A COMPARISON OF THE METABOLISM OF EIGHTEEN CARBON $^{13}$C-
UNSATURATED FATTY ACIDS IN HEALTHY WOMEN

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Running Title: $^{13}$C-fatty acid partitioning in women

Abbreviations: AP - atom percent, APE - atom percent excess, AUC - area under the curve, BMI - body mass index, CE - cholesteryl ester, FFA - free fatty acid, GC - Gas Chromatography, GC-
C-IRMS – GC-Combustion-Isotope Ratio Mass Spectrometry, PL - Phospholipid, U-$^{13}$C -
uniformly carbon-13 labelled.
Abstract

Altered utilisation of different dietary fatty acids may contribute to several chronic diseases including obesity, non-insulin dependent diabetes mellitus and cardiovascular disease. However, few comparative data are available to support this link so the goal of the present study was to compare the metabolism of $^{13}$C-oleate, $^{13}$C-$\alpha$-linolenate, $^{13}$C-elaidate, and $^{13}$C-linoleate through oxidation and incorporation into plasma lipid fractions and adipose tissue. Each tracer was given as a single oral bolus to six healthy women. Samples were collected over 8 days and $^{13}$C was analysed using isotope ratio mass spectrometry. At 9 h post-dose, cumulative oxidation was similar for $^{13}$C-elaidate, $^{13}$C-oleate and $^{13}$C-$\alpha$-linolenate (19 ± 1, 20 ± 4, 19 ± 3 % dose, respectively). Significantly lower oxidation of $^{13}$C-linoleate (12 ± 4 % dose; p<0.05) was accompanied by its higher incorporation into plasma phospholipids and cholesteryl esters. Abdominal adipose tissue was enriched with $^{13}$C-$\alpha$-linolenate, $^{13}$C-elaidate or $^{13}$C-linoleate within 6 h. Percent linoleate in plasma phospholipids positively correlated with $^{13}$C-linoleate and $^{13}$C-elaidate oxidation, indicating a potential role of background diet. Conversion of $^{13}$C-linoleate and $^{13}$C-$\alpha$-linolenate to longer chain polyunsaturates was a quantitatively minor route of utilisation.

Key words: Carbon-13, linoleic acid, $\alpha$-linolenic acid, elaidic acid, oleic acid, long chain polyunsaturated fatty acid, body fat, blood lipids, adipose tissue, fatty acid oxidation, dietary fat.
Linoleate (18:2n-6) and oleate (cis 18:1n-9) are the two predominant unsaturated fatty acids in the diet and are associated with several health benefits (1). α-Linolenate (cis 18:3n-3) is much less common in the diet, but also has well known health benefits, particularly against coronary artery disease and mortality (2,3,4,5). Elaidate, the main trans isomer of oleate (trans 18:1n-9), which is mainly formed by food industry hydrogenation of vegetable oils, is associated with health concerns, particularly a raised risk of hypercholesterolemia (6,7,8). Despite their different abundances in the diet and different health implications, structurally, these four fatty acids are broadly similar in having 18 carbons and 1-3 double bonds. Nevertheless, comprehensive, comparative information is lacking about their metabolism that could help account for their differing health attributes in humans. This could be especially helpful in identifying potential reasons why trans fatty acids may be hypercholesterolemic.

The overall objective of this study was to gain insight into the differential health effects of these four common 18 carbon dietary unsaturated fatty acids by tracing their metabolism in healthy women using uniformly carbon-13 (U-13C) labelled stable isotope tracers. This was a crossover study in which free living healthy subjects consuming a self-selected diet orally ingested a tracer dose of each of uniformly carbon-13 labelled (U-13C)-oleate, elaidate, linoleate, and α-linolenate in random sequential order with washout periods in between each tracer. 13C was measured in breath (β-oxidation), plasma fatty acids and adipose tissue (storage) by high precision gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). Thus, the amount of each tracer used for energy, esterified to different lipid classes in plasma, desaturated and chain elongated to long chain polyunsaturated fatty acids (PUFA) or stored in adipose tissue was measured over a period of eight days. MRI was used to quantify adipose
tissue volumes to obtain pool size. Relationships between abdominal subcutaneous fat volume, blood lipids, plasma fatty acid profiles and metabolism of these tracers were also determined to identify contributions of lifestyle, body composition, and background diet (dietary fatty acids).

MATERIALS AND METHODS

Subjects. Six healthy, normolipidemic, pre-menopausal women participated in the study (Table 1). Subjects were excluded if they had any known endocrine conditions, a body mass index (BMI) outside the healthy range of 20-27, or if they smoked. One subject was taking oral contraceptives, and another was taking milder hormonal therapy for acne control. Five of the six subjects had regular menstrual cycles (26-30 days) so were timed to be in the same phase of the cycle for all tracers. Approval for the study was obtained from the Research Ethics Board at the Hospital for Sick Children, Toronto. Written informed consent was obtained from all subjects and remuneration was provided.

Experimental Design. Baseline blood, breath and adipose tissue samples were collected at 8 am after a minimum 12 h fast and before tracer dosing. Subjects were then sequentially administered an oral bolus of [U-13C]-oleate (Isotec, Miamisburg, Ohio), [U-13C]-linoleate, [U-13C]-α-linolenate (both from Martek Biosciences, Columbia, MD), or [U-13C]-elaidate incorporated into a breakfast meal in random order with a three week washout between each. The [U-13C] elaidate was originally purchased as [U-13C]-oleic acid from Isotec and then isomerized by Dr. N. Ratnayake (Health Canada, Ottawa) according to the procedure of Snyder and Scholfield (9). With the exception of 13C-elaidate, all tracers were given as the free fatty acid, which was placed directly on a bagel containing a cream cheese spread via a 100 µl syringe (Hamilton, Reno, Nevada). 13C-Elaidate was administered as the methyl ester so that it would be liquid at room
temperature. The bagel and cream cheese (with tracer) were part of a breakfast meal which included fruit juice and a banana. The macronutrient composition of the breakfast was 17% fat, 9% protein, and 74% carbohydrate. On the first day of each study, identical, standardized, weighed, regularly timed meals were provided for each subject and each tracer study. The meals were consumed at 0 h (test meal) and 4, 7, and 10 h post-dose. The energy value of the meals given at these time points was 525, 653, 229, and 869 kcal, respectively. Starting the weekend before the study, subjects were given a list of foods that are naturally enriched in $^{13}$C, especially corn-based products, that were to be avoided during the study period (10). The effect of the natural $^{13}$C content in the meals provided on background breath $^{13}$CO$_2$ excretion was measured on two separate days in one subject and these background values were subtracted from the dosed values in all subjects.

On the first study day, five ml of blood was collected at two hour intervals through a heated intravenous hand vein line flushed with saline and heparin, drawn into 5 ml syringes and immediately transferred to EDTA-coated tubes (Becton Dickinson, Franklin Lakes, NJ) and placed on ice. Plasma was separated from erythrocytes by centrifuging at 2500 rpm at 4°C for 10 min and stored for no longer than 2 weeks at -20°C prior to analysis. Breath samples were collected hourly, in duplicate, directly into evacuated glass tubes using a breath collection device (EasySampler™, Quintron Instrument Co., Milwaukee, WI). The rate of CO$_2$ production was determined from a variable-flow indirect calorimeter (Vmax 29n; Sensormedics Corp., Yorba Linda, CA). This was performed 90 min after breakfast in the post-prandial period rather than at fasting due to the mainly post-prandial sampling points throughout the study day. Fasting samples of blood and breath were also collected at 24, 48, 72, and 168 h post-dose. Samples of subcutaneous fat (5-20mg) were taken at 6, 24, and 168 h post-dose from the anterior abdominal
wall near the umbilicus using gentle suction with a 18 gauge needle and 5 ml syringe (11), and transferred to a glass test tube filled with hexane until analysed.

**Tracer Protocol.** The chemical purity (measured in our lab) of the tracers was 93.9% for [U-$^{13}$C]-α-linolenate, 95.2% for [U-$^{13}$C]-linoleate, 94.4% [U-$^{13}$C]-oleate, and 74% for [U-$^{13}$C]-elaidate. Each tracer had an isotopic purity of >99%. The volume administered (55 µl) corresponded to 3.06 mmol $^{13}$C for α-linolenate, 2.91 mmol $^{13}$C for oleate, 2.99 mmol $^{13}$C for linoleate, and 2.73 mmol $^{13}$C for elaidate (0.69-0.91mg/kg body weight). These values include all $^{13}$C, including the contaminating fatty acids, and were used in the breath CO$_2$ calculations. However, for plasma and adipose tissue calculations from the GC-C-IRMS data, the actual mass of the $^{13}$C fatty acids of interest was used, excluding any contaminating fatty acids. This corresponded to 33.7 mg elaidate, 47.0 mg α-linolenate, 47.0 mg linoleate, and 46.7 mg oleate. The test meal contained 2665 mg of oleate, 152 mg α-linolenate, 860 mg linoleate, and 27 mg elaidate, resulting in tracer:tracee ratios of 0.018, 0.315, 0.055, and 1.25, respectively.

**Analytical Methods.** Plasma total cholesterol was determined using a colorimetric assay (Diagnostic Chemicals Limited, Charlottetown, PEI). Plasma triglyceride (TG) concentration was determined from GC analysis of plasma TG fatty acids using triheptadecanoic acid as the internal standard (Sigma Chemical Co., St. Louis, MO). Fatty acid composition of the freeze-dried test meal was determined in quadruplicate portions of the bagel and cream cheese (12). Plasma total lipids were extracted using a modified Folch procedure (13) with internal standards added before thin layer chromatography to separate the plasma lipid classes. Plasma phospholipids (PL) and cholesteryl esters (CE) were saponified separately, and all fatty acids were methylated with BF$_3$ in methanol. Fatty acid methyl esters were analysed using a GC (Hewlett Packard, Palo Alto, CA, USA, model 5890A) equipped with a fused capillary column.
(30 m x 0.25 mm x 0.25 μm DB-23; J&W Scientific, Folsom, CA, USA) as described previously (14). The identity of individual fatty acids was determined by comparing retention times with standard mixtures of fatty acids (NuChek 68A, NuChek 96; NuChek Prep Inc., Elysian, MN, USA and Supelco PUFA2, Sigma-Aldrich Canada Ltd., Mississauga, ON, Canada). The concentration of 13C-labelled fatty acid in the plasma lipid classes was calculated by multiplying the percent 13C enrichment above baseline (atom % excess, APE) by the concentration of dosed fatty acid.

Adequate separation of trans isomers was obtained by GC (Hewlett Packard, Palo Alto, CA, USA, model 6890) using a fused capillary column (100 m x 0.25 mm x 0.2 μm; SP2560; Supelco, Inc. Bellafonte, PA, USA) as described by Ratnayake et al (15). Samples were run at a constant flow, with an injection split ratio of 10:1. Isotopic analysis was performed by GC-C-IRMS with 100% CO2 as the reference gas. The GC (Hewlett Packard, Palo Alto, CA, USA, model 6890) was connected to a 800°C combustion interface (Orchid GC Interface Module, PDZ Europa Ltd., Crewe, UK) which was linked to the mass spectrometer (20-20 Stable Isotope Analyser, PDZ Europa Ltd., Crewe, UK). Isotope enrichment linearity and precision were determined by analysing serial dilutions of [1-13C]-palmitate (16:0) combined with increasing amounts of palmitate at natural 13C abundance. Fatty acid enrichment, with the exception of samples enriched with 13C-elaidate, was analysed with a fused capillary 30 m x 0.32 mm x 0.32 μm DB-23 column (J & W Scientific, Folsom, CA, USA) with an injection split ratio of 5:1. Injection and detector temperatures were 230°C and 240°C, respectively. Starting temperature was 135°C for 2 min, ramped at 4°C/min to 160°C for 5 min, then 6°C/min with 195°C the final temperature. 13C enrichment values were computed by the Orchid software (GC Post Processor v.2.3c; Europa Scientific, Crewe, UK) in units of atom percent (AP);
\[ AP = \left( \frac{[^{13}\text{C}]}{[^{13}\text{C}]+[^{12}\text{C}]} \right) \times 100 \]

APE was calculated by subtracting baseline AP from dosed values at each time point (t):

\[ \text{APE} = \text{AP}_t - \text{AP}_{\text{baseline}}. \]

Percent of dose /L of plasma was calculated using the tracer concentration at each time point, and the tracer dose:

\[ \% \text{ Dose/L Plasma} = \frac{\mu g^{13}\text{C fatty acid/L plasma} \times 100\%}{\text{Dose administered (}\mu g^{13}\text{C fatty acid)}} \]

The % dose/L plasma was calculated for each timepoint and was plotted against time (h).

The total area under the curve was calculated using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA, USA) to give a relative measure of the amount of label appearing over the 168 h time period of the study. The time to reach the maximum % dose/L for each individual subject in each lipid fraction was also averaged to give a measure of the peak enrichment.

For the $^{13}$C-elaidate-enriched samples, a \textit{cis/trans} capillary GC column was used (HP 23, 60 m x 0.32 mm x 0.25 μm) with an injection split ratio of 5:1. Injection and detector temperatures were 240°C and 250°C, respectively. The temperature program started at 170°C for 2 min, than increased at a rate of 4°C/min to 230°C, where it was held for 5 min. The chromatograms (for all but the plasma PL) had just one \textit{trans} fatty acid shoulder, which was assumed to be the total of 6-11t-18:1, with 12-14t-18:1 co-eluting with oleate. These assumptions were based on the chromatograms using the 100 m column, which showed a near baseline separation of 11t-18:1 from 12t -18:1. Plasma PL, unlike the other plasma lipids and adipose tissue, had two \textit{trans} fatty acid shoulders on the main \textit{trans} fatty acid isomer peak, due to the presence of 10t-18:1 as a small portion of the total \textit{trans} 18:1 isomers in plasma PL.
Comparison of the GC and the GC-C-IRMS chromatograms indicated that the first peak contained 6-9t-18:1 and the second contained 10t and 11t-18:1. A correction was then made to accommodate dilution of enrichment from the co-eluting peaks on the GC-C-IRMS chromatography. Calculated APE of $^{13}$C-elaidate was obtained by dividing the APE of the total trans peak (obtained from the GC-C-IRMS) by the % of elaidate of all the co-eluting trans isomers ($8t + 9t + 10t + 11t$ 18:1, obtained from the trans fatty acid analysis done by GC). Since only 8t and 9t-18:1 co-eluted in plasma PL, the fraction used was the proportion of 9t/8t+9t 18:1;

$$APE_{9t-18:1} = \frac{APE_{total}}{\frac{9t-18:1}{8t+9t+10t+11t 18:1}}$$

The concentration of $^{13}$C-elaidate in individual plasma lipid classes was then calculated as for the other tracers using this calculated APE value of elaidate.

The enrichment of $^{13}$C in breath CO$_2$ was analysed by continuous flow-IRMS with 5% CO$_2$ as the reference gas (20-20 stable isotope analyser, PDZ Europa Ltd., Crewe, UK). Samples were collected into evacuated tubes (Labco Ltd., Buckinghamshire, England). Software (Ancant System Ver.1.999s) calculated the AP relative to the previously calibrated 5% CO$_2$. Linearity and precision of isotope enrichment was assessed using serial dilutions and combustion of $^{13}$C-glycine. Precision was routinely very good at 0.04. Results are expressed as a % of administered dose expired per hour. Cumulative oxidation was calculated from the AUC of the % dose/hour versus time as was done for plasma (GraphPad Prism, v3.0):

$$% \text{dose/hour} = \left(\frac{APE \times \text{mmol total CO}_2 \text{ expired}/h}{\text{mmol }^{13}\text{C administered}}\right) \times 100\%$$

where mmol CO$_2$ = 22.4 mol/L $\times$ L CO$_2$ expired/h (indirect calorimetry), and
mmol $^{13}$C administered = mol wt fatty acid $\times$ mg fatty acid administered $\times$ number of $^{13}$C-labelled carbons.

**Magnetic Resonance Imaging.** To obtain total and regional body fat and lean tissue composition, whole body magnetic resonance images (MRI) taken with a General Electric 1.5 Tesla whole body scanner. From the feet to the fingertips, 41 images were taken while the subjects lay in a supine position (16). Ten mm thick images were acquired every 40 mm to obtain total, subcutaneous, visceral, and abdominal subcutaneous adipose tissue as well as lean tissue, and skeletal muscle volumes. Changes in body weight which occurred during the delay between the MRI and the tracer studies (0-18 months, -3.8 to +2.8 kg) was corrected for by assuming that 80% of weight gain or loss was from adipose tissue, with an even distribution over the whole body. Tracer enrichment in adipose tissue was calculated as:

$$\text{% dose present in abdominal subcutaneous adipose tissue (ASAT);}$$

$$= \frac{\text{APE}/100\times \%FA (ASAT biopsy)\times\text{ASAT(L)} \times 0.724 (\text{kg fatty acid/L adipose tissue})}{10^6} \text{mg}^{13}\text{C fatty acid administered}$$

where % fatty acid was determined by GC, volume of abdominal subcutaneous adipose tissue (ASAT) was determined by MRI, and 0.724 kg/L is the fatty acid density of adipose tissue (11).

**Plasma Tracer Kinetics.** The disappearance of $^{13}$C-labelled fatty acids from plasma was fitted to a one phase exponential decay curve using a non-linear regression formula (GraphPad Prism v3.0). The rate of disappearance was the rate constant, $K$, and the half-life ($h$) of the decay was $0.6932/K$. The time to peak enrichment was taken from the maximum calculated % dose/l for each individual subject in each tracer pool.

**Statistics.** All statistics were performed using the software package SigmaStat (v. 2.0, Jandell Scientific, San Rafael, CA). To determine differences in tracer oxidation, one way repeated
measures analysis of variance was performed, and Tukey’s test was used as the post hoc test.

Plasma fatty acid and adipose tissue enrichment were not normally distributed, therefore all other comparisons were analysed using the non-parametric Friedman Repeated Measures ANOVA on Ranks with Student-Newman-Keuls as the Post Hoc test. All correlations were performed using the non-parametric Spearman Rank Order Correlation test.

**Results**

**Tracer Oxidation.** $^{13}$C fatty acid oxidation peaked about 5 h post-dose for all tracers (Figure 1). The peak rate of oxidation was 4.9 % dose/h for $^{13}$C-elaidate, 4.4 % dose/h for $^{13}$C-oleate, 3.0 % dose/h for $^{13}$C-linoleate, and 4.5 % dose/h for $^{13}$C–α-linolenate, with $^{13}$C-linoleate values being significantly lower than those of the other three tracers (p<0.05; Figure 1A). Detectable $^{13}$C enrichment was present in breath CO$_2$ at 168 h in 22 of the 24 individual tracer studies.

Cumulative fatty acid oxidation determined from AUC results, was significantly lower for $^{13}$C-linoleate than for $^{13}$C–α-linolenate, $^{13}$C-elaidate or $^{13}$C-oleate at 9, 12, and 24 h (p<0.05) but not after that (Figure 1B). The % linoleate in plasma PL fatty acids (Table 2) correlated positively with cumulative oxidation of both $^{13}$C-elaidate and $^{13}$C-linoleate at 9 h post-dose ($r = 0.94$, p = 0.02, for both) and also at 12 h and 24 h post-dose for linoleate. The % α-linolenate in plasma PL fatty acids correlated with cumulative oxidation of $^{13}$C-elaidate at 12 and 24 h post dose ($r = 0.89$, p = 0.03).

**Tracer in Plasma Lipids.** The time course of $^{13}$C enrichment in plasma lipids is expressed as % dose/L plasma (Figure 2). AUC values over the 168 h study period are in Table 3. At the time of peak enrichment, the order of enrichment in plasma TG was $^{13}$C-oleate = $^{13}$C-elaidate > $^{13}$C-linoleate = $^{13}$C–α-linolenate (p<0.05). However, the AUC values in plasma TG differed
somewhat and were in the order of $^{13}$C- elaidate > $^{13}$C- oleate > $^{13}$C-linoleate > $^{13}$C-α-linolenate (Table 3). From the time of peak enrichment, tracer disappearance from plasma TG was exponential, with a half-life of 4-6 h, and no significant difference between tracers (Table 4). At 4 h post-dose (which was close to peak enrichment in plasma TG), $^{13}$C-linoleate in plasma TG and $^{13}$C-linoleate oxidation rate were inversely related ($r = -0.94$, $p = 0.02$), as was $^{13}$C-oleate oxidation compared $^{13}$C-oleate enrichment in the combined plasma lipid classes ($r = -0.94$, $p = 0.02$). Higher fasting plasma TG levels were associated with higher $^{13}$C tracer in plasma TG at 4 h post dose for $^{13}$C-linoleate ($r = 0.90$, $p = 0.03$), $^{13}$C-oleate ($r = 0.93$, $p = 0.02$) and $^{13}$C-elaidate ($r = 0.90$, $p = 0.02$).

In plasma PL, $^{13}$C enrichment of all four tracers peaked 6-10 h post-dose and then followed a one phase exponential decay pattern in all subjects ($r^2$ between 0.93-0.99; Figure 2). Time to peak enrichment was significantly shorter for α-linolenate (6 h) than for the other three tracers (8-10 h; $p<0.05$). Peak $^{13}$C-linoleate incorporation into plasma PL was 5-10 fold that of the other three tracers (1.46 ± 0.46 % dose/L plasma versus 0.15 ± 0.03 for oleate, 0.34 ± 0.12 for elaidate, and 0.13 ± 0.05 for $^{13}$C-α-linolenate). The rank order of AUC for these tracers in plasma PL, was: $^{13}$C-linoleate > $^{13}$C-elaidate > $^{13}$C-oleate > $^{13}$C-α-linolenate ($p<0.05$; Table 3). $^{13}$C-linoleate incorporation into plasma PL was 18 fold more than for $^{13}$C-α-linolenate, 8 fold more than for $^{13}$C-oleate, and 3.5 fold more than for $^{13}$C-elaidate (Table 2). $^{13}$C-Elaidate had the longest half-life in plasma PL and the slowest rate of decay (K), while $^{13}$C-α-linolenate had the shortest half-life (Table 4). The AUC for $^{13}$C-linoleate enrichment in plasma PL was negatively correlated with whole body lean tissue volume ($r = -0.94$, $p = 0.02$).

In plasma CE, there was both slower enrichment and slower non-exponential decay of all the tracers than in the other plasma lipid classes (Figure 2). Far more $^{13}$C-linoleate than the other
three tracers was incorporated into plasma CE (p<0.05; Table 3). Time to reach peak enrichment in plasma CE was significantly shorter for $^{13}$C-elaidate than the other three tracers (median of 6 h versus 12-24 h for other tracers). There was an inverse correlation between $^{13}$C-elaidate enrichment in CE and the percent elaidate in both plasma PL ($r = -1.00$, $p = 0.003$) and in adipose tissue fatty acids ($r = -0.94$, $p = 0.02$). The AUC for $^{13}$C-linoleate enrichment in plasma CE negatively correlated with both total ($r = -0.89$, $p = 0.03$) and subcutaneous ($r = -0.94$, $p = 0.02$) adipose tissue volume.

$^{13}$C enrichment in plasma FFA peaked 2-4 h post-dose, with peak values significantly higher for $^{13}$C-oleate than for $^{13}$C-linoleate or $^{13}$C-α-linolenate (Figure 2; no data were obtained for $^{13}$C-elaidate in plasma FFA). Based on the AUC, $^{13}$C-α-linolenate had the lowest enrichment in FFA (p<0.05; Table 3).

When data from each of the three individual esterified plasma lipid classes studied were combined (TG+PL+CE), the total AUC for $^{13}$C-linoleate was 4-10 fold higher than for the other three tracers, with $^{13}$C-elaidate more than $^{13}$C-oleate, followed by $^{13}$C-α-linolenate (p<0.05). The decay curves for $^{13}$C in plasma total lipids were exponential, with $^{13}$C-linoleate having about a four fold longer half-life (24 h) than the other three tracers (Table 4).

**Tracer Desaturation and Chain Elongation.** Amongst the ω3 long chain PUFA derived from $^{13}$C-α-linolenate, peak $^{13}$C enrichments in plasma TG were highest for $^{13}$C-eicosapentaenoate (0.05 % dose/L plasma) and lowest for $^{13}$C-docosahexaenoate (0.006 % dose/L plasma; Figure 3). $^{13}$C enrichment values for the intermediate n-3 long chain PUFA, 20:3n-3 and 20:4n-3, could not be calculated due to low or undetected tracee concentrations. In plasma PL, peak $^{13}$C enrichments (% dose/L plasma) ranged from 0.012 for $^{13}$C-eicosapentaenoate to 0.0028 for $^{13}$C-docosahexaenoate (Figure 3). In plasma CE, the only $^{13}$C-enriched long chain PUFA derived
from $^{13}\text{C}-\alpha$-linolenate that was detected was $^{12}\text{C}$-eicosapentaenoate. When the n-3 long chain
PUFA in all four plasma lipid classes were combined at each time point, the AUC was 1.53, 0.56, 0.34 % dose/L plasma for $^{13}\text{C}$-eicosapentaenoate, $^{13}\text{C}$-ω3 docosapentaenoate and $^{13}\text{C}$-docosahexaenoate, respectively. Summing the plasma lipid enrichments together, $^{13}\text{C}$-eicosapentaenoate reached a plateau at 24-48 h post-dose, $^{13}\text{C}$-ω3-docosapentaenoate plateaued 48-72 h post-dose, while enrichment in $^{13}\text{C}$-docosahexaenoate were still increasing at the last time point studied (168 h post-dose), leading to a potential underestimation of $^{13}\text{C}$-docosahexaenoate formation.

For n-6 long chain PUFA derived from $^{13}\text{C}$-linoleate in plasma PL, $^{13}\text{C}$ enrichment was 0.010 % dose/L plasma for $^{13}\text{C}$-dihomo-γ-linolenate (20:3ω6) and 0.005 for $^{13}\text{C}$-arachidonate (20:4n-6), which was either still increasing or plateauing at the last time point (168 h post-dose; Figure 3). In plasma CE in four of the six subjects, $^{13}\text{C}$ enrichment was detected in $^{13}\text{C}$-γ-linolenic acid (18:3ω6), which first appeared at 10 h, and peaked at 24-48 h post-dose. The mean peak $^{13}\text{C}$ enrichment (% dose/L plasma) was 0.004 for $^{13}\text{C}$-γ-linolenic acid, 0.002 for $^{13}\text{C}$-dihomo-γ-linolenate, and 0.004 for $^{13}\text{C}$-arachidonate (Figure 3). AUC values (% dose/L plasma) for $^{13}\text{C}$-enriched n-6 long chain PUFA derived from $^{13}\text{C}$-linoleate were 0.33 for $^{13}\text{C}$-γ-linolenic acid, 1.35 for $^{13}\text{C}$-dihomo-γ-linolenate, and 0.94 for $^{13}\text{C}$-arachidonate. Significant enrichment was observed for $^{13}\text{C}$-ω6 eicosadienoate (20:2n-6). However, this peak co-eluted with 20:3n-9, so quantitative enrichments could not be obtained. No $^{13}\text{C}$ was detected in adrenate (22:4n-6) or ω6 docosapentaenoate (22:5n-6) during the time course of this study.

**Adipose Tissue.** $^{13}\text{C}$-enrichment in adipose tissue was measured for each tracer except $^{13}\text{C}$-oleate, for which the high oleate content in adipose tissue caused excess $^{13}\text{C}$ dilution (Table 3). Adipose tissue enrichment after dosing with $^{13}\text{C}$-α-linolenate is reported in detail because it had
the highest tracer/tracee ratio (Figure 4). Maximal $^{13}$C enrichment at 6 h post-dose was seen in all subjects, after which it either plateaued ($n=4$) or started to decrease ($n=2$). Between 2 and 11% of the $^{13}$C-$\alpha$-linolenate was present as such in abdominal fat at 6 h post-dose. Based on the calculated fat content of the whole body, this would have been 15-81% of the administered dose. At 24 h post-dose, 2-9% of the $^{13}$C-$\alpha$-linolenate dose was in the abdominal fat (13-70% on a whole body fat basis). By 168 h post-dose, 0.6-8% of the $^{13}$C-$\alpha$-linolenate dose was in the abdominal region (or 4-57% on a whole body fat basis). The whole body fat calculations make the potentially inaccurate assumption that the tracer was taken up similarly in all adipose tissue regions.

$^{13}$C-elaidate enrichment in abdominal adipose tissue increased throughout the 168 h study period, averaging 4% of dose at 6 h, 6% at 24 h, and 8% at 168 h post-dose. There was a trend towards greater $^{13}$C enrichment at 168 h after $^{13}$C-elaidate than after either $^{13}$C-linoleate or $^{13}$C-$\alpha$-linolenate ($p=0.11$) with no statistical difference at 6 or 24 h post-dose (Figure 5). At 6 h post-dose, although adipose tissue $^{13}$C enrichment was 2-3 times higher for $^{13}$C-linoleate than the other two tracers, these values were not significantly different. At 24 h post-dose, adipose tissue $^{13}$C enrichment differences between tracers were small (4, 6, and 6% dose for $^{13}$C-$\alpha$-linolenate, $^{13}$C-linoleate, and $^{13}$C-elaidate, respectively). There was no significant relationship between abdominal adipose tissue volume and tracer enrichments in plasma or CO$_2$. However, at 24 h post-dose, both total adipose tissue ($r = -1.00$, $p = 0.003$) and total subcutaneous adipose tissue volumes ($r = -0.94$, $p = 0.02$) correlated negatively with $^{13}$C-elaidate enrichment in adipose tissue. $^{13}$C-Elaidate enrichment in adipose tissue at 6 h post-dose correlated negatively with cumulative $^{13}$C-elaidate oxidation at 12 h post-dose ($r = -0.89$, $p = 0.03$) which was not seen for linoleate, or $\alpha$-linolenate.
Figure 5 shows the whole body distribution of $^{13}$C as % dose for all tracers (excluding oleate) in breath, total esterified plasma lipids, and abdominal adipose tissue. At both 6 and 24 h post-dose, the plasma and adipose tissue compartments made up a higher proportion of whole body enrichment in $^{13}$C-elaidate and $^{13}$C-linoleate than for $^{13}$C-α-linolenate. Significantly lower $^{13}$C-linoleate oxidation at +6 h was offset by its higher incorporation in both plasma and adipose tissue; however, these differences were not significant. By 168 h post-dose, most of the $^{13}$C from all three tracers had disappeared as $^{13}$CO$_2$ in breath, and little remained in plasma lipids.

**DISCUSSION**

This is the first description of the metabolism of labelled fatty acids that combines analysis of $^{13}$C appearing in adipose tissue, plasma lipid fractions and excretion in breath. Although the comparative oxidation (17), and the simultaneous measurement of incorporation into plasma lipid classes as well as the conversion of linoleate and α-linolenate to their desaturated and chain elongated products (18,19,20,21) has previously been described, tracing enrichment in humans of all three compartments at once (plasma, adipose tissue and breath CO$_2$) has not been done, limiting the opportunity to understand the whole body utilization of these fatty acids. Our results show that after oral ingestion of these common fatty acids, adipose tissue is rapidly and extensively labelled. Under our experimental conditions, no major differences in abdominal subcutaneous adipose tissue uptake of linoleate, elaidate or α-linolenate were discernible.

Our results concerning the quantitative recovery of $^{13}$C in breath CO$_2$ during the first 24 h post-dose and the rank order of oxidation of these four common dietary fatty acids agree with similar previously published results (22,23,24,25). Tracer studies reporting breath data do not...
often extend beyond 24 h but in 22 of the 24 breath tracer profiles in the present study, $^{13}$C enrichment in breath CO$_2$ was still above baseline at 168 h (8 days) post-dose. In fact, as reported elsewhere (26,27,28), the period between 24-168 h post-dose accounts for a significant proportion of the excretion on breath of common dietary fatty acids. Although much of this slow $^{13}$C recovery in breath could be due to retention of labelled CO$_2$ in the bicarbonate pool, the continued detectable enrichment in plasma and adipose tissue demonstrates that significant amounts of utilizable tracer fatty acid was still present in the body long after the first 24 hours post-dose. It is also important to note that some $\beta$-oxidised fatty acids may have been recycled to other fatty acids or cholesterol, such that the $^{13}$C would not appear in breath CO$_2$.

The slower oxidation of linoleate compared to these other 18 carbon unsaturated fatty acids deserves comment. It is intermediate in pool size between oleate (higher) and $\alpha$-linolenate or elaidate (both much lower), yet was oxidized more slowly than these other fatty acids (Figure 1). Data from Bretillon et al (25), who compared the oxidation of TG enriched with [1-$^{13}$C]-$\alpha$-linolenate and [1-$^{13}$C]-linoleate as well as their trans counterparts, [1-$^{13}$C]-9cis, 12trans-linoleate and [1-$^{13}$C]-9cis, 12cis, 15trans- $\alpha$-linolenate in healthy men, implicates a stereochemical effect in the slower oxidation of linoleate. The trans forms of $\alpha$-linolenate were oxidized similarly to the all cis form, however, the trans and cis forms of linoleate were oxidized differently. As well, in the present study, oxidation of oleate and its trans isomer elaidate were not different. Either the shape of linoleate seems to enhance it ability to be esterified, or reduces its affinity for oxidative pathway. Emken et al (29), showed that the incorporation of deuterated 12cis, 15trans-linoleate into plasma CE and phosphatidylcholine was more similar to the saturated fatty acids and oleate than to that of all cis-linoleate. Therefore, unlike the other fatty acids, conversion of a
cis to a trans double bond in linoleate dramatically affects its metabolism, thus indicating that its particular affinity for incorporation into plasma PL probably depends on its stereochemistry.

In the present study, the percent of linoleate and α-linolenate in plasma PL fatty acids correlated positively with the oxidation of 13C-elaidate, and linoleate was associated with its own oxidation. Since higher linoleate and α-linolenate in plasma PL is usually due to their higher dietary intake, diets rich in these two PUFA would seem to increase oxidation and reduce adipose tissue retention of trans fatty acids. Previously, consumption of a high versus low polyunsaturated:saturated 30% fat diet led to a higher oxidation of labelled palmitate and decanoate (30). In addition, postprandial fatty acid oxidation, as measured by indirect calorimetry, was higher on a high linoleic acid: saturated fat diet in eight normal weight adults (31). As a potential mechanism, high PUFA diets are known to regulate gene transcription (32) by simultaneously inducing the transcription of genes involved in thermogenesis and fatty acid oxidation (e.g. mitochondrial uncoupling protein-3, CPT, acyl CoA oxidase) while suppressing the transcription of lipogenic genes (e.g. acetyl CoA carboxylase, fatty acid synthase). Our results suggest that diet rich in linoleate, or α-linolenate should promote more fatty acid oxidation and should help reduce the tissue accumulation of trans fatty acids like elaidate. A health consequence would be that the fatty acid composition of the rest of the diet may ameliorate some of the deleterious health effects of elaidate by increasing its removal in the postprandial period.

As shown previously (20,21), a lower number of double bonds in the fatty acids we studied was inversely related to their abundance in plasma TG, i.e. oleate > linoleate > α-linolenate (Figure 2, Table 3). Other experimental models, including human hepatoma cells (33), hepatocytes from neonatal and adult rats (34) and perfused rat liver (35) have also shown
an inverse relationship between amount of enrichment in TG with degree of unsaturation of long
chain fatty acids. In terms of cis/ trans conformation, elaidate is incorporated into TG to a
greater extent than oleate when an intestinal cell line is used (36), whereas in a hepatic cell line,
similar or lower incorporation occurs (37,38), which may indicate differences in chylomicron
incorporation. With these differences in TG fatty acid enrichment, the potential implication is
that either high linoleate or \(\alpha\)-linolenate diets would chronically induce lower fasting plasma TG
levels than high elaidate or oleate diets. Also, in response to test meals with either high LA or
ALA a lower postprandial TG response than diets high in elaidate or oleate would be seen (short
term). Although feeding trials have examined both of these chronic and acute responses to
dietary fatty acids, much of the results are inconclusive.

Our present results demonstrate that, compared to \(\alpha\)-linolenate, oleate, or elaidate,
linoleate is preferentially esterified to plasma PL and CE. This agrees with the higher proportion
of linoleate than these other fatty acids that is normally found in plasma PL and CE (Table 2).
Since plasma fatty acid profiles are a marker of tissue fatty acid profiles, greater acylation into
plasma (and tissue) PL and CE could explain the greater whole body retention rather than
oxidation of linoleate. Whether this decreased oxidation of linoleate is due to its higher affinity
for the glycerolipid pathway or lower affinity for carnitine palmitoyl transferase, either of which
would lead to less linoleate being available for oxidation, is unknown. Fatty acyl CoAs are
partitioned between either oxidation or glycerolipid (PL and TG) synthesis with the latter being
formed from a common substrate, diacylglycerol (39,40). Therefore, the results from the present
study indicate that linoleate, perhaps through a combination of being a poorer substrate for
oxidation, or being a better substrate for PL acyltransferases, is diverted towards the PL pathway,
without particularly high incorporation into TG.
Previously, only two in vivo studies in humans, both using $^{14}$C fatty acids, have reported tracer enrichment in adipose tissue. Ormsby et al (41) injected intravenous [1-$^{14}$C]-linoleate bound to albumin into three men and measured $^{14}$C enrichment in adipose tissue. The average enrichment was 9% of dose at 10 min, 20% at 4.5 h, 10% at 10 h, and 11% at 24h, values that are similar to ours (Figure 5). Marin et al (42) reported the pre- and post-prandial femoral and abdominal adipose tissue uptake of [U-$^{14}$C] oleate in 16 healthy premenopausal women. At 4 h post-dose, 23% was incorporated into adipose tissue (average of both sites), up to 36% at 24 h, with a further slight increase up to one month post-dose. Both of these studies demonstrate that uptake of fatty acids in human adipose tissue in the postprandial phase is rapid but that mobilisation of tracer fatty acids from fat is relatively slow. Importantly, different adipose tissue regions may take up these fatty acids in different amounts; as well as having a higher turnover, abdominal adipose tissue had about 15% higher uptake of tracer at 4 h post-dose than did femoral adipose tissue (42). In the present study, the $\alpha$-linolenate data were the most reliable, since its tissue pool size is fairly small, creating less dilution of the tracer and consequently higher $^{13}$C $\alpha$-linolenate enrichments above baseline. On a whole body basis, we observed similar time courses and % incorporation of $^{13}$C -$\alpha$-linolenate (33% at +6 h, 29% at +24 h and 24% at +168 h) as these other two studies (41,42).

The partitioning of dietary fat between oxidation and storage in adipose tissue potentially plays a role in the mechanism underlying hyperlipidemia and obesity. In support of this, hyperlipidemic men had four times more $^{14}$C LA in TG and also oxidised 30% less of the tracer than normolipidemics (43). Similarly, in this study for three of four tracers, there was an association between fasting TG and tracer presence in TG. In terms of obesity, adipose tissue postprandially clears and stores more fat from non-diabetic obese women than from lean
females, accompanied by a higher postprandial insulin and an earlier acylation stimulating protein (ASP) response (44).

In summary, under our study conditions, the major route of utilization of all four of the tracer fatty acids was oxidation, followed by storage in adipose tissue. Breath CO₂ accounted for between 46 and 71% of the administered dose. Abdominal adipose tissue accumulated between 5-12% of dose administered, which translates to ~30-70% on a whole body adipose tissue basis.

Maximal incorporation of tracer into plasma ranged from 3-8% depending on the tracer administered. The ω3 and ω6 long chain PUFA in plasma had a maximum value of <0.1% of the administered dose, values which agree with other recent data (45,46). These net desaturation-chain elongation values are derived from the plasma pool but should at least qualitatively mirror the tissue incorporation of these fatty acids and their long chain products.

Beynan and Katan (47) have previously suggested that fatty acids which are retained rather than oxidised have a hyperlipidemic effect, due to being available for VLDL output by the liver, which consequently may result in lowered plasma LDL. This hypothesis results primarily from a comparison of the metabolism of saturated and unsaturated fatty acids in the literature. In the present study, elaidate, which has not been studied as extensively as some of the saturated fatty acids, particularly 16:0, was studied rather than a saturated fatty acid as a “hyperlipidemic” fatty acid. The results in the present study indicate that this hypothesis is perhaps a simplistic view. Linoleic acid, which has long been known to have potent blood cholesterol lowering effects and HDL-C raising effects, was less oxidised, particularly in the postprandial period, and was retained more in plasma lipid than the other three tracers. Conversely, a higher plasma phospholipid linoleic acid was associated with greater oxidation of fatty acids. However, the strength of the present study, is the examination of tracer incorporation into the other plasma
lipid fractions. Linoleic acid was markedly incorporated into plasma PL, which may be a marker of tissue membrane uptake and subsequently into CE. However, as compared to the other fatty acids, it was not incorporated to a great extent into plasma TG. Elaidate, on the other hand, which was oxidised similarly to oleate and \(\alpha\)-linolenate, had the highest incorporation into plasma TG. Also, it was esterified quite poorly to cholesterol, which may be a factor in reverse cholesterol transport and elaidate’s HDL-C lowering effect.
ACKNOWLEDGEMENTS

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linoleic, and linolenic acids by male subjects. *Lipids.* **34**: 785-791.


acid chain length and saturation on the gastrointestinal handling and metabolic disposal


Figure Captions.

Figure 1. Time course of $^{13}$C enrichment in breath CO$_2$ after an oral dose of [U-$^{13}$C] linoleate, oleate, α-linolenate, and elaidate given to six healthy women. Panel A is the % of administered dose recovered hourly in breath CO$_2$. Panel B is the cumulative oxidation determined from area under the curve calculations from the hourly % oxidation. Data points are means ± SD.

Figure 2. Time course of $^{13}$C enrichment data in plasma triglyceride (TG), phospholipids (PL), Cholesteryl esters (CE) and free fatty acids (FFA) in six healthy women given [U-$^{13}$C] linoleate, (●), oleate (■), α-linolenate (▲), and elaidate(◇). Data are normalised for the concentration of fatty acids in plasma and the tracer dose administered. Data points are means ± SD. Note that the axes are scaled differently.

Figure 3. Time course of $^{13}$C enrichment in desaturation-chain elongation products in plasma triglyceride (TG), phospholipids (PL) and cholesteryl esters (CE) after an oral dose of [U-$^{13}$C] linoleate (Left side) or [U-$^{13}$C] α-linolenate (Right side) in six healthy women. Data points are means ± SD.

Figure 4. Time course of $^{13}$C enrichment in abdominal adipose tissue after an oral dose of [U-$^{13}$C] α-linolenate showing data for individual subjects. Panel A shows the mean atom % excess at 6, 24, and 168 h after dosing. Data are means ± SD for between 2-4 sample injections. Panel B is the $^{13}$C enrichment data normalised for the concentration of fatty acids in adipose tissue and the amount of tracer administered.
Figure 5. Distribution of $^{13}$C-labelled fatty acids between total plasma lipids, adipose tissue, and breath $^{13}$CO$_2$ 6, 24, and 168 h after an oral dose of three fatty acid tracers. Data are means ± SD for 6 subjects.
Table 1

Characteristics of study subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>28.7 ± 4.9 (22-36)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.0 ± 6.3 (54-71)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164 ± 3.8 (159-168)</td>
</tr>
<tr>
<td>BMI²</td>
<td>24.1 ± 2.3 (20.5-27)</td>
</tr>
<tr>
<td>Plasma Triglyceride (mmol/L)³</td>
<td>0.6 ± 0.3 (0.4-1.1)</td>
</tr>
<tr>
<td>Plasma Total Cholesterol (mmol/L)⁴</td>
<td>4.5 ± 0.6 (3.8-5.2)</td>
</tr>
<tr>
<td>% Body Fat⁵</td>
<td>25.1 ± 2.5 (20.7-28.2)</td>
</tr>
</tbody>
</table>

¹Data are the mean ± SD, range in brackets.

²Body Mass Index = weight (kg) / height (m²)

³Plasma triglyceride values are the means of all fasting samples.

⁴Plasma cholesterol values are the average of fasting blood samples taken in the first week of the study.

⁵Determined from magnetic resonance images, assuming adipose tissue is 82% water (Cunnane et al 2001)
Table 2

Percent composition of fatty acids in plasma lipid fractions and adipose tissue.

<table>
<thead>
<tr>
<th>Fatty Acid&lt;sup&gt;1,2,3&lt;/sup&gt;</th>
<th>Phospholipid</th>
<th>Triglyceride</th>
<th>Cholesteryl Ester</th>
<th>Free Fatty Acid</th>
<th>Adipose Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Σ Saturates</td>
<td>39.77 ± 4.31</td>
<td>27.68 ± 3.98</td>
<td>13.62 ± 2.33</td>
<td>35.85 ± 4.69</td>
<td>25.16 ± 1.59</td>
</tr>
<tr>
<td>18:1n-9cis</td>
<td>10.41 ± 0.56</td>
<td>36.78 ± 2.97</td>
<td>18.48 ± 1.73</td>
<td>38.21 ± 2.54</td>
<td>41.69 ± 1.37</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>13.39 ± 0.60</td>
<td>44.27 ± 3.56</td>
<td>22.77 ± 2.70</td>
<td>44.40 ± 2.36</td>
<td>49.49 ± 1.53</td>
</tr>
<tr>
<td>18:1n-9trans&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.42 ± 0.21</td>
<td>0.74 ± 0.27</td>
<td>0.049 ± 0.02</td>
<td>ND</td>
<td>1.11 ± 0.29</td>
</tr>
<tr>
<td>Σ 18:1trans&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2.18 ± 1.12</td>
<td>2.55 ± 0.92</td>
<td>ND</td>
<td>ND</td>
<td>4.26 ± 1.48</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>24.31 ± 5.21</td>
<td>18.33 ± 3.17</td>
<td>52.89 ± 6.01</td>
<td>13.33 ± 0.90</td>
<td>15.43 ± 1.80</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>3.37 ± 0.62</td>
<td>0.30 ± 0.03</td>
<td>0.79 ± 0.15</td>
<td>0.17 ± 0.03</td>
<td>0.17 ± 0.09</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>10.34 ± 2.25</td>
<td>1.13 ± 0.31</td>
<td>6.44 ± 1.48</td>
<td>0.61 ± 0.17</td>
<td>0.33 ± 0.06</td>
</tr>
<tr>
<td>Σ n-6 PUFA</td>
<td>39.33 ± 4.98</td>
<td>20.31 ± 3.20</td>
<td>60.98 ± 5.31</td>
<td>14.69 ± 0.97</td>
<td>16.25 ± 1.96</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.42 ± 0.25</td>
<td>1.64 ± 0.53</td>
<td>0.96 ± 0.43</td>
<td>1.21 ± 0.15</td>
<td>1.45 ± 0.33</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>1.51 ± 1.19</td>
<td>0.48 ± 0.73</td>
<td>0.85 ± 0.32</td>
<td>0.09 ± 0.10</td>
<td>0.04 ± 0.05</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>3.82 ± 1.17</td>
<td>0.92 ± 1.04</td>
<td>0.60 ± 0.27</td>
<td>0.41 ± 0.37</td>
<td>0.13 ± 0.05</td>
</tr>
<tr>
<td>Σ n-3 PUFA</td>
<td>6.64 ± 1.71</td>
<td>3.87 ± 1.41</td>
<td>2.60 ± 0.68</td>
<td>2.17 ± 0.76</td>
<td>2.11 ± 0.41</td>
</tr>
</tbody>
</table>

<sup>1</sup>Data are calculated as the percent of identified fatty acids.

<sup>2</sup>Only fatty acids of interest are shown, however sums of subclasses include all fatty acids.

<sup>3</sup>Data are means ± SD of all 6 subjects, taken at baseline on day 1 of the study.

<sup>4</sup>The trans fatty acid data were analysed on a different column using blood samples from the elaidate tracer study only (see Methods).

ND, Not Determined; trans analysis was not performed on the FFA fraction. Also a total 18:1t value was not obtained for CE.
Table 3

Area under the curve (AUC) for plasma tracer incorporation.

<table>
<thead>
<tr>
<th></th>
<th>mg $^{13}$C/ L Plasma/mg $^{13}$C administered (AUC)(^1)^ (^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phospholipid</td>
</tr>
<tr>
<td>Linoleate</td>
<td>40.2(^a) (31.9-46.4)</td>
</tr>
<tr>
<td>$\alpha$-Linolenate</td>
<td>2.4(^b) (2.0-2.6)</td>
</tr>
<tr>
<td>Oleate</td>
<td>5.1(^c) (3.9-6.5)</td>
</tr>
<tr>
<td>Elaidate</td>
<td>11.9(^d) (9.1-12.5)</td>
</tr>
</tbody>
</table>

\(^1\)Values are Medians, bracketed values are the 25\(^{th}\)-75\(^{th}\) percentiles.

\(^2\)Values with different numbered superscripts within each column are significantly different p < 0.05 (Friedman Repeated Measures ANOVA on Ranks with Student-Newman-Keuls as the post-hoc test).
Table 4

Kinetics of Tracer disappearance (1/2 life, h)\textsuperscript{1-4}.

<table>
<thead>
<tr>
<th></th>
<th>Phospholipid</th>
<th>Triglyceride</th>
<th>Total Plasma (PL+TG+CE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linoleate</strong></td>
<td>13.8\textsuperscript{a}</td>
<td>4.7</td>
<td>24.2\textsuperscript{a}</td>
</tr>
<tr>
<td>(12.7-14.7)</td>
<td>(4.5-5.1)</td>
<td>(19.4-28.8)</td>
<td></td>
</tr>
<tr>
<td><strong>α-Linolenate</strong></td>
<td>9.7\textsuperscript{b}</td>
<td>6.4</td>
<td>6.2\textsuperscript{b}</td>
</tr>
<tr>
<td>(7.3-12.3)</td>
<td>(5.6-6.9)</td>
<td>(5.8-10.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Oleate\textsuperscript{5}</strong></td>
<td>16.6\textsuperscript{a}</td>
<td>4.9</td>
<td>7.1\textsuperscript{b}</td>
</tr>
<tr>
<td>(14.0-18.0)</td>
<td>(3.8-5.9)</td>
<td>(5.8-9.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Elaidate\textsuperscript{6}</strong></td>
<td>19.1\textsuperscript{c}</td>
<td>4.0</td>
<td>5.9\textsuperscript{b}</td>
</tr>
<tr>
<td>(15.4-22.7)</td>
<td>(3.3-4.1)</td>
<td>(4.8-6.2)</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1}Values are medians, bracketed values are the 25\textsuperscript{th}-75\textsuperscript{th} percentiles.

\textsuperscript{2}Values with different lettered superscripts within each column are significantly different, p < 0.05 (Friedman Repeated Measures ANOVA on Ranks with Student-Newman-Keuls as the post-hoc test).

\textsuperscript{3}Exponential decay curves were performed on a per subject basis, calculations were performed using a one phase exponential decay model, where K is the disappearance rate constant, and the half-life is 0.6932/K.

\textsuperscript{4}Clearance of tracer in CE and FFA lipid fractions did not fit an exponential decay pattern.
Linoleate is significantly lower at 9, 12, and 24h, p<0.05

Figure 1
Figure 2
Figure 3

Time Post-Dose (h)

n-3 Polyunsaturates

TG

n-6 Polyunsaturates

TG

% Dose / L Plasma (x10^-3)

PL

CE

PL

CE

% Dose Administered / L Plasma

0 12 24 36 48 60 72 170

0 12 24 36 48 60 72 170

0 12 24 48 72 96 120 144 168

0 12 24 48 72 96 120 144 168

0 24 48 72 96 120 144 168

0 24 48 72 96 120 144 168

0 24 48 72 96 120 144 168

0 24 48 72 96 120 144 168
figure 4
Figure 5