Conjugated linoleic acid supplementation for 8 weeks fails to impact body composition, lipid profile, or safety parameters in overweight, hyperlipidemic men [1, 2, 3]

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[8]Abbreviations used: β-OHB, β-hydroxybutyrate; *c, cis*; CRP, C-reactive protein; CVD, cardiovascular disease; hs, high sensitive; Ox-LDL, oxidized LDL; RCFFN, Richardson Center for Functional Foods and Nutraceuticals; *t, trans*.

ABSTRACT

The usefulness of conjugated linoleic acid as a nutraceutical remains ambiguous. Our objective was, therefore, to investigate the effect of CLA on body composition, blood lipids and safety parameters in overweight, hyperlipidemic men. A double-blinded, 3-phase crossover trial was conducted in overweight (BMI ≥ 25 kg/m^2); borderline hypercholesterolemic (LDL-C ≥ 2.5 mmol/L) men aged 18-60 y. During three 8-wk phases, each separated by a 4-wk washout period, 27 subjects consumed under supervision in random order 3.5 g/d of (i) safflower oil: Control, (ii) 50:50 mixture of *trans* 10, *cis* 12 (*t*10, *c*12) and *cis* 9, *trans* 11 (*c*9, *t*11) CLA: Clarinol G-80® and (iii) *c*9, *t*11 isomer: *c*9, *t*11 CLA. At baseline and endpoint of each phase body weight, body fat mass and lean body mass were measured by DXA. Blood lipid profiles and safety biomarkers, including insulin sensitivity, blood concentrations of adiponectin, and inflammatory [hs-C-reactive protein (CRP), TNF-α, IL-6] and oxidative (oxidized-LDL) molecules were measured. Effect of CLA consumption on fatty acid oxidation was also assessed. Results show that CLA treatments did not affect body weight, body composition or blood lipids compared to control, either at phase end or as change from baseline to endpoint. CLA failed to impact β-oxidation rate of fatty acids. No significant alterations were observed in any of the safety parameters tested. In conclusion, while no detrimental effects were observed with supplementation, these results fail to confirm a role for CLA in either body weight or blood lipid regulation in humans.

INTRODUCTION

Nutraceutical and functional food approaches are being investigated as viable options to help combat the obesity epidemic (1). Positional and geometric isomers of linoleic acid, known as conjugated linoleic acid, have shown promise with respect to modulation of body composition, but the majority of the evidence is from *in vitro* and animal models. In mice, CLA induces as large as a 60 % reduction in body fat mass (2). Less dramatic, results have been observed in rats (3), pigs (4), and hamsters (5). It has been established that the *trans* 10, *cis* 12 isomer (*t*10, *c*12 CLA), rather than the *cis* 9, *trans* 11 (*c*9, *t*11 CLA; 'Rumenic acid') isomer, is primarily responsible for these effects (Reviewed in 6, 7). CLA supplements for humans promoting weight loss and increased lean body mass are currently being marketed in the form of a 50:50 mixture of the *t*10, *c*12 and *c*9, *t*11 isomers based on these animal data and on limited clinical evidence (8-10). However, CLA does not perform consistently in humans, and therefore its purported anti-obesity effects remain unconfirmed. In fact, a growing number of studies suggest a lack of effect of CLA on body composition (11-13). In addition, CLA's effect on risk markers of cardiovascular disease (CVD) also remains inconclusive. CLA has been shown to have hypo-cholesterolemic, hypo-triglyceridemic and anti-atherosclerotic properties in animals (14). CLA supplementation for 8 wk in normolipidemic men and women lowered plasma TG and VLDL-C (15). However, other studies showed that CLA supplemented at varying doses either in the form of naturally enriched foods or industrially produced supplements, failed to affect blood lipids (11, 13, 16, 17). The discrepancy observed between the effectiveness of CLA in animal and *in vitro* models, and human studies may be due to differences in dose and duration of supplementation, species-specific physiology, gender, as well as the initial metabolic status of the study sample (18).

In addition, controversy surrounds the safety of CLA as a dietary supplement. In mice, CLA causes liver enlargement due to steatosis (19, 20). In humans, CLA has been shown to induce insulin resistance in overweight men (21), possibly due to increases in inflammatory molecules [C-reactive protein (CRP), TNF- α and IL-6] (22-24).

The primary objective of this study was to evaluate the effectiveness of two forms of CLA in modulating body weight and body composition, as well blood lipids, in overweight, hyperlipidemic men in a free living environment. Our secondary objective was to study the effect of the CLA supplementation on selected inflammatory and oxidative markers and insulin sensitivity, considered as safety parameters in the sample population.

SUBJECTS AND METHODS

Subjects. Healthy male volunteers aged 18 to 60 y, with a BMI ≥ 25 kg/m^2 were recruited from the city of Winnipeg, Manitoba. Men responding to radio and newspaper advertisements were initially screened for eligibility by answering a phone-based questionnaire. Individuals were considered ineligible for participation in the study if they were smokers, were consuming 2 or more alcoholic drinks per day, were taking medication that affects lipid metabolism such as cholestyramine, colestipol, niacin, clofibrate, gemfibrozil, probucol, HMG-CoA reductase inhibitors, high dose (4 g/d) dietary supplements, as well as fish oil capsules within 6 mo prior to the start of the intervention. The diagnosis of diabetes mellitus, liver disease, kidney disease or heart disease precluded participation in the study. Potential subjects from this initial stage were invited to the Richardson Center for Functional Foods and Nutraceuticals (RCFFN) at the University of Manitoba to undergo further screening based on anthropometry and a blood draw to determine fasting blood lipid profile. Subsequently, suitable candidates were invited back for assessment of their overall health and fitness involving a second blood draw for routine biochemistry and hematology, and a complete history and physical examination taken by the study physician. Signed informed consent was obtained from subjects prior to any procedure performed on them for study purposes. The study protocol was approved by Health Canada, and the Research Ethics Boards of the University of Manitoba and Dalhousie University. This study was registered at www.clinicaltrials.gov, ID NCT01047280 (January 8, 2010).

Diet and treatments. The three treatments tested in the study were (i) 3.5 g/d of safflower oil (Control) (ii) 3.5 g/d of 50:50 mixture of *t*10, *c*12 + *c*9, *t*11 CLA oil (Clarinol G-80®, containing 2.8 g of total CLA)], and (iii) 3.5 g/d of *c*9, *t*11 CLA (*c*9, *t*11 CLA oil, containing 2.7 g of total CLA). The three experimental oils were kindly provided by Lipid Nutrition (Wormerveer, The

Netherlands). The fatty acid composition of the experimental fats is presented in **Table 1**. The CLA and control treatments were administered to subjects in the form of liquid oil, which was mixed into a constant amount (150 g) of fat-free, sugar-free fruit yogurt. This study was conducted under free-living conditions wherein subjects were able to maintain their usual dietary habits and physical activity level during the intervention phases, as well as washout periods. Study volunteers were required to be present at the RCFFN during week day evening mealtimes when treatment yogurts were administered under supervision of the clinical coordinator, ensuring strict compliance. Subjects were strongly encouraged to partake of a complementary supper buffet that was offered to them during their daily visits. Weekend treatments were provided as 'take away', and empty or unused containers were returned on the following Monday to monitor compliance on the weekends.

Study Protocol. This study was designed as a double-blind crossover clinical intervention in which subjects received the two experimental CLA treatments and a control treatment in random order. The clinical trial comprised 3 treatment phases of 8 consecutive wk each, alternated with 4-wk wash out periods. The crossover nature of the study ensured minimal subject variability as each participant served as his own control. Subjects were instructed by the clinical coordinator to maintain their regular dietary habits and physical activity during the treatment phases, as well as during the washout periods. Physical activity questionnaires and 24 h dietary recalls provided a general idea of any changes in diet and physical activity that may have occurred during the study. Blood biochemistry and hematology were conducted at the end of each phase to ensure that no health abnormalities had occurred as a result of the experimental treatments.

Measurement of body weight and body composition. Subjects weighed themselves on a clinical weighing scale during their daily visits to the RCFFN. BMI was calculated as weight (kg)/height (m^2). Body composition (including overall body fat mass and lean mass) was analyzed at the beginning and end of each phase using DXA. DXA scan series was conducted using General Electric's Lunar Digital Prodigy Advance and the General Electric Prodigy Body Composition software program, EnCore 2005, was used to analyze the scans and generate body composition data.

Blood sampling. On days 1, 2, 56 and 57 of each phase, venous blood samples were collected following a 12 h overnight fast. Subjects were also asked to abstain from alcohol 24 h prior to blood draw. Evacuated blood collection tubes containing disodium EDTA were used for obtaining plasma, whereas serum samples were obtained from blood drawn into uncoated tubes. Blood samples were centrifuged at 1500 rpm for 20 min, and plasma and serum were separated and stored at -80 °C until further analysis. All analyses on the stored samples were completed within 24 mo of initial collection.

Estimation of plasma lipids and lipoproteins. Commercially available enzymatic assays were used to quantify cholesterol (Roche Diagnostics) and triglycerides (Sigma-Aldrich) in plasma samples and lipoprotein gradient fractions. Plasma lipoproteins in 1000 μL of sample were separated by density gradient unltracentrifugation at 55,000 rpm for 20 h in a SW60Ti rotor following pre-treatment of samples as described previously (25). Twenty fractions of 200 μL each were then collected starting from the top of the gradients. Fractions were defined as VLDL=1-4, and HDL=11-20. The cholesterol concentration of each class of lipoproteins was

calculated as the area under the curve of the corresponding density gradient profile (25). LDL-C concentrations were calculated using the Friedewald equation (26).

Assessment of atherogenicity of LDL-C. Oxidation of LDL-C is considered to be one of the key events in the development of atherosclerosis. There is some evidence that CLA can beneficially influence the susceptibility of LDL to oxidation. Plasma concentration of oxidized LDL (Ox-LDL) was measured using a commercially available solid phase two-site enzyme immunoassay (Mercodia, Sweden) with murine monoclonal antibody mAb-4E6. The assay was performed according to the manufacturer's instructions.

Assessment of insulin sensitivity (HOMA index). Insulin sensitivity of subjects was determined by the homeostasis model insulin resistance index (HOMA-IR) (27). HOMA index was calculated using the formula: fasting insulin concentration (μU/mL) x fasting glucose concentration (mmol/L)/22.5. Plasma insulin and glucose concentrations were measured by radioimmunoassay and enzymatic methods, respectively (28, 29).

Estimation of inflammatory biomarkers [hs (high sensitive)-CRP, TNF-∝ and IL-6]. Plasma concentrations of hs-CRP were estimated using Behring latex enhanced high-sensitivity assays, which were conducted on a Behring BN-100 nephelometer (Behring Diagnostic, Westwood, Massachusetts), using calibrators provided by the manufacturer (N rheumatology standards SL). Serum IL-6 and TNF-α concentrations were measured with the help of commercially available ELISA kits (Quantikinine HS immunoassay kits; R&D Systems Inc., Minneapolis, MN).

Measurement of plasma adiponectin. Plasma concentrations of adiponectin were measured with a commercially available ELISA kit according to manufacturer's instructions (Linco Research, St. Charles, MO).

Estimation of ^{13}C-linoleate β-oxidation. CLA is known to be a ligand for PPAR-α (30), and therefore might increase the rate of β-oxidation of fatty acids in muscles. For this purpose, a subset of 10 men from the study were orally administered a dose of uniformly labeled linoleic acid on day 56 of each phase. During the breakfast mealtime subjects consumed a single dose of 50 mg of ^{13}C-linoleate (Cambridge Isotope Laboratories Inc., Andover, USA), which was mixed into butter and spread on half a bagel. β-oxidation of the tracer was measured toward recovery of $^{13}CO_2$ in breath samples collected in duplicate at baseline and every hour for the next 12 h using a breath collection device (Easysampler, Quintron Instrument Company, Milwaukee, WI) and 10 mL evacuated glass tubes (Exetainer, Labco Ltd, Buckinghamshire, UK). A final breath sample was collected at 24h after ^{13}C-linoleate administration. Enrichment of ^{13}C in breath CO_2 following the ingestion of ^{13}C-linoleate was analyzed by isotope ratio mass spectrometry (Europa 20-20, Sercon Ltd, Crewe, Cheshire, UK) using He as the carrier gas (Praxair Canada Inc. Mississauga, ON, Canada). 100% CO_2 gas was used as the reference. Additionally, fasting plasma concentrations of FFA, TG, and β-hydroxybutyrate (β-OHB), the most stable plasma ketone and possibly another marker of β-oxidation, were estimated at the end of each phase. Commercially available reagent kits were used for the analysis of β-OHB (RX Daytona kit; Randox Laboratories Ltd., Antrim, UK), FFA (Wako Diagnostics, Richmond, VA) and TG (Dade Behring Inc., Newark, DE) using an automated clinical chemistry analyzer (Dimension XPand Plus, Dade Behring Inc., Newark, DE).

Statistical analyses. A sample size of 28 was determined based on a power level corresponding to 80 % in detecting an anticipated difference in our primary outcome, which was body fat mass, to a probability level of < 0.05 (31). Taking into account a dropout rate of approximately 25 %, the target sample size for recruitment was determined to be 36. We therefore recruited a final sample of 36. Sample size for the measurement of fatty acid oxidation rate was calculated to be 8 based on the change in the area under the curve with an alpha of 0.05 and a beta of 0.8 (32). A total sample of 10 men, randomly selected from the main study group of 27, ensured that we obtained complete information on beta oxidation of at least 8 participants.

Data were subjected to repeated measures ANOVA based on the comparison between the final data from the end of each phase with the data from the start of the phase. Due to the crossover nature of the study, possible carryover effects were tested by including a term in the statistical model referring to the sequence in which the experimental treatments were administered. However, a repeated-measures ANOVA was used to identify significant differences between the effects induced by the three treatments on total triglycerides, cholesterol, glucose, FFA and β-OHB, and fatty acid oxidation of ^{13}C-linoleic acid at the end of phases only, in a subset of 10 men. Data that were not normally distributed were transformed with the PROC RANK procedure of SAS prior to analysis (hs-CRP, TNF-α, IL-6, HOMA-IR index, triglycerides, total cholesterol, HDL-C, Ox-LDL and adiponectin). Spearman's correlation coefficient was used to identify significant associations between dependent variables. Statistical significance was set at $\alpha=0.05$ for all analyses, and data are presented as their mean ± standard error of mean (SEM). Data were analyzed with SPSS versions 11.5 and 12.0 for Windows (SPSS Inc., Chicago, IL), GraphPad Prism (GraphPad Software Inc. La Jolla, CA) and SAS (SAS Institute Inc., Cary NC).

RESULTS

***Subject characteristics*.** A total of 36 men were recruited. Participants were randomly assigned to one of 6 treatment sequences. Eight subjects withdrew from the study due to reasons described as 'inconveniences of a personal nature' (phase 1-n=6; phase 2-n=2). A total of 28 men successfully completed the entire 3-phase protocol. However, the data of one subject who was diagnosed with mononucleosis during his second washout period have been excluded from the entire statistical analysis. The final sample size was 27. Additionally, one subject's data was removed only from HOMA-IR analysis due to highly unstable fasting plasma glucose concentration. The baseline characteristics of the subject population are presented in **Table 2**. Subjects were overweight or obese (BMI = 30.8 ± 0.8 kg/m^2) (33), with plasma LDL-C concentrations categorized, based on American Heart Association criteria, as being either optimal (<2.6 mmol/L, n=1), near optimal (2.6-3.3 mmol/L, n=9), borderline high (3.4-4.1 mmol/L, n=11), high (4.15-4.9, n=5), or very high (> 4.9, n=1) (34).

***Effect of CLA supplementation on body weight and body composition*.** At the end of the 8-wk supplementation period no significant differences were observed in the changes in body weight, BMI, body fat mass, or lean body mass, either at end of phase or over time from baseline to endpoint, as a result of CLA supplementation compared to control, or between the two CLA treatments (**Table 3**).

***Effect of CLA supplementation on plasma lipids and lipoproteins*.** Compared to control, and between the two CLA treatments, no significant changes were observed in circulating concentrations of total cholesterol and triglycerides at the end of 8 wk of CLA supplementation Plasma lipoprotein concentrations in response to treatments are presented in (**Table 4**).

Consumption of CLA supplements for 8 wk did not alter plasma concentrations of VLDL-C, LDL-C and HDL-C when compared to control treatment and between the two CLA treatments (Table 4).

Effect of CLA supplementation on β-oxidation of linoleic acid. There was no statistical difference between the three treatment phases for the fasting plasma measures in a subgroup of our subjects, as presented in **Table 5**. Consuming CLA as the *c*9, *t*11 CLA or *t*10, *c*12 and *c*9, *t*11 CLA mixture did not increase FFA and β-OHB concentrations compared to the control, and between CLA treatments. Although there seems to be a link between certain variables such as BMI, plasma FFA and β-OHB, we did not find any such significant correlation in this trial among the men consuming any of the treatments (data not shown). These results indicate that CLA supplementation did not increase β-oxidation of ^{13}C-linoleate as seen by the cumulative β-oxidation curves of ^{13}C-linoleate following 8 wk of supplementation (**Figure 1**); neither did it increase ketone production.

Effect of CLA supplementation on inflammatory markers. No significant differences between treatments were observed in change from baseline to endpoint in the blood concentrations of either hs-CRP, TNF-α or IL-6 (**Table 6**).

Effect of CLA supplementation on plasma adiponectin. No significant change in circulating adiponectin concentration was observed as a result of CLA supplementation when compared to control treatment, as well as between the two CLA supplements (Table 6).

Effect of CLA supplementation on plasma oxidized-LDL. At the end of 8 wk of supplementation, no significant effect of CLA treatments was observed on plasma concentrations of oxidized-LDL when compared to control (**Figure 2**).

Effect of CLA supplementation on insulin sensitivity. Determination of the HOMA-IR index as a surrogate marker of insulin resistance indicated that no significant differences between treatments, in change from baseline to endpoint, occurred following 8 wk of supplementation (Table 6).

DISCUSSION

Functional foods such as CLA may aid in controlling the increasing prevalence of obesity and related diseases. The amount of CLA in the diet ingested from ruminant meats, milk and dairy products has been estimated to be approximately 152 mg/d in women and 212 mg/day in men (35), and may be somewhat higher due to the endogenous conversion of vaccenic acid from these foods to CLA (36). However, dietary CLA alone is not sufficient to attain therapeutic levels (~ 3-6 g/d), necessitating the use of CLA supplements and CLA-enriched foods.

The present study shows that supplementation of CLA in overweight, hyperlipidemic men for a period of 8 wk failed to alter body weight or body composition. Similarly, none of the CLA treatments improved blood lipid profiles. These results agree with other human data demonstrating a lack of effect of CLA (as either *c*9, *t*11 isomer, *t*10, *c*12 isomer or *c*9, *t*11 + *t*10, *c*12 CLA mixture) on body mass or the different body compartments from recent clinical trials (11-13, 16, 17, 37, 38). However, a meta-analysis of 18 studies concluded that CLA supplementation (mainly as a 50:50 mixture of *c*9, *t*11 and *t*10, *c*12 isomers) at a dose of 3.2 g/d does reduce fat mass at a rate of 50 g/wk for up to 2 y in both men and women (39). More

283 recently, the ability of CLA to elicit significant reductions in total (40) and regional (trunk and
284 legs) fat mass was observed in a sample of women (40-42). Our study population consisted of
285 only males, which could partly explain the lack of efficacy, but further work in females is
286 required to establish if gender plays a role in determining the effectiveness of CLA in humans.
287 Nevertheless, the results of the current study add to the growing body of evidence that CLA
288 might not be a useful tool for weight management in men.

289 It has also been suggested that CLA possesses cardio-protective potential by regulating
290 markers that are associated with the development of heart disease. However, the failure to elicit a
291 response in the plasma lipid and lipoprotein profiles following CLA supplementation in
292 overweight, hyperlipidemic men in this study is in keeping with recent data indicating that CLA
293 does not impact blood lipids (13, 37). Furthermore, whether CLA in mixed or pure form, is either
294 naturally incorporated into dairy products such as milk (1.3 g CLA/d) (13) and butter (2.59 g
295 CLA/d) (11), or is chemically synthesized, the results on blood lipids are apparently the same
296 suggesting no impact of the CLA dietary form. In an overweight male population similar to the
297 current study (37), consumption of 4.5 g/d of *c*9, *t*11 and *t*10, *c*12 CLA mixture did not improve
298 blood lipid profile which supports our finding.

299 Proposed mechanisms by which CLA, mainly the *t*10, *c*12 isomer, have been shown to
300 reduce fat mass and body weight in animal and *in vitro* studies include decreased enzymatic
301 activity of lipoprotein lipase (43) and stearoyl CoA desaturase (44) leading to decreased TG
302 uptake by adipocytes. Inhibitory effects of CLA have been documented on preadipocyte
303 differentiation via reduction in the expression of transcription factors regulating adipogenesis,
304 such as PPAR-γ (45). In addition, CLA induces higher fat β-oxidation leading to smaller
305 adipocyte size (46) perhaps by increasing the activity of the rate limiting enzyme, carnitine
306 palmitoyl transferase. We, therefore, investigated if CLA consumption would increase linoleic

acid β-oxidation in a subset of 10 subjects. Our results failed to support our hypothesis since neither of the two CLA treatments significantly increased cumulative β-oxidation. Similar results were observed in a recent study in men and women given 4 g/d of a *c*9, *t*11 + *t*10, *c*12 CLA mixture (47). Since cumulative β-oxidation was unchanged after treatment, the concentration of β-OH in the blood also remained unaffected by treatment. However, it is possible that the low number of subjects may have contributed to the lack of a significant effect.

A few studies have reported that CLA supplementation in humans, specifically with the *t*10, *c*12 isomer, increases concentrations of CRP, which is a marker of systemic inflammation (22, 48, 49). The current study did not show such an increase in circulating hs-CRP after 8 wk of both CLA treatments in comparison to control. This difference could be due to our use of safflower oil, instead of olive oil as the control, which has been shown to have strong anti-oxidative property as well as the ability to lower CRP concentrations (50). It is to be noted that olive oil was used as the control in studies in which CLA supplementation increased circulating CRP concentrations (22, 48, 49). None of the two other serum inflammatory markers analyzed in this study varied upon CLA intake and confirm observations reported in previous clinical trials (13, 48, 49). Taken together with the absence of an influence on blood lipid profile, the lack of an effect on inflammatory markers, and adiponectin concentrations provides further evidence that CLA may have no negative impact on CVD risk.

There has been some evidence that CLA increases *in vivo* oxidant stress status. Ox-LDL, formed by the exposure of LDL to oxidizing agents, is also a widely used marker of *in vivo* oxidative stress. Ox-LDL particles are known to play a key role in atherosclerosis by being pro-inflammatory in nature; their concentrations in plasma have been correlated with the presence of insulin resistance and clinical CVD (51). In the present study, a positive correlation was indeed

found between baseline to phase end change in Ox-LDL concentration and change in HOMA index in the study subjects (n=76, r=0.347, p=0.002). However, CLA supplementation for 8 wk did not modify Ox-LDL, suggesting that under the present experimental conditions CLA supplementation does not affect oxidative status in men.

The presence of insulin resistance represents an important step in the sequence of events leading to the development of type 2 diabetes. Early studies conducted in animals indicated that CLA can improve insulin sensitivity; however, these results have since been shown to be exclusive to rat models of diabetes (52, 53). In fact, in both mice (19, 20) and men (21), supplementation with a CLA mixture or *t*10, *c*12 isomer reportedly resulted in insulin resistance. In our study, 8 wk of supplementation with the two CLA treatments did not modify the HOMA index, a marker used to assess insulin sensitivity. These data suggest that under the current experimental conditions, CLA (*c*9, *t*11 isomer and *c*9, *t*11 + *t*10, *c*12 CLA mixture) appears not to affect glycemic control.

In light of the findings from our study, although we did not control the dietary intake of subjects, no changes in body weight or in body composition were observed, suggesting that habitual dietary intake was maintained among the three dietary interventions. Physical activity was maintained as well during the study period (data not shown). Of particular interest in the context of the current study is that there is some evidence that additional physical exercise in conjunction with CLA supplementation could lead to beneficial changes in body composition (54, 55).

In conclusion, the present study failed to provide evidence to support the purported anti-obesity and anti-CVD effects of CLA, which is in agreement with a substantial number of studies showing that CLA indeed does not impact body weight and body composition, or blood lipid concentrations. However, 8 wk of CLA intake did not alter any of the safety parameters that were

354 tested, supporting the concept that CLA-rich oil supplementation at a dose of 3.5 g/d (2.7-2.8 g/d
355 of CLA isomers) is safe. Overall, the results of this study did not support a role for CLA *per se* as
356 an effective weight loss nutraceutical for overweight or obese men.

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TABLE 1 Fatty acid composition of Control and CLA treatments[1]

Fatty acid	Treatment		
	Control	*c*9, *t*11 + *t*10, *c*12 CLA	*c*9, *t*11 CLA
		Relative area %	
C14:0	0.1	0	0.1
C16:0	6.7	3.7	4.4
C16:1 *cis*	0.1	0.1	0.2
C18:0	2.4	2.1	0.8
C18:1 *trans*	0	0.1	0
C18:1 *cis*	12.9	10.8	13.5
C18:2 *cis*	76.3	1.1	1.7
C20:0	0.4	0.3	0
C20:1 *cis*	0.2	0.2	0
C22:0	0.3	0.2	0
C18:2 all *cis, trans*	76.3	76.4	76.5
C18:2 *cis* 9, *trans* 11	0	37	66.2
C18:2 *trans* 10, *cis* 12	0	38.3	8.6
Proportion of *c*9, *t*11:*t*10, *c*12	NA[2]	49.1:50.9	88.5:11.5

[1] According to certificate of analysis provided by Lipid Nutrition.

[2] NA, not available.

TABLE 2 Baseline characteristics of study population[1]

	Value
Age, *y*	44.8 ± 1.5
Weight, *kg*	96.9 ± 2.9
Body mass index, kg/m^2	30.9 ± 0.9
Triglycerides, *mmol/L*	2.2 ± 0.2
Total Cholesterol, *mmol/L*	5.7 ± 0.2
LDL-cholesterol, *mmol/L*	3.6 ± 0.1
HDL-cholesterol, *mmol/L*	1.1 ± 0.0

[1] Values represent means ± SEM , n=27.

TABLE 3 Effect of 8 wk of CLA supplementation on body weight and body composition[1]

	Treatment			
	Control	*c*9, *t*11 + *t*10, *c*12 CLA	*c*9, *t*11 CLA	P^2
Body weight, *kg*				
Baseline	98.2 ± 3.0	99.0 ± 3.0	98.7 ± 3.0	
Phase end	98.9 ± 3.1	99.1 ± 3.1	99.0 ± 3.1	
Δ[3]	0.7 ± 0.4	0.1 ± 0.4	0.3 ± 0.4	0.432
Body mass index, kg/m^2				
Baseline	31.3 ± 0.8	31.5 ± 0.8	31.4± 0.8	
Phase end	31.5 ± 0.8	31.5± 0.8	31.5 ± 0.8	
Δ	0.2 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.533
Body fat mass, *kg*				
Baseline	34.5 ± 2.2	34.8 ± 2.2	34.9 ± 2.2	
Phase end	34.7 ± 2.1	34.8 ± 2.1	34.6 ± 2.1	
Δ	0.2 ± 0.3	0.0 ± 0.3	-0.3 ± 0.3	0.584
Lean body mass, *kg*				
Baseline	60.1 ± 1.2	60.6 ± 1.2	60.2 ± 1.2	
Phase end	60.7 ± 1.3	60.7 ± 1.3	60.8 ± 1.3	
Δ	0.6 ± 0.3	0.1 ± 0.3	0.6 ± 0.3	0.437

[1] Data are presented as means ± SEM, n=27.

[2] *P* values refer to comparisons between absolute changes from baseline to phase end elicited by experimental treatments (repeated measures ANOVA).

[3] Change from baseline to end of phase.

TABLE 4 Effect of 8 wk of CLA supplementation on plasma lipid and lipoprotein concentrations[1]

Plasma lipid	Treatment			P^2
	Control	*c*9, *t*11 + *t*10, *c*12 CLA	*c*9, *t*11 CLA	
		mmol/L		
Total cholesterol				
Baseline	6.31 ± 0.22	6.26 ± 0.22	6.02 ± 0.22	
Endpoint	6.31 ± 0.28	6.04 ± 0.28	6.02 ± 0.28	
Δ^3	0.00 ± 0.22	-0.22 ± 0.22	0.00 ± 0.22	0.343
Triglycerides				
Baseline	3.23 ± 0.30	2.87 ± 0.30	2.95 ± 0.28	
Endpoint	2.96 ± 0.26	2.68 ± 0.26	2.76 ± 0.26	
Δ	-0.27 ± 0.16	-0.19 ± 0.12	-0.19 ± 0.12	0.581
VLDL-cholesterol				
Baseline	1.16 ± 0.15	1.13 ± 0.15	1.14 ± 0.15	
Endpoint	0.69 ± 0.09	0.84 ± 0.09	0.86 ± 0.09	
Δ	-0.46 ± 0.14	-0.29 ± 0.14	-0.28 ± 0.14	0.515
LDL-cholesterol				
Baseline	2.28 ± 0.16	2.44 ± 0.16	2.32 ± 0.16	
Endpoint	2.24 ± 0.13	2.27 ± 0.13	2.21 ± 0.13	
Δ	-0.04 ± 0.12	-0.17 ± 0.12	-0.11 ± 0.12	0.728
HDL-cholesterol				
Baseline	0.54 ± 0.05	0.57 ± 0.05	0.55 ± 0.05	
Endpoint	0.49 ± 0.04	0.48 ± 0.04	0.50 ± 0.04	
Δ	-0.05 ± 0.05	-0.09 ± 0.05	-0.05 ± 0.05	0.597

[1] Data are presented as means ± SEM, n=27.

[2] *P* values refer to comparisons between absolute changes from baseline to phase end elicited by experimental treatments (repeated measures ANOVA).

[3] Change from baseline to end of phase.

TABLE 5 Phase-end anthropometric and fasting plasma measures of a subset of participants[1]

	Treatment			
	Control	*c*9, *t*11 + *t*10, *c*12 CLA	*c*9, *t*11 CLA	P^2
BMI, *kg/m²*	29.5 ± 1.4	30.1 ± 1.6	29.9 ± 1.6	0.269
Fat mass, *%*	34.5 ± 1.8	35.1 ± 2.2	34.8 ± 2.5	0.798
Lean mass, *%*	65.5 ± 1.8	64.9 ± 2.2	65.3 ± 2.5	0.798
Triglycerides, *mmol/L*	1.8 ± 0.4	1.8 ± 0.4	1.9 ± 0.3	0.731
Total cholesterol, *mmol/L*	5.9 ± 0.4	5.6 ± 0.4	6.0 ± 0.3	0.190
Glucose, *mmol/L*	5.6 ± 0.2	5.4 ± 0.2	5.7 ± 0.1	0.212
FFA, *mmol/L*	0.37 ± 0.02	0.43 ± 0.03	0.36 ± 0.04	0.305
β–OHB, *μmol/L*	21.5 ± 5.5	20.7 ± 3.3	10.3 ± 5.6	0.192

[1] Data are presented as means ± SEM, n=10.

[2] *P* values refer to comparisons between changes elicited by experimental treatments at end of phases only (repeated measures ANOVA).

TABLE 6 Effect of 8 wk of CLA supplementation on plasma markers of insulin sensitivity and inflammation[1]

	Treatment			
	Control	*c*9, *t*11 + *t*10, *c*12 CLA	*c*9, *t*11 CLA	*P*[2]
hs-CRP, *mg/L*				
Baseline	1.89 ± 0.29	2.17 ± 0.33	2.21 ± 0.45	
Phase end	2.01 ± 0.50	2.35 ± 0.43	3.14 ± 0.96	
Δ[3]	0.12 ± 0.41	0.18 ± 0.31	0.96 ± 0.58	0.559
TNF-α, *pg/mL*				
Baseline	1.63 ± 0.19	1.38 ± 0.17	1.32 ± 0.11	
Phase end	1.35 ± 0.21	1.38 ± 0.19	1.48 ± 0.19	
Δ	-0.28 ± 0.19	-0.0 ± 0.14	0.16 ± 0.15	0.087
IL-6, *pg/mL*				
Baseline	1.15 ± 0.20	1.40 ± 0.25	1.32 ± 0.18	
Phase end	1.29 ± 0.26	1.05 ± 0.17	1.32 ± 0.22	
Δ	0.14 ± 0.13	-0.35 ± 0.15	0.00 ± 0.18	0.159
HOMA-IR				
Baseline	4.6 ± 0.7	4.1 ± 0.4	4.7 ± 0.8	
Phase end	4.0 ± 0.5	3.8 ± 0.5	4.6 ± 0.6	
Δ	-0.6 ± 0.4	-0.3 ± 0.3	-0.1 ± 0.8	0.208
Adiponectin, *pg/mL*				
Baseline	12.1 ± 1.2	12.3 ± 1.1	12.3 ±1.3	
Phase end	12.1 ± 1.2	11.5 ± 1.0	11.8 ± 1.2	
Δ	0.0 ± 0.5	-0.8 ± 0.7	-0.5 ± 0.3	0.840

[1] Data are presented as means ± SEM, n=27; except for HOMA-IR, n=26.

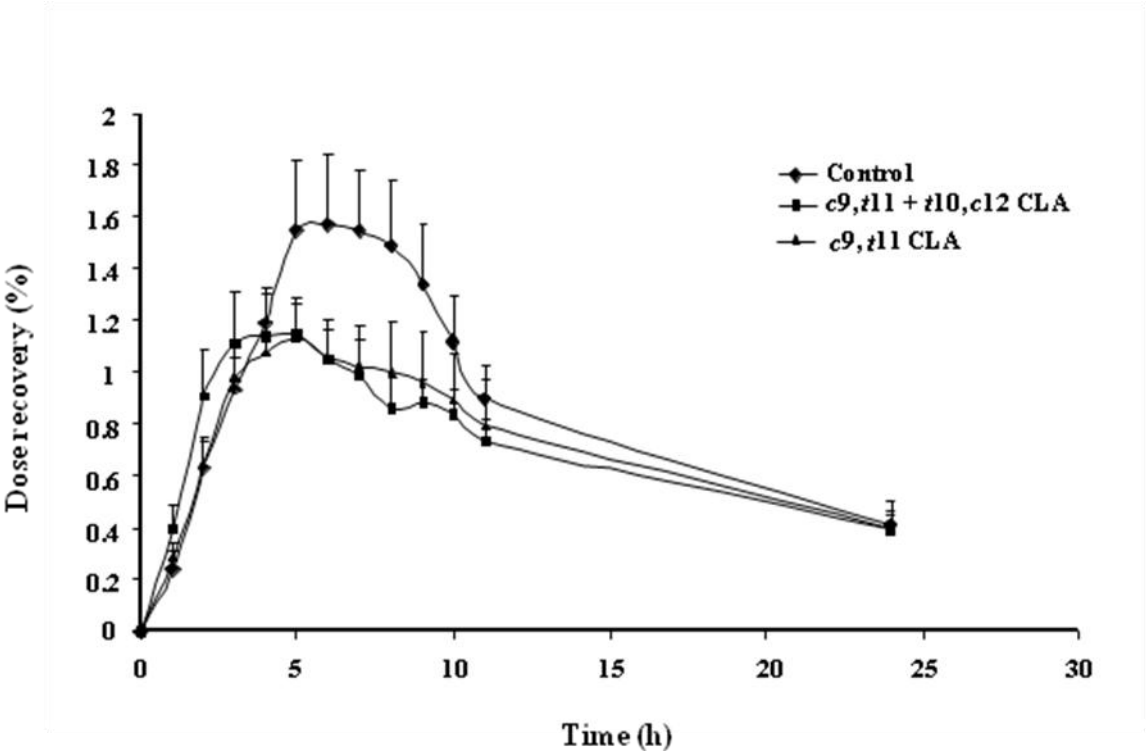
[2] *P* values refer to comparisons between absolute changes from baseline to phase end elicited by experimental treatments (repeated measures ANOVA).

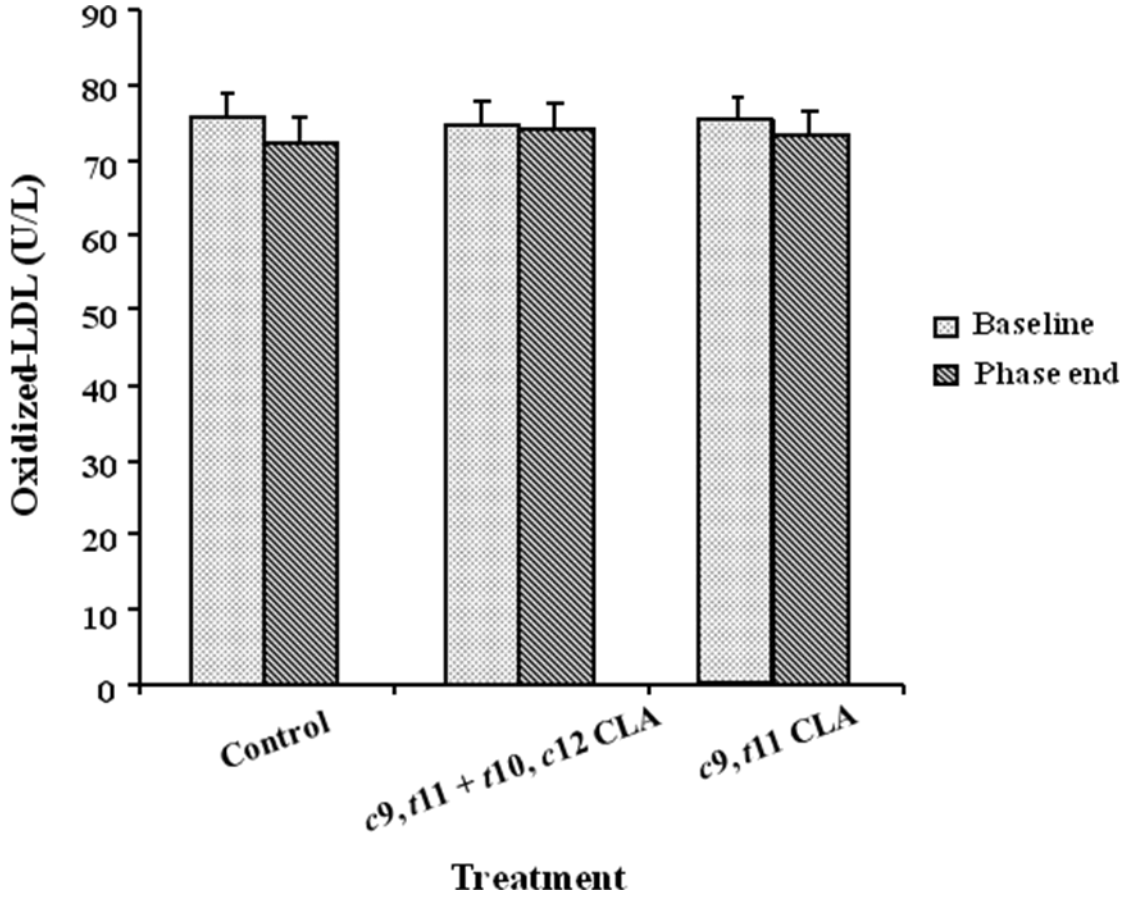
[3] Change from baseline to end of phase.

FIGURE LEGENDS

FIGURE 1 Cumulative oxidation of ^{13}C linoleate over 24 h following 8 wk of experimental treatments. Data represent means ± SEM, n=10. Repeated measures ANOVA was used to analyze data at the end of phases only; overall P=0.28.

FIGURE 2 Concentration of plasma oxidized-LDL following 8 wk of experimental treatments. Bars represent means ± SEM, n=27. Repeated measures ANOVA was used to compare absolute changes from baseline to end of phase elicited by the experimental treatments; overall P=0.61.


Dose recovery (%)
Time (h)
Control
c9,t11 + t10,c12 CLA
c9,t11 CLA
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0
5
10
15
20
25
30


Oxidized-LDL (U/L)
90
80
70
60
50
40
30
20
10
0
Control
c9,t11 + t10,c12 CLA
c9,t11 CLA
Treatment
Baseline
Phase end