In vivo distribution and turnover of \textit{trans}- and \textit{cis}-10-octadecenoic acid isomers in human plasma lipids

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Triacylglycerols containing deuterium-labeled \textit{trans}-10- and \textit{cis}-10-octadecenoic acid (10t-18:1, 10c-18:1) plus the triacylglycerol of deuterated \textit{cis}-9-octadecenoic acid (9c-18:1) were fed as a mixture to two young, adult male subjects. Analysis by mass spectroscopy of the labeled fats in blood samples collected periodically for 48 h allowed the uptake, distribution and turnover of both 10-octadecenoic acid isomers to be directly compared to 9c-18:1. A feature of this study is that actual weight data for the labeled fats were obtained. These data allowed plasma triacylglycerol turnover rates of 3.47–5.13 mg/min per kg to be estimated. Plasma and chylomicron triacylglycerol data also provided evidence that absorption of the deuterated fats mobilized 10–12 g of a triacylglycerol pool present in the intestinal cells. Other results are summarized as follows: (1) the 10t-, 10c- and 9c-18:1 fatty acids were equally well absorbed, (2) both \textit{Δ}10-18:1 isomers were oxidized more rapidly than 9c-18:1, (3) conversion of the \textit{Δ}10-18:1 isomers into their corresponding 16:1 isomers was about 3-times faster than for 9c-18:1, (4) the \textit{Δ}10-18:1 isomers were preferentially incorporated at the 1-acyl and excluded from the 2-acyl position of phosphatidylcholine, (5) esterification of cholesterol with the \textit{Δ}10-18:1 fatty acids was 2.5–4.3-times slower than for 9c-18:1 and (6) desaturation and elongation rates for the \textit{Δ}10-18:1 acids were very low.

Introduction

Partially hydrogenated vegetable oils are extensively used in a wide variety of processed food products, supply a significant portion of the total dietary fat consumed and are the main source of the 8 to 13 positional \textit{cis}- and \textit{trans}-octadecenoic acid isomers in human tissues [1,2].

Essentially all existing metabolic data for the 10c- and 10t-18:1 isomers are based on in vitro and animal studies and indicate the metabolism of the \textit{Δ}10-18:1 isomers are significantly different from other 18:1 isomers. For example, studies with the rat [3–11] and laying hen [12,13] indicate that the \textit{Δ}10-18:1 isomers are much more strongly excluded from certain tissue lipids than are the other 18:1 fatty acid isomers. In contrast, analysis of human tissue lipids [14,15] suggests that the \textit{Δ}0 isomers are incorporated at levels more closely proportional to their percentages in dietary fat. In vitro experiments also indicate that the \textit{Δ}0-18:1
isomers are poor substrates for specific enzymes [16,17]. Evidence for chain shortening, elongation and desaturation of these isomers has raised the question of whether their metabolic products are of nutritional significance and whether 16:1 isomer levels increase because of the differences observed for in vitro oxidation rates [18–22].

Objectives of this study were to obtain metabolic data for normal adults which could be compared to animal in vivo data and to provide some basis for determining whether the utilization of the Δ10 isomers is sufficiently unusual in humans to warrant further investigation of their properties with regard to health and nutrition. In addition, the data provided additional insight into the absorption and transport of dietary fat in humans.

**Experimental**

**Multiple deuterium isotope methodology**

Previous reports have illustrated the advantage of utilizing mixtures of triacylglycerol containing both deuterium-labeled isomeric fatty acids and deuterium-labeled 9c-18:1 as an internal control for human metabolic studies with isomeric fatty acids [23–26].

In this study, two subjects were each fed a mixture of triacylglycerols containing primarily three different deuterium-labeled fatty acids. The amount and identity of the deuterated fatty acids in the triacylglycerol mixtures are summarized in Table I. The small amounts of 16:1 and 17:1 deuterated fatty acids, listed in Table I, were formed in conjunction with the synthesis of the labeled 18:1 fatty acids. Synthesis of trans-10- [14,15-2H]octadecenoic acid (10t-18:1-d₄), cis-10- [13,14-2H]octadecenoic acid (10c-18:1-d₄), trans-10-[14,14,15,15-2H]octadecenoic acid (10t-18:1-d₄), cis-10-[14,14,15,15-2H]octadecenoic acid (10c-18:1-d₄) and cis-9-[14,14,15,17,18-2H]octadecenoic acid (9c-18:1-d₄) fatty acids has been described previously [27,28].

Quantitation of the deuterium-labeled fatty acids incorporated into plasma lipids was achieved by use of computer-assisted gas chromatography-mass spectrometry (GC–MS) techniques developed specifically for these analyses. These techniques have been described previously [29]. The methodology is based on GC separation of the plasma lipid fatty methyl esters according to chain length and number of double bonds, followed by quantitation of the deuterium-labeled and non-labeled methyl esters in each GC peak by chemical ionization mass spectroscopy. Quantitation and weight data were obtained by use of an added 17:0 internal standard and standard mixtures containing weighted amounts of methyl esters. The various deuterium-labeled fatty acids and corresponding unlabeled endogenous fatty acids present in the plasma lipid were distinguished by differences in their molecular weights.

**Subjects, sampling and sample preparation**

The subjects were two Caucasian males, ages 24 and 26, with no history of congenital ailments. Medical histories, examinations and clinical blood profile data indicated that they were in excellent health and had not taken any medication for two weeks before the study. Weight/height (83.6 kg/185 cm and 61.7 kg/162 cm), blood pressure (110/70 and 110/70), serum cholesterol (160 and

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**Table I**

DEUTERIUM-LABELED FATTY ACIDS USED IN TRIACYLGlycerol (TG) MIXTURES FED

<table>
<thead>
<tr>
<th>Fatty acids in mixture fed</th>
<th>Melting point of TG (°C)</th>
<th>wt. (g)</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10t-18:1-d₄</td>
<td>51</td>
<td>8.898</td>
<td>32.88</td>
</tr>
<tr>
<td>10c-18:1-d₄</td>
<td>19</td>
<td>8.527</td>
<td>31.51</td>
</tr>
<tr>
<td>9c-18:1-d₄</td>
<td>5</td>
<td>7.999</td>
<td>32.52</td>
</tr>
<tr>
<td>9t-17:1-d₄</td>
<td>0.158</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>9c-17:1-d₄</td>
<td>0.405</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>8c-17:1-d₄</td>
<td>0.140</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>8r-16:1-d₄</td>
<td>0.036</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>8c-16:1-d₄</td>
<td>0.048</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>7c-16:1-d₄</td>
<td>0.049</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>27.060</td>
<td></td>
</tr>
<tr>
<td>Subject 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10t-18:1-d₄</td>
<td>51</td>
<td>8.935</td>
<td>31.54</td>
</tr>
<tr>
<td>10c-18:1-d₂</td>
<td>19</td>
<td>9.245</td>
<td>32.64</td>
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<tr>
<td>9c-18:1-d₆</td>
<td>5</td>
<td>9.236</td>
<td>32.60</td>
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<td>9t-17:1-d₄</td>
<td>0.454</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>9c-17:1-d₂</td>
<td>0.164</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>8c-17:1-d₆</td>
<td>0.158</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>8r-16:1-d₂</td>
<td>0.051</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>8c-16:1-d₂</td>
<td>0.041</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>7c-16:1-d₆</td>
<td>0.064</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>28.328</td>
<td></td>
</tr>
</tbody>
</table>
fasting triacylglycerol (66 and 74 mg/dl) and high-density lipoprotein levels (41 and 68 mg/dl) were considered to be within normal ranges. Both subjects were placed on a 2200 kcal/day diet similar in composition to the standard American Diabetic Association's diet for diabetics, for 1 week prior to feeding. No food was consumed for 10 h before the experimental meal was fed. The diabetic diet was used to achieve a caloric intake of approx. 40% fat, 40% carbohydrate and 20% protein. No measurable weight change was noted for either subject while on the diabetic diet and previous dietary histories were judged to be typical of a U.S. diet. Both subjects normally drank 8-10 cups of coffee per day, were non-smokers and neither exercised regularly.

The deuterated triacylglycerol mixtures (approx. 28 g) were emulsified with 30 g calcium-sodium cassinate (Savortone®, 100, Western Dairy Products, San Francisco, CA), 30 g dextrose, 15 g sucrose and 200 ml water. The labeled fats were fed at 8:00 a.m. in place of the subjects' normal breakfast, a light lunch was allowed at 12:45 p.m. and an evening meal at about 6:30 p.m.

Blood samples (38 ml) were obtained by venipuncture at 0, 2, 4, 6, 8, 12, 15, 24 and 48 h for plasma lipid class analyses. Blood samples (38 ml) were also collected at 2, 4, 6, 8, 12, 15 and 24 h for lipoprotein lipid class analyses. Other details of the feeding and sampling procedures have been described previously [23-26].

Analysis of blood lipids

Plasma and lipoprotein triglycerides, free fatty acids, cholesteryl ester, phosphatidylethanolamine (PE), phosphatidylserine phosphatidylinositol (PS/PI), phosphatidylcholine (PC), lysophosphatidylcholine (lysoPC), sphingomyelin, 1-acylphosphatidylcholine (1-acylPC) and 2-acyl phosphatidylcholine (2-acylPC) fractions were isolated, separated and derivatized by standard methods. Total lipids were extracted with 2:1 CHCl₃-MeOH [30], neutral and phospholipid classes were separated by preparative TLC [31,32] and methyl esters were prepared using HCl-MeOH [33]. Phospholipase A₂ hydrolysis of PC with Ophiophagus hannah venom (Ross Allen Reptile Institute, Silver Springs, FL), followed by TLC separation of the reaction products, was used to determine the distribution of fatty acids in the 1- and 2-acyl positions [34].

Methyl esters of the various lipid classes were analyzed for deuterated fatty methyl ester content by GC-MS. The GC was equipped with a fused silica capillary column (DB-1, 0.032 mm × 30 m) J&W Scientific) and a packed OV 101 column (3% OV 101 on chromasorb P, 0.25 in. × 6 ft.). Chemical ionization-mass spectroscopy with isobutane as the reagent gas was used to obtain data for quantitation of the various lipid class fatty acid methyl esters. The mass spectroscopy methodology involved selective-ion monitoring of each GC peak, followed by integration of the peak areas at appropriate mass numbers. The mass spectrometry data were corrected for carbon-13 natural abundance, and response factors were obtained from spectra of pure fatty methyl esters (Applied Science and Nu-Chek Prep., Inc.) and weighed standard mixtures containing all the major deuterium and non-deuterium-labeled fatty methyl esters present in the esterified plasma samples. The accuracy of the GC-MS analyses was estimated at 2% relative standard deviation from analysis of the weighed standard mixtures.

Fatty acid composition of plasma lipid classes was also determined by GC. Gas chromatographs used were a Packard 7400 series (20 ft. × 4 mm, glass column, 15% OV 275), Packard Model 428 (DB-1 fused silica capillary column, 0.032 mm × 30 m) and Perkin-Elmer model 3920 (Silar 10C glass capillary column 0.02 mm × 50 m). Methyl ester peaks were identified, and quantification was confirmed by analysis of authentic standards and weighed standard mixtures. Known weights of an internal fatty acid or methyl ester standard (17:0) were added to all plasma samples after isolation of the lipid classes by TLC [35]. Calculation of lipid class concentration (mg/ml) was based on the 17:0 internal standard weights and the peak areas determined by GC and GC-MS.

Selectivity values for 10t-18:1 are defined as the logarithm of the result obtained by dividing the ratio of deuterated 10t-18:1 to deuterated 9c-18:1 in the plasma lipid fractions by the ratio in the fed mixture. Selectivity values for 10c-18:1 were calculated in a similar manner. Total area selectivity values were calculated by the same procedure, except that the isomer-to-deuterated 9c-18:1 ratio was obtained from the total area of the curve.
produced by plotting percent deuterated fatty acid vs. sampling time. This calculation yields negative selectivity values when the percent deuterated 10e- or 10t-18:1 is lower than the percent 9c-18:1-d₆ and positive values when percent deuterated isomer is higher than 9c-18:1-d₆.

Results

Absorption and turnover of 10t- and 10c-18:1 isomers

The incorporations of deuterium-labeled 10t- and 9c-18:1 acids into the octadecenoic acid component of plasma chylomicron triacylglycerol samples from both subjects are compared in Fig. 1. Curve shape and maximum levels of the deuterated fatty acid incorporated are similar for both subjects and indicate that the Δ10-18:1 isomers and 9c-18:1 were equally well absorbed. The chylomicron triacylglycerol fractions contain about 70% total deuterated fat, whereas the 18:1 component contained 75–85% deuterated monoenes and 15–25% undeuterated 18:1 fatty acids. Only deuterated fat was fed to fasting subjects and the source of endogenous undeuterated fatty acids in these samples will be discussed later.

Incorporation of 10t- and 10c-18:1 isomers into plasma lipids

The percentages of deuterium-labeled 10t, 10c and 9c-18:1 present in the octadecenoic acid fraction of plasma triacylglycerol, free fatty acid PE and PC samples from both subjects are plotted in Fig. 2. The actual weights of the deuterated 18:1 acids in plasma triacylglycerol, free fatty acid, cholesteryl ester, PE, PC and lysoPC samples from subject 2 are plotted on a µg/ml basis in Fig. 3.

The percent deuterated 18:1 data in Fig. 2 compares the relative incorporation and turnover of the 10t- and 10c-18:1 isomers to 9c-18:1 and gives a visual impression of selective incorporation and discrimination for individual plasma lipids.
The weight (µg/ml) data in Fig. 3 provide an appreciation of the major differences in the actual level and rate of incorporation and turnover of the isotope-labeled fatty acids for specific lipid classes. For example, the plasma triacylglycerol, free fatty acid, lysoPC and PE curves in Fig. 3 indicate that incorporation and turnover of the deuterium-labeled fats are much more rapid than for PC and cholesteryl ester. This information is not obvious from the curves in Fig. 2.

The curves in Fig. 2 allow comparison of the difference in metabolism of the 10r-18:1 isomers relative to 9c-18:1 for the two subjects. There were no consistent differences in the utilization of 10r- and 10c-18:1 compared to 9c-18:1 in plasma triacylglycerol and free fatty acids samples from subject 1. In contrast, for subject 2, the levels of 9c-18:1-d₈ in the 8–24 h plasma triacylglycerol samples were consistently 25–30% higher than for the ∆10-18:1 isomers, which indicate higher rates of removal of the 10-18:1 isomers. For the plasma free fatty acid samples from subject 2, uptake and disappearance rates for the 10c- and 9c-18:1 fatty acids were similar, but levels of 10r-18:1 were 25–35% higher, which indicates discrimination against this isomer by the mechanisms responsible for the removal of plasma free fatty acids.

Plasma PE data from both subjects were consistent. Little difference was found in the percentage of 10r- and 10c-18:1, relative to 9c-18:1 incorporated into the PE samples. The PC samples from both subjects were consistently high in 10c-18:1 and low in 10r-18:1. The differences were more pronounced (15–30% greater) for samples from subject 2. There was no obvious explanation for the differences in these PC data from the two subjects.

Selective hydrolysis of plasma PC by phospholipase A₂ was used to determine the relative distribution of the isotope-labeled 18:1 acids in the 1- and 2-acyl positions of PC. The plasma lipid selectivity values for seven 18:1 isomers are summarized in Fig. 4. The 1-acylPC data for 10r- and 10c-18:1 indicate a strong preference for incorporation of both isomers. These data are consistent with previous 1-acylPC data for other positional 18:1 isomers which are included for comparison. This preferential incorporation of 18:1 isomers into the 1-acyl position of PC is also consistent with in vitro rat data. The 2-acylPC values for 10r- and 10c-18:1 indicate discrimination against incorporation at the 2-acyl position relative to 9c-18:1. This selective exclusion is much greater for the 10r-18:1 isomer compared to the 10c-18:1...
isomer. The negative values are generally consistent with results for other isomers (see Fig. 4). with the notable exception of 12c-18:1.

Change in plasma triacylglycerol concentration during absorption of deuterated triacylglycerol

Individual unlabeled fatty acid concentrations (μg/ml) and total deuterium fatty acid levels for plasma triacylglycerol and PC samples are listed in Table II. The 4 h and 6 h triacylglycerol samples from subjects 1 and 2, respectively, contained both the maximum level of deuterated fatty acids and the highest total triacylglycerol concentration. The concentration of the unlabeled triacylglycerol fatty acids, at peak absorption of the deuterated fats, was 2-3-times the concentration in the 0 h samples. Since only labeled fats were fed, this dramatic increase in unlabeled triacylglycerol concentration must result from mobilization of a stored triacylglycerol pool. The percent change in concentration of individual fatty acid concentrations varied and reflects the composition of the mobilized triacylglycerol. In contrast to the triacylglycerol data, the change in concentration of unlabeled PC fatty acids was insignificant. Changes in concentrations of plasma free fatty acid and PE fatty acids (data not shown) were also minimal.

Distribution of deuterium-labeled 10t-, 10c- and 9c-18:1 in lipoprotein lipid classes

The percent deuterium-labeled 10t, 10c and 9c-18:1 in the total fatty acid and the 18:1 fatty acid fraction of chylomicron, very-low-density (VLDL), low-density (LDL) and high-density lipoprotein (HDL) triacylglycerol, cholesteryl ester, free fatty acid, PE and PC lipid classes are summarized in Table III. The percentages listed are for samples which contained the maximum total percent of deuterium-labeled fatty acids. The area selectivity values reflect differences in the overall incorporation and disappearance of the 10t- and 10c-18:1 isomers relative to deuterated 9c-18:1.

Three major trends are obvious. First, the percentage of the deuterium-labeled fat incorporated into the various lipoproteins varies considerably depending on the lipid class. (For example, triacylglycerols and cholesteryl esters, the selectivity values are generally more negative for LDL and HDL fractions compared to VLDL fractions. Third, the various fractions exhibit definite differences in the overall incorporation and disappearance of the 10t- and 10c-18:1 isomers relative to 9c-18:1.

Fig. 4. Summary of plasma lipid class area selectivity values for cis and trans positional octadecenoic acid isomers. Data plotted are the average for two subjects. Selectivity values for 9t, 12t, 12c, 13t and 13c-18:1 are from Refs. 24-26. TG, triacylglycerol; CE, cholesteryl ester; FFA, free fatty acid; LPC, lysoPC; SM, sphingomyelin; PC-1, 1-acylPC; PC-2, 2-acylPC.
TABLE II
EFFECT OF OCTADECENOIC ACIDS ON PLASMA LIPID CLASS FATTY ACID CONCENTRATIONS

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Subject 1</th>
<th>Subject 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (μg/ml)</td>
<td>Change (μg/ml) (%)</td>
</tr>
<tr>
<td></td>
<td>0 h</td>
<td>6 h</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>16:0</td>
<td>74.5</td>
</tr>
<tr>
<td></td>
<td>16:1</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>18:0</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>18:1</td>
<td>118.7</td>
</tr>
<tr>
<td></td>
<td>18:2</td>
<td>51.0</td>
</tr>
<tr>
<td></td>
<td>20:4</td>
<td>5.3</td>
</tr>
<tr>
<td>Other</td>
<td>8.4</td>
<td>8.6</td>
</tr>
<tr>
<td>Total (no label)</td>
<td>277.8</td>
<td>852.7</td>
</tr>
<tr>
<td>Total (label)</td>
<td>0.0</td>
<td>460.0</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>16:0</td>
<td>170.0</td>
</tr>
<tr>
<td></td>
<td>16:1</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>18:0</td>
<td>71.5</td>
</tr>
<tr>
<td></td>
<td>18:1</td>
<td>75.4</td>
</tr>
<tr>
<td></td>
<td>18:2</td>
<td>131.0</td>
</tr>
<tr>
<td></td>
<td>20:4</td>
<td>65.0</td>
</tr>
<tr>
<td>Other</td>
<td>30.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Total (no label)</td>
<td>546.8</td>
<td>460.8</td>
</tr>
<tr>
<td>Total (label)</td>
<td>0.0</td>
<td>27.6</td>
</tr>
</tbody>
</table>

Retroconversion, desaturation and elongation of deuterium-labeled 10r-, 10c- and 9c-18:1

Evidence for retroconversion (chain-shortening) of the 10r- and 10c-18:1 isomers to 16:1 is pro-
vided by the data plotted in Fig. 5 for total plasma, VLDL, LDL triacylglycerols. The 16:1 component of these triacylglycerol fractions contained from 2 to 6-times more 16:1-d₂ and 16:1-d₄ than 16:1-d₆. Since the fed mixture contained approximately equal amounts of these deuterated 16:1 fatty acids, these data are clear evidence for either preferential chain-shortening of the 10r-18:1-d₂ and 10c-18:1-d₄ compared to 9c-18:1-d₆, or for inhibition of further β-oxidation of the 16:1 isomers formed from the 10r- and 10c-18:1 isomers. However, the concentrations (μg/ml) of these deuterated 16:1 fatty acids in the plasma triacylglycerol samples indicate that conversion rates were low. For example, the 6 h plasma triacylglycerol sample contained 2.3, 2.3 and 1.2 μg/ml of 16:1-d₂, -d₄ and -d₆, respectively, compared to 193.3, 189.5 and 202.1 μg/ml of 18:1-d₂, -d₄ and -d₆. The levels of deuterated 16:1 fatty acids identified in plasma free fatty acid, PC, sphingomyelin, lysoPC, VLDL free fatty acid, HDL cholesteryl ester and HDL-PC samples were too low to allow reliable quantitation.

Elongation products (20:1-d₂ and -d₄) of 10r-
TABLE III
LIPOPROTEIN LIPIDS: DISTRIBUTION OF LABELED 18:1 AT MAXIMUM INCORPORATION

Percent and selectivity values are average of data for samples from both subjects.

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>Sample (h)</th>
<th>Percent of total fatty acids</th>
<th>Percent of 18:1 fraction</th>
<th>Area selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sub. 1</td>
<td>sub. 2</td>
<td>10r 10c 9c</td>
<td>10r 10c 9c</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chylomicron</td>
<td>4 6</td>
<td>23.5 23.5 22.2</td>
<td>27.2 26.3 26.6</td>
<td>0.02 0.04</td>
</tr>
<tr>
<td>VLDL</td>
<td>6 6</td>
<td>7.3 6.9 9.4</td>
<td>16.1 16.5 17.0</td>
<td>0.14 0.14</td>
</tr>
<tr>
<td>LDL</td>
<td>6 8</td>
<td>4.6 4.7 7.2</td>
<td>9.2 9.3 14.1</td>
<td>0.19 0.21</td>
</tr>
<tr>
<td>HDL</td>
<td>4 8</td>
<td>5.0 5.2 7.1</td>
<td>10.5 11.5 16.1</td>
<td>0.27 0.23</td>
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<tr>
<td>Cholesteryl ester</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chylomicron</td>
<td>15 24</td>
<td>0.6 0.6 1.3</td>
<td>1.5 2.1 6.2</td>
<td>0.48 0.53</td>
</tr>
<tr>
<td>VLDL</td>
<td>12 12</td>
<td>0.9 0.6 1.3</td>
<td>3.0 2.5 6.3</td>
<td>0.22 0.37</td>
</tr>
<tr>
<td>LDL</td>
<td>24 24</td>
<td>0.3 0.4 1.1</td>
<td>2.4 1.5 5.7</td>
<td>0.36 0.64</td>
</tr>
<tr>
<td>HDL</td>
<td>12 24</td>
<td>0.4 0.5 1.4</td>
<td>1.9 2.6 6.0</td>
<td>0.45 0.46</td>
</tr>
<tr>
<td>Free fatty acid</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chylomicron</td>
<td>6 6</td>
<td>10.4 7.5 7.0</td>
<td>20.1 15.3 15.0</td>
<td>0.04 0.10</td>
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<td>VLDL</td>
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and 10c-18:1-d_2 and -d_4 fatty acids were detected in some plasma free fatty acid, and triacylglycerol samples. Desaturation products (18:2-d_2 and -d_4) were detected in a few plasma cholesteryl ester and phospholipid samples but the amounts of deuterated 20:1 and 18:2 were below 0.02 percent.

Discussion

Multiple-label techniques

The experimental design used in this study employed the approach of simultaneous feeding of the deuterium-labeled 10r- and 10c-18:1 isomers (experimental fatty acids) plus a deuterium-labeled 9c-18:1 (internal control). This approach is an extension of the dual-labeled radioisotope technique commonly used in animal studies. Advantages of the multiple isotope-labeled approach compared to single-isotope studies are (1) the subject serves as his own control and (2) difficult to control experimental parameters, such as diet, activity and subject variation, have identical influence on the experimental and control fatty acids, and analytical errors are compensated for by the use of ratio measurements. Thus, this technique is much better than single-isotope experiments for determination of differences in metabolism because it significantly reduces the problems associated with biological variation.
Assessment of the subjects metabolic and health status

The subjects used in this study were normal, young adult males with no health or metabolic problems. This judgment was based on standard clinical data which included blood cell populations, protein, glucose, triacylglycerol, total cholesterol, HDL cholesterol and lipoprotein distribution. Weight/height ratio, blood pressure medical history and a general physical examination were normal and did not indicate any medical problems existed. Dietary histories indicated that food selection was typical of American diets based on HANES and USDA food surveys [36,37]. The rate of absorption and disappearance of the control fatty acid (9c-18:1-d₆) and the maximum levels of incorporation of 9c-18:1-d₆ into plasma lipids were similar to those observed previously for seven other subjects [24-26]. In addition, the data for the Δ10-18:1 isomers were similar for both subjects (Fig. 2). These combined data indicate that the lipid metabolism of the subjects was representative of the norm for young adult males.

Absorption of 10t- and 10c-18:1 compared to 9c-18:1

The percentage of 10t-, 10c and 9c-18:1 in chylomicron triacylglycerol samples was nearly identical and provides good evidence that the absorption process does not significantly discriminate for or against the 10t- and 10c-18:1 isomers. The wide difference in melting points (see Table I) of the triacylglycerols containing the 10t-, 10c- and 9c-18:1 fatty acids was not a factor in their hydrolysis by pancreatic lipase and subsequent uptake by the intestinal mucosa cells. Substantially greater amounts of deuterium-labeled fats were found in the 2 h chylomicron triacylglycerol fraction from subject 1 compared to subject 2 and indicate that absorption and/or lymphatic transport of dietary fat was more rapid for subject 1.

Maximum total percent deuterated fatty acid incorporated into the 18:1 chylomicron triacylglycerol fractions were 75 and 85%. Since only deuterated fats were fed, the source of the 15-25% undeuterated 18:1 in these fractions is uncertain. The levels of dietary fat in the stomach, intestinal tract, lymphatic system and chylomicron triacylglycerols of serum should have been low, since the subjects were fasted for 10-12 h before feeding. Fatty acid composition data indicate that about 70% of the total chylomicron triacylglycerol fatty acids was labeled. This ratio of 1:2.33 would require dilution of the 26.6 g of labeled fats fed with 11.4 g of endogenous non-labeled fats.

Total fatty acids in plasma is approx. 0.11% for a fasting subject and total plasma volume is normally 39 ml/kg of body weight, or 2398 ml for subject 2 [38]. Thus, total plasma lipids for subject 2 was estimated at approx. 2.4 g based on the body weight (61.7 kg) of this subject. Since 27.4 g of deuterated fat (mainly as chylomicron triacylglycerol), would be expected to enter the circulation system, exchange between chylomicron and plasma triacylglycerol or contamination by traces of VLDL triacylglycerol due to incomplete separation of the lipoproteins could not provide sufficient triacylglycerol to explain the 30% undeuterated fat observed in the 6 h chylomicron triacylglycerol sample. Thus, the chylomicron triacylglycerol data are consistent with the concept of intestinal mucosa cells and/or lacteal spaces retaining significant quantities of triacylglycerol from earlier meals which mix with the isotope-labeled triacylglycerol during absorption and formation of chylomicron particles.

Incorporation of 10t- and 10c-18:1 compared to 9c-18:1 by plasma lipid classes

The area selectivity values for the major plasma lipid classes summarized in Fig. 4 represent incorporation of 10t- and 10c-18:1 relative to the internal standard (c-18:1-d₆). Negative values indicate discrimination against the 10-18:1 isomers and positive values indicate preferential incorporation relative to 9c-18:1. Discrimination against incorporation of 10t- and 10c-18:1 relative to 9c-18:1 occurred in six of the eight plasma fractions. This result requires that either β-oxidation or incorporation into tissue is higher for the Δ10-18:1 isomers. Since the isomeric fatty acid content in human tissue lipid classes reported by Ohlrogge et al. [15] did not indicate selective accumulation of the Δ10-18:1 isomers, higher β-oxidations rates are the most likely explanation for the negative selectivity values.

Selectivity values for the 1-acyl and 2-acyl positions of PC reflect a strong positive incorporation
for both 10r- and 10c-18:1 into the 1-acyl position and a strong discrimination against incorporation into the 2-acyl position. These results are summarized in Fig. 4 along with values obtained from previous studies with seven other 18:1 isomers [24-26]. The selective incorporation of 18:1 isomers into 1-acylPC is observed for all isomers and agrees well with in vitro and in vivo rat data [5-11,16,17]. Selectivity values for human plasma 2-acylPC indicate that discrimination against the 10r-18:1 isomer is about 3-times greater than for the 10c-18:1 isomer. The selectivity values for plasma cholesteryl ester are more negative than for 2-acylPC which is the substrate for lecithin:cholesterol acyl transferase. Thus, a comparison of the 2-acylPC and cholesteryl ester selectivity values for the 10c-18:1 isomers indicates lecithin:cholesterol acyl transferase activity for 10c-18:1 is significantly lower than for 10r-18:1 and is in contrast to data for the other cis positional isomers.

**Turnover of plasma lipids**

In addition to the percent enrichment data obtained in previous human studies with deuterium-labeled fatty acids, the improved MS methodology used in this study also provided actual concentration data. This data allowed a comparison between lipid class and isomeric fatty acid turnover on a weight basis which rigorously confirms the differences between the metabolism of the 10r-18:1 isomer vs. 9c-18:1 based on percent and ratio data. In addition, the weight data in Fig. 3 for the plasma lipid classes illustrate the two dramatically different sets of turnover rates for plasma lipid class fatty acids. Turnover rates for fatty acids in plasma triacylglycerol, free fatty acid and PE are similar and obviously significantly higher than rates for PC and cholesteryl ester fatty acids. In general, the selective utilization of the 10c- and 10r-18:1 isomers is more pronounced for lipid classes when turnover rates are relatively low. This difference in turnover rates and selectivities may reflect the different metabolic roles of these lipid classes. For example, triacylglycerol and free fatty acid are utilized mainly in lipid transport and as energy sources which require high turnover rates. In contrast, the PC and cholesteryl ester lipids are important structural components of membranes which require that their physical properties and, therefore, their fatty acid compositions be selectively controlled.

Calculation of turnover rates from these curves is complicated by the possibility of interchange of deuterated fats between the various lipid classes and lipoprotein fractions. For example, a major problem encountered with the calculation of plasma triacylglycerol turnover rates is that chylomicron triacylglycerols entering the circulatory system have short half-lives of 5-15 min [39,40]. Since the chylomicrons are a significant source of total plasma triacylglycerol, the sampling period of 2 h is not frequent enough to provide the data necessary to describe precisely the early portion of the plasma triacylglycerol curve.

However, an estimate of the total plasma triacylglycerol turnover rate is possible if the entire 27.4 g of triacylglycerol fed is assumed to enter the circulatory system over a relatively short (2 h) period. For subject 2, the calculated plasma volume was approx. 2400 ml which, when multiplied by the triacylglycerol concentration in the 6 h sample (1.1 mg/ml) and percent deuterated fat content (53%) at 6 h (see Fig. 2, subject 2), gives a calculated total deuterated triacylglycerol content of about 1.4 g. This value represents approx. 5% of the fed mixture. Based on isotope dilution of the chylomicron triacylglycerol fraction, (see earlier discussion), 11.4 g of undeuterated triacylglycerol is assumed to enter the circulation system along with the deuterated fats for a total of approx. 38.8 g of triacylglycerol which must be cleared over a 120 min period. This requires a minimum turnover rate for deuterated plus undeuterated triacylglycerol of around 320 mg/min or 5.18 mg/min per kg or 0.133 mg/min per ml plasma. If a 3 h total clearance time is assumed, a turnover rate of 3.47 mg/min per kg is required. These data for triacylglycerol turnover are higher than reported rates obtained by steady-state infusion of tritium-labeled glycerol. 14C- or 13C-labeled oleic and palmitic acids in humans and dogs [40-50]. However, low concentrations of fatty acids were infused in these studies and it is well recognized that
fatty acid turnover rates are linearly dependent on plasma fatty acid concentration which may also be true for triacylglycerols.

Change in plasma triacylglycerol concentration during dietary fat absorption

Our recent success in determining the absolute weight of both the labeled and unlabeled fatty acids in various plasma lipid classes produced some unexpected data on dietary fat absorption. The total labeled fatty acid concentrations listed in Table II for plasma triacylglycerols are as expected but the increase in the levels of non-labeled fatty acids was 1-2-times greater than the labeled fatty acid concentration. This large increase in the non-labeled fatty acids was unexpected, since only labeled fats were fed and the subjects had fasted for 10-12 h before consuming the meal containing the deuterated fats. The concentration of total undeuterated fatty acids in the 4 h plasma triacylglycerol samples (subject 1) and the 6 h plasma triacylglycerol samples (subject 2) were also 2-3-times the concentration at 0 h. To our knowledge, this effect has not been previously observed and it is difficult to explain, since the body is rapidly removing deuterated triglyceride from the blood system and there would appear to be no physiological need to mobilize triacylglycerol stores at this time.

A probable explanation for this result is that the intestinal mucosa cells contain a pool of triacylglycerol which is displaced from the cells by the deuterated fat meal. This concept is consistent with the percent enrichment data for the chylomicron triacylglycerol data (Fig. 1 and Table III). Based on the weight of deuterated fats fed and the enrichment data, the size of the intestinal triacylglycerol pool can be estimated at 10-12 g.

Lipoprotein data

The lipoprotein selectivity values and labeled fatty acid distribution data presented in Table III are provided for comparison to total plasma data. The selectivity values in Table III for specific lipoprotein lipids generally followed the same trends as values calculated for the corresponding plasma lipid classes and confirm the accuracy of the plasma lipid data. However, comparison of selectivity values and percent labeled 10r- and 10c-18:1 incorporated into the chylomicron, VLDL, LDL and HDL lipid classes indicate that the individual lipoprotein classes into which the isomeric fatty acid are incorporated have a definite but varying influence and depend on the lipid class as well as the specific isomer.

The result is a complex three-way interaction in which lipoprotein class, lipid class and double-bond configuration and position are factors. In addition, no one factor predominately controls the metabolic fate of an isomer but rather various factors have a varying impact which is apparently dependent on the specific metabolic process involved. For example, comparison of 10r and 10c selectivity data for the lipoprotein free fatty acid and PC fractions indicates that the double-bond configuration dominates the distribution of these isomers, whereas HDL has a major impact on triacylglycerol selectivity values and the lipid class is the major factor involved in determining the cholesteryl ester selectivity values.

Chain-shortening, desaturation and elongation of 10-, 10c- and 9c-18:1

The fed mixtures contained 35 to 65 mg of 16:1-d2, -d4 and -d6 which was about 0.15-0.20% of the total mixture. Consequently, the amounts of deuterated 16:1 fatty acids incorporated into the plasma lipoprotein lipids were very small and could only be quantitated in the triacylglycerol fractions. The curves in Fig. 5 for the individual lipoprotein triacylglycerol fractions and the total plasma triacylglycerol samples illustrate the quality of the GC-MS data for following the uptake and disappearance of the labeled 16:1 isomers in these samples. The higher 16:1-d2 and -d4 to 16:1-d6 ratios in these fractions compared to the ratios in the fed mixture indicate that chain-shortening of the correspondingly labeled 10r- and 10c-18:1 isomer occurs at a higher rate than for 9c-18:1-d6. A significantly lower turnover rate for the chain-shortened 16:1 isomer is an alternative but not a plausible explanation because the curves in Fig. 5 indicate that relative turnover rates for the deuterated 16:1 fatty acids are similar. This higher rate of chain-shortening of the Δ10-18:1 isomers to 16:1 is consistent with in vivo data reported for rats and chickens [6.16-19]. However, the wt./ml data demonstrate that the conversion rate of the Δ10-
18:1 fatty acids to 16:1 isomers is low, and that the amounts of 16:1 isomers formed are not sufficient to have significant nutritional impact.

In comparison to the 16:1 isomers, which were easily followed in the triacylglycerol fractions, the levels of deuterated 18:2 desaturation and 20 carbon chain-elongation products were much lower. In fact, the desaturase and elongase products were not detected consistently in any series of lipid class samples, although they were qualitatively identified in a number of samples. The interconversion of Δ10-18:1 isomers to 18:2-d and -d4 isomers and 20:1-d2 and -d4 isomers is consistent with in vitro data reported for rat studies using radioisotope-labeled fats [9-13], but our in vivo data suggest that the rates of desaturation and elongation of the 10-18:1 isomers are too low to have physiological significance.

A general conclusion from these data is that the metabolism of the Δ10-18:1 isomer was significantly different from 9c-18:1, but the differences are not as large as has been reported for animal models. It was also apparent that both subjects efficiently absorbed and metabolized the Δ10-18:1 isomers and that the enzymatic activities are within the limits observed for most other 18:1 isomers in hydrogenated vegetable oil.

Acknowledgements

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