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

Jean-Michel Gaullier, Johan Halse, Kjetil Høye, Knut Kristiansen, Hans Fagertun, Hogne 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/m²) 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 (± SD) BFM in the CLA-triacylglycerol and CLA-FFA groups was 8.7 ± 9.1% and 6.9 ± 9.1%, respectively, lower than that in the placebo group (P < 0.001). Subjects receiving CLA-FFA had 1.8 ± 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.


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 F₂-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 F₂-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-
TABLE 1
Capsule composition of the free fatty acid (FFA) and triacylglycerol forms of conjugated linoleic acid (CLA)

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

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/m²) 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, Hovdebygda, 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 (Fürt 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 A₁c), 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 ≥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, Wisconsin) 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 ± 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 ≥1 × 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 × 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 ≥4.5% from month 0 to month 12. A subject was thus categorized as a treatment responder on the basis of a BFM reduction ≥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 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 ≥ 0.05). The effects of CLA-triacylglycerol on weight and BMI did not differ significantly from the effects of CLA-FFA (P ≥ 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 ≥ 0.05). A discriminant analysis showed that the best responders to CLA (≥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 ≥ 0.05; Table 3). Within-group analyses showed significant increases

<p>| TABLE 2  |
| Characteristics of the study population at month 0 |</p>
<table>
<thead>
<tr>
<th>Placebo</th>
<th>CLA-FFA</th>
<th>CLA-triacylglycerol</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.72</td>
</tr>
<tr>
<td>Male (n)</td>
<td>12</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Female (n)</td>
<td>47</td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td>Age (y)</td>
<td>45 ± 9.5</td>
<td>44.5 ± 10.7</td>
<td>48.0 ± 10.7</td>
</tr>
<tr>
<td>Alcohol use (%)</td>
<td>71</td>
<td>69</td>
<td>61</td>
</tr>
<tr>
<td>Tobacco use (%)</td>
<td>20</td>
<td>32</td>
<td>17</td>
</tr>
<tr>
<td>Exercise (%)</td>
<td>52</td>
<td>51</td>
<td>50</td>
</tr>
</tbody>
</table>

1 CLA, conjugated linoleic acid; FFA, free fatty acid.
2 ± 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.

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Effects of CLA on weight and BMI

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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 = 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.

### 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 A1c concentrations between the groups, but all 3 groups had significantly higher Hb A1c 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.
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).

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Laboratory blood analyses for subjects taking either placebo (olive oil), CLA-FFA, or CLA-triacylglycerol at month 0 and month 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo group (n = 59)</td>
<td>CLA-FFA group (n = 61)</td>
</tr>
<tr>
<td><strong>Hb A1c (%)</strong></td>
<td>Month 0</td>
</tr>
<tr>
<td>5.4 ± 0.31</td>
<td>5.6 ± 0.21</td>
</tr>
<tr>
<td><strong>Glucose (mmol/L)</strong></td>
<td>5.1 ± 0.42</td>
</tr>
<tr>
<td><strong>Triacylglycerol (mmol/L)</strong></td>
<td>2.9 ± 0.58</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/L)</strong></td>
<td>5.9 ± 1.27</td>
</tr>
<tr>
<td><strong>LDL cholesterol (mmol/L)</strong></td>
<td>1.5 ± 0.38</td>
</tr>
<tr>
<td><strong>Lp(a) (mg/L)</strong></td>
<td>3.3 ± 0.80</td>
</tr>
<tr>
<td><strong>Leukocytes (10^9/L)</strong></td>
<td>275 ± 256</td>
</tr>
<tr>
<td><strong>Thrombocytes</strong></td>
<td>258.2 ± 56.2</td>
</tr>
<tr>
<td><strong>ALT (U/L)</strong></td>
<td>26.2 ± 13.1</td>
</tr>
<tr>
<td><strong>AST (U/L)</strong></td>
<td>23.6 ± 8.0</td>
</tr>
</tbody>
</table>

1 All values are x ± SD. CLA, conjugated linoleic acid; FFA, free fatty acid; Δ, change; Hb A1c, 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).

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 adipocytic apoptosis (45), reduced accumulation of fatty acids in adipocytes due to an inhibition of lipoprotein lipase and increase in carnitine palmitoyltransferase (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) concentration 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 A1c concentrations in all 3 groups. The fact that the placebo group Hb A1c values did not differ from those of the other 2 groups suggests that the higher Hb A1c 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 Skinningsrud 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.

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CLA REDUCES BODY FAT MASS IN OVERWEIGHT HUMANS

Effect of changes in fruit and vegetable intake on plasma antioxidant defenses in humans

Dear Sir:

In a recent issue of the Journal, Dragsted et al (1) investigated whether fruit and vegetable intake affects biomarkers of oxidative stress or antioxidant defenses. They conducted a well-designed, 25-d, randomized, partly blinded intervention trial. Some of their conclusions related to an apparent lack of effect on markers of total antioxidant capacity [TAC; namely, the ferric-reducing ability of plasma (FRAP) and Trolox-equivalent antioxidant capacity (TEAC)], most of the enzymatic antioxidant defenses (superoxide dismutase, catalase, glutathione reductase, and glutathione S-transferase), and lipid oxidation (isoprostanes and malondialdehyde) in the fruit and vegetable (fruveg) group compared with the placebo group.

TAC measurement, representing the cumulative action of all electron-donating antioxidants present in body fluids, is increasingly being used to monitor redox status in vivo in intervention, bioavailability, and epidemiologic studies (2, 3). However, different studies have indicated that there may be a physiologic modulation of the redox status of body fluids (4, 5), and results from the SU.VI.MAX intervention trial indicate the importance of baseline plasma concentrations on the effectiveness of antioxidant supplementation (6). Therefore, dietary effects on the redox status of healthy subjects may be small and difficult to discern, especially if nonoptimized assay conditions are used. We suggest that the lack of significant variation in plasma antioxidant defenses observed by Dragsted et al may be a consequence of these factors. First, the dietary change failed to modify the redox status of the healthy subjects during the experimental period (see Table 6 in reference 1) and, second, the plasma TAC data could have been adversely affected by suboptimal measurement conditions.

The data of Dragsted et al clearly show that none of the measured redox markers were affected by the withdrawal of fruit and vegetables from the control diet. A decrease in plasma antioxidant concentrations was observed only with vitamin C and carotenoids, which in humans are modest contributors to plasma TAC (7, 8). We speculate that this indicates that 25 d was not an adequate time period to impair plasma TAC in healthy subjects. Because of the ability to cope with light dietary stress, plasma antioxidant defenses may need >25 d or specific and stronger dietary stresses, such as a high-fat diet, to be challenged significantly. We believe that the lack of change in plasma TAC concentrations in the placebo and fruveg groups could have been due to a physiologic regulatory mechanism that in the short term buffers against significant variation in plasma TAC in healthy young subjects (26 ± 6 y for the fruveg group and 29 ± 8 y for the placebo group).

The lack of observed changes in plasma FRAP and TEAC could also be the result of a decrease in the sensitivity of the TAC measurements as the result of nonoptimized assay techniques. The wavelength used by Dragsted et al to measure both FRAP and TEAC was 620 nm. The correct reference wavelengths are 595 nm for the FRAP assay and 734 nm for the TEAC assay (9, 10). Experiments conducted in our laboratories indicate that measurement at 620 nm results in a decrease in sensitivity of ≈40% and 66% for TEAC and FRAP, respectively. This is borne out by the uncharacteristically high CVs (16.6% and 8.8%, respectively, for TEAC and FRAP) obtained by Dragsted et al compared with reference studies (9, 10). The difference in vitamin C concentration between the fruveg and the placebo group at the end of the supplementation period was ≈60 μmol/L (Figure 2 in reference 1). The expected relative difference in TAC, based on the stoichiometry of ascorbic acid, should have been ≈10% for FRAP (10). This small, but generally discernable, effect on TAC, may have been masked by the reduced sensitivity of the TAC protocols applied in this study.

In conclusion, this interesting and valuable study by Dragsted et al (1) highlights both a requirement for optimized assay conditions and the need to consider the possibility of dynamic mechanisms of control of the body’s redox defenses when designing human intervention studies with dietary antioxidants. Measurements of TAC (the sum of the parts) and of single antioxidants (parts of the sum) are useful biomonitoring tools in supplementation and health-related studies of redox balance. However, an understanding of the physiologic mechanisms of control of the body’s redox defenses is an important issue that must be addressed to clarify the role of dietary antioxidants in disease prevention.

None of the authors had any conflict of interest.

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Dear Sir:

We appreciate the comments on our paper (1) made by Serafini et al, who highlight some important problems in the interpretation and power of biomarker-based human intervention studies. Serafini et al’s letter contains 2 major points of criticism. The first concerns the power of biomarker-based human intervention studies. Serafini et al, who highlight some important problems in the interpretation and power of biomarker-based human intervention studies.

In conclusion to this point, we agree that our assay sensitivity was probably not optimal and that our absolute values for TEAC may have been offset by the shortcomings of our automated equipment. However, we disagree that this seriously affected our power to detect a real change in measures of antioxidant capacity. The major source of noise in the measurement of plasma antioxidant capacity is the interindividual variation, which was similar in our study to that of the measurement of plasma antioxidant capacity.

In conclusion, we can speculate that prolonged dietary changes are necessary to affect antioxidant capacity. For example, the lifestyle factors leading to type 2 diabetes may also result in chronic decreases in plasma antioxidant capacity, apparently as the result of changes in uric acid metabolism (9, 10). Whether fruit and vegetables would counteract this effect in the long run remains to be investigated. Therefore, our conclusion that a large intake of fruit and vegetables does not affect fasting plasma measures of antioxidant capacity seems valid and in accordance with the literature.
observed by others, including the cited reference studies. Consequently, we still conclude that there was no significant effect of fruit and vegetables on fasting plasma antioxidant capacity within the 25-d study period.

None of the authors had any conflict of interest related to the results and discussion published in this letter.

FIGURE 1. Mean (±SE) ferric-reducing ability of plasma (FRAP) and fasting plasma Trolox-equivalent antioxidant capacity (TEAC) determined according to reference 1 in samples collected before (day 0), during (days 3–25), and after (days 33 and 54) intervention with 600 g fruits and vegetables (●); a corresponding supplement containing nutrients, vitamins, and minerals (▲); or a placebo pill plus an energy-balancing drink (■). The start and end of the intervention are marked with vertical arrows. None of the groups differed significantly at any time point by repeated-samples analysis of covariance.
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REFERENCES

Body mass index and survival in incident dialysis patients: the answer depends on the question

Dear Sir:

In a recent issue of the journal, Johansen et al (1) examined an important question—What is the association of body size with survival adjusted for muscle mass in incident dialysis patients? However, there are really 3 questions: 1) What is the independent association between muscle mass and mortality, 2) What is the independent association between BMI and mortality, and 3) How does mortality vary across different levels of BMI and muscle mass combined. Based on the answer to the question posed by Johansen et al, inferences on whether body composition influences the survival of incident dialysis patients with a high BMI could not be drawn.

We reexamined the data from our earlier study (2), which the authors graciously discussed. Details on study population, inclusion criteria, data collection, and statistical methods were described earlier (2). In 70 028 incident hemodialysis patients in the United States, from 1 January 1995 to 31 December 1999, the associations of BMI categories described by Johansen et al with survival were examined in a multivariable parameteric proportional hazards survival model adjusted for urinary creatinine, demographics, comorbid conditions, serum albumin, and functional status. The results (Figure 1) are similar to those reported by Johansen et al.

To further examine the influence of body composition on survival in high-BMI patients, each of the BMI groups was divided into subgroups on the basis of muscle mass: low (urinary creatinine <25th percentile), normal, or high (urinary creatinine >25th percentile) subgroups. The hazard ratios from the multivariable parameteric proportional hazards survival model, adjusted for all of the above factors except urinary creatinine, are presented in Figure 2.

At first glance, Figures 1 and 2 appear contradictory, but, in reality, they are not. Adjustment for urinary creatinine in the multivariable model (Figure 1) does not mean that the hazard of death is constant across the spectrum of urinary creatinine values in any
given BMI group (Figure 2). Whether the association of BMI with survival is confounded by muscle mass is examined in Figure 1. Whether those with a large body size but low muscle mass have a survival advantage over “healthy” patients with a normal BMI and a normal or high muscle mass is examined in Figure 2.

In our study we summarized the findings in Figure 2 as “the survival advantage conferred by high BMI in dialysis patients is limited to patients with normal or high muscle mass.” We understand the concerns of Johansen et al that this could be construed as independence. We rephrase our conclusions as follows. Patients with a
high BMI and low muscle mass have a higher mortality than do 
“healthy” incident dialysis patients with a normal BMI and normal 
or high muscle mass. On the other hand, patients with a high BMI and 
normal or high muscle mass have a lower mortality than do “healthy” 
incident dialysis patients with a normal BMI and normal or high 
muscle mass. Thus, compared with “healthy” incident dialysis pa-
tients with a normal BMI and normal or high muscle mass, those with 
a high BMI have a lower mortality only if their muscle mass is 
normal or high.

In conclusion, the questions addressed in the 2 studies were re-
lated but had different emphases. We absolutely agree with Johansen 
et al that body size is an important determinant of survival in incident 
dialysis patients. However, we stand by our earlier conclusion that, 
in incident dialysis patients, body size and body composition influ-
ence survival. In incident dialysis patients, adiposity confers a sur-
vival advantage over undernutrition, but higher muscle mass is better 
than higher body fat. We agree that, given the current data, incident 
dialysis patients should not be encouraged to lose weight but should 
be encouraged to increase muscle mass rather than fat mass.

None of the authors had a conflict of interest.

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and body composition on survival in hemodialysis patients. J Am Soc 

Reply to S Beddu et al
Dear Sir:

We appreciate the comments of Beddu et al regarding our recent 
publication that examined the relation between body size and out-
comes among incident hemodialysis patients (1). In particular, we 
agree with the idea that body composition, and perhaps muscle mass 
in particular, is important to consider for patients receiving dialysis. 
However, it is important that, in our discussion of the “best” way to 
adjust for muscle mass in these patients, we not lose sight of the 
larger issues at hand. First, although analyses using large data sets are 
often constrained to the use of body mass index or similar weight-
for-height indexes as the primary indicator of body size, they are

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None of the authors had a conflict of interest.

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Diet and risk of ischemic heart disease in India

Dear Sir:

We would like to point out some problems with the interesting 
article by Rastogi et al (1) that was recently published in the Journal. 
In this study, only 12% of the subjects were women; the remaining 
88% were men. Generally speaking, in India, men are not involved 
in cooking. Hence, the men in this study may not have been able to 
correctly specify the amount of cooking oil that would be used.

In Table 4, there are some factors that were not significant in the 
univariate analysis but that were significant in the multivariate anal-
ysis because of an interaction among the variables. A better way of 
presenting these data would have been to present data for only those 
variables that were significant in the univariate analysis and then 
subjected to multivariate analysis to determine the variation in rel-
ative risk. Another problem with the study was that type of personal-
ality and stress were not taken into consideration, which may have 
confounded the results.

None of the authors had a conflict of interest.
LETTERS TO THE EDITOR

Reply to TV Chacko et al

Dear Sir:

We appreciate the comments provided by Chacko et al regarding our recent article on diet and risk of ischemic heart disease in India (1). Most of our study participants were indeed men, despite the fact that we recruited only incident myocardial infarction cases from 8 different hospitals in New Delhi and Bangalore. Although it is true that women in India are primarily involved in cooking foods, we qualitatively observed that men are often involved in the shopping for food items, particularly those items purchased in bulk, such as flour, rice, and cooking oils. Thus, men are knowledgeable about the oils used in food preparation. Moreover, our analysis did not consider the amount of oil used in cooking but simply the type of oil used.

Concerning the comment about Table 4, it is important to consider the possibility that some variables may not have been significant in the univariate analyses because of confounding. Ignoring variables that are not significant in univariate analyses from further consideration could, thus, lead to biased results. Because this was one of the first epidemiologic studies of diet and heart disease in India, we believed it prudent to present the findings for all food groups because these preliminary findings could stimulate further research.

Stress is an important factor in coronary heart disease risk. Unfortunately, we did not have the opportunity to examine the association between stress and risk of coronary heart disease in our study. However, we believe it unlikely that stress would have confounded our results because the diets of individuals in our study population were determined, to a large extent, by the overall dietary pattern of their families.

None of the authors had a conflict of interest.

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Serum cholesterol and visuomotor speed: inverse or direct association?

Dear Sir:

The article by Zhang et al (1) contains an apparent contradiction in its findings. In the abstract, the authors first state that “...we found inverse linear associations of serum total cholesterol and non-HDL cholesterol with visuomotor speed...”. However, the conclusion of the abstract then states, “Low serum total cholesterol and non-HDL cholesterol are associated with slow visuomotor speed...” which implies a direct, not an inverse, association. Similarly, a portion of the title of the article, “Serum cholesterol concentrations are associated with visuomotor speed,” and the statement in the discussion that “...we documented that low serum TC [total cholesterol] and low serum NHDLC [non-HDL cholesterol] concentrations are significantly associated with slow visuomotor speed...” imply a direct association.

The source of the confusion is the incorrect way in which Zhang et al define “visuomotor speed.” They state that “Visuomotor speed was measured by the SRT [simple reaction time] test,” and that “The measured response was the latency (in ms)...”. Consistent with this definition, their Table 2 reports latency values in ms under the heading “Visuomotor speed.” However, the term “speed” refers to rate of response, conventionally defined as distance traveled divided by time (2); thus, visuomotor speed would be the reciprocal of latency (ms^-1), not latency itself. In the first statement quoted above in which Zhang et al declare their finding an inverse relation, they use “visuomotor speed” as they have incorrectly defined it, namely as latency. However, in the remaining quoted statements, they seem to be using it in its conventional and correct sense, as the reciprocal of latency. Hence, their statements appear contradictory.

The solution is to consistently use the term “visuomotor speed” to refer to the reciprocal of latency. Then their finding, when clearly stated, is that the higher the cholesterol concentration, the higher the visuomotor speed—a direct association. This is an interesting and provocative finding. It would be regrettable if their inconsistent use of an incorrect definition prevented this important finding from being readily appreciated.

The author had no conflict of interest to declare.

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Reply to SL Black

Dear Sir:

We appreciate Black’s comments and thank him for providing a detailed explanation. It is true that many neuropsychological tests involve timed responses, such that longer response latencies correspond to poorer performance (1). In describing test results, however, there is a tendency to refer to the “speed” of the subject’s response, which is essentially the inverse of response latency. Black’s suggestion that data be scored and reported as the inverse of response latency, corresponding more directly to the concept of speed, is quite appropriate and would have enabled us to avoid confusion among readers (2).

None of the authors had a conflict of interest to declare.

Matthew F Muldoon

Erratum


In Table 4 of this article, the Month 0 LDL-cholesterol concentrations are incorrect. The correct concentrations (all in mmol/L) are as follows: Placebo group, 3.7 ± 1.15; CLA-FFA group, 3.3 ± 0.80; and CLA-triacylglycerol group, 3.6 ± 0.97.

Erratum


The first author’s name is incorrectly printed in the journal. It should appear as SoJung Lee.