Conjugated Linoleic Acid Impairs Endothelial Function

Justin S.W. Taylor, Simon R.P. Williams, Rhian Rhys, Phillip James, Michael P. Frenneaux

Objectives—To determine the effect of dietary supplementation with conjugated linoleic acid (CLA) on body mass index (BMI), body fat distribution, endothelial function, and markers of cardiovascular risk.

Methods and Results—Forty healthy volunteers with BMI > 27 kg/m² were randomized to receive a CLA isomeric mixture or olive oil in a 12-week double-blind study. Subcutaneous body fat and abdominal/hepatic fat content were assessed using skin-fold thicknesses and computed tomography scanning, respectively. Endothelial function was assessed by brachial artery flow-mediated dilatation (FMD). Plasma isoprostanes were measured as an index of oxidative stress. CLA supplementation did not result in a significant change in BMI index or total body fat. There was a significant decrease in limb (−7.8 mm, \(P<0.001\)), but not torso skin-fold thicknesses or abdominal or liver fat content. Brachial artery FMD declined (−1.3%, \(P=0.013\)), and plasma F2-isoprostanes increased (+91 pg/mL, \(P=0.042\)).

Conclusions—A CLA isomeric mixture had at most modest effects on adiposity and worsened endothelial function. On the basis of these results, the use of the isomeric mixture of CLA as an aid to weight loss cannot be recommended.

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Key Words: body composition • conjugated linoleic acid • endothelial function • obesity • oxidative stress

Abdominal obesity¹ and the associated dysmetabolic syndrome² confer increased cardiovascular risk. Dietary modification with n-3 polyunsaturated fatty acids appears to reduce the risk of coronary artery disease and improve mortality.³–⁵ Conjugated linoleic acid (CLA) is a naturally occurring fatty acid and is found in dairy products and meat from ruminants. It differs from the better known linoleic acid by having an extra carbon-carbon double bond. It has been widely promoted in the lay press,⁶ with claims that it can prevent and treat cancer,⁷ prevent heart disease,⁸ improve immune function,⁹ and treat obesity.¹⁰ Whereas some of these effects are supported by studies in animals,⁸,¹¹ there is conflicting published human research on CLA, and in particular there have been no conclusive studies measuring its effect on markers of cardiovascular risk. Furthermore, it is now realized that the different isomers of CLA may have very different biological properties and may have different mechanisms of action.¹² The 2 main CLA isomers that have been studied are 9,11 and 10,12 CLA. Other studies have suggested that CLA supplementation may increase oxidative stress, although this was not proven.¹⁶,¹⁷ Furthermore, the small number of studies thus far published have suggested that in humans, weight loss produced by CLA supplementation is at most modest.¹⁰,¹³,¹⁶,¹⁸ To assess the efficacy of CLA as an aid to weight loss and its effect on cardiovascular risk factors, we undertook a double blind study examining the effects of a commercially available isomeric mixture of CLA on body weight, body fat mass and distribution, endothelial function, insulin sensitivity, and markers of oxidative stress in overweight middle-aged men.

Methods

Subjects

Forty nonsmoking white men, aged 35 to 60, without diabetes, hypertension, or cardiovascular disease, with a body mass index (BMI) > 27 kg/m² were recruited from the local community through media advertisements. All subjects gave informed written consent and the protocol was approved by the local research and ethics committee.

Protocol

Subjects were randomly assigned to receive 4.5 g/d of CLA(isomeric mixture 60 calories/d) or olive oil(54 calories/d). The randomization was performed by an independent observer who also matched subjects by age and BMI. The isomeric mixture contained 35% 9c,11t-CLA, 36% t10,c12-CLA, 1% to 2% 9c,11c and 10c,12c CLA, 1.5% 9c,11t and 10t,11t-CLA, and ≤1% 8c,10 and c11,t13-CLA. The CLA and olive oil capsules were supplied by Natural Lipids (Hovdebygda, Norway). All vascular measurements were made in the morning after an overnight fast, at the beginning of the study, and after 12 weeks of supplementation.

Body Composition

Body weight and height were measured and BMI calculated. Skinfold thicknesses were measured according to standard guidelines¹⁹ with Harpenden skinfold calipers (Holtain Ltd, Crymych, UK) at the following sites: biceps, triceps, front mid-thigh, medial calf, subscapular, mid-axillary, and abdominal. Abdominal, waist, and hip girths were measured. All measurements were made in triplicate and averaged. Bioelectrical impedance analysis was performed using the

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tetrapolar method and a Bodysat 1500 analyser (Bodysat Ltd, Isle of Man). Abdominal adipose tissue and liver fat were measured using two computed tomography (CT) images as described previously. Abdominal adipose tissue is presented as a surface area at the level of the fourth lumbar vertebrae, and hepatic and splenic fat is presented as radiographic density in Hounsfield units (HU). Images were acquired using a Somatom Plus 4 scanner. CT slices were 10 mm in thickness and were obtained at 120 kV and 200 mA, with a 42-cm field of view and a 512x512 matrix. Image analysis was performed using dedicated software (SliceOmatic Version 4.2; Tomovision, Montreal, Canada).

Endothelial Function
Changes in brachial artery diameter in response to reactive hyperemia (FMD) were measured noninvasively using a high-resolution ultrasonic wall-tracking system (Vadirec Wall-track System™ as previously validated. Studies were performed at a controlled temperature of 21°C, with subjects supine and their arm held outstretched on a cushion. Baseline measurements of internal brachial artery diameter were taken after 15 minutes of rest. Reactive hyperemia was produced by releasing a pediatric sphygmomanometer wrist cuff inflated to systolic pressure plus 50 mm Hg for 5 minutes. Internal brachial artery diameter was measured every minute after cuff release, and the maximum change from baseline was used to calculate FMD. Data are presented as the percentage diameter change from baseline in the brachial artery.

Laboratory Measurements
Venous blood samples were freshly analyzed for glucose, insulin, cholesterol (including low-density lipoprotein and high-density lipoprotein), and C-reactive protein. Further samples were centrifuged and the supernatant frozen at −80°C. These samples were analyzed later using enzyme immuno-linked assays for leptin (Alexis), adiponectin (Biogenesis UK), F2-isoprostanes (Alexis), and tumor necrosis factor-α (paired antibody enzyme-linked immunosorbent assay). Insulin sensitivity was measured indirectly using HOMA-IR [fasting serum insulin (μU/mL) x fasting plasma glucose (mmol/L)/22.5].

Statistical Analysis
Analysis was performed using SPSS v11.5. Baseline values are presented as mean±SD. Variables with a skewed distribution were logarithmically transformed to achieve a normal distribution prior to analysis. Baseline comparison between the CLA and olive oil groups was assessed by Student t test. The effects of treatment with CLA versus olive oil were assessed by using ANCOVA using baseline values as covariates and are presented with 95% confidence intervals. To avoid type I errors, post-hoc Bonferroni corrections were applied to the groups of primary objective measurements. Therefore, 2-tailed P<0.025, P<0.005, P<0.001, P<0.025, and P<0.008 were regarded as significant for blood pressure, skin-folds, girths, FMD, and abdominal CT measurements, respectively. A 2-tailed P<0.05 was regarded as significant for primary objective measurements weight, and bioimpedance, and the exploratory blood analyses.

Results
Baseline measurements did not differ significantly in the olive oil (19 subjects) and CLA (21 subjects) groups (Table 1). All subjects completed the study. The effect of 12 weeks of supplementation is shown in Table 2. The important changes are also displayed in the Figure. There were no significant changes in parameters in the olive oil group except for a decline in tumor necrosis factor-α (−61pg/mL [95% CI, −3 to −120]; P=0.04). In the CLA group there was no significant change in body mass (−1.1 kg [95% CI, −2.3 to 0.4]; P=0.06), BMI (−0.4kg/m² [95% CI, −0.8 to 0.03]; P=0.07), or total body fat (−1% [95% CI, −2.5 to 0.5];

### Table 1. Characteristics of Olive Oil and CLA Groups at the Start of the Study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Olive Oil (n=19)</th>
<th>CLA (n=21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>47±8</td>
<td>45±6</td>
<td>NS</td>
</tr>
<tr>
<td>Mass, kg</td>
<td>97±13</td>
<td>101±9</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>33±3</td>
<td>33±3</td>
<td>NS</td>
</tr>
<tr>
<td>Fat, % (Bioimpedance)</td>
<td>29±3</td>
<td>28±4</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>128±13</td>
<td>122±10</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>85±7</td>
<td>80±8</td>
<td>NS</td>
</tr>
</tbody>
</table>

### Anthropometric measurements
- Skin-folds: Biceps, mm: 12±4 vs 11±4 NS
- Skin-folds: Triceps, mm: 20±5 vs 19±5 NS
- Skin-folds: Mid-thigh, mm: 23±7 vs 25±8 NS
- Skin-folds: Medial calf, mm: 16±4 vs 16±5 NS
- Skin-folds: Subscapular, mm: 32±6 vs 32±7 NS
- Skin-folds: Mid-axillary, mm: 23±4 vs 24±6 NS
- Skin-folds: Abdominal, mm: 33±7 vs 35±6 NS
- Skin-folds: Limb, mm: 72±17 vs 72±18 NS
- Skin-folds: Torso, mm: 123±13 vs 128±16 NS
- Skin-folds: Torso:Limb ratio: 1.8±0.4 vs 1.9±0.3 NS
- Girth: Abdomen, cm: 107±7 vs 107±6 NS
- Girth: Hip, cm: 110±6 vs 110±5 NS
- Girth: Waist:Hip ratio: 1.02±0.03 vs 1.01±0.04 NS
- Girth: Abdominal:Hip ratio: 0.98±0.05 vs 0.97±0.05 NS
- Brachial artery diameter, mm: 3.8±0.5 vs 3.7±0.4 NS
- Brachial artery FMD, %: 2.1±2.1 vs 2.2±2.7 NS
- Liver, HU: 60±13 vs 66±10 NS
- Spleen, HU: 64±2 vs 61±2 NS
- Liver:Spleen ratio: 0.93±0.18 vs 1.08±0.18 NS
- Visceral area, cm²: 242±82 vs 204±70 NS
- Subcutaneous fat area, cm²: 341±80 vs 357±93 NS
- Visceral:Subcutaneous ratio: 0.75±0.38 vs 0.63±0.37 NS
- Total cholesterol, mmol/L: 3.6±0.9 vs 3.4±0.8 NS
- HDL cholesterol, mmol/L: 1.0±0.3 vs 1.1±0.3 NS
- Triglycerides, mmol/L: 5.6±0.9 vs 5.4±0.8 NS
- CRP, mg/L: 5.2±2.9 vs 4.8±2.7 NS
- Glucose, mmol/L: 5.2±0.6 vs 5.1±0.7 NS
- Insulin, mU/L: 18±22 vs 13±7 NS
- HOMA-IR: 4.6±6.4 vs 3.1±1.8 NS
- Leptin, ng/mL: 21±12 vs 22±16 NS
- Adiponectin, pg/mL: 8±6 vs 9±6 NS
- F2-isoprostanes, pg/mL: 135±67 vs 99±103 NS
- Tumor necrosis factor-α, pg/mL: 122±97 vs 109±145 NS

NS indicates not significant.

P=0.18. There was a significant decrease in limb (−7.8 mm [95% CI, −11.1 to −4.5]; P<0.001), but not torso (−4.0 mm [95% CI, −11.5 to 3.5]; P=0.29) skin folds, and a significant increase in the total torso-to-limb skin-fold ratio (+0.13 [95%
There was no significant change in abdominal, waist, or hip girths, or in subcutaneous abdominal fat and liver fat measured by CT. However, there was a significant decrease in brachial artery FMD (−1.3% [95% CI, −2.4 to −0.3]; \( P=0.013 \)), and a significant increase in plasma F2-isoprostanes (91 pg/mL [95% CI, 3 to 178]; \( P=0.042 \)). There was no change in estimated insulin sensitivity, total cholesterol, low-density lipoprotein cholesterol,
CRP and an improvement in insulin resistance. A study that examined the effect of the Mediterranean diet in patients with the metabolic syndrome found a reduction in body mass index (BMI) after 2 years of following the Mediterranean diet on cardiovascular risk. A 2-year study has been a great deal of interest in the effect of the Mediterranean diet in patients with increased cardiovascular risk, and intentional weight loss associated with improved cardiovascular risk. Recently there has been a great deal of interest in the effect of the Mediterranean diet on cardiovascular risk.

Discussion

Obesity, and in particular abdominal obesity, is associated with increased cardiovascular risk, and intentional weight reduction improves cardiovascular risk. Recently there has been a great deal of interest in the effect of the Mediterranean diet on cardiovascular risk. A 2-year study examining the effect of the Mediterranean diet in patients with the metabolic syndrome found a reduction in body weight, an improvement in endothelial function, a decrease in CRP and an improvement in insulin resistance. A study that tried to identify which component of the Mediterranean diet was responsible for improving cardiovascular risk paradoxically found that olive oil impaired endothelial function, although this was inversely correlated with changes in triglycerides. The conclusion of the study was that it was the antioxidant and omega-3–rich foods that conferred cardiovascular benefit. Nevertheless, these studies supported the view that dietary modification or supplementation may have a significant impact on obesity and, in particular, cardiovascular risk.

Experimental evidence in animal models suggests that CLA supplementation, in particular the 10,12 CLA isomer, induces fat mass loss. On the basis of this initial evidence, there has been a great deal of interest in its use as an aid to lose fat and weight in humans. Blankson et al reported that 12-week supplementation with >3.4 g/d isomeric CLA significantly reduced body fat mass in overweight volunteers, although there was no change in weight or BMI, and Riserus et al found that only 4-week supplementation with 4.2 g/d isomeric CLA significantly improved sagittal abdominal diameter. However, Zambell et al found no significant change in weight, BMI, or fat mass after 3-g/d supplementation with isomeric CLA (in women who were not overweight). The observation in two studies of an impairment of insulin sensitivity have raised concerns. This study was therefore designed to assess the effect of CLA supplementation on BMI, body fat distribution, and markers of cardiovascular risk, including endothelial function.

Our study found that an isomeric mixture of CLA did not cause significant weight loss (although there was a trend to weight loss of 1.1 kg). This is consistent with the 0.24 to 0.46 kg weight loss reported in previous studies. Although CLA reduced limb fat, it had no effect on abdominal fat or liver fat (although there was a nonsignificant trend to an increase in liver fat, measured as a decrease in liver density in Hounsfield units). This finding is in contrast to a previous report that found a decrease in sagittal abdominal diameter after 4 weeks of 4.2 g/d CLA. However, the suggestion that CLA may have a lipodystrophic effect is not new. A study supplementaling mice with isomeric CLA found a reduction in fat mass, liver hypertrophy, and an increase in insulin resistance, whereas mice fed the 10,12 CLA isomer had hyperinsulinemia and an increase in liver fat develop. The mechanism for this is not clear, although a rapid decrease in leptin and adiponectin has been observed in mice only 2 days after starting CLA supplementation. A decrease in leptin has also been observed in rats. This hypothesis is supported by the observation that hyperinsulinemia and liver steatosis are partially reversed when hypoleptinemia is normalized by leptin infusion in CLA lipoatrophic mice. However, there is conflicting evidence regarding the effects of CLA supplementation on plasma leptin in humans. One study supplementaling patients with type 2 diabetes with CLA found a decrease in leptin, but another study supplementaling obese men with CLA found no change in leptin. No clinical studies in humans have measured adiponectin after CLA supplementation. In contrast to these findings, a study supplementaling Zucker diabetic fatty rats with CLA found that the previous impaired glucose tolerance improved.

Cell culture and animal studies have suggested other potential mechanisms by which CLA may reduce body fat, including reducing apolipoprotein B secretion in HepG2 cells, increasing carnitine palmitoyltransferase activity and decreasing lipoprotein lipase activity, and increasing tumor necrosis factor and uncoupling protein levels. The role of peroxisome proliferator-activated receptor gamma (PPARγ) activity is not clear. PPARγ activity was increased in Zucker diabetic fatty rats and genetically obese mice fed CLA, although several in vitro studies have found that 10,12 CLA downregulates PPARγ activity in mice adipocytes. In vivo and in vitro studies in pigs have also found that CLA induced an increase in PPARγ activity. However, human studies have found an opposite effect on PPARγ. Human adipocytes cultured in vitro with 10,12 CLA decreased the expression of PPARγ and diabet patients treated with PPARγ agonists (glitazones) experience decreased central fat and increased peripheral fat.
Our observations are important because reducing nonabdominal fat is less likely to reduce cardiovascular risk, and an increase in hepatic fat will increase insulin requirements.47 Thus any weight loss with this regime is at most modest and the pattern of weight loss is not metabolically favorable.

Furthermore, we found that CLA significantly impaired brachial artery endothelial function, consistent with an adverse impact on cardiovascular risk.51 The mechanism of this effect is not clear. We and others16 found an increase in F2 isoprostanes, a lipid peroxidation product generally considered to be a marker of increased oxidative stress. Another previous study reported an increase in plasma CRP, a marker of inflammation.17 Taken together, these data suggest that this CLA regime impairs endothelial function and that this may, at least in part, be caused by increased oxidative stress. It is possible that the observed change in FMD in this study has been caused by the change in limb skin-fold thicknesses interfering with the FMD technique (for example, by altering wrist arterial occlusion pressure or changing depth from probe to brachial artery), thus giving a false measurement. However, we feel that this is unlikely because a high wrist arterial occlusion pressure was used and a change in depth to the brachial artery of 1 to 2 mm is well within the capability of the ultrasound probe. Brachial artery FMD measurements vary markedly from laboratory to laboratory, dependant on the exact technique used.48 In this study, we used the wrist cuff technique, which results in lower values of brachial artery FMD than those obtained using the upper arm or mid-femoral techniques. We did not observe an adverse impact on insulin sensitivity. However, the study may have been under-powered to detect differences using the HOMA technique. We did not identify any change in plasma lipid profiles.

The effect of CLA on other cardiovascular risk factors has been examined. One study reported that CLA decreased platelet aggregability,53 but another reported no change in platelet aggregability.54 A small decrease in total, low-density lipoprotein, and high-density lipoprotein cholesterol was found in overweight men taking 1.7 g/d CLA and 3.4 g/d CLA, although this was not maintained at higher doses.10 A further study in obese men using 10,12 CLA and a CLA mixture lowered high-density lipoprotein cholesterol, although no change was observed in total or low-density lipoprotein cholesterol or triglycerides.13 No change in lipids was found in a study with healthy women supplemented with 3.9 g/d CLA.55

Consistent with our observations, 4.2 g/d CLA for 1 month was found to increase urinary isoprostanes in men with abdominal obesity.16 Isoprostanes are produced from peroxidation of lipids, and it was suggested that the increase in isoprostanes might be simply a result of increased fat lipolysis, rather than indicating increased oxidative stress. However, more recent studies have found that 10,12 CLA increases insulin resistance13 and plasma CRP.17 Taken together with our observation of an impairment of endothelial function, it seems highly likely that the increase in isoprostanes does indeed imply an increase in oxidative stress.

Conclusions

CLA supplementation for 12 weeks using the regime used in this study had no significant effect on BMI. Even if this represents a type 2 error, the reduction is at most modest, consistent with previous reports in man. Furthermore, the pattern of fat loss is peripheral rather than central. Importantly, the observed impairment of endothelial function and increase in markers of oxidative stress raise concerns about the widespread use of this agent until further studies demonstrate its cardiovascular safety or otherwise.

References


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