ELSEVIER

Journal of Nutritional Biochemistry 12 (2001) 585–594

THE JOURNAL OF Nutritional Biochemistry

Effect of supplementation with conjugated linoleic acid on human serum lipids and body fat

Vassilis Mougios[a,*], Antonis Matsakas[a], Anatoli Petridou[a], Susanne Ring[b], Angelos Sagredos[c], Anastasia Melissopoulou[a], Nikos Tsigilis[a], Michalis Nikolaidis[a]

[a]*Department of Physical Education and Sport Science, Aristotle University of Thessaloniki, Thessaloniki, Greece*
[b]*Institute of Sport Sciences, University of Salzburg, Salzburg, Austria*
[c]*Consulting, Hamburg, Germany*

Received 11 January 2001; received in revised form 28 May 2001; accepted 8 June 2001

Abstract

Conjugated linoleic acid (CLA) is a natural component of meat and dairy products with anticarcinogenic, fat lowering, antiatherogenic and anticatabolic activity in animals. The purpose of this study was to examine the effect of CLA supplementation to humans on body fat, certain biochemical parameters of serum, and the CLA content of serum lipids. Twenty-two volunteers were divided into a study group and a control group in a doubly blind design. The study group received 0.7 g of CLA for four weeks and 1.4 g of CLA for the next four weeks, while the control group received placebo. Diet was controlled and no significant differences in energy or macronutrient intake were found between the two groups. Measurements were taken at baseline, four weeks, and eight weeks. The sum of the thickness of ten skinfolds, percentage body fat calculated from it and fat mass was significantly reduced in the CLA group during the second period ($P < 0.004$) but not overall during the study. Serum HDL-cholesterol decreased significantly ($P < 0.001$) and triacylglycerols as well as total cholesterol tended to decrease in the CLA group during the first period. The CLA content of serum non-esterified fatty acids, triacylglycerols, phospholipids, and cholesteryl esters increased gradually with supplementation; the CLA content of total serum lipids doubled at the end of the study compared to baseline. Phospholipids had the highest CLA content regardless of supplementation. These data indicate that supplementation with 0.7–1.4 g CLA daily for 4–8 weeks may modulate body fat and serum lipids, as well as increase the CLA content of serum lipids in humans.

Keywords: Cholesterol; Cholesteryl esters; HDL; Non-esterified fatty acids; Phospholipids; Triacylglycerols

1. Introduction

Conjugated linoleic acid (CLA) is a natural substance found mainly in the fat of ruminants and dairy products in quantities averaging 0.5 and 0.7% of total fat, respectively [1]. The term CLA covers several constitutional isomers and stereoisomers of octadecadienoic acid having conjugated double bonds. Studies on animals have shown that CLA inhibits carcinogenesis [2–5], lowers body fat [6–12], increases lean body mass [6,7,9,10,12], decreases atherogenesis [13–15], prevents the catabolic effects of immune stimulation [16,17], and exhibits antidiabetic characteristics [18], although its anticarcinogenic and antiatherogenic effects have been questioned [19,20]. Biological activity has been attributed to *cis*,*trans*-9,11-octadecadienoic acid [21] and *trans*,*cis*-10,12-octadecadienoic acid [22,23], the two major CLA isomers.

CLA is being marketed as a nutritional supplement for humans accompanied by claims for fat lowering, hypolipidemic, bodybuilding, and anticatabolic effects. These claims extrapolate the findings mentioned above but have not been substantiated with regard to humans. The aim of the present study was to investigate certain aspects of CLA's activity in the human body. In particular, we examined the effect of supplementation with moderate doses of CLA on (i) body fat, (ii) biochemical parameters of serum related to findings of animal studies, and (iii) the CLA content of individual serum lipid classes. Preliminary reports on our findings have been presented.[1,2]

* Corresponding author. Tel.: +3031-992237, 992238; fax: +3031-992183.
E-mail address: mougios@phed.auth.gr (V. Mougios).

0955-2863/01/$ – see front matter
PII: S0955-2863(01)00177-2

2. Materials and methods

2.1. Subjects

Fourteen men and ten women, aged 19–24, who responded to a public invitation participated in the study initially. Subjects were not obese (body mass index <30 kg m^{-2}), were not suffering from any chronic or acute illness, and were not taking 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.

2.2. Design

Each participant was given a balanced isoenergetic weekly dietary plan (based on estimated basal metabolic rate and physical activity) for the duration of the study. The participants were asked to record their dietary intake and the quantity of experimental capsules taken daily, as well as any medication they might be obliged to take in order to treat acute illness during the study. Finally, they were asked not to modify their usual way of life (including physical activity) in any other respect.

The participants were divided into a study group and a control group in a doubly blind design. Each group consisted of seven males and five females. The study group received 500-mg soft gelatin capsules of CLA-70 manufactured by TrofoCell (Hamburg, Germany). According to the manufacturer, each capsule contained 350 mg of CLA divided equally between the *cis,trans*-9,11 and *trans,cis*-10,12 isomers. This was confirmed by our own gas chromatographic analysis (as described below), which showed CLA to constitute 69% of each capsule, and the distribution of the two isomers to be 49 and 51%, respectively. The dosage recommended by the manufacturer was 2–4 capsules per diem. We therefore prescribed two capsules daily for 4 weeks and four capsules daily for the next 4 weeks to each member of the study group. The control group received the same dosage of identically looking capsules containing soybean oil as placebo.

2.3. Measurements

The participants visited the laboratory on three occasions: before starting receiving the capsules, immediately after the end of the low dosage, and immediately after the end of the high dosage. All measurements were taken between 9 and 11 a.m. after an overnight fast. During each visit we measured body weight, stature, and thickness of ten skinfolds for the estimation of percentage body fat [24]. All skinfold thickness measurements were performed by the same highly experienced researcher with Harpenden metal calipers from British Indicators (West Sussex, United Kingdom).

2.4. 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 triacylglycerols (TG), cholesterol, creatine kinase (CK, an index of muscle cell damage), and cortisol (a catabolic hormone), as well as fatty acid analysis of lipid classes.

2.5. Assays

TG, cholesterol, and CK were assayed by enzymic spectrophotometric methods through the use of reagent kits from Randox (Crumlin, Co. Antrim, United Kingdom). High density lipoprotein (HDL) cholesterol was determined after treatment of serum aliquots with a precipitant from Böhringer (Mannheim, Germany). Cortisol was measured by enzyme immunoassay through the use of a reagent kit from Radim (Rome, Italy).

Determination of the fatty acid composition of serum lipid classes was initiated by the addition of a mixture of heptadecanoic acid, triheptadecanoin, diheptadecanoyl phosphatidylcholine, and cholesteryl heptadecanoate (all from Sigma, St Louis, MO) as internal standards to 0.5 ml of serum. Lipids were extracted as described [25], and separated by thin-layer chromatography on silica gel plates. The developer was petroleum ether-diethyl ether-acetic acid 90:10:1 (v/v/v). Lipid spots were located under ultraviolet light after spraying with a solution of dichlorofluorescein in ethanol, and the spots corresponding to non-esterified fatty acids (NEFA), TG, phospholipids (PL), and cholesteryl esters (CE) were excised and incubated in methanolic potassium hydroxide (1 mol/L) at room temperature overnight. The fatty acids thus produced were methylated by incubating in 10% boron trifluoride in methanol (Fluka, Buchs, Switzerland) at 60°C for 5 min. After extraction with hexane, the methyl esters were separated by gas chromatography 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 fatty acid methyl esters 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). Fatty acid methyl esters in the samples were quantified by comparing the area under their peaks in the chromatogram to that of methyl heptadecanoate (derived from the

Table 1
Analysis of daily energy intake during the two periods of the study

	CLA group (n = 10)		Placebo group (n = 12)	
	1st period	2nd period	1st period	2nd period
Energy (MJ)	11.04 ± 2.95	11.78 ± 2.92	9.94 ± 2.38	10.36 ± 2.93
Carbohydrate (g)	308 ± 64	334 ± 92	277 ± 73	304 ± 92
Carbohydrate (% energy)	47.4 ± 5.5	47.4 ± 6.0	46.9 ± 7.8	49.1 ± 3.4
Fat (g)	111 ± 46	121 ± 36	101 ± 30	101 ± 29
Fat (% energy)	37.0 ± 6.3	38.8 ± 5.5	38.3 ± 6.7	36.9 ± 4.2
Protein (g)	102 ± 24	96 ± 26	90 ± 32	88 ± 33
Protein (% energy)	15.6 ± 2.1	13.8 ± 2.6	14.8 ± 2.5	14.0 ± 3.0

internal standards) with the aid of the HP 3365 ChemStation software from Hewlett-Packard.

The fatty acid composition of total serum lipids was determined by the addition of a mixture of the internal standards mentioned above to 10 μl of serum, and incubation in methanolic KOH and $BF_{3,}$ followed by gas chromatography as described above.

2.6. 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 [26].

2.7. Statistical analysis

Results are reported as the mean ± standard deviation. Significant differences between measurements for each parameter were detected by performing multiple *t* tests (paired or independent, as appropriate) and by adjusting the α level according to the Bonferroni inequality so that the overall familywise type I error be $\leq$0.05. Nine such tests were performed for each anthropometric and biochemical parameter (three within the CLA group, three within the placebo group, and three between the two groups at each visit). Thus the level of statistical significance regarding these parameters was set at $\alpha = 0.0056$ (0.05/9). Four tests were performed for energy and nutrient intakes (one between the periods of low and high dosage within the CLA group, one between the two periods within the placebo group, and two between the two groups at each period). Thus the level of statistical significance regarding these parameters was set at $\alpha = 0.0125$ (0.05/4).

3. Results

Before the end of the study one male volunteer withdrew due to inability to swallow the capsules and one female volunteer withdrew due 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 two volunteers who had withdrawn were found to be members of the CLA group. Therefore data referring to this group are derived from ten individuals (six male and four female).

The age of the members of the CLA group was 22.4 ± 1.7 years, whereas that of the members of the placebo group, 22.0 ± 1.3 years (not significant). Most participants received all capsules regularly but a few omitted some. The total compliance with the regimen was 97 ± 4% for the CLA group and 99 ± 2% for the placebo group.

Table 1 lists the analysis of energy intake by the participants during the two periods of the study. No significant differences in energy, carbohydrate, lipid or protein intake were found between the two groups in any of the two periods or between the two periods in any of the two groups.

Anthropometric data are presented in Table 2. No significant differences in body mass or body mass index were

Table 2
Anthropometric data of participants

	CLA group			Placebo group		
	Baseline	4 weeks	8 weeks	Baseline	4 weeks	8 weeks
Body mass (kg)	73.1 ± 7.9	72.6 ± 7.7	72.1 ± 7.5	68.3 ± 15.0	67.9 ± 14.9	67.9 ± 15.3
Height (m)	1.75 ± 0.09	1.76 ± 0.08	1.76 ± 0.08	1.73 ± 0.09	1.72 ± 0.08	1.73 ± 0.09
Body mass index (kg m^{-2})	23.8 ± 2.7	23.6 ± 2.5	23.4 ± 2.5	22.7 ± 3.3	22.7 ± 3.4	22.5 ± 3.4
Sum of 10 skinfolds (mm)	101.1 ± 36.8	103.3 ± 33.7	96.8 ± 30.7[a]	94.4 ± 23.9	96.1 ± 29.0	93.6 ± 27.6
Body fat (%)	16.6 ± 5.2	17.0 ± 5.1	16.1 ± 4.8[a]	15.4 ± 3.5	15.5 ± 3.9	15.1 ± 3.9
Fat mass (kg)	12.0 ± 3.7	12.2 ± 3.4	11.5 ± 3.3[a]	10.6 ± 3.6	10.7 ± 4.0	10.5 ± 4.1

[a] Significantly different from the second measurement ($P < 0.0056$).

Table 3
Biochemical analyses

	CLA group			Placebo group		
	Baseline	4 weeks	8 weeks	Baseline	4 weeks	8 weeks
Triacylglycerols (mmol/L)	0.95 ± 0.40	0.75 ± 0.23	0.82 ± 0.37	0.97 ± 0.54	0.88 ± 0.38	0.90 ± 0.36
Total cholesterol (mmol/L)	4.49 ± 0.98	4.16 ± 0.77	4.32 ± 0.87	4.38 ± 0.61	4.24 ± 0.70	4.58 ± 0.74
HDL-cholesterol (mmol/L)	1.42 ± 0.29	1.26 ± 0.30[a]	1.25 ± 0.32[a]	1.28 ± 0.29	1.25 ± 0.30	1.26 ± 0.30
Total/HDL-cholesterol	3.2 ± 0.8	3.4 ± 0.7	3.6 ± 0.9	3.6 ± 0.9	3.6 ± 1.1	3.8 ± 1.1
Creatine kinase (U/L, 37°C)	158 ± 85	189 ± 160	215 ± 171	164 ± 118	161 ± 129	182 ± 106
Cortisol (nmol/L)	693 ± 126	700 ± 143	716 ± 67	692 ± 202	656 ± 207	695 ± 204

[a] Significantly different from the first measurement ($P < 0.0056$).

observed, although both parameters decreased gradually during CLA supplementation. The sum of the thickness of ten skinfolds and percentage body fat calculated from it decreased significantly during the high CLA intake period ($P = 0.003$ and 0.004, respectively). It is remarkable that all ten members of the CLA group experienced these decrements. Additionally, fat mass (calculated from percentage body fat and body mass) decreased significantly during the same period ($P = 0.003$). However, none of these parameters changed significantly during the entire study period. No significant differences in any anthropometric parameter were found within the placebo group or between the two groups.

The results of the hematological and biochemical analyses except fatty acid analysis are shown in Table 3. The only significant change detected was a decrease in HDL-cholesterol during the low CLA intake period and overall during CLA intake ($P = 0.001$). There was also a tendency toward decreased triacylglycerols and total cholesterol during the first period ($P = 0.05$ and 0.10, respectively), as well as a tendency toward increased total/HDL-cholesterol overall ($P = 0.04$). No significant differences were found within the placebo group or between the two groups.

Gas chromatography of the fatty acid methyl esters derived from serum NEFA, TG, PL, CE, and total lipids of both experimental groups revealed the presence of both *cis,trans*-9,11- and *trans,cis*-10,12-octadecadienoic acid as separate peaks. A third peak was also visible which comigrated with *trans,trans*-9,11-octadecadienoic acid and which was, at least in part, produced from the former two CLA isomers during methylation as judged from its appearance in their chromatograms when the pure isomers were used to set retention times, and as reported in the literature [27,28]. Therefore the sum of the three peaks is presented as CLA below.

The fatty acid composition of serum lipid classes and total lipids is listed in Tables 4–8. Besides CLA, fourteen fatty acids were detected, namely, myristate (14:0), palmitate (16:0), palmitoleate (16:1ω7), stearate (18:0), oleate (18:1ω9), vaccenate (18:1ω7), linoleate (18:2ω6), γ-lino-

Table 4
Serum concentrations (mmol/L) of non-esterified fatty acids

Fatty acid	CLA group			Placebo group		
	Baseline	4 weeks	8 weeks	Baseline	4 weeks	8 weeks
14:0	0.0103 ± 0.0054	0.0091 ± 0.0045	0.0081 ± 0.0029	0.0080 ± 0.0034	0.0078 ± 0.0046	0.0068 ± 0.0030
16:0	0.1282 ± 0.0355	0.1371 ± 0.0429	0.1192 ± 0.0216	0.1301 ± 0.0370	0.1245 ± 0.0468	0.1120 ± 0.0280
16:1ω7	0.0078 ± 0.0043	0.0088 ± 0.0040	0.0075 ± 0.0043	0.0080 ± 0.0053	0.0074 ± 0.0041	0.0072 ± 0.0046
18:0	0.0547 ± 0.0169	0.0575 ± 0.0346	0.0577 ± 0.0413	0.0607 ± 0.0197	0.0604 ± 0.0350	0.0500 ± 0.0125
18:1ω9	0.1269 ± 0.0583	0.1379 ± 0.0565	0.1095 ± 0.0384	0.1468 ± 0.0789	0.1286 ± 0.0561	0.1236 ± 0.0669
18:1ω7	0.0065 ± 0.0029	0.0071 ± 0.0024	0.0052 ± 0.0014	0.0076 ± 0.0043	0.0066 ± 0.0026	0.0066 ± 0.0031
18:2ω6	0.0694 ± 0.0238	0.0752 ± 0.0163	0.0604 ± 0.0164	0.0748 ± 0.0293	0.0669 ± 0.0283	0.0596 ± 0.0181
CLA	0.0007 ± 0.0003	0.0009 ± 0.0004	0.0009 ± 0.0004	0.0007 ± 0.0005	0.0007 ± 0.0004	0.0006 ± 0.0003
18:3ω6	0.0003 ± 0.0002	0.0005 ± 0.0004	0.0004 ± 0.0003	0.0009 ± 0.0020	0.0005 ± 0.0009	0.0002 ± 0.0002
18:3ω3	0.0020 ± 0.0015	0.0026 ± 0.0016	0.0020 ± 0.0009	0.0037 ± 0.0043	0.0019 ± 0.0009	0.0019 ± 0.0009
20:3ω6	0.0019 ± 0.0011	0.0019 ± 0.0010	0.0018 ± 0.0013	0.0017 ± 0.0011	0.0014 ± 0.0008	0.0016 ± 0.0008
20:4ω6	0.0049 ± 0.0015	0.0046 ± 0.0010	0.0044 ± 0.0012	0.0063 ± 0.0019	0.0057 ± 0.0026	0.0060 ± 0.0019
20:5ω3	0.0002 ± 0.0004	0.0001 ± 0.0003	0.0002 ± 0.0003	0.0002 ± 0.0004	0.0006 ± 0.0010	0.0002 ± 0.0004
22:5ω3	0.0001 ± 0.0002	0.0005 ± 0.0006	0.0003 ± 0.0004	0.0009 ± 0.0013	0.0012 ± 0.0015	0.0007 ± 0.0007
22:6ω3	0.0004 ± 0.0004	0.0005 ± 0.0006	0.0005 ± 0.0005	0.0011 ± 0.0008	0.0012 ± 0.0010	0.0011 ± 0.0009
Sum	0.4144 ± 0.1284	0.4443 ± 0.1311	0.3780 ± 0.0745	0.4513 ± 0.1641	0.4153 ± 0.1455	0.3781 ± 0.1240

Table 5
Serum concentrations (mmol/L) of triacylglycerol acyl groups

Fatty acid	CLA group			Placebo group		
	Baseline	4 weeks	8 weeks	Baseline	4 weeks	8 weeks
14:0	0.032 ± 0.022	0.017 ± 0.006	0.024 ± 0.016	0.037 ± 0.040	0.029 ± 0.024	0.044 ± 0.053
16:0	0.473 ± 0.304	0.354 ± 0.130	0.394 ± 0.167	0.533 ± 0.385	0.483 ± 0.220	0.526 ± 0.309
16:1ω7	0.035 ± 0.023	0.024 ± 0.014	0.032 ± 0.019	0.035 ± 0.031	0.033 ± 0.017	0.042 ± 0.025
18:0	0.081 ± 0.091	0.045 ± 0.017	0.051 ± 0.019	0.101 ± 0.106	0.064 ± 0.036	0.089 ± 0.070
18:1ω9	0.637 ± 0.402	0.465 ± 0.221	0.513 ± 0.263	0.681 ± 0.521	0.627 ± 0.285	0.655 ± 0.311
18:1ω7	0.028 ± 0.011	0.026 ± 0.012	0.030 ± 0.014	0.034 ± 0.028	0.027 ± 0.014	0.034 ± 0.021
18:2ω6	0.309 ± 0.243	0.264 ± 0.135	0.240 ± 0.104	0.371 ± 0.302	0.345 ± 0.248	0.286 ± 0.204
CLA	0.002 ± 0.001	0.003 ± 0.002	0.006 ± 0.003	0.004 ± 0.006	0.003 ± 0.002	0.003 ± 0.004
18:3ω6	0.002 ± 0.002	0.002 ± 0.001	0.002 ± 0.001	0.005 ± 0.005	0.004 ± 0.004	0.004 ± 0.004
18:3ω3	0.009 ± 0.011	0.007 ± 0.007	0.005 ± 0.003	0.012 ± 0.015	0.007 ± 0.007	0.007 ± 0.007
20:3ω6	0.005 ± 0.003	0.003 ± 0.001	0.004 ± 0.003	0.005 ± 0.006	0.004 ± 0.004	0.004 ± 0.004
20:4ω6	0.009 ± 0.006	0.007 ± 0.002	0.007 ± 0.003	0.015 ± 0.016	0.013 ± 0.016	0.013 ± 0.013
20:5ω3	0.001 ± 0.001	0.000 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.000 ± 0.000	0.001 ± 0.001
22:5ω3	0.001 ± 0.002	0.001 ± 0.001	0.001 ± 0.001	0.002 ± 0.003	0.002 ± 0.002	0.001 ± 0.002
22:6ω3	0.002 ± 0.003	0.001 ± 0.001	0.001 ± 0.002	0.002 ± 0.002	0.003 ± 0.005	0.002 ± 0.003
Sum	1.626 ± 1.058	1.219 ± 0.494	1.310 ± 0.583	1.836 ± 1.426	1.645 ± 0.837	1.710 ± 0.942

lenate (18:3ω6), α-linolenate (18:3ω3), dihomo-γ-linolenate (20:3ω6), arachidonate (20:4ω6), eicosapentaenoate (20:5ω3), docosapentaenoate (22:5ω3), and docosahexaenoate (22:6ω3).

No significant differences were found in the concentrations of individual NEFA (Table 4), although CLA tended to be higher in the study group as compared to the control group at the end of the study ($P = 0.04$). Likewise, no significant differences were found in the concentrations of TG acyl groups (Table 5), although CLA tended to increase during the high-dosage period and overall ($P = 0.04$ and 0.01, respectively).

The total PL acyl group concentration in the CLA group decreased significantly ($P = 0.003$) during the study (Table 6). In terms of individual fatty acids, palmitate decreased significantly during the low-dosage CLA administration

Table 6
Serum concentrations (mmol/L) of phospholipid acyl groups

Fatty acid	CLA group			Placebo group		
	Baseline	4 weeks	8 weeks	Baseline	4 weeks	8 weeks
14:0	0.020 ± 0.011	0.015 ± 0.008	0.014 ± 0.006	0.017 ± 0.008	0.013 ± 0.005	0.012 ± 0.005
16:0	0.885 ± 0.154	0.781 ± 0.164[a]	0.797 ± 0.148	0.898 ± 0.177	0.842 ± 0.148	0.838 ± 0.140
16:1ω7	0.011 ± 0.006	0.008 ± 0.006	0.010 ± 0.004	0.012 ± 0.005	0.009 ± 0.002	0.011 ± 0.005
18:0	0.570 ± 0.119	0.520 ± 0.105	0.509 ± 0.110	0.599 ± 0.146	0.567 ± 0.093	0.598 ± 0.100
18:1ω9	0.377 ± 0.087	0.317 ± 0.064	0.314 ± 0.070	0.417 ± 0.116	0.393 ± 0.099	0.403 ± 0.110
18:1ω7	0.042 ± 0.008	0.038 ± 0.008	0.035 ± 0.007	0.047 ± 0.012	0.043 ± 0.010	0.045 ± 0.011
18:2ω6	0.664 ± 0.149	0.622 ± 0.113	0.600 ± 0.126	0.716 ± 0.156	0.683 ± 0.110	0.676 ± 0.106
CLA	0.007 ± 0.005	0.011 ± 0.007	0.012 ± 0.006	0.009 ± 0.004	0.007 ± 0.004	0.008 ± 0.006
18:3ω6	0.002 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.002 ± 0.001	0.001 ± 0.001	0.001 ± 0.001
18:3ω3	0.004 ± 0.002	0.003 ± 0.002	0.002 ± 0.002	0.004 ± 0.003	0.004 ± 0.002	0.004 ± 0.002
20:3ω6	0.080 ± 0.032	0.067 ± 0.021	0.074 ± 0.024	0.067 ± 0.028	0.073 ± 0.019	0.084 ± 0.024
20:4ω6	0.204 ± 0.065	0.194 ± 0.045	0.192 ± 0.049[b]	0.266 ± 0.081	0.254 ± 0.076	0.289 ± 0.081
20:5ω3	0.006 ± 0.003	0.005 ± 0.006	0.006 ± 0.008	0.009 ± 0.004	0.009 ± 0.005	0.008 ± 0.003
22:5ω3	0.010 ± 0.005	0.014 ± 0.008	0.013 ± 0.003	0.018 ± 0.007	0.017 ± 0.006	0.017 ± 0.008
22:6ω3	0.054 ± 0.023	0.057 ± 0.020	0.050 ± 0.023	0.063 ± 0.018	0.063 ± 0.020	0.067 ± 0.019
Sum	2.936 ± 0.536	2.651 ± 0.470	2.631 ± 0.474[a]	3.143 ± 0.633	2.979 ± 0.516	3.062 ± 0.492

[a] Significantly different from the first measurement ($P < 0.0056$).
[b] Significantly different from the placebo group at the same time ($P < 0.0056$).

Table 7
Serum concentrations (mmol/L) of cholesteryl ester acyl groups

Fatty acid	CLA group			Placebo group		
	Baseline	4 weeks	8 weeks	Baseline	4 weeks	8 weeks
14:0	0.032 ± 0.026	0.025 ± 0.021	0.028 ± 0.023	0.022 ± 0.009	0.024 ± 0.008	0.027 ± 0.010
16:0	0.395 ± 0.109	0.383 ± 0.082	0.379 ± 0.099	0.409 ± 0.073	0.407 ± 0.087	0.433 ± 0.087
16:1ω7	0.067 ± 0.041	0.059 ± 0.050	0.065 ± 0.051	0.053 ± 0.019	0.056 ± 0.017	0.063 ± 0.020
18:0	0.040 ± 0.022	0.036 ± 0.013	0.036 ± 0.016	0.035 ± 0.012	0.037 ± 0.015	0.038 ± 0.011
18:1ω9	0.678 ± 0.245	0.629 ± 0.210	0.681 ± 0.252	0.672 ± 0.213	0.645 ± 0.227	0.701 ± 0.215
18:1ω7	0.040 ± 0.012	0.042 ± 0.015	0.039 ± 0.017	0.041 ± 0.013	0.043 ± 0.012	0.045 ± 0.014
18:2ω6	2.175 ± 0.689	2.168 ± 0.671	2.088 ± 0.793	2.167 ± 0.613	2.140 ± 0.672	2.232 ± 0.661
CLA	0.005 ± 0.003	0.007 ± 0.009	0.008 ± 0.007	0.004 ± 0.003	0.004 ± 0.003	0.004 ± 0.002
18:3ω6	0.021 ± 0.013	0.015 ± 0.010	0.019 ± 0.011	0.024 ± 0.012	0.019 ± 0.010	0.022 ± 0.016
18:3ω3	0.011 ± 0.005	0.013 ± 0.010	0.010 ± 0.006	0.010 ± 0.004	0.007 ± 0.003[a]	0.010 ± 0.004
20:3ω6	0.021 ± 0.012	0.017 ± 0.013	0.015 ± 0.012	0.020 ± 0.006	0.018 ± 0.007	0.022 ± 0.009
20:4ω6	0.138 ± 0.061	0.135 ± 0.049	0.136 ± 0.071	0.162 ± 0.048	0.138 ± 0.053	0.176 ± 0.074[b]
20:5ω3	0.004 ± 0.005	0.005 ± 0.005	0.005 ± 0.006	0.006 ± 0.005	0.006 ± 0.005	0.006 ± 0.006
22:5ω3	0.001 ± 0.002	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.001	0.001 ± 0.002
22:6ω3	0.005 ± 0.005	0.005 ± 0.005	0.004 ± 0.004	0.005 ± 0.005	0.005 ± 0.004	0.006 ± 0.005
Sum	3.632 ± 1.128	3.538 ± 1.055	3.513 ± 1.270	3.630 ± 0.871	3.548 ± 0.962	3.787 ± 0.944

[a] Significantly different from the first measurement ($P < 0.0056$).
[b] Significantly different from the second measurement ($P < 0.0056$).

($P = 0.004$), and arachidonate was significantly lower in the CLA group as compared to the placebo group at the end of the study ($P = 0.005$). Additionally, the overall increase in the CLA of the study group's serum PL tended to be significant ($P = 0.03$).

No significant differences were found in the concentrations of CE acyl groups in the CLA group (Table 7), although CLA increased overall ($P = 0.04$). In the placebo group there was a significant decrease in α-linolenate ($P = 0.005$) during the first period, and a signif-

Table 8
Serum concentrations (mmol/L) of acyl groups in total lipids

Fatty acid	CLA group			Placebo group		
	Baseline	4 weeks	8 weeks	Baseline	4 weeks	8 weeks
14:0	0.103 ± 0.053	0.089 ± 0.026	0.094 ± 0.041	0.138 ± 0.106	0.149 ± 0.207	0.170 ± 0.201
16:0	2.984 ± 0.638	2.719 ± 0.524	2.768 ± 0.499	3.207 ± 0.801	3.216 ± 0.791	3.334 ± 0.929
16:1ω7	0.133 ± 0.085	0.117 ± 0.075	0.122 ± 0.069	0.133 ± 0.053	0.126 ± 0.044	0.156 ± 0.063
18:0	1.077 ± 0.370	0.923 ± 0.268	1.004 ± 0.311	1.221 ± 0.457	1.173 ± 0.446	1.276 ± 0.515
18:1ω9	2.103 ± 1.151	1.727 ± 0.930	1.790 ± 0.843	2.279 ± 0.990	2.151 ± 0.715	2.328 ± 0.865
18:1ω7	0.155 ± 0.070	0.128 ± 0.059	0.126 ± 0.049	0.160 ± 0.072	0.152 ± 0.049	0.178 ± 0.073
18:2ω6	3.535 ± 1.475	3.274 ± 1.411	3.018 ± 1.474	3.745 ± 1.122	3.727 ± 1.221	3.744 ± 1.190
CLA	0.019 ± 0.009	0.027 ± 0.015	0.033 ± 0.014[a]	0.020 ± 0.012	0.019 ± 0.009	0.020 ± 0.009
18:3ω6	0.031 ± 0.026	0.020 ± 0.014	0.022 ± 0.016	0.034 ± 0.023	0.030 ± 0.023	0.053 ± 0.058
18:3ω3	0.042 ± 0.028	0.033 ± 0.023	0.025 ± 0.016	0.036 ± 0.018	0.030 ± 0.018	0.035 ± 0.021
20:3ω6	0.134 ± 0.055	0.109 ± 0.041	0.112 ± 0.048	0.133 ± 0.064	0.132 ± 0.050	0.153 ± 0.060[b]
20:4ω6	0.411 ± 0.171	0.359 ± 0.146	0.377 ± 0.163	0.544 ± 0.201	0.513 ± 0.236	0.596 ± 0.247
20:5ω3	0.021 ± 0.015	0.023 ± 0.019	0.019 ± 0.015	0.075 ± 0.168	0.081 ± 0.177	0.077 ± 0.179
22:5ω3	0.019 ± 0.010	0.021 ± 0.012	0.016 ± 0.009[c]	0.028 ± 0.011	0.028 ± 0.015	0.031 ± 0.010
22:6ω3	0.061 ± 0.035	0.056 ± 0.032	0.054 ± 0.029	0.087 ± 0.037	0.094 ± 0.053	0.099 ± 0.043
Sum	10.829 ± 3.612	9.625 ± 2.884	9.580 ± 2.592	11.841 ± 3.477	11.621 ± 3.315	12.251 ± 3.760

[a] Significantly different from the first measurement ($P < 0.0056$).
[b] Significantly different from the second measurement ($P < 0.0056$).
[c] Significantly different from the placebo group at the same time ($P < 0.0056$).

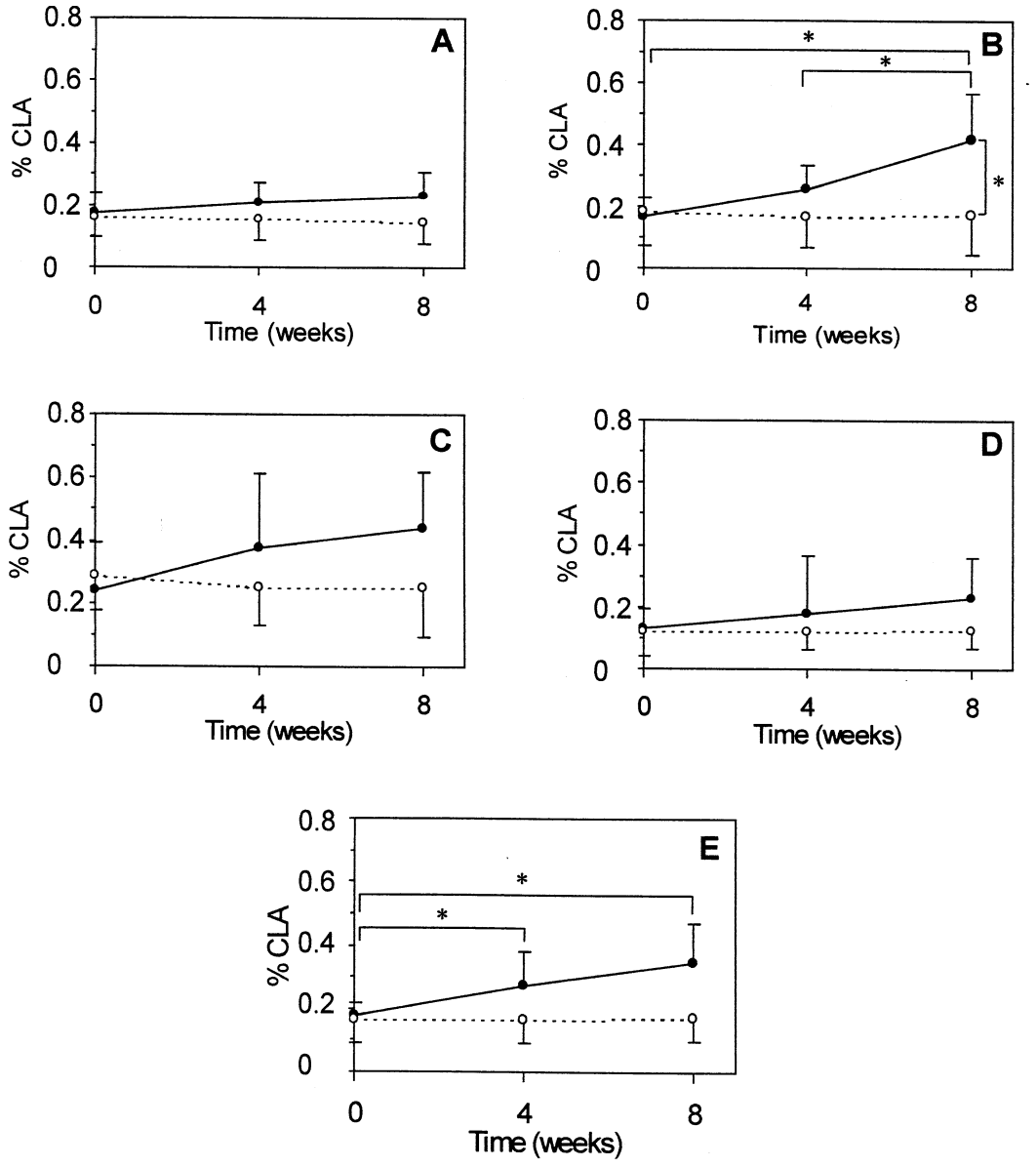

Fig. 1. Means and standard deviations of the molar percentage of CLA in serum non-esterified fatty acids (A), and in the acyl groups of triacylglycerols (B), phospholipids (C), cholesteryl esters (D), as well as total lipids (E) of the CLA (●) and placebo group (○). Asterisks denote significant differences ($P < 0.0056$).

icant increase in arachidonate during the second period ($P = 0.004$).

Following the trend observed in the individual lipid classes, the CLA content of total serum lipids increased gradually in the CLA group (Table 8) and was significantly higher at the end as compared to the beginning of the study ($P = 0.001$). In the placebo group there was a significant increase in dihomo-γ-linolenate ($P = 0.003$) during the second period. Finally, docosapentaenoate was significantly lower at the end of the study in the CLA group as compared to the placebo group ($P = 0.001$).

As can be calculated from Tables 4–7, the sum of acyl groups in the four lipid classes determined was less than that in total lipids (Table 8). A percentage of total acyl groups ranging from 18 (at the second and third measurements of the CLA group) to 27 (at the third measurement of the placebo group), and averaging 23 was unaccounted for. This may correspond to acyl groups incorporated into minor serum lipids (e.g. monoacylglycerols and diacylglycerols), as well as proteolipids.

The molar percentages of CLA in serum NEFA, TG, PL, CE, and total lipids of both experimental groups are depicted in Figure 1. The percentage of CLA in the NEFA of the CLA group increased gradually from 0.17 at the beginning to 0.23 at the end of the study ($P = 0.01$, Fig. 1A). The percentage of CLA in the TG acyl groups of the CLA group at the end of the study (0.42) was significantly higher as compared to the beginning (0.17, $P = 0.003$) or the middle

Table 9
Comparison of studies reporting reduction of body fat with CLA supplementation

Study	Park et al., 1997 (ref 6)	West et at., 1998 (ref 8)	Present
Subjects	Mice (female)	Mice (male, low-fat diet)	Humans
% total energy intake from CLA	1.16	2.21	0.43
% total fat intake as CLA	9.1	14.8	1.1
Daily dose of CLA (g/kg body mass at onset)	0.70	1.19	0.019
% reduction in percentage body fat	60	62	5

Data have been calculated from mean values reported in the studies.

of the study ($P = 0.005$, Fig. 1B). Additionally, it was significantly higher as compared to the placebo group at the end of the study ($P = 0.001$). Gradual increases were also observed in the percentages of CLA within the PL (from 0.24 to 0.44, $P = 0.01$, Fig. 1C) and CE (from 0.13 to 0.23, $P = 0.006$, Fig. 1D) of the CLA group. Finally, the percentage of CLA in the total lipids of the CLA group increased significantly from 0.17 at the beginning to 0.27 in the middle ($P = 0.003$), and 0.35 at the end of the study ($P < 0.001$, Fig. 1E).

4. Discussion

In the present study we examined the effect of supplementation with CLA (0.7 g per day for 4 weeks followed by 1.4 g per day for 4 weeks) *vs* placebo on human body fat, biochemical parameters of serum, and the CLA content of serum lipids. We found a statistically significant decrease in the sum of the thickness of ten skinfolds and, concomitantly, percentage body fat and fat mass during the high CLA intake period, the relative magnitude of the decrements being 5–6% (Table 2). Similar studies on the effect of CLA supplementation on human body composition are scarce and provide contradictory results. Supplementation of lean women with 3 g of CLA per day for 64 days was reported to have no significant effect on body composition or energy expenditure compared to placebo, although percentage body fat decreased by 0.67 with CLA *vs* an increase by 0.05 with placebo [29]. On the other hand, supplementation of overweight or obese men and women with 1.7–6.8 g of CLA daily for 12 weeks reduced body fat mass significantly as compared to the placebo group [30]. However, the reduction of body fat within the groups receiving different doses of CLA was significant only for the 3.4- and 6.8-g groups, and not for the 1.7- and 5.1-g groups [30]. Finally, reports not peer-reviewed as yet suggest an ability of CLA to modulate human body composition in a favorable way 31].[3]

Our finding of body fat reduction agrees qualitatively with the results of animal studies, although the latter have reported higher decrements in body fat with CLA supplementation [6–10,12]. One should consider, however, that those studies employed higher dosages of CLA, expressed as either percentage of total energy intake or percentage of total fat intake or g per kg body mass per day. A comparison of our study with the two studies having reported the highest reductions in body fat is presented in Table 9.

A trend toward lower TG and total cholesterol, as well as a significant decrease in HDL-cholesterol was observed during the low CLA intake period (Table 3). Notably, a decrease in HDL-cholesterol was the only significant change in serum lipids consistently observed in all groups receiving CLA in the aforementioned human study [30]. The fact that similar changes were not seen during the high CLA intake period in our study may indicate that CLA exhausted this facet of its action during the first period. Interestingly, a similar effect (i.e., a significant decrease in total cholesterol after 3 or 4 weeks but not after 6, 8, or 12 weeks of CLA administration) was observed in hamsters [23] and rats [32]. Studies on rabbits [13], hamsters [14,23, 33], mice [20], rats [32,34], and pigs [35], have yielded mixed results with regard to serum lipids. When compared to controls, CLA-fed animals had significantly lower [13, 14,20,33], significantly higher [23], or not significantly different [32,34,35] TG levels. Total cholesterol was either significantly decreased [14,23,32,33,34], or not significantly different [13,20,23,32,35]. Finally, HDL-cholesterol was either significantly decreased [23,34], or not significantly different [13,14,20,33,35]. This variety of responses of the lipidemic profile to CLA supplementation and the rather unusual concomitant decrease in TG and HDL-cholesterol found in the present study warrants further investigation.

Our study found body fat to be affected by a higher dose of CLA as compared to the dose that affected serum lipids. This differential dose response of stored *vs* circulating lipids may be due to different biochemical mechanisms activated by different CLA concentrations. Relevant studies have attributed the fat-lowering ability of CLA to reduced lipoprotein lipase activity in mouse adipocytes [6,22], increased carnitine palmitoyltransferase activity in adipose tissue of mice [6] and rats [36], reduced expression of stearoyl coenzyme A desaturase in mouse liver [37], and an inhibition of differentiation as well as proliferation of mouse adipocytes [38]. On the other hand, the ability of CLA to reduce blood lipids has been attributed to reduced secretion of apolipoprotein B and TG by human hepatocytes [39]. Our data do not allow testing of these hypotheses, which requires further research.

Advertisements of CLA supplements have used the findings of a protective action of CLA against the catabolic

effect of endotoxin injection to animals [16,17] to suggest an "anticatabolic effect" on humans. Although this term is vague, the implication is made that CLA supplementation may benefit athletes by mitigating proteolysis in muscle. To test this hypothesis, we measured an index of muscle fiber breakdown (CK), and a hormone promoting proteolysis in muscle (cortisol). The absence of significant changes in these parameters (Table 3) lends no support to such claims.

The CLA content of serum lipids of unsupplemented subjects averaged 0.020 mmol/L (Table 8) or 0.17% of total fatty acids (Fig. 1). This agrees with the average values of 0.021 mmol/L reported for healthy men and women [40], and 0.17% reported after a diet not enriched in dairy foods or *trans* fatty acids ("stearic acid diet") [41]. On the other hand, it is higher than the average concentration of 0.007 mmol/L reported by Herbel and coworkers [42], and lower than the percentages of 0.30–0.54[43] and 0.25[44] reported for the *cis*,*trans*-9,11 isomer alone. These differences may reflect differences in the intake of dietary sources of CLA and/or in the methodology of CLA determination.

We report for the first time data on the concentration of CLA in the major lipid classes of human serum, with and without CLA supplementation. PL had the highest CLA content in both absolute and relative terms (average 0.26% of total fatty acids before supplementation). Considering the fact that linoleate, the common isomer of CLA, has its highest presence in CE rather than PL (60 *vs* 23% in the present study), whereas CLA was minimal in CE (0.12%), it becomes evident that these fatty acids have quite different metabolic fates.

After 8 weeks of supplementation with CLA its molar percentage in the fatty acids of total lipids doubled (from 0.17 to 0.35%). The lipid class where the most remarkable increase occured was TG (from 0.17 to 0.42%), although PL remained the class with the highest CLA content.

No consistent changes after CLA supplementation were observed in the other fourteen fatty acids detected in the serum samples. Worthy of attention is the significantly lower concentration of arachidonate in the PL of the CLA group as compared to the placebo group at the end of the study (Table 6). Taken together with reports of decreased arachidonate concentration in tissues of CLA-fed chicks, rats, and mice [16,17,45], as well as in the PL of CLA-treated cell cultures [46], our finding suggests interference of CLA with arachidonate metabolism. This, in turn, may have consequences on membrane composition and dynamics. The decreased arachidonate content of membrane PL and the subsequent decreased synthesis of arachidonate-derived prostaglandin PGE_2 has been suggested as a mechanism for the inhibition of immune stimulation and skin tumor promotion by CLA in animals [17,46]. Finally, the significant decrease in total PL concentration after CLA supplementation (Table 6) may indicate inhibition of PL biosynthesis as a whole.

In conclusion, our data provide evidence for a fat lowering effect of CLA on healthy humans and a tendency toward lowering serum lipids, although only the undesirable decrease in HDL-cholesterol was statistically significant. Additionally, supplementation with as little as 0.7 g CLA daily for 4 weeks was sufficient to increase its incorporation into serum total lipids significantly. Further controlled studies on larger population samples should make it possible to clarify the effects of this interesting nutrient on human metabolism, and examine their dependence on dose, duration of administration, and subject characteristics.

Notes

1. Matsakas, A., Ring, S., Petridou, A., Melissopoulou, A., Tsigilis, N., and Mougios, V. (1999). Effect of supplementation with conjugated linoleic acid on human body composition and blood lipids. *Proceedings of the 4th Annual Congress of the European College of Sport Science*, 46.
2. Petridou, A., Matsakas, A., Sagredos, A., and Mougios, V. (2000). Incorporation of conjugated linoleic acid (CLA) into human serum lipid classes after supplementation with CLA. *4th International Conference on Nutrition and Fitness–Abstracts*, 58–59.
3. Vessby, B., and Smedman, A. (1999). Conjugated linoleic acid (CLA) reduces the body fat content in humans. *Chem Phys Lipids* **101**, 152.

References

[1] J. Fritsche, H. Steinhart, Analysis, occurrence and physiological properties of trans fatty acids (TFA) with particular emphasis on conjugated linoleic acid isomers (CLA) - a review, Fett/Lipid 100 (1998) 190–210.
[2] Y.L. Ha, N.K. Grimm, M.W. Pariza, Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid, Carcinogenesis 8 (1987) 1881–1887.
[3] M.A. Belury, Conjugated dienoic linoleate: a polyunsaturated fatty acid with unique chemoprotective properties, Nutr. Rev. 53 (1995) 83–89.
[4] C. Ip, Review of the effects of *trans* fatty acids, oleic acid, n-3 polyunsaturated fatty acids, and conjugated linoleic acid on mammary carcinogenesis in animals, Am. J. Clin. Nutr. 66 (1997) 1523S–1529S.
[5] A. Cesano, S. Visonneau, J.A. Scimeca, D. Kritchevsky, D. Santoli, Opposite effects of linoleic acid and conjugated linoleic acid on human prostatic cancer in SCID mice, Anticancer Res. 18 (1998) 1429–1434.
[6] Y. Park, K.J. Albright, W. Liu, J.M. Storkson, M.E. Cook, M.W. Pariza, Effect of conjugated linoleic acid on body composition in mice, Lipids 32 (1997) 853–858.
[7] M.E.R. Dugan, J.L. Aalhus, A.L. Schaefer, J.K.G. Kramer, The effect of conjugated linoleic acid on fat to lean repartitioning and feed conversion in pigs, Can. J. Anim. Sci. 77 (1997) 723–725.
[8] D.B. West, J.P. Delany, P.M. Camet, F. Blohm, A.A. Truett, J. Scimeca, Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse, Am. J. Physiol. 275 (1998) 667–672.
[9] J.P. DeLany, F. Blohm, A.A. Truett, J.A. Scimeca, D.B. West, Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake, Am. J. Physiol. 276 (1999) 1172–1179.

[10] E. Ostrowska, M. Muralitharan, R.F. Cross, D.E. Bauman, F.R. Dunshea, Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs, J. Nutr. 129 (1999) 2037–2042.
[11] O. Simon, K. Männer, K. Schäffer, A. Sagredos, K. Eder, Effects of conjugated linoleic acid on protein to fat proportions, fatty acids, and plasma lipids in broilers, Eur. J. Lipid Sci. Technol. 102 (2000) 402–410.
[12] G.I. Stangl, Conjugated linoleic acids exhibit a strong fat-to-lean partitioning effect, reduce serum VLDL lipids and redistribute tissue lipids in food-restricted rats, J. Nutr. 130 (2000) 1140–1146.
[13] K. N. Lee, D. Kritchevsky, M.W. Pariza, Conjugated linoleic acid and atherosclerosis in rabbits, Atherosclerosis 108 (1994) 19–25.
[14] R.J. Nicolosi, E.J. Rogers, D. Kritchevsky, J.A. Scimeca, P.J. Huth, Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters, Artery 22 (1997) 266–277.
[15] D. Kritchevsky, S.A. Tepper, S. Wright, P. Tso, S.K. Czarnecki, Influence of conjugated linoleic acid (CLA) on establishment and progression of atherosclerosis in rabbits, J. Am. Coll. Nutr. 19 (2000) 472S–477S.
[16] M.E. Cook, C.C. Miller, Y. Park, M.W. Pariza, Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression, Poultry Sci. 72 (1993) 1301–1305.
[17] C.C. Miller, Y. Park, M.W. Pariza, M.E. Cook, Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection, Biochem. Biophys. Res. Commun. 198 (1994) 1107–1112.
[18] K.L. Houseknecht, J.P. Vanden Heuvel, S.Y. Moya-Camarena, C.P. Portocarrero, L.W. Peck, K.P. Nickel, M.A. Belury, Dietary conjugated linoleic normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat, Biochem. Biophys. Res. Commun. 244 (1998) 678–682.
[19] M.W. Wong, B.P. Chew, T.S. Wong, H.L. Hosick, T.D. Boylston, T.D. Shultz, Effects of dietary conjugated linoleic acid on lymphocyte function and growth of mammary tumors in mice, Anticancer Res. 17 (1997) 987–993.
[20] J.S. Munday, K.G. Thompson, K.A. James, Dietary conjugated linoleic acids promote fatty streak formation in the C57BL/6 mouse atherosclerosis model, Br. J. Nutr. 81 (1999) 251–255.
[21] Y.L. Ha, J. Storkson, M.W. Pariza, Inhibition of benzo(a)pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid, Cancer Res. 50 (1990) 1097–1101.
[22] Y. Park, J.M. Storkson, K.J. Albright, W. Liu, M.W. Pariza, Evidence that the *trans*-10,*cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice, Lipids 34 (1999) 235–241.
[23] E.A. de Deckere, J.M. van Amelsvoort, G.P. McNeill, P. Jones, Effects of conjugated linoleic acid (CLA) isomers on lipid levels and peroxisome proliferation in the hamster, Br. J. Nutr. 82 (1999) 309–317.
[24] J. Parizkova, Body composition and physical fitness, Curr. Anthropol. 9 (1968) 273–287.
[25] V.P. Dole, A relation between non-esterified fatty acids in plasma and the metabolism of glucose, J. Clin. Invest. 35 (1956) 150–154.
[26] B. Holland, A.A. Welch, I.D. Unwin, D.H. Buss, A.A. Paul, D.A.T. Southgate, McCance and Widdowson's the composition of foods, Royal Society of Chemistry, Cambridge, UK, 1991.
[27] S.A. Werner, L.O. Luedecke, T.D. Shultz, 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 (1992) 1817–1821.
[28] J.K.G. Kramer, V. Fellner, M.E.R. Dugan, F.D. Sauer, M.D. Mossoba, M.P. Yurawecz, 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 (1997) 1219–1228.
[29] K.L. Zambell, N.L. Keim, M.D. Van Loan, B. Gale, P. Benito, D.S. Kelley, G.J. Nelson, Conjugated linoleic acid supplementation in humans: effects on body composition and energy expenditure, Lipids 35 (2000) 777–782.
[30] H. Blankson, J.A. Stakkestad, H. Fagertun, E. Thom, J. Wadstein, O. Gudmundsen, Conjugated linoleic acid reduces body fat mass in overweight and obese humans, J. Nutr. 130 (2000) 2943–2948.
[31] L.D. Whigham, M.E. Cook, R.L. Atkinson, Conjugated linoleic acid: Implications for human health, Pharm. Res. 42 (2000) 503–510.
[32] M. Yamasaki, K. Mansho, Y. Ogino, M. Kasai, H. Tachibana, K. Yamada, Acute reduction of serum leptin level by dietary conjugated linoleic acid in Sprague-Dawley rats, J. Nutr. Biochem. 11 (2000) 467–471.
[33] V.C. Gavino, G. Gavino, M.J. Leblanc, B. Tuchweber, An isomeric mixture of conjugated linoleic acids but not pure *cis*-9,*trans*-11-octadecadienoic acid affects body weight gain and plasma lipids in hamsters, J. Nutr. 130 (2000) 27–29.
[34] G.I. Stangl, Conjugated linoleic acids exhibit a strong fat-to-lean partitioning effect, reduce serum VLDL lipids and redistribute tissue lipids in food-restricted rats, J. Nutr. 130 (2000) 1140–1146.
[35] G.I. Stangl, H. Muller, M. Kirchgessner, Conjugated linoleic acid effects on circulating hormones, metabolites and lipoproteins, and its proportion in fasting serum and erythrocyte membranes of swine, Eur. J. Nutr. 38 (1999) 271–277.
[36] J.-C. Martin, S. Gregoire, M.-H. Siess, M. Genty, J.-M. Chardigny, O. Berdeaux, P. Juaneda, J.-L. Sebedio, Effects of conjugated linoleic acid isomers on lipid-metabolizing enzymes in male rats. Lipids 35 (2000) 91–98.
[37] K.N. Lee, M.W. Pariza, M. Ntambi, Conjugated linoleic acid decreases hepatic stearoyl-CoA desaturase mRNA expression, Biochem. Biophys. Res. Commun. 248 (1998) 817–821.
[38] A.E. Brodie, V.A. Manning, K.R. Ferguson, D.E. Jewell, C.Y. Hu, Conjugated linoleic acid inhibits differentiation of pre- and post-confluent 3T3–L1 preadipocytes but inhibits cell proliferation only in preconfluent cells, J. Nutr. 129 (1999) 602–606.
[39] H. Yotsumoto, E. Hara, S. Naka, R.O. Adlof, E.A. Emken, T. Yanagita, 10*trans*,12*cis*-linoleic acid reduces apolipoprotein B secretion in HepG2 cells, Food Res. Int. 31 (1999) 403–409.
[40] R. Fink, M.R. Clemens, D.H. Marjot, P. Patsalos, P. Cawood, A.G. Norden, S.A. Iversen, T.L. Dormandy, Increased free-radical activity in alcoholics, Lancet 2 (1985) 291–294.
[41] I. Salminen, M. Mutanen, M. Jauhiainen, A. Aro, Dietary *trans* fatty acids increase conjugated linoleic acid levels in human serum, J. Nutr. Biochem. 9 (1998) 93–98.
[42] B.K. Herbel, M.K. McGuire, M.A. McGuire, T.D. Shultz, Safflower oil consumption does not increase plasma conjugated linoleic acid concentrations in humans, Am. J. Clin. Nutr. 67 (1998) 332–337.
[43] A.C. Fogerty, G.L. Ford, D. Svoronos, Octadeca-9,11-dienoic acid in foodstuffs and in the lipids of human blood and breast milk, Nutr. Rep. Int. 38 (1988) 937–944.
[44] J. Jiang, A. Wolk, B. Vessby, Relation between the intake of milk fat and the occurrence of conjugated linoleic acid in human adipose tissue, Am. J. Clin. Nutr. 70 (1999) 21–27.
[45] M.A. Belury, A. Kempa-Steczko, Conjugated linoleic acid modulates hepatic lipid composition in mice, Lipids 32 (1997) 199–204.
[46] K.L. Liu, M.A. Belury, Conjugated linoleic acid reduces arachidonic acid content and PGE_2 synthesis in murine keratinocytes, Cancer Lett. 127 (1998) 15–22.