity of helium, 21 cm/s; detector and injection temperature, 235°C. Methyl ester GC peaks were identified and quantitation of peak areas was confirmed by analysis of authentic standards and mixtures of known composition.

Statistical analysis and calculations. Data were analyzed with the SAS-PC statistical software package from Statistical Analysis System Institute (Cary, NC). For lipid classes, least square means were compared by analysis of variance and 18:1n-9[d2] vs. 20:3n-6[d4] comparisons used a two-tailed, pairwise t-test (20).

The concentrations (μg/mL plasma) of the deuterated fatty acids and their metabolites were calculated by integrating the areas under the time-course curves produced by plotting the deuterated fatty acid data for the eight samples collected between 0 and 72 h, as described previously (21). The values obtained are weighted averages of the eight plasma lipid samples collected over the 72-h time period. Examples of these time-course plots for plasma TG and PL samples from a subject fed the H120:4 diet are shown in Figures 1 and 2. Plots are included also for percentage enrichment times 100. When the concentration of a lipid class is relatively constant between samples, the curves for percentage enrichment and μg/mL data are generally similar (see Fig. 2), but for TG the concentration of the lipid class varies considerably and percentage enrichment data underestimate the incorporation of the deuterated fatty acids. For this reason, concentration data were used rather than enrichment data to compare the effect of the H120:4 and LO20:4 diets on incorporation and conversion of the deuterium-labeled fatty acids. The μg/mL data were converted to μmol/mL data and corrected for the differences in the body weight of the subjects and the amounts of...
RESULTS

The percentage of deuterated fatty acids in chylomicron TL samples was 57.2 ± 1.77% 18:1n-9[d2] and 42.8 ± 1.77% 20:3n-6[d4] for the subjects fed the HI20:4 diet and 62.7 ± 2.54% 18:1n-9[d2] and 37.3 ± 2.54% 20:3n-6[d4] for the subjects fed the LO20:4 diet. The difference between these chylomicron data for the HI20:4 vs. LO20:4 diet groups was not significant at P < 0.05. The mean for the chylomicron data from all subjects was 59.9 ± 3.1% for 18:1n-9[d2] and was significantly different (P < 0.002) from the mean of 40.1 ± 3.1% for 20:3n-6[d4]. This difference indicates that ethyl 20:3n-6[d4] was about 33% less well absorbed than ethyl 18:1n-9[d2].

The deuterated fatty acid concentrations (μmole/mL) in plasma TL, TG, PL, and CE are plotted in Figure 3. Concentrations of 18:1n-9[d2] and 20:3n-6[d4] in plasma lipid classes for subjects fed the LO20:4-6 diet were higher than the data for subjects in the HI20:4 diet group, but the data were not significantly different at P < 0.01. This lack of a statistically significant effect of diet on the concentrations of the deuterated fatty acids may be the result of a type II error due to the small number of subjects. If a 4-subject per diet group and the same variance as the actual data are assumed, diet would have had a significant effect (P < 0.01) on incorporation of 18:1n-9[d2] into TG, PL, and TL but not CE. Also the 20:3n-6[d4] concentrations shown in Figure 3 for plasma TL and PL would have been significantly lower (P < 0.01) for the subjects fed the HI20:4 diet compared to the data for the subjects fed the LO20:4 diet. The 20:3n-6[d4] data are consistent with the fatty acid composition data for plasma, red cell, and platelet. Only the total red cell lipids from subjects in the HI20:4 diet group contained a significantly lower (P < 0.05) percentage of 20:3n-6 (22). The 20:3n-6[d4] results when combined with the fatty acid composition results indicate that 20:4n-6 dietary supplementation has a limited effect on the incorporation of 20:3n-6 in plasma lipids.

For comparison of 18:1n-9[d2] vs. 20:3n-6[d4] in plasma lipid classes, the concentration of 18:1n-9[d2] was greater than 20:3n-3[d4] in the TG (P < 0.04) and CE (P < 0.012) fractions, and the concentration of 20:3n-6[d4] in PL was greater (P < 0.02) than 18:1n-9[d2]. For TL, the concentrations of 18:1n-9[d2] and 20:3n-6[d4] were not significantly different. The concentrations for 18:1n-9[d2] and 20:3n-6[d4] in plasma TL from subjects fed the LO20:4 diet were significantly higher (P < 0.005) than the concentrations of these deuterated fatty acids in TL from subjects fed the HI20:4 diet.

The mean fasting plasma TL concentration of fatty acids was 52% greater (average of 24- and 48-h samples) for subjects from the LO20:4 diet group (3.2 mg/mL) than for subjects from the HI20:4 diet group (2.1 mg/mL). In comparison, the concentrations for the total deuterated fatty acid methyl
esters in plasma TL were 51% greater in subjects from the LO20:4 diet group (27.0 µg/mL) than for the subjects from the HI20:4 diet group (17.9 µg/mL). Since the differences in total plasma lipid concentrations and deuterated fatty acid concentrations were proportional, the difference in the concentration of the deuterated fatty acid concentrations between groups can be explained by the difference between the plasma lipid concentrations of the subjects in the HI20:4 and LO20:4 diet groups.

The µmole/mL data in Figure 3 are replotted as a 100% stacked bar chart in Figure 4 to illustrate the differences between incorporation of the deuterated fatty acids into plasma lipid fractions. The patterns for the concentration data for 18:1n-9[d2] and 20:3n-6[d4] (Fig. 3) and the percentage data (Fig. 4) for the plasma lipid fractions were not influenced by dietary 20:4n-6. The percentage 18:1n-9[d2] in all lipid classes was different from 20:3n-6 at P < 0.015. These results suggest that fatty acid structure influenced incorporation and that dietary 20:4n-6 did not influence the selectivity of the acyltransferases for these deuterated fatty acids.

The concentrations of deuterated 20:4n-6 (µmole 20:4n-6[d4]/mL plasma per µmole of 20:3n-6[d4] fed/kg body weight) in plasma TG, PL, CE, and TL are compared in Figure 5 for subjects fed the HI20:4 and LO20:4 diets. For individual lipid classes (TG, PL, CE), the concentrations of 20:4n-6[d4] were two to four times higher in samples from subjects fed the HI20:4 diet than in samples from subjects fed the LO20:4 diet. The differences were significant for PL (P < 0.03) and CE (P < 0.05) but not for TG. The concentration of 20:4n-6[d4] was about seven times higher in plasma total lipid from subjects fed the HI20:4 diet than in plasma lipids from the subjects fed the LO20:4 diet (P < 0.001). In contrast to the concentration data in Figure 3, the difference in the concentration of 20:4n-6[d4] for subjects from the two diet groups was not proportional to the difference in plasma lipid concentration. If the 20:4n-6[d4] data are adjusted for the 50% lower plasma lipid concentration in the subjects fed the HI20:4 diet compared to the subjects fed the LO20:4 diet, the concentration of 20:4n-6[d4] is about 14 times greater for the HI20:4 diet group and reflects the “true” effect of diet.

The effect of diet on the percentage conversion of 20:3n-6[d4] to 20:4n-6[d4] is shown in Figure 6. The percentages for 20:3n-6[d4] conversion were calculated by dividing the 20:4n-6[d4] µmole/mL plasma data by the sum of the 20:3n-6[d4] plus 20:4n-6[d4] µmole/mL plasma data. Percentage conversion based on plasma TL data was 17.7 ± 0.79% for the subjects receiving the HI20:4 diet and 2.1 ± 1.44% for the subjects fed the LO20:4 diet. The 8.3-fold difference in percentage conversion of 20:3n-6[d4] to 20:4n-6[d4] was significant at P < 0.006.

The 12-h total PL samples contained the highest concentrations of 20:3n-6[d4]. The percentage enrichment for 20:3n-6[d4] in the 12-h PL samples was 16.8 ± 1.7% for subjects fed the HI20:4 diet and 6.8 ± 1.2% for samples from subjects fed the LO20:4 diet. These percentage enrichment data for 20:3n-6[d4] were significantly different at P < 0.02. The 72-h total PL samples contained the highest concentration of 20:4n-6[d4] and the percentage enrichment for
20:4n-6[d4] in these 72-h samples was 0.34 ± 0.07% for subjects fed the HI20:4 diet and 0.07 ± 0.054% for subjects fed the LO20:4-6 diet. The 20:4n-6[d4] percentage enrichment data for the HI20:4 and LO20:4 diets were significantly different at P < 0.05.

The chain-shortened products (18:3n-6[d4], 18:2n-6[d4], 16:2n-6[d4]) of 20:3n-6[d4] were detected in the plasma lipids of all subjects. The sum of these three products in total plasma lipid from the subjects fed the HI20:4 diet was 0.11 ± 0.01 µmole/mL or 1.1 ± 0.25% of the 20:3n-6[d4]. The total concentration of the chain-shortened products in plasma TL from the subjects fed the LO20:4 diet was 0.37 ± 0.14 or 2.76 ± 0.95% of the 20:3n-6[d4]. The difference between the percent of retroconversion products in plasma TL was not significant. Trace amounts of the 22:3n-6[d4] elongation product of 20:3n-6[d4] and the 22:4n-6[d4] elongation product of 20:4n-6[d4] were detected in 10–15% of the samples. A few samples contained measurable amounts of 22:5n-6[d4], and none of the samples contained detectable amounts of 24:4n-6[d4] or 24:5n-6[d4].

**DISCUSSION**

**Absorption.** The absorption of 18- and 20-carbon n-6 and n-3 fatty acids as their ethyl esters, free fatty acids, or TG has been the subject of numerous studies (23). The results from studies that have compared the absorption of unsaturated 18- and 20-carbon fatty acids are not consistent. Studies in humans and rats indicate that 20:5n-3 and 18:1n-9 are equally well absorbed when fed either as their ethyl esters or as TG (24–27) and that 20:4n-6 and 18:1n-9 are equally well absorbed (28), but others have concluded that 20:5n-3 is 30–50% less well absorbed than 18:3n-3 or corn oil (29,30).

The conclusion from this study is that the absorption of ethyl 20:3n-6[d4] was 35% lower than 18:1n-9[d2] which is consistent with results from studies that have compared absorption of 20- to 18-carbon unsaturated fatty acids. Since this conclusion is based on chylomicron TL, there are explanations other than absorption differences that could explain the results. One is that the chylomicron data are a reflection of a more rapid removal of 20:3n-6[d4] than 18:1n-9[d2] from the chylomicron samples. Other possible explanations are that a greater portion of the 20:3n-6[d4] than 18:1n-9[d2] may have been incorporated into intestinally derived very low density lipoprotein TG rather than chylomicron particles or that the absorption of 20:3n-6[d4] was delayed relative to 18:1n-9[d2] in a manner similar to that described for 20:4n-6 (28).

**Incorporation.** Owing to the similarity of the patterns for the deuterated 18:1n-9 and 20:3n-6 plasma lipid class data (Figs. 3 and 4), it appears that dietary supplementation with 1.7 g/d of 20:4n-6 for 50 d did not affect the uptake and accumulation of 18:1n-9[d2] relative to 20:3n-6[d4] into plasma lipid classes. For all subjects, the concentration of deuterated fatty acids in the plasma lipid classes was proportional to the concentration of the subject’s plasma lipids. Thus the difference shown in Figure 3 for the concentrations of the deuterated fats from the subjects fed the HI20:4 and LO20:4 diets is not believed to be due to the difference in the 20:4n-6 contents of the diets.

The difference in the concentrations of 18:1n-9[d2] and 20:3n-6[d4] in plasma TG, CE, and PL (Fig. 3) and the percentage data shown in Figure 4 illustrate the difference in the selectivity of the TG, PL, and lecithin:cholesterol acyltransferases (LCAT) for 18:1n-9 and 20:3n-6. The difference for accumulation of 18:1n-9[d2] and 20:3n-6n-6[d4] in plasma PL primarily reflects LCAT selectivity and is in contrast to the nonselective incorporation of carbon-14 labeled 18:2n-6 and 20:3n-6 in PL of rat liver microsomes, which reflects acyl CoA cholesterol acyltransferase selectivity (31). Previous results from human subjects fed deuterated 18:1n-9 and 18:2n-6 showed that in vivo PL acyltransferase was about 5-fold more selective for 18:2n-6 than 18:1n-9 (32). In this study, selectivity was 2.44-fold higher for 20:3n-6[d4] than for 18:1n-9(d2). The combined results from previous studies and this study show that fatty acid chainlength influences n-6 fatty acid accumulation in PL. The high 20:3n-6[d4] to 18:1n-9(d2) ratio of 2.44 in plasma PL is consistent with the recognized high selectivity of PL acyltransferase for n-6 fatty acids. The low 20:3n-6[d4] to 18:1n-9(d2) ratio of 0.34 in plasma CE is consistent with the higher selectivity of LCAT and acyl CoA cholesterol acyltransferase for 18:1n-9 relative to 20:3n-6 and 20:4n-6 (33) and is in contrast to the 18:2n-6 to 18:1n-9 ratio of about 3 in plasma CE for subjects fed deuterated 18:2n-6 and 18:1n-9 (32). The results show that supplementation of diets with 1.5 g/d of 20:4n-6 did not influence significantly the selectivity of the acyltransferases involved in the incorporation of 18:1n-9[d2] and 20:3n-6[d4] into plasma lipids.

**Retroconversion.** Considerable retroconversion of 20:3n-3 to 18:3n-3 has been observed in mice (18,34), but the retroconversion in vivo of 20:3n-6 has not been reported. The approximate 35% retroconversion of deuterated 20:3n-3 observed in mice (18) is in marked contrast to the approximately 2% retroconversion of 20:3n-6[d4] in the human subjects used in this study. The estimate of retroconversion is based on the sum of the deuterated 18-carbon n-6 retroconversion products of 20:3n-6[d4] in total plasma lipid, and it likely underestimates retroconversion because much of the 18:2n6[d4] and 18:3n-6[d4] chain-shortened products are probably oxidized to acetyl CoA or carbon dioxide. These data indicate also that retroconversion of 20:3n-6[d4] was not significantly influenced by supplementation of the diet with 1.5 g/d of 20:4n-6 for 50 d.

**Desaturation.** The diet of the LO20:4 subject group contained 0.21 g/d of 20:4n-6 and is probably typical of normal U.S. diets. For these subjects, conversion of 20:3n-6[d4] to 20:4n-6[d4] was low (2.12 ± 1.44%) which is consistent with other reports that humans do not extensively desaturate 20:3n-6 (6,7). The results are also consistent with the low percentage conversion of 18:2n-6 to 20:4n-6 observed in subjects fed deuterated 18:2n-6 (32).

In contrast to the results discussed above, the concentrations of the desaturated product, 20:4n-6[d4], in plasma TL,
TG, PL, and CE were significantly higher for the subjects fed the H120:4 diet (Fig. 5). Plasma TL percentage conversion data (Fig. 6) suggest that total conversion of 20:3n-6[d4] to 20:4n-6[d4] increased by 8.3-fold when the 20:4n-6 content of the diet was increased to 1.7 g/d. Plasma TL concentration data show a 7.2-fold difference in the amount of 20:4n-6[d4]. The size of this difference for total conversion of 20:3n-6[d4] to 20:4n-6[d4] indicates that a relative small increase (1.5 g/d) in 20:4n-6 intake may markedly influence the 20:3n-6 to 20:4n-6 ratio in tissues and has the potential to produce a change in the amount of eicosanoids synthesized.

The reason the relative pattern of the percentage conversion data in Figure 6 for the individual lipid classes is different from the pattern for the concentration data in Figure 5 is that calculation of the estimated percentage conversion is dependent on the amount of both 20:4n-6[d4] and 20:3n-6[d4]. For example, in Figure 6 the percentage conversion for CE is larger than for PL because the concentration of 20:3n-6[d4] in CE is small rather than because 20:4n-6[d4] is large.

For subjects fed the LO20:4 diet, the μmole/mL plasma data and percentage data in Figures 5 and 6 are consistent with the low conversion of 20:3n-6 observed with rabbit and hamster models relative to rat and mouse models which have higher Δ5-desaturase activity than rabbits, hamsters, and humans (6). Comparison of the H120:4 and LO20:4 diet data indicates that, in humans, conversion of 20:3n-6[d4] to 20:4n-6[d4] by Δ5 desaturation was stimulated by dietary supplementation with 1.5 g/d of 20:4n-6 for 50 d. These in vivo data in humans are supported by results from cell culture studies that report a 40–50% higher conversion of 18:2n-6[14C] to 20:4n-6[14C] with cells incubated in media containing added 20:4n-6 (35–37).

Classical enzyme kinetic theory suggests that dietary 20:4n-6 intake should influence the metabolism of 20:3n-6 due to the substrate-product relationship. Thus classical theory would predict that an increase in dietary 20:4n-6 should reduce conversion of 20:3n-6[d4] to 20:4n-6[d4] because of product feedback inhibition. Since stimulation of 20:3n-6 conversion to 20:4n-6 by dietary 20:4n-6 supplementation cannot be explained by simple enzyme kinetic data, our results suggest that conversion in vivo of 20:3n-6 to 20:4n-6 is controlled by mechanisms other than or in addition to the rate of Δ5 desaturation.

It is known that Δ5 desaturase prefers the CoA thioester derivative of 20:3n-6 as a substrate rather than 1-acyl-2-eicosatrienoyl-sn-glycerol-3-phosphorylcholine (38). Also, most of the 20:3n-6 in tissue lipids is in the 2-acyl position of PL (1,5,32), and 20:3n-6, like 20:4n-6, is conserved by an efficient deacylation–reacylation mechanism that rapidly reincorporates these fatty acids into PL after hydrolysis (39). The combined effect of these observations suggests that most of the 20:3n-6 and 20:4n-6 in tissue lipids are "locked" into PL structures, and only small amounts are available as substrates for other reactions.

Thus, we propose as a working hypothesis that an increase in dietary 20:4n-6 increases the PL deacylation–reacylation reaction rates or the turnover rate for 20:3n-6 and 20:4n-6. A higher turnover rate would increase the availability in the free fatty acid pool of 20:3n-6[d4] that had been incorporated into PL and thus allow formation of its CoA derivative and its subsequent desaturation. This sequence could explain the increased conversion of 20:3n-6[d4] to 20:4n-6[d4] that was observed. The possibility that dietary 20:4n-6 greatly increased the direct desaturation of the 20:3n-6[d4] incorporated into the PL pool is unlikely because supplementation with 20:4n-6 did not have a significant effect on the μmole of 20:3n-6[d4] incorporated per mg of total PL. In contrast, the percentage enrichment for 20:3n-6[d4] in plasma total PL for the H120:4 diet group subjects was 2.5-fold higher than for the LO20:4 diet group. This difference indicates that dietary supplementation with 20:4n-6 increased the turnover rate of 20:3n-6 in the PL pool. The percentage enrichment for 20:4n-6[d4] was 5-fold higher for samples from subjects fed the H120:4 diet which is additional evidence in support of a higher turnover rate.

The implication of the results from this study is that, if dietary 20:4n-6 intake is low, dietary 20:3n-6 should increase the 20:3n-6 to 20:4n-6 ratio in tissue lipids because the 20:3n-6 levels would increase, but the 20:4n-6 levels would not greatly increase. This change in the 20:3n-6 to 20:4n-6 ratio might in turn influence the production or balance of eicosanoids synthesized from these fatty acids. If the dietary intake of 20:4n-6 is high, supplementation with 20:3n-6 could paradoxically lower rather than increase the 20:3n-6 to 20:4n-6 ratio owing to enhanced conversion of 20:3n-6 to 20:4n-6. However, the effect of dietary supplementation with large amounts of 20:3n-6 on 20:3n-6 conversion to 20:4n-6 and on 20:4n-6 metabolism is not known. Nevertheless, the results from this study on the incorporation, absorption, and conversion of 20:3n-6[d4] to 20:4n-6[d4] illustrate that there is still much that needs to be learned about the effect of dietary n-6 fatty acids and the regulation of their metabolism.

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REFERENCES


