

Effects of *cis*-9,*trans*-11 conjugated linoleic acid supplementation on insulin sensitivity, lipid peroxidation, and proinflammatory markers in obese men^{1–3}

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ABSTRACT

Background: We recently showed that *trans*-10,*cis*-12 (*t*10,*c*12) conjugated linoleic acid (CLA) causes insulin resistance in obese men. However, metabolic effects of the *c*9,*t*11 CLA isomer are still unknown in obese men. Because *c*9,*t*11 CLA is the predominant CLA isomer in foods and is included in dietary weight-loss products, it is important to conduct randomized controlled studies that use *c*9,*t*11 CLA preparations.

Objective: We investigated the effects of *c*9,*t*11 CLA supplementation on insulin sensitivity, body composition, and lipid peroxidation in a group at high risk for cardiovascular disease.

Design: In a randomized, double-blind, placebo-controlled study, 25 abdominally obese men received 3 g *c*9,*t*11 CLA/d or placebo (olive oil). Before and after 3 mo of supplementation, we assessed insulin sensitivity (hyperinsulinemic euglycemic clamp), lipid metabolism, body composition, and urinary 8-*iso*-prostaglandin F_{2α} (a major F₂-isoprostane) and 15-*keto*-dihydro-prostaglandin F_{2α}, markers of in vivo oxidative stress and inflammation, respectively.

Results: All subjects completed the study. Compared with placebo, *c*9,*t*11 CLA decreased insulin sensitivity by 15% ($P < 0.05$) and increased 8-*iso*-prostaglandin F_{2α} and 15-*keto*-dihydro-prostaglandin F_{2α} excretion by 50% ($P < 0.01$) and 15% ($P < 0.05$), respectively. The decreased insulin sensitivity was independent of changes in serum lipids, glycemia, body mass index, and body fat but was abolished after adjustment for changes in 8-*iso*-prostaglandin F_{2α} concentrations. There were no differences between groups in body composition.

Conclusions: A CLA preparation containing the purified *c*9,*t*11 CLA isomer increased insulin resistance and lipid peroxidation compared with placebo in obese men. Because *c*9,*t*11 CLA occurs in commercial supplements as well as in the diet, the present results should be confirmed in larger studies that also include women. *Am J Clin Nutr* 2004;80:279–83.

KEY WORDS *trans* Fatty acids, fatty acids, conjugated linoleic acid, dairy fat, diet, insulin resistance, abdominal obesity, inflammation, oxidative stress, lipid peroxidation

INTRODUCTION

Conjugated linoleic acid (CLA) is a group of dietary fatty acid isomers that have received considerable attention because of their antiobesity actions and antidiabetic effects in certain animal models (1). CLA is naturally found in fat from dairy and beef

foods and in hydrogenated vegetable oils (2, 3). The main biologically active isomers are *cis*-9,*trans*-11 (*c*9,*t*11) CLA and *t*10,*c*12 CLA. *c*9,*t*11 CLA is the predominant isomer in the diet ($\approx 90\%$ of dietary CLA is *c*9,*t*11 CLA and $< 10\%$ is *t*10,*c*12 CLA) (2). In addition, CLA is found in weight-loss products sold over the counter as isomer mixtures, usually containing *c*9,*t*11 CLA and *t*10,*c*12 CLA in equal proportions. Furthermore, *c*9,*t*11 CLA seems to be endogenously formed in humans from *trans* vaccenic acid (4) found in dairy fat.

Metabolic effects of CLA in animals are divergent, which is partly explained by isomer-specific properties of CLA as well as differences in phenotypes studied (1). There is little information available about isomer-specific effects on glucose and lipid metabolism in humans because CLA mixtures were used in most studies (5–8). Our recent studies showed that *t*10,*c*12 CLA caused marked insulin resistance, oxidative stress, and dyslipidemia in obese men (9, 10). Because *c*9,*t*11 CLA is the major dietary CLA isomer (2), it is relevant to investigate the metabolic effects of *c*9,*t*11 CLA in obese men. Because of the prooxidative (10–12) and HDL-lowering effects (9) of CLA mixtures, a study that uses purified *c*9,*t*11 CLA is needed to gain more information about this particular CLA isomer in humans.

In the present study, we hypothesized that *c*9,*t*11 CLA could improve glucose metabolism and serum lipid profile on the basis of recent data. First, results in obese mice suggest that *c*9,*t*11 CLA, in contrast to *t*10,*c*12 CLA, reduced serum lipids without impairing insulin action (13). Second, in a recent 8-wk controlled study in healthy normolipidemic subjects there was a significant decrease in plasma VLDL cholesterol after *c*9,*t*11 CLA supplementation (14). To date, that study provides the only data on the effect of purified *c*9,*t*11 CLA in humans. Because that study included only lean healthy subjects, it would be interesting to investigate the effects of *c*9,*t*11 CLA on lipid and glucose metabolism in a high-risk group of abdominally obese men. Thus, in

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a randomized, double-blind, controlled trial, we investigated the effects of *c9,t11* CLA on insulin sensitivity and lipid peroxidation in vivo by using direct methods for determining these 2 variables, euglycemic hyperinsulinemic clamp (15) and F_2 -isoprostane production (16, 17), respectively.

SUBJECTS AND METHODS

Abdominally obese white men (35–65 y old) were recruited through local advertisement in Uppsala, Sweden. At screening, inclusion criteria were waist girth > 102 cm, body mass index (BMI; in kg/m^2) = 27–35, and triacylglycerol concentration > 1.7 mmol/L or HDL-cholesterol concentration < 1.1 mmol/L. Subjects taking antidiabetic or lipid-lowering drugs, nonsteroidal antiinflammatory drugs, or antioxidant supplements or with previously diagnosed diabetes or heart, liver, or renal disease were excluded. All study participants provided informed consent. The protocol was approved by the Ethics Committee of Uppsala University.

Protocol and study design

A randomized, placebo-controlled, double-blind study design with 2 parallel groups was used. Twenty-five men were randomly assigned to 1 of 2 groups receiving 3 g/d (6 capsules/d; $\approx 1\%$ of total energy intake) as *c9,t11* CLA (83% triacylglycerol) or placebo (olive oil; 83% triacylglycerol). The isomer composition of the CLA preparation was assessed with the use of high-resolution gas chromatography of fatty acid ethyl esters (sodium ethylate method) as described in detail by Saebo (18). The isomer content of the CLA preparation was 83.3% *c9,t11* CLA; 7.3% *t10,c12* CLA; 5.5% *c9-18:1*; 0.46% *c9,c11* CLA; 0.2% *c10,c12* CLA; and 1.4% *t9,t11+t10,t12* CLA, which provided 2.5 g *c9,t11* CLA/d and ≈ 0.2 g *t10,c12* CLA/d. The major fatty acid content of placebo was 82.7% *c9-18:1*; 5.6% *c9-18:2,c12*; 3.6% 16:0; and 3.3% 18:0. The preparations did not contain any added antioxidants. Capsules were kept cold and in the dark in sealed boxes before the study. During the study, all participants were instructed to keep the boxes in the refrigerator to avoid any possible oxidation. All preparations (identical in appearance) were prepared by Natural Lipids Ltd (Hovebygda, Norway), which also generated randomization numbers and blinding.

Primary outcome measures were insulin sensitivity, serum lipoproteins, and free radical and cyclooxygenase-dependent lipid peroxidation in vivo (urinary 8-*iso*-prostaglandin $F_{2\alpha}$ and 15-*keto*-dihydro-prostaglandin $F_{2\alpha}$, respectively). Secondary outcome measures were anthropometric variables. All variables were determined after 12-h fast, before and after 3-mo supplementation. Subjects were asked to restrain from smoking, physical activity, and alcohol within 48 h preceding clinical investigations. Subjects were encouraged to maintain their usual diet and physical activity habits during the study.

Nonenzymatic lipid peroxidation

Concentrations of free 8-*iso*-prostaglandin $F_{2\alpha}$ (a major F_2 -isoprostane) were analyzed in morning urine without any extraction, with the use of a highly specific and sensitive radioimmunoassay as previously described (19). 8-*iso*-Prostaglandin $F_{2\alpha}$ concentrations were adjusted for creatinine values measured with a commercial kit (IL Test; Monarch Instrument, Amherst, NH).

Enzymatic lipid peroxidation

Urinary samples were analyzed for 15-*keto*-dihydro-prostaglandin $F_{2\alpha}$, a major metabolite of prostaglandin $F_{2\alpha}$ and

proinflammatory marker, without any extraction, by using a radioimmunoassay as described previously (20).

Body composition

Sagittal abdominal diameter was measured at the L_{4-5} level in the supine position, and waist girth was measured as previously described in detail (7). Anthropometric variables were measured by a single investigator. Bioelectrical impedance analysis was measured with the use of a multifrequency analyzer (Xitron Technologies Inc, San Diego). From the estimation of body water, body fat content was calculated, with the assumption that fat-free mass contains 73.2% water (21). Lean body mass was calculated with the formula provided by the manufacturer.

Hyperinsulinemic clamp

Hyperinsulinemic euglycemic clamp was used to determine insulin sensitivity in vivo according to DeFronzo et al (15), slightly modified as previously described (9). Blood glucose was assayed with the use of a HemoCue Analyzer (HemoCue AB, Ängelholm, Sweden) and an enzymatic method. The glucose infusion rate (M ; $\text{mg} \cdot \text{kg body wt}^{-1} \cdot \text{min}^{-1}$) was calculated during the last hour of the clamp (15). Insulin sensitivity (M/I) was calculated by dividing M by the mean plasma insulin concentration (in mU/L) during the last 60 min of the clamp multiplied by 100 (I) (15).

Biochemical analyses

Venous blood was drawn into evacuated tubes, coagulated, centrifuged ($2500 \times g$, 10 min, room temperature), and then frozen at -20°C . Serum samples were stored at -70°C . Serum insulin was measured with the use of an enzyme-linked immunosorbent assay kit (Mercodia AB, Uppsala, Sweden) in a Bio-Rad Coda automated electroimmunoassay analyzer (Bio-Rad Laboratories AB, Hercules, CA). Lipoproteins were isolated from fresh serum by a combination of preparative ultracentrifugation (22) and precipitation with a sodium phosphotungstate and magnesium chloride solution (23). Serum lipoproteins were assayed by using enzymatic techniques with a Monarch 2000 centrifugal analyzer (Instrumentation Laboratories, Lexington, MA).

Statistics

Values are means \pm SDs. Shapiro-Wilk W tests were used to test distributions. Variables with skewed distributions [all measures except cholesterol and free fatty acid (FFA)] were logarithmically transformed. All measures were normally distributed after logarithmic transformation. The mean changes within groups from baseline to 12 wk were assessed with the use of the paired t test. Analysis of covariance (ANCOVA) was used to compare changes between groups when baseline values and change (Δ) from baseline values for each outcome measure were taken into account. It was calculated that 13 subjects per group would be needed to detect a mean difference between groups in insulin sensitivity index (M/I) of 1 unit with a power of 0.80 at a significance level of 0.05 with the use of the unpaired t test.

Pearson's correlation coefficient was determined from pairwise correlations. Partial correlation analysis for Δ values was assessed with the use of both baseline values and Δ values as covariates. A two-tailed P value < 0.05 was regarded as significant. For statistical analyses, the JMP software package (version 3.2) was used (SAS Institute Inc, Cary, NC).

TABLE 1Anthropometric and metabolic variables at baseline and changes over 12 wk of supplementation with placebo or *cis-9,trans-11* (c9,t11) CLA¹

	Baseline		Change from baseline	
	Placebo	c9,t11 CLA	Placebo	c9,t11 CLA
Weight (kg)	100.2 ± 10.2	98.2 ± 12.8	0.53 ± 1.22	1.40 ± 1.34 ²
BMI (kg/m ²)	30.4 ± 2.5	30.6 ± 2.0	0.15 ± 0.36	0.42 ± 0.41 ²
Waist girth (cm)	114.2 ± 6.3	112.2 ± 7.1	0.49 ± 2.50	-0.06 ± 2.0
Sagittal diameter (cm)	28.3 ± 2.5	27.8 ± 1.9	-0.46 ± 1.24	0.18 ± 0.66
Total percentage body fat (%)	37.5 ± 2.1	35.7 ± 4.5	-0.87 ± 2.63	0.62 ± 1.86
Lean body mass (kg)	61.0 ± 6.3	61.6 ± 7.4	1.22 ± 2.85	0.14 ± 1.70
Serum cholesterol (mmol/L)	5.6 ± 0.9	5.2 ± 0.7	-0.17 ± 0.40	0.08 ± 0.50
LDL cholesterol (mmol/L)	3.8 ± 0.7	3.4 ± 0.8	-0.12 ± 0.35	-0.07 ± 0.45
Serum triacylglycerols (mmol/L)	1.7 ± 0.9	1.7 ± 1.4	0.24 ± 1.0	0.32 ± 0.83
VLDL cholesterol (mmol/L)	0.6 ± 0.4	0.7 ± 1.0	0.02 ± 0.38	0.14 ± 0.28
HDL cholesterol (mmol/L)	1.1 ± 0.2	1.1 ± 0.3	-0.05 ± 0.20	-0.04 ± 0.10
Free fatty acids (mmol/L)	0.59 ± 0.17	0.66 ± 0.21	0.04 ± 0.13	-0.001 ± 0.24
Blood glucose (mmol/L)	4.9 ± 0.4	5.3 ± 0.7	-0.19 ± 0.50	-0.05 ± 0.55
Insulin sensitivity index (M/I)	4.2 ± 2.2	4.3 ± 2.5	0.13 ± 1.48	-0.62 ± 0.72 ³
Serum insulin (mU/L)	10.7 ± 4.4	12.1 ± 7.9	-0.70 ± 4.65	-0.12 ± 9.12
8-iso-PGF _{2α} (nmol/mmol creatinine)	0.23 ± 0.04	0.20 ± 0.05	0.004 ± 0.07	0.10 ± 0.08 ^{4,5}
15-keto-dihydro-PGF _{2α} (nmol/mmol creatinine)	0.25 ± 0.04	0.28 ± 0.12	0.0008 ± 0.05	0.04 ± 0.05 ^{2,3}

¹ All values are $\bar{x} \pm SD$. $n = 12$ in the placebo group and 13 in the c9,t11 CLA group. M/I, glucose infusion rate (M) divided by mean plasma insulin concentration (in mU/L) during the last 60 min of the clamp multiplied by 100 (I); PG, prostaglandin. ANCOVA was used for all tests, and changes were adjusted for age and baseline values for each variable. Change in insulin sensitivity (ANCOVA) was also adjusted for changes in BMI, percentage body fat, and serum free fatty acid concentration.

² $P < 0.05$ (paired t test).

^{3,4} Significantly different from placebo (ANCOVA): ³ $P < 0.05$, ⁴ $P < 0.01$.

⁵ $P < 0.01$ (paired t test).

RESULTS

There were no significant differences between groups at baseline (Table 1). The age of the subjects was 56 ± 6.0 y in the placebo group and 54 ± 5.5 y in the c9,t11 CLA group. All 25 men completed the study. Complete data from the euglycemic clamp test were available from 23 men: 1 subject in each treatment group did not complete the clamp test at follow-up. All other metabolic measures were obtained in these 2 subjects at baseline and at follow-up. Compliance (capsule count) was 91% ($n = 25$) with no differences between groups. No side effects were reported, and no adverse effects on liver enzymes occurred (data not shown).

Lipid metabolism

Compared with placebo, c9,t11 CLA had no significant effects on lipoprotein, triacylglycerol, or FFA concentrations (Table 1).

Glucose metabolism

Insulin sensitivity (M/I) was significantly lower after c9,t11 CLA than after placebo (-15% ; $P < 0.05$; Table 1). This effect remained significant after control for baseline values of insulin sensitivity (ANCOVA). The mean insulin-mediated glucose uptake (M) was also lower after c9,t11 CLA than after placebo ($P < 0.05$; data not shown). No significant differences were found between the groups in fasting blood glucose or serum insulin concentrations. The significant impairment of insulin sensitivity was abolished when $\Delta 8$ -iso-prostaglandin $F_{2\alpha}$ and $\Delta 15$ -keto-dihydro-prostaglandin $F_{2\alpha}$ were adjusted for (ANCOVA) but remained significant after the addition of age, Δ BMI, Δ body fat, Δ waist girth, Δ FFA, Δ triacylglycerols, or Δ blood glucose as covariates.

Lipid peroxidation and inflammation

Concentrations of 8-iso-prostaglandin $F_{2\alpha}$ and 15-keto-dihydro-prostaglandin $F_{2\alpha}$ were higher [50% ($P < 0.01$) and 15% ($P < 0.05$), respectively] after c9,t11 CLA than after placebo. These measures remained unchanged in the placebo group (Table 1). The significant increase in 8-iso-prostaglandin $F_{2\alpha}$ remained significant after adjustment for all other variables, including Δ -insulin sensitivity (ANCOVA).

Body composition

No significant differences were found between the groups in weight, BMI, total body fat, lean body mass, sagittal diameter, or waist girth after adjustments (ANCOVA) for baseline values (Table 1). Within the c9,t11 CLA group (paired t test), there was a significant increase in body weight and BMI from baseline to 12 wk (Table 1).

Correlation analysis (whole-group analysis)

The changes in insulin sensitivity were inversely correlated with $\Delta 8$ -iso-prostaglandin $F_{2\alpha}$ ($r = -0.43$, $P < 0.05$, $n = 23$), Δ VLDL cholesterol ($r = -0.63$, $P < 0.01$), and Δ total body fat ($r = -0.46$, $P < 0.05$). The changes in 8-iso-prostaglandin $F_{2\alpha}$ concentrations were not correlated with changes in other measures, except for Δ insulin sensitivity (r and P values presented above) and $\Delta 15$ -keto-dihydro-prostaglandin $F_{2\alpha}$ ($r = 0.40$, $P < 0.05$).

DISCUSSION

To our knowledge, this is the first randomized, placebo-controlled study that investigated the effects of c9,t11 CLA

supplementation in obese men. *c9,t11* CLA is the predominant CLA isomer in foods and is present in significant amounts in commercial supplements. In contrast to our hypothesis, *c9,t11* CLA supplementation decreased insulin sensitivity and increased lipid peroxidation compared with placebo. Interestingly, after adjustment for change in lipid peroxidation, the difference between the groups in insulin sensitivity did not remain significant. This finding is in agreement with our previous study (10), which suggests that CLA-induced lipid peroxidation might mediate insulin resistance. In the present study, there was also a significant inverse correlation between insulin sensitivity and lipid peroxidation. A potential mechanism mediating such a relation could include impaired cellular insulin signaling caused by increased free-radical generation (24, 25). We previously suggested that CLA-induced lipid peroxidation was mainly caused by the *t10,c12* CLA isomer (10), but the present study indicates that *c9,t11* CLA also could increase lipid peroxidation. Furthermore, lipid peroxidation increases after supplementation with CLA mixtures (11, 12). Thus, CLA seems to be a unique group of prooxidative fatty acids. Whether CLA-induced lipid peroxidation is proatherogenic in humans is unknown but should be further investigated in experimental studies because CLA supplements are used among obese subjects at high cardiovascular risk.

Interestingly, there was a significant difference between groups in insulin sensitivity but not in serum lipids or glucose concentrations. In contrast, *t10,c12* CLA was previously shown to aggravate both dyslipidemia and glycemia (9). This finding is accordant with isomer-specific effects in mice and hamsters, whereby metabolic disorders occurred after *t10,c12* CLA but not after *c9,t11* CLA (13, 26, 27).

The cyclooxygenase-mediated lipid peroxidation (15-*keto*-dihydro-prostaglandin $F_{2\alpha}$), which reflects a proinflammatory response (20), also increased after *c9,t11* CLA. The changes were related to free radical-mediated lipid peroxidation (8-*iso*-prostaglandin $F_{2\alpha}$).

On the basis of previous data (10), we assume that the very small *t10,c12* CLA content (≈ 200 mg) in the present *c9,t11* CLA preparation does not explain the increased lipid peroxidation, although a small effect of *t10,c12* CLA cannot be excluded. Our assumption is supported by evidence that shows increased lipid peroxidation (8-*iso*-prostaglandin $F_{2\alpha}$) after dietary supplementation with *trans* vaccenic acid, a precursor of *c9,t11* CLA in the human body (4). Furthermore, it is not likely that the small amount of *t10,c12* CLA in the *c9,t11* CLA preparation solely mediated the current lowering of insulin sensitivity, considering that CLA mixtures containing ≈ 1 g *t10,c12* CLA do not significantly alter insulin sensitivity (9). However, a possible antagonistic effect of *t10,c12* CLA and *c9,t11* CLA present in CLA mixtures would complicate interpretations because effects of a single isomer might be different from those of the 2 isomers combined. In addition, when comparing the amount of *t10,c12* CLA in the *t10,c12* CLA concentrate and CLA isomer mixture, respectively, the previous study (10) indicated a possible dose-response effect of *t10,c12* CLA on lipid peroxidation, which was not apparent for insulin sensitivity (9). Thus, before we can conclude that *c9,t11* CLA alone increases lipid peroxidation, future studies that use entirely pure *c9,t11* CLA preparations are needed to clarify the isomer-specific effects of CLA.

No significant effect of *c9,t11* CLA on VLDL-cholesterol concentrations was observed, which stands in contrast to the

results of a recent controlled study in lean and healthy subjects that reported decreased VLDL cholesterol after *c9,t11* CLA (14). In that study, a slightly less purified *c9,t11* CLA preparation (3 g/d; 80:20 CLA isomer blend of *c9,t11* and *t10,c12*) was used. However, in agreement with the results by Noone et al (14), there were no effects on BMI or on lipid, glucose, or insulin concentrations compared with placebo. In contrast with our data, BMI did not increase within the *c9,t11* CLA group in the study by Noone et al (14). Because our study included obese men and the duration of the intervention period and CLA preparations differed slightly, caution should be taken when comparing these 2 studies. Unfortunately, insulin sensitivity and lipid peroxidation were not assessed by Noone et al (14).

Our results suggest that *c9,t11* CLA has no antiobesity effects and are in accord with evidence in mice (28), which suggests that *t10,c12* CLA is the antiadipogenic isomer (29). It is unlikely that insulin resistance was mediated by the modest increase in mean body weight (30) that occurred in both groups, because change in BMI and body fat was adjusted for. Nor did adjustments for insulin sensitivity and age at baseline or changes in body composition affect the significant effect of *c9,t11* CLA on insulin sensitivity when compared with placebo.

A limitation of this study could be the restricted sample size. The power calculation suggested that the present sample size was large enough to detect significant differences between groups in insulin sensitivity. However, before firm conclusions can be drawn about the effect of *c9,t11* CLA on body composition and metabolic variables, the present results should be confirmed by conducting large studies that include both obese men and women.

Another limitation was that we did not assess dietary intake or physical activity during the study. Thus, the possibility that changes in dietary habits affected insulin sensitivity cannot be completely excluded, although the randomized design should have clearly lowered the risk of such bias, and all subjects were instructed to maintain their usual lifestyle habits. From a nutritional viewpoint, the CLA preparation used in the present study (83% *c9,t11* CLA, 7% *t10,c12* CLA) mirrors dietary CLA isomer distribution quite well (2). However, note that the absolute dose of *c9,t11* CLA was ≥ 8 times as high as the average dietary CLA intake in a similar population (7), which indicates that it is unlikely that possible variations in dietary CLA intake (ie, from high amounts of dairy fat) significantly influenced the present results.

The clinical relevance of the present results is uncertain, but the 15% reduction in insulin sensitivity, which corresponds to ≈ 5 –10 kg of weight gain as estimated from dose-response clamp studies (26), and the 50% increase in lipid peroxidation are pronounced effects that might be proatherogenic in obese subjects having high, long-term CLA intakes from dairy foods (2), hydrogenated vegetable oils (3), or dietary supplements. However, with regard to the metabolic syndrome, epidemiologic data suggest no hazardous effects of consuming dairy fat. If anything, it seems as if a high intake of dairy products can protect against developing the metabolic syndrome (31, 32). The present study suggests that such associations could not be explained by *c9,t11* CLA present in dairy fat.

These results could have important implications for human nutrition and the food industry. At the present time, attempts in several countries are being made to enrich the amount of *c9,t11* CLA in foods by altering cattle feeding (33). Future epidemiologic studies

together with large long-term trials could help answer the important question of whether relatively small, but apparently potent, amounts of dietary CLA are safe with regard to cardiovascular disease risk.



Each author contributed intellectually or practically to this research. UM recruited the subjects, conducted the intervention study at the clinic, analyzed the data, and wrote the study together with BV. BV was the principal investigator who initiated the study and provided supervision. SB was responsible for sample collection of biochemical markers of lipid peroxidation and provided supervision. JÄ was responsible for statistical issues and provided advice. None of the authors had a financial or personal interest in any company or organization sponsoring this study.

REFERENCES

1. Belury MA. Dietary Conjugated linoleic acid and health: physiological effects and mechanisms of action. *Annu Rev Nutr* 2002;22:505–31.
2. Chin S, Liu W, Storkson J, Ha Y, Pariza M. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J Food Comp Anal* 1992;5:185–97.
3. Jung MY, Ha YL. Conjugated linoleic acid isomers in partially hydrogenated soybean oil obtained during nonselective and selective hydrogenation processes. *J Agric Food Chem* 1999;47:704–8.
4. Turpeinen AM, Mutanen M, Aro A, et al. Bioconversion of vaccenic acid to conjugated linoleic acid in humans. *Am J Clin Nutr* 2002;76:504–10.
5. Medina EA, Horn WF, Keim NL, et al. Conjugated linoleic acid supplementation in humans: effects on circulating leptin concentrations and appetite. *Lipids* 2000;35:783–8.
6. Blankson H, Stakkestad JA, Fagertun H, Thom E, Wadstein J, Gudmundsen O. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. *J Nutr* 2000;130:2943–8.
7. Riserus U, Berglund L, Vessby B. Conjugated linoleic acid (CLA) reduced abdominal adipose tissue in obese middle-aged men with signs of the metabolic syndrome: a randomised controlled trial. *Int J Obes Relat Metab Disord* 2001;25:1129–35.
8. Smedman A, Vessby B. Conjugated linoleic acid supplementation in humans—metabolic effects. *Lipids* 2001;36:773–81.
9. Riserus U, Arner P, Brismar K, Vessby B. Treatment with dietary *trans*-10/*cis*-12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. *Diabetes Care* 2002;25:1516–21.
10. Riserus U, Basu S, Jovinge S, Fredrikson GN, Arnlov J, Vessby B. Supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein: a potential link to fatty acid-induced insulin resistance. *Circulation* 2002;106:1925–9.
11. Basu S, Smedman A, Vessby B. Conjugated linoleic acid induces lipid peroxidation in humans. *FEBS Lett* 2000;468:33–6.
12. Basu S, Riserus U, Turpeinen A, Vessby B. Conjugated linoleic acid induces lipid peroxidation in men with abdominal obesity. *Clin Sci* 2000;99:511–6.
13. Roche HM, Noone E, Sewter C, et al. Isomer-dependent metabolic effects of conjugated linoleic acid: insights from molecular markers sterol regulatory element-binding protein-1c and LXRalpha. *Diabetes* 2002;51:2037–44.
14. Noone EJ, Roche HM, Nugent AP, Gibney MJ. The effect of dietary supplementation using isomeric blends of conjugated linoleic acid on lipid metabolism in healthy human subjects. *Br J Nutr* 2002;88:243–51.
15. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979;237:E214–23.
16. Patrono C, FitzGerald GA. Isoprostanes: potential markers of oxidant stress in atherothrombotic disease. *Arterioscler Thromb Vasc Biol* 1997;17:2309–15.
17. Roberts LJ, Morrow JD. Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. *Free Radic Biol Med* 2000;28:505–13.
18. Saebo A. Commercial synthesis of conjugated linoleate. In: Sebedio J-L, Christie WW, Adlof RO, eds. *Advances in conjugated linoleic acid research*. Vol 2. Champaign, IL: AOCS Press, 2003:344:71–81.
19. Basu S. Radioimmunoassay of 8-iso-prostaglandin F2alpha: an index for oxidative injury via free radical catalysed lipid peroxidation. *Prostaglandins Leukot Essent Fatty Acids* 1998;58:319–25.
20. Basu S. Radioimmunoassay of 15-keto-13,14-dihydro-prostaglandin F2alpha: an index for inflammation via cyclooxygenase catalysed lipid peroxidation. *Prostaglandins Leukot Essent Fatty Acids* 1998;58:347–52.
21. Pace N, Rathbun E. Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. *J Biol Chem* 1945;158:685–91.
22. Havel R, Eder H, Bragdon J. The determination and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* 1955;34:1345–53.
23. Siegler L, Wu W. Separation of serum high-density lipoprotein for cholesterol determination: ultracentrifugation vs precipitation with sodium phosphotungstate and magnesium chloride. *Clin Chem* 1981;27:838–41.
24. Tirosh A, Potashnik R, Bashan N, Rudich A. Oxidative stress disrupts insulin-induced cellular redistribution of insulin receptor substrate-1 and phosphatidylinositol 3-kinase in 3T3-L1 adipocytes. A putative cellular mechanism for impaired protein kinase B activation and GLUT4 translocation. *J Biol Chem* 1999;274:10595–602.
25. Rudich A, Kozlovsky N, Potashnik R, Bashan N. Oxidant stress reduces insulin responsiveness in 3T3-L1 adipocytes. *Am J Physiol* 1997;272:E935–40.
26. Clement L, Poirier H, Niot I, et al. Dietary *trans*-10, *cis*-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse. *J Lipid Res* 2002;43:1400–9.
27. Bouthegourd JC, Even PC, Grippo D, et al. A CLA mixture prevents body triglyceride accumulation without affecting energy expenditure in Syrian hamsters. *J Nutr* 2002;132:2682–9.
28. Pariza MW, Park Y, Cook ME. The biologically active isomers of conjugated linoleic acid. *Prog Lipid Res* 2001;40:283–98.
29. Park Y, Storkson JM, Albright KJ, Liu W, Pariza MW. Evidence that the *trans*-10, *cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids* 1999;34:235–41.
30. Bonadonna RC, Groop L, Kraemer N, Ferrannini E, Del Prato S, DeFronzo RA. Obesity and insulin resistance in humans: a dose-response study. *Metabolism* 1990;39:452–9.
31. Pereira MA, Jacobs DR Jr, Van Horn L, Slattery ML, Kartashov AI, Ludwig DS. Dairy consumption, obesity, and the insulin resistance syndrome in young adults: the CARDIA Study. *JAMA* 2002;287:2081–9.
32. Wirfalt E, Hedblad B, Gullberg B, et al. Food patterns and components of the metabolic syndrome in men and women: a cross-sectional study within the Malmo Diet and Cancer cohort. *Am J Epidemiol* 2001;154:1150–9.
33. Kelly ML, Berry JR, Dwyer DA, et al. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. *J Nutr* 1998;128:881–5.