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Safety profile of conjugated linoleic acid in a 12-month trial in obese humans

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Abstract

Conjugated linoleic acid (CLA) is marketed in numerous commercially available dietary supplements, but few studies have looked at the long-term safety of this product. The current study evaluated the safety of one CLA product (Clarinol™) over a one-year period in obese humans who were generally healthy. This was a randomized, double-blind study consisting of three phases in which subjects were given 6 g/day of CLA or placebo. Phase 1 was a low calorie diet (13 kcal/kg desirable weight) for 12 weeks or until 10–20% of initial body weight was lost. In phase 2, from weeks 12 to 28, subjects were re-fed a diet providing 25–30 kcal/kg of desirable body weight. Phase 3 was open label, with subjects from both groups taking CLA from weeks 28 to 52. At biweekly visits, subjects completed a questionnaire evaluating side effects and adverse events. Blood was taken for assay of liver function, glucose, insulin, serum lipids, blood counts, and general chemistry. Overall, body composition did not differ between groups. Laboratory tests showed no adverse effects of CLA. Adverse events and side effects were less in the CLA group compared to placebo. We conclude that CLA as Clarinol™ is safe for use in obese humans for at least one year.

Keywords: Hepatic function; Glucose; Insulin; Insulin resistance; Dietary supplement; Low calorie diet; Adverse events

1. Introduction

Conjugated linoleic acid (CLA) is present in the normal human diet in small quantities, coming mainly from meat and dairy products of ruminant animals (Parodi, 1994; Lin et al., 1995; Ritzenthaler et al., 2001). Estimated average daily intake of CLA from dietary sources is about 200 mg/day (Ritzenthaler et al., 2001). The predominant isomer of CLA in those dietary sources is the *c*9, *t*11 form, amounting to as much as 90% of the total CLA content of dairy foods (Chin et al., 1992). CLA can be produced industrially by partial hydrogenation of linoleic acid. The majority of dietary supplements sold on the market contain industrially produced CLA, and consist predominantly of two isomers, *c*9, *t*11 and *t*10, *c*12 in equal amounts. When given separately, these isomers have different effects. In animals, CLA has been shown to have effects on multiple systems, including body composition, immune function, and hormone action and metabolism (Whigham et al., 2000; O'Shea et al., 2004). There have been relatively few studies of CLA in humans, and questions of its safety in supplemental form still remain. Despite the paucity of data in humans, CLA is sold over-the-counter, and quantities far larger than can be obtained in the diet are consumed.

Abbreviations: AE, adverse event; CLA, conjugated linoleic acid; BMI, body mass index; LCD, low calorie diet; EKG, electrocardiogram; RMR, resting metabolic rate; RQ, respiratory quotient; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglycerides; CBC, complete blood count; WBC, white blood cells

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The main areas of concern regarding safety in humans are focused on glucose/insulin metabolism and liver function because of adverse changes in some animal studies. Mice develop increasing insulin resistance and lipodystrophy with increasing doses of CLA, particularly the *t*10, *c*12 isomer (Tsuboyama-Kasaoka et al., 2000; Delany et al., 1999; Clement et al., 2002; Roche et al., 2002). Mice also develop triglyceride accumulation and liver enzyme elevations on CLA, and the effects are dose-related (Delany et al., 1999; Clement et al., 2002; Belury and Kempa-Steczko, 1997; Takahashi et al., 2003). Trends for insulin resistance occur in other animal species including hamsters and pigs (Bouthegourd et al., 2002; Stangl et al., 1999). Pigs are reported to have trends for higher insulin levels and lower white blood cell counts (Stangl et al., 1999).

In humans, insulin resistance was reported with ingestion of a supplement enriched with the *t*10, *c*12 isomer, but not with a mixed preparation of predominantly *c*9, *t*11 and *t*10, *c*12 isomers (Riserus et al., 2002). In a recent double-blind placebo controlled study, the same enriched *t*10, *c*12 CLA isomer was given to 81 healthy men and women for a period of 18 weeks at 3 g/day and did not show any effect in relation to insulin resistance (Malpuech-Brugere et al., 2004). No liver function abnormalities have been reported in humans, but studies to date have been short-term, usually three months or less (Smedman and Vessby, 2001; Larsen et al., 2003). Evaluation of immune function after three months of CLA treatment did not reveal any adverse changes (Kelley et al., 2000; Albers et al., 2003). Additional long-term studies are needed to determine the safety of CLA. The current study evaluated the safety of 6 g per day of CLA (as Clarinol™ G80) over 12 months in humans.

2. Methods

2.1. Subjects

The inclusion criteria for subjects for this study were a body mass index (BMI) between 27 and 35 kg/m^2, age between 18 and 50, weight stable, no serious medical or psychiatric illness, no interfering drugs, and not pregnant or lactating. Sixty-four subjects were enrolled at baseline. Because the protocol involved a series of perturbations over a period of a year, the data analyses were meaningful only for subjects who remained in the protocol for at least the first phase of the study, which was a weight loss intervention. A total of 50 subjects met these criteria. The baseline characteristics of the "completers" are listed in Table 1, and reasons for dropout are listed in Table 2. The protocol and consent form were approved by the Human Subjects Committee at the University of Wisconsin, Madison.

Table 1
Baseline characteristics of subjects

	Placebo	CLA	Early drops
Females	15	20	11
Males	8	7	2
Race	1 H[a], 22 C	1 AA, 26 C	13 C
Age	41.2 ± 5.9	43.4 ± 4.8	36 ± 8.7
Total wt. (kg)	91.4 ± 12.5	93.4 ± 13.8	88.9 ± 8.8
BMI	31.4 ± 2.3	32.0 ± 2.1	31.9 ± 2.4

[a] H=Hispanic, C=Caucasian, AA=African American. Early drops are those subjects who did not complete first phase (12 weeks) of the study.

Table 2
Description of subjects who dropped out of study

Week of drop	Reason for drop	Treatment
Baseline	Lost to follow-up	CLA
Baseline	Too busy	CLA
Baseline	Thyroid levels	CLA
Baseline	Too busy	CLA
Week 2	AEs related to LCD	CLA
Week 2	AE-Rash	CLA
Week 4	Could not take LCD	CLA
Week 4	Lack of commitment	CLA
Week 4	Too busy	CLA
Week 8	Too busy	CLA
Baseline	Too busy	Placebo
Baseline	Too busy	Placebo
Baseline	Could not take LCD	Placebo
Week 16	Pregnant	Placebo
Week 18	AE-hair loss	Placebo
Week 28	AE-lump in neck	Placebo
Week 38	Too busy	Placebo

2.2. Research design

The main outcome objective of this protocol was to determine if CLA could prevent fat regain in obese subjects after a period of significant weight loss. Subjects were randomized in a double-blinded fashion to 6 g/day of CLA mixed isomers (as 7.5 g/day Clarinol™, 37.3% *c*9, *t*11 and 37.6% *t*10, *c*12) or placebo (high oleic sunflower oil) (Table 3). Thus, subjects received six 1.25 g capsules per day containing either Clarinol™ or placebo (provided by Loders-Croklaan, Wormerveer, The Netherlands). There were three phases of the protocol. For the first two phases, subjects and investigators were blinded to treatment group. Fifty subjects completed phase I, which consisted of a low calorie diet (LCD) for rapid weight loss. Subjects were supplied with a primarily liquid LCD providing 13 kcal/kg of desirable body weight (subject's weight at a BMI of 22 kg/m^2) over 12 weeks. Phase I ended when subjects had lost between 10% and 20% of initial body weight, or at 12 weeks, whichever came first. Forty-eight subjects completed phase II, which was a weight-maintenance/weight-regain phase lasting for an additional 16 weeks.

Table 3
Typical values for analysis of CLA composition

Compound (%)	High oleic sunflower oil	CLA (50:50 *c*9, *t*11: *t*10, *c*12)
*c*9, *t*11 CLA isomer	–	37.3
*t*10, *c*12 CLA isomer	–	37.6
t, *t* CLA isomers	–	1.3
Total CLA isomers	–	79.4
Total unsaturated fatty acids	91.2	92.4
Saturated fatty acids	8.8	6.9

During this time subjects initially were gradually reintroduced to solid food over a two-week transition period. They then ate a "maintenance" diet designed to preserve or extend weight loss, but many subjects had difficulty adhering to this diet, so weight regain was common. Subjects were prescribed a calorie intake of about 25–30 kcal/kg of body weight at a BMI of 22 kg/m^2 for this maintenance diet. Forty-six subjects completed phase III, which was an open label study for five months to continue to assess long-term safety. All subjects were given 6 g of CLA/day during this phase, which extended the study to 12 months total. Subjects were instructed to continue to follow the "maintenance diet."

2.3. Clinical evaluation and follow up

Subjects were recruited from the Clinical Nutrition Center database and by local advertisement. At baseline, volunteers gave informed consent, then underwent a complete history and physical examination, EKG, and fasting lab tests (see below) to determine if they met all inclusion criteria. At the baseline visit to the clinic, qualifying subjects underwent body composition analysis, determination of resting metabolic rate (RMR) and respiratory quotient (RQ), submitted baseline exercise and food intake diaries from the previous two weeks, and filled out an adverse event questionnaire. They were randomized to placebo or CLA and sent home with a 16-day supply of capsules and the LCD. They were scheduled for clinic visits every two weeks for the remainder of the trial. Complete lab tests, body composition by deuterium water dilution, RMR and RQ determination, and EKG were done at all major time points (baseline, weeks 12, 28, and 52). In addition, limited lab tests (insulin, glucose, liver function) were done at weeks 2, 6, 16, 22, 36, and 44. Body composition by BodPod® was performed monthly. At every clinic visit, vital signs, weight, and body composition by BIA were recorded, the adverse event questionnaire was completed by subjects, and subjects met with a study dietician to discuss weight loss progress and adherence to the protocol. Food intake and exercise diaries were submitted by subjects at every clinic visit during the LCD phase and monthly thereafter.

2.4. Procedures

Body composition analysis was performed by body density determination by air displacement using BodPod® (Life Measurements, Inc., Concord, CA) (McCrory et al., 1995; Sardinha et al., 1998; Wells and Fuller, 2001), deuterium dilution for total body water (Clark et al., 2003), and bioelectrical impedance analysis (Tanita Model 310, Chicago, IL) (Jebb et al., 2000). EKG tracings were analyzed by our consultant, Dr. Patrick McBride, Cardiologist at the University of Wisconsin. RMR and RQ were determined by metabolic cart (DeltaTrac II, Sensor Medics, Yorba Linda, CA).

2.5. Laboratory tests

Lab tests performed at the times noted above included a standard chemistry panel with electrolytes, blood urea nitrogen, creatinine, liver function tests (ALT, AST, alkaline phosphatase), serum total protein and albumin, uric acid, and magnesium. A complete blood count (CBC) consisted of a hematocrit, hemoglobin, red blood cell indices, and differential. A serum lipid panel consisted of total cholesterol, triglycerides, HDL, and a calculated LDL. Glucose and insulin assays were done separately, and HOMA calculations performed using the formula: fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5 (Matthews et al., 1985). All lab tests were performed by the Penn Medical Laboratories, MedStar Research Institute, Washington, DC.

2.6. Statistical analyses

Data were analyzed using repeated measures ANOVA models. All models included treatment assignment, week and a treatment-by-week interaction term as fixed effects and subject as a random effect. Models were fit with and without adjustment for sex. Analyses were performed using Proc Mixed in SAS (SAS Institute, Cary, NC). A nominal p-value of $p < 0.05$ was regarded as statistically significant.

3. Results

3.1. Laboratory data

As noted above, the elements of particular concern from a laboratory test safety standpoint were insulin, glucose, and liver function tests (ALT, AST, alkaline phosphatase). There were no significant differences between groups in insulin levels at any of the time points (Fig. 1). CLA subjects had a significantly higher serum glucose level compared to placebo subjects at week 2 (93.1 ± 1.5 vs. 87.2 ± 1.6 mg/dl, $p < 0.007$), but

differences were not significant at any other time points (Fig. 2). The difference at week 2 resulted from a decrease in mean glucose levels in the control group of 1 mg/dl, coupled with an increase of 2 mg/dl in the CLA group. No subject with a normal baseline glucose developed glucose intolerance (⩾110 mg/dl), but one CLA subject with a baseline glucose of 112 mg/dl increased to 118 mg/dl. Using HOMA as a measure of insulin resistance (Matthews et al., 1985) there were no differences between groups at any time point throughout the study (Table 4).

Alkaline phosphatase levels were similar between groups throughout the trial (Fig. 3). Placebo subjects had significantly higher AST levels at week 12 (Fig. 4) and higher ALT levels at weeks 6 and 12 (Fig. 5), but not at any other time points. There were no significant differences between groups in any of the other laboratory measures at baseline and week 12 (Table 5).

At week 28, CLA subjects had significantly higher TG (154.7 ± 11.4 vs. 114.9 ± 12.3 mg/dl, $p = 0.02$) and WBC (6.6 ± 0.3 vs. 5.5 ± 0.3 K/μl, $p = 0.02$), and lower HDL (53.2 ± 2.4 vs. 62.3 ± 2.5 mg/dl, $p = 0.02$). The

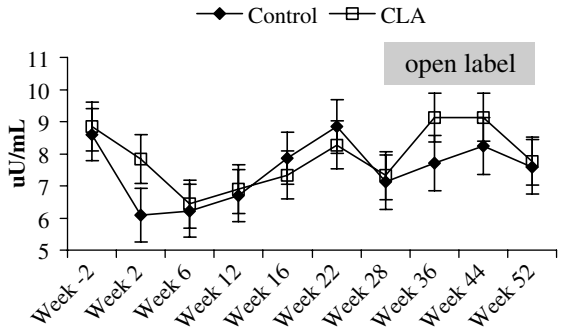

Fig. 1. Serum insulin. There were no significant differences between CLA and placebo groups. Weeks 28–52 were open label with all subjects receiving CLA. * $p < 0.05$.

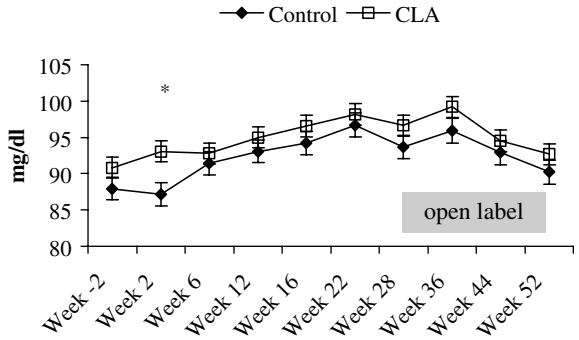

Fig. 2. Serum glucose. At week 2, glucose was significantly higher in the CLA vs. placebo group, but this was not felt to be clinically significant. Glucose went up from baseline by 2 mg/dl in CLA and down by 1 mg/dl in placebo. Weeks 28–52 were open label with all subjects receiving CLA. * $p < 0.05$.

Table 4
HOMA values calculated for each time point measured (±SEM)

	Placebo	CLA
Week 0	1.86 ± 0.15	2.00 ± 0.15
Week 2	1.33 ± 0.10	1.83 ± 0.17
Week 6	1.45 ± 0.17	1.49 ± 0.19
Week 12	1.56 ± 0.14	1.64 ± 0.16
Week 16	1.84 ± 0.24	1.77 ± 0.16
Week 22	2.22 ± 0.42	2.01 ± 0.15
Week 28	1.69 ± 0.17	1.77 ± 0.16
Week 36	1.84 ± 0.15	2.32 ± 0.35
Week 44	1.89 ± 0.18	2.19 ± 0.32
Week 52	1.78 ± 0.19	1.83 ± 0.20

There were no significant differences between groups throughout the study.

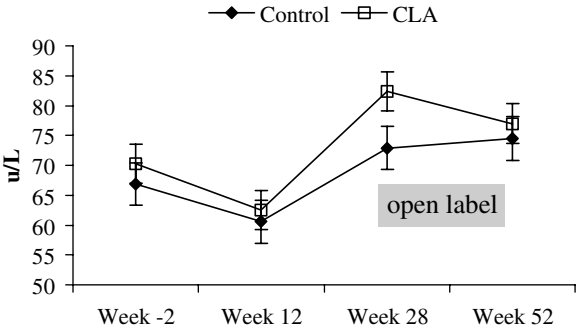

Fig. 3. Serum alkaline phosphatase. There were no significant differences between CLA and placebo groups. Weeks 28–52 were open label with all subjects receiving CLA. * $p < 0.05$.

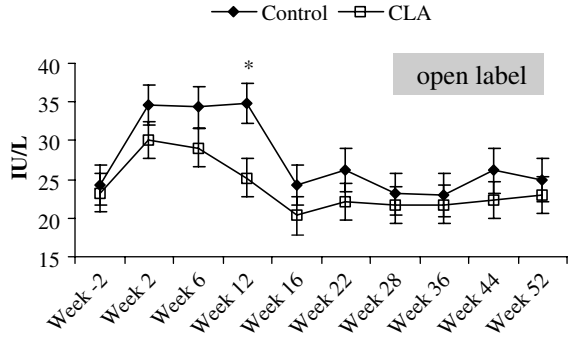

Fig. 4. Serum AST. At week 12, serum AST was significantly lower in the CLA group vs. placebo group. Weeks 28–52 were open label with all subjects receiving CLA. * $p < 0.05$.

CLA subjects started with higher TG and WBC at baseline (both p = NS compared to placebo), and the patterns of change with diet were approximately similar (Table 5). Therefore, the differences at week 28 were felt not to be clinically meaningful. HDL cholesterol levels in CLA and placebo groups were virtually identical at baseline and week 12, so the dramatic rise at week 28 for placebo is difficult to explain.

At week 52, CLA subjects had higher TG (150.5 ± 11.4 vs. 118.2 ± 12.5 mg/dl, $p = 0.053$) and

pulse (66.6 ± 1.67 vs. 61.2 ± 1.9 beats/min, $p = 0.03$). All other measures at weeks 28 and 52 were not significantly different (Table 5).

When the data were compared as an analysis of change in measures between major time points (Table 6), the CLA group had a significantly smaller increase in cholesterol levels from weeks 12 to 28 (19.2 ± 4.8 vs. 33.1 ± 5.3, $p = 0.054$). The control group had a significantly greater rise in HDL from weeks 12 to 28 (10.3 ± 2.4 vs. 2.1 ± 2.1 mg/dl, $p = 0.01$), but then had a decrease from weeks 28 to 52 while the CLA group increased HDL levels during that same time (−8.3 ± 2.4 vs. 1.5 ± 2.1, $p = 0.003$). All other changes between major time points were not significantly different. No adverse event was significantly greater in the CLA group compared to the placebo group (data not shown).

3.2. Dropout rate and compliance

Of the 63 subjects who were initially randomized into the study, 17 (10 CLA, 7 placebo) dropped out before the end of the study at 52 weeks (Table 2). A summary of these subjects and their reasons for dropout are listed in Table 2. Nine subjects dropped out at or before the first follow-up visit. Of these, the most frequent reason given for dropping out was that the subjects were "too busy", but conversations with some of these subjects suggested that they had difficulties remaining on the liquid formula LCD, and two subjects dropped out specifically for this reason. One subject was belatedly found to have abnormal thyroid function tests from baseline laboratory evaluations, and was dropped from the study. Four subjects (2 CLA, 2 placebo) dropped out for adverse events. A CLA subject had a skin rash that the investigators thought was unlikely to be due to the CLA, but possibly due to the ingredients in the liquid formula diet. One CLA subject complained of AEs at two weeks that were attributed by the subject to chocolate in the liquid LCD, but the investigators felt this was unlikely to be due to the CLA. A placebo subject

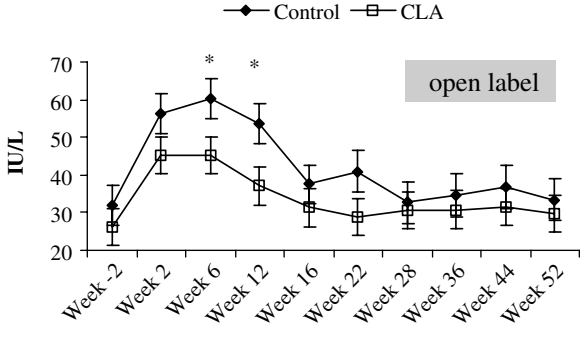

Fig. 5. Serum ALT. At weeks 6 and 12, serum ALT was significantly lower in the CLA group vs. placebo group. Weeks 28–52 were open label with all subjects receiving CLA. * $p < 0.05$.

Table 5
Laboratory results at baseline, weeks 12, 28, and 52 (±SEM)

	Baseline			Week 12			Week 28			Week 52		
	Placebo	CLA	p	Placebo	CLA	p	Placebo	CLA	p	Placebo	CLA	p
Cholesterol (mg/dl)	214.9 ± 7.9	207.2 ± 7.6	NS	174.7 ± 7.9	179.5 ± 7.6	NS	207.8 ± 8.0	198.7 ± 7.6	NS	205.1 ± 8.1	204.4 ± 7.6	NS
LDL (mg/dl)	135.9 ± 7.2	125.0 ± 6.9	NS	102.3 ± 7.2	102.5 ± 6.9	NS	122.6 ± 7.3	114.7 ± 6.9	NS	127.6 ± 7.3	119.5 ± 6.9	NS
TG (mg/dl)	139.3 ± 12.0	160.6 ± 11.4	NS	102.3 ± 12.0	128.4 ± 11.4	NS	114.9 ± 12.3	154.7 ± 11.4	0.02	118.2 ± 12.5	150.5 ± 11.4	0.053
HDL (mg/dl)	51.8 ± 2.5	51.4 ± 2.4	NS	52.0 ± 2.5	51.1 ± 2.4	NS	62.3 ± 2.5	53.2 ± 2.4	0.01	54.0 ± 2.6	54.7 ± 2.4	NS
Hematocrit (%)	44.3 ± 0.7	45.4 ± 0.7	NS	43.4 ± 0.7	44.7 ± 0.7	NS	43.5 ± 0.8	44.0 ± 0.7	NS	41.1 ± 0.8	42.8 ± 0.7	NS
WBC (cells/m^3)	5.66 ± 0.30	6.16 ± 0.29	NS	4.63 ± 0.30	5.19 ± 0.29	NS	5.53 ± 0.32	6.55 ± 0.29	0.02	5.01 ± 0.32	5.48 ± 0.29	NS
Lymphocytes (%)	36.8 ± 2.3	41.2 ± 2.2	NS	38.6 ± 2.3	41.5 ± 2.2	NS	44.5 ± 2.4	41.2 ± 2.2	NS	45.0 ± 2.4	49.4 ± 2.2	NS
Neutrophils (%)	50.4 ± 2.4	47.7 ± 2.3	NS	49.3 ± 2.4	45.4 ± 2.3	NS	44.1 ± 2.6	47.9 ± 2.3	NS	41.1 ± 2.6	38.5 ± 2.3	NS
Monocytes (%)	7.3 ± 1.3	6.4 ± 1.2	NS	7.1 ± 1.3	6.3 ± 1.2	NS	5.2 ± 1.4	7.9 ± 1.2	NS	7.2 ± 1.4	5.8 ± 1.3	NS

NS = not significant ($p > 0.05$).

Table 6
Change in laboratory results in phases 1, 2, and 3 (±SEM)

	Change from baseline to week 12			Change from weeks 12 to 28			Change from weeks 28 to 52		
	Placebo	CLA	*p*	Placebo	CLA	*p*	Placebo	CLA	*p*
Cholesterol (mg/dl)	−40.1 ± 5.1	−27.7 ± 4.8	NS	33.1 ± 5.3	19.2 ± 4.8	0.054	−2.7 ± 5.5	5.7 ± 4.8	NS
LDL (mg/dl)	−33.5 ± 4.9	−22.5 ± 4.5	NS	20.2 ± 5.0	12.1 ± 4.5	NS	5.1 ± 5.2	4.9 ± 4.5	NS
TG (mg/dl)	−37.0 ± 11.2	−32.2 ± 10.3	NS	12.6 ± 11.6	26.3 ± 10.3	NS	3.3 ± 11.9	−4.2 ± 10.3	NS
HDL (mg/dl)	0.3 ± 2.3	−0.3 ± 2.1	NS	10.3 ± 2.3	2.1 ± 2.1	0.01	−8.3 ± 2.4	1.5 ± 2.1	0.003
Hematocrit (%)	−1.0 ± 0.8	−0.8 ± 0.7	NS	0.1 ± 0.8	−0.7 ± 0.7	NS	−2.4 ± 0.9	−1.2 ± 0.8	NS
WBC (cells/m^3)	−1.03 ± 0.33	−0.97 ± 0.31	NS	0.90 ± 0.34	1.36 ± 0.31	NS	−0.52 ± 0.35	−1.06 ± 0.31	NS
Lymphocytes (%)	1.8 ± 2.8	0.3 ± 2.6	NS	5.9 ± 3.0	−0.3 ± 2.6	NS	0.5 ± 3.1	8.2 ± 2.7	NS
Neutrophils (%)	−1.2 ± 3.2	−2.3 ± 2.9	NS	−5.2 ± 3.3	2.5 ± 2.9	NS	−3.1 ± 3.4	−9.4 ± 3.0	NS
Monocytes (%)	−0.3 ± 1.8	−0.1 ± 1.6	NS	−1.9 ± 1.8	1.6 ± 1.6	NS	2.0 ± 1.9	−2.1 ± 1.6	NS

NS = not significant ($p > 0.05$).

dropped out because of hair loss, which is a common side effect of LCD. Another placebo subject dropped out because of a subcutaneous lump in the neck.

3.3. Self-reported adverse events

Adverse events recorded by subjects on the follow-up questionnaires that were completed at each biweekly visit overall were lower in the CLA group compared to placebo. Significantly lower frequencies of skin rash, depression, irritability/anger, hair loss, and infection were noted in the CLA group vs. placebo.

3.4. Changes in body composition

There were no significant changes overall in body weight or body fat between the CLA group and placebo.

4. Discussion

The major conclusions from this study are that CLA as Clarinol™ appears safe for use in obese humans for periods of up to one year. Specifically, CLA did not affect glucose and insulin levels or liver function tests in a negative fashion over the long term. Our data show a small, but significant, difference in glucose levels after two weeks on a LCD, but these differences disappear quickly and neither glucose nor insulin were different thereafter. Assessment of insulin resistance by HOMA (Matthews et al., 1985) showed no differences between groups throughout the study. Likewise, liver function tests are not adversely affected at any time, and in fact, AST and ALT levels were higher in the placebo group at some time periods during the study. There was no evidence of a difference in white blood cells (WBC), including any component of the WBC count. Finally, adverse events were significantly fewer in CLA subjects.

These variables in humans are of interest because of the effects of CLA noted in some previous animal studies. In mice, adding CLA to the diet, particularly the *t*10, *c*12 CLA isomer, resulted in hyperinsulinemia and insulin resistance (Tsuboyama-Kasaoka et al., 2000; Delany et al., 1999; Clement et al., 2002; Roche et al., 2002). A similar trend was observed in CLA fed hamsters (Bouthegourd et al., 2002) and pigs (Stangl et al., 1999). However, increasing the dietary fat content of the diet attenuated the phenomenon of CLA-induced insulin resistance and lipodystrophy in mice (Tsuboyama-Kasaoka et al., 2003). Some studies report a positive effect of CLA on glucose metabolism. Using a glucose tolerance test, CLA fed to male Zucker diabetic fatty rats has been shown to normalize impaired glucose tolerance and improve insulin sensitivity, perhaps by increasing adiponectin levels (Houseknecht et al., 1998; Nagao et al., 2003). The *t*10, *c*12 isomer of CLA was associated with a significant reduction of plasma leptin concentrations in rats (Yamasaki et al., 2000, 2003). Leptin infusions are reported to reduce the hyperinsulinemia associated with CLA (Tsuboyama-Kasaoka et al., 2000). However, CLA produced apoptosis of adipocytes in mice (Tsuboyama-Kasaoka et al., 2000; Hargrave et al., 2002), so the interactions of each CLA isomer, insulin resistance, leptin, and apoptosis are not clear and seem to be species dependent.

Studies of insulin–glucose metabolism in humans are mixed, but the type of CLA isomer appears to play a central role. A small, double-blind clinical trial looked at CLA supplementation (6.0 g/day) or placebo oil in the management of type 2 diabetes over a period of six weeks (Belury et al., 2003). An improved serum insulin was noted in 64% of subjects on CLA supplementation compared with an improvement in 40% of subjects taking placebo supplements. Similarly, Eyjolfson et al. (2004) found an improvement in insulin sensitivity index (ISI) and decrease in fasting insulin in young sedentary subjects given 4 g/day of mixed isomer CLA for eight weeks. Other studies in nondiabetic obese/overweight humans (Blankson et al., 2000; Berven et al., 2000; Smedman and Vessby, 2001; Kamphuis et al., 2003; Malpuech-Brugere et al., 2004; Gaullier et al., 2004), and in healthy humans (Noone et al., 2002; Medina

et al., 2000) found no negative effect on serum glucose and insulin with CLA.

Riserus et al. reported that CLA did not negatively affect glucose metabolism in nondiabetic overweight humans (2001). Humans fed a mixture of the two CLA isomers containing 0.7–2.0 g of each isomer did not show significant changes in plasma insulin concentrations, and they had a significant decrease in sagittal abdominal diameter. However, Riserus et al. (2002) reported isomer-specific insulin resistance with CLA. In a randomized, controlled trial in 60 overweight viscerally obese men with insulin resistance syndrome, subjects received either 2.42 g/day of CLA containing both the *c*9, *t*11 and the *t*10, *c*12 CLA isomers in a 50:50 ratio, placebo (olive oil), or 2.6 g/day of *t*10, *c*12 CLA isomer alone for 12 weeks. Euglycemic, hyperinsulinemic clamps were used to evaluate treatment effects on insulin–mediated glucose disposal. The mixture of the two CLA isomers had no effect on glucose metabolism, but the product enriched in the *t*10, *c*12 CLA isomer increased insulin resistance compared with the control. Plasma insulin and glucose concentrations were significantly increased from baseline in the *t*10, *c*12 CLA group, but this effect was not significant when compared with the control group. Our HOMA data did not show any evidence of insulin resistance and support the safety of CLA when consumed as a mixture of the *c*9, *t*11 and the *t*10, *c*12 CLA isomers in a 50:50 ratio.

Gaullier et al. (2004) treated overweight subjects for 12 months with CLA as either free fatty acids or triglycerides and found no significant changes in glucose or insulin levels from baseline or from placebo. Hemoglobin A1c was increased in all three groups, but there was no difference between groups. In contrast to our study, these authors found a modest reduction in body fat, but no significant difference in body weight.

The second main concern was alterations in liver function tests. In CLA-fed mice, cytoplasmic vacuolization in hepatocytes typical of lipid accumulation was observed (Tsuboyama-Kasaoka et al., 2000; Delany et al., 1999). A positive association has also been found between increasing amounts of dietary CLA and total lipid extracted per gram liver tissue in mice (Belury and Kempa-Steczko, 1997). Furthermore, liver weights were higher in CLA-fed mice compared to controls (Delany et al., 1999). Leptin treatment has been shown to partially reverse the vacuolization and decrease liver weight (Tsuboyama-Kasaoka et al., 2000). In hamsters, liver histology revealed that increased liver weights with CLA supplementation were due to hypertrophy (DeDeckere et al., 1999). However, increases in liver weight were reduced in one group of hamsters consuming *c*9, *t*11 CLA. It was not possible to determine if this was an effect of *c*9, *t*11 CLA or due to a lower intake of *t*10, *c*12 CLA (DeDeckere et al., 1999). Chin et al. (1992) reported no effect on liver weight in rats, but O'Hagan and Menzel (2003) found hepatocellular toxicity at massive doses of 15% by weight of the diet as CLA. A no observed adverse effect level (NOAEL) of 2433 mg/kg bw/day for male and 2728 mg/kg bw/day female rats was identified in the study, amounts much greater than would be feasible for humans. A possible explanation for the resistance of rats to the hepatic effects of CLA compared to mice and hamsters is the higher induction of hepatic PPAR-α by CLA in mice than in rats (Moya-Camarena et al., 1999a,b).

In humans, no adverse effects on liver safety parameters are reported. Blankson et al. (2000) investigated the effect of CLA (1.7, 3.4, 5.1 and 6.8 g/day) vs. placebo for 12 weeks in overweight or obese subjects, and reported no significant differences between the groups regarding the frequency of side effects. In another 12-week study investigating the effect of CLA (3.4 g/day for 12 weeks), Berven et al. (2000) also reported no clinically significant changes in liver safety parameters. The authors concluded that the use of CLA does not impair liver function in humans. Pure isomers of CLA at 3 g/day for 18 weeks did not demonstrate any adverse effect (Malpuech-Brugere et al., 2004). Gaullier et al. (2004) found no significant changes in liver function tests over a 12-month period of treatment with CLA.

Finally, in pigs there was a trend for a decreased WBC count in one study (Stangl et al., 1999), but Bassaganya-Riera et al. (2001) found that CLA actually increased lymphocytes and reduced infection. Gaullier et al. (2004) found an increase in leukocytes in subjects on CLA as either free fatty acids or triglycerides after 12 months of treatment. We did not notice an increase in lymphocytes in the humans in our study, but we did find a significant decrease in infection rate (as assessed by adverse events questionnaire, data not shown) in CLA treated subjects compared to those on placebo.

In summary, the results of this study suggest that CLA as Clarinol™ is safe for use up to 12 months in obese humans. However, because CLA has been shown to decrease breast milk fat, it should not be used in pregnant and lactating women (Masters et al., 2002). We found no clinically significant differences between CLA and placebo in all screening laboratory tests. Specifically, for liver function, glucose and insulin levels, insulin resistance by HOMA analysis, and white blood cell counts that were areas of concern based on prior animal or human studies, results in CLA subjects were either not clinically worse or were better compared to placebo. Subjects on CLA had significantly fewer clinical complaints and adverse events than did subjects on placebo. There were no overall differences between CLA and placebo in measures of total body weight and body composition. Responses of humans to CLA are different than in animals in multiple areas of physiology and biochemistry. Because dietary supplements on the market are highly variable in quality and even amounts of total

CLA (Gaullier et al., 2002), the results of this study may not be applicable for all CLA products.

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