Effect of Dietary Conjugated Linoleic Acid (CLA) on Metabolism of Isotope-Labeled Oleic, Linoleic, and CLA Isomers in Women

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ABSTRACT: The purpose of this study was to investigate the effect of dietary CLA on accretion of 9*c*-18:1, 9*c*,12*c*-18:2, 10*t*,12*c*-18:2, and 9*c*,11*t*-18:2 and conversion of these FA to their desaturated, elongated, and chain-shortened metabolites. The subjects were six healthy adult women who had consumed normal diets supplemented with 6 g/d of sunflower oil or 3.9 g/d of CLA for 63 d. A mixture of 10*t*,12*c*-18:2-d_4, 9*c*,11*t*-18:2-d_6, 9*c*-18:1-d_8, and 9*c*,12*c*-18:2-d_2, as their ethyl esters, was fed to each subject, and nine blood samples were drawn over a 48-h period. The results show that dietary CLA supplementation had no effect on the metabolism of the deuterium-labeled FA. These metabolic results were consistent with the general lack of a CLA diet effect on a variety of physiological responses previously reported for these women. The ^{2}H-CLA isomers were metabolically different. The relative percent differences between the accumulation of 9*c*,11*t*-18:2-d_6 and 10*t*,12*c*-18:2-d_4 in plasma lipid classes ranged from 9 to 73%. The largest differences were a fourfold higher incorporation of 10*t*,12*c*-18:2-d_4 than 9*c*,11*t*-18:2-d_6 in 1-acyl PC and a two- to threefold higher incorporation of 9*c*,11*t*-18:2-d_6 than 10*t*,12*c*-18:2-d_4 in cholesterol esters. Compared to 9*c*-18:1-d_8 and 9*c*,12*c*-18:2-d_2, the 10*t*,12*c*-18:2-d_4 and 9*c*,11*t*-18:2-d_6 isomers were 20–25% less well absorbed. Relative to 9*c*-18:1, incorporation of the CLA isomers into 2-acyl PC and cholesterol ester was 39–84% lower and incorporation of 10*t*,12*c*-18:2 was 50% higher in 1-acyl PC. This pattern of selective incorporation and discrimination is similar to the pattern generally observed for *trans* and *cis* 18:1 positional isomers. Elongated and desaturated CLA metabolites were detected. The concentration of 6*c*,10*t*,12*c*-18:3-d_4 in plasma TG was equal to 6.8% of the 10*t*,12*c*-18:2-d_4 present, and TG was the only lipid fraction that contained a CLA metabolite present at concentrations sufficient for reliable quantification. In conclusion, no effect of dietary CLA was observed, absorption of CLA was less than that of 9*c*-18:1, CLA positional isomers were metabolically different, and conversion of CLA isomers to desaturated and elongated metabolites was low.

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The geometrical and positional isomers of conjugated octadecadienoic acid are commonly referred to as CLA and have been reported to produce a variety of physiological effects. Examples include body fat loss, increased lean body mass, anticarcinogenic activity, antiatherogenic effects, reduced glucose levels, and enhanced immune function. Studies related to these and other physiological and health effects of CLA have recently been reviewed (1–3). The metabolism of CLA isomers in rats and yeast cells has been investigated (4–8), but no definitive data are available on the metabolism of CLA isomers in human subjects.

Three recent human studies have reported that supplementation with 0.36 to 3.2 g/d of CLA reduced the percent body fat compared to the control group (9–11). Differences in plasma lipid FA composition following dietary CLA supplementation suggested that CLA decreased Δ-6 and Δ-9 desaturase and increased Δ-5 desaturase activity (11). The results from a fourth study with adult women receiving 3.9 g/d of CLA for 63 d have been reported recently in a series of papers (12–18). In this study, CLA supplementation did not produce measurable effects on body composition, energy expenditure, lipolysis, plasma leptin concentrations, appetite, platelet function, blood coagulation, immune response, and mononuclear cell function (12–15). Diet did not influence cytokines and eicosanoid secretion *in vitro* and did not alter plasma cholesterol, LDL, HDL, or TG levels (16,18). An almost fourfold increase in the weight percentage of CLA in plasma lipid occurred, but there was no change in the CLA content of adipose tissue (18).

The purpose of this study was to address the following questions that relate to the metabolism of CLA isomers in a subgroup of adult women from the above dietary study. Does dietary CLA supplementation influence accretion and turnover of oleic acid, linoleic acid, and CLA isomers in plasma lipids? To what degree does conjugation of a *trans* double bond with a *cis*-9 or a *cis*-12 double bond influence unsaturated FA metabolism? Are there significant differences between the metabolism of the two CLA isomers (9*c*,11*t*-18:2 and 10*t*,12*c*-18:2) most prevalent in diets?

EXPERIMENTAL PROCEDURES

Study design. Six Caucasian female subjects enrolled in a CLA diet study volunteered to participate in this stable isotope study. Three of the women were from the control group that had received a sunflower oil supplement (6 g/d), and three were from the experimental group that had received 6

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Abbreviations: AUC, area under the curve; CE, cholesterol ester; Chylo-TL, chylomicron total lipid; PC-1, 1-acyl phosphatidylcholine; PC-2, 2-acyl phosphatidylcholine; PL Total, total phospholipid; THP, tetrahydropyranyl; TL, total lipid.

TABLE 1
Physical Characteristics and Plasma Lipid Profiles of Female Subjects[a]

Diet group[b]	Subject number	Body wt (kg)	BMI[c] (kg/m^2)	Plasma lipids (mg/dL)[d]		
				TG	Cholesterol	Total lipid[e]
Cntrl	1	63.6	21.32	67	174	139
	2	87.2	28.39	46	212	177
	3	54.0	20.43	53	211	214
Exp	4	55.6	21.71	64	200	214
	5	65.1	23.16	44	155	167
	6	54.5	21.98	77	216	231

[a]Nonsmokers, ages 23 to 41, normal hypertensive.
[b]Cntrl, control diet. Exp, experimental CLA diet.
[c]BMI, body mass index.
[d]Fasting plasma lipid concentrations at end of 60 d diet period.
[e]mg of total lipid FA per 100 mL of plasma.

g/d of a commercially produced CLA supplement for 60 d. The CLA supplement contained 65% CLA (3.9 g/d of CLA). The subjects were between the ages of 23 and 41 yr and were housed in a metabolic ward. Physical examinations and clinical blood profile data indicated that all subjects were in good health. Medical histories indicated no evidence of congenital ailments. The subjects' height/weight ratios, blood pressures, and fasting serum cholesterol and TG concentrations were within normal ranges. Physical characteristics and plasma lipid profiles for the subjects participating in this isotope study are summarized in Table 1. Plasma lipid data are for blood samples drawn at the end of the diet study. Institutional ethical approval was obtained for the study protocol from the Agricultural Research Service's Human Studies Institutional Review Committee and the University of California at Davis, Human Subjects Committee. Informed consent was obtained from each subject before initiation of the study. Complete information on the study design, diet compositions, and other details has been described previously (12,14).

Deuterated FA. The synthesis of deuterium-labeled methyl *cis*-9,*cis*-12-octadecadienoate-12,13-d_2 (9*c*,12*c*-18:2-d_2) and methyl *trans*-10,*cis*-12-octadecadienoate-15,15,16,16-d_4 (10*t*,12*c*-18:2-d_4) were synthesized and purified as previously reported (19,20). Methyl *cis*-9-octadecenoate-11,11,12,12,17,17,18,18-d_8 (9*c*-18:1-d_8) was prepared in a manner analogous to that described for 9*c*-18:1-14,14,15,15,17,18-d_6 (21). To prepare the -d_8 analog, 5-hexyn-1-ol was converted to the tetrahydropyranyl (THP) ether, reduced ($^2H_2$; Wilkinson's catalyst) to yield the THP ether of hexanol-5,5,6,6-d_4, and the THP ether was converted to the iodide (H_3PO_4/P_2O_5/KI). The C6 iodide was coupled (lithium metal/liquid NH_3) with 2-propyn-1-ol to yield 2-nonyn-1-ol-8,8,9,9-d_4. The nine-carbon acetylenic alcohol-d_4 was converted to the THP ether and reduced (Wilkinson's catalyst; $^2H_2$) to form the THP ether of nonanol-2,2,3,3,8,8,9,9-d_8. The THP ether of nonanol-d_8 was converted to 1-iodononane-d_8 (H_3PO_4/P_2O_5/KI), then to 1-nonyl-d_8-triphenylphosphonium iodide, and the phosphonium salt was coupled with methyl 9-oxononanoate (Wittig reaction) to yield 9*c*-18:1-d_8. The synthesis of methyl *cis*-9,*trans*-11-octadecadienoate-14,14,15,15,17,18-d_6 (9*c*,11*t*-18:2-d_6) was analogous to preparation of 9*c*,11*t*-18:2-17,17,18,18-d_4 (20) except that the THP ether of hexanol-2,2,3,3,5,6-d_6 [2-(hexyloxy-2,2,3,3,5,6-d_6) tetrahydropyran] was utilized as the deuterium-containing fragment. The hexanol-d_6 was prepared by reduction (Wilkinson's catalyst/D_2 gas) of the THP ether of 2-hexyn-5-ene-1-ol. The "ene-yne-ol" THP ether was prepared by coupling (Grignard) 1-bromo-prop-2-ene with the THP ether of 2-propyn-1-ol. The THP ether of hexanol-2,2,3,3,5,6-d_6 was converted to the iodide (H_3PO_4/P_2O_5/KI), then coupled with 2-propyn-1-ol to yield 2-nonyn-1-ol-d_6, which was reduced (lithium metal/liquid NH_3) to *trans*-2-nonen-1-ol-d_6, converted to 1-bromo-*trans*-2-nonen-d_6 (with Ph_3PBr_2). This triphenylphosphonium salt was subsequently coupled (Wittig reaction) with methyl 9-oxononanoate to yield 9*c*,11*t*-18:2-d_6. The deuterated FAME were converted to their ethyl esters using sodium metal in ethanol as previously described (22). The isotopic purity of the deuterated FA was: 85.8% for 9*c*-18:1-d_8, 73.4% for 9*c*,12*c*-18:2-d_2, 83.5% for 9*c*,11*t*-18:2-d_6, and 83.7% for 10*t*,12*c*-18:2-d_4. Chemical purity was 90.6% for 9*c*-18:1-d_8, 78.9% for 9*c*,12*c*-18:2-d_2, 91.6% for 9*c*,11*t*-18:2-d_6, and 89.9% for 10*t*,12*c*-18:2-d_4. Impurities were small amounts (1 to 5%) of deuterated odd chain-length and *trans* FA isomers. Nondeuterated 16:0, 18:0, and 9*c*-18:1 (13% total) were also present in 9*c*,12*c*-18:2-d_2.

Stable isotope study design. A mixture of deuterated ethyl esters (62.4 g) containing 9*c*-18:1-d_8, 9*c*,12*c*-18:2-d_2, 9*c*,11*t*-18:2-d_6, and 10*t*,12*c*-18:2-d_4 was blended for 1 min with 950 g of no-fat yogurt and 92.7 g of powdered sugar in a blender blanketed with nitrogen. The six subjects were fasted overnight (12 h), and each subject was fed a one-sixth portion of the deuterated ethyl ester mixture. The first subject was fed at 7:00 A.M. and the remaining subjects were fed at 10-min intervals in order to allow time for blood draws. The amount of deuterated ethyl esters fed to each subject was 1.77 g 9*c*-18:1-d_8, 3.30 g 9*c*,12*c*-18:2-d_2, 2.66 g 9*c*,11*t*-18:2-d_6, and 2.68 g 10*t*,12*c*-18:2-d_4 after adjusting the actual weights for chemical and isotopic purity. Subjects were provided a no-fat breakfast at 8:00 A.M. A low-fat (*ca.*15% fat calories) lunch was provided at 12:00 noon. Following the noon meal, subjects returned to their respective control or CLA diet schedule.

Sample collection. Blood samples (14 mL each) were collected by venipuncture at 0, 2, 4, 6, 8, 12, 24, 36, and 48 h and

used for isolation of plasma lipid classes. Additional blood samples (*ca.* 14 mL) were collected at 2, 4, and 6 h and used for isolation of chylomicron total lipid (Chylo-TL). Chylomicron fractions were isolated by conventional preparative ultracentrifuge methods (23). Representative chylomicron samples were analyzed by electrophoresis to confirm the purity of the chylomicron fractions (24).

Analysis of plasma lipid FA. Plasma lipids were extracted with 2:1 chloroform/methanol (25). Preparative TLC was used to isolate TG, cholesterol ester (CE), and phospholipid fractions from plasma total lipid (TL) (26). Known weights of triheptadecanoin, cholesterol heptadecanoate, and diheptadecanyl-*sn*-phosphatidylcholine (Applied Science, State College, PA) were added as internal standards to the TL extract. Methanolic sodium methoxide was used to prepare methyl esters from the isolated lipid classes (22). The FA at the 1-acyl and 2-acyl positions of PC were determined by isolation and analysis of products produced following treatment of PC with *Ophiophagus hannah* venom (Kentucky Reptile Zoo, Slade, KY) (27).

The percentages of labeled and unlabeled FA in the plasma lipid classes were obtained by GC–MS analysis of their methyl esters (28). Weights of the 17:0 internal standards added were used to determine the concentrations (mg/mL) of each deuterated and nondeuterated FA. A Hewlett-Packard model 5988A quadrupole mass spectrometer operated in a positive CI mode with isobutane as the ionization reagent was used to analyze the methyl ester samples. The GC–MS methodology utilized selected ion monitoring of the appropriate ion masses for the FA in each GC peak. The areas for each of the ion masses monitored were obtained by integration of the selected ion peaks. The gas chromatograph–mass spectrometer was equipped with a SUPELCOWAX 10 fused-silica column (30 m × 0.25 mm; Supelco Inc., Bellefonte, PA). The column was temperature-programmed from 165 to 265°C at 5°C/min with a 20-min final hold. The specific operating conditions and computer-assisted storage and processing of the MS data have been described previously (28,29). 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 analysis of plasma TG, phospholipid, and CE samples containing known weights of 9*c*-18:1-d_8, 9*c*,12*c*-18:2-d_2, 9*c*,11*t*-18:2-d_6, and 10*t*,12*c*-18:2-d_4. The weight for each of the deuterated fatty esters added was equal to about 0.3% of the total unlabeled FA in the samples. SD were based on three replicate analyses. The SD for 9*c*-18:1-d_8, 9*c*,12*c*-18:2-d_2, 9*c*,11*t*-18:2-d_6, and 10*t*,12*c*-18:2-d_4 in the TG, phospholipid, and CE-spiked samples ranged from ±0.002 to 0.004.

The accuracy of the GC–MS data for unlabeled methyl esters was confirmed by analysis of a subset of samples using a Varian model 3400 gas chromatograph. The GC was equipped with a 100 m × 0.25 mm SP2560 fused-silica capillary column (Supelco) and an FID. Operating conditions were: split ratio, 1:100; linear velocity of helium, 21 cm/s; detector and injection temperature, 235°C. The identification and accuracy of the GC peaks areas were confirmed by comparison to data for authentic standards and mixtures of known composition.

Statistical analysis and calculations. A two-tailed, unpaired *t*-test was used to test for significant differences between control and experimental diet group data for the same FA. A two-tailed, paired *t*-test was used to test for significant differences between data for different deuterium-labeled FA (30). For comparisons of different labeled FA, the data for the individual control and experimental samples were pooled since the data for the two groups were not significantly different. The concentration data (μg/mL) for the deuterated FA and their metabolites were normalized to compensate for differences in plasma lipid concentrations of the subjects and for differences between the weights of ^{2}H-FA in the fed mixture. Normalization of the data to a per gram of deuterated FA fed basis was achieved by dividing the mg of deuterated FA per mL plasma data by the total concentration (mg/mL plasma) of the sample. The μg of deuterated FA per mg of TL data were then divided by the weights for the deuterated FA in the fed mixture. Time-course curves were produced by plotting the normalized μg deuterated FA/mg of TL data for the nine samples collected over the 48-h study period. The total area-under-curve (AUC) data were obtained by calculating the total area under the time-course curve, as described previously (31,32), and are similar to weighted-average data.

RESULTS

Absorption. The time-course curves for Chylo-TL are shown in Figure 1 for 9*c*,11*t*-18:2-d_6, 10*t*,12*c*-18:2-d_4, 9*c*,12*c*-18:2-d_2 and 9*c*-18:1-d_8. These curves provide a comparison of the relative absorption and clearance of these FA. Clearance of the labeled FA appears to be faster for the CLA diet group, but there were no significant differences between AUC data from the control and experimental groups. The Chylo-TL results show that 10*t*,12*c*-18:2-d_4 was about 10% less well absorbed than 9*c*,11*t*-18:2-d_6 ($P < 0.001$ for pooled control plus experimental data) and that both CLA isomers were 20–25% less well absorbed than oleic and linoleic acid ($P < 0.001$).

Effect of diet. Time-course curves for the major lipid classes in plasma are shown in Figures 2–6. Comparison of data plots from women fed the control and CLA-supplemented experimental diets show that dietary CLA had little influence on incorporation of the deuterium-labeled FA used in this study. The total areas for these curves are summarized in Table 2. The control vs. experimental diet data were not significantly different except for 9*c*,11*t*-18:2-d_6 CE and 9*c*-18:1-d_8 PE data. Accumulation of 9*c*,11*t*-18:2-d_6 in CE was low, but it was 65.5% higher ($P < 0.02$) for subjects fed the control diet than for the experimental group subjects. In contrast, control vs. experimental group CE data for the 10*t*,12*c*-18:2-d_4 CLA isomer are not significantly different. The concentration of 9*c*-18:1-d_8 in PE was 6.5 μg/mg/g higher for the CLA-supplemented diet than for the control diet ($P < 0.02$),

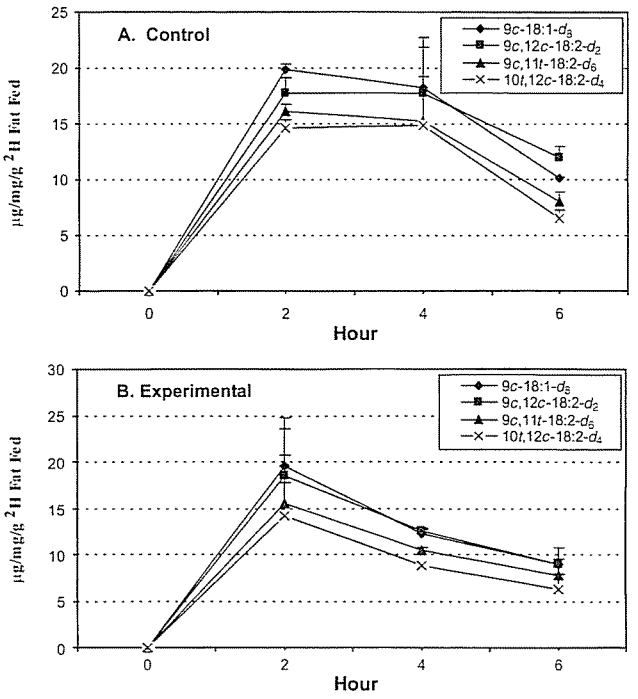

FIG. 1. Time-course plots for uptake and clearance of 9*c*-18:1-d_8, 9*c*,12*c*-18:2-d_2, 9*c*,11*t*-18:2-d_6 and 10*t*,12*c*-18:2-d_4 in chylomicron total lipids of subjects fed the control and experimental diets. Each data point is the average for data from three subjects. Error bars equal SE. Error bars are not shown when less than the size of the symbol.

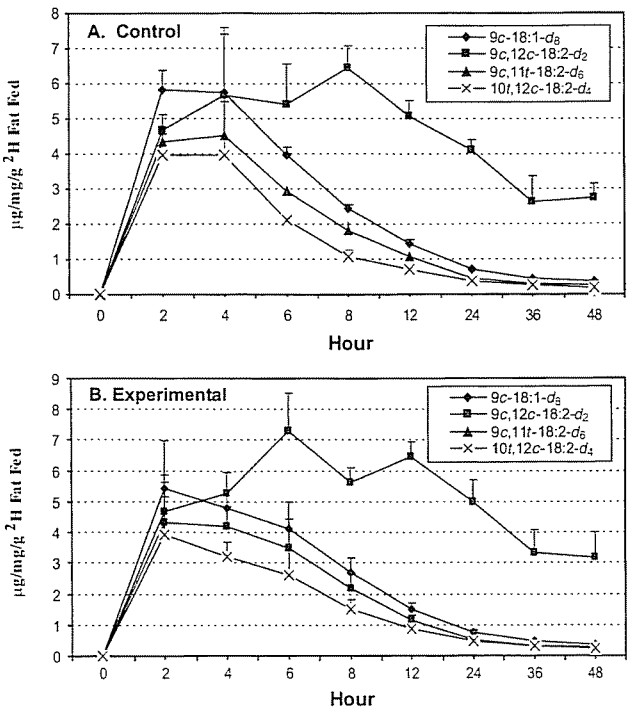

FIG. 2. Time-course plots for uptake and clearance of 9*c*-18:1-d_8, 9*c*,12*c*-18:2-d_2, 9*c*,11*t*-18:2-d_6, and 10*t*,12*c*-18:2-d_4 in plasma total lipid of subjects fed the control and experimental diets. Each data point is the average for data from three subjects. Error bars equal SE. Error bars are not shown when less than the size of the symbol.

but the time-course curves show this minor difference was due to the 8-h sample data. Overall, the effect of dietary supplementation with 3.9 g/d of CLA on the accumulation and turnover of 9*c*,11*t*-18:2-d_6, 10*t*,12*c*-18:2-d_4, 9*c*,12*c*-18:2-d_2 and 9*c*-18:1-d_8 in plasma lipids was limited and consistent with the absence of a dietary supplementation effect.

Comparisons of FA accretion in plasma lipid classes. An overview of differences between the lipid class data is provided by the percent ^{2}H-FA data in Figure 7. The percentage data illustrate the relative difference between incorporation of 9*c*,11*t*- and 10*t*,12*c*-18:2, the general overall discrimination against incorporation of the CLA isomers, and the obvious (expected) preference for 9*c*,12*c*-18:2-d_2 incorporation. The differences between the percentages shown for Chylo-TL and the other lipid classes are a reflection of enzyme specificity for incorporation of the deuterated FA. For example, 78% of all the deuterated FA incorporated in total PC was 9*c*,12*c*-18:2-d_2, which is consistent with the high 9*c*,12*c*-18:2-d_2 content observed for human plasma PC. The percentages for 9*c*-18:1-d_8 (7.5%) and 9*c*,11*t*-18:2-d_6 (6.0%) were not significantly different, but the percentage for 10*t*,12*c*-18:2-d_4 (8.4%) was significantly higher ($P < 0.001$) than 9*c*-18:1-d_8 and 9*c*,11*t*-18:2-d_6 due to its selective incorporation in the 1-acyl PC (PC-1) position (34% of total deuterated FA). The lower percentage for the CLA isomers in plasma TL and TG than for 9*c*-18:1-d_8 suggests that the β-oxidation rates for the CLA isomers were higher than for 9*c*-18:1.

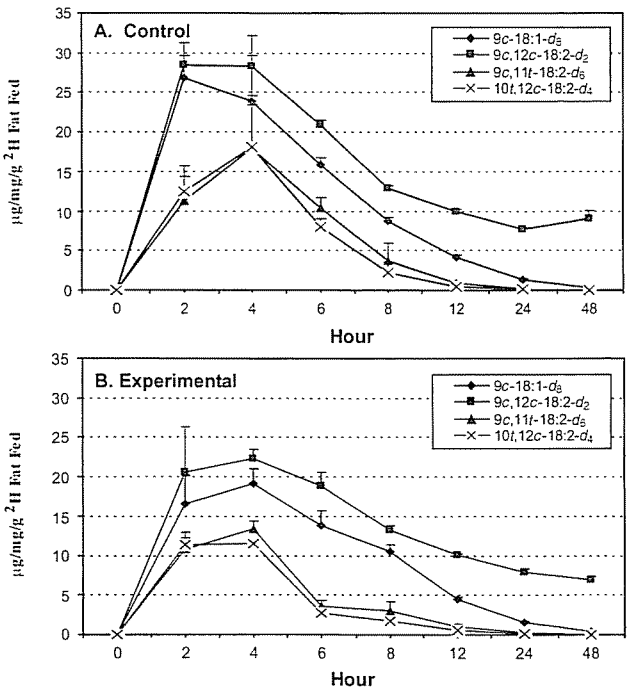

FIG. 3. Time-course plots for uptake and clearance of 9*c*-18:1-d_8, 9*c*,12*c*-18:2-d_2, 9*c*,11*t*-18:2-d_6, and 10*t*,12*c*-18:2-d_4 in plasma TG of subjects fed the control and experimental diets. Each data point is the average for data from three subjects. Error bars equal SE. Error bars are not shown when less than the size of the symbol.

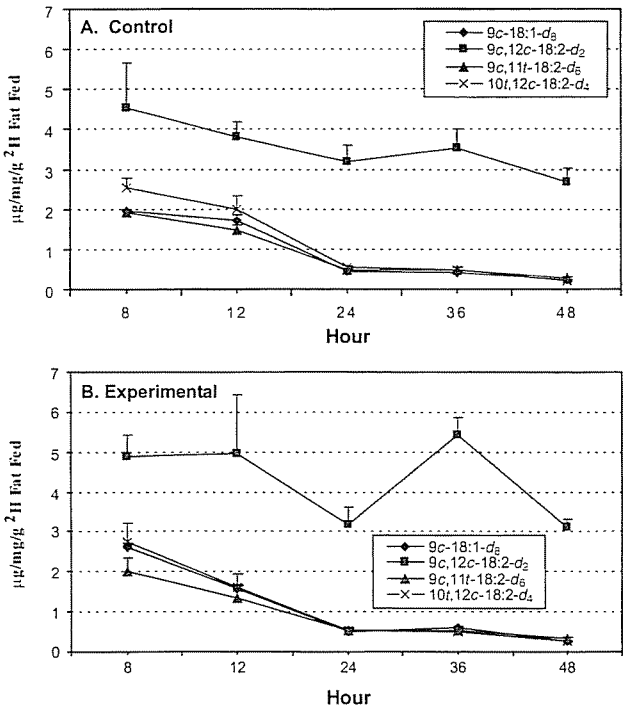

FIG. 4. Time-course plots for uptake and clearance of 9*c*-18:1-d_8, 9*c*,12*c*-18:2-d_2, 9*c*,11*t*-18:2-d_6, and 10*t*,12*c*-18:2-d_4 in plasma PE of subjects fed the control and experimental diets. Each data point is the average for data from three subjects. Error bars equal SE. Error bars are not shown when less than the size of the symbol.

The selectivity ratios shown in Table 3 for 9*c*,11*t*-18:2-d_6, 10*t*,12*c*-18:2-d_4, and 9*c*,12*c*-18:2-d_2 are a measure of the acyltransferase selectivity for incorporation of these FA into the various plasma lipid classes. Previously reported (33–38) selectivity values for *trans*- and *cis*-18:1 positional isomers and 18:0 were recalculated as simple ratios and are included for comparison. Note that 9*c*-18:1 is the reference FA and has a selectivity value of 1.0. Values less than 1.0 indicate discrimination and values greater than 1.0 indicate preferential incorporation. The selectivity values within a specific lipid class for 9*c*,11*t*-18:2-d_6 and 10*t*,12*c*-18:2-d_4 are in the same range as values listed for the positional 18:1 isomers. The exception is the PC-1 value for 9*c*,11*t*-18:2-d_6 (0.46), which is much smaller than values listed for other isomers. The selectivity value of 0.46 for 9*c*,11*t*-18:2-d_6 indicates that 9*c*,11*t*-18:2-d_6 incorporation at the PC-1 position was about 50% lower than for 9*c*-18:1-d_8 and 5–9 times less than for the other 18:1 isomers listed in Table 3. These results suggest that, compared to most other FA isomers, 9*c*,11*t*-18:2-d_6 has an atypical configuration and is not a good substrate for PC transferase. However, a comparison of the selectivity values for accretion of 9*c*,11*t*-18:2-d_6 and 10*t*,12*t*-18:2-d_4 into all other plasma lipid classes shows that both CLA isomers are more metabolically characteristic of *trans*- and *cis*-18:1 positional isomers than of 18:0, 9*c*-18:1, or 9*c*,12*c*-18:2.

AUC data for the control and experimental diet groups listed in Table 2 were not significantly different and were

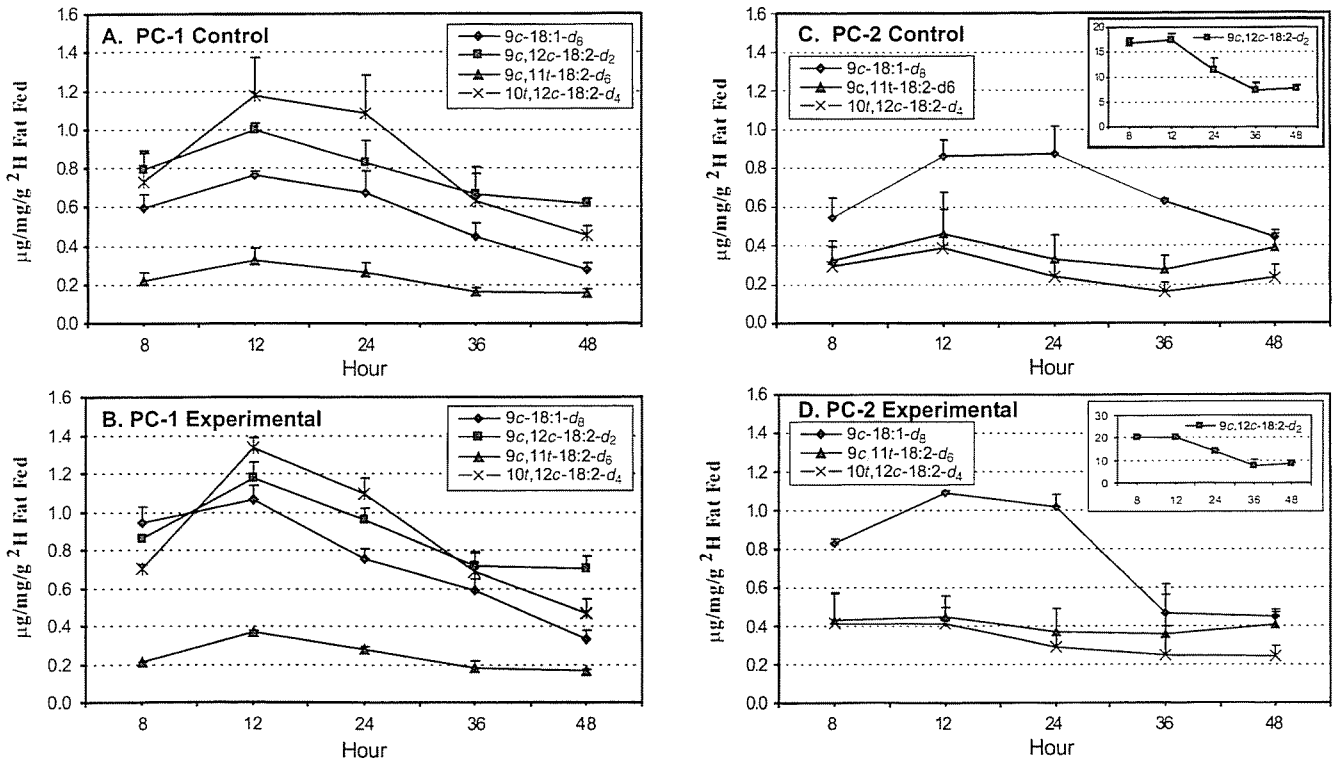

FIG. 5. Time course plots for uptake and clearance of 9*c*-18:1-d_8, 9*c*,12*c*-18:2-d_2, 9*c*,11*t*-18:2-d_6, and 10*t*,12*c*-18:2-d_4 in plasma 1-acyl phosphatidylcholine (PC-1) and 2-acyl phosphatidylcholine (PC-2) of subjects fed the control and experimental diets. The plots for the 9*c*,12*c*-18:2-d_2 PC-2 data are shown as inserts. Each data point is the average for data from three subjects. Error bars equal SE. Error bars are not shown when less than the size of the symbol.

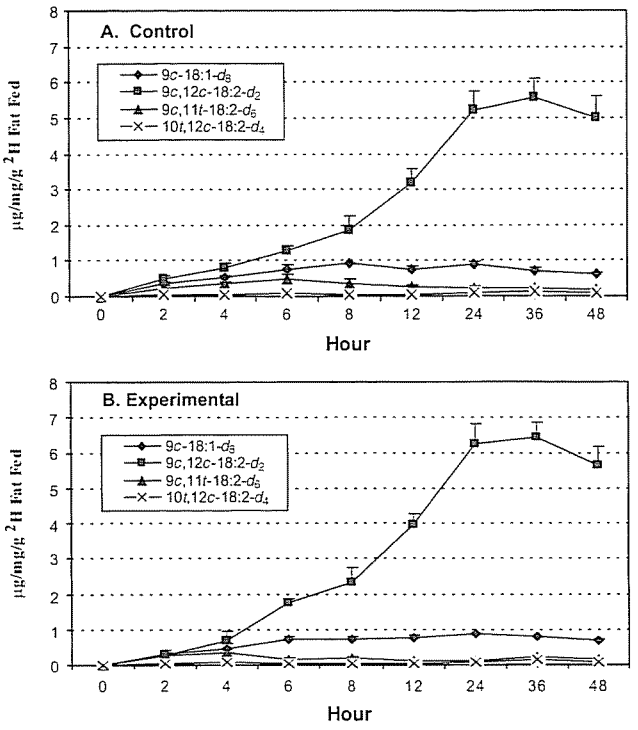

FIG. 6. Time-course plots for uptake and clearance of 9*c*-18:1-d_8, 9*c*,12*c*-18:2-d_2, 9*c*,11*t*-18:2-d_6, and 10*t*,12*c*-18:2-d_4 in plasma cholesterol ester of subjects fed the control and experimental diets. Each data point is the average for data from three subjects. Error bars equal SE. Error bars are not shown when less than the size of the symbol.

therefore combined to test for statistical differences between individual FA within a lipid class. The following are some selected comparisons.

(i) TG. Incorporation of 9*c*-18:1-d_8 was 50–70% higher ($P < 0.001$) than for the labeled CLA isomers, and the difference between the 9*c*,11*t*-18:2-d_6 and 10*t*,12*c*-18:2-d_4 isomers was small (13%) but significant ($P < 0.001$).

(ii) CE. Incorporation of 9*c*,12*c*-18:2-d_2 was about 7.7 times higher than 9*c*-18:1-d_8, 33 times higher than 9*c*,11*t*-18:2-d_6, and 70 times higher than 10*t*,12*c*-18:2-d_4 (P values all <0.001). The concentration of ^{2}H-CLA in CE was the lowest for any plasma lipid class. AUC data for 10*t*,12*c*-18:2-d_4 are 48 to 65% lower than for 9*c*,11*t*-18:2-d_6 ($P < 0.001$). This difference is both a reflection of LCAT selectivity and the lower concentrations of 10*t*,12*c*-18:2 in 2-acylphosphatidylcholine (PC-2).

(iii) PC-1. Accumulation of 10*t*,12*c*-18:2-d_4 in PC-1 was three to four times higher than 9*c*,11*t*-18:2-d_6 but was not different from 9*c*,12*c*-18:2-d_2. More (25–30%) 10*t*,12*c*-18:2-d_4 than 9*c*-18:1-d_8 ($P < 0.001$) was incorporated. In contrast, incorporation of 9*c*,11*t*-18:2-d_6 in PC-1 was 60–70% less than 9*c*-18:1-d_8 ($P < 0.001$). The striking difference between the incorporation in PC-1 for these two CLA isomers is evidence to support the suggestion that these CLA isomers have different physiological effects (39,40). The results are consistent with cultured cell and yeast studies that found these CLA isomers have different effects on apolipoprotein B secretion, TG levels, and stearoyl-CoA desaturase activity (39–41).

TABLE 2
Total Area-Under-Curve Data for Deuterium-Labeled FA in Plasma Lipids of Adult Female Subjects[a]

		Deuterium-labeled FA			
Lipid class	Diet group[b]	9*c*-18:1-d_8 μg/mg/g ± SD[c]	9*c*,12*c*-18:2-d_2 μg/mg/g ± SD	9*c*,11*t*-18:2-d_6 μg/mg/g ± SD	10*t*,12*c*-18:2-d_4 μg/mg/g ± SD
Chylo-TL[d]	Cntrl	86.5 ± 17.1	83.2 ± 13.9	70.7 ± 16.7	65.5 ± 18.0
	Exp	81.3 ± 7.5	82.2 ± 7.0	67.0 ± 2.0	59.0 ± 2.1
TG	Cntrl	207.1 ± 18.1	502.6 ± 20.6	98.1 ± 25.1	87.7 ± 23.7
	Exp	188.0 ± 29.7	460.8 ± 49.0	73.8 ± 11.4	59.6 ± 10.5
CE	Cntrl	33.6 ± 6.5	193.9 ± 34.4	11.2 ± 1.1[e]	4.0 ± 1.1
	Exp	34.9 ± 3.9	230.4 ± 30.6	8.0 ± 0.4	4.1 ± 0.6
Total PL[d]	Cntrl	2.1 ± 0.6	15.7 ± 3.1	1.9 ± 0.6	2.6 ± 0.9
	Exp	2.2 ± 0.2	21.3 ± 2.0	2.0 ± 0.3	3.1 ± 0.01
PCf	Cntrl	34.2 ± 4.2	370.0 ± 34.5	26.6 ± 2.2	38.6 ± 5.2
	Exp	38.6 ± 0.7	383.0 ± 6.9	31.6 ± 4.9	42.0 ± 5.0
PC-1	Cntrl	21.6 ± 3.4	30.1 ± 3.8	8.8 ± 2.0	33.2 ± 7.4
	Exp	27.9 ± 3.8	34.9 ± 0.9	9.8 ± 0.9	35.9 ± 0.3
PC-2	Cntrl	27.8 ± 2.6	423.9 ± 25.5	13.5 ± 2.0	9.4 ± 2.5
	Exp	26.5 ± 7.3	438.6 ± 96.3	13.8 ± 4.0	10.6 ± 3.2
PE	Cntrl	26.6 ± 2.2	130.4 ± 13.5	26.4 ± 0.2	31.0 ± 1.2
	Exp	33.1 ± 1.9	171.2 ± 36.8	29.6 ± 3.8	32.4 ± 2.8
TL	Cntrl	65.5 ± 8.8	189.1 ± 3.3	48.1 ± 9.8	37.3 ± 9.2
	Exp	65.6 ± 9.1	222.0 ± 50.8	51.5 ± 9.8	41.7 ± 6.2

[a]For comparisons of individual FA in a lipid class, data for Cntrl and Exp subjects are combined ($n = 6$). All FA comparisons are significantly different at $P < 0.001$except for PC (9*c*,11*t*-18:2-d_6 vs. 9*c*-18:1-d_8), PE (9*c*,11*t*-18:2-d_6 vs. 9*c*-18:1-d_8), PE (10*t*,12*c*-18:2-d_4 vs. 9*c*-18:1-d_8) and PE (10*t*,12*c*-18:2-d_4 vs. 9*c*,11*t*-18:2-d_6).

[b]Cntrl = control diet group; Exp = experimental diet group.

[c]μg/mg/g = μg deuterated FA per mg of total FA in lipid class per g of deuterated fat fed ($n = 3$).

[d]Chylomicron total lipid (Chylo-TL) and total phospholipid (Total PL) values represent area-under-curve data for 0-, 2-, 4-, and 6-h samples.

[e]Cntrl vs. Exp diet group for cholesterol esters 9*c*,11*t*-18:2-d_6 ($P < 0.01$). No other diet group comparisons for the same deuterium-labeled FA are significantly different.

[f]Values for PC represent area-under-curve data for the 8-, 12-, 24-, 36-, and 48-h samples. PC-1, 1-acyl phosphatidylcholine; PC-2, 2-acyl phosphatidylcholine; TL, total lipid.

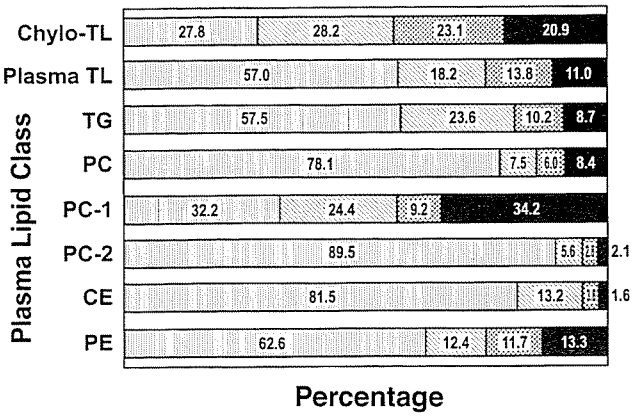

FIG. 7. Percentage of 9*c*-18:1-d_8 (diagonal lines), 9*c*,12*c*-18:2-d_2 (vertical lines), 9*c*,11*t*-18:2-d_6, (cross-hatched), and 10*t*,12*c*-18:2-d_4 (solid) in human plasma lipid classes. Percentages are calculated from the combined average of area concentration data for subjects fed the control and experimental diets. Chylo-TL, chylomicron total lipid; TL, total lipid; PC-1, 1-acyl PC; PC-2, 2-acyl PC; CE, cholesterol ester.

(iv) PC-2. The concentration of 9*c*-18:1-d_8 was 2.0 to 2.7 times higher than that of the CLA isomers ($P < 0.001$), and 9*c*,11*t*-18:2-d_6 was 25% higher than 10*t*,12*c*-18:2-d_4 ($P < 0.001$). The concentration of 9*c*,12*c*-18:2-d_2 in the PC-2 position is about 16 times greater than 9*c*-18:1-d_8 and is typical of the high selectivity previously observed for linoleic acid (31,32,42).

(v) PE. Data for the CLA isomers were not significantly different, and 9*c*,12*c*-18:2-d_2 concentrations were about five times higher than for any other labeled FA. PE is the only lipid class where accumulation of 9*c*-18:1-d_8 was not significantly greater than that for 9*c*,11*t*-18:2-d_6 or 10*t*,12*c*-18:2-d_4.

(vi) TL. Accumulation and turnover of 9*c*,12*c*-18:2-d_2 in plasma TL were significantly higher ($P < 0.001$) than for the CLA isomers and reflect the marked difference in the shapes of the time-course curves for plasma TL (Fig. 2). AUC TL values for 9*c*,12*c*-18:2-d_2 are four to five times larger ($P < 0.001$) than for the CLA isomers and three times larger than for 9*c*-18:1-d_8 ($P < 0.001$). Curve shape for 9*c*-18:1-d_8 incorporation and disappearance was similar to those of the CLA isomers, but total curve area for 9*c*-18:1-d_8 was 25% higher than for 9*c*,11*t*-18:2-d_6 ($P < 0.001$) and 40% higher than for 10*t*,12*c*-18:2-d_4 ($P < 0.001$). The 9*c*,11*t*-18:2-d_6 data were 21% higher than for 10*t*,12*c*-18:2-d_4 ($P < 0.001$).

Metabolites. Only two deuterium-labeled FA metabolites were present at levels sufficient for accurate measurement. The chain-shortened 9*c*-18:1-d_8 metabolite (16:1-d_8) was 1.8% in TL and 1.3% in TG of the 9*c*-18:1-d_8 present. The Δ6-desaturated 10*t*,12*c*-18:2 metabolite (6*c*,10*t*,12*c*-18:3-d_4) was equal to 5% in TL and 6.8% in TG of the 10*t*,12*c*-18:2-d_4 present. Little or no 6*c*,10*t*,12*c*-18:3-d_4 was detected in the samples of other plasma lipid classes, and the Δ6-desaturated 9*c*,11*t*-18:2-d_6 metabolite (6*c*,9*c*,11*t*-18:3-d_6) was not detected. Trace amounts of labeled 20:2, 20:3, and 20:4 containing a conjugated diene structure were present in a few (<5%) samples. Elongated and desaturated 9*c*,12*c*-18:2-d_2 metabolites were present in all plasma lipid classes, but concentrations of the dideuterated n-6 metabolites were not sufficiently higher than the m + 2 natural ^{13}C background levels to allow accurate quantification.

DISCUSSION

Accumulations of labeled FA in all plasma lipid classes were not significantly different for women receiving the control and CLA-supplemented diets, with one exception. The concentrations of 9*c*,11*t*-18:2-d_6 in CE were statistically different, but the difference probably does not indicate a biologically important effect of diet because the absolute difference is very small. Comparison of the control and experimental diet results shows that dietary CLA does not alter oleic acid or linoleic acid metabolism and suggests that dietary CLA would not produce physiological effects associated with oleic and linoleic acid metabolism. The lack of a measurable effect of dietary CLA on 9*c*-18:1-d_8 and 9*c*,12*c*-18:2-d_2 metabolism contrasts with results for 20:4n-6- and 22:6n-3-supplemented diets that showed both 20:4n-6 and 22:6n-3 have a major influence on the metabolism of deuterium-labeled 9*c*-18:1 and 9*c*,12*c*-18:2 (31,32,43). The results from this study are consistent with results from a study that found incorporation of

TABLE 3
Selectivity Values for Incorporation of CLA Isomers Relative to Oleic Acid in Plasma Lipid Classes of Adult Women[a]

	FA selectivity values										
Lipid class	9*c*,11*t*-18:2	10*t*,12*c*-18:2	9*c*,12*c*-18:2	9*t*-18:1	10*t*-18:1	10*c*-18:1	11*t*-18:1	11*c*-18:1	12*t*-18:1	12*c*-18:1	18:0
TG	0.53	0.50	2.48	0.89	0.87	0.79	0.66	0.85	0.51	0.71	0.96
CE	0.34	0.16	6.26	0.19	0.29	0.18	0.06	0.45	0.20	0.69	0.01
PC	0.97	1.50	10.54	1.35	0.54	1.29	1.00	1.15	1.48	3.8	4.56
PC-1	0.46	1.90	1.34	3.63	2.19	3.98	3.89	1.62	5.37	3.80	13.13
PC-2	0.61	0.50	16.14	0.61	0.23	0.71	0.14	0.85	0.13	4.47	0.04
PE	1.15	1.44	5.11	1.95	0.91	0.89	1.41	1.26	1.02	1.29	ND[b]

[a]Area-under-curve (AUC; μg/mg/g) data used for calculation of selectivity values. Selectivity values equal FA AUC data divided by the AUC data for 9*c*-18:1. Reference value for 9*c*-18:1 = 1.00. CLA and 9*c*,12*c*-18:2 selectivity values are the average of control plus experimental groups. All selectivity ratios are adjusted for reduced absorption of FA if appropriate. Selectivity values for *cis*- and *trans*-18:1 positional isomers and for 18:0 are from previously published studies.

[b]ND, not data; for other abbreviations see Table 2.

nonconjugated unsaturated FA in rat liver was not altered by dietary CLA (8).

The apparent lower absorption for the ^{2}H-CLA isomers compared to 9*c*-18:1-d_8 was unexpected. Previous studies showed absorption of ^{2}H-20:3n-6 and ^{2}H-20:4n-6 was lower than ^{2}H-18:1 (43,44), but significantly lower absorption of 18-carbon FA was not observed for labeled *cis*- and *trans*-18:1 FA isomers (45) nor for ^{2}H-18:0 (38). A possible explanation for this result is that 20–30% of the ^{2}H-CLA was absorbed *via* the portal transport and is consistent with human jejunum data for linolenic acid (46). Alternatively, one or both of the pathways (MAG and glycerol-3-phosphate) responsible for FA incorporation into TG during absorption may partially discriminate against CLA isomers. Hydrolysis of CLA TG by pancreatic lipase *in vitro* was reported to be lower than for trilinolein (47), and may have been a factor with the deuterated CLA ethyl esters used in the present study. However, rat data showed that CLA and 9*c*,12*c*-18:2 TG were equally well absorbed (47), which is not consistent with the results from this study in humans.

Comparison of incorporation data shows that metabolism of the ^{2}H-CLA isomers is clearly different from that for oleic and linoleic acids. These results suggest that conjugation of the *cis*-9 and the *cis*-12 double bonds with a *trans* double bond has a major impact on the selectivity of the acylation enzymes that are responsible for incorporation. Conjugation does not appear to influence turnover rates since all plasma lipid class time-course curves show that disappearance of the CLA isomers is similar to 9*c*-18:1. The selectivity values listed in Table 3 show that there was discrimination against incorporation of the ^{2}H-CLA isomers in TG and CE and preferential incorporation in phospholipid, PC, and PE relative to 9*c*-18:1-d_8 and 9*c*,12*c*-18:2-d_2. Comparison of selectivity values for the CLA isomers to 9*c*,12*c*-18:2-d_2 values and values previously reported for *cis*- and *trans*-18:1 positional isomers and 18:0 (34–39) shows that 9*c*,11*t*-18:2 and 10*t*,12*c*-18:2 are metabolized more like *trans*-18:1 isomers than 18:0, 9*c*-18:1, or 9*c*,12*c*-18:2. For example, the selectivity ratio of 1.90 for 10*t*,12*c*-18:2 is between the values for 11*c*- and 10*t*-18:1 and indicates preferential incorporation at the PC-1 position. The PC-1 selectivity ratio of 0.46 indicates discrimination against 9*c*,11*t*-18:2 incorporation and is unusual because 9*c*,11*t*-18:2 is the only *trans* FA in which discrimination has been observed to occur at the PC-1 position. The CE selectivity values for 9*c*,11*t*-18:2 (0.34) and 10*t*,12*c*-18:2 (0.16) reflect a strong discrimination against acylation of cholesterol, which is a consistent characteristic of *trans*- and *cis*-18:1 positional isomers.

The lower concentrations of CLA isomers in TG and TL compared to oleic acid are generally consistent with the selectivity ratios for 18:1 isomers. The 10*t*,12*c*-18:2/9*c*,11*t*-18:2 in human plasma TL was 0.78 and indicated an overall 22% preferential accretion of 9*c*,11*t*-18:2. The TL selectivity values of 0.81 for 10*t*,12*c*-18:2 and 0.92 for 9*c*,11t-18:2 represent the entire plasma lipid pool and suggest that, like ^{13}C-labeled 9*t*-18:1 (48), a larger portion of the CLA isomers than oleic acid are β-oxidized. This suggestion is supported by results from rats, where β-oxidation of ^{14}C-CLA isomers was about 17% higher than 9*c*,12*c*-18:2 (49). Overall, the results for the metabolism of CLA isomers showed many similarities to our previously reported results for *trans*-18:1 positional isomers. This observation is an interesting anomaly since a plethora of positive health benefits have been reported for CLA (1–3) but *trans*-18:1 isomers are considered to have a negative effect on serum cholesterol and to constitute a risk for coronary heart disease (45).

Studies in rats and yeast cell models have reported chain-shortened, elongated, and desaturated metabolites of CLA (4–8). In rat liver, the two main metabolites of 10*t*,12*c*-18:2 were 8*t*,10*c*-16:2 and 6*c*,10*t*,12*c*-18:3, and the main metabolite of 9*c*,11*t*-18:2 was 8*c*,11*c*,13*t*-20:3 (5). In this study with adult women, 6*c*,10*t*,12*c*-18:3-d_4 was the only CLA metabolite present in measurable amounts, and it does not appear to be elongated to 8*c*,12*t*,14*c*-20:3. The detection of 6*c*,10*t*,12*c*-18:3 in plasma TG but not in phospholipid samples is consistent with results for rat liver lipids (5). The importance of the *cis*-12 double bond is well recognized, but conjugation of the *cis*-12 double bond with a *trans* double bond would reduce the π electron density of the *cis*-12 double bond and alter its spatial arrangement or conformation compared to 9*c*,12*c*-18:2. Thus, the relatively high conversion of 10*t*,12*c*-18:2-d_4 is an unexpected result.

For these women, 9*c*,11*t*-18:2-d_6 clearly was not a good substrate for Δ6-desaturation, and neither CLA isomer was a good substrate for elongation, but why CLA metabolite concentrations were much lower than reported for rats was not clear. The higher amount of dietary CLA fed per kilogram of body weight in the rat studies and the higher desaturase activity of rats compared to humans would account for some of the observed difference. A possible contributing factor is that desaturase and elongase activities for women appear to be considerably lower than for men. For example, in comparable previous studies with adult men, the concentrations of 9*c*,12*c*-18:2-d_2 desaturation and elongation products were sufficient for accurate measurements (31,32,42). In this study with adult females, levels of elongated and desaturated 9*c*,12*c*-18:2-d_2 metabolites were usually too low for accurate quantification.

We found no effect of dietary CLA supplementation on the metabolism of the ^{2}H-FA investigated. CLA isomers were not as well absorbed as oleic and linoleic acids. The 9*c*,11*t*-18:2 and 10*t*,12*c*-18:2 isomers were metabolically different, and their accretion in plasma lipids was characteristic of *cis*- and *trans*-18:1 positional isomers. Conversion of the ^{2}H-CLA isomers to 18- and 20-carbon PUFA was negligible, except for the conversion of 10*t*,12*c*-18:2 to 6*c*,10*t*,12*c*-18:2.

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