Conjugated Linoleic Acid Supplementation in Humans—Metabolic Effects

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ABSTRACT: Supplementation with conjugated linoleic acid (CLA) induces a number of physiological effects in experimental animals, including reduced body fat content, decreased aortic lipid deposition, and improved serum lipid profile. Controlled trials on the effects of CLA in humans have hitherto been scarce. The aim of this study was to evaluate the effects of supplementation with CLA in healthy humans on anthropometric and metabolic variables and on the fatty acid composition of serum lipids and thrombocytes. Fifty-three healthy men and women, aged 23–63 yr, were randomly assigned to supplementation with CLA (4.2 g/d) or the same amount of olive oil during 12 wk in a double-blind fashion. The proportion of body fat decreased (–3.8%, $P < 0.001$) in the CLA-treated group, with a significant difference from the control group ($P = 0.050$). Body weight, body mass index, and sagittal abdominal diameter were unchanged. There were no major differences between the groups in serum lipoproteins, nonesterified fatty acids, plasma insulin, blood glucose, or plasminogen activator inhibitor 1 (PAI-1). In the CLA group the proportions of stearic, docosatetraenoic, and docosapentaenoic acids increased in serum lipids and thrombocytes, while proportions of palmitic, oleic, and dihomo-γ-linolenic acids decreased, causing a decrease of the estimated Δ-6 and Δ-9 and an increase in the Δ-5 desaturase activities. These results suggest that supplementation with CLA may reduce the proportion of body fat in humans and that CLA affects fatty acid metabolism. No effects on body weight, serum lipids, glucose metabolism, or PAI-1 were seen.

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Conjugated linoleic acid (CLA) is the common name of a group of fatty acids found in dairy products and meat from ruminants (1). CLA is an octadecadienoic acid (18:2) with two conjugated double bonds, predominantly found in the 9 and 11 or 10 and 12 positions. Each of the double bonds can be in *cis* or *trans* conformation. Lately, CLA has received considerable attention due to its metabolic and chemoprotective properties in experimental animals. These effects include reduced body fat content, improved serum lipid profile, decreased aortic lipid deposition, enhanced glucose metabolism and inhibited tumorigenesis (2,3). Whereas there are a large number of data from animal experiments, there are hitherto few reports on the effects of CLA in humans with inconclusive results (4–6).

The aim of the present study was to investigate the effects of CLA supplementation in humans under doubly-blinded placebo-controlled conditions and evaluate the effects on anthropometric variables, body composition, serum lipids, and fatty acid composition of serum lipids and thrombocytes. We previously reported the effects of CLA on lipid peroxidation in humans (7), which was also evaluated in this trial.

MATERIALS AND METHODS

Subjects. Fifty-three healthy subjects, 27 men and 26 women, between 23 and 63 yr of age were included and randomly assigned to either a CLA-treated group or a control group before entering the study. Table 1 shows baseline characteristics of the participants. At the baseline there were no statistical differences between the groups with regard to the variables in Table 1. All subjects gave their informed consent, and the study was approved by the Ethical Committee of the Faculty of Medicine at Uppsala University.

Study design. During the initial 2 wk all subjects were given control capsules containing olive oil. For the following 12 wk, in a double-blind design, the subjects in the CLA group were given capsules containing 4.2 g/d of CLA while the control group continued taking capsules containing the corresponding amount of the control oil consisting of olive oil. The CLA capsules contained 75.9% CLA with equal amounts of the CLA isomers *cis*-9,*trans*-11 and *trans*-10,*cis*-12, respectively, and only minor amounts of other isomers. In addition to CLA, the capsules contained small amounts of oleic acid (18:1n-9) (14.0%), palmitic acid (16:0) (4.4%), stearic acid (18:0) (1.5%), and linoleic acid (18:2n-6) (0.4%). All capsules were provided by Natural Ltd. A/S (Oslo, Norway). The examinations and blood samplings were done in the morning after an overnight fast. The main investigations, on which the calculations were based, were on the first and the last days of the trial. Minor examinations were performed during the fourth and eighth weeks of the trial. The participants were requested not to change their habits regarding diet and physical activity and to abstain from any dietary supplementation with vitamins, minerals, or fatty acids prior to and during the study.

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Abbreviations: Apo(a), apolipoprotein(a); Apo A-I, apolipoprotein A-I; Apo B, apolipoprotein B; BMI, body mass index; CLA, conjugated linoleic acid; HDL, high density lipoprotein; LDL, low density lipoprotein; PAI-1, plasminogen activator inhibitor 1; PPAR, peroxisome proliferator activator receptor; VLDL, very low density lipoprotein.

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TABLE 1
Baseline Characteristics of the Participants[a]

	Control group (n = 24)		CLA group (n = 26)	
	Mean (SD)	Range	Mean (SD)	Range
Sex (men/women)	10/14	—	15/11	—
Age (yr)	47.6 (10.2)	27.4–59.9	42.8 (13.1)	23.0–63.4
TG (mmol/L)	1.3 (0.6)	0.7–3.4	1.4 (0.8)	0.3–3.2
Serum cholesterol (mmol/L)	5.9 (1.1)	3.9–7.4	5.4 (1.0)	3.4–6.9
VLDL cholesterol (mmol/L)	0.32 (0.24)	0.06–0.94	0.36 (0.25)	0.04–0.93
LDL cholesterol (mmol/L)	4.0 (1.1)	2.0–5.9	3.6 (1.0)	1.6–4.8
HDL cholesterol (mmol/L)	1.3 (0.3)	0.8–2.1	1.2 (0.3)	0.9–2.2
VLDL-TG (mmol/L)	0.66 (0.51)	0.14–2.34	0.85 (0.66)	0.08–2.51
LDL-TG (mmol/L)	0.41 (0.14)	0.18–0.75	0.37 (0.11)	0.16–0.59
HDL-TG (mmol/L)	0.13 (0.08)	0.05–0.38	0.12 (0.06)	0.05–0.24
NEFA (mmol/L)	0.47 (0.21)	0.19–1.00	0.38 (0.16)	0.13–0.69
LDL/HDL	3.2 (1.2)	0.9–5.5	3.1 (1.0)	0.7–4.5
Apo A1 (g/L)	1.39 (0.24)	1.11–2.12	133 (17)	108–175
Apo B (g/L)	0.95 (0.26)	0.57–1.47	92 (26)	41–133
Apo(a) (U/L)	404 (366)$^{n=22}$	39–1192	234 (246)	31–924
Waist (cm)	83 (12)	66–111	85 (14)$^{n=24}$	64–114
Hip (cm)	101 (7)	93–116	101 (8)$^{n=24}$	89–122
SAD (cm)	22 (3)	18–28	22 (3)$^{n=24}$	18–31
BMI (kg/m^2)	24.5 (4.3)	19.1–34.5	25.5 (3.9)	19.5–33.5
Weight (kg)	73.8 (15.5)	54.0–109.0	77.1 (15.1)	53.0–105.0
Body fat (%)	29.6 (6.9)	15.9–46.2	29.3 (7.1)	11.4–46.7
WHR	0.8 (0.1)	0.7–1.0	0.8 (0.1)$^{n=24}$	0.7–1.0
Fasting insulin (mU/L)	8.3 (14.5)	2.7–75.4	8.4 (8.0)	2.4–40.0
Fasting blood glucose (mmol/L)	4.7 (0.5)	3.7–5.6	4.4 (0.8)	3.1–7.6
SBP (mm Hg)	122 (12)	94–146	121 (14)	106–170
DBP (mm Hg)	72 (10)	54–92	72 (7)	60–84
PAI-1 (U/mL)	8.5 (11.5)	0.1–37.2	15.7 (17.2)	0.1–48.4

[a]Apo, apolipoprotein; BMI, body mass index; HDL, high density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; NEFA, nonesterified fatty acids; PAI-1, plasminogen activator inhibitor 1; SAD, sagittal abdominal diameter; SD, standard deviation; TG, triglycerides; VLDL, very low density lipoprotein cholesterol; WHR, waist to hip.

Dietary assessment. On three occasions, before start and during the fifth and ninth weeks of the test period, the subjects were asked to perform 3-d weighed dietary records, including two weekdays and one weekend day. Dietary data from the registrations were computerized using the personal computer software MATs (MATs program version 4_03e, Rudans Lättdata, Västerås, Sweden). Calculations were made using the Swedish National Food Administration Food Database 2_97, PC version (Swedish National Food Administration, Uppsala, Sweden). The mean dietary intake at baseline is shown in Table 2. The fat provided by the CLA and control capsules was not included in the calculations of intakes.

Anthropometric measurements. Body weight was determined to the nearest kilogram and height to the nearest centimeter, wearing light indoor clothing and no shoes. The body mass index (BMI) was calculated as the body weight in kilograms divided by the square of the height in meters. The waist circumference was measured midway between the lowest rib and the iliac crest and the hip circumference at the widest part of the hip; and from these the waist-to-hip ratio was calculated. The sagittal abdominal diameter was measured as the height of the stomach when lying on the back on a firm bed with the knees bent. The percentage body fat was calculated from the three-compartment model based on measurement of skin fold thickness, measured with Harpenden skin fold calipers (John Bull, British Indicators Ltd., St Albans, Great Britain), and body water volume was estimated by a multifrequency bioelectric impedance analyzer Hydra 4200 (Xitron Technologies Inc., San Diego, CA) as described by Forslund *et al.* (8).

Fatty acid composition of serum lipids. The extraction, separation, and methylation of the plasma lipids were performed as described in detail by Boberg *et al.* (9). In short, plasma lipids were extracted with chloroform. Butylated hydroxytoluene and NaH_2PO_4 were added prior to evaporation under nitrogen. Phospholipids, triglycerides, and cholesterol esters were separated by thin-layer chromatography. The lipids esters were transmethylated with methanol and H_2SO_4. The fatty acid methyl esters were separated with gas chromatography using a Hewlett-Packard GC system (Avondale, PA) consisting of an HP 5890 Series II GC apparatus, HP 7673 automatic sampler, HP 3365A Series II Chemstation integrator software, and a 50 m × 0.25 mm CP-Sil 88 Chrompack capillary column, with helium as carrying gas. Standards from Nu-Chek-Prep (Elysian, MN) were used for identification of the individual fatty acids and as a control of the GC system. The technique used is not optimal for resolving different CLA isomers. Thus, the peaks identified as CLA when using a reference standard (Sigma Chemical, St. Louis, MO) were added, and the sum of the total CLA is presented here as CLA. The proportions of fatty acids are given as the relative percentage of the sum of the fatty acids analyzed. The desaturase

TABLE 2
Dietary Intake Before Start and During the Fifth and Ninth Weeks, $n = 50$[a]

		Control group (n = 24)		CLA group (n = 26)	
	Week	Mean (SD)	Range	Mean (SD)	Range
Energy (kJ)	0	10,110 (2115)	5,430–14000	9,710 (1810)	6,860–13360
	5	10,270 (2153)	6,250–15240	9,990 (2400)	5,910–15990
	9	9,480 (1609)	5,580–12030	9,870 (2240)	5,300–15470
Energy (kCal)	0	2,420 (506)	1,300–3350	2,320 (430)	1,640–3200
	5	2,460 (515)	1,500–3650	2,390 (580)	1,410–3830
	9	2,270 (390)	1,340–2880	2,360 (540)	1,270–3700
Fat (g)	0	87 (27)	48–153	81 (27)	39–134
	5	88 (24)	50–160	89 (32)	28–161
	9	81 (17)	38–115	87 (28)	26–150
Carbohydrate (g)	0	299 (70)	137–435	282 (44)	183–358
	5	303 (82)	176–555	282 (55)	180–407
	9	277 (63)	144–378	271 (56)	173–412
Protein (g)	0	93 (21)	48–137	88 (17)	58–114
	5	91 (20)	59–148	88 (20)	46–124
	9	89 (16)	52–116	86 (21)	41–123
Fiber (g)	0	25 (6)	13–37	25 (9)	10–44
	5	24 (6)	16–36	24 (9)	9–44
	9	23 (7)	10–33	22 (8)	11–41
Alcohol (g)	0	9 (10)	0–27	15 (15)	0–47
	5	11 (13)	0–37	15 (19)	0–83
	9	9 (10)	0–30	20 (20)	0–84
Fat (E%)	0	31 (5)	22–43	30 (6)	18–45
	5	31 (4)	21–43	32 (6)	18–41
	9	31 (4)	25–39	32 (6)	18–42
Carbohydrate (E%)	0	50 (7)	36–63	50 (7)	39–67
	5	50 (6)	37–62	49 (8)	36–68
	9	50 (6)	32–58	48 (8)	32–68
Protein (E%)	0	16 (3)	12–24	16 (2)	11–20
	5	15 (3)	10–20	15 (2)	11–18
	9	16 (3)	11–23	15 (2)	10–19
Saturated fat (E%)	0	13 (3)	9–20	12 (3)	4–20
	5	13 (3)	7–21	13 (4)	3–19
	9	13 (2)	9–19	13 (3)	4–18
Monounsaturated fat (E%)	0	12 (2)	8–17	11 (2)	8–17
	5	11 (2)	8–16	12 (2)	7–17
	9	12 (2)	9–15	12 (2)	6–17
Polyunsaturated fat (E%)	0	4 (1)	3–9	4 (1)	3–7
	5	5 (2)	3–11	5 (1)	3–10
	9	5 (1)	3–9	5 (1)	3–9

[a]Assessed using a 3-d weighed dietary record. SD, standard deviation; E%, energy percentage; CLA, conjugated linoleic acid.

activities were assessed by calculating product to precursor ratios as follows: 20:4n-6/20:3n-6 for Δ-5 desaturase, 18:3n-6/18:2n-6 for Δ-6 desaturase in triglycerides and cholesterol esters, 20:3n-6/18:2n-6 for Δ-6 desaturase in phospholipids and thrombocytes, and 18:1n-9/18:0 for Δ-9 desaturase activities.

Fatty acid composition of the thrombocytes. Blood was drawn using minimal venous pressure. A $16 \times g$ butterfly cannula was used to collect blood drop by drop. Thrombocyte clot was received through gentle centrifugation $120 \times g$ for 20 min, washed in saline, and dissolved with a Polytron mixer. From the dissolved clot the total platelet lipids were extracted with chloroform/methanol, including butylated hydroxytoluene. The fatty acid composition was analyzed as in the serum lipids.

Serum lipoprotein analyses. Serum lipoproteins (very low density lipoprotein, VLDL; low density lipoprotein, LDL; and high density lipoprotein, HDL) were isolated by a combination of preparative ultracentrifugation (10) and precipitation with a sodium phosphotungstate and magnesium chloride solution (11). Triglyceride and cholesterol concentrations in serum and in the isolated lipoprotein fractions were measured by enzymatic methods in a Monarch 2000 centrifugal analyzer. The concentrations of apolipoprotein A-I (Apo A-I) and apolipoprotein B (Apo B) were measured by immunoturbidimetry in a Monarch apparatus. Apolipoprotein(a) [Apo(a)] was determined by a Coda Automated EIA automatic enzyme-linked immunosorbent assay analyzer (Bio-Rad Laboratories, Hercules, CA) using Mercodia Apo(a) reagents (Mercodia AB, Uppsala, Sweden). Serum free fatty acids were measured by an enzymatic colorimetric method (Wako Chemicals, Neuss, Germany).

Glucose and insulin. Blood glucose was measured by the glucose dehydrogenase method (Gluc-DH; Merck,

Darmstadt, Germany). Plasma insulin was analyzed using an enzymatic-immunological assay (Enzymmun; Boehringer Mannheim) in an ES300 automatic analyzer (Boehringer Mannheim, Germany).

Plasminogen activator inhibitor-1 (PAI-1) activity. The amount of active PAI-1 was analyzed in citrate plasma using a commercially available bioimmunoassay (Chromolize PAI-1 kits; Biopool AB, Umeå, Sweden).

Statistical analyses. Two groups of subjects including 25 persons each was calculated to give a power of 80% to detect a difference in serum or LDL cholesterol of 9% if $P < 0.05$. For analyses of differences between the changes in the two groups, unpaired t-tests were used. Changes within each group were analyzed using paired t-tests. Variables with a skewed distribution ($W < 0.95$ in Shapiro-Wilk's W test for normality) were logarithmically transformed prior to the t-test. Variables that were not normally distributed after logarithmic transformation were analyzed using Wilcoxon Mann-Whitney two-sample test. Results with a P-value less than or equal to 0.05 were considered as significant. Percent change was calculated as [(mean value after – mean value before)/mean value before] × 100. The main statistical analyses were performed according to protocol, i.e., excluding participants with a low compliance (who had taken less than 80% of the prescribed number of capsules). Additional analyses were also made according to intention to treat, i.e. all subjects were included irrespective of their degree of compliance. Results from the latter analyses are shown in the text when differing from analyses according to the protocol. The statistical analyses were performed using the software systems Statistical Analysis System and STATA (Stata Corporation, College Station, TX).

RESULTS

Compliance and tolerance. All participants fulfilled the trial. The compliance, counted as percentage of eaten capsules out of those prescribed, was more than 91% in 46 of the participants, between 81 and 90% in three, and 80% or less of the capsules in three of the participants. The participants with a compliance of 80% or less were excluded from the main statistical analyses. The capsules were well tolerated, and only a few subjects reported mild diarrhea at some occasions. No effect on serum levels of the liver enzymes aspartate amino transferase and alanine amino transferase was found. Fifty out of the 53 subjects completed the three 3-d weighed dietary registrations. The participants did not statistically change their dietary intake during the study with regard to energy, fat, carbohydrates, or protein (data not shown).

Anthropometry. As shown in Table 3 there was no significant change in body weight, BMI, waist-to-hip ratio, and sagittal abdominal diameter. The proportion of body fat was reduced by 3.8% in the CLA group, $P = 0.0006$ ($P = 0.05$ for difference between the groups) (Table 3). When analyzing according to intention to treat (including all participants), reduction of body fat in the CLA group was 3.7%, $P < 0.001$ ($P = 0.07$ for difference between the groups). There was no relation between change in body fat and the proportion of body fat at start (data not shown).

TABLE 3
Change of Anthropometric Variables[a]

	Absolute change (range)	Percentage change[b]	P for difference within group	P for difference between groups
Weight (kg)				
Control	0.21 (–2–3)	0.28	0.487	0.664
CLA	0.4 (–6–4)	0.55	0.282	
BMI (kg/m$_2$)[c]				
Control	0.06 (–0.7–1.1)	0.25	0.409	0.655
CLA	0.14 (–1.9–1.5)	0.53	0.181	
WHR[(n=48)]				
Control	0.0 (–0.1–0.1)	1.04	0.311	0.560
CLA	0.0 (–0.1–0.1)	0.23	0.811	
SAD (cm)[d(n=48)]				
Control	0.1 (–2.5–2.0)	0.57	0.493	0.423
CLA	0.0 (–2.0–2.5)	–0.19	0.674	
Body fat (%)				
Control	–0.4 (–3.8–1.9)	–1.23	0.150	0.050
CLA	–1.1 (–3.9–1.7)	–3.84	0.0006	

[a]$n = 50$ unless otherwise stated. BMI, body mass index; SAD, sagittal abdominal diameter; WHR, waist-to-hip ratio.
[b]Percentage change is calculated as: [(value after – value before)/value before] × 100, using mean values.
[c]Logarithmically transformed prior to t-test.
[d]Analyzed using Wilcoxon Mann-Whitney two-sample test.

Serum lipids and apolipoproteins. As shown in Table 4, an increase in Apo B was observed in the CLA-treated group ($P = 0.009$) with a significant difference between the two groups ($P = 0.044$). Within the control group we observed a decreased LDL triglyceride concentration ($P = 0.033$) and a significant difference between the groups ($P = 0.039$). When including all participants in the statistical analyses, there were no significant differences between the changes in serum lipid and apolipoprotein concentrations in the two groups.

Plasma variables. There was a borderline significant difference between the changes of fasting blood glucose in the groups ($P = 0.053$), as shown in Table 4. When including all participants, there were no significant differences between the two groups with regard to changes in fasting glucose, insulin, nonesterified fatty acids, or PAI-1.

Fatty acid composition of serum lipids and thrombocytes. In the serum phospholipids the proportion of stearic acid (18:0), docosatetraenoic acid (22:4n-6), and docosapentaenoic acid (22:5n-3) increased, and palmitic acid (16:0), oleic acid (18:1n-9), γ-linolenic acid (18:3n-6) ($P = 0.065$), and dihomo-γ-linolenic acid (20:3n-6) decreased in the CLA group, as compared to the control group (Table 5). The fatty acid composition in the thrombocytes changed with an increase of docosapentaenoic acid (22:5n-3) and a decrease of oleic acid (18:1n-9) and dihomo-γ-linolenic acid (20:3n-6) as shown in Table 5. The changes of fatty acid composition of serum cholesterol esters and triglycerides were similar to the changes of the serum phospholipids (data not shown). The changed proportions of the fatty acid composition in the serum lipids correspond to an increase in the estimated Δ-5 desaturase activity (20:4n-6/20:3n-6) in the CLA group as com-

TABLE 4
Changes of Serum Lipids, Blood Glucose, Plasma Insulin, and PAI-1[a]

	Absolute change (range)	Percentage change[b]	P for difference within group	P for difference between groups
Total cholesterol (mmol/L)				
Control	0.17	2.82	0.197	0.432
CLA	0.29	5.35	0.006	
HDL cholesterol[c] (mmol/L)				
Control	0.16	12.1	<0.0001	0.204
CLA	0.09	7.35	0.004	
LDL cholesterol (mmol/L)				
Control	0.09	2.25	0.386	0.415
CLA	0.20	5.46	0.022	
LDL/HDL				
Control	−0.30	−9.25	0.018	0.168
CLA	−0.10	−3.40	0.193	
TG[c] (mmol/L)				
Control	−0.23	−17.8	0.006	0.445
CLA	−0.07	−4.82	0.184	
HDL-TG[c] (mmol/L)				
Control	−0.02	−16.1	0.186	0.869
CLA	−0.01	−7.69	0.401	
LDL-TG (mmol/L)				
Control	-0.04	-8.84	0.033	0.039
CLA	0.01	2.06	0.566	
VLDL cholesterol[c] (mmol/L)				
Control	0.01	1.19	<0.0001	0.577
CLA	0.11	13.0	<0.0001	
VLDL-TG[c] (mmol/L)				
Control	−0.13	−20.0	0.224	0.785
CLA	−0.01	−0.45	0.479	
NEFA[c] (mmol/L)				
Control	0.02	3.40	0.926	0.941
CLA	0.01	3.02	0.980	
Apo A1[d] (g/L)				
Control	−0.62	−0.45	0.303	0.472
CLA	−2.88	−2.16	0.109	
Apo B (g/L)				
Control	−1.0	−1.06	0.702	0.044
CLA	5.77	6.24	0.009	
Apo(a)[d] (U/L)$^{(n=48)}$				
Control	16.9	4.20	0.833	0.482
CLA	12.8	5.47	0.208	
Blood glucose[c] (mmol/L)$^{(n=49)}$				
Control	−0.06	−1.30	0.159	0.053
CLA	0.11	2.45	0.174	
Plasma insulin[d] (mU/L)				
Control	−2.30	−27.81	0.875	0.600
CLA	1.16	−13.7	0.424	
PAI-1[c] (U/mL)$^{n=50}$				
Control	3.69	43.2	0.184	0.575
CLA	5.08	32.4	0.385	

[a] n = 50 unless otherwise stated. For abbreviations see Table 1.
[b] Percentage change is calculated as: [(value after – value before)/value before] × 100, using mean values.
[c] Logarithmically transformed prior to t-test.
[d] Analyzed using Wilcoxon Mann-Whitney two-sample test.

pared to the control group, whereas the estimated activities of Δ-6 desaturase (18:3n-6/18:2n-6 in triglycerides and cholesterol esters and 20:3n-6/18:2n-6 in phospholipids) and Δ-9 desaturase (18:1n-9/18:0) decreased as compared to the control group (data not shown). Also in the thrombocytes, there was an increase in Δ-5 (20:4n-6/20:3n-6) and decrease in Δ-6 (20:3n-6/18:2n-6) estimated activities in the CLA group as compared to the control group while Δ-9 (18:1n-9/18:0) was unchanged (data not shown). No changes were seen in the activity of Δ-9 desaturase calculated as the ratio between 16:1n-7 and 16:0 in any of the compartments analyzed.

The effects of CLA and control supplementation on any of the parameters analyzed were not significantly different in men and women and were not changed when including mean fat intake in the statistical analyses as a possible confounder (data not shown)

DISCUSSION

In this study the proportion of body fat in the subjects given CLA decreased significantly within the CLA-treated group by 3.8% with a borderline significant decrease as compared to the control group ($P = 0.050$). BMI, body weight, waist-to-hip ratio, and sagittal abdominal diameter were unchanged (Table 3).

Hitherto, there are two published studies on the effects of CLA on human body composition (4,6). In the study by Zambell *et al.*, where healthy normal weight women were fed 3 g of CLA, consisting of minor amounts of several different isomers, no effect on body weight or composition was found (4). Contrastingly, Blankson *et al.* (6) observed a decreased body fat mass in overweight or moderately obese men and women when supplementing with 3.4 or 6.8 g of a CLA preparation with equal proportions of the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers. The diverging results between the two studies mentioned and the present could possibly be due to the different amounts and isomer compositions of the CLA preparation because different isomers and different study designs have been hypothesized to have different effects (12,13). It could also be due to the different study designs. Another interesting possibility is that there might be diverging effects of CLA in obese compared to normal-weight subjects. There could possibly also be gender and/or genetically determined difference. CLA supplementation to animals has been observed to reduce body fat (14). Little work has been done on the mechanisms of action of CLA on energy metabolism and the explanations of the effects are as yet not known. CLA has been suggested to affect the rate of *de novo* lipogenesis and/or the rate of lipolysis. Increased lipolysis and decreased lipoprotein lipase activity have been observed *in vitro* in adipocytes when CLA was added to the medium (14). An increased carnitine palmitoyltranferase activity giving an increased fatty acid oxidation in adipose and skeletal muscle tissue was found in mice after CLA supplementation (14). However, no effects on energy expenditure or fat oxidation was observed after CLA supplementation in healthy women (4).

In the present study, we observed an increase in Apo B and LDL triglycerides in the CLA group when compared to the control group (Table 4). Within the CLA group there were increases in total and LDL cholesterol, although not different from the changes in the control group, as also observed in a recent study of healthy women (15). In another study, reduc-

TABLE 5
Fatty Acid Composition of Serum Phospholipids and Thrombocytes[a]

	Serum phospholipids			Thrombocytes		
	Mean at baseline	Change (%)[b]	*P* for difference between groups	Mean at baseline	Change (%)	*P* for difference between groups
14:0						
Control	0.46	−8.0	0.926	0.67[c]	−7.1	0.885
CLA	0.43	−7.8		0.63[c]	−1.9	
15:0						
Control	0.22	−5.0	0.669	0.23[d]	−8.5	0.533$^{(n=44)}$
CLA	0.20	−7.4*		0.22[d]	−14.7**	
16:0						
Control	31.3	0.3	0.045	23.3	−0.4	0.920
CLA	31.2	−1.2*		23.5	−0.6	
16:1n-7						
Control	0.52[c]	−8.4	0.160$^{(n=46)}$	0.42[c]	7.0	0.507$^{(n=48)}$
CLA	0.55[c]	−14.8**		0.42[c]	14.3	
17:0						
Control	0.43[d]	−6.8	0.595	0.38[c]	−3.9	0.829$^{(n=48)}$
CLA	0.39[d]	4.9		0.38[c]	−5.3	
18:0						
Control	14.2	−0.3	<0.0001	15.7	−0.9	0.467
CLA	13.8	6.3****		16.2	−2.5	
18:1n-9						
Control	12.1	1.7	<0.0001	19.1	0.7	0.015
CLA	12.8	−8.9****		19.5	−3.1*	
18:2n-6						
Control	20.5	−1.0	0.461	6.8	-2.1	0.112
CLA	21.0	1.2		6.7	3.4	
18:3n-6						
Control	0.08[c]	−9.3	0.065$^{(n=27)}$	ND	ND	ND
CLA	0.09[c]	−24.2**		ND	ND	
18:3n-3						
Control	0.49[c]	2.2	0.778	ND	ND	ND
CLA	0.55[c]	0.9		ND	ND	
20:3n-6						
Control	2.91	−1.5	0.0001	1.45	0.7	<0.0001
CLA	3.25	−16.0****		1.64	−11.5****	
20:4n-6						
Control	8.26	−0.8	0.645	22.1	−0.6	0.441
CLA	7.95	−2.1		21.5	−2.3	
20:5n-3						
Control	1.87[c]	2.3	0.746	1.14[c]	7.9	0.663
CLA	1.60[c]	8.3		1.00[c]	17.8*	
22:4n-6						
Control	0.25	-3.3	0.039	2.08	−6.0*	0.476
CLA	0.25	6.0*		2.08	−3.5	
22:5n-3						
Control	1.05	−1.0	<0.001	1.87	1.1	0.002
CLA	0.98	16.3****		1.78	12.3****	
22:6n-3						
Control	4.85[c]	4.4	0.983	2.65	3.9	0.143
CLA	4.31[c]	6.8		2.48	11.3**	
CLA						
Control	0.07	4.4	<0.0001$^{(n=32)}$	ND	ND	ND
CLA	0.07	609****		ND		

[a]n = 49 unless otherwise stated. *P < 0.05 for a change within the group, **P < 0.01 for a change within the group, ***P < 0.001 for a change within the group, ****P < 0.0001 for a change within the group.
[b]Percentage change is calculated as: [(mean value after − mean value before)/mean value before] × 100.
[c]Logarithmically transformed prior to t-test.
[d]Analyzed using Wilcoxon Mann-Whitney two-sample test.

tions of total, HDL, and LDL cholesterol, but no changes in triglycerides and lipoprotein(a), were seen within the treatment groups after CLA treatment (6). CLA has, in studies of experimental animals, been suggested to affect serum lipoprotein concentrations. In a recent study in hamsters, the levels of total, LDL, and HDL cholesterol decreased and triglycerides increased after treatment with a mixture of CLA isomers (16). Other animal studies have shown decreased plasma LDL cholesterol (17), decreased total and non-HDL cholesterol and triglycerides (18), and decreased levels of total cholesterol and triglycerides (19) after CLA supplementation. The diverging results between the present study in humans and earlier studies in experimental animals could be due to species differences, but should be further investigated.

Insulin and nonesterified fatty acid levels were unchanged after CLA supplementation in the present study, while there was a tendency to an increased glucose concentration in the CLA group as compared to the controls (Table 4). In another human study, no changes in insulin or glucose concentrations were observed (5). Belury and coworkers (20), on the other hand, have found that dietary CLA normalizes glucose tolerance and prevents the progression to hyperglycemia and diabetes in diabetic fatty fa/fa Zucker rat, with an effect similar to that of the insulin-sensitizing drug thiazolidindione. The insulin-sensitizing effects are suggested to be mediated *via* activation of peroxisome proliferator activator receptor (PPAR) γ and a subsequent stimulation of adipocyte differentiation. In the same study, they also found a reduction of plasma nonesterified fatty acid concentration after CLA supplementation. This effect was assumed to be caused by an increased β-oxidation, *via* an activation of hepatic PPAR γ.

The PAI-1 activity was not significantly changed in any of the groups. PAI-1 has been found to be elevated in obesity, hypertension, glucose intolerance, insulin resistance, and type 2 diabetes, all included in the metabolic syndrome (21). In the adipose tissue of humans, mice, and rats, relatively high levels of PAI-1 have been detected, with increasing amounts in obese subjects (22). One could anticipate a decreased PAI-1 activity in the CLA supplemented group as body fat was reduced in this group and as PAI-1 levels have been observed to decrease when reducing body fat or body weight (21,23), but no such effect was seen in the present study.

In contrast to our findings (Table 5), no changes in the fatty acid composition of plasma lipids, thrombocytes, or adipose tissue were observed in another study of the effects of CLA in humans (15,24). The authors suggested that this could be due to a low number of participants ($n = 17$), but it could also be due to the lower amounts of several different isomers. In two studies of CLA feeding to rats, different tissues showed decreased proportions of dihomo-γ-linolenic acid (20:3n-6) and increased docosatetraenoic (22:4n-6) and docosapentaenoic acid (22:5n-3) (25,26), as also observed in the present study. However, in the rats the proportions of γ-linolenic (18:3n-6) and arachidonic acid (20:4n-6) decreased, which was not observed in the present study. Decreased proportions of linoleic (27) and arachidonic acid (25,27) were found in mouse liver and rat mammary gland after supplementation with CLA. In the present study the proportions of linoleic acid (18:2n-6) were unchanged, possibly indicating that CLA is not displacing linoleic acid to any larger extent or is not metabolized in the same way, as suggested by Banni *et al.* (25). The decrease in oleic acid (18:1n-9) can be interpreted as a decreased Δ-9 activity, as observed previously after CLA supplementation in experimental animals (28,29). A decrease in the activity of Δ-9 desaturase has been suggested to be due to an inhibitory effect of CLA on the mRNA expression of stearoyl-CoA desaturase, an enzyme catalyzing the Δ-9 desaturation (28,29). The estimated Δ-5 desaturase activity increased in the CLA group compared to the control group. The Δ-5 desaturase activity in humans has been suggested to be inversely related to the proportion of body fat (30), insulin levels (31), and risk for myocardial infarction (32). The Δ-6 desaturase activity (18:3n-6/18:2n-6) decreased after CLA treatment, as observed earlier in *in vitro* rat liver microsomes (29). The increased proportion of docosapentaenoic acid (22:5n-3) and the unchanged docosahexaenoic acid (22:6n-3) supports the indications of decreased Δ-6 desaturase activity.

Although the method used here was not optimal for analysis of the different CLA isomers, the proportions of total CLA increased in serum phospholipids (Table 5), cholesterol esters, and triglycerides (data not shown) in the CLA-treated group as compared to the control group. This supports earlier findings in humans (15) and animals (25). The fact that the proportions of CLA increased only in the CLA-treated group could be regarded as a verification of compliance.

The altered fatty acid profile of the serum lipids and thrombocytes, especially the decreased proportions of dihomo-γ-linolenic acid (20:3n-6) and increased activity of Δ-5 desaturase may lead to an altered eicosanoid metabolism. We have elsewhere reported on increased urinary levels of 8-iso-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and 15-keto-dihydro-$PGF_{2\alpha}$ in humans after CLA supplementation (7). In some contrast to this Kavanaugh *et al.* (33) reported reduced PGE_2 levels in CLA-treated mice.

The intake of CLA in 123 Swedish men and women was estimated to be on average 160 mg/d (*cis*-9,*trans*-11) (34). CLA intakes in German men and women was estimated to 430 and 350 mg/d (*cis*-9,*trans*-11), respectively (35), and in 12 American men and women CLA intake was 127 mg/d (*cis*-9,*trans*-11) (36).

Of the known isomers of CLA, *cis*-9,*trans*-11 is the most abundant in natural food products (1). The two isomers considered most biologically active are *cis*-9,*trans*-11 and *trans*-10,*cis*-12. For example, *trans*-10,*cis*-12 has been suggested to be responsible for body composition changes in mice (37) and affect lipid metabolism in hamsters (16), and it has been observed to reduce milk fat synthesis in dairy cows (38). In the present study we used a mix of equal proportions of *cis*-9,*trans*-11 and *trans*-10,*cis*-12. It would be interesting to investigate the metabolic effects of the individual isomers.

The participants in the present study consisted of a rather heterogeneous group of healthy, nonobese, normolipidemic

men and women aged 23 to 63 yr. It is possible that the moderate changes seen after supplementation with CLA might be due to the heterogeneity of the group and to difficulties in changing physiological parameters within the normal range. The magnitude of a possible effect of CLA on, for example, body composition was unknown when designing the study. Thus the number of participants needed was estimated by a calculation of statistical power based on a change of serum lipid concentrations comparable to what has been seen when changing the fatty acid quality of the diet. Possibly, a larger number of participants is needed to detect changes in nonobese subjects. It would be interesting to study the effects of CLA supplementation in subjects with, for example, hyperlipidemia or abdominal obesity. An interesting view of this study is that the participants were weight-stable adult humans, as opposed to several studies where CLA has been given to growing animals. Judging from the effect on body composition in the present study and from the effects observed in growing animals, it could be hypothesized that CLA may be useful in inhibition of weight gain rather than in weight reduction *per se*.

These results indicate that supplementation with CLA for a limited period of time may cause reduction of the proportions of body fat and alter the fatty acid metabolism in healthy humans. However, CLA appeared to have no major effects on body weight, serum lipids, glucose metabolism, or PAI-1 in this group of subjects, in apparent contrast to results of earlier studies in animals. Whether the low concentrations of CLA naturally occurring in milk fat could have metabolic effects when eaten habitually for a long time still remains to be investigated.

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