Supplementation with CLA: Isomer Incorporation into Serum Lipids and Effect on Body Fat of Women

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ABSTRACT: Animal studies have suggested that CLA, a natural component of meat and dairy products, may confer beneficial effects on health. However, human studies using supplementation with CLA have produced contradictory results. The aim of the present study was to further investigate the effect of CLA supplementation on human body fat, serum leptin, and serum lipids, as well as the incorporation of CLA isomers into serum lipids classes. Sixteen young healthy nonobese sedentary women received 2.1 g of CLA (divided equally between the *cis,trans*-9,11 and *trans,cis*-10,12 isomers) daily for 45 d and placebo for 45 d in a randomized double-blind crossover design. Body fat was estimated (by measurement of skinfold thickness at 10 sites), and blood was sampled at the beginning, middle, and end of the entire intervention period; an additional blood sample was obtained 2 wk thereafter. No significant differences in energy, carbohydrate, lipid, or protein intake existed between the CLA and placebo intake periods. No significant differences were found in body fat or serum leptin, TAG, total cholesterol, HDL-cholesterol, and alanine aminotransferase between CLA and placebo. The CLA isomer content of serum TAG, phospholipids, and total lipids increased 2–5 times with CLA supplementation ($P < 0.05$). In contrast, the CLA content of cholesteryl esters did not change significantly. The period of 2 wk after the end of CLA supplementation was sufficient for its washout from serum lipids. These data indicate that supplementation with 2.1 g of CLA daily for 45 d increased its levels in blood but had no effect on body composition or the lipidemic profile of nonobese women.

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CLA is a family of constitutional isomers and stereoisomers of octadecadienoic acid having conjugated double bonds. Several of these isomers occur naturally, mainly in the fat of ruminants and dairy products, and two (*cis,trans*-9,11 and *trans,cis*-10,12) are known to possess biological activity. As has been shown in animals studies, both isomers inhibit carcinogenesis, whereas the latter lowers body fat and increases lean body mass (1). Other reported actions of CLA in animals include decrement of atherogenesis and prevention of the catabolic effects of immune stimulation (1).

Of the aforementioned physiological effects, that on body composition has been the one most extensively studied in humans so far. Results from the relevant articles are contradictory, with some (including one from our laboratory) indicating that supplementation with CLA causes body fat reduction (2–6) and others reporting no significant effect on body fat (7–9). Results with regard to the effect of CLA supplementation on human serum lipids are also equivocal (2–5,9,10). The aim of the present study was to further investigate the effect of CLA supplementation on body fat and serum biochemical parameters related to lipid metabolism by using a higher dose and longer supplementation compared to our previous study (3), as well as controlling for interindividual and gender differences. An additional aim was to determine the incorporation of individual CLA isomers into serum lipid classes.

MATERIALS AND METHODS

Subjects. Seventeen sedentary women, aged 19–24, who responded to a public invitation participated in the study initially. Subjects were eumenorrheic, not obese (body mass index < 30 kg/m^2), not suffering from any acute or chronic illness, and not taking any medication or dietary supplements. They were informed orally and in writing of the design and probable risks of the research and consented to participate. The study was designed and carried out according to the guidelines of the University of Thessaloniki Ethics Committee.

Design. Participants received CLA for 45 d and placebo for 45 d, with no washout period in between, in a randomized double-blind crossover design, thus forming two groups, CLA-placebo and placebo-CLA. The daily dose of CLA was 2.1 g in the form of six soft gelatine capsules from TrofoCell (Hamburg, Germany). Each capsule contained 500 mg of oil, 70% of which was CLA, divided equally between the *cis,trans*-9,11 and *trans,cis*-10,12 isomers. Placebo was in the form of identical-appearing capsules containing soybean oil. Subjects were asked not to modify their nutritional habits and physical activity for the duration of the study and to record their dietary intake as well as the quantity of experimental capsules taken daily. As an additional measure of compliance, they were asked to return any remaining capsules at the end of each supplementation period.

Measurements. The participants visited the laboratory at the onset, middle (change of regimen), and end of the entire intervention period, between 9 and 11 A.M., after an overnight fast. During each visit we measured body weight, height, and thickness of 10 skinfolds for the estimation of percentage

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Abbreviations: ALT, alanine aminotransferase; CE, cholesteryl esters; PL, phospholipids.

body fat (11). All skinfold thickness measurements were performed by the same highly experienced researcher using Harpenden metal calipers from British Indicators (West Sussex, United Kingdom). This method of estimating body fat has recently been reported to differ by 1.4 ± 2.2 (mean ± SD) percentage body fat units from the reference method, hydrodensitometry, in a large sample (n = 114) of women (12).

Blood sampling. At each of the three visits, the volunteers provided a blood sample from an antecubital vein into an evacuated test tube while seated. After clotting, serum was prepared by centrifugation and was stored at −20°C for the determination of TAG, total cholesterol, HDL cholesterol, leptin, and alanine aminotransferase (ALT), as well as for the FA analysis of lipid classes. In addition, the volunteers provided a blood sample during a follow-up visit to the laboratory 2 wk after the end of the intervention period for the examination of the washout of CLA from serum lipids.

Assays. TAG, cholesterol, and ALT were assayed by enzymic spectrophotometric methods through the use of reagent kits from Randox (Crumlin, Co. Antrim, United Kingdom). HDL cholesterol was determined after treatment of serum aliquots with a precipitant from Roche (Mannheim, Germany). Leptin was measured by ELISA through the use of a kit from DRG (Marburg, Germany).

Determination of the FA composition of serum lipid classes was initiated by the addition of a mixture of triheptadecanoin, diheptadecanoyl PC, and cholesteryl heptadecanoate (all from Sigma, St. Louis, MO) as internal standards to 0.5 mL of serum. Lipids were extracted and separated by TLC as described (3). Lipid spots were located under UV light after spraying with a solution of dichlorofluorescein in ethanol, and the spots corresponding to TAG, phospholipids (PL), and cholesteryl esters (CE) were excised and incubated with 0.5 mol/L $NaOCH_3$ in methanol (Sigma) at 50°C for 10 min (13). After extraction with hexane, the methyl esters were separated by GC in a Hewlett-Packard 5890 Series II chromatograph (Waldbronn, Germany) equipped with a 30-m-long BPX70 capillary column from SGE (Ringwood, Victoria, Australia). The column temperature was programmed from 140 to 240°C at 5°C/min. The carrier gas was helium at a flow rate of 0.67 mL/min (at 140°C). Methyl esters were identified by comparing their retention times to those of pure FAME purchased from Sigma. Additionally, *cis,trans*-9,11-, *trans,cis*-10,12-, and *trans,trans*-9,11-octadecadienoic acid were purchased from Cayman (Ann Arbor, MI). FAME in the samples were quantified by comparing the area under their peaks in the chromatogram to that of methyl heptadecanoate (derived from the internal standards) with the aid of the HP 3365 ChemStation software from Hewlett-Packard.

The FA composition of total serum lipids was determined by the addition of a mixture of the internal standards mentioned above to 10 μL of serum, followed by preparation of FAME and GC as described above.

Dietary analysis. Dietary records were analyzed in Microsoft® Access by the use of a food database created in our laboratory on the basis of published data (14).

Statistical analysis. Results are reported as the mean ± SD. Significant differences between supplementation with CLA and placebo were detected by ANOVA based on a counterbalanced Latin square design (15), which controls for possible carryover effects. The level of statistical significance was set at $\alpha = 0.05$. SPSS (version 10.0) was used for all analyses (SPSS Inc., Chicago, IL).

RESULTS

Before the end of the study, one volunteer withdrew owing to acute illness (influenza) and the demand of her physician that she discontinue the experimental capsules. No other adverse effect was reported by any of the participants. When the study was unblinded, the volunteer who had withdrawn was found to be member of the placebo-CLA group. Of the 16 volunteers who completed the study, 9 belonged to the CLA-placebo group and 7 to the placebo-CLA group. Compliance with the regimen was 99.1 ± 2.4% for CLA and 99.2 ± 3.2% for placebo.

The age of the participants was 22.3 ± 1.8 yr. There were no significant differences in energy, carbohydrate, lipid, or protein intake between the periods of CLA and placebo supplementation (data not shown). Daily energy intake averaged 1975 kcal and was derived from 43% carbohydrate, 41% fat, and 16% protein.

Anthropometric data are presented in Table 1. There were no significant differences between supplementation with CLA and placebo in regard to body mass, body mass index, sum of skinfold thickness, or percentage body fat. The observed power for these parameters was low, ranging from 0.05 to 0.14.

The results of the biochemical analyses except FA analysis are shown in Table 2. There was no significant effect of CLA supplementation on TAG, total cholesterol, HDL cholesterol, total/HDL cholesterol, leptin, or ALT.

TABLE 1
Anthropometric Data of Participants

	CLA-placebo group			Placebo-CLA group		
	Baseline	CLA	Placebo	Baseline	Placebo	CLA
Body mass (kg)	66.3 ± 9.5	66.2 ± 9.0	67.1 ± 9.9	66.7 ± 4.7	66.9 ± 5.1	67.4 ± 6.0
Height (m)	1.69 ± 0.08	1.69 ± 0.07	1.69 ± 0.07	1.68 ± 0.08	1.68 ± 0.08	1.68 ± 0.08
Body mass index (kg/m^2)	23.1 ± 2.4	23.2 ± 2.4	23.5 ± 2.5	23.7 ± 2.9	23.8 ± 3.0	24.0 ± 3.3
Sum of 10 skinfolds (mm)	189.4 ± 56.6	199.2 ± 40.0	186.0 ± 48.7	204.4 ± 44.1	223.8 ± 41.6	203.1 ± 50.4
Body fat (%)	28.1 ± 5.7	29.4 ± 3.6	28.0 ± 4.6	29.9 ± 3.5	31.5 ± 3.1	29.7 ± 4.0
Fat mass (kg)	19.0 ± 5.8	19.7 ± 4.6	19.1 ± 5.5	20.0 ± 3.4	21.1 ± 3.1	20.1 ± 4.0

TABLE 2
Biochemical Analyses

	CLA-placebo group			Placebo-CLA group		
	Baseline	CLA	Placebo	Baseline	Placebo	CLA
TAG (mg/dL)	65.0 ± 26.1	54.8 ± 18.0	51.8 ± 9.6	47.4 ± 14.2	41.2 ± 10.7	47.2 ± 6.7
Total cholesterol (mg/dL)	181.1 ± 17.3	172.0 ± 19.6	165.8 ± 17.0	168.2 ± 20.2	157.2 ± 18.3	159.3 ± 11.6
HDL cholesterol (mg/dL)	56.9 ± 11.1	55.2 ± 10.0	56.0 ± 11.9	56.2 ± 6.0	53.4 ± 6.5	50.9 ± 7.2
Total/HDL cholesterol	3.3 ± 0.6	3.2 ± 0.7	3.1 ± 0.6	3.0 ± 0.4	3.0 ± 0.4	3.2 ± 0.5
Leptin (ng/mL)	15.6 ± 8.9	17.7 ± 7.5	19.6 ± 16.3	24.4 ± 10.5	22.2 ± 13.1	25.5 ± 12.4
ALT[a] (U/L, 37°C)	9.6 ± 4.0	8.4 ± 3.8	12.6 ± 4.4	7.5 ± 4.3	7.8 ± 3.3	11.8 ± 4.4

[a]ALT, alanine aminotransferase.

GC revealed the presence, in considerable amounts, of 19 FA including the *cis,trans*-9,11, *trans,cis*-10,12, and the *trans,trans*-9,11 isomers of CLA, although a fourth isomer, *trans,trans*-10,12-CLA, has been reported to comigrate with the latter (16,17). The FA composition of serum TAG, PL, CE, and total lipids is listed in Tables 3–6.

No significant differences between placebo and CLA supplementation were found in the preceding FA except CLA. Compared to the placebo, CLA supplementation resulted in a significant increase in the concentration of all CLA isomers in serum TAG ($P < 0.05$), PL ($P < 0.001$), and total lipids ($P < 0.05$). The CLA content of CE increased to a lesser degree with CLA supplementation, and the difference from the placebo was not significant.

Concerning the individual CLA isomers, there was a significant increase in the percentage of *cis,trans*-9,11-CLA within the acyl groups of serum TAG (from 0.32 to 0.76, $P = 0.003$, mean of all 16 volunteers), PL (from 0.20 to 0.40, $P < 0.001$), and total lipids (from 0.18 to 0.40, $P = 0.001$) with CLA supplementation (Fig. 1). Likewise, the percentage of *trans,cis*-10,12-CLA increased significantly in TAG (from 0.05 to 0.24, $P = 0.021$), PL (from 0.07 to 0.15, $P < 0.001$), and total lipids (from 0.08 to 0.19, $P = 0.003$). Finally, the percentage of *trans,trans*-9,11-CLA increased significantly in TAG (from 0.20 to 0.30, $P = 0.001$) and PL (from 0.21 to 0.31, $P = 0.013$). There was no significant difference between placebo and CLA supplementation in the percentages of any of the three isomers or their sum in CE. On the contrary, the sum of the percentages of the three isomers increased significantly in TAG (from 0.57 to 1.29, $P = 0.004$), PL (from 0.48 to 0.87, $P < 0.001$), and total lipids (from 0.45 to 0.81, $P = 0.001$). Two weeks after the end of supplementation the CLA content of serum lipids had returned to the values before CLA supplementation (Fig. 1).

TABLE 3
Serum Concentrations (mmol/L) of TAG Acyl Groups

	CLA-placebo group			Placebo-CLA group		
FA	Baseline	CLA	Placebo	Baseline	Placebo	CLA
14:0	0.0337 ± 0.0344	0.0310 ± 0.0280	0.0204 ± 0.0127	0.0177 ± 0.0090	0.0113 ± 0.0011	0.0231 ± 0.0107
16:0	0.5366 ± 0.3030	0.4226 ± 0.1470	0.4331 ± 0.1211	0.3359 ± 0.0941	0.2817 ± 0.0801	0.3540 ± 0.0614
16:1n-7	0.0511 ± 0.0496	0.0379 ± 0.0288	0.0439 ± 0.0375	0.0300 ± 0.0124	0.0227 ± 0.0081	0.0269 ± 0.0068
18:0	0.1186 ± 0.0577	0.0955 ± 0.0264	0.0949 ± 0.0601	0.0700 ± 0.0286	0.0548 ± 0.0151	0.0722 ± 0.0132
18:1n-9	0.6244 ± 0.2887	0.5312 ± 0.1487	0.5889 ± 0.1813	0.4431 ± 0.1303	0.4089 ± 0.1067	0.4974 ± 0.0954
18:1n-7	0.0316 ± 0.0128	0.0279 ± 0.0109	0.0339 ± 0.0168	0.0218 ± 0.0052	0.0214 ± 0.0065	0.0227 ± 0.0036
18:2n-6	0.2448 ± 0.0779	0.2161 ± 0.0786	0.2379 ± 0.0495	0.1752 ± 0.0703	0.1873 ± 0.0330	0.1969 ± 0.0871
cis,trans-9,11-CLA[a]	0.0050 ± 0.0037	0.0109 ± 0.0091	0.0050 ± 0.0036	0.0041 ± 0.0018	0.0039 ± 0.0027	0.0100 ± 0.0076
trans,cis-10,12-CLA[b]	0.0006 ± 0.0003	0.0042 ± 0.0055	0.0005 ± 0.0003	0.0005 ± 0.0005	0.0006 ± 0.0005	0.0027 ± 0.0031
trans,trans-9,11-CLA[a]	0.0030 ± 0.0010	0.0039 ± 0.0025	0.0029 ± 0.0010	0.0024 ± 0.0009	0.0021 ± 0.0009	0.0039 ± 0.0010
CLA sum[a]	0.0086 ± 0.0044	0.0191 ± 0.0162	0.0084 ± 0.0043	0.0070 ± 0.0022	0.0066 ± 0.0032	0.0166 ± 0.0104
18:3n-6	0.0040 ± 0.0025	0.0023 ± 0.0016	0.0031 ± 0.0017	0.0028 ± 0.0023	0.0020 ± 0.0007	0.0024 ± 0.0020
18:3n-3	0.0047 ± 0.0016	0.0055 ± 0.0080	0.0043 ± 0.0020	0.0025 ± 0.0009	0.0022 ± 0.0012	0.0029 ± 0.0014
20:1n-9	0.0042 ± 0.0017	0.0038 ± 0.0015	0.0041 ± 0.0020	0.0029 ± 0.0007	0.0026 ± 0.0007	0.0034 ± 0.0007
20:3n-6	0.0028 ± 0.0010	0.0033 ± 0.0027	0.0031 ± 0.0019	0.0021 ± 0.0009	0.0018 ± 0.0004	0.0021 ± 0.0009
20:4n-6	0.0056 ± 0.0026	0.0050 ± 0.0029	0.0077 ± 0.0031	0.0055 ± 0.0018	0.0055 ± 0.0013	0.0054 ± 0.0023
20:5n-3	0.0002 ± 0.0002	0.0002 ± 0.0004	0.0003 ± 0.0006	0.0003 ± 0.0004	0.0002 ± 0.0003	0.0004 ± 0.0005
22:5n-3	0.0010 ± 0.0008	0.0009 ± 0.0007	0.0012 ± 0.0008	0.0009 ± 0.0008	0.0008 ± 0.0005	0.0007 ± 0.0005
22:6n-3	0.0008 ± 0.0005	0.0010 ± 0.0007	0.0014 ± 0.0008	0.0009 ± 0.0004	0.0009 ± 0.0009	0.0007 ± 0.0003
24:0	0.0008 ± 0.0007	0.0009 ± 0.0008	0.0009 ± 0.0007	0.0006 ± 0.0002	0.0008 ± 0.0003	0.0007 ± 0.0005
FA sum	1.6734 ± 0.7158	1.4041 ± 0.3845	1.4876 ± 0.3562	1.1193 ± 0.3146	1.0115 ± 0.2351	1.2287 ± 0.1664

[a]$P \leq 0.01$, significantly higher after CLA supplementation compared to placebo.
[b]$P < 0.05$, significantly higher after CLA supplementation compared to placebo.

TABLE 4
Serum Concentrations (mmol/L) of Phospholipid Acyl Groups

	CLA-placebo group			Placebo-CLA group		
FA	Baseline	CLA	Placebo	Baseline	Placebo	CLA
14:0	0.0171 ± 0.0167	0.0183 ± 0.0114	0.0139 ± 0.0073	0.0109 ± 0.0039	0.0075 ± 0.0033	0.0118 ± 0.0035
16:0	0.9696 ± 0.2966	0.8342 ± 0.1370	0.8657 ± 0.1865	0.8027 ± 0.1045	0.6840 ± 0.0914	0.7547 ± 0.1335
16:1n-7	0.0220 ± 0.0110	0.0191 ± 0.0079	0.0181 ± 0.0100	0.0183 ± 0.0047	0.0143 ± 0.0033	0.0149 ± 0.0019
18:0	0.9652 ± 0.5752	0.7739 ± 0.2016	0.8320 ± 0.4451	0.7454 ± 0.2606	0.6091 ± 0.0884	0.7063 ± 0.1968
18:1n-9	0.4260 ± 0.1144	0.3849 ± 0.0698	0.3701 ± 0.0553	0.3637 ± 0.0463	0.3267 ± 0.0531	0.3377 ± 0.0356
18:1n-7	0.0428 ± 0.0071	0.0408 ± 0.0098	0.0439 ± 0.0074	0.0340 ± 0.0048	0.0336 ± 0.0046	0.0305 ± 0.0027
18:2n-6	0.5902 ± 0.1009	0.5230 ± 0.1408	0.6206 ± 0.1489	0.4786 ± 0.1029	0.4689 ± 0.0746	0.5309 ± 0.1432
cis,trans-9,11-CLA[a]	0.0060 ± 0.0026	0.0123 ± 0.0038	0.0053 ± 0.0034	0.0048 ± 0.0019	0.0054 ± 0.0015	0.0093 ± 0.0020
trans,cis-10,12-CLA[a]	0.0021 ± 0.0017	0.0040 ± 0.0016	0.0022 ± 0.0013	0.0019 ± 0.0005	0.0016 ± 0.0010	0.0044 ± 0.0019
trans,trans-9,11-CLA[a]	0.0073 ± 0.0030	0.0082 ± 0.0026	0.0045 ± 0.0026	0.0048 ± 0.0023	0.0045 ± 0.0011	0.0084 ± 0.0038
CLA sum[a]	0.0154 ± 0.0063	0.0245 ± 0.0054	0.0120 ± 0.0061	0.0115 ± 0.0039	0.0115 ± 0.0028	0.0221 ± 0.0055
18:3n-6	0.0007 ± 0.0021	0.0001 ± 0.0003	0.0005 ± 0.0016	0.0011 ± 0.0017	0.0006 ± 0.0010	0.0004 ± 0.0004
18:3n-3	0.0114 ± 0.0152	0.0107 ± 0.0070	0.0122 ± 0.0118	0.0088 ± 0.0046	0.0078 ± 0.0042	0.0086 ± 0.0047
20:1n-9	0.0064 ± 0.0020	0.0066 ± 0.0011	0.0059 ± 0.0023	0.0050 ± 0.0017	0.0063 ± 0.0012	0.0063 ± 0.0014
20:3n-6	0.0608 ± 0.0224	0.0531 ± 0.0335	0.0704 ± 0.0309	0.0436 ± 0.0151	0.0425 ± 0.0154	0.0452 ± 0.0136
20:4n-6	0.1261 ± 0.0484	0.1205 ± 0.0592	0.1640 ± 0.0699	0.1213 ± 0.0605	0.1336 ± 0.0643	0.1295 ± 0.0552
20:5n-3	0.0044 ± 0.0018	0.0075 ± 0.0086	0.0082 ± 0.0055	0.0092 ± 0.0106	0.0053 ± 0.0040	0.0055 ± 0.0041
22:5n-3	0.0076 ± 0.0041	0.0129 ± 0.0079	0.0107 ± 0.0061	0.0077 ± 0.0040	0.0077 ± 0.0041	0.0081 ± 0.0036
22:6n-3	0.0325 ± 0.0246	0.0354 ± 0.0288	0.0427 ± 0.0276	0.0280 ± 0.0239	0.0290 ± 0.0273	0.0317 ± 0.0286
24:0	0.0104 ± 0.0059	0.0069 ± 0.0035	0.0085 ± 0.0038	0.0063 ± 0.0044	0.0107 ± 0.0100	0.0081 ± 0.0025
FA sum	3.3072 ± 0.9684	2.8695 ± 0.4466	3.0976 ± 0.7621	2.6949 ± 0.4246	2.3962 ± 0.2454	2.6497 ± 0.4752

[a]Significantly higher after CLA supplementation compared to placebo ($P < 0.001$).

DISCUSSION

In the present study we examined the effect of supplementation with CLA (2.1 g for 45 d) vs. a placebo on women's body fat, biochemical parameters of serum, and the CLA isomer content of individual serum lipid classes. We found no effect of CLA on body mass, body fat, or serum leptin, the latter considered an index of fat mass (18). These findings are in agreement with results published in three articles (7–9), which showed no significant change in body mass or body fat

TABLE 5
Serum Concentrations (mmol/L) of Cholesteryl Ester Acyl Groups

	CLA-placebo group			Placebo-CLA group		
FA	Baseline	CLA	Placebo	Baseline	Placebo	CLA
14:0	0.0212 ± 0.0079	0.0208 ± 0.0110	0.0165 ± 0.0122	0.0205 ± 0.0062	0.0155 ± 0.0054	0.0185 ± 0.0093
16:0	0.4424 ± 0.0650	0.3969 ± 0.0632	0.3919 ± 0.0382	0.3794 ± 0.0521	0.3513 ± 0.0582	0.3684 ± 0.0587
16:1n-7	0.0677 ± 0.0462	0.0634 ± 0.0506	0.0667 ± 0.0603	0.0584 ± 0.0132	0.0457 ± 0.0147	0.0461 ± 0.0098
18:0	0.0779 ± 0.0564	0.0588 ± 0.0300	0.0468 ± 0.0155	0.0446 ± 0.0182	0.0413 ± 0.0225	0.0658 ± 0.0458
18:1n-9	0.6136 ± 0.1334	0.6050 ± 0.1591	0.5744 ± 0.1107	0.5414 ± 0.0762	0.4956 ± 0.1006	0.4971 ± 0.0646
18:1n-7	0.0346 ± 0.0064	0.0324 ± 0.0110	0.0359 ± 0.0041	0.0287 ± 0.0078	0.0290 ± 0.0052	0.0282 ± 0.0055
18:2n-6	1.3732 ± 0.2956	1.2204 ± 0.1942	1.4136 ± 0.2294	1.2437 ± 0.1948	1.2054 ± 0.2182	1.3273 ± 0.2773
cis,trans-9,11-CLA	0.0020 ± 0.0020	0.0036 ± 0.0033	0.0024 ± 0.0026	0.0025 ± 0.0029	0.0032 ± 0.0027	0.0038 ± 0.0029
trans,cis-10,12-CLA	0.0014 ± 0.0010	0.0019 ± 0.0018	0.0015 ± 0.0010	0.0018 ± 0.0013	0.0020 ± 0.0013	0.0038 ± 0.0039
trans,trans-9,11-CLA	0.0051 ± 0.0027	0.0051 ± 0.0024	0.0045 ± 0.0022	0.0066 ± 0.0040	0.0044 ± 0.0019	0.0050 ± 0.0015
CLA sum	0.0085 ± 0.0052	0.0106 ± 0.0073	0.0084 ± 0.0056	0.0109 ± 0.0060	0.0096 ± 0.0050	0.0126 ± 0.0075
18:3n-6	0.0102 ± 0.0072	0.0070 ± 0.0048	0.0122 ± 0.0106	0.0110 ± 0.0076	0.0119 ± 0.0109	0.0084 ± 0.0040
18:3n-3	0.0074 ± 0.0079	0.0037 ± 0.0022	0.0059 ± 0.0024	0.0047 ± 0.0015	0.0063 ± 0.0045	0.0038 ± 0.0011
20:1n-9	0.0006 ± 0.0002	0.0004 ± 0.0001	0.0006 ± 0.0002	0.0005 ± 0.0002	0.0007 ± 0.0005	0.0006 ± 0.0006
20:3n-6	0.0096 ± 0.0041	0.0086 ± 0.0049	0.0115 ± 0.0041	0.0079 ± 0.0014	0.0073 ± 0.0026	0.0084 ± 0.0033
20:4n-6	0.0407 ± 0.0160	0.0368 ± 0.0101	0.0543 ± 0.0191	0.0447 ± 0.0112	0.0514 ± 0.0169	0.0475 ± 0.0170
20:5n-3	0.0042 ± 0.0015	0.0048 ± 0.0034	0.0069 ± 0.0019	0.0049 ± 0.0020	0.0039 ± 0.0013	0.0042 ± 0.0015
22:5n-3	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000
22:6n-3	0.0044 ± 0.0020	0.0045 ± 0.0012	0.0052 ± 0.0033	0.0039 ± 0.0031	0.0038 ± 0.0030	0.0036 ± 0.0027
24:0	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000
FA sum	2.7169 ± 0.4874	2.4736 ± 0.4311	2.6508 ± 0.3696	2.4054 ± 0.3176	2.2787 ± 0.3820	2.4406 ± 0.4284

TABLE 6
Serum Concentrations (mmol/L) of Acyl Groups in Total Lipids

	CLA-placebo group			Placebo-CLA group		
FA	Baseline	CLA	Placebo	Baseline	Placebo	CLA
14:0	0.1010 ± 0.0828	0.0948 ± 0.0779	0.0733 ± 0.0521	0.0764 ± 0.0341	0.0587 ± 0.0303	0.0809 ± 0.0470
16:0	2.9500 ± 1.1592	2.6060 ± 0.9496	2.4593 ± 0.7720	2.3379 ± 0.6222	2.0516 ± 0.6210	2.0639 ± 0.6721
16:1n-7	0.2139 ± 0.1770	0.1939 ± 0.1417	0.2036 ± 0.1842	0.1636 ± 0.0591	0.1201 ± 0.0358	0.1220 ± 0.0316
18:0	1.5616 ± 1.0664	1.2220 ± 0.5900	1.0193 ± 0.4286	1.1376 ± 0.7004	0.9250 ± 0.3762	1.0267 ± 0.3785
18:1n-9	2.4941 ± 0.8858	2.3071 ± 0.6639	2.3250 ± 0.7348	1.9870 ± 0.5036	1.7843 ± 0.4916	1.9007 ± 0.4179
18:1n-7	0.1744 ± 0.0528	0.1610 ± 0.0737	0.1732 ± 0.0525	0.1422 ± 0.0417	0.1321 ± 0.0336	0.1236 ± 0.0400
18:2n-6	3.6316 ± 1.1366	3.3498 ± 1.1104	3.5880 ± 1.0820	3.1951 ± 1.2566	3.0691 ± 1.1100	3.2180 ± 1.3070
cis,trans-9,11-CLA[a]	0.0208 ± 0.0096	0.0401 ± 0.0228	0.0194 ± 0.0081	0.0205 ± 0.0105	0.0187 ± 0.0145	0.0386 ± 0.0266
trans,cis-10,12-CLA[a]	0.0091 ± 0.0029	0.0160 ± 0.0104	0.0073 ± 0.0045	0.0093 ± 0.0052	0.0069 ± 0.0049	0.0217 ± 0.0141
trans,trans-9,11-CLA[b]	0.0206 ± 0.0062	0.0204 ± 0.0066	0.0144 ± 0.0052	0.0211 ± 0.0087	0.0165 ± 0.0100	0.0212 ± 0.0101
CLA sum[a]	0.0504 ± 0.0117	0.0766 ± 0.0340	0.0410 ± 0.0118	0.0508 ± 0.0236	0.0422 ± 0.0266	0.0815 ± 0.0460
18:3n-6	0.0360 ± 0.0326	0.0260 ± 0.0226	0.0311 ± 0.0198	0.0464 ± 0.0355	0.0245 ± 0.0204	0.0369 ± 0.0366
18:3n-3	0.0268 ± 0.0163	0.0230 ± 0.0158	0.0312 ± 0.0250	0.0277 ± 0.0221	0.0236 ± 0.0133	0.0230 ± 0.0127
20:1n-9	0.0142 ± 0.0054	0.0137 ± 0.0049	0.0150 ± 0.0063	0.0114 ± 0.0028	0.0127 ± 0.0077	0.0145 ± 0.0059
20:3n-6	0.0972 ± 0.0650	0.0930 ± 0.0885	0.1139 ± 0.0932	0.0837 ± 0.0501	0.0670 ± 0.0414	0.0741 ± 0.0449
20:4n-6	0.2828 ± 0.1721	0.2830 ± 0.1906	0.3382 ± 0.2024	0.3065 ± 0.1616	0.3082 ± 0.2130	0.3104 ± 0.1962
20:5n-3	0.0169 ± 0.0065	0.0228 ± 0.0268	0.0288 ± 0.0166	0.0384 ± 0.0346	0.0259 ± 0.0161	0.0257 ± 0.0118
22:5n-3	0.0154 ± 0.0087	0.0158 ± 0.0129	0.0167 ± 0.0125	0.0164 ± 0.0092	0.0150 ± 0.0088	0.0244 ± 0.0207
22:6n-3	0.0446 ± 0.0397	0.0471 ± 0.0441	0.0660 ± 0.0630	0.0531 ± 0.0441	0.0430 ± 0.0351	0.0506 ± 0.0534
24:0	0.0170 ± 0.0158	0.0126 ± 0.0114	0.0112 ± 0.0070	0.0120 ± 0.0128	0.0114 ± 0.0112	0.0092 ± 0.0035
FA sum	11.7279 ± 3.6029	10.5482 ± 3.2376	10.5350 ± 2.8161	9.6862 ± 2.7535	8.7144 ± 2.2703	9.1862 ± 2.4528

[a]$P \leq 0.001$, significantly higher after CLA supplementation compared to placebo.
[b]$P < 0.05$, significantly higher after CLA supplementation compared to placebo.

after CLA supplementation in humans. On the other hand, our findings contrast those of five other articles (2–6), which have found that CLA reduced human body fat.

The equivocal findings on the effect of CLA on body fat may be attributed to differences in design, subject characteristics, dosage, and duration of supplementation. In particular, compared to our previous study (3), in which we found a significant reduction in body fat with 1.4 g of CLA for 28 d, the present study used a cautious increase in dosage (owing to concern about safety) and duration of supplementation. This, along with the use of the same method of body fat estimation, led us to expect a positive outcome in the present study as well. Our negative findings may be attributed to the different design of the present study, i.e., the use of each subject as a control of herself (to protect the validity of the results from differences in subject characteristics of different groups) and the use of an homogeneous sample in terms of gender. Interestingly, the participants in each of the studies that has found body fat reduction were of both sexes or men only (2–6), whereas two out of the four studies that showed no significant change in body fat (Ref. 8; present study) have used only women. Additionally, the effect of CLA may depend on the degree of fatness, as most of the studies that have found body fat reduction have used nonobese subjects (2,3,5,6), whereas two of the studies that have not have used obese subjects (7,8).

Concerning leptin, our data contrast the finding of a significant reduction after CLA supplementation despite no effect on body fat (19). This difference may be attributed to differences in dosage (3 vs. 2.1 g/d), supplementation period (64 vs. 45 d), and subject characteristics (obese vs. nonobese women) between the aforementioned and the present study.

The absence of a significant effect of CLA supplementation on the lipidemic profile in the present study is in agreement with the majority of the relevant articles. Specifically, studies have found no effect of CLA on human serum TAG (3–5,9,10), with the exception of the report by Noone *et al.* (20), who found decreased TAG levels after CLA supplementation. Likewise, studies have found no cholesterol lowering effect of CLA (3–5,9,10,20) except for that of Blankson *et al.* (2). Finally, HDL and LDL cholesterol have been reported to be unaffected by CLA supplementation (4,5,9,10,20) with two exceptions where reductions were found (2,3). As pointed out elsewhere, careful scrutiny of the literature suggests that at present it is premature to assign any beneficial role of CLA in terms of its ability to affect blood lipids (21).

Motivated by the implication of CLA in increased risk for liver tumor promotion in mice (22), we measured ALT as an index of liver damage. The finding of no significant change with CLA supplementation provides no evidence of such damage with the regimen used in the present study.

We have determined the incorporation of CLA isomers into lipid classes of human serum before and after CLA supplementation. To be certain that no modification of CLA isomers takes place we have used base-catalyzed transesterification of acyl groups. Isomer modification has been reported to occur with acid catalysis (13), although there is no agreement on the issue (16). Avoidance of possible artifacts was

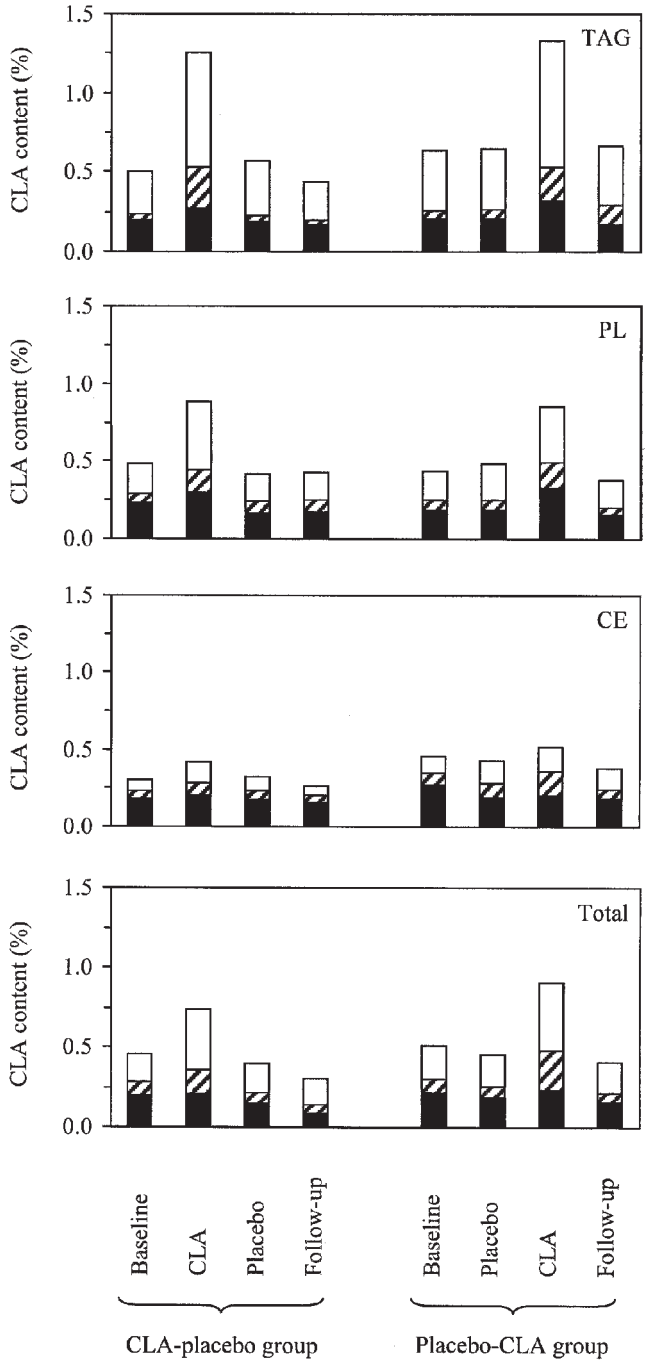

FIG. 1. Molar percentages of *cis,trans*-9,11-CLA (open bars), *trans,cis*-10,12-CLA (hatched bars), and *trans,trans*-9,11-CLA (solid bars) within the acyl groups of serum TAG, phospholipids (PL), cholesteryl esters (CE), and total lipids of the CLA-placebo and the placebo-CLA groups at baseline, end of CLA supplementation, end of placebo supplementation, and follow-up visit.

achieved at the cost of not determining two minor lipid components, nonesterified FA and sphingomyelin, which are not (trans)esterified through base catalysis (13).

The main CLA isomers of serum lipids at baseline were *cis,trans*-9,11 and *trans,trans*-9,11/10,12. These have been reported to exist in dairy products and ruminant fat (17, 23,24). CLA supplementation resulted in significant increases in the percentages of CLA isomers in serum lipids, in agreement with data from similar studies (3,5,10,20). TAG were the lipid class where the most remarkable increase occurred (from 0.57 to 1.29%) and the class with the highest CLA content. In accordance with our previous study (3), CLA was lowest in CE, where linoleate (the common isomer of CLA) is most abundant, thus reiterating the different metabolic fates of these FA.

The incorporation of the two CLA isomers of the experimental capsules (i.e., *cis,trans*-9,11 and *trans,cis*-10,12) into serum lipids increased two- to fivefold with supplementation (specifically, 2.4- and 4.8-fold in TAG, 2.0- and 2.1-fold in PL, and 2.2- as well as 2.4-fold in total lipids, respectively). Interestingly, the percentage of the *trans,trans*-9,11/10,12 isomers (which were not present in the supplement) also increased significantly, although only 1.5-fold and only in TAG and PL. This suggests that part of these isomers may be produced endogenously (probably from the other two isomers).

For the first time, we report on the washout of CLA supplement from human serum lipids. Our data show that a period of 2 wk after cessation of supplementation was sufficient for the return of CLA concentrations to baseline values (Fig. 1).

In conclusion, supplementation of healthy nonobese women with 2.1 g of CLA daily for 45 d caused a two- to fivefold increase in its incorporation into serum TAG, PL, and total lipids. In contrast, the CLA content of CE did not change significantly. Despite the significant increase of CLA levels in blood, there was no evidence of fat reduction. Additionally, there was no change in serum leptin and lipids. Further controlled studies should allow us to settle the discrepancies of the current literature on the effects of this interesting nutrient on human metabolism and the factors that may modulate these effects.

REFERENCES

1. Pariza, M.W., Park, Y., and Cook, M.E. (2001) The Biologically Active Isomers of Conjugated Linoleic Acid, *Progr. Lipid Res. 40,* 283–298.
2. Blankson, H., Stakkestad, J.A., Fagertun, H., Thom, E., Wadstein, J., and Gudmundsen, O. (2000) Conjugated Linoleic Acid Reduces Body Fat Mass in Overweight and Obese Humans, *J. Nutr. 130,* 2943–2948.
3. Mougios, V., Matsakas, A., Petridou, A., Ring, S., Sagredos, A., Melissopoulou, A., Tsigilis, N., and Nikolaidis, M. (2001) Effect of Supplementation with Conjugated Linoleic Acid on Human Serum Lipids and Body Fat, *J. Nutr. Biochem. 12,* 585–592.
4. Riserus, U., Berglund, L., and Vessby, B. (2001) Conjugated Linoleic Acid (CLA) Reduced Abdominal Adipose Tissue in Obese Middle-Aged Men with Signs of the Metabolic Syndrome: A Randomised Controlled Trial, *Int. J. Obes. Relat. Metab. Disord. 25,* 1129–1135.
5. Smedman, A., and Vessby, B. (2001) Conjugated Linoleic Acid Supplementation in Humans—Metabolic Effects, *Lipids 36,* 773–781.
6. Thom, E., Wadstein, J., and Gudmundsen, O. (2001) Conjugated Linoleic Acid Reduces Body Fat in Healthy Exercising Humans, *J. Int. Med. Res. 29,* 392–396.
7. Atkinson, R.L. (1999) Conjugated Linoleic Acid for Altering Body Composition and Treating Obesity, in *Advances in Conju-*

gated Linoleic Acid Research, Vol. 1 (Yurawecz, M.P., Mossoba, M.M., Kramer, J.K.G., Pariza, M.W., and Nelson, G., eds.), pp. 348–353, AOCS Press, Champaign.
8. Zambell, K.L., Keim, N.L., Van Loan, M.D., Gale, B., Benito, P., Kelley, D.S., and Nelson, G.J. (2000) Conjugated Linoleic Acid Supplementation in Humans: Effects on Body Composition and Energy Expenditure, *Lipids 35,* 777–782.
9. Kreider, R.B., Wilson, M., Ferreira, M.P., Greenwood, M., and Almada, L.A. (2002) Effects of Conjugated Linoleic Acid Supplementation During Resistance Training on Body Composition, Bone Density, Strength, and Selected Hematological Markers, *J. Strength Cond. Res. 16,* 325–334.
10. Benito, P., Nelson, G.J., Kelley, D.S., Bartolini, G., Schmidt, P.C., and Simon, V. (2001) The Effect of Conjugated Linoleic Acid on Plasma Lipoproteins and Tissue Fatty Acid Composition in Humans, *Lipids 36,* 229–236.
11. Parizkova, J. (1968) Body Composition and Physical Fitness, *Curr. Anthropol. 9,* 273–287.
12. Williams, C.A., and Bale, P. (1998) Bias and Llimits of Agreement Between Hydrodensitometry, Bioelectrical Impedance and Skinfold Calipers Measures of Percentage Body Fat, *Eur. J. Appl. Physiol. Occup. Physiol. 77,* 271–277.
13. Kramer, J.K.G., Fellner, V., Dugan, M.E.R., Sauer, F.D., Mossoba, M.D., and Yurawecz, M.P. (1997) Evaluating Acid and Base Catalysts in the Methylation of Milk and Rumen Fatty Acids with Special Emphasis on Conjugated Dienes and Total *trans* Fatty Acids, *Lipids 32,* 1219–1228.
14. Holland, B., Welch, A.A., Unwin, I.D., Buss, D.H., Paul, A.A., and Southgate, D.A.T. (1991) *McCance and Widdowson's the Composition of Foods,* Royal Society of Chemistry, Cambridge, United Kingdom.
15. Fellingham, G.W., Bryce, G.R., and Carter, M.W. (1978) Latin Square Changeover Design in Physical Education Research, *Res. Quart. 49,* 125–134.
16. Park, S.J., Park, C.W., Kim, S.J., Kim, J.K., Kim, Y.R., Park, K.A., Kim, J.O., and Ha, Y.L. (2002) Methylation Methods for the Quantitative Analysis of Conjugated Linoleic Acid (CLA) Isomers in Various Lipid Samples, *J. Agric. Food Chem. 50,* 989–996.
17. Werner, S.A., Luedecke, L.O., and Shultz, T.D. (1992) Determination of Conjugated Linoleic Acid Content and Isomer Distribution in Three Cheddar-type Cheeses: Effects of Cheese Cultures, Processing, and Aging, *J. Agric. Food Chem. 40,* 1817–1821.
18. Lönnqvist, F., Nordfors, L., Jansson, M., Thörne, A., Schalling, M., and Arner, P. (1997) Leptin Secretion from Adipose Tissue in Women. Relationship to Plasma Levels and Gene Expression, *J. Clin. Invest. 99,* 2398–2404.
19. Medina, E.A., Horn, W.F., Keim, N.L., Havel, P.J., Benito, P., Kelley, D.S., Nelson, G.J., and Erickson, K.L. (2000) Conjugated Linoleic Acid Supplementation on Humans: Effects on Circulating Leptin Concentrations and Appetite, *Lipids 35,* 783–788.
20. Noone, E.J., Roche, H.M., Nugent, A.P., and Gibney, M.J. (2002) The Effects of Dietary Supplementation Using Isomeric Blends of Conjugated Linoleic Acid on Lipid Metabolism in Healthy Human Subjects, *Br. J. Nutr. 88,* 243–251.
21. Khosla, P., and Fungwe, T.V. (2001) Conjugated Linoleic Acid: Effects on Plasma Lipids and Cardiovascular Function, *Curr. Opin. Lipidol. 12,* 31–34.
22. Belury, M.A., Moya-Camarena, S.Y., Liu, K.L., and Vanden-Heuvel, J.P. (1997) Dietary Conjugated Linoleic Acid Induces Peroxisome-Specific Enzyme Accumulation and Ornithine Decarboxylase Activity in Mouse Liver, *J. Nutr. Biochem. 8,* 579–584.
23. Fogerty, A.C., Ford, G.L., and Svoronos, D. (1988) Octadeca-9,11-dienoic Acid in Foodstuffs and in the Lipids of Human Blood and Breast Milk, *Nutr. Rep. Int. 38,* 937–944.
24. Ha, Y.L., Grimm, N.K., and Pariza, M.W. (1989) Newly Recognized Anticarcinogenic Fatty Acids: Identification and Quantification in Natural and Processed Cheeses, *J. Agric. Food Chem. 37,* 75–81.

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