International Journal of Obesity (2007) 31, 481–487
 0307-0565/07 $30.00
www.nature.com/ijo

ORIGINAL ARTICLE

The role of conjugated linoleic acid in reducing body fat and preventing holiday weight gain

AC Watras[1], AC Buchholz[2], RN Close[1], Z Zhang[1] and DA Schoeller[1]

[1]*Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, WI, USA and* [2]*Family Relations and Applied Nutrition, University of Guelph, Ontario, Canada*

Objective: The incidence of obesity and overweight in the US has increased considerably during the past two decades and currently affects 65% of the adult population. Research has indicated that small, yet irreversible, gains during the holiday season contribute to increases in weight during adulthood. Conjugated linoleic acid (CLA), a naturally occurring dietary fatty acid, has been found to reduce weight gain and dramatically decrease fat mass in animals. Although research in humans has shown inconsistent results, most studies have been of insufficient duration or have utilized body composition methods that are less accurate than the currently accepted criterion.
Design: Randomized, double-blind, placebo-controlled study of 3.2 g/day CLA for 6 months.
Subjects: Forty healthy, overweight subjects (age: 18–44 years; body mass index: 25–30 kg/m^2).
Measurements: Body composition by the four-compartment model, resting metabolic rate (RMR) by indirect calorimetry, self-reported physical activity and dietary intake, and blood chemistries were determined at baseline and after 6 months. Body weight was measured monthly during the pre-holiday season (August–October), holiday season (November–December) and post-holiday season (January–March). Adverse events were assessed monthly.
Results: Compared to CLA, the placebo group showed a greater rate of weight gain during the holiday season ($P=0.01$). Within the placebo group, holiday weight change was significantly greater compared to the pre-holiday period (August–October) ($P=0.03$). Six-month change in body composition was improved with CLA compared to placebo ($P=0.02$), and body fat was significantly reduced within the CLA group (-1.0 ± 2.2 kg, $P=0.05$). CLA had no effect on RMR, physical activity or dietary intake. The rate of reported negative emotions decreased significantly with CLA, although there was no difference in any other category of adverse event. In comparison to the placebo, CLA did not affect insulin resistance, blood lipids and markers of liver function or markers of inflammation, with the exception of a significant decrease in a biomarker of endothelial dysfunction.
Conclusion: CLA supplementation among overweight adults significantly reduced body fat over 6 months and prevented weight gain during the holiday season. Although no adverse effects were seen, additional studies should evaluate the effect of prolonged use of CLA.
International Journal of Obesity (2007) **31,** 481–487. doi:10.1038/sj.ijo.0803437; published online 22 August 2006

Keywords: CLA; body composition; four-compartment model; body weight; overweight

Introduction

Recent estimates indicate that 65% of the US adult population is overweight or obese, part of a continuing trend that began in the mid-1980s.[1] Overweight and obesity increase all-cause mortality; raise the risk of morbidities including heart disease, stroke, diabetes, osteoarthritis and certain types of cancer; and are primary contributors to preventative death in the US.[2] Therefore, determining effective strategies for weight loss or prevention of weight gain is essential.

Conjugated linoleic acid (CLA), an isomer of linoleic acid found in food products of ruminant animals and available as a dietary supplement, was identified as possibly having anticarcinogenic activity by Pariza *et al*. in 1978.[3] Research with the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers of CLA has shown additional effects in animals, including a decrease in body fat and a reduction in weight gain.[4] CLA's effect on body fat was first demonstrated almost a decade ago in mice, and research has since confirmed and extended these findings to include several species, with some variation by animal type and CLA isomer.[5] By far, the most dramatic results have been shown in mice with decreases in body fat between 40 and 80% compared to controls.[6] Proposed

Correspondence: Dr D Schoeller, Department of Nutritional Sciences, University of Wisconsin-Madison, 1415 Linden Drive, Madison, WI 53706, USA.
E-mail: dschoell@nutrisci.wisc.edu
Received 18 February 2006; revised 18 June 2006; accepted 27 June 2006; published online 22 August 2006

mechanisms have included increased fat mobilization and oxidation, reduced adipocyte proliferation and differentiation, and increased apoptosis in pre-adipocytes.[6]

Despite clear evidence that CLA reduces body fat in animals, the effect of CLA supplementation on body composition in humans has been inconsistent: of seven studies that have evaluated body composition change with mixed-isomer CLA among overweight or obese adults,[7–13] only three have shown a significant reduction in fat mass.[9–11] The conflicting results in human versus animal studies have been attributed to factors such as lower doses per unit body weight; the use of young, growing animals versus adult humans; and the limited sensitivity and accuracy of *in vivo* body composition measures.[6] In fact, a commonality among previous human studies has been the assessment of body composition with techniques, such as bioelectrical impedance analysis (BIA), dual-energy X-ray absorptiometry (DXA) and underwater weighing, that may be less sensitive in detecting change when compared to criterion multicompartment models.

The aim of the current study was to determine whether CLA supplementation reduces body fat in overweight adults. To address limitations in previous study designs, we utilized the criterion four-compartment model of body composition to assess change in body fat mass. We were also able to determine the effect of CLA on body weight during the holiday season, a period that is commonly associated with weight gain. Research has shown that holiday weight gain contributes considerably to the annual weight gain that may be associated with incremental gains during adulthood, and that overweight and obese individuals are susceptible to greater holiday gains.[14]

Subjects and methods

Subjects

Eligible participants were 18- to 44-year-old men and women with body mass index (BMI) values between 25 and 30 kg/m^2 at the time of initial screening. Subjects provided medical history and underwent a physical exam, including electrocardiograph and routine blood chemistry analysis. The study was approved by the University of Wisconsin–Madison Institutional Review Board, and all subjects provided written informed consent.

Of the 48 randomized subjects, 40 subjects completed the study protocol and were included in the current data analyses. Eight subjects were excluded: three subjects left the study owing to a lack of interest in participation at months 1, 5 and 6; one subject moved out of the area during month 1; two subjects became ineligible during the course of the study (one started medication to treat depression at month 5 and one resumed smoking during month 1); and two subjects were removed after the completion of the study for noncompliance (one did not meet supplement compliance criteria of 75% and one initiated an intense aerobic training program).

Experimental design

The study design was double-blind, randomized and stratified by gender and age (<30 and $\geqslant 30$ years). Subjects were randomly assigned to 4 g/day of placebo (safflower oil) or 4 g/day of 78% active CLA isomers of safflower oil (3.2 g/day CLA: 39.2% *cis*-9, *trans*-11 and 38.5% *trans*-10, *cis*-12). Each supplement was prepared from the same lot and capsules were identical in color, size and taste (Cognis Corporation, La Grange, IL, USA). Subjects were instructed to take four soft gel capsules each morning with food. At months 0 and 6, subjects arrived in the morning after a 12 h fast and underwent testing for blood chemistry, resting metabolic rate (RMR) and body composition. Body weight, capsule compliance and adverse events (AE) were assessed monthly.

Body composition and body weight

Four-compartment model. Selinger's four-compartment equation was used to calculate the criterion body fat mass:

$$\%\mathrm{BF} = ((2.747/(\mathrm{BD}/\mathrm{Wt}) - (0.714(\mathrm{TBW}/\mathrm{Wt})) + (1.146 * (\mathrm{TBM}/\mathrm{Wt}) - 2.0503) \times 100,$$

where %BF is percent body fat, BD is body density (kg/l) determined by underwater weighing, Wt is body mass (kg), TBW is total body water (kg) determined by ^{18}O isotope dilution and TBM is total body mineral (kg) calculated from DXA.[15]

Body Density. BD was determined by underwater weighing using procedures described previously.[16] Residual lung volume was measured by the closed-circuit oxygen dilution method,[17] using a 13.5 l respirometer (Collins Medical, Braintree, MA, USA) and a Med Science nitrogen analyzer (Model 505; St Louis, MO, USA). At month 6, the mean of the month 0 and month 6 residual volumes was used to minimize within-subject variability.

Total Body Water. TBW was measured by ^{18}O dilution using 10.3% ^{18}O-enriched water (Rotem Industries Ltd, Beer Sheva, Israel) and continuous-flow isotope ratio mass spectrometry (Delta S, Finnigan MAT, Bremen, Germany) as described by Schoeller and Luke.[18] TBW was calculated from the dilution of the isotopic water using the difference in enrichment between the predose sample and the final (3–4 h) urine sample. Each sample was analyzed on 2 separate days and the average enrichment of the 2 days was used (within-subject standard deviation (s.d.) = 0.16 permil)[1]. The ^{18}O dilution space was assumed to be 0.7% higher than TBW.[19]

[1]$\delta(\mathrm{permil}) = ((\mathrm{R}_{\mathrm{unknown}} - \mathrm{R}_{\mathrm{standard}})/\mathrm{R}_{\mathrm{standard}}) \times 1000$, where R is the ratio of the abundance of heavy isotope to that of the light isotope.

Total Body Mineral. Bone ash was determined by whole-body DXA using the Norland XR-36 bone densitometer with software version 3.7.4/2.1.0 (Norland Corporation, Ft. Atkinson, WI, USA). Because 4.2% of osseous mineral is lost during the ashing process and non-osseous mineral is estimated to be 23.5% of total body bone ash, TBM was calculated by multiplying total body bone ash as determined by DXA by 1.279.[20]

Height and weight. Height was measured within 0.5 cm on a custom wall-mounted stadiometer and weight within 0.1 kg on a calibrated beam balance platform scale (Continental Scale Corp., Bridgeview, IL, USA).

Abdominal fat mass. DXA was used to assess the change in abdominal fat mass from month 0 to 6 using the Norland XR-36 bone densitometer. The abdominal region was bounded superiorly by the lowest rib and inferiorly by the pubic symphysis.

Energy expenditure and intake
RMR was measured at months 0 and 6 by indirect calorimetry using a ventilated hood (Deltatrac II; Sensor-Medics Corp., Yorba Linda, CA, USA). RMR was calculated using minute-by-minute O_2 and CO_2 according to the equation of Weir.[21] Physical activity was determined at months 0 and 6 by the 7-day physical activity recall questionnaire according to the methods of Sallis *et al*.[22] Total hours per day of moderate, hard and very hard activity were determined and multiplied by metabolic equivalents (MET) to yield an activity score in MET h/day. Dietary intake was assessed with a 3-day food record at months 0 and 6. Dietary records were analyzed for total energy intake using Food Processor Nutrition Software (version 8.4; ESHA Research, Salem, OR, USA).

Safety and compliance
Blood chemistry. Blood samples were collected after a 12 h fast on the morning of body composition testing at months 0 and 6. Samples were analyzed for alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), bilirubin, glucose, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total cholesterol and triglycerides (TGs) (General Medical Laboratories, Madison, WI, USA). Insulin and leptin levels were measured at the University of Wisconsin–Madison Primate Center Assay Laboratory using double-antibody liquid-phase radioimmunoassays. Insulin resistance was determined using the homeostasis model assessment as follows: ((fasting plasma insulin (μIU/ml) $\times$ fasting plasma glucose (mmol/l)/22.5).[23] Plasma biomarkers of inflammation and endothelial dysfunction were analyzed, including C-reactive protein (CRP), interleukin-6 (IL-6), soluble tumor necrosis factor receptor, E-selectin, soluble intercellular adhesion molecule (sICAM-1) and soluble vascular cell adhesion molecule (sVCAM-1) (Clinical & Epidemiologic Research Laboratory, Boston Children's Hospital).

Adverse events and compliance. AE were recorded monthly throughout the study utilizing a 36-item questionnaire. Symptoms from the questionnaire were then grouped into four categories: cold or flu; general symptoms (nausea, headache); orthopedic symptoms (joint pain, back pain); and emotional symptoms (depression, irritability). Supplement compliance was evaluated monthly by questionnaire and capsule counts. Compliance for inclusion was established *a priori* as $\geqslant$75%.

Statistical analysis
The sample size was selected based on 80% power to detect a 1 kg difference in fat mass, with $P \leqslant 0.05$. The primary outcome variable was the change in body fat mass as measured by the four-compartment model. The *t*-test procedure was used to determine differences between the groups at month 0 for all variables. Within-group change over 6 months was tested with a paired *t*-test. Between-group comparisons of the 6-month change values for body composition, blood chemistries, energy expenditure and physical activity were completed using a regression model that adjusted for the covariates of age, sex and baseline BMI. To assess seasonal weight change, weight change was expressed as the average rate per month for the periods of pre-holiday (August–October), holiday (November–December) and post-holiday (January–March). Between-group comparisons in the rate of weight change by season were analyzed with the *t*-test procedure. Longitudinal weight change by month was analyzed using repeated measures. Differences in the frequency of reported AE were assessed by repeated measures analysis with Poisson regression log-linear model. SAS was used for all analyses (version 8.2; SAS Institute Inc., Cary, NC, USA).

Results

Baseline characteristics, body composition and body weight
There were no baseline differences between the groups in age (CLA, 34 $\pm$ 8 years; placebo, 32 $\pm$ 7 years) or gender (CLA, five men and 17 women; placebo, three men and 15 women). Body weight, BMI and body composition also did not differ between groups at month 0 (Table 1). The 6-month loss in body fat, expressed either in kilograms ($P = 0.02$) or as a percentage ($P = 0.02$), was significantly greater with CLA as compared to placebo. Within-group comparisons showed significant decreases in fat mass ($P = 0.05$) and body fat percentage ($P = 0.02$) in the CLA group only. There was no difference between the groups in the change in fat-free mass ($P = 0.8$) or abdominal fat mass, although a trend for reduction of abdominal adiposity with CLA was observed

Table 1 Change in body weight and body composition after 6 months of CLA or placebo

	Placebo (n = *18*)		*CLA* (n = *22*)	
	Month 0	*Δ (6–0)*	*Month 0*	*Δ (6–0)*
Body weight (kg)	79.0±10.9	1.1±3.2	80.0±9.1	−0.6±2.5[a]
BMI (kg/m^2)	28.0±2.2	0.4±1.1	27.6±1.8	−0.2±0.9[a]
4-C FM (kg)	28.4±5.0	0.7±3.0	26.6±5.5	−1.0±2.2[a,b]
4-C FM (%)	36.0±4.2	0.2±2.3	33.6±7.4	−1.0±1.8[a,b]
4-C FFM (kg)	50.6±8.1	0.4±1.0	53.4±10.4	0.4±1.3
Abd. FM (kg)	7.5±1.5	0.2±1.2	6.9±1.5	−0.2±1.0

Abbreviatons: Abd. FM, abdominal fat mass (DXA); BMI, body mass index; CLA, conjugated linoleic acid; FFM, fat-free mass (four-compartment model); FM, fat mass (four-compartment model). All values are x±s.d. [a]Significant between-group comparison, $P \leqslant 0.05$. [b]Significant within-group comparison, $P \leqslant 0.05$.

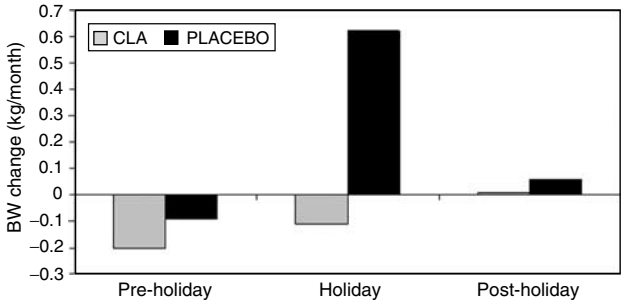

Figure 1 Seasonal change in body weight. Rate of change in body weight (kg/month) during the pre-holiday period (August–October), holiday season (November–December) and post-holiday period (March–January). Compared to CLA, the placebo group had a significantly greater rate of weight gain during the holiday season ($P = 0.01$). The placebo group also had a significantly greater rate of weight gain during the holiday season compared to the pre-holiday period ($P = 0.03$).

($P = 0.1$). There was a significant difference between groups in the 6-month change in body weight ($P = 0.04$) and BMI ($P = 0.05$). The placebo group had a higher rate of weight gain during the holiday season as compared to the pre-holiday period ($P = 0.03$) and as compared to the CLA group during the holiday season ($P = 0.01$) (Figure 1).

Energy expenditure and intake

There were no differences at month 0 between the groups in RMR, respiratory exchange ratio (RER) or physical activity. Reported energy intake was lower in the placebo group, and this was close to significant ($P = 0.06$). There were no significant differences between the groups in the 6-month change in RMR, RER, reported physical activity or reported total energy intake (Table 2). Within-group changes showed significant increases in RER within the placebo group ($P = 0.03$), and significant increases in RMR within the CLA group ($P = 0.002$). Compared to baseline, reported physical activity decreased in the placebo group by 33% ($P = 0.07$) and in the CLA group by 40% ($P = 0.001$). Both groups also reduced reported energy intake as measured by 3-day food records, with a significant decrease found only within the placebo group ($P = 0.02$).

Table 2 Change in resting energy expenditure, physical activity and reported energy intake after 6 months of CLA or placebo

	Placebo (n = *18*)		*CLA* (n = *22*)	
	Month 0	*Δ (6–0)*	*Month 0*	*Δ (6–0)*
RMR (kcal/day)	1443±206	37±110	1476±211	50±64[a]
RER	0.82±0.05	0.04±0.07[a]	0.82±0.05	0.02±0.06
PA MET (h/day)	7.2±5.3	−2.4±4.6	9.2±5.6	−3.7±4.4[a]
EI (kcal/day)	2020±497	−322±497[a]	2408±649	−199±493

Abbreviations: EI, energy intake from 3-day food record; MET, metabolic equivalents; PA, sum of moderate, hard and vigorous activity hours per day from 7-day physical activity recall multiplied by MET intensity level; RER, respiratory exchange ratio; RMR, resting metabolic rate. All values are x±s.d. [a]Significant within-group comparison, $P \leqslant 0.05$.

Safety and compliance

Blood chemistry. At month 0, there were no significant differences between the groups with one exception: TG levels were higher in the CLA group (Table 3). When two subjects from the CLA group with elevated TGs at month 0 were excluded, the difference between the groups was no longer significant but still showed a tendency toward a difference ($P = 0.07$). Between groups, the only marker that changed significantly was soluble vascular cell adhesion molecule (sVCAM-1), which showed a greater decrease with CLA compared to placebo ($P = 0.02$). Both groups significantly decreased bilirubin, LDL sVCAM-1 cholesterol and total cholesterol, and increased glucose and leptin. Within CLA only, AST decreased and CRP increased. There was one subject within the CLA group with CRP values that increased more than 3 s.d. above the mean, and when this subject was excluded, the within-group increase in CRP was only a trend ($P = 0.08$). In addition, there was no significant difference between the groups in CRP. It is important to note that all within-group changes remained within normal ranges and therefore were not considered clinically significant.

Adverse events and compliance. There was no difference between groups in symptom rate at month 0 (not shown). The frequency of reported AE in the categories of cold or flu, general symptoms and orthopedic symptoms was not significantly different between the groups during the study period. The rate of cold and flu symptoms increased significantly with CLA ($P = 0.02$) and placebo ($P < 0.0001$), which may be a seasonal occurrence, as both groups started in the late summer to early fall and finished in the late winter to early spring. There was a decrease in the frequency of reported emotional symptoms (e.g., anxiety, depression and irritability) in the CLA group that was significantly

Table 3 Change in blood chemistry variables after 6 months of CLA or placebo

	Placebo (n = *18*)		*CLA* (n = *22*)	
	Month 0	*Δ (6–0)*	*Month 0*	*Δ (6–0)*
GLU (mg/dl)	85.6±7.2	6.1±5.9[a]	88.5±6.5	2.8±4.9[a]
INS (μU/ml)	12.1±4.4	−0.6±6.2	13.3±4.1	2.1±11.6
HOMA-IR	2.6±1.0	0±1.4	2.9±1.0	0.6±2.8
Leptin (ng/ml)	16.1±10.6	5.6±8.5[a]	14.9±8.5	3.9±5.2[a]
Liver function markers				
ALT (U/l)	13.4±6.5	6.6±17.3	19.8±20.1	−2.5±20.7
ALP (U/l)	60.8±12.1	1.3±8.1	63.2±29.3	−4.7±35.3
AST (U/l)	23.7±3.6	−3.0±7.4	27.6±23.5	−4.2±4.8[a]
BILI (mg/dl)	0.6±0.2	−0.2±0.2[a]	0.7±0.4	−0.1±0.2[a]
Cardiovascular markers				
HDL (mg/dl)	56±13	0±11	56±11	1±7
LDL (mg/dl)	122±27	−17±18[a]	122±28	−16±20[a]
TC (mg/dl)	197±31	−14±24[a]	203±32	−13±23[a]
TG (mg/dl)	95±36[b]	9±53	133±73[b]	12±70
Markers of inflammation				
CRP (mg/l)	1.7±1.6	0.4±1.8	1.7±1.7	0.9±1.9[a]
IL-6 (pg/ml)	1.7±1.3	−0.1±1.1	1.1±0.6	0.6±2.2
sTNFR-2 (pg/ml)	1902±305	6.5±425	2003±315	−38.3±211
Markers of endothelial dysfunction				
E-selectin (ng/ml)	33.8±12.8	2.9±8.3	37.3±10.6	1.0±4.7
sICAM-1 (ng/l)	241±48.3	−2.6±28.6	231.5±40.8	2.3±26.2
sVCAM-1 (ng/l)	585±157	−74.1±114[a,b]	626±129	−141±87.5[a,b]

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BILI, total bilirubin; CRP, C-reactive protein; GLU, glucose; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; IL-6, interleukin-6; INS, insulin; LDL, low-density lipoprotein; sICAM-1, soluble intercellular adhesion molecule; sVCAM-1, soluble vascular cell adhesion molecule; sTNFR-2, soluble tumor necrosis factor receptor; TC, total cholesterol; TG, triglycerides. All values are x±s.d. [a]Significant within-group comparison, $P \leqslant 0.05$. [b]Significant between-group comparison, $P \leqslant 0.05$.

different from the placebo group ($P = 0.04$). Given the small number of observations, however, it is unlikely that this is a meaningful finding. Six-month compliance rates were 97 and 95% in the placebo group and CLA group, respectively, and there was no significant difference between the groups.

Discussion

Our results indicate that CLA plays a role in mitigating weight gain associated with the holiday season. CLA significantly reduced body weight compared to placebo during the 6-month study, and during the holiday season in particular. In November and December, the placebo group had a significantly higher rate of weight gain compared to the CLA group and compared to their rate of weight change in the pre-holiday period. A study in the New England Journal of Medicine found that although holiday weight gain averages only 0.4 kg – less than often claimed in the popular press – the weight that is gained during this 6-week period is not reversed during the remainder of the year.[14] Thus, holiday weight gain may contribute to the cumulative weight gain that is seen with aging. The study also showed that among overweight and obese individuals, there was a trend toward a large (>2.3 kg) weight gain during the holiday period.[14] In our study, CLA versus placebo prevented weight gain during the holiday season among overweight adults and therefore may play a role in reducing the cumulative weight and fat gain that occurs with age.

This is the first study utilizing the criterion four-compartment body composition method to demonstrate changes in body composition due to CLA supplementation in overweight adults. Over 6 months, CLA significantly reduced body fat mass, despite concurrent decreases in reported physical activity. Of seven studies that have examined the effect of mixed-isomer CLA on body composition among overweight or obese subjects,[7–13] three have found similar results.[9–11] Gaullier *et al*.[11] found fat mass losses of 2.4 and 1.7 kg during 1 year of supplementation with CLA in TG and free-fatty acid forms, respectively. In a 1-year, open-label extension of Gaullier's study[10] with all three groups taking CLA TG, there was a significant decrease in fat mass in the placebo-TG group (−1.7 kg), whereas the remaining two groups maintained their previous losses and showed additional, but non-significant, decreases in fat mass. During 12 weeks of supplementation, Blankson *et al*.[9] showed that fat mass decreased by 1.2, 1.7 and 1.3 kg for CLA doses of 1.7, 3.4 and 6.8 g/day, respectively.

Conversely, four previous studies of CLA in overweight or obese adults have not shown significant effects on fat mass.[7,8,12,13] Berven *et al*.[8] found no significant effect on fat mass with a protocol of 3.4 g/day CLA for 12 weeks, although a trend was observed for body fat to decrease with CLA (0.9 kg) and increase with placebo (0.3 kg). In this study, body composition was assessed with BIA, a method that may be less precise in overweight adults.[24] In addition, the study length was possibly too short for an effect to be observed. A commonality among the three remaining studies in overweight adults that did not find an effect of CLA on body fat was the inclusion of a weight loss component: Whigham *et al*.[13] included a 12-week very low-calorie diet (VLCD); Kamphius *et al*.[12] utilized a 3-week VLCD; and Atkinson[7] included a diet and exercise regimen during 6 months of treatment. Possibly, the effect of calorie restriction for weight loss in prior studies introduced variability in body weight and body composition change and masked the effect of CLA.

With respect to the safety and tolerance of CLA supplementation, we found no significant effects of CLA on the 6-month change in blood chemistry variables or the frequency of reported AE. Although some animal studies have shown negative effects with CLA, this is possibly attributable to greater doses: animals often receive 10–50 times the dosage per unit body weight compared to humans.[25] The effect of CLA on blood glucose and insulin sensitivity is controversial,

although reported deleterious effects appear to be primarily related to the *trans*-10, *cis*-12 CLA isomer.[6] Riserus *et al.*[26]showed detrimental isomer-specific effects on insulin sensitivity in abdominally obese men: supplementation with 3.4 g/day *trans*-10, *cis*-12 CLA, but not mixed-isomer CLA, significantly increased fasting glucose and insulin sensitivity as measured by a euglycemic–hyperinsulinemic clamp. Conversely, Eyjolfson[27] used an oral glucose tolerance test to demonstrate an improved insulin sensitivity index with 3 g/day mixed-isomer CLA over 8 weeks. Although our study found no effect of mixed-isomer CLA on plasma glucose or insulin levels, a result that has also been supported by several human studies using mixed-isomer supplementation,[8,9,11–13] we did not find the expected reductions in glucose or insulin in the CLA-treated group that usually accompanies small weight losses.[28] The longest human trial of CLA to date reported no detectable change in glucose and insulin levels during 2 years of supplementation with 3.4 g/day mixed-isomer CLA in the free fatty acid form; yet these individuals failed to show any improvements in glucose and insulin, which might have been expected for their weight loss, and there was a significant increase in insulin within the group taking CLA in the TG form.[10] We therefore cannot rule out the potential for small, subclinical effects to develop with prolonged use.

In some animal studies, CLA has altered liver metabolism resulting in increased liver weights and fatty liver.[25] We did not see a change in liver function tests including AST, ALT, ALP and total bilirubin. Of six recent studies including similar measures of liver function, only Gaullier *et al.*[10,11] showed significant within-group increases in AST.[8–11,13,29] However, this increase in AST was not significantly different from the control group and was not accompanied by changes in ALT or bilirubin.[10,11] Malpuech *et al.*[29] measured liver size by ultrasound and concluded that CLA had no effect on liver ultrastructure or morphology and no evidence of hepatic lipodystrophy.

CLA has also been shown to reduce cardiovascular disease risk factors such as atherosclerosis and blood lipids in animals, although results have been conflicting.[25] In humans, however, the majority of studies have shown that supplementation with CLA mixtures does not have an effect on plasma cholesterol or TG levels compared to controls.[5] Similarly, we found no difference between groups in the change in total cholesterol, LDL, HDL or TGs. We also assessed the effect of CLA on biomarkers of inflammation and endothelial dysfunction that have been linked to the development of cardiovascular disease: CRP, IL-6 and TNFR-2 are markers of systemic inflammation, whereas E-selectin, sICAM-1 and s-VCAM-1 are overexpressed in the presence of inflammatory stimuli to the endothelium.[30] Although we found a trend for CRP to increase within the CLA group, it is important to note that the CRP value remained within the 'Average Risk' category (1–3 mg/l). We also found that s-VCAM-1, which has been described as a predictor of atherosclerotic processes, decreased significantly with CLA.[30]

The frequency of AE also did not differ between the CLA and placebo groups in our study, although one subject with less than 75% capsule compliance reported difficulty in tolerating the CLA supplement. In the largest human trial of CLA to date ($n = 157$), Gaullier *et al.*[11] also reported no significant difference in the frequency of AE between groups.

There are several limitations to our study design. First, our subjects consisted primarily of overweight women (80%), and although it may not be appropriate to generalize our findings to men, two previous studies found a significant effect of CLA among samples that included a greater percentage of men (33 and 51%).[9,31] Second, we assessed energy intake using a 3-day diet record, and the results of the self-reported energy intakes were counterintuitive. Among the placebo group, the 6-month change in reported energy intake decreased despite a concurrent increase in body weight. In addition, the reported energy intakes among both groups are lower than would be necessary for weight maintenance given the weight and body composition of the subjects.[32] It is likely that these self-reported energy intakes are confounded by energy underreporting and should be interpreted with extreme caution.[33]

In conclusion, we found supplementation with 3.2 g/day of a 50:50 mixture of CLA to be well tolerated and effective in reducing body fat among overweight adults, and we addressed a limitation of previous studies by utilizing the criterion measure of body composition. Further, we found that CLA prevented the seasonal gain of weight that occurred in control subjects, suggesting that CLA may be useful for long-term weight and body composition maintenance by preventing the annual gains that may be especially problematic among overweight individuals. As the longest CLA trial to date is 2 years, however, additional studies are needed before prolonged use of CLA can be recommended.

Acknowledgements

We thank Randall Clark and Jude Sullivan of the University of Wisconsin Hospitals and Clinics Exercise Science Laboratory for conducting body composition analysis; The University of Wisconsin General Clinical Research Center for providing in-patient support and dietary analysis; and Dr Earl Shrago for completing subject medical history and physical examinations. We acknowledge Cognis Nutrition and Health for providing test materials and sponsorship.

ACW contributed to study design, recruited subjects, completed data collection, contributed to data interpretation and wrote the manuscript. ACB contributed to study design and revised the manuscript. RNC assisted with data collection and ZZ provided assistance with statistical analysis. DAS established the study design, obtained study funding, interpreted data and revised the manuscript. None

of the authors had or have any financial or personal interest in the sponsor of this research.

References

1 Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999–2002. *JAMA* 2004; **291**: 2847–2850.
2 Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary Expert Panel on the Identification, Evaluation, and Treatment of Overweight in Adults. *Am J Clin Nutr* 1998; **68**: 899–917.
3 Pariza MW. Perspective on the safety and effectiveness of conjugated linoleic acid. *Am J Clin Nutr* 2004; **79**: 1132S–1136S.
4 Wang YW, Jones PJ. Conjugated linoleic acid and obesity control: efficacy and mechanisms. *Int J Obes Relat Metab Disord* 2004; **28**: 941–955.
5 Terpstra AH. Effect of conjugated linoleic acid on body composition and plasma lipids in humans: an overview of the literature. *Am J Clin Nutr* 2004; **79**: 352–361.
6 Wahle KW, Heys SD, Rotondo D. Conjugated linoleic acids: are they beneficial or detrimental to health? *Prog Lipid Res* 2004; **43**: 553–587.
7 Atkinson RL. Conjugated linoleic acid for altering body composition and treating obesity. Advances in conjugated linoleic acid research, volume 1. In: Yurawecz MP, Mossoba MM, Kramer JKG, Pariza MW, Nelson GJ (eds). AOCS Press: Champagne, IL, 1999. pp. 348–353.
8 Berven G, Bye A, Hals O. Safety of conjugated linoleic acid (CLA) in overweight or obese volunteers. *Eur J Lipid Sci Technol* 2000; **102**: 455–462.
9 Blankson H, Stakkestad JA, Fagertun H, Thom E, Wadstein J, Gudmundsen O. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. *J Nutr* 2000; **130**: 2943–2948.
10 Gaullier JM, Halse J, Hoye K, Kristiansen K, Fagertun H, Vik H *et al*. Supplementation with conjugated linoleic acid for 24 months is well tolerated by and reduces body fat mass in healthy, overweight humans. *J Nutr* 2005; **135**: 778–784.
11 Gaullier JM, Halse J, Hoye K, Kristiansen K, Fagertun H, Vik H *et al*. Conjugated linoleic acid supplementation for 1 y reduces body fat mass in healthy overweight humans. *Am J Clin Nutr* 2004; **79**: 1118–1125.
12 Kamphuis MM, Lejeune MP, Saris WH, Westerterp-Plantenga MS. The effect of conjugated linoleic acid supplementation after weight loss on body weight regain, body composition, and resting metabolic rate in overweight subjects. *Int J Obes Relat Metab Disord* 2003; **27**: 840–847.
13 Whigham LD, O'Shea M, Mohede IC, Walaski HP, Atkinson RL. Safety profile of conjugated linoleic acid in a 12-month trial in obese humans. *Food Chem Toxicol* 2004; **42**: 1701–1709.
14 Yanovski JA, Yanovski SZ, Sovik KN, Nguyen TT, O'Neil PM, Sebring NG. A prospective study of holiday weight gain. *N Engl J Med* 2000; **342**: 861–867.
15 Bartok-Olson CJ, Schoeller DA, Sullivan JC, Clark RR. The 'B' in the Selinger Four-Compartment body composition formula should be body mineral instead of bone mineral. *Ann NY Acad Sci* 2000; **904**: 342–344.
16 Behnke A, Wilmore J. *Evaluation and Regulation of Body Build and Composition*. Prentice-Hall: Englewood Cliffs, NJ, 1974.
17 Wilmore H. A simplified method for determination of residual lung volumes. *J Appl Physiol* 1969; **27**: 98–100.
18 Schoeller D, Luke A. Rapid ^{18}O analysis of CO_2 samples by continuous-flow isotope ratio mass spectrometry. *J Mass Spec* 1997; **32**: 1332–1336.
19 Schoeller D. Hydrometry. In: Heymsfield S, Lohman T, Wang Z, Going S (eds). *Human Body Composition*. Human Kinetics: Champagne, IL, 2005. pp 35–49.
20 Heymsfield S, Lichtman S, Baumgartner R, Wang J, Kamen Y, Aliprantis A *et al*. Body composition of humans: comparison of two improved four-compartment models that differ in expense, technical complexity, and radiation exposure. *Am J Clin Nutr* 1990; **52**: 52–58.
21 Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 1949; **109**: 1–9.
22 Sallis J. Seven day physical activity recall. *Med Sci Sports Exerc* 1997; **29**: S89–S103.
23 Conwell LS, Trost SG, Brown WJ, Batch JA. Indexes of insulin resistance and secretion in obese children and adolescents: a validation study. *Diabetes Care* 2004; **27**: 314–319.
24 Sun G, French CR, Martin GR, Younghusband B, Green RC, Xie YG *et al*. Comparison of multifrequency bioelectrical impedance analysis with dual-energy X-ray absorptiometry for assessment of percentage body fat in a large, healthy population. *Am J Clin Nutr* 2005; **81**: 74–78.
25 Larsen TM, Toubro S, Astrup A. Efficacy and safety of dietary supplements containing CLA for the treatment of obesity: evidence from animal and human studies. *J Lipid Res* 2003; **44**: 2234–2241.
26 Riserus U, Arner P, Brismar K, Vessby B. Treatment with dietary *trans*10*cis*12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. *Diabetes Care* 2002; **25**: 1516–1521.
27 Eyjolfson V, Spriet LL, Dyck DJ. Conjugated linoleic acid improves insulin sensitivity in young, sedentary humans. *Med Sci Sports Exerc* 2004; **36**: 814–820.
28 Klein S, Sheard NF, Pi-Sunyer X, Daly A, Wylie-Rosett J, Kulkarni K *et al*. Weight management through lifestyle modification for the prevention and management of type 2 diabetes: rationale and strategies. A statement of the American Diabetes Association, the North American Association for the Study of Obesity, and the American Society for Clinical Nutrition. *Am J Clin Nutr* 2004; **80**: 257–263.
29 Malpuech-Brugere C, Verboeket-van de Venne WP, Mensink RP, Arnal MA, Morio B, Brandolini M *et al*. Effects of two conjugated linoleic acid isomers on body fat mass in overweight humans. *Obes Res* 2004; **12**: 591–598.
30 Lopez-Garcia E, Schulze MB, Meigs JB, Manson JE, Rifai N, Stampfer MJ *et al*. Consumption of *trans* fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. *J Nutr* 2005; **135**: 562–566.
31 Smedman A, Vessby B. Conjugated linoleic acid supplementation in humans – metabolic effects. *Lipids* 2001; **36**: 773–781.
32 Schulz LO, Schoeller DA. A compilation of total daily energy expenditures and body weights in healthy adults. *Am J Clin Nutr* 1994; **60**: 676–681.
33 Subar AF, Kipnis V, Troiano RP, Midthune D, Schoeller DA, Bingham S *et al*. Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: the OPEN study. *Am J Epidemiol* 2003; **158**: 1–13.