

Conjugated linoleic acid supplementation for 1 y reduces body fat mass in healthy overweight humans¹⁻³

Jean-Michel Gaullier, Johan Halse, Kjetil Høyve, Knut Kristiansen, Hans Fagertun, Høgne Vik, and Ola Gudmundsen

ABSTRACT

Background: Short-term trials showed that conjugated linoleic acid (CLA) may reduce body fat mass (BFM) and increase lean body mass (LBM), but the long-term effect of CLA was not examined.

Objective: The objective of the study was to ascertain the 1-y effect of CLA on body composition and safety in healthy overweight adults consuming an ad libitum diet.

Design: Male and female volunteers ($n = 180$) with body mass indexes (in kg/m^2) of 25–30 were included in a double-blind, placebo-controlled study. Subjects were randomly assigned to 3 groups: CLA-free fatty acid (FFA), CLA-triacylglycerol, or placebo (olive oil). Change in BFM, as measured by dual-energy X-ray absorptiometry, was the primary outcome. Secondary outcomes included the effects of CLA on LBM, adverse events, and safety variables.

Results: Mean (\pm SD) BFM in the CLA-triacylglycerol and CLA-FFA groups was $8.7 \pm 9.1\%$ and $6.9 \pm 9.1\%$, respectively, lower than that in the placebo group ($P < 0.001$). Subjects receiving CLA-FFA had $1.8 \pm 4.3\%$ greater LBM than did subjects receiving placebo ($P = 0.002$). These changes were not associated with diet or exercise. LDL increased in the CLA-FFA group ($P = 0.008$), HDL decreased in the CLA-triacylglycerol group ($P = 0.003$), and lipoprotein(a) increased in both CLA groups ($P < 0.001$) compared with month 0. Fasting blood glucose concentrations remained unchanged in all 3 groups. Glycated hemoglobin rose in all groups from month 0 concentrations, but there was no significant difference between groups. Adverse events did not differ significantly between groups.

Conclusion: Long-term supplementation with CLA-FFA or CLA-triacylglycerol reduces BFM in healthy overweight adults. *Am J Clin Nutr* 2004;79:1118–25.

KEY WORDS Conjugated linoleic acid, body fat mass, lean body mass, weight, body mass index, dual-energy X-ray absorptiometry, overweight, humans

INTRODUCTION

Conjugated linoleic acid (CLA) is a mixture of linoleic acid isomers with conjugated double bonds. CLA was first identified when extracts from fried beef were found to be anticarcinogenic (1). This effect was confirmed in animal and in vitro models of carcinogenesis (2–7). Later studies in animals showed other beneficial health roles for CLA, including protection against atherosclerosis (8, 9), immune stimulation (10, 11), and the normalization of impaired glucose tolerance and improvement of

hyperinsulinemia in ZDF rats (12). Numerous studies in mice, rats, hamsters, rabbits, and pigs showed that CLA supplementation causes changes in body composition, such as a reduction in body fat mass (BFM) and an increase in lean body mass (LBM; 13–23).

In humans, only short-term clinical studies with small numbers of subjects have been conducted with CLA (24). Some CLA studies performed with a mixture of the bioactive isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12, showed reductions in BFM and in some cases increases in LBM (25–27). Other short-term studies performed with the use of different methods and technology, such as body-composition measurements, daily dosage, CLA composition, and study design, did not show any effects on body composition (28–32), which raises questions about the consistency of the effects of CLA on BFM and LBM in humans. After correction for differences in metabolic rate, similar effects are observed in humans and in mice, which suggests that the mechanisms for reducing BFM in animals and humans may be similar (33).

Previous short-term studies concluded that CLA supplementation was safe. The only adverse events (AEs) reported in these studies were gastrointestinal complaints (25, 27). Two published clinical studies showed that CLA may induce lipid peroxidation (34, 35). Riserus et al (32, 36) showed that a preparation with high concentrations of the *trans*-10, *cis*-12 CLA isomer causes increases in F_2 -isoprostane excretion and in insulin resistance in men with the metabolic syndrome. Men with the metabolic syndrome receiving a mixture of the 2 isomers (*cis*-9, *trans*-11 and *trans*-10, *cis*-12) had greater F_2 -isoprostane excretion than did those in the placebo group, but the CLA mixture had no effect on insulin resistance (32, 36).

The present study was designed primarily to investigate the long-term effects of CLA (as a 50:50 mixture of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers) on BFM and LBM in a randomized, double-blind, placebo-controlled study. Because CLA is mar-

¹ From the Scandinavian Clinical Research AS (JMG, KK, and OG) and the Scandinavian Statistical Services AS (HF), Kjeller, Norway; the Betanien Medical Center, Oslo (JH); the Helsetorget Medical Center, Elverum, Norway (KH); and the Matforsk (Norwegian Food Research Institute), Ås, Norway (HV).

² Supported by Natural LTD and Cognis Nutrition and Health.

³ Address reprint requests to J-M Gaullier, Scandinavian Clinical Research AS, Gåsevikeveien 8, PO Box 135, 2027 Kjeller, Norway. E-mail: j-m@scr.no.

Received June 16, 2003.

Accepted for publication December 4, 2003.

TABLE 1

Capsule composition of the free fatty acid (FFA) and triacylglycerol forms of conjugated linoleic acid (CLA)¹

| Ingredient | CLA-FFA | CLA-triacylglycerol |
|---|---------|---------------------|
| Fatty acid composition (g/100 g fatty acid) | | |
| 16:0 | 1.3 | 2.7 |
| 18:0 | 2.3 | 2.6 |
| 18:1 | 9.4 | 10.6 |
| 18:2 | 0.7 | 0.9 |
| Others | 2.3 | 3.3 |
| CLA isomers | | |
| Total CLA | 84 | 80 |
| <i>cis</i> -9, <i>trans</i> -11 | 39 | 38 |
| <i>trans</i> -10, <i>cis</i> -12 | 41 | 38 |

¹ Materials and analyses (gas chromatography columns) provided by Natural Lipids, Hovdebygda, Norway.

keted either as triacylglycerol or free fatty acids (FFA), we also wanted to ascertain whether either of the 2 forms of CLA is more efficacious and to evaluate the safety of both CLA forms in a study of longer duration.

SUBJECTS AND METHODS

Subjects

Healthy volunteer men and women ($n = 180$) aged 18–65 y and with a body mass index (BMI; in kg/m^2) of 25–30 were recruited by 2 research centers (Betanien Medical Center, Oslo, $n = 100$; Helsetorget Medical Center, Elverum, Norway, $n = 80$). All subjects gave written informed consent before inclusion in the study. Subjects could not be included in the study if they were receiving drug therapy, consuming a special diet, or taking dietary substitutes for weight loss; in addition, the female subjects were excluded if they were pregnant or lactating. Subjects with type 1 or type 2 diabetes according to American Diabetes Association criteria (37) were also excluded from the study. Subjects with renal, liver, pancreatic, or chronic inflammatory or infectious diseases; hypertension; cardiac failure; or malignant tumors were excluded. Subjects who had active thyroid disease or who were receiving thyroid hormone substitution, subjects taking adrenergic agonists, subjects with known or suspected drug or alcohol abuse or with any clinical condition rendering them unfit to participate, and as subjects who did not sign the informed-consent document were also excluded from participation. The study was approved by the Region I (East Norway) Ethics Committee and conducted in agreement with the Declaration of Helsinki of 1975 as revised in 1983 and in accordance with the International Conference on Harmonization guidelines.

Study design

This was a randomized, double-blind, placebo-controlled study stratified only by center. The subjects were randomly assigned to receive either 4.5 g olive oil (placebo, $n = 59$), 4.5 g 80% CLA-FFA (3.6 g active CLA isomers, $n = 61$), or 4.5 g 76% CLA-triacylglycerol (3.4 g active isomers, $n = 60$). The fatty acid composition of CLA-FFA and CLA-triacylglycerol is shown in **Table 1**. Each supplement was prepared from a single batch. Daily doses were taken as 6 opaque, soft gel capsules, all identical in taste and in appearance (Natural Lipids, Hovde-

bygda, Norway). The eligible subjects were randomly assigned to treatment with the use of a simple block randomization (12 subjects per block). Both centers followed the study's randomization procedure and did not break the code at any time of the study. The randomization list was kept confidential and was opened only after the closure of the database. Because the purpose of the study was to follow the effects of CLA on body composition in healthy overweight subjects consuming an ad libitum diet, no restrictions in lifestyle or in caloric intake were implemented. However, at the start of the study, the study nurse gave the subjects dietary advice of a general nature and exercise recommendations on request.

Clinical assessments

Characteristics (including smoking and drinking habits) and demographic data were recorded when subjects entered the study (at month 0). Weight, BMI, vital signs, and AEs were recorded every 3 mo, and serious AEs were monitored continuously throughout the study. Body composition was analyzed at months 0, 6, 9, and 12. Blood samples were obtained from fasting subjects between 0800 and 0900 and were analyzed in accredited laboratories (Først Laboratory and Aker University Hospital, Oslo) at 0, 3, and 12 mo. Analyses were performed in serum samples for the following variables: alanine aminotransferase, aspartate aminotransferase, hemoglobin, bilirubin, chloride, creatine phosphokinase, creatinine, erythrocytes, γ -glutamyltransferase, leukocytes, potassium, sodium, thyroid-stimulating hormone, thrombocytes, thyroxin, glycated hemoglobin (Hb A_{1c}), glucose, HDL and LDL cholesterol, total cholesterol, insulin-like growth factor 1, insulin, insulin C-peptide, leptin, lipoprotein(a) [Lp(a)], and triacylglycerols. The LDL concentration was calculated (38). Compliance was measured every 3 mo by a comparison of the number of unused capsules with the number of capsules that should have been used. A subject was considered compliant when he or she took $\geq 75\%$ of the supplement provided.

Diet and exercise

Diet and exercise were assessed at 0, 6, and 12 mo. Each participant was given detailed instruction on how to complete a questionnaire (a total of 418 questions). All returned questionnaires were reviewed by the medical staff and a clinical nutritionist. Each subject completed diet records for 14 consecutive days before the visit at the medical center, according to a previously evaluated and validated method (39). This method provides information on the quantity and types of food consumed. Completed questionnaires were returned by 81.7% of the subjects. Nonresponders were defined as subjects who failed to complete or did not return 1 of the 3 questionnaires on at least one occasion. The nonresponders were evenly distributed among all groups (placebo group: $n = 13$; CLA-FFA group: $n = 11$; and CLA-triacylglycerol group: $n = 9$). A specially designed software program, BEREGN (Oslo University, Norway), was used to convert the food intake to caloric intake. Exercise was assessed as the product of the number of 20-min training sessions per week and their intensity (high or low), according to a validated method (40).

Measurement of body composition and body weight

Dual-energy X-ray absorptiometry (DXA; Lunar Radiation Corp, Madison, WI) was used to determine body composition with LUNAR PRODIGY software (version 5.6; Lunar Radiation

Corp). At month 0, the Oslo center used the Lunar IQ absorptiometer, but, before the 6-mo visit, a change was made to the Lunar Prodigy model because of mechanical problems with the Lunar IQ model. Data from the Oslo center at month 0 were therefore adjusted by a factor of 4.5% by using a sample of placebo-treated subjects (5 F, 4 M; age <50 y) who had no weight change between 0 and 6 mo and by assuming no BFM change, as was observed in a matching group of placebo-treated subjects at the Elverum center.

Repeated measurements ($n > 20$) performed with the use of a Hologic whole-body phantom (WB-1406; Hologic Inc, Waltham, MA) at each medical center showed no significant difference between the centers. The subjects were weighed on digital scales (TBF-305; Tanita, Yiewsley, United Kingdom) in their underwear. No subtractions for clothes were performed.

Statistical analysis

Results are shown as means \pm SDs in the tables and as means and 95% CIs in the figure. The primary outcome variable was the change in BFM, as ascertained with the use of DXA. A test power of 80% was planned, on the basis of a relative difference in BFM reduction between each CLA group and placebo of $\geq 1 \times$ SD. Testing between the 3 treatment groups to investigate comparability at 0 mo was done by using analysis of variance (treatment and center as factors). Comparisons between treatment groups with regard to changes between month 0 and month 12 for DXA variables and weight were performed by using analysis of covariance (treatment, center, and sex as factors; month 0 value, total energy intake, exercise, and drug \times energy intake and drug \times training score interactions as covariates). The model was chosen to avoid potential regression-to-the-mean effects, and hence a nonsignificant higher BFM in the CLA-triacylglycerol group at 0 mo was adjusted for by using potential covariates. The variables were normally distributed, and no transformations were performed before analysis. Tukey's test was applied for pairwise comparisons of changes in all 3 groups between month 0 and month 12 (41). Because treatment groups interacted with effect over time, differences from month 0 to month 12 within treatment groups were tested by using a paired t test. Categorical variables were analyzed by using Fisher's exact test (42). According to Fisher's linear discriminant function (43), the median BFM decreased by $\geq 4.5\%$ from month 0 to month 12. A subject was thus categorized as a treatment responder on the basis of a BFM reduction $\geq 4.5\%$ and as a nonresponder on the basis of a BFM reduction of $< 4.5\%$. The intention-to-treat criterion was applied by extrapolating results from month 0 ($n = 180$), 3 ($n = 167$), 6 ($n = 159$), or 9 ($n = 158$) to month 12 ($n = 157$) for the efficacy variables (DXA measurements and weight) relating to the 180 subjects who were randomly assigned. DXA measurements were performed at months 0, 6, 9, and 12, and the last value carried forward was therefore applied to missing DXA data from months 6–12. A significance level of 5% was used in tests, and all tests were two-tailed.

RESULTS

Study subjects

Of the original 180 subjects, 157 (87.2%) completed the study. Ten subjects withdrew from the study because of AEs and 1 did so because of pregnancy, and the remaining subjects withdrew

TABLE 2

Characteristics of the study population at month 0¹

| | Placebo | CLA-FFA | CLA-triacylglycerol | <i>P</i> |
|------------------------------|---------------------------|-----------------|---------------------|----------|
| Sex | | | | 0.72 |
| Male (<i>n</i>) | 12 | 10 | 9 | |
| Female (<i>n</i>) | 47 | 51 | 51 | |
| Age (y) | 45 \pm 9.5 ² | 44.5 \pm 10.7 | 48.0 \pm 10.7 | 0.35 |
| Alcohol use (%) ³ | 71 | 69 | 61 | 0.69 |
| Tobacco use (%) ³ | 20 | 32 | 17 | 0.23 |
| Exercise (%) ⁴ | 52 | 51 | 50 | 0.77 |

¹ CLA, conjugated linoleic acid; FFA, free fatty acid.

² $\bar{x} \pm$ SD (all such values); recorded within 2 wk of subject's inclusion in the study.

³ The percentage of subjects who answered these questions positively.

⁴ The percentage of subjects training ≥ 1 time/wk with sweating.

for reasons other than AEs. Compliance was 88.3% in the placebo group, 88.1% in the CLA-FFA group, and 90.8% in the CLA-triacylglycerol group. Withdrawal rates were also similar in all groups (placebo, $n = 9$; CLA-FFA, $n = 9$; CLA-triacylglycerol, $n = 5$). There were no differences in age, alcohol use, tobacco use, or exercise between the groups at month 0 (Table 2), nor were there differences between the groups in medical history.

Effects of CLA on weight and BMI

There were no differences between the groups for either weight or BMI at month 0 (Table 3). Compared with month 0, body weight and BMI decreased significantly in both CLA groups during 12 mo of supplementation (CLA-FFA: $P = 0.02$; CLA-triacylglycerol: $P < 0.001$), whereas there was no change in the placebo group ($P = 0.59$). The reductions in weight and BMI in the CLA-triacylglycerol group were significantly different from those in the placebo group ($P < 0.05$), but weight and BMI reductions in the CLA-FFA group did not differ significantly from those in the placebo group ($P \geq 0.05$). The effects of CLA-triacylglycerol on weight and BMI did not differ significantly from the effects of CLA-FFA ($P \geq 0.05$; data not shown).

Effects of CLA on body composition

BFM and LBM did not differ between the groups at month 0 (Table 3). After 12 mo, BFM was significantly ($P < 0.05$) lower in both groups of CLA-supplemented subjects than in placebo-supplemented subjects (Table 3). In fact, this significant reduction in BFM was observed after 6 mo of supplementation with CLA-FFA and CLA-triacylglycerol. This difference between the CLA groups and the placebo group was progressively higher through the last 6 mo of the study ($P < 0.05$; Figure 1). Compared with month 0 values, BFM was significantly different in the CLA-FFA and CLA-triacylglycerol groups at months 6, 9, and 12 ($P < 0.001$), whereas that in the placebo group remained unchanged ($P = 0.56$). CLA-triacylglycerol was not significantly more efficient in reducing BFM than was CLA-FFA ($P \geq 0.05$). A discriminant analysis showed that the best responders to CLA ($\geq 4.5\%$ BFM reduction) were women and subjects with a higher BMI at month 0. After 12 mo of supplementation, the CLA-FFA group had significantly higher LBM than did the placebo group ($P < 0.05$), whereas LBM in the CLA-triacylglycerol group did not differ significantly from that in the placebo group ($P \geq 0.05$; Table 3). Within-group analyses showed significant increases

TABLE 3

Body weight, body composition, daily caloric intake, and exercise measurements in subjects taking either placebo (olive oil), CLA-FFA, or CLA-triacylglycerol at month 0 and month 12¹

| | Placebo group (n = 59) | | | CLA-FFA group (n = 61) | | | CLA-triacylglycerol group (n = 60) | | |
|----------------------------|------------------------|-------------|---------------------------|------------------------|-------------|---------------------------|------------------------------------|-------------|---------------------------|
| | Month 0 | Month 12 | Δ 12 - 0 | Month 0 | Month 12 | Δ 12 - 0 | Month 0 | Month 12 | Δ 12 - 0 |
| Body weight (kg) | 80.1 ± 9.4 | 80.4 ± 10.5 | 0.2 ± 3.0 | 81.0 ± 9.3 | 79.9 ± 9.5 | -1.1 ± 3.7 ² | 80.7 ± 9.5 | 78.9 ± 9.9 | -1.8 ± 3.4 ^{2,3} |
| BMI (kg/m ²) | 27.7 ± 1.7 | 27.7 ± 1.8 | 0.0 ± 1.0 | 28.1 ± 1.5 | 27.7 ± 1.7 | -0.4 ± 1.2 ² | 28.3 ± 1.6 | 27.6 ± 1.6 | -0.6 ± 1.2 ^{2,3} |
| BFM (kg) | 30.2 ± 5.7 | 30.4 ± 5.6 | 0.2 ± 3.3 | 31.6 ± 5.2 | 29.9 ± 5.6 | -1.7 ± 3.0 ^{2,3} | 31.6 ± 5.6 | 29.2 ± 5.5 | -2.4 ± 3.0 ^{2,3} |
| LBM (kg) | 47.1 ± 9.6 | 47.1 ± 9.6 | 0.0 ± 1.5 | 46.5 ± 8.5 | 47.2 ± 7.8 | 0.7 ± 2.0 ^{2,3} | 46.4 ± 8.4 | 47.0 ± 8.0 | 0.6 ± 1.8 ² |
| BMM (kg) | 2.82 ± 0.48 | 2.83 ± 0.51 | 0.01 ± 0.12 | 2.88 ± 0.43 | 2.84 ± 0.44 | -0.04 ± 0.11 ² | 2.72 ± 0.42 | 2.71 ± 0.47 | -0.01 ± 0.12 |
| Diet (kcal/d) ⁴ | 1926 ± 441 | 1758 ± 446 | -168.1 ± 384 ² | 2045 ± 578 | 1761 ± 462 | -283.8 ± 445 ² | 2018 ± 592 | 1745 ± 436 | -273.8 ± 525 ² |
| Capsules (kcal/d) | 0.0 | 35.8 | 35.8 | 0.0 | 35.7 | 35.7 | 0.0 | 36.8 | 36.8 |
| Exercise ⁵ | 4.6 ± 3.3 | 5.0 ± 3.4 | 0.4 ± 2.7 | 4.0 ± 3.3 | 4.5 ± 3.2 | 0.5 ± 3.0 | 3.9 ± 2.5 | 3.8 ± 2.1 | 0.0 ± 3.1 |

¹ All values are $\bar{x} \pm SD$. CLA, conjugated linoleic acid; FFA, free fatty acid; BFM, body fat mass; LBM, lean body mass; BMM, bone mineral mass; Δ, change. There was no significant difference between the groups at month 0 (except for BMM in the CLA-triacylglycerol group as compared with the placebo and CLA-FFA groups).

² Change from month 0 to month 12 within the groups was significant, $P < 0.05$ (paired t test).

³ Change within the CLA group was significantly different from that within the placebo group, $P < 0.05$ (Tukey's t test).

⁴ Daily caloric intake from capsules was calculated as (4.5 g oil \times 9 kcal/g = 40.5 kcal/d) \times (compliance/group).

⁵ Assessed as the product of the number of 20-min training sessions and their intensity (high or low) and expressed in arbitrary units.

from month 0 in LBM in subjects given CLA-FFA ($P = 0.009$) or CLA-triacylglycerol ($P = 0.008$), but there was no significant change in the placebo group ($P = 0.81$). Changes in LBM did not differ significantly between the 2 CLA groups ($P \geq 0.05$; data not shown). Whereas the bone mineral mass (BMM) of the CLA-triacylglycerol group was lower than that of the placebo and CLA-FFA groups at month 0 ($P < 0.05$), there was no significant difference in BMM between any of the groups at month 12 ($P = 0.62$; Table 3). The CLA-FFA group had a small reduction in

BMM from month 0 to month 12 ($P = 0.01$), but BMM did not change significantly in the placebo group ($P = 0.55$) or CLA-triacylglycerol group ($P = 0.47$) from month 0 to month 12.

Diet and exercise

There were no differences between the 3 groups at month 0 or month 12, but caloric intake decreased significantly in all groups compared with month 0 (Table 3). Exercise estimates remained unchanged between month 0 and month 12 and were unchanged within each group and between the groups ($P = 0.23$; Table 3).

Safety

There were no significant between- or within-group differences at month 12 for the following clinical chemistry variables: bilirubin, chloride, creatine phosphokinase, creatinin, erythrocytes, γ -glutamyltransferase, thyroid-stimulating hormone, thyroxin, insulin-like growth hormone 1, insulin, and insulin C-peptide (data not shown). Hemoglobin, potassium, sodium, and leptin concentrations also did not differ significantly between the groups at month 12, but there were significant within-group changes from the values at month 0: CLA-triacylglycerol lowered both hemoglobin and leptin ($P < 0.05$), the sodium concentrations were higher in the placebo and CLA-triacylglycerol groups ($P < 0.05$), and the potassium concentrations were higher in all 3 groups ($P < 0.05$) (data not shown).

There were no significant differences in Hb A_{1c} concentrations between the groups, but all 3 groups had significantly higher Hb A_{1c} concentrations than at month 0 (Table 4). All subjects had normal values for fasting blood glucose at month 0 and month 12, and fasting blood glucose concentrations did not differ significantly between the groups at month 12 (Table 4).

Triacylglycerol and total cholesterol concentrations did not differ significantly between the groups at month 12 (Table 4). HDL-cholesterol concentrations also did not differ significantly between the groups at month 12, but, in the CLA-triacylglycerol group, HDL cholesterol decreased from the month 0 values.

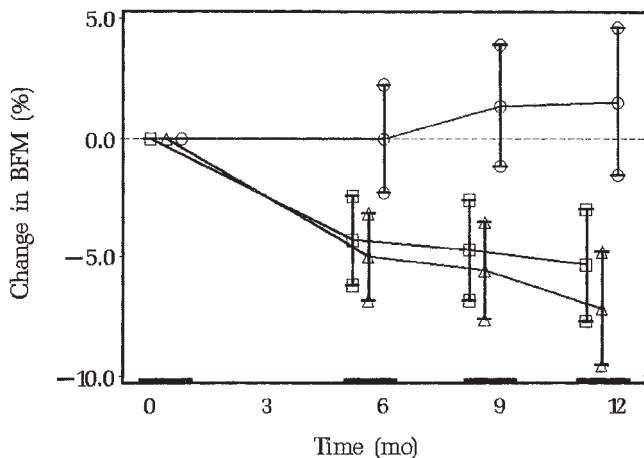


FIGURE 1. Mean (95% CI) percentage change in body fat mass (BFM) in subjects taking placebo (○), CLA-free fatty acids (FFA; □), or CLA-triacylglycerol (△) for 12 mo. All values were measured at the same points (ie, 0, 6, 9, and 12 mo) in all 3 groups. Intervals not including 0 are significant within the group. Between-group comparisons of changes from month 0 in DXA and weight variables were performed by using ANCOVA (treatment, center, and sex as factors; month 0 value, total energy intake, exercise, and drug \times energy intake and drug \times training score interactions as covariates). A significant time \times treatment interaction was found ($P = 0.001$). Differences between both CLA groups and the placebo group were significant at 6, 9, and 12 mo ($P < 0.05$). There was no difference between the CLA-FFA and CLA-triacylglycerol groups ($P \geq 0.05$).

TABLE 4Laboratory blood analyses for subjects taking either placebo (olive oil), CLA-FFA, or CLA-triacylglycerol at month 0 and month 12¹

| | Placebo group (n = 59) | | | CLA-FFA group (n = 61) | | | CLA-triacylglycerol group (n = 60) | | |
|-----------------------------------|------------------------|--------------|--------------------------|------------------------|--------------|----------------------------|------------------------------------|--------------|---------------------------|
| | Month 0 | Month 12 | Δ 12 - 0 | Month 0 | Month 12 | Δ 12 - 0 | Month 0 | Month 12 | Δ 12 - 0 |
| Hb A _{1c} (%) | 5.4 ± 0.31 | 5.6 ± 0.21 | 0.16 ± 0.28 ² | 5.5 ± 0.26 | 5.7 ± 0.3 | 0.21 ± 0.23 ² | 5.5 ± 0.25 | 5.6 ± 0.26 | 0.14 ± 0.22 ² |
| Glucose (mmol/L) | 5.1 ± 0.42 | 5.1 ± 0.43 | -0.10 ± 0.44 | 5.1 ± 0.53 | 5.2 ± 0.75 | 0.08 ± 0.60 | 5.1 ± 0.49 | 5.1 ± 0.58 | -0.05 ± 0.44 |
| Triacylglycerol (mmol/L) | 1.29 ± 0.58 | 1.24 ± 0.6 | -0.02 ± 0.47 | 1.39 ± 0.81 | 1.46 ± 1.13 | 0.01 ± 0.77 | 1.29 ± 0.58 | 1.38 ± 0.72 | 0.08 ± 0.61 |
| Total cholesterol (mmol/L) | 5.9 ± 1.27 | 5.7 ± 1.09 | -0.03 ± 0.82 | 5.4 ± 0.94 | 5.5 ± 1.00 | 0.15 ± 0.64 | 5.7 ± 0.94 | 5.7 ± 0.94 | -0.04 ± 0.68 |
| HDL cholesterol (mmol/L) | 1.5 ± 0.38 | 1.5 ± 0.45 | 0.00 ± 0.27 | 1.4 ± 0.32 | 1.4 ± 0.38 | -0.03 ± 0.24 | 1.5 ± 0.34 | 1.4 ± 0.33 | -0.09 ± 0.23 ² |
| LDL cholesterol (mmol/L) | 3.3 ± 0.80 | 3.6 ± 0.97 | -0.03 ± 0.75 | 3.6 ± 0.97 | 3.5 ± 0.84 | 0.22 ± 0.58 ² | 3.7 ± 1.15 | 3.6 ± 0.85 | 0.02 ± 0.63 |
| Lp(a) (mg/L) | 275.5 ± 256 | 261 ± 235 | 6.6 ± 46.6 | 321 ± 390 | 346.8 ± 448 | 39.5 ± 71.6 ^{2,3} | 244.1 ± 267 | 284.8 ± 292 | 33.1 ± 66.6 ² |
| Leukocytes (10 ⁹ /L) | 5.8 ± 1.61 | 5.9 ± 1.77 | 0.02 ± 1.26 | 5.6 ± 1.63 | 6.5 ± 1.71 | 0.47 ± 1.5 ² | 5.3 ± 1.62 | 6.0 ± 1.69 | 0.51 ± 1.12 ² |
| Thrombocytes (10 ⁹ /L) | 258.2 ± 56.2 | 259.1 ± 54.7 | -0.24 ± 25.3 | 265.7 ± 61.4 | 280.1 ± 65.5 | 15.1 ± 24.4 ^{2,3} | 263.9 ± 62.9 | 272.5 ± 68.3 | 7.36 ± 31.4 |
| ALT (U/L) | 26.2 ± 13.1 | 26.4 ± 12.3 | 0.30 ± 11.06 | 24.3 ± 14.3 | 26.6 ± 14.7 | 1.71 ± 11.8 | 23.9 ± 9.7 | 24.9 ± 12.2 | 0.73 ± 10.41 |
| AST (U/L) | 23.6 ± 8.0 | 23.2 ± 5.2 | -0.32 ± 5.06 | 22.4 ± 5.5 | 24.8 ± 8.2 | 2.35 ± 7.00 ^{2,3} | 23.1 ± 5.3 | 23.4 ± 5.9 | 0.25 ± 5.33 |

¹ All values are $\bar{x} \pm \text{SD}$. CLA, conjugated linoleic acid; FFA, free fatty acid; Δ, change; Hb A_{1c}, glycated hemoglobin; Lp(a), lipoprotein(a); ALT, alanine aminotransferase; AST, aspartate aminotransferase. There were no significant differences between the groups at month 0.

² Change from month 0 to month 12 within the group was significant, $P < 0.05$ (paired *t* test).

³ Change within the CLA group was significantly different from that within the placebo group, $P < 0.05$ (Tukey's *t* test).

There was no significant difference in HDL-cholesterol concentrations in the CLA-FFA group from the month 0 values or the concentrations in the placebo group (Table 4).

Lp(a) concentrations were higher in the CLA-FFA group than in the placebo group after 12 mo and were higher in both CLA groups than at month 0 (Table 4). Leukocyte counts did not differ significantly between the CLA groups and the placebo group at month 12, but both CLA groups had higher leukocyte counts at month 12 than at month 0 (Table 4). Thrombocytes were significantly higher in the CLA-FFA group at month 12 than at month 0 and than in the placebo group, whereas CLA-triacylglycerol had no effect on thrombocytes at month 12 or in comparison with placebo (Table 4). Alanine aminotransferase concentrations did not differ significantly between the groups at month 12 (Table 4). Aspartate aminotransferase concentrations in the CLA-FFA group were significantly higher at month 12 than at month 0 and in comparison with the placebo group, whereas CLA-triacylglycerol had no effect on aspartate aminotransferase at month 0 or in comparison with placebo (Table 4).

Systolic and diastolic blood pressures decreased in all groups between month 0 and month 12, but these changes did not differ significantly between the groups (data not shown). Heart rate did not differ significantly between the groups, but heart rate in the CLA-triacylglycerol group at month 12 was significantly lower than that at month 0 ($P = 0.02$). Heart rate was unchanged in the CLA-FFA and placebo groups (data not shown).

Adverse events

AEs were reported by 68% of all randomly assigned subjects and with similar frequency in all 3 study groups ($P = 0.68$). Of 264 single events, the investigators considered 30 to be drug related. The drug-related AEs were evenly distributed among the 3 study arms. All AEs were rated as either "mild" or "moderate," and the symptoms were transient. Ten subjects (5.5% of the total) left the study because of musculoskeletal ailments or gastroin-

testinal symptoms such as abdominal discomfort, diarrhea, or nausea. The gastrointestinal events were judged by the study investigators as probably related to the tested drug. Abdominal discomfort or pain, loose stools, and dyspepsia were the most frequently reported drug-related AEs. Three subjects experienced serious AEs not related to the use of study drugs: 2 had accidents requiring hospitalization, and 1 underwent surgical correction of a genital prolapse.

DISCUSSION

This is the first clinical study documenting the long-term (12 mo) safety and efficacy of CLA supplementation in healthy overweight subjects consuming an ad libitum diet and without specific lifestyle restrictions. In the present study, DXA technology was used to assess changes in body composition. This method has been thoroughly evaluated, even in subjects with small changes in body weight (44).

Supplementation with CLA, either as FFA or triacylglycerol, for 12 mo significantly lowered BFM in comparison with BFM in the placebo group and tended to induce higher LBM. The results of this study corroborate and expand on the findings of previous short-term studies that suggested that CLA reduces BFM and increases or maintains LBM (24–27). The 2 CLA forms, CLA-FFA and CLA-triacylglycerol, were equally efficacious in BFM reduction. Best-responder analysis in subjects with a BMI from 25 to 30 suggests that the effect is greatest in those with the highest BMI and in women, who have a relatively greater contribution of fat mass to body weight than do men. This may explain why obese subjects in a short-term study had larger BFM reduction than did our study subjects (25).

The mechanism or mechanisms by which CLA decreases BFM and increases LBM are not completely understood. CLA is known to accumulate in tissues of animals and humans, where it is readily metabolized. In vitro and in vivo studies suggested that

the ability of CLA to reduce adipose tissue could be explained by one or more of the following mechanisms: the induction of adipocyte apoptosis (45), reduced accumulation of fatty acids in adipocytes due to an inhibition of lipoprotein lipase and increase in carnitine palmityltransferase (46), the binding to peroxisome proliferator-activated receptor γ present in fat tissue and modification of the signaling cascade to down-regulate the expression of leptin (47) and the prevention of the triacylglycerol accumulation in adipocytes (48), or the modification of the energy expenditure, the metabolic rate, or both (22, 33).

A small decrease in BMM observed in the DXA analysis of the CLA-FFA-supplemented subjects is not readily explained by site differences and group differences in BMM. This decrease borders on the smallest possible difference observable with DXA technology.

Daily caloric intake did not differ significantly between groups at either month 0 or month 12, and, in accordance with the intention of the study, a small reduction in caloric intake was observed during the study in all 3 groups. This strongly suggests that the observed effects of CLA on body composition (ie, BFM and LBM) were independent of diet. In addition, the observed decrease in daily energy intake from diet may result in part from a compensation for the energy intake from capsules, from a reduced appetite, or both. It is also likely from the narrowing of variance and closeness of mean caloric intake after 12 mo that a learning effect may be present in the recording of the food intake, as was observed in other studies (39). Exercise, another possible confounder, did not differ significantly between the groups, and therefore it most likely did not play a role in the body-composition changes observed in the CLA groups.

The current study monitored the long-term safety of CLA supplementation in healthy, overweight subjects over a 12-mo period. High compliance and a low dropout rate indicate good tolerance of CLA supplementation. Only 11.4% of the reported AEs were related to the supplementation. These AEs were mostly gastrointestinal, as were most of the AEs reported in previous short-term studies (25, 27, 49, 50), and likely resulted from the daily ingestion of oil or of the gelatin capsules alone. The lack of difference in AE reports between the CLA groups and the placebo group indicates that CLA was tolerated as well as was olive oil.

Previous short-term clinical studies showed that the effect of CLA on blood lipids was diverse, including a reduction of HDL (25, 32), a reduction of VLDL without effect on HDL or LDL (51), and no effect on cholesterol lipids (27). In the current study, we observed no effect on total cholesterol or triacylglycerol concentrations, but the CLA-triacylglycerol group had lower HDL concentrations and the CLA-FFA group had higher LDL than at the start of the study. The changes in these measures, however, were small, within the normal range, and not significantly different from the values in the placebo group. The introduction of the mean values of LDL, HDL, age, sex, blood pressure, diabetes, and smoking after 12-mo CLA supplementation, as taken from a table of values from the Framingham Study (52), showed that the cardiovascular disease (CVD) risk prediction scores in 10 y in the CLA-FFA group (+3.6%) and in the CLA-triacylglycerol group (+3.3%) are lower than those in an average population (+5%) matched for age and sex. Furthermore, when LDL and HDL are examined independently in the Framingham Study table, there is no increase in CVD risk.

At month 12, both CLA forms had higher Lp(a) concentrations than did placebo and than at month 0. Elevated Lp(a) concentra-

tion is thought to be a risk factor for CVD, but the use of Lp(a) as a routine test has been questioned (53). In addition, at month 12, the CLA-FFA group had higher leukocytes and thrombocytes than did the placebo and than at month 0, whereas the CLA-triacylglycerol group had higher leukocytes than at month 0. As observed with the lipid profiles, the mean values for these changes were not outside of the normal range. Higher Lp(a) concentrations and numbers of leukocyte and thrombocyte suggest that CLA may increase CVD risk and may promote an inflammatory response. Previous studies on the effect of CLA on CVD risk have been divergent. A proatherogenic effect of CLA mixture has been shown in mice (54), and LDL and apolipoprotein B concentrations higher than those in the placebo group have been reported in persons supplemented with CLA (26). Other studies showed a reduction in atherosclerosis in rabbits (55), an anti-inflammatory role for CLA in animals (56–58), and an enhancement in immune response in animals and humans with CLA (10, 11, 59–61).

Epidemiologic studies showed that higher weight (62), greater BMI (63), and greater fat mass (64) are all related to increased CVD and all-cause mortality. In contrast, intentional weight loss is associated with reduced mortality (65). In the present study, no reduction in CVD risk factors other than the changes in vital signs were observed, despite a significant reduction in body fat mass. Further studies with appropriate endpoints and design (eg, larger population and longer time) are required to investigate the effect of CLA on CVD risk factors other than BFM, weight, and BMI.

Previous studies by Riserus et al (32) showed that supplementation with 2.6 g pure *trans*-10, *cis*-12 isomer for 12 wk increased insulin resistance in a male population with metabolic syndrome, whereas the men who were supplemented with a mixture of CLA isomers (1.20 g *cis*-9, *trans*-11 and 1.22 g *trans*-10, *cis*-12 isomers), which is similar to the supplement used in the present study (1.31 g *cis*-9, *trans*-11 and 1.39 g *trans*-10, *cis*-12), had no significant increase in insulin resistance. In the current study, fasting serum glucose concentrations were not affected by CLA supplementation, but there was a slight increase in Hb A_{1c} concentrations in all 3 groups. The fact that the placebo group Hb A_{1c} values did not differ from those of the other 2 groups suggests that the higher Hb A_{1c} concentrations were not mediated by CLA. All study subjects had fasting serum glucose concentrations within the normal range throughout the study, according to the American Diabetes Association criteria, which indicates that CLA supplementation was not diabetogenic in this population of healthy subjects.

In a similar study, Basu et al (35) showed that men with the metabolic syndrome had an increase in F₂-isoprostane excretion after supplementation with 4.2 g mixed CLA isomers that returned to baseline 2 wk after the CLA supplementation stopped, without effect on serum α - and γ -tocopherol concentrations or on urinary 2,3-dinor-thromboxane B₂ excretion. These findings suggest that CLA may induce lipid peroxidation, but the long-term effects of lipid peroxidation are not known. The current study was not designed to measure lipid peroxidation, and therefore it is not possible at this time to ascertain the role of CLA in oxidative stress in healthy overweight people.

In conclusion, a CLA mixture containing 80% *trans*-10, *cis*-12 and *cis*-9, *trans*-11 isomers, administered either in the triacylglycerol or FFA form to healthy overweight adults for 1 y, results

in a significant decrease in BFM. Future studies are needed to address the role of CLA in CVD, diabetes, and oxidative stress.



We are very thankful to Mette Bogen, who monitored all diet forms and collected data from analyses. Particular thanks go to clinical nurses Oddrun Kulvedrøsten, Lill Johannessen, and Linda Magnor for their active contributions to the success of this study. We also thank Heather Nelson-Cortes and Kari Skiningsrud for reviewing the manuscript and for their fruitful comments.

J-MG coordinated and monitored the study. JH was the main investigator at the Betanien Medical Center. KH was the main investigator at the Helse-torget Medical Center. KK monitored the study, analyzed the adverse events, and functioned as the safety officer. HF performed statistical analyses. HV and OG were overall responsible for the project. All authors participated in protocol development, result evaluation, and writing and editing of the manuscript. None of the authors had any financial or personal interest in any company or organization sponsoring the research, including advisory board affiliations.

REFERENCES

- Pariza M, Ashoor S, Chu F, Lund D. Effects of temperature and time on mutagen formation in pan-fried hamburger. *Cancer Lett* 1979;7:63–9.
- Cunningham DC, Harrison LY, Shultz TD. Proliferative responses of normal human mammary and MCF-7 breast cancer cells to linoleic acid, conjugated linoleic acid and eicosanoid synthesis inhibitors in culture. *Anticancer Res* 1997;17:197–203.
- Liew C, Schut HA, Chin SF, Pariza MW, Dashwood RH. Protection of conjugated linoleic acids against 2-amino-3-methylimidazo[4,5-f]quinoline-induced colon carcinogenesis in the F344 rat: a study of inhibitory mechanisms. *Carcinogenesis* 1995;16:3037–43.
- Ip C, Singh M, Thompson HJ, Scimeca JA. Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. *Cancer Res* 1994;54:1212–5.
- Schonberg S, Krokan HE. The inhibitory effect of conjugated dienoic derivatives (CLA) of linoleic acid on the growth of human tumor cell lines is in part due to increased lipid peroxidation. *Anticancer Res* 1995;15:1241–6.
- Wong MW, Chew BP, Wong TS, Hosick HL, Boylston TD, Shultz TD. Effects of dietary conjugated linoleic acid on lymphocyte function and growth of mammary tumors in mice. *Anticancer Res* 1997;17:987–93.
- Zu HX, Schut HA. Inhibition of 2-amino-3-methylimidazo[4,5-f]quinoline-DNA adduct formation in CDF1 mice by heat-altered derivatives of linoleic acid. *Food Chem Toxicol* 1992;30:9–16.
- Nicolosi RJ, Rogers EJ, Kritchevsky D, Scimeca JA, Huth PJ. Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. *Artery* 1997;22:266–77.
- Lee KN, Kritchevsky D, Pariza MW. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* 1994;108:19–25.
- Cook ME, Miller CC, Park Y, Pariza M. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. *Poult Sci* 1993;72:1301–5.
- Miller CC, Park Y, Pariza MW, Cook ME. Feeding conjugated linoleic acid. *Biochem Biophys Res Commun* 1994;198:1107–12.
- Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY, et al. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat. *Biochem Biophys Res Commun* 1998;244:678–82. (Published erratum appears in *Biochem Biophys Res Commun* 1998;247:911.)
- DeLany JP, Blohm F, Truett AA, Scimeca JA, West DB. Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. *Am J Physiol* 1999;276:R1172–9.
- Ostrowska E, Suster D, Muralitharan M, et al. Conjugated linoleic acid decreases fat accretion in pigs: evaluation by dual-energy X-ray absorptiometry. *Br J Nutr* 2003;89:219–29.
- Akahoshi A, Goto Y, Muraio K, et al. Conjugated linoleic acid reduces body fats and cytokine levels of mice. *Biosci Biotech Biochem* 2002;66:916–20.
- Corino C, Mourot J, Magni S, Pastorelli G, Rosi F. Influence of dietary conjugated linoleic acid on growth, meat quality, lipogenesis, plasma leptin and physiological variables of lipid metabolism in rabbits. *J Anim Sci* 2002;80:1020–8.
- Takahashi Y, Kushiro M, Shinohara K, Ide T. Dietary conjugated linoleic acid reduces body fat mass and affects gene expression of proteins regulating energy metabolism in mice. *Comp Biochem Physiol B Biochem Mol Biol* 2002;133:395–404.
- Rahman S, Wang Y, Han S, et al. 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* 2001;34:515–20.
- Dugan MER, Aalhus JL, Schaefer AL, Kramer JKG. The effects of conjugated linoleic acid on fat to lean repartitioning and feed conversion in pigs. *Can J Anim Sci* 1997;77:723–5.
- Gavino VC, Gavino G, Leblanc MJ, Tuchweber B. 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* 2000;130:27–9.
- 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.
- West DB, DeLany JP, Camet PM, Blohm F, Truett AA, Scimeca J. Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. *Am J Physiol* 1998;275:R667–72.
- Szymczyk B, Pisulewski PM, Szczurek W, Hanczakowski P. Effects of conjugated linoleic acid on growth performance, feed conversion efficiency, and subsequent carcass quality in broiler chickens. *Br J Nutr* 2001;85:465–73.
- Gaullier J-M, Berven G, Blankson H, Gudmundsen O. Clinical trial results support a preference for using CLA preparations enriched with two isomers rather than four isomers in human studies. *Lipids* 2002;37:1019–25.
- 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.
- Smedman A, Vessby B. Conjugated linoleic acid supplementation in humans—metabolic effects. *Lipids* 2001;36:773–81.
- Berven G, Bye A, Hals O, et al. Safety of conjugated linoleic acid (CLA) in overweight or obese human volunteers. *Eur J Lipid Sci Technol* 2000;102:455–62.
- Zambell KL, Keim NL, Van Loan MD, et al. Conjugated linoleic acid supplementation in humans: effects on body composition and energy expenditure. *Lipids* 2000;35:777–82.
- Atkinson RL. Conjugated linoleic acid for altering body composition and treating obesity. In: Yurawecz MP, Mossoba MM, Kramer JKG, Pariza MW, Nelson GJ, eds. *Advances in conjugated linoleic acid research*. Vol 1. Champaign, IL: AOCS Press, 1999:348–53.
- Kreider RB, Ferreira MP, Greenwood M, Wilson M, Almada AL. Effects of conjugated linoleic acid supplementation during resistance-training on body composition, bone density, strength, and selected hematological markers. *J Strength Cond Res* 2002;3:325–34.
- Lowery LM, Appicelli PA, Lemon PWR. Conjugated linoleic acid enhances muscle size and strength gains in novice bodybuilders. *Med Sci Sports Exerc* 1998;30:182(abstr).
- Riserus U, Arner P, Brismar K, Vessby B. Treatment with dietary trans10cis12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. *Diabetes Care* 2002;25:1516–21.
- Terpstra AH. Differences between humans and mice in efficacy of the body fat lowering effect of conjugated linoleic acid: role of metabolic rate. *J Nutr* 2001;131:2067–8.
- Basu S, Smedman A, Vessby B. Conjugated linoleic acid induces lipid peroxidation in humans. *FEBS Lett* 2000;468:33–6.
- Basu S, Riserus U, Turpeinen A, Vessby B. Conjugated linoleic acid induces lipid peroxidation in men with abdominal obesity. *Clin Sci (Lond)* 2000;99:511–6.
- Riserus U, Basu S, Jovinge S, Fredrikson GN, Arnlöv 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.
- Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 1997;20:1183–97.
- Friedewald W, Levy R, Fredrickson D. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.

39. Nes M, Andersen LF, Solvoll K, et al. Accuracy of a quantitative food frequency questionnaire applied in elderly Norwegian women. *Eur J Clin Nutr* 1992;46:809–21.
40. Reseland J, Anderssen S, Solvoll K, et al. Effect of long-term changes in diet and exercise on plasma leptin concentrations. *Am J Clin Nutr* 2001;73:240–5.
41. Montgomery D. Design and analysis of experiments. 2nd edition. New York: John Wiley & Sons, 1984.
42. Gresti A. Categorical data analysis. New York: John Wiley & Sons, 1990.
43. Kendall M, Stuart A. The advance theory of statistics. Vol 3. London: Charles Griffin & Co Ltd, 1979.
44. Tylavsky F, Lohman T, Dockrell M, et al. Comparison of the effectiveness of 2 dual-energy X-ray absorptiometers with that of total body water and computed tomography in assessing changes in body composition during weight change. *Am J Clin Nutr* 2003;77:356–63.
45. Evans M, Geigerman C, Cook J, Curtis L, Kuebler B, McIntosh M. Conjugated linoleic acid suppresses triglyceride accumulation and induces apoptosis in 3T3-L1 preadipocytes. *Lipids* 2000;35:899–910.
46. Park Y, Pariza MW. The effects of dietary conjugated nonadecadienoic acid on body composition in mice. *Biochim Biophys Acta* 2001;1533:171–4.
47. Kallen C, Lazar M. Antidiabetic thiazolidinediones inhibit leptin ob gene expression in 3T3-L1 adipocytes. *Proc Natl Acad Sci U S A* 1996;93:5793–6.
48. Granlund L, Juvet L, Pedersen J, Nebb H. Trans10, cis12-conjugated linoleic acid prevents triacylglycerol accumulation in adipocytes by acting as a PPARgamma modulator. *J Lipid Res* 2003;44:1441–52.
49. Thom E, Wadstein J, Gudmundsen O. Conjugated linoleic acid reduces body fat in healthy exercising humans. *J Int Med Res* 2001;29:392–6.
50. Vessby B, Smedman A. Conjugated linoleic acid (CLA) reduces the body fat content in humans. *Chem Phys Lipids* 1999;101:152(abstr).
51. 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.
52. Wilson P, D'Agostino R, Levy D, Belanger A, Silbershatz H, Kannel W. Prediction of coronary heart disease using risk factor categories. *Circulation* 1998;97:1837–47.
53. Hackam D, Anand SS. Emerging risk factors for atherosclerotic vascular disease: a critical review of the evidence. *JAMA* 2003;290:932–40.
54. Munday JS, Thompson KG, James KA. Dietary conjugated linoleic acids promote fatty streak formation in the C57BL/6 mouse atherosclerosis model. *Br J Nutr* 1999;81:251–5.
55. Kritchevsky D, Tepper SA, Wright S, Tso P, Czarnecki SK. Influence of conjugated linoleic acid (CLA) on establishment and progression of atherosclerosis in rabbits. *J Am Coll Nutr* 2000;19:472S–7S.
56. Hontecillas R, Wannemeulher MJ, Zimmerman DR, et al. Nutritional regulation of porcine bacterial-induced colitis by conjugated linoleic acid. *J Nutr* 2002;132:2019–27.
57. Whigham LD, Higbee A, Bjorling DE, Park Y, Pariza MW, Cook ME. Decreased antigen-induced eicosanoid release in conjugated linoleic acid-fed guinea pigs. *Am J Physiol Regul Integr Comp Physiol* 2002;282:R1104–12.
58. Turek J, Li Y, Schoenlein I, Allen K, Watkins B. Modulation of macrophage cytokine production by conjugated linoleic acids is influenced by the dietary n–6:n–3 fatty acid ratio. *J Nutr Biochem* 1998;9:258–66.
59. Bassaganya-Riera J, Hontecillas R, Zimmerman DR, Wannemuehler MJ. Dietary conjugated linoleic acid modulates phenotype and effector functions of porcine CD8(+) lymphocytes. *J Nutr* 2001;131:2370–7.
60. Bassaganya-Riera J, Hontecillas-Magarzo R, Bregendahl K, Wannemuehler MJ, Zimmerman DR. 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* 2001;79:714–21.
61. Albers R, Van der Wielen RP, Brink EJ, Hendriks HF, Dorovska-Taran VN, Mohede IC. Effects of cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid (CLA) isomers on immune function in healthy men. *Eur J Clin Nutr* 2003;57:595–603.
62. Rissanen A, Heliovaara M, Knekt P, Aromaa A, Reunanen A, Maatela J. Weight and mortality in Finnish men. *J Clin Epidemiol* 1989;42:781–9.
63. Jonsson S, Hedblad B, Engström G, Nilsson P, Berglund G, Janzon L. Influence of obesity on cardiovascular risk. Twenty-three-year follow up of 22,025 men from an urban Swedish population. *Int J Obes Relat Metab Disord* 2002;26:1046–53.
64. Heitman B, Erikson H, Ellsinger B, Mikkelsen K, Larsson B. Mortality associated with body fat, fat-free mass and body mass index among 60-year old Swedish men—a 22-year follow up. The study of men born in 1913. *Int J Obes Relat Metab Disord* 2000;24:33–7.
65. Gregg E, Gerzoff R, Thompson T, Williamson D. Intentional weight loss and death in overweight and obese US adults 35 years of age and older. *Ann Intern Med* 2003;138:383–9.