

Supplementation with Conjugated Linoleic Acid for 24 Months Is Well Tolerated by and Reduces Body Fat Mass in Healthy, Overweight Humans¹

Jean-Michel Gaullier,² Johan Halse,* Kjetil Høye,[†] Knut Kristiansen, Hans Fagertun,** Hogne Vik,[‡] and Ola Gudmundsen

Scandinavian Clinical Research AS, NO-2027 Kjeller, Norway; *Betanien Medical Center, NO-0172 Oslo, Norway; [†]Helsetorget Medical Center, NO-2408 Elverum, Norway; **Capturo AS, NO-2027 Kjeller, Norway; and [‡]MATFORSK, Norwegian Food Research Institute, NO-1430 Ås, Norway

ABSTRACT After 12 mo in a randomized, double-blind, placebo-controlled trial of conjugated linoleic acid (CLA) supplementation (2 groups received CLA as part of a triglyceride or as the free fatty acid, and 1 group received olive oil as placebo), 134 of the 157 participants who concluded the study were included in an open study for another 12 mo. The goals of the extension study were to evaluate the safety [with clinical chemistry analyses and reported adverse events (AEs)] and assess the effects of CLA on body composition [body fat mass (BFM), lean body mass (LBM), bone mineral mass (BMM)], body weight, and BMI. All subjects were supplemented with 3.4g CLA/d in the triglyceride form. Circulating lipoprotein(a) and thrombocytes increased in all groups. There was no change in fasting blood glucose. Aspartate amino transferase, but not alanine amino transferase, increased significantly. Plasma total cholesterol and LDL cholesterol were reduced, whereas HDL cholesterol and triglycerides were unchanged. The AE rate decreased compared with the first 12 mo of the study. Body weight and BFM were reduced in the subjects administered the placebo during the initial 12 mo study (-1.6 ± 3.2 and -1.7 ± 2.8 kg, respectively). No fat or body weight changes occurred in the 2 groups given CLA during the initial 12 mo. LBM and BMM were not affected in any of the groups. Changes in body composition were not related to diet and/or training. In conclusion, this study shows that CLA supplementation for 24 mo in healthy, overweight adults was well tolerated. It confirms also that CLA decreases BFM in overweight humans, and may help maintain initial reductions in BFM and weight in the long term. *J. Nutr.* 135: 778–784, 2005.

KEY WORDS: • conjugated linoleic acid • body composition • body mass index • weight reduction

Conjugated linoleic acid (CLA)³ refers to a mixture of linoleic acid isomers with conjugated double bonds. CLA in extracts from fried ground beef was found to be anticarcinogenic (1). Further research revealed beneficial roles for CLA in immune function, atherosclerosis, and blood glucose maintenance (2–6). The effect of CLA on body composition has been studied extensively, specifically the loss of body fat mass (BFM) and a possible increase in lean body mass (LBM). These effects of CLA were demonstrated in animal studies (7–16) and in a few short-term studies in humans (17–19). Although these studies reported no adverse events (AEs) related to CLA supplementation, determination of the long-term safety of CLA is extremely important because CLA is currently sold as a dietary supplement for weight management,

and the longest study completed to date had a duration of 12 mo (20).

Previously, we demonstrated that daily supplementation with a 1:1 mixture of the CLA isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12, in either the FFA (CLA-FFA) and/or the triglyceride (CLA-TG) form for 12 mo reduced BFM, body weight, and BMI without affecting lean mass in overweight subjects consuming food ad libitum (20). No safety issue was encountered during this 12-mo randomized, double-blind, placebo-controlled trial; however, we did observe slight increases in lipoprotein (a) [Lp(a)], and LDL cholesterol, as well as, a minor decrease in HDL cholesterol with CLA supplementation. Glycosylated hemoglobin (HbA1c) was also slightly elevated in all groups, including the placebo group, whereas fasting blood glucose and insulin levels did not change in any of the groups. All changes were small and were not considered clinically relevant.

This is in contrast to other short-term studies in which the safety of CLA was questioned. Riserus et al. (21) demonstrated that the purified *trans*-10, *cis*-12 CLA isomer increased insulin resistance in subjects with the metabolic syndrome, whereas there was no effect of the CLA isomer mixture on insulin resistance compared with the placebo group. Another study from the same group reported an increase in urinary excretion

¹ Supported by Cognis Nutrition and Health Ltd.

² To whom correspondence should be addressed. E-mail: j-m@scr.no.

³ Abbreviations used: AE, adverse event; ALAT, alanine amino transferase; ASAT, aspartate amino transferase; BFM, body fat mass; BMM, body mineral mass; CLA, conjugated linoleic acid; CPK, creatinine phosphokinase; DXA, dual energy X-ray absorptiometry; γ -GT, γ -glutamyl transferase; HbA1c, glucohemoglobin; IGF-1, insulin-like growth factor-1; LBM, lean body mass; Lp(a), lipoprotein (a); SAE, serious adverse event; TG, triglycerides; TSH, thyroid-stimulating hormone.

of F_2 -isoprostanes, a possible indicator of lipid peroxidation. That finding was consistent whether supplementation was with the purified *trans*-10, *cis*-12 isomer or a mixture of the CLA isomers (22).

To further investigate the long-term safety of the 1:1 CLA isomer mixture (*cis*-9, *trans*-11 and *trans*-10, *cis*-12), we conducted a 12-mo open-study extension of the original 12-mo randomized, double-blind, placebo-controlled study, i.e., ~70% of the initial subjects were exposed to CLA for 24 mo. In the present study, all participants were administered the CLA isomers in the triglyceride form (CLA-TG).

SUBJECTS AND METHODS

Subjects. Of the 157 subjects completing the 12-mo placebo-controlled study, 134 (24 men and 110 women) signed new informed consent forms before being enrolled in the 12-mo study extension (Fig. 1). The present study was approved by the regional Ethics Committee and conducted in agreement with the Declaration of Helsinki of 1975 as revised in 1983, and performed in accordance with the International Conference on Harmonization (ICH) guidelines.

Study design. The subjects remained in the original 3 groups of the earlier study; all were administered daily 6 opaque soft gel capsules of CLA-TG 4.5 g (3.4 g CLA isomers; Natural Lipids) for the 12-mo open extension. The composition of CLA-TG was similar to the CLA-TG used during the first 12 mo (20). CLA-TG was chosen as the supplement during the extension period because TGs are the natural form of lipids.

Depending on their supplementation during the first 12 mo, group CLAFFA consisted of 46 subjects previously supplemented with CLA-FFA; group CLATG included 47 subjects from the initial CLA-TG group, and group PLAC had 41 subjects from the placebo (olive oil) group (Fig. 1). As in the original study, the subjects consumed their food ad libitum without energy restrictions or changes in lifestyle, including exercise habits. There was no gap in treatment between the original study and the present open extension. Thus, to record changes within each group during the 12–24 mo extension period, results were compared with mo 0 as baseline for groups CLAFFA and CLATG and with mo 12 as baseline for group PLAC.

Clinical assessments. Demographic characteristics (including smoking and drinking habits) were recorded upon entry into the

12-mo open study extension. At mo 0, the subjects had a BMI of 25–30 kg/m² and were 18–65 y of age. Weight, BMI, vital signs, and AEs were recorded every 3 mo, whereas serious adverse events (SAEs) were monitored continuously throughout the study. Body composition was analyzed at mo 12, 18, and 24. Blood samples were analyzed in accredited laboratories: alanine amino transferase (ALAT), aspartate amino transferase (ASAT), hemoglobin, bilirubin, chloride, creatinine phosphokinase (CPK), creatinine, erythrocytes, γ -glutamyl transferase (γ -GT), leukocytes, potassium, sodium, thyroid-stimulating hormone (TSH), thrombocytes, thyroxin, HbA_{1c}, glucose, HDL and LDL cholesterol, total cholesterol, and triglycerides at the Fürst Laboratory (Oslo, Norway), and insulin-like growth factor-1 (IGF-1), insulin, insulin c-peptide, leptin, and Lp(a) at the Aker University Hospital (Oslo, Norway). Compliance was measured every 3 mo by comparing the number of returned capsules with the number of capsules delivered. A subject was considered compliant when taking $\geq 75\%$ of the CLA capsules dispensed.

Diet and exercise. Diet and exercise were assessed at mo 12, 18, and 24. Each participant completed diet and exercise records for 14 consecutive days before their visit to the medical center, following a previously evaluated method (23), which was also used during the first 12 mo of the study (20).

Measurement of body composition and body weight. Dual-energy X-ray absorptiometry (DXA) was used to determine body composition (Lunar Prodigy, software version 5.6) as described in the study publication from the first 12 mo (20). Repeated measures ($n > 20$) with a Hologic whole-body phantom (WB-1406) at each medical center showed no difference between the centers. The subjects were weighed on digital scales (TBF-305, Tanita) in their underwear.

Statistical analysis. The efficacy of an outcome variable was the change in DXA BFM from mo 0 to 24. A test power of 80% was planned, based on a relative mean difference in BFM reduction between each CLA group and the placebo group of at least $1 \times SD$. Testing among the 3 treatment groups for comparability at mo 0 was done by ANOVA (treatment and center as factors). Comparisons among treatment groups for change from mo 0 in DXA and weight were performed using analysis of covariance (with treatment, center and gender as factors; and with mo 0 value, total energy intake, exercise, drug \times energy intake, and drug \times training score as covariates). The model included the BFM at mo 0 to avoid potential regression-to-the-mean effects. Tukey's studentized *t* test was applied for pairwise comparisons of all 3 groups (24). Because treatment groups interacted with effect over time, additional testing within groups regarding changes from mo 12 to mo 24 was justified. Categorical variables were analyzed using Fisher's exact test (25). A treatment responder was defined as a subject who in the previous 1-y study had a $\geq 4.5\%$ median BFM reduction from mo 0 to 12 (20); responders were classified using Fisher's linear discriminant function (26). All tests were two-tailed and differences were considered significant at $P < 0.05$.

RESULTS

Study subjects. Of the 134 subjects that began the 12-mo extension study, 125 (93%) completed it (Fig. 1). Withdrawal rates were similar in the 3 groups (Group CLAFFA, $n = 2$; Group CLATG, $n = 3$; Group PLAC, $n = 4$). Three subjects discontinued the study due to AEs, whereas 6 subjects subsequently withdrew from the study (lost to follow-up, $n = 3$; too low compliance, $n = 1$; without justification, $n = 2$). Compliance was 95% in group CLAFFA, and 94% in groups CLATG and PLAC. There were no differences in the characteristics of the groups at mo 0 (20) or 12 (Table 1).

Adverse events. AEs were reported by 50% of all randomized subjects with similar frequencies in the 3 study groups ($P = 0.26$). Of 124 single events, we considered 7 AEs to be drug related (Group CLAFFA, $n = 5$; Group CLATG, $n = 1$; Group PLAC, $n = 1$), and all were rated as "mild." Three of these subjects (2.2% of the total number of subjects) left the

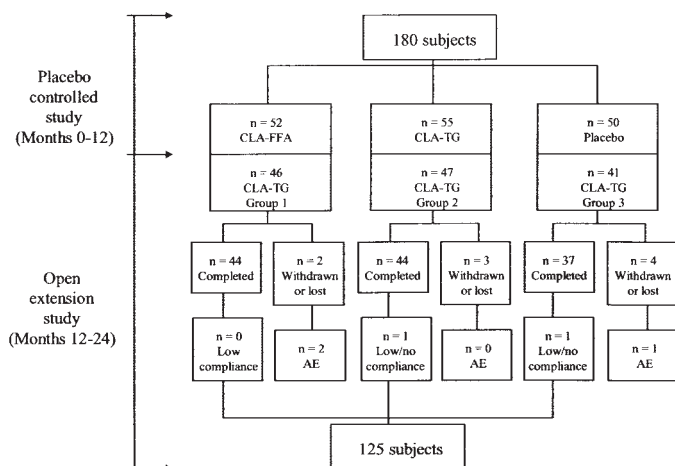


FIGURE 1 In the first 12-mo study, 180 subjects were randomly assigned to one of 3 groups in a double-blind parallel study. The subjects were either supplemented with CLA as a triglyceride (CLA-TG), CLA as a free fatty acid (CLA-FFA) or placebo (olive oil). Of the 157 subjects who finished this study, 134 entered the extension open study in which all subjects were administered CLA-TG for an additional 12 mo, and 125 completed the entire 24-mo study.

TABLE 1

Characteristics of the healthy, overweight study population entering the 12-mo extension with CLA supplementation¹

	Group CLAFFA ²	Group CLATG ²	Group PLAC ²
Gender, <i>n</i>			
Men	10	6	8
Women	36	41	33
Age, ³ y	45.1 ± 10.5	48.6 ± 10.6	45.1 ± 8.8
Alcohol use, ⁴ %	67	67	71
Tobacco use, ⁴ %	34	15	19
Exercise, ⁵ %	45	52	53

¹ Values are for subjects who started the 12-mo study.

² Group CLAFFA: subjects randomly assigned to the CLA-FFA group during the first 12 mo and administered CLA-TG in mo 12–24; Group CLATG: subjects randomly assigned to the CLA-TG group during the first 12 mo who continued taking CLA-TG in mo 12–24; Group PLAC: subjects randomly assigned to the placebo group during the first 12 mo and administered CLA-TG in mo 12–24.

³ Values are means ± SD.

⁴ Alcohol and tobacco uses are expressed as the percentage of subjects who answered positively to these questions (%Yes).

⁵ Exercise is expressed as the percentage of subjects training at least once a week with sweating (% with sweating).

trial due to AE. The most frequently reported drug-related AEs were various gastrointestinal complaints.

Eight subjects had serious adverse events (SAE) during the extension period, all of them were judged to be unrelated to the use of CLA. These included: 1) a 50-y-old woman suffering acute abdominal pain/possible gallstone, with a prolonged course presenting elevated serum-amylase. However, CLA was not withdrawn and the subject recovered after some time; 2) a 56-y-old woman was hospitalized due to acute colitis, CLA was not suspended, and the subject recovered quickly; 3) a 57-y-old woman presented a diagnosis of gallstones in biliary tract/bladder, which was treated surgically. In this case the CLA was permanently withdrawn. The other 5 SAEs included myoma uteri, acute subendocardial infarction, protracted pneumonia, and 2 cases of skin infection after preplanned surgery. Except for the subject suffering from protracted pneumonia and who withdrew from the study, CLA was not suspended for the other subjects.

Safety. Groups CLAFFA and CLATG did not change from mo 0 or group PLAC from mo 12 for the following clinical chemistry variables: hemoglobin, bilirubin, chloride, CPK, creatinine, erythrocytes, γ -GT, potassium, sodium, TSH, thyroxin, and IGF-1 (data not shown).

Serum total cholesterol was lower at mo 24 compared with mo 0 in group CLAFFA ($P = 0.04$) but not in group CLATG. Serum total cholesterol tended to be lower ($P = 0.053$) in group PLAC at mo 24 than at mo 12 (Table 2). The HDL cholesterol level did not change between mo 0 and 24 in group CLAFFA; however, it decreased in group CLATG ($P = 0.026$). Four subjects had decreases in HDL cholesterol to a level below normal at mo 24 compared with mo 0 in group CLAFFA ($n = 1$) and group CLATG ($n = 3$), respectively, whereas all other subjects from both groups had normal levels of HDL cholesterol at mo 24. Serum HDL cholesterol did not change in group PLAC from mo 12 to 24. In group PLAC, 2 subjects had decreases in HDL cholesterol to a level below normal at mo 24 compared with mo 12, and 1 subject had an increase in HDL cholesterol to a level above normal at mo 24. Serum LDL cholesterol and triglyceride concentrations did not change in groups CLAFFA and CLATG compared with mo 0,

or in group PLAC compared with mo 12 (Table 2). However, 3 subjects in the CLAFFA group with abnormal levels at mo 0 had decreases in LDL cholesterol to within normal levels at mo 24 and 1 subject in the CLATG group had a decrease in LDL cholesterol to a level below normal at mo 24 compared with mo 0. In group PLAC, 1 subject with abnormal levels at mo 12 had a decrease in LDL cholesterol to a normal level at mo 24.

Lp(a) levels were increased at mo 24 in groups CLAFFA and CLATG compared with mo 0 and were also increased in group PLAC compared with mo 12 ($P = 0.005$, $P = 0.008$, and $P = 0.010$, respectively) (Table 2). In groups CLAFFA and CLATG, 2 subjects in each group had increases in Lp(a) to above normal levels at mo 24 compared with mo 0; 16 subjects had Lp(a) levels above normal from mo 0 to 24 in group CLAFFA ($n = 7$) and group CLATG ($n = 9$), respectively, whereas 1 subject in group CLAFFA with abnormal levels at mo 0 had a decrease in LDL cholesterol to normal levels at mo 24. In group PLAC, 2 subjects had increases in Lp(a) levels above normal at mo 24 compared with mo 12 and 4 subjects had Lp(a) levels above normal from mo 12 to 24.

Blood glucose levels did not differ in groups CLAFFA and CLATG at mo 24 compared with mo 0 or in group PLAC compared with mo 12 (Table 2). HbA1c levels, which had increased significantly from 0 to 12 mo in all groups, returned to mo 0 levels by mo 24. HbA1c levels did not differ in groups CLAFFA and CLATG at mo 24 compared with mo 0, whereas they were lower in group PLAC compared with mo 12 ($P < 0.001$) (Table 2). Four subjects had decreases in HbA1c level to below normal at mo 24 compared with mo 0 in group CLAFFA ($n = 3$) and group CLATG ($n = 1$), respectively, whereas 1 subject with abnormal levels at mo 0 had a decrease in HbA1c level to within normal at mo 24 in group CLAFFA. In group PLAC, 1 subject had a decrease in HbA1c level to below normal at mo 24 compared with mo 12.

Insulin levels at mo 24 were not different in group CLAFFA compared with mo 0, but insulin levels were slightly increased in group CLATG ($P = 0.01$). Two subjects in group CLATG had increases in insulin at mo 24 compared with mo 0. Insulin levels did not differ at mo 24 compared with mo 12 in group PLAC (Table 2). In group CLAFFA, insulin c-peptide at mo 24 was reduced compared with mo 0 ($P = 0.015$), whereas there was no change in group CLATG. Group PLAC insulin c-peptide levels at mo 24 were not different from mo 12.

Leptin levels were reduced in groups CLAFFA and CLATG compared with mo 0 ($P < 0.001$), and in group PLAC compared with mo 12 ($P < 0.001$) (Table 2).

At mo 24, ALAT levels in groups CLAFFA and CLATG did not differ from mo 0, or in group PLAC from mo 12. ASAT levels were increased at mo 24 in groups CLAFFA and CLATG compared with mo 0 ($P = 0.002$ and $P = 0.009$, respectively), whereas there no change occurred in group PLAC compared with mo 12 (Table 2). One subject in each of groups CLAFFA and CLATG had an increase in ASAT to above the normal level at mo 24 compared with mo 0, whereas 3 subjects in group PLAC had an increase in ASAT to above the normal level at mo 24 compared with mo 12. Among these, 2 subjects (1 from group CLATG and 1 from group PLAC) with normal levels at mo 12 had increases in ASAT and ALAT to above the normal level at mo 24 (2–3 times above the upper limit).

Leukocytes were increased in groups CLAFFA and CLATG at mo 24 compared with mo 0 ($P = 0.028$ and $P < 0.001$, respectively). Five subjects in group CLAFFA ($n = 3$) and group CLATG ($n = 2$) with abnormal low levels at mo 0 had

TABLE 2

Laboratory blood analyses of the healthy, overweight study population after 24 mo of CLA supplementation¹

	Group ²	mo 0	mo 12	mo 24	Δ 24–12	Δ 24–0
Serum total cholesterol, mmol/L	CLAFFA	5.39 ± 0.89	5.50 ± 0.97	5.16 ± 0.84	-0.34 ± 0.82#	-0.23 ± 0.73#
	CLATG	5.71 ± 1.10	5.70 ± 0.86	5.54 ± 0.87	-0.16 ± 0.55	-0.17 ± 0.95
	PLAC	5.82 ± 1.36	5.80 ± 1.14	5.53 ± 0.95	-0.27 ± 0.83	NA
Serum HDL cholesterol, mmol/L	CLAFFA	1.44 ± 0.31	1.37 ± 0.33	1.38 ± 0.33	+0.01 ± 0.27	-0.06 ± 0.22
	CLATG	1.51 ± 0.31	1.43 ± 0.33	1.42 ± 0.29	-0.01 ± 0.19	-0.09 ± 0.26#
	PLAC	1.55 ± 0.40	1.57 ± 0.50	1.51 ± 0.49	-0.06 ± 0.25	NA
Serum LDL cholesterol, mmol/L	CLAFFA	3.27 ± 0.77	3.44 ± 0.80	3.14 ± 0.66	-0.30 ± 0.72#	-0.12 ± 0.63
	CLATG	3.56 ± 0.98	3.65 ± 0.79	3.49 ± 0.81	-0.16 ± 0.55	-0.08 ± 0.89
	PLAC	3.66 ± 1.29	3.62 ± 1.01	3.42 ± 0.89	-0.20 ± 0.78	NA
Serum triglycerides, mmol/L	CLAFFA	1.42 ± 0.84	1.47 ± 1.19	1.34 ± 0.99	-0.13 ± 0.92	-0.08 ± 0.62
	CLATG	1.30 ± 0.57	1.29 ± 0.51	1.28 ± 0.47	-0.01 ± 0.43	-0.01 ± 0.48
	PLAC	1.24 ± 0.63	1.26 ± 0.63	1.28 ± 0.79	+0.02 ± 0.53	NA
Serum Lp(a), mg	CLAFFA	318 ± 427	359 ± 468	372 ± 480	+13 ± 94	+54 ± 122#
	CLATG	246 ± 282	283 ± 301	304 ± 369	+21 ± 115	+58 ± 138#
	PLAC	220 ± 219	223 ± 220	270 ± 305	+47 ± 106#	NA
Serum glucose, mmol/L	CLAFFA	5.14 ± 0.47	5.16 ± 0.64	5.30 ± 0.63	+0.14 ± 0.49	+0.16 ± 0.54
	CLATG	5.14 ± 0.46	5.06 ± 0.58	5.25 ± 0.54	+0.19 ± 0.52#	+0.12 ± 0.47
	PLAC	5.18 ± 0.43	5.06 ± 0.43	5.16 ± 0.51	+0.10 ± 0.52	NA
Whole blood HbA1c, %	CLAFFA	5.48 ± 0.28	5.69 ± 0.29	5.51 ± 0.28	-0.18 ± 0.25#	+0.03 ± 0.29
	CLATG	5.49 ± 0.26	5.70 ± 0.27	5.51 ± 0.28	-0.19 ± 0.21#	+0.02 ± 0.23
	PLAC	5.42 ± 0.33	5.60 ± 0.20	5.42 ± 0.23	-0.18 ± 0.23#	NA
Serum insulin, pmol/L	CLAFFA	72.2 ± 26.7	70.8 ± 28.5	77.7 ± 32.8	+6.81 ± 22.8	+4.95 ± 27.5
	CLATG	75.0 ± 33.7	81.9 ± 71.7	90.6 ± 55.1	+8.71 ± 41.4	+14.9 ± 39.2#
	PLAC	64.5 ± 23.2	77.7 ± 78.1	74.9 ± 29.0	-2.82 ± 73.3	NA
Serum insulin c-peptide, pmol/L	CLAFFA	859 ± 222	805 ± 309	772 ± 238	-33 ± 237	-86 ± 227#
	CLATG	813 ± 216	811 ± 299	816 ± 293	+5 ± 216	+3 ± 282
	PLAC	773 ± 239	734 ± 337	690 ± 234	-44 ± 311	NA
Serum leptin, pmol/L	CLAFFA	1118 ± 553	1051 ± 798	887 ± 478	-164 ± 644	-221 ± 431#
	CLATG	1169 ± 499	993 ± 459	822 ± 364	-171 ± 410#	-347 ± 448#
	PLAC	1068 ± 575	1149 ± 712	766 ± 411	-383 ± 460#	NA
Serum ALAT, U/L	CLAFFA	24.3 ± 14.9	26.4 ± 15.1	26.2 ± 17.3	-0.22 ± 13.7	+1.93 ± 12.8
	CLATG	24.3 ± 10.3	24.8 ± 11.7	26.0 ± 11.9	+1.20 ± 9.7	+1.66 ± 10.1
	PLAC	24.8 ± 12.6	26.5 ± 13.6	31.7 ± 35.6	+5.26 ± 35.1	NA
Serum ASAT, U/L	CLAFFA	21.9 ± 5.7	24.0 ± 8.3	25.0 ± 7.4	+0.96 ± 7.15	+3.07 ± 6.28#
	CLATG	23.1 ± 5.4	23.5 ± 5.6	25.3 ± 5.8	+1.82 ± 4.85#	+2.25 ± 5.44#
	PLAC	22.5 ± 5.9	22.6 ± 5.2	28.1 ± 20.0	+5.45 ± 19.4	NA
Whole blood leucocytes, 10 ⁹ /L	CLAFFA	6.03 ± 1.58	6.50 ± 1.78	6.63 ± 1.88	+0.13 ± 1.70	+0.60 ± 1.76#
	CLATG	5.30 ± 1.57	5.96 ± 1.68	6.19 ± 1.65	+0.23 ± 1.75	+0.92 ± 1.21#
	PLAC	5.92 ± 1.77	5.94 ± 1.82	6.29 ± 1.56	+0.35 ± 1.40	NA
Whole blood thrombocytes, 10 ⁹ /L	CLAFFA	265 ± 61.6	281 ± 69.1	297 ± 65.9	+15.7 ± 34.7#	+31.8 ± 37.1#
	CLATG	264 ± 60.6	273 ± 67.6	299 ± 76.9	+26.3 ± 49.2#	+35.8 ± 48.3#
	PLAC	257 ± 53.1	259 ± 54.8	283 ± 57.0	+24.1 ± 30.8#	NA

¹ Values are means ± SD, *n* = 46 (CLAFFA); *n* = 47 (CLATG); *n* = 37 (PLAC).

² See Table 1 for group descriptions.

Change from mo 0 to 24 (groups CLAFFA and CLATG) or from mo 12 to 24 (group PLAC); paired *t* test, *P* < 0.05. NA, not applicable.

an increase in leukocytes to within normal levels at mo 24, whereas 2 subjects had an increase in leukocytes above the normal level at mo 24 compared with mo 0 in group CLATG. Leukocytes in group PLAC did not differ compared with mo 12. Thrombocytes levels at mo 24 had increased in groups CLAFFA and CLATG compared with mo 0 and in group PLAC compared with mo 12 (*P* < 0.001) (Table 2). Six subjects in group CLAFFA (*n* = 1) and group CLATG (*n* = 5) had an increase in thrombocyte to above normal levels at mo 24 compared with mo 0. All subjects in group PLAC had changes in thrombocyte levels within the normal range from mo 12 to mo 24.

Vital signs. Systolic and diastolic blood pressures and heart rates were all within normal ranges and did not differ between or within the groups throughout the 24-mo study (data not shown).

Weight and BMI. Body weight and BMI decreased in groups CLAFFA and CLATG at mo 24 compared with mo 0 (*P* = 0.013 and *P* < 0.001, respectively) (Table 3). Group

PLAC at mo 24 also had a reduction in body weight and consequently a reduction in BMI compared with mo 12 (*P* = 0.003) (Table 3). Reductions in body weight and BMI were observed during the first 6 mo of supplementation with CLA (mo 0–6 for groups CLAFFA and CLATG, and mo 12–18 for group PLAC) (20). During mo 12–24, there was no further reduction in weight and BMI in groups CLAFFA and CLATG (data not shown).

Body composition. BFM in groups CLAFFA and CLATG was reduced at mo 24 compared with mo 0 (*P* < 0.001); however, the greatest loss of body fat was observed during the first 6 mo of CLA supplementation (mo 0–6) (Fig. 2). During mo 12–24, there was no further reduction in BFM in groups CLAFFA and CLATG. BFM was reduced in group PLAC at mo 24 compared with mo 12 (*P* < 0.001). As observed in groups CLAFFA and CLATG, the greatest amount of fat loss occurred during the first 6 mo (mo 12–24) of CLA supplementation (Fig. 2). LBM did not change in groups CLAFFA and CLATG at mo 24 compared with mo 0, and in group

TABLE 3

Body weight, body composition, daily energy intake, and exercise measurements of the healthy, overweight study population during 24 mo of CLA supplementation¹

	Group ²	mo 0	mo 12	mo 24	Δ 24–12	Δ 24–0
Body weight, kg	CLAFFA	81.9 ± 9.8	80.9 ± 9.9	80.4 ± 9.3	-0.5 ± 3.7	-1.5 ± 4.1#
	CLATG	80.9 ± 8.3	78.9 ± 9.2	78.5 ± 8.9	-0.4 ± 2.7	-2.4 ± 3.4#
	PLAC	79.6 ± 9.2	79.9 ± 10.0	78.3 ± 10.4	-1.6 ± 3.2#	NA
BMI, kg/m ²	CLAFFA	28.1 ± 1.4	27.7 ± 1.7	27.5 ± 1.6	-0.2 ± 1.3	-0.6 ± 1.4#
	CLATG	28.3 ± 1.5	27.6 ± 1.6	27.4 ± 1.7	-0.2 ± 0.9	-0.9 ± 1.2#
	PLAC	27.4 ± 1.7	27.4 ± 1.8	26.8 ± 2.0	-0.6 ± 1.1#	NA
BFM, kg	CLAFFA	31.3 ± 5.3	29.5 ± 5.8	29.4 ± 5.5	-0.1 ± 3.2	-1.8 ± 3.7#
	CLATG	31.6 ± 5.6	28.9 ± 5.5	28.9 ± 5.3	0.0 ± 2.4	-2.7 ± 3.4#
	PLAC	29.4 ± 6.0	29.7 ± 6.1	28.0 ± 5.7	-1.7 ± 2.8#	NA
LBM, kg	CLAFFA	47.7 ± 9.3	48.5 ± 8.2	48.0 ± 5.5	-0.5 ± 1.9	+0.3 ± 2.1
	CLATG	46.6 ± 7.9	47.3 ± 7.5	46.9 ± 7.6	-0.4 ± 1.7	+0.3 ± 2.0
	PLAC	47.4 ± 9.4	47.4 ± 9.3	47.5 ± 9.4	+0.1 ± 1.5	NA
BMM, kg	CLAFFA	2.93 ± 0.43	2.88 ± 0.46	2.93 ± 0.45	+0.05 ± 0.09#	0.00 ± 0.09
	CLATG	2.71 ± 0.42	2.68 ± 0.47	2.70 ± 0.45	+0.02 ± 0.11	-0.01 ± 0.10
	PLAC	2.84 ± 0.48	2.83 ± 0.52	2.84 ± 0.54	+0.01 ± 0.09	NA
Energy intake, kJ/d	CLAFFA	8326 ± 2251	7000 ± 1674	7456 ± 1795	+456 ± 1719	-870 ± 1510#
	CLATG	8268 ± 2138	6962 ± 1397	6979 ± 1761	+17 ± 1447	-1289 ± 2159#
	PLAC	8125 ± 1941	7410 ± 1999	7351 ± 1853	-59 ± 1656	NA
Exercise, ³ AU	CLAFFA	4.0 ± 3.1	4.5 ± 2.9	4.2 ± 3.3	-0.3 ± 2.5	+0.2 ± 2.8
	CLATG	4.0 ± 2.1	4.3 ± 2.1	5.2 ± 3.7	+1.0 ± 3.5	+1.3 ± 3.8
	PLAC	4.4 ± 3.1	5.0 ± 3.2	4.8 ± 2.8	-0.3 ± 2.7	NA

¹ Values are means ± SD, *n* = 46 (CLAFFA); *n* = 47 (CLATG); *n* = 37 (PLAC).

² See Table 1 for group descriptions.

³ Exercise was assessed as the product of the number of training sessions (20 min) with intensity (high or low) and expressed with arbitrary units (AU).

Change from mo 0 to 24 (groups CLAFFA and CLATG) or from mo 12 to 24 (group PLAC); paired *t* test, *P* < 0.05. NA, not applicable.

PLAC compared with mo 12 (Table 3). Bone mineral mass (BMM) did not differ in groups CLAFFA and CLATG at mo 24 compared with mo 0 or in group PLAC compared with mo 12 (Table 3).

In groups CLAFFA and CLATG, responders to CLA during the first 12 mo (*n* = 64), defined as a >4.5% reduction in BFM, had stable values for BFM, LBM, and body weight during the extension study. The nonresponders in groups

CLAFFA and CLATG during the first 12-mo study (*n* = 70) had a significant loss in body weight and BFM during the 12-mo extension (data not shown). Discrimination analysis of group PLAC during the 12-mo extension revealed that responders had higher BFM (*P* = 0.002) and were older than nonresponders at mo 12 (data not shown).

Diet and exercise. Groups CLAFFA and CLATGA had a significant reduction in energy intake at mo 24 compared with mo 0, whereas group PLAC did not compared with mo 12 (Table 3). Estimates of exercise amounts did not change throughout the study in all groups (Table 3). There was no correlation between the changes in body weight and body composition with changes in diet or exercise.

DISCUSSION

The good compliance and low drop-out rates indicate that long-term CLA supplementation was well tolerated by the subjects. The incidence of CLA-related AEs was lower during the second 12 mo which may be due in part to the loss of subjects at mo 12 who experienced AEs during the first 12 mo. As observed in other short-term studies (17,18) and in the first 12 mo (20), most of the AEs that were considered to be drug related were gastrointestinal events.

Lp(a) levels were consistently increased in all groups supplemented with CLA. Increased Lp(a) levels are thought to be an independent predictor of cardiovascular disease risk; however, the clinical relevance of individual changes in Lp(a) levels is still unclear (27). In addition to Lp(a), we observed increases in leukocyte and thrombocyte counts. These changes were within the normal ranges but were consistent over time and suggest the presence of an inflammatory or immunological response to CLA supplementation. The clinical relevance of these changes is unclear. However, the possibility that an

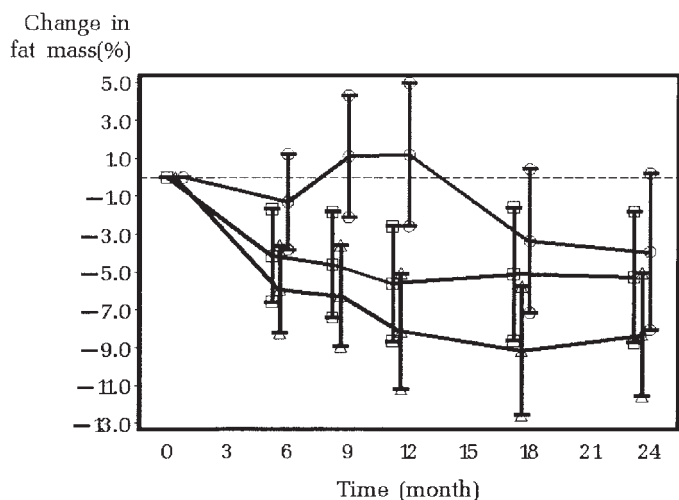


FIGURE 2 The percentage change in BFM in subjects administered placebo (○), CLA-FFA (□), or CLA-TG (Δ) during the first 12 mo. From mo 12–24, all subjects were given CLA-TG. Values are means with 95% CI, *n* = 134, and were measured at the same periods for all 3 groups (i.e., mo 0, 6, 9, 12, 18 and 24). Intervals not including 0 are significant within the group.

increase in these markers is related to the loss of BFM remains to be elucidated.

The role of CLA in cardiovascular risk is equivocal. Although we observed no effect on blood lipid levels (no changes in triglyceride and very small changes in total, HDL, or LDL cholesterol in all groups), there was an increase in some markers associated with inflammation and cardiovascular disease risk. Studies on the effects of CLA in cardiovascular disease and inflammation in animals have also demonstrated mixed results including a proatherogenic effect in mice (28), an antiatherogenic effect in rabbits (29) an anti-inflammatory effect in animals (30,31), and stimulation of the immune system (32,33). Short-term clinical studies on CLA supplementation also yielded inconsistent findings on cardiovascular disease risk factors. In a study by Smedman et al. (19), an increase in LDL cholesterol and apolipoprotein B levels was reported, whereas Noone et al. (34) reported no effect on total, LDL, or HDL cholesterol levels and a decrease in triglycerides with CLA supplementation. Further studies are warranted to determine whether there is an effect of CLA on cardiovascular risk and inflammation in humans.

During the first 12 mo of the study, there were no changes in blood glucose, insulin, or insulin c-peptide levels, although there was a slight increase in HbA1c levels in all groups including the placebo group. HbA1c levels at mo 24 had returned to mo 0 values. None of the subjects developed diabetes as defined by the American Diabetic Association (35). The role of CLA in diabetes has yet to be determined, and the results from animal and human studies have been conflicting. In a small study of type 2 diabetics, Belury et al. (36) demonstrated that CLA supplementation with a commercially available CLA mixture for 8 wk led to a decrease in fasting blood glucose in 81% of the diabetics, suggesting that CLA may be beneficial in type 2 diabetics. These findings differ from the results of a study by Riserus et al. (21) that demonstrated that men with metabolic syndrome, who were supplemented with the pure *trans*-10, *cis*-12 isomer, had an increase in insulin resistance compared with the placebo group. Subjects in that study who were supplemented with a commercially available mixture of the 2 bioactive CLA isomers (*cis*-9, *trans*-11 and *trans*-10, *cis*-12) did not have an increase in insulin resistance. The latter finding is in agreement with our results. Because supplementation did not affect blood glucose, HbA1c, insulin, and insulin c-peptide, we contend that long-term CLA supplementation is not diabetogenic.

An accumulation of lipids in the liver was observed in female Sencar mice fed CLA-enriched diets (up to 1.5%) for 6 wk (37), in male AKR/J mice treated with CLA (up to 1.2% by weight) for 6 wk (16), and in hamsters fed CLA for 8 wk (38). To date, liver hypertrophy was reported only in some of the studies performed in mice and hamsters, and the response thus seems to be species specific. In the present study, 2 of 134 subjects had increased levels of transaminases (ASAT and ALAT) at the end of the study. The transaminases levels returned to normal values 4 wk after ending the CLA treatment, indicating that a relation to CLA treatment cannot be excluded. However, other explanations cannot be dismissed. There were no other safety issues raised by the other blood variables because they remained stable overall during the 24-mo study.

Throughout the 24-mo study period, serum leptin levels decreased 20–35% as the subjects lost BFM. Leptin is a protein that is synthesized and secreted by adipocytes (39). Studies have demonstrated that leptin acts centrally to regulate appetite and energy expenditure, as well as peripherally to regulate

whole-body energy metabolism. High levels of leptin are associated with increased adiposity (40–42). During the 24-mo study, there was a direct correlation ($r = 0.43$, $P < 0.0001$) between BFM reduction and a decrease in leptin levels in the subjects administered CLA without changes in energy intake. This relation was confirmed in the 12-mo extension. This supports the effects of CLA on leptin as seen in other studies in animals (7,43–47) and humans (36,48). The subjects given CLA during the first 12 mo also had a significant decrease in BFM and leptin levels. The findings of the present study provide further evidence for using leptin as a biochemical marker of body fat reduction with no effect on energy intake; it constitutes the only biochemical variable reflecting the loss of BFM and body weight in the current study.

This 12-mo extension study also provided the opportunity to further evaluate the efficacy of long-term CLA supplementation. Supplementation with CLA for 24 mo led to a 6–8% reduction in BFM compared with baseline. These changes in body composition were not related to diet and exercise. The loss in BFM was also confirmed in the group of subjects, who were previously supplemented with the placebo, because they lost 6% of their body fat during the 12-mo extension study. Discriminant analysis of responders revealed that subjects with higher BFM were more likely to lose fat with CLA supplementation than those with lower BFM. This latter finding corroborates the results of our responder analysis during the previous 12-mo placebo-controlled study (20). As in the first 12 mo, most of the effect on BFM was observed during the first 6 mo of CLA supplementation. We do not know whether this was due to a dose-response pattern (higher dosage causing a greater initial or more sustained effect), or the result of adaptation or the loss of efficacy. It is unlikely that there was a loss of efficacy given that the subjects maintained a stable BFM and LBM for 18 mo after the initial loss of BFM, despite no changes in diet or exercise.

Weight loss studies in overweight and obese subjects have demonstrated that most subjects will regain the lost weight within the next 1–2 y (49). In the present study, we demonstrated long-term maintenance of BFM and LBM, accompanied by a reduction in body weight, suggesting that CLA may be beneficial in preventing weight regain, which is part of the yo-yo effect often observed with many diet plans. Loss of LBM usually accompanies dietary or drug-induced weight loss, resulting in an unchanged or decreased LBM:BFM ratio (50). Because loss of LBM is associated with a reduction in basal metabolic rate (51), this may contribute to the resurgence of body weight with time. In contrast, CLA supplementation prevented loss of LBM during weight reduction, thus likely maintaining the basal metabolic rate and subsequently reducing the risk of weight increase.

In the present study, we demonstrated that supplementation with a commercially available CLA mixture for 24 mo in healthy, overweight subjects led to a significant reduction in BFM and body weight, while maintaining LBM. These changes in body composition were not related to diet or exercise. CLA was well tolerated and the observed changes in the safety variables were all within the normal range, suggesting that CLA supplementation in healthy, overweight subjects for 24 mo is safe. Based on the findings of these studies, we suggest that CLA may have merit as a weight loss supplement when combined with another weight-reducing treatment or may singularly promote selective loss of BFM and maintenance of LBM with a subsequent reduced risk of weight regain.

ACKNOWLEDGMENTS

The authors are very grateful to Mette Bogen, clinical nutritionist, who monitored all of the diet forms and collected data from analysis. Particular thanks to the clinical nurses Oddrun Kulvedrøsten, Linda Magnor, Lill Johannessen, Maj Granmark, and Janne Nyborg for their active contribution to the success of this study. Finally the authors thank Heather Nelson-Cortes for her fruitful comments and evaluation of the manuscript.

LITERATURE CITED

- Ha, Y. L., Grimm, N. K. & Pariza, M. W. (1987) Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. *Carcinogenesis* 8: 1881–1887.
- Chin, S. F., Storkson, J. M., Liu, W., Albright, K. J. & Pariza, M. W. (1994) Conjugated linoleic acid (9,11- and 10,12-octadecadienoic acid) is produced in conventional but not germ-free rats fed linoleic acid. *J. Nutr.* 124: 694–701.
- Houseknecht, K. L., Van den Heuvel, J. P., Moya-Camarena, S. Y., Portocarrero, C. P., Peck, L. W., Nickel, K. P. & Belury, M. A. (1998) Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty *fa/fa* rat. *Biochem. Biophys. Res. Commun.* 244: 678–682.
- Lee, K. N., Kritchevsky, D. & Pariza, M. W. (1994) Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* 108: 19–25.
- Miller, C. C., Park, Y., Pariza, M. W. & Cook, M. E. (1994) Feeding conjugated linoleic acid. *Biochem. Biophys. Res. Commun.* 198: 1107–1112.
- Nicolosi, R. J., Rogers, E. J., Kritchevsky, D., Scimeca, J. A. & Huth, P. J. (1997) Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. *Artery* 22: 266–277.
- Akahoshi, A., Goto, Y., Mura, K., Miyazaki, T., Yamasaki, M., Nonaka, M., Yamada, K. & Sugano, M. (2002) Conjugated linoleic acid reduces body fats and cytokine levels of mice. *Biosci. Biotechnol. Biochem.* 66: 916–920.
- DeLany, J. P., Blohm, F., Truett, A. A., Scimeca, J. A. & West, D. B. (1999) Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. *Am. J. Physiol.* 276: R1172–R1179.
- Dugan, M.E.R., Aalhus, J. L., Schaefer, A. L. & Kramer, J.K.G. (1997) The effects of conjugated linoleic acid on fat to lean repartitioning and feed conversion in pigs. *Can. J. Anim. Sci.* 77: 723–725.
- Gavino, V. C., Gavino, G., Leblanc, M. J. & Tuchweber, B. (2000) 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: 27–29.
- Ostrowska, E., Suster, D., Muralitharan, M., Cross, R. F., Leury, B. J., Bauman, D. E. & Dunshea, F. R. (2003) Conjugated linoleic acid decreases fat accretion in pigs: evaluation by dual-energy X-ray absorptiometry. *Br. J. Nutr.* 89: 219–229.
- Park, Y., Storkson, J. M., Albright, K. J., Liu, W. & Pariza, M. W. (1999) Evidence that the *trans*-10, *cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids* 34: 235–241.
- Rahman, S., Wang, Y., Han, S. Y., Cha, J. Y., Fukuda, N., Yotsumoto, H. & Yanagita, T. (2001) Effects of short-term administration of conjugated linoleic acid on lipid metabolism in white and brown adipose tissues of starved/refed Otsuka Long-Evans Tokushima Fatty rats. *Food Res. Int.* 34: 515–520.
- Szymczyk, B., Pisulewski, P. M., Szczurek, W. & Hanczakowski, P. (2001) Effects of conjugated linoleic acid on growth performance, feed conversion efficiency, and subsequent carcass quality in broiler chickens. *Br. J. Nutr.* 85: 465–473.
- Takahashi, Y., Kushiro, M., Shinohara, K. & Ide, T. (2002) Dietary conjugated linoleic acid reduces body fat mass and affects gene expression of proteins regulating energy metabolism in mice. *Comp. Biochem. Physiol. B* 133: 395–404.
- West, D. B., DeLany, J. P., Camet, P. M., Blohm, F., Truett, A. A. & Scimeca, J. (1998) Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. *Am. J. Physiol.* 275: R667–R672.
- Berven, G., Bye, A., Hals, O. et al. (2000) Safety of conjugated linoleic acid (CLA) in overweight or obese human volunteers. *Eur. J. Lipid Sci. Technol.* 102: 455–462.
- Blankens, H., Stakkestad, J. A., Fagertun, H., Thom, E., Wadstein, J. & Gudmundsen, O. (2000) Conjugated linoleic acid reduces body fat mass in overweight and obese humans. *J. Nutr.* 130: 2943–2948.
- Smedman, A. & Vessby, B. (2001) Conjugated linoleic acid supplementation in humans—metabolic effects. *Lipids* 36: 773–781.
- Gaullier, J. M., Halse, J., Høye, K., Kristiansen, K., Fagertun, H., Vik, H. & Gudmundsen, O. (2004) Conjugated linoleic acid (CLA) supplementation for one year reduces body fat mass in healthy, overweight humans. *Am. J. Clin. Nutr.* 79: 1118–1125.
- Riserus, U., Amer, P., Brismar, K. & Vessby, B. (2002) Treatment with dietary *trans*10*cis*12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. *Diabetes Care* 25: 1516–1521.
- Basu, S., Riserus, U., Turpeinen, A. & Vessby, B. (2000) Conjugated linoleic acid induces lipid peroxidation in men with abdominal obesity. *Clin. Sci. (Lond.)* 99: 511–516.
- Nes, M., Andersen, L. F., Solovoll, K., Sandstad, B., Hustvedt, B. E., Løvd, A. & Drevon, C. A. (1992) Accuracy of a quantitative food frequency questionnaire applied in elderly Norwegian women. *Eur. J. Clin. Nutr.* 46: 809–821.
- Montgomery, D. (1984) *Design and Analysis of Experiments*. Wiley, New York, NY.
- Gresti, A. (1990) *Categorical Data Analysis*. Wiley, New York, NY.
- Kendall, M. & Stuart, A. (1979) *The Advanced Theory of Statistics*. Charles Griffin & Co., London, UK.
- Hackam, D. & Anand, S. S. (2003) Emerging risk factors for atherosclerotic vascular disease: a critical review of the evidence. *J. Am. Med. Assoc.* 290: 932–940.
- Munday, J. S., Thompson, K. G. & James, K. A. (1999) Dietary conjugated linoleic acids promote fatty streak formation in the C57BL/6 mouse atherosclerosis model. *Br. J. Nutr.* 81: 251–255.
- Kritchevsky, D., Tepper, S. A., Wright, S., Tso, P. & Czarniecki, S. K. (2000) Influence of conjugated linoleic acid (CLA) on establishment and progression of atherosclerosis in rabbits. *J. Am. Coll. Nutr.* 19: 472S–477S.
- Hontecillas, R., Wannemuehler, M. J., Zimmerman, D. R., Hutto, D. L., Wilson, J. H., Ahn, D. U. & Bassaganya-Riera, J. (2002) Nutritional regulation of porcine bacterial-induced colitis by conjugated linoleic acid. *J. Nutr.* 132: 2019–2027.
- Whigham, L. D., Higbee, A., Bjorling, D. E., Park, Y., Pariza, M. W. & Cook, M. E. (2002) Decreased antigen-induced eicosanoid release in conjugated linoleic acid-fed guinea pigs. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 282: R1104–R1112.
- Bassaganya-Riera, J., Hontecillas-Magarzo, R., Bregendahl, K., Wannemuehler, M. J. & Zimmerman, D. R. (2001) Effects of dietary conjugated linoleic acid in nursery pigs of dirty and clean environments on growth, empty body composition, and immune competence. *J. Anim. Sci.* 79: 714–721.
- Cook, M. E., Miller, C. C., Park, Y. & Pariza, M. (1993) Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. *Poult. Sci.* 72: 1301–1305.
- Noone, E. J., Roche, H. M., Nugent, A. P. & Gibney, M. J. (2002) The effect of dietary supplementation using isomeric blends of conjugated linoleic acid on lipid metabolism in healthy human subjects. *Br. J. Nutr.* 88: 243–251.
- American Diabetes Association (1997) Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 20: 1197.
- Belury, M. A., Mahon, A. & Banni, S. (2003) The conjugated linoleic acid (CLA) isomer, *t10c12*-CLA, is inversely associated with changes in body weight and serum leptin in subjects with type 2 diabetes mellitus. *J. Nutr.* 133: 257S–260S.
- Belury, M. A. & Kempa-Steczko, A. (1997) Conjugated linoleic acid modulates hepatic lipid composition in mice. *Lipids* 32: 199–204.
- De Deckere, E. A., van Amelsvoort, J. M., McNeill, G. P. & Jones, P. (1999) Effects of conjugated linoleic acid (CLA) isomers on lipid levels and peroxisome proliferation in the hamster. *Br. J. Nutr.* 82: 309–317.
- Lonnqvist, F., Arner, P., Nordfors, L. & Schalling, M. (1995) Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. *Nat. Med.* 1: 950–953.
- Hamilton, B., Paglia, D. & Kwan, A.D.M. (1995) Increased obese mRNA expression in omental fat cells from massively obese humans. *Nat. Med.* 1: 953–956.
- Maffei, M., Halaas, J., Ravussin, E., Pratley, R. E., Lee, G. H., Zhang, Y., Fei, H., Kim, S., Lallone, R., Ranganathan, S., et al. (1995) Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat. Med.* 1: 1155–1161.
- Considine, R., Sinha, M., Heiman, M. L., Kriauciunas, A., Stephens, T. W., Nyce, M. R., Ohannesian, J. P., Marco, C. C., McKee, L. J., Bauer, T. L., et al. (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N. Engl. J. Med.* 334: 292–294.
- Rahman, S. M., Wang, Y., Yotsumoto, H., Cha, J., Han, S., Inoue, S. & Yanagita, T. (2001) Effects of conjugated linoleic acid on serum leptin concentration, body-fat accumulation, and beta-oxidation of fatty acid in OLETF rats. *Nutrition* 17: 385–390.
- Tsuboyama-Kasaoka, N., Takahashi, M., Tanemura, K., Kim, H. J., Tange, T., Okuyama, H., Kasai, M., Ikemoto, S. & Ezaki, O. (2000) Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. *Diabetes* 49: 1534–1542.
- Faulconnier, Y., Arnal, M., Patureau Mirand, P., Chardigny, J. & Chilliard, Y. (2004) Isomers of conjugated linoleic acid decrease plasma lipids and stimulate adipose tissue lipogenesis without changing adipose weight in postprandial adult sedentary or trained Wistar rat. *J. Nutr. Biochem.* 15: 741–748.
- Nagao, K., Inoue, N., Wang, Y. M., Hirata, J., Shimada, Y., Nagao, T., Matsui, T. & Yanagita, T. (2003) The 10-*trans*,12-*cis* isomer of conjugated linoleic acid suppresses the development of hypertension in Otsuka Long-Evans Tokushima fatty rats. *Biochem. Biophys. Res. Commun.* 306: 134–138.
- Yamasaki, M., Ikeda, A., Oji, M., Tanaka, Y., Hirao, A., Kasai, M., Iwata, T. & Yamada, K. (2003) Modulation of body fat and serum leptin levels by dietary conjugated linoleic acid in Sprague-Dawley rats fed various fat-level diets. *Nutrition* 19: 30–35.
- Medina, E. A., Horn, W. F., Keim, N. L., Havel, P. J., Benito, P., Kelley, D. S., Nelson, G. J. & Erickson, K. L. (2000) Conjugated linoleic acid supplementation in humans: effects on circulating leptin concentrations and appetite. *Lipids* 35: 783–788.
- Borg, P., Kukkonen-Harjula, K., Fogelholm, M. & Pasanen, M. (2002) Effects of walking or resistance training on weight loss maintenance in obese, middle-aged men: a randomized trial. *Int. J. Obes. Relat. Metab. Disord.* 26: 676–683.
- Cox, K., Burke, V., Morton, A., Beilin, L. & Pudley, I. (2003) The independent and combined effect of 16 weeks of vigorous exercise and energy restriction on body mass and composition in free-living overweight men—a randomized controlled trial. *Metabolism* 52: 107–115.
- Wyatt, H., Grunwald, G., Seagle, H. M., Klem, M. L., McGuire, M. T., Wing, R. R. & Hill, J. O. (1999) Resting energy expenditure in reduced-obese subjects in the National Weight Control Registry. *Am. J. Clin. Nutr.* 69: 1189–1193.