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 (+91pg/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.

Key Words: body composition ■ conjugated linoleic acid ■ endothelial function ■ obesity ■ oxidative stress
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.21,49,50 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 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 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)×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 log-transformed for a normal distribution prior to analysis. Baseline comparison between the CLA and olive oil groups was 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 (−61μg/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 0.04]; 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]; 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]; \( P=0.017 \)). There was also 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 (+91pg/mL [95% CI, 3 to 178]; \( P=0.042 \)). There was no change in estimated insulin sensitivity, total cholesterol, low-density lipoprotein cholesterol,
Discussion

Obesity, and in particular abdominal obesity, is associated with increased cardiovascular risk,23–24 and intentional weight reduction improves cardiovascular risk.25,26 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 increased peripheral fat.46 However, several in vitro studies have found that 10,12 CLA supplementation on plasma leptin in humans. One study supplementing patients with type 2 diabetes with CLA found a decrease in leptin,38 but another study supplementing obese men with CLA found no change in leptin.13 No clinical studies in humans have measured adiponectin after CLA supplementation. In contrast to these findings, a study supplementing Zucker diabetic fatty rats with CLA found that the previous impaired glucose tolerance improved.39

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,40 increasing carnitine palmitoyltransferase activity and decreasing lipoprotein lipase activity,30 and increasing tumor necrosis factor and uncoupling protein levels.31 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,39,41,42 although several in vitro studies have found that 10,12 CLA downregulates PPARγ activity in mice adipocytes.43,44 In vivo and in vitro studies in pigs have also found that CLA induced an increase in PPARγ activity.45 However, human studies have found an opposite effect on PPARγ. Human adipocytes cultured in vitro with 10,12 CLA decreased the expression of PPARγ,41,43 and diabetic patients treated with PPARγ agonists (glitazones) experience decreased central fat and increased peripheral fat.46

There was a significant negative correlation between change in F2-isoprostanes and change in total limb skin folds (ie, loss of limb skin-fold thickness was associated with an increase in F2-isoprostanes) for the entire group (CLA + olive oil) (P = 0.012) but no significant correlation when each group was analyzed separately. There was no significant correlation between change in endothelial function and change in F2-isoprostanes or change in limb skin-fold thicknesses.
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. 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. The mechanism of this effect is not clear. We and others found an increase in F$_2$ 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. 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. 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-forearm 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, but another reported no change in platelet aggregability. 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. 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. No change in lipids was found in a study with healthy women supplemented with 3.9 g/d CLA.

Consistent with our observations, 4.2 g/d CLA for 1 month was found to increase urinary isoprostanes in men with abdominal obesity. 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 resistance and plasma CRP. 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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