

High Dose *trans*-10,*cis*-12 CLA Increases Lean Body Mass in Hamsters, but Elevates Levels of Plasma Lipids and Liver Enzyme Biomarkers

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Abstract The current study examined the efficacy of graded doses of *c9,t11* and *t10,c12* CLA isomers on body composition, energy expenditure, hepatic and serum lipid liver biomarkers in hamsters. Animals ($n = 105$) were randomized to seven treatments (control, 1, 2, 3% of *c9,t11*; 1, 2, 3% of *t10,c12*) for 28 days. After 28 days treatment, 1–3% of *t10,c12* lowered ($p < 0.05$) body fat mass compared to the control group. The 1–3% *t10,c12* and 3% *c9,t11* fed groups showed higher ($p < 0.05$) lean mass compared to other groups. We observed unfavorable changes in plasma total cholesterol and non-HDL cholesterol levels in animals fed with 3% *t10,c12* CLA isomers. The 2%, 3% *t10,c12* groups presented elevated ($p < 0.05$) ALT levels. The present data suggest that a diet enriched with more than 2% *t10,c12* led to liver malfunction and poses unfavorable changes on plasma lipid profiles. The 1% *t10,c12* CLA lowered ($p < 0.05$) body fat mass and

increased ($p < 0.05$) lean body mass. The *c9,t11* CLA has less potent actions than *t10,c12* CLA. We conclude that the actions of CLA on energy and lipid metabolism are form and dose dependent in the hamster model.

Keywords *trans*-10,*cis*-12 CLA · *cis*-9,*trans*-11 CLA · Body composition · Lipids · Hamster · Liver biomarker · Safety

Abbreviations

DEXA Dual energy X-ray absorptiometry
AST Aspartate aminotransferase
ALT Alanine aminotransferase
GGT γ -Glutamyltranspeptidase

Introduction

Major isomers of CLA include *cis*-9,*trans*-11 (*c9,t11*) CLA and *trans*-10,*cis*-12 (*t10,c12*) CLA [1]. Over the last decade, health benefits of CLA have received a great deal of attention in various animal models and humans. Results have shown that *c9,t11* and *t10,c12* CLA mixtures possess anticancer effects both in vitro and in vivo [2–5]. Other effects including body composition modulation, atherosclerosis prevention, as well as immune system enhancement, have also been suggested in animal models [6–9]. CLA isomer enriched diets also effectively reduce body fat and increase lean body mass in several species [7, 10, 11]. However, previous studies have mainly focused on the effect of *c9,t11* and *t10,c12* CLA mixtures and conflicting results have been reported in many studies (Supplemental Table 1). Such sizable variation in results may due to the variability in animal model, duration of feeding as well as form, and the dosage of each isomer used. Indeed, the two

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CLA isomers may play different roles in body composition changes as well as lipid metabolism. The favorable modulation of body composition is mainly ascribed to the *t10,c12* CLA isomer [12], while a less potent effect has been reported for the *c9,t11* CLA isomer. Lack of effect on *c9,t11* CLA consumption on body composition modulation may due to the use of mixture of isomers, where the effect of one isomer may overshadow another, or to the relative low dosage used in different trials.

In terms of lipid metabolism and safety, previous investigations have shown that CLA lowers total cholesterol and triglyceride levels in plasma [13, 14]; nevertheless, these appear to be highly species dependent. Less consistent results have been observed in hamster and human studies [13, 15]. Moreover, safety issues have been raised over CLA supplementation since increased spleen and liver weights have been observed in some animal studies [16, 17]. Indeed, a systematic comparison of the two major isomers at graded dosages has not been previously conducted. Very few studies have investigated the effect of highly enriched *c9,t11* and *t10,c12* CLA isomer effect solely (Supplement Table 1). Therefore, the current investigation was aimed to examine both isomer and dosage effect of *c9, t11* and *t10, c12* CLA on body composition changes and serum lipid content modulation in Golden Syrian hamsters. Hamsters are considered to be a suitable model for studying lipid changes due to their similar lipoprotein profile and metabolism compared to humans [18]. Additionally, safety evaluation was also assessed in the present study.

Materials and Methods

Animals and Study Design

One hundred and five male Golden Syrian hamsters (Charles River Laboratories, Montreal, Quebec) weighing between 90 and 100 g were housed individually in plastic cages and subjected to a 12 h light/dark cycle at constant room temperature 25 °C. Upon arrival, the hamsters were provided with free access to rodent chow diet (Nestle, Purina, USA) and water for 3 weeks, and then switched to a semi-purified hypercholesterolemic diet containing 5% fat and 0.25% cholesterol for 3 more weeks. The study used a completely randomized design. Hamsters were randomized into 7 groups of 15 animals. Seven experimental diets were tested, including a control diet with no CLA and treatment diets enriched with 1, 2, 3% of *c9, t11* CLA as well as 1, 2, 3% of *t10,c12* CLA, each provided for 4 weeks. CLA isomers were provided in free fatty acid form and prepared by low temperature crystallization. Both CLA isomers were supplied by Lipid Nutrition

(Wormerveer, Netherlands). Table 1 presents the macronutrient and fatty acid composition of experimental diets. Dietary ingredients were purchased from Harland Laboratories Inc. (Indiana, USA) except cornstarch and sucrose which were purchased locally. Butylated hydroxytoluene (Sigma-Aldrich, Inc. ON, Canada) was added as an antioxidant. Food intake was measured every 2 days and body weight was measured weekly. On day 28, hamsters were anesthetized by isoflurane inhalation. Blood samples were taken by cardiac puncture then transferred into pre-coated heparin tubes. Hamsters were sacrificed followed by evisceration. Organ samples were wrapped and snap frozen by liquid nitrogen then stored at -80°C until further analysis. The study was approved by the University of Manitoba Animal Care Protocol Review Committee, and carried out in accordance with the guidelines of the Canadian Council on Animal Care [19].

Body Composition Measurement

Body composition was measured by DEXA from Lunar Prodigy Advance. Tissue analyses were conducted by software Encore version 9.30.044. Percentage body fat, body fat mass and lean body mass were assessed using this software.

Lipid Profile and Hepatic Biomarker Measurement

Blood samples were thawed at room temperature then centrifuged at 3,500 rpm for 20 min. Red blood cells (RBC), plasma and serum samples were collected separately. Serum samples were used for lipid profile and liver enzyme assessment. Analyses were conducted by the Vitro Chemistry System 350 (Ortho-Clinical Diagnostics, Inc. Rochester, NY, USA). Triglyceride, total cholesterol and HDL-cholesterol levels were measured directly, whereas non-HDL cholesterol levels were calculated by subtracting HDL-cholesterol from total cholesterol content. Serum concentrations of liver enzymes AST, ALT and GGT were also measured.

Cholesterol Synthesis Measurement

Hamsters received 0.5 ml of deuterium oxide (D_2O , 99.9%, Cambridge Isotope Laboratories, Inc MA, USA) by intraperitoneal injection 2 h prior to sacrifice to assess cholesterol synthesis rate. Deuterium-enriched cholesterol was used as an indication of cholesterol synthesis [20]. Isotope ratios of DH/H_2 of plasma cholesterol were expressed in δ per mil, relative to Vienna Standard Mean Ocean Water. Enrichment of deuterium in both plasma water and RBC cholesterol were measured. Cholesterol samples extracted from RBCs were analyzed by online GC/pyrolysis/isotope

Table 1 Experimental diet composition (% w/w)

	Control	1% <i>c9,t11</i> ^a	2% <i>c9,t11</i> ^a	3% <i>c9,t11</i> ^a	1% <i>t10,c12</i> ^b	2% <i>t10,c12</i> ^b	3% <i>t10,c12</i> ^b
Casein	200	200	200	200	200	200	200
Cornstarch	280	280	280	280	280	280	280
Sucrose	360.3	360.3	360.3	360.3	360.3	360.3	360.3
Cellulose	50	50	50	50	50	50	50
DL-methionine	5	5	5	5	5	5	5
Mineral mix (AIN-93G Hamster)	40	40	40	40	40	40	40
Vitamin (AIN-76A)	10	10	10	10	10	10	10
Choline bitartrate	2	2	2	2	2	2	2
BHT	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Cholesterol	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Lard ^c	25	20	15	10	20	15	10
Safflower oil ^d	25	20	15	10	20	15	10
<i>c9,t11</i> CLA ^a	0	10	20	30	0	0	0
<i>t10,c12</i> CLA ^b	0	0	0	0	10	20	30

^a *c9,t11* CLA:0.2% C14:0, 5.1% C16:0, 0.4% C16:1, 1.6% C18:0, 17.1% C18:1, 3% C18:2, 57.5% *c9,t11*, 10.7% *t10,c12*

^b *t10,c12* CLA:0.2% C14:0, 2.6% C16:0, 0.1% C16:1, 1.6% C18:0, 4.3% C18:1, 0.4% C18:2, 13.2% *c9,t11*, 70.5% *t10,c12*

^c Lard: 2% C14:0, 26% C16:0, 3% C16:1, 14.% C18:0, 44% C18:1, 10% C18:2

^d Safflower oil:7.0% C16:0, 2.0% C18:0, 13% C18:1, 78% C18:2

ratio mass spectrometry (IRMS) equipped with an Agilent 6890N GC and Finnigan Delta V Plus IRMS (Bremen, Germany) through a Finnigan combustion interface (Combustion Interface III, Bremen, Germany). Cholesterol fractional synthesis rates were measured and calculated as previously described [20].

Energy Expenditure Assessment

On day 25, energy expenditure was assessed by the MM100-metabolic monitor system (CWE, Inc Ardmore, USA). Animals were kept in individual air chambers. Oxygen consumption was measured indirectly by monitoring oxygen and carbon dioxide concentrations in the chamber for 2 h per animal.

Hepatic Lipid Content Analysis

Liver lipids were extracted according to the Folch method [21] by chloroform and methanol in a ratio of 2:1 (v/v). Analysis of hepatic triglyceride and cholesterol concentrations was conducted by commercial available enzymatic kits (Roche Diagnostics, QC, Canada).

Statistics Analysis

All statistical analyses were performed by Statistical Analysis System (version 8.1; SAS Institute Inc Cary, NC). Data from different diet groups were analyzed by one-way ANOVA for overall significance then followed by Tukey's

post-hoc tests to identify the significant difference between treatment groups. Results were expressed as means \pm SEM (standard error mean). Regression analysis was also performed. Treatment effects and differences between means were considered significant when $p < 0.05$.

Results

Effects of CLA Consumption on Food Intake, Body Composition and Energy Expenditure

Hamsters fed with *t10,c12* CLA enriched diets showed a lower ($p < 0.05$) food intake in comparison to control and other groups (Table 2). No differences were noted in average daily food intake between the control animals and *c9,t11* treatment groups. Energy expenditure data suggested a strong tendency towards animals on three dosages of *t10,c12* CLA supplementation having greater oxygen consumption rate compared to control group and others ($p = 0.0657$). At the end of the 28-day experimental period, hamsters in all seven treatment groups exhibited similar body weights. Body composition results are presented in Table 2. DEXA analyses data indicate that hamsters fed with 1, 2, and 3% *t10,c12* CLA enriched diets showed 27, 30 and 23% less ($p < 0.0001$) body fat mass compared to control animals, respectively. Additional, higher lean body mass was found in groups fed with all three dosages of *t10,c12* CLA supplemented diets. Such effects were also observed in animals fed with 3% *c9,t11*

CLA compared to control ($p = 0.0002$). Both 1 and 3% $t10,c12$ CLA fed animals had a lower body weight gain over 4 weeks, whereas opposite effects were observed in animals on 3% $c9,t11$ CLA enriched diet (Fig. 1).

Effect of CLA Consumption on Lipid Profiles

Serum lipid content was affected by $t10,c12$ CLA isomer in hamsters (Table 3). Neither the serum HDL-cholesterol concentration nor the triglyceride concentration differed among treatment groups. However, animals consuming 3% $t10,c12$ CLA enriched diets displayed the highest total cholesterol and non-HDL cholesterol levels, in contrast to the rest of the treatment groups after 28 days of dietary intervention ($p < 0.05$).

Effect of CLA Consumption on Liver Weight and Hepatic Lipid Content

Data on effects of CLA consumption on liver weight and hepatic lipid content are presented in Table 4. Hamsters in the 2 and 3% $t10,c12$ CLA diet groups exhibited higher liver weight ($p = 0.001$). With regard to hepatic lipid content, neither liver cholesterol nor the triglyceride concentration differed between groups after 28 days of consumption of CLA enriched diets. No differences were observed between dosages. Moreover, there was no difference in serum total protein level across the treatment groups (data not shown).

Effect of CLA Consumption on Hamster Liver Enzyme Level

After the 4-week experimental period, elevated ALT levels were observed in hamsters fed 2 and 3% $t10,c12$ CLA diets ($p < 0.0001$), however, such results were not noted in any of the other treatment groups. Additionally, ALT levels in animals on 3% $t10,c12$ CLA enriched diet were higher ($p = 0.0155$) than those in animals fed with 2% $t10,c12$ CLA enriched diet. No differences were noticed in hepatic

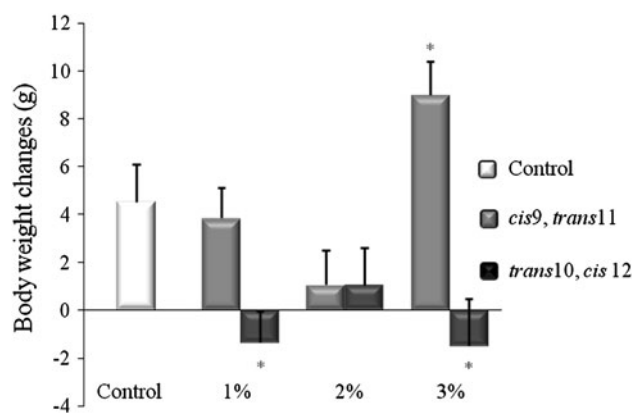


Fig. 1 Body weight changes in hamsters fed diets enriched with 1, 2, 3% $c9,t11$ CLA or 1, 2, 3% $t10,c12$ CLA for 28 days $n = 15$

AST or GGT levels across the seven experimental diets (Table 5).

Effect of CLA Consumption on Cholesterol Synthesis

Hamsters displayed similar cholesterol synthesis rates regardless of CLA intervention after 28 days (Fig. 2). Regression analysis suggested that the elevated serum cholesterol level of 2 and 3% $t10,c12$ CLA treatment groups occurred independently of the individual rates of cholesterol synthesis across animals.

Discussion

The primary finding of the present study was that 1% $t10,c12$ CLA effectively altered body composition without exhibiting potential adverse effects on the lipid profile and liver health. Similar body composition changes were also observed at 2 and 3% of $t10,c12$ CLA. However, data from both serum lipid profiles and hepatic biomarkers suggest that 3% $t10,c12$ CLA led to unfavorable increases in total as well as non-HDL cholesterol levels. Moreover, both 2 and 3% $t10,c12$ CLA treatment induced elevated ALT

Table 2 Food intake, body weight and body composition of hamster fed diets enriched with 1, 2, 3% $c9,t11$ CLA or 1, 2, 3% $t10,c12$ CLA for 28 days

Diets	Food intake (g/day)	Energy expenditure (ml/h)	Final body weight (g)	Fat body mass (g)	Lean body mass (g)
Control	6.5 ± 0.18	0.8 ± 0.1	145.4 ± 3.6	58.2 ± 2.3	65.5 ± 2.8
$c9,t11$ -1%	6.5 ± 0.2	0.8 ± 0.0	144.8 ± 2.6	57.3 ± 2.3	64.9 ± 1.8
$c9,t11$ -2%	6.2 ± 0.1	0.8 ± 0.1	141.9 ± 2.9	51.0 ± 1.7	68.9 ± 1.6
$c9,t11$ -3%	6.3 ± 0.2	0.8 ± 0.1	149.9 ± 3.3	53.4 ± 2.6	73.8 ± 2.8*
$t10,c12$ -1%	5.7 ± 0.1*	0.9 ± 0.1	139.5 ± 3.1	42.5 ± 2.6*	74.8 ± 2.6*
$t10,c12$ -2%	5.9 ± 0.2*	0.9 ± 0.1	141.9 ± 2.7	41.0 ± 1.7*	77.7 ± 1.5*
$t10,c12$ -3%	5.7 ± 0.2*	0.9 ± 0.1	139.5 ± 2.9	44.5 ± 2.1*	71.7 ± 2.0*

Values are expressed as means ± SEM

* $p < 0.05$ vs. control

Table 3 Serum cholesterol, HDL cholesterol and non-HDL cholesterol level of hamster fed with diets enriched with 1, 2, 3% *c9,t11* CLA or 1, 2, 3% *t10,c12* CLA for 28 days

Diets	Cholesterol (mmol/L)	HDL cholesterol (mmol/L)	non-HDL cholesterol (mmol/L)	Triglycerides (mmol/L)
Control	5.9 ± 0.3	3.3 ± 0.1	2.6 ± 0.2	4.4 ± 0.4
<i>c9,t11</i> -1%	6.0 ± 0.3	3.3 ± 0.1	2.6 ± 0.1	4.3 ± 0.3
<i>c9,t11</i> -2%	5.8 ± 0.3	3.2 ± 0.1	2.5 ± 0.1	4.1 ± 0.2
<i>c9,t11</i> -3%	5.9 ± 0.2	3.3 ± 0.1	2.9 ± 0.2	4.4 ± 0.3
<i>t10,c12</i> -1%	5.5 ± 0.2	3.1 ± 0.1	2.3 ± 0.2	4.3 ± 0.4
<i>t10,c12</i> -2%	6.4 ± 0.3	3.2 ± 0.1	3.3 ± 0.2	4.6 ± 0.3
<i>t10,c12</i> -3%	7.5 ± 0.5*	3.4 ± 0.1	3.9 ± 0.2*	4.0 ± 0.3

Values are expressed as means ± SEM

* $p < 0.05$ vs. control

Table 4 Liver cholesterol concentration and triglyceride content fed diets enriched with 1, 2, 3% *c9,t11* CLA or 1, 2, 3% *t10,c12* CLA for 28 days

Diets	Liver weight (g)	Hepatic cholesterol concentration (μmol/g)	Hepatic triglyceride concentration (μmol/g)
Control	7.8 ± 0.2	139.5 ± 26.8	4.8 ± 0.3
<i>c9,t11</i> -1%	8.3 ± 0.2	111.6 ± 10.7	6.0 ± 0.5
<i>c9,t11</i> -2%	8.0 ± 0.2	156.6 ± 22.7	4.9 ± 0.4
<i>c9,t11</i> -3%	8.9 ± 0.3	146.2 ± 30.0	5.8 ± 0.7
<i>t10,c12</i> -1%	8.5 ± 0.3	124.3 ± 19.8	4.7 ± 0.4
<i>t10,c12</i> -2%	9.3 ± 0.4*	105.3 ± 19.0	4.8 ± 0.3
<i>t10,c12</i> -3%	9.4 ± 0.4*	82.3 ± 13.4	5.6 ± 0.5

Values are expressed as means ± SEM

* $p < 0.05$ vs. control

Table 5 Serum concentration of liver enzymes of hamster fed diets enriched with 1, 2, 3% *c9,t11* CLA or 1, 2, 3% *t10,c12* CLA for 28 days

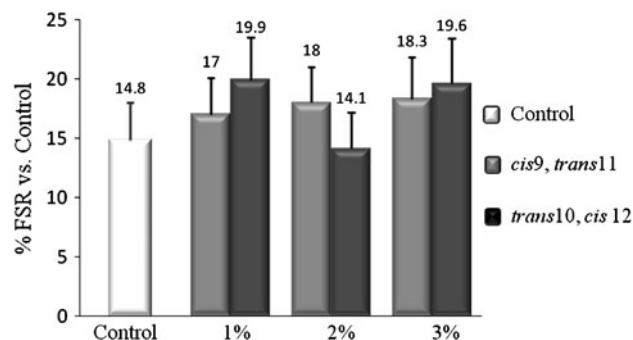
Diets	ALT (U/L)	AST (U/L)	GGT (U/L)
Control	104.8 ± 11.2	151.9 ± 24.5	6.6 ± 0.4
<i>c9,t11</i> -1%	127.7 ± 18.2	131.9 ± 20.4	5.8 ± 0.3
<i>c9,t11</i> -2%	128.6 ± 21.2	148.7 ± 23.5	5.6 ± 0.2
<i>c9,t11</i> -3%	105.0 ± 9.2	123.0 ± 20.5	5.7 ± 0.4
<i>t10,c12</i> -1%	129.3 ± 14.8	126.7 ± 28.9	5.9 ± 0.2
<i>t10,c12</i> -2%	197.8 ± 30.7*	194.9 ± 21.1	6.0 ± 0.3
<i>t10,c12</i> -3%	306.2 ± 50.9**	182.1 ± 35.7	5.4 ± 0.2

Values are expressed as means ± SEM

* $p < 0.05$ vs. control; ** $p < 0.0001$ vs. control

levels. Such changes may suggest health concerns associated with consuming high dosages of *t10,c12* CLA.

As a secondary effect of CLA, data from the current study suggest that *t10,c12* CLA decreases energy intake in hamsters. These data are in line with results reported by others [12, 22, 23]. Data from the present study show that animals on the *t10,c12* CLA treatment had a lower fat body

**Fig. 2** Cholesterol synthesis rate in hamsters fed diets enriched with 1, 2, 3% *c9,t11* CLA or 1, 2, 3% *t10,c12* CLA for 28 days $n = 15$

mass which was in accordance with previous observations [10, 22, 24, 25]. In earlier studies, *t10,c12* CLA isomers were identified as being responsible for biological effects on body composition [13]. Results from the current study also provide evidence supporting a physiological effect of CLA on fat mass in a manner that is isomer dependent. Several possible mechanisms may explain body fat reduction effects in response to CLA supplementation. Much evidence suggests that *t10,c12* CLA may induce fat mass reduction by decreasing energy intake, inhibiting adipogenesis or lipogenesis by suppressing gene expression of sterol regulatory element binding protein (SREBP), liver X-receptor (LXR) α , PPAR- α and PPAR- γ target genes [26–29]. Additional evidence supports a *t10,c12* CLA-induced down regulation of the hypothalamic appetite regulating gene expression which suppresses appetite, leading to reduced energy intakes [30, 31]. Data from current study indicate that reductions in fat mass following the three doses of *t10,c12* CLA were associated with decreased energy intake as well as a tendency towards increased energy expenditure.

Notably, animals fed with 3% *c9,t11* CLA displayed increased lean body mass. The same observation was also noticed in animals fed all three dosages of *t10,c12* CLA. To our knowledge, the current study is the first evidence supporting an effect on hamster body composition by consumption of the *c9,t11* CLA isomer solely. Other work

suggests that a CLA mixture including 25% *c9,t11* CLA induced increased carcass lean tissue in growing pigs [32]. Previous works reported that CLA consumption increases lean body mass in several species (reviewed in [33]). However, the lack of evidence of such an effect on *c9,t11* CLA isomer may be explained by the relative low dose of *c9,t11* CLA isomer or *c9,t11* containing mixture used in previous studies. Recently, Nall et al. [34] demonstrated that CLA and arginine increased lean body mass, due possibly to a depression in muscle protein turnover, however, the mechanism of CLA supplementation increasing lean body mass remains to be fully understood and requires further investigation.

The effects of *c9,t11* and *t10,c12* CLA on serum lipid profiles vary between animals and humans [14, 35]. In the present study, animals fed 3% *t10,c12* enriched diet exhibited the highest total as well as non-HDL cholesterol levels, in comparison with the rest of the groups. These results are in accordance with some studies published by other authors when animals were fed diets with either a CLA mixture or the *t10,c12* CLA isomer. Bissonauth et al. [36] reported an increase in LDL-cholesterol levels induced by 2% highly enriched *t10,c12* CLA. Kritchevsky et al. [9] demonstrated elevated total cholesterol levels induced by CLA mixtures (43:44%, *c9,t11:t10,c12*) in rabbits. In contrast, evidence supports either a positive action or no effects on total cholesterol and non-HDL cholesterol levels by either pure or mixed CLA consumption [37–39]. Current data fail to provide any further positive indication of CLA improving serum lipid profile. Most human studies report no effect of supplementation with CLA mixtures on lipid profiles [35]. Tricon et al. [40] reported that serum concentration of total cholesterol, LDL cholesterol, TG and the ratio of total to HDL cholesterol were elevated after consumption of CLA capsules containing 80% *t10, c12* in healthy subjects. Moloney et al. [41] also demonstrated an increased LDL to HDL ratio in subjects with type II diabetes. Moreover, a recent review has suggested that all fatty acids with a double bond in the *trans*-configuration raise the LDL to HDL ratio [15]. Data from the present work partially reflects this adverse effect of high dose *t10,c12* CLA isomer on serum lipid content. The isotope work indicates that increased total serum cholesterol is not the result of augmented endogenous cholesterol synthesis. Navarro et al. [42] found that *t10,c12* CLA significantly reduced the LDL-receptor number when expressed as an arbitrary value of per mg of protein in hamster liver. One possible theory offered was that *t10,c12* CLA induced an increase in free cholesterol pool size, perhaps related to down regulation of the LDL receptor. In comparison with the aforementioned study, the high dosage of *t10,c12* CLA provided in the current study significantly increased serum total and non-HDL cholesterol levels, with

a lack of change in hepatic lipid content. Taken together, growing evidence suggests that *t10,c12* CLA does not favorably change serum lipid levels.

Recently, the safety of CLA consumption has become a concern. In the present study, the high dosages of *t10,c12* CLA (2 and 3%) increased hamster liver weights compared with control and *c9,t11* CLA treatments. This finding is consistent with those of a number of other studies [13, 21, 43]. Along with this observation, our study has shown that CLA supplementation did not affect lipogenesis in liver since the triglyceride concentrations failed to differ across treatment groups. The increased liver weight in animals fed with *t10,c12* isomer are not necessarily due to liver lipid accumulation but rather from a greater number of hepatocytes [13, 44]. Future histological analysis may provide a better understanding of the underlying cause of elevated liver weight.

It is well known that serum liver enzyme activity exists as an indicator of liver function. In our study, elevated ALT concentrations in liver were observed in animals fed 2 and 3% *t10,c12* CLA. Such results suggest that high doses of *t10,c12* may lead to liver malfunction since ALT serves as a biomarker for hepatocellular necrosis [45]. Elevated ALT level may also indicate increased inflammation and oxidation subsequent to *t10,c12* CLA consumption. In contrast, animal studies conducted by Macarulla et al. [44] reported no changes in hepatic ALT concentration after 6-week consumption of *t10,c12* CLA. The discrepancy could be attributed to the dosage variances between studies (0.5 vs. 2% and higher).

In a recent investigation on the safety of dietary CLA consumption, Iwata et al. [46] reported slight increases in ALT activities in the high dose CLA (6.8/d) group after 12-week intervention in healthy overweight Japanese subjects. Taken together, *c9,t11* CLA and 1% *t10,c12* CLA did not exert any adverse effect on liver health in the hamster model. Present data show clearly that the degree of impact of CLA supplementation on liver health is isomer dependent. Recently, the European Food Safety Authority has issued positive safety opinions about two CLA products as a safe ingredient for food and beverage [47]. In the present study, 1, 2 and 3% CLA-enriched diet correspond to human diet equivalents (2,500 kcal intake daily) of 2, 4 and 6 g/d. These levels of intakes are very similar to what EFSA has recommended. The 3% CLA treatment represented the higher dosages than what has been recommended by EFSA (6 g at 3% vs. 3.5–4.5 g) which provided a better idea for safety evaluation for CLA consumption. Iwata et al. [46] reported mild to moderate adverse effects in overweight male Japanese subjects supplemented with either 3.4 or 6.8 g/d CLA (50:50, *c9,t11:t10,c12*), where slight increases in AST and ALT activity levels were observed at 12 weeks. The author indicated that such elevations were

small and within the normal range. Moreover, one of the most recent reviews conducted by Katan and colleagues [15] has suggested that CLA exerts a negative effect on the circulation lipid profile.

In summary, the present study provided a systematic comparison of two CLA isomers at three different dosages. The most important finding is that low dose intakes of *t10,c12* CLA effectively lower body fat mass and increase lean body mass without posing unfavorable changes in serum lipid profile. Higher dosages (2 and 3%) of *t10,c12* CLA supplementation produce adverse effects in liver function and 3% *t10,c12* CLA has negative effects on serum lipid content. 3% *c9,t11* CLA also increased lean body mass; however, no body fat lowering effects of the *c9,t11* CLA isomers were seen at any of the three dosages. Additionally, *c9,t11* CLA did not have any adverse effect in liver function in the hamster model. Data from the present study suggests that 1% *t10,c12* CLA, which was close to the EFSA recommended level, effectively modulates body composition whereas 2 and 3% cause unfavorable physiological changes.

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