Dosage Ranging Effect and Safety Evaluation of Conjugated Linoleic Acid (CLA) in a Hamster Model

By

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DEDICATION

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ABSTRACT

The objectives of this study was to examine the efficacy and safety of graded doses of *c*9, *t*11, *t*10, *c*12 CLA isomers on body composition, energy expenditure, lipid profile and hepatic biomarkers in hamsters. Male Golden Syrian hamsters (n=105) were randomized to seven treatments (control; 1, 2, 3% of *c*9, *t*11; 1, 2, 3% of *t*10, *c*12) for 28 days. Compared with control, 1% and 3% *t*10, *c*12 had lowered food intake with all three doses of *t*10, *c*12 lowering ($p<0.0001$) body fat mass (g). Groups fed with 1, 2, 3% *t*10, *c*12 and 3% *c*9, *t*11 treatments showed higher lean mass compared to control and other treatment groups. However, neither body weights, nor serum HDL or triglyceride levels differed across treatment groups. The 3% *t*10, *c12* groups exhibited higher ($p<0.0001$) cholesterol and LDL-C levels compared to control or other treatment groups. The 2% and 3% *t*10, *c*12 groups also presented elevated ALT level ($p<0.05$). The present data suggest that 3% *t*10, *c*12 possess potential adverse effects on liver and posing unfavorable change in lipid profile.

ABBREVIATIONS

AST, aspartate aminotransferase

ALT, alanine aminotransferase

CHOL, cholesterol

cis-9, *trans*-11, *c*9, *t*11

CLA, conjugated linoleic acid

Dual energy X-ray absorptiometry, DEXA

FFA, free fatty acid

FM, fat mass

GGT, γ-glutamyltranspeptidase

HDL, high density lipoprotein

High density lipoprotein, HDL

LA, linoleic acid

LDL, low density lipoprotein

LM, lean mass

Low density lipoprotein, LDL

NS, not significant as compared to control diet

ND, note determined

TBM, total body mass

trans-10, *cis*-12, *t*10, *c*12

trans-9, *trans*-11, *t*9, *t*11

trans-10, *trans*-12, *t*10, *t*12

TRIG, triglycerol

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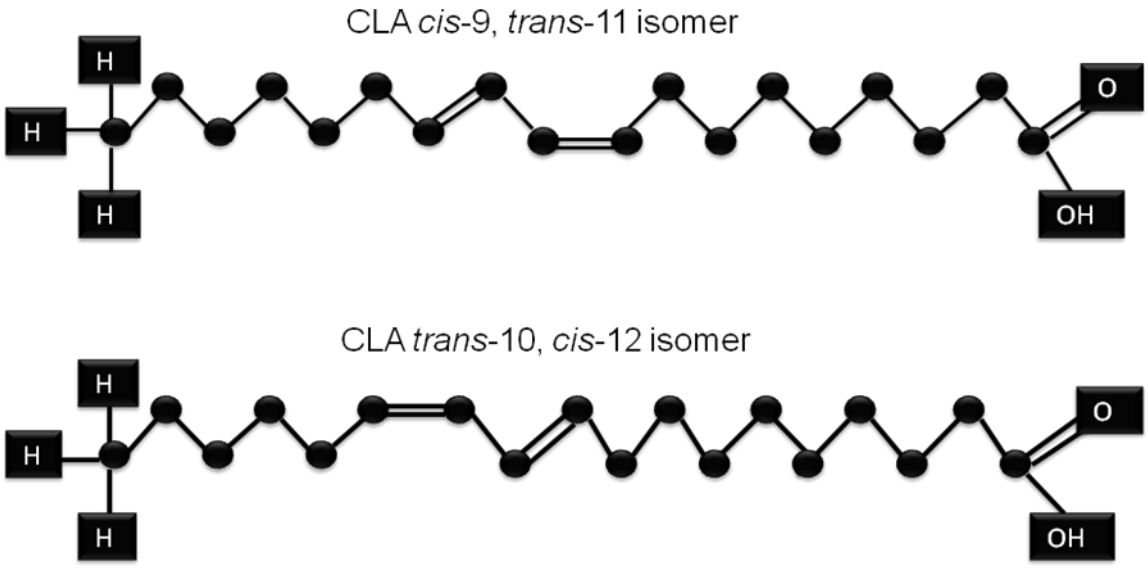
INTRODUCTION

Conjugated linoleic acid (CLA) refers to a collection of geometric and positional isomers of linoleic acid (18:2*n*-6; LA). Recently, CLA has attracted considerable attention. CLA was first discovered by Pariza *et al*. (1979) while they intended to investigate the temperature and time effect on mutagen formation in pan-fried hamburger. Unexpectedly, these researchers found antimutagenic components existing in both uncooked and fried hamburgers. Afterward, Pariza and Hargreaves suggested such antimutagenic components were from ground beef which may have positive effect on inhibiting chemically induced epidermal tumor initiation in mouse (Pariza & Hargreaves, 1985). Later, Ha, *et al*. (1987) identified that these antimutagenic active components are a mixture of four isomeric derivatives from linoleic acid, namely, *c*9, *t*11; *t*9, *t*11; *t*10, *c*12 and *t*10, *t*12 based on their chemical configuration.

CLA is also naturally produced in ruminant animals by fermentative bacteria. Such fermentative bacteria isomerize linoleic acid to CLA. The common food source of CLA is ruminant meats, dairy products from ruminant animals. The predominant (>90%) CLA isomer found naturally in food product is *c*9, *t*11. Only a small amount of *t*10, *c*12 CLA isomer exists in food. **Figure 1** presents the chemical structure of *c*9, *t*11 and *t*10, *c*12 CLA isomers.

Figure 1: Chemical structure of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA isomers



During the past two decades, numerous investigations about biological functions and health benefits of CLA have been conducted immensely. Beneficial effects of CLA such as anti-obese, anti-hypertension, and arthrosclerosis inhibitory have been reported in both animals and humans. More recently, dietary CLA has shown to possess a body composition alternation effect. Favorable body composition changes have been observed in various animal models (Plourde *et al.*, 2008). However, the data regarding most of the beneficial effects of CLA, especially its effect on body composition modulation still remain controversial. Moreover, contrasting functionalities between isomers are also frequently reported on circulating lipid profiles in both human and animal studies. Inconclusive effects of CLA have also been reported on total, LDL and HDL cholesterol levels between studies. Safety concerns also become an issue with CLA consumption. Such concern major focus on CLA enriched diet may induce increased liver weight,

lipodystrophy, elevated levels of inflammatory markers (Pariza, 2004). In addition, CLA purification technique was not available until 2004; hence, most of the earlier research was focused on the mixture form containing different ratios of *c*9, *t*11 and *t*10, *c*12 CLA isomers. Therefore, limited information has been available examining the effects of single CLA isomers at varying dosages. The aim of current study was therefore to evaluate the efficacy and safety of either *c*9, *t*10 or *t*10, *c*12 CLA on body composition change, serum lipid profile and hepatic biomarkers in hamsters.

LITERATURE REVIEW

I Origin and production of CLA

There is increasing interest in producing CLA as a food ingredient and health supplementation because of its potential health benefits. CLA is a mixture of positional and geometric isomers of linoleic acid. CLA is formed as intermediate by rumen biohydrogenation from linoleic acid. Bacteria are largely responsible for such biohydrogenation such as *Butyrivirio fibrisolvens* and other rumen bacteria (Harfoot and Hazlewood, 1988).In addition, the *c*9, *t*11 CLA is also synthesized in animal tissue through endogenous synthesis from vaccenic acid which is another intermediate of biohydrogenation by Δ-9 desaturase which is present in mammary tissue and adipose tissue.

Several methods are using to produce CLA, such as dehydration of ricinoleic acid (Yang *et al*., 2002), photoisomerization of LA-rich vegetable oil (Gangidi and Proctor, 2004) or alkaline isomerization of LA or LA-rich oils (Kim *et al*., 2003, Ma *et al*., 1999). Alkali isomerization of high linoleic acid plant oil such as sunflower oil and soybean oil is usually used for commercial production of CLA (Choi *et al*., 2004). Such product contains both *c*9, *t*11 (43-45%) and *t*10, *c*12 (43-45%) CLA isomers and some other isomers (Wang and Jones 2004). Purification technique such as urea inclusion crystallization usually employed to concentrate CLA in edible oils (Kapoor *et al*., 2005).

II Body Weight and Body Composition Changes and body composition in animals

2.1 Effect of CLA on body weight in animals

Effects of CLA on body weight have been investigated in different animal models. Most of these studies used mixture of different CLA isomers that synthetically prepared. Commercially available CLA supplementation normally contains both *c*9, *t*11 and *t*10, *c*12 CLA isomers in a 50:50 ratio. Varying doses have been tested on rodent models in evaluating the effect of CLA on body weight (**Table 1**). Some studies shown consistent results on its effect on reducing body weight; however, contrasting results were also reported that there was no effect of CLA supplementation on body weight in rodent models.

West *et al.* (1998) reported after fed AKR/J mice CLA mixture enriched high or low fat diet for 42 days, significant body weight loss were observed. Similar results were also reported by other groups (Delany *et al*. 1999; Takahashi *et al*. 2002; Martins *et al*. 2008). On the other hand, contrasting results were suggested CLA consumption did not have effect on body weight changes. Ryder *et al*. (2001) suggested that 14 days diet intervention with 1.5% CLA did not cause any changes on body weight in Zucker diabetic fatty (ZDF) rats. Similarly, 1.5% CLA mixture contained diet did not cause body weight changes in Sprague-Dawley rats after 24 days supplementation (Yamasaki *et al*. 2003). One of the most recent study conducted by Joseph *et al*. (2010) also suggested that after 28 days dietary intervention, CLA enriched hypercholestrolimic diet did not show any effect on body weight changes (Joseph *et al*. 2010).

2.2 Effect of CLA on body composition in animals

In several animal studies, CLA has been shown to modulate body composition include reducing adiposity and increasing lean body mass (Plourde *et al.*, 2008). Dietary CLA was first reported to modulate mice body composition by Park and colleagues in 1997. In this study, by additional 0.5% CLA isomer mixture (50% *c*9, *t*11; 50% *t*10, *c*12) to an animals' diet resulted in decreased body fat mass as well as increased lean body mass. Later, West *et al*. 1998 examined the effect of CLA consumption on body fat accumulation and energy metabolism in mice. In their study male AKR/J mice were fed high-fat diets supplemented with 1.2% CLA isomer mixture (41% *t*10, *c*12; 39% *c*9, *t*11) and a low-fat diet supplemented with a 1.0% CLA isomer mixture for 6 week. After 42 days of intervention, animals on the CLA treatment presented significantly lowered energy intake and adipose depot weight compared with the high-fat control group and low-fat control groups, respectively (West *et al.*, 1998). Later, the same group conducted another study in AKR/male mice; using the same composition of CLA. A potential CLA dose ranging effect on body composition was evaluated in this study. Results suggested after 39 days of dietary intervention, animals fed 0.5%, 0.75% and 1.0% CLA enriched high-fat diet had significantly lowered body fat mass in comparison to animals in the control group and the 0.25% CLA group (DeLany *et al.*, 1999). Body weight also fluctuated considerably during the experimental period. Animal body weight was significantly decreased in the 0.75% and 1.0% CLA groups on days 18 and 21, respectively. Such effects remained throughout the study.

Similar results were observed by Takahashi *et al*. 2002 in ICR and C57BL/6J mice. Animal were offered experimental diets containing either 2% CLA or LA as

control for 21 days. The CLA mixture contained 34% *t*10, *c*12 and 33% *c*9, *t*11 CLA isomers. In contrast to the control group, mice in the CLA treatment group had significantly decreased adipose tissue weight. Latter research in mice showed that animals fed *t*10, *c*12 CLA enriched diets exhibited a significant reduction in body fat mass (Park *et al.*, 1999). In contrast, *c*9, *t*11 CLA has no such effect. The same group conducted another study and data indicated that 0.5% CLA mixture (44 % *t*10, *c*12; 42% *c*9, *t*11) supplemented diet inhibited body weight gain in ICR mice (Park *et al.,* 1999). However, a CLA mixture containing 3 % *t*10, *c*12 and 29% *c*9, *t*11 failed to produce a similar trend. By feeding ZDF rats 5% fat added diet, supplemented with 1.5% CLA containing 48% *t*10, *c*12 and 47% *c*9, *t*11 led to reduced of body weight gain (Ryder *et al.*, 2001). Conversely, in the same study, CLA containing 91% *c*9, *t*11 and only 1% *t*10, *c*12 did not show any effect on the body weight (Ryder *et al.*, 2001).

In hamsters, Navarro *et al*. (2003) demonstrated that by feeding animal atherogenic diet enriched with 0.5% *t*10, *c*12 CLA significantly reduced weights of white adipose tissue, but no change in total body weight was observed. In a later study, the same group provided more evidence that CLA has less effect on body weight changes when relatively low dosages (0.5% or 1%) *t*10, *c*12 CLA were incorporated in a hypercholesterolemic diet in hamsters (Navarro *et al.*, 2007). A study done by Bissonauth *et al*. (2008) shown 2% CLA mixture at 50:50 ratio of *c*9, *t*11 and *t*10, *c*12 did not present any effect on body weight and body weight gain in hamster after 28 days intervention.

As a summary, previous studies have examined various dosages (0.25%-2%) of CLA in different animal models. Inconsistent results were reported. Evidence suggested

that the effect of CLA is highly isomer dependent. Endpoints are sensitive to the isomeric form of CLA include physiological changes such as reduction of adipose deposition. *t*10, *c*12 CLA isomer has been suggested being more effective on body weight changes in comparison with other isomers. The effect of CLA on body composition and body weight also varies between animal species. Other possible factors such as animal age, experimental design and duration may also cause some of the discrepancies between studies. In previous studies, growing stage animals were chosen the most. It has been suggested that the accumulation rate of adipose deposition rate between growing phase animal and mature animal is different. For example, previous study presented that mice at growing stage accumulate less body fat (up to 70%) when animal consumed CLA supplemented diet compared to control diet (Park *et al*.1997).

Table 1: Effect of CLA on body weight and body composition in animals

Authors	Animal model	CLA supplement	Dosage & Duration	Body weight	Fat mass
Park, *et al*. 1997	mice	50% *c*9,*t*11; 50% *t*10,*c*12	0.5% for 30 days	↓	↓
West, *et al*. 1998	AKR/J mice	39% *c*9,*t*11; 41% *t*10,*c*12	HF diet +1.2% LF diet +1.0% for 42 days	↓ ↓	NS NS
DeLany *et al*. 1999	AKR/J mice	39% *c*9,*t*11; 41% *t*10,*c*12	0.25%-1.0% for 39 days	↓	↓
Park *et al*. 1999	ICR mice	42% *c*9,*t*11; 44% *t*10,*c*12	0.5% for 4 wks	NS	↓
Ryder *et al*. 2001	ZDF rats	91% *c*9,*t*11; 1% *t*10,*c*12 47% *c*9,*t*11; 48% *t*10,*c*12	1.5% for 14 days	NS NS	NS NS
Takahashi *et al*. 2002	C57BL/6J mice ICR mice	33% *c*9,*t*11; 34% *t*10,*c*12	1.5% for 21 days	↓ ↓	↓ ↓
Yamasaki *et al*.2003	Sprague-Dawley rats	46% *c*9,*t*11; 47% *t*10,*c*12	1.5% for 24 days	NS	NS
Navarro *et al*. 2003	Hamster	*c*9, *t*11 *t*10, *c*12	0.5% for 6 wks 0.5% for 6 wks	NS NS	NS ↓
Navarro *et al*. 2007	Hamster	*t*10, *c*12	0.5% for 6wks 1% for 6 wks	NS NS	NA NA
Martins *et al*. 2008	Zucker rat	50% *c*9,*t*11; 50% *t*10,*c*12	1% for 14 weeks	↓	NS
Bissonauth *et al*.2008	Hamster	50% *c*9,*t*11; 50% *t*10,*c*12	2% for 28 days	NS	NA
Joseph *et al*. 2010	Hamster	70% *t*10, *c*12	2% for 28 days	NS	↓

2.3 Effect of CLA on body weight and body composition in human

In human studies, results of the effect of CLA on body composition remain inconsistent (**Table 2**). Zambell *et al*. (2000) reported that after 64 days consumption of 3g/day CLA (17.6% *c*9, t11; 22.6% *t*10, *c*12), seventeen healthy female subjects remained unchanged in body weight, body fat mass and energy expenditure. Similar results were also observed by Benito *et al*. (2001) as there was no significant difference

between the control group and the CLA treatment group in body composition after 9 week of 3 g daily consumption of a CLA mixture (22.6% *t*10, *c*12; 23.6% *c*11, *t*13,) in ten female healthy, normolipidemic subjects . Lack of effect of CLA consumption on body composition changes was also reported by Kelley *et al*. (2000). Seventeen subjects were all issued 1g of placebo for the first 30 days of this trial; ten subjects were then switched to the treatment diet which contained 3.9g CLA (1g *c*9, *t*11 and 1g *t*10, *c*12) for the following 64 days while the rest of subjects remained on the placebo diet. No changes were observed between the placebo group and the CLA treated group on body weight, fat mass and lean body mass (Kelley *et al.*, 2000).

In contrast, a number of studies indicate that CLA supplementation exerts a positive effect on body composition without altering overall body weight. Twenty healthy exercising volunteers were offered either a placebo or 0.6g CLA treatment that containing equal amount of *c*9, *t*11 and *t*10, *c*12 CLA isomers for 12 weeks. Results showed that CLA reduced body fat but not body weight in healthy subjects (Thom *et al.*, 2001). Similar observations have also been reported by Mougius *et al* (2001). In their study, subjects were offered different dosages of CLA (50% *c*9, *t*11; 50% *t*10, *c*12) from 0.7-1.4 g per day. After 8 weeks intervention, high dosage (1.4g per day) of CLA induced significantly decreased body fat mass and percentage body fat. A loss of body fat mass from 4% to 20% in normal weight subjects was reported by several studies after CLA intake in the range 0.7-4.2g on a daily basis (Smedman & Vessby, 2001; Colakoglu *et al.*, 2006; Pinkoski *et al.*, 2006). Blackson *et al*. (2000) reported that 42 overweight subjects had a reduction of fat mass of up to 6% after they consumed CLA (1.7-6.8g/d) over 12 weeks. Similar results were also reported by Risérus *et al*. (2002). After 12 weeks of 3.4g

daily CLA supplementation, 3% fat loss was observed in obese human subjects. Gaullier *et al*. (2005) reported a reduction of both body weight (-1%) and body fat mass (-5%) in overweight subject after a daily 3.6g consumption of CLA for 52 weeks. These contradictory findings between human studies may due to different experiment design such as 1) dosages of CLA used in studies 2) CLA mixture versus individual isomer 3) gender, age, health status of subjects (Plourde *et al.*, 2008).

Table 2: Effect of CLA on body weight and body composition in humans

Authors	Subjects (n)	CLA Supplement	Dose (g/d) & Duration	Body weight	Fat mass
Blackson *et al*. 2000	52	50% *c*9,*t*11, 50% *t*10,*c*12	1.7-6.8 for 12 wks	NS	↓
Kelly *et al*. 2000	17	25.6% *c*9,*t*11, 25.6% *t*10,*c*12	3.9 for 64 days	NS	NS
Zambell *et al*. 2000	17	17.6% *c*9,*t*11, 22.6% *t*10,*c*12	3 for 64 days	NS	NS
Benito *et al*. 2001	10	11.4% *c*9,*t*11, 10.8% *t*8,*c*10 15.3% *c*11,*t*13,14.7% *t*10,*c*12	3 for 9wks	NS	ND
Mougios *et al*. 2001	12	50% *c*9,*t*11, 50% *t*10,*c*12	0.7-1.4 for 8wks	NS	↓
Smedman *et al*.2001	26	CLA mixture	4.2 for 12 wks	NS	↓
Thom *et al*. 2001	20	50% *c*9,*t*11, 50% *t*10,*c*12	1.8 for 12 wks	NS	↓
Gaullier *et al*. 2004	180	39% *c*9,*t*11, 41% *t*10,*c*12	3.6 for 52 wks	NS	↓
Gaullier *et al*. 2005	134	50% *c*9,*t*11, 50% *t*10,*c*12	3.4 for 52 wks	↓	↓
Colakoglu *et al*. 2006	44	CLA mixture	3.6 for 6 wks	NS	↓
Pinkoski *et al*. 2006	38	CLA mixture	5 for 7 wks	NS	↓
Watras *et al*. 2007	40	39.2% *c*9,*t*11, 38.5%*t*10,*c*12	3.2 for 24 wks	NS	↓
Racine, *et al*. 2010	53	50% *c*9,*t*11, 50% *t*10,*c*12	3 for 28 wks	NS	↓

III Circulating Lipid Profile

3.1 Effect of CLA on plasma or serum lipid profile in animals

There is much evidence to support CLA as having a beneficial effect on cardiovascular risk through modulation the plasma lipid metabolism. However, inconsistent results were reported with regard to lipid profile modulation by CLA consumption in both human and animal studies. Lack of convincing data of such effects may be caused by the varying animal models, different dosages of single isomer or distribution of CLA isomers in mixture. Experimental design and background diet may also contribute to the inconclusive results.

A study done by Nicolosi *et al*. (1997) on F_1B hamsters showed that animals fed with 0.025%, 0.05% or 0.5% CLA mixtures in an enriched diet had unchanged total triglyceride, reduced plasma total and non-HDL cholesterol concentrations after 11 weeks in comparison with control animals. Morphometric analysis of aortas from the same study indicated that CLA and LA fed hamsters showed less early atherosclerosis in comparison to the control group. In contrast, Wilson *et al*. (2000) reported that F_1B hamster fed chow-based foods supplemented with 1% mixed isomer CLA (*c*9, *t*11; *t*9, *c*11; *t*10, *c*12) exhibited lower total and non-HDL cholesterol concentration in plasma. Total triglyceride and HDL-cholesterol concentrations remained unchanged. Mitchell *et al*. (2005) reported unchanged plasma cholesterol concentration when Syrian Golden hamster were offered high fat, high cholesterol diet enriched with either 1% *c*9, *t*11 CLA or *t*10, *c*12 CLA compared to diet supplemented with 1% LA. In the same study, higher

plasma HDL concentrations were observed in animal consuming *t*10, *c*12 CLA enriched diet.

Ledoux *et al*. (2007) reported that hamsters fed with 1% *c*9, *t*11 CLA enriched semi purified diet had significantly lowered plasma total cholesterol, LDL, HDL concentrations compared to animals in control the group. In same study, 1% *t*10, *c*12 CLA and 1% CLA mixture (50% *c*9, *t*11; 50% *t*10, *c*12) did not demonstrate any effect on plasma lipid content. Valeille *et al*. (2005) observed elevated plasma triglyceride concentrations in Syrian Golden hamsters fed with a 1% CLA (90% *c*9, *t*11) supplemented high fat diet. In the same treatment group, 1% *c*9, *t*11 CLA also induced a reduction in the ratio of non-HDL to HDL-cholesterol. Bissonauth *et al*. (2006) reported significant increases in LDL-cholesterol induced by *t*10, *c*12 CLA in comparison to *c*9, *t*11 CLA. The lack of effect of *t*10, *c*12 isomer on serum lipid profile (total, HDL, LDL-cholesterol) was also bserved by Navarro *et al*. (2007) in hypercholesterolaemic hamsters when animals were fed with 0.5%-1% t10, c12 CLA for 6 weeks.

In New Zealand White (NZW) rabbits, Kritchevsky *et al*. (2000) reported elevated total cholesterol level, triglyceride level and decreased HDL-cholesterol level after a range of 0.1%-1% CLA mixture supplementation for 13 weeks. Later, the same group reported no changes on total cholesterol, triglyceride as well as HDL-cholesterol in NZW rabbits, when they adjusted the dosage of mixed isomer CLA as low as 0.05%-0.5% (Kritchevsky *et al.*, 2002). Martins *et al*. (2008) observed after feeding obese Zucker rats an atherogenic diet enriched with 1% CLA (50% *c*9, *t*11; 50% *t*10, *c*12) induced elevated total and LDL-cholesterol in serum, however the same treatment did not affect serum triglyceride after 14 weeks of experimental period.

3.2 Effect of CLA on plasma or serum lipid profile in humans

In human studies, evidence of CLA favorably altering lipid profiles remains inconclusive. Blackson, *et al*. (2000) reported that there was no change on lipid profile in obese subjects after a daily basis consumption of CLA mixture (50% *c*9, *t*11; 50% *t*10, *c*12) for 12 weeks at the dosages from 1.7g to 6.8g. Similar results were observed by other groups. Benito *et al*. (2001) also reported that supplementation with 3.9g CLA isomer mixture (22.6% *t*10, *c*12; 17.6% *c*9, *t*11) did not cause any change in, plasma cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides after 63 days. Noone *et al*. (2002) and Kamphuis *et al*. (2003) also suggested there was no effect of a CLA isomer mixture (50% *c*9, *t*11; 50% *t*10, *c*12) on lipid profile in human subjects (Noone *et al.*, 2002; Kamphuis *et al.*, 2003). Taylor *et al*. (2006) demonstrated similar results that after 4.5g of CLA isomer mixture (36% *t*10, *c*12; 35%*c*9, *t*11) was consumed for 12 weeks; there was no significant differences on total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride levels between subjects on CLA treatment group and control group.

On the other hand, there exists evidence suggesting that lipid profile can be modulated by CLA supplementation. Risérus, *et al*. (2002) compared a CLA mixture, a purified *t*10, *c*12 CLA isomer and a placebo treatment in a randomized, controlled trial in 60 obese men with insulin resistance syndrome. Subjects received either 2.42 g/day of CLA mixture containing both *c*9, *t*11 and *t*10, *c*12 CLA isomers in a 50:50 ratio, or 2.6 g/day of *t*10, *c*12 CLA or placebo (olive oil) for 12 weeks. Results indicated *t*10, *c*12 CLA reduced HDL-cholesterol level in contrast with other groups (Risérus *et al.*, 2002). Following in the same path, a 52-week clinical trial was conducted by Whigham *et al*.

(2004) to evaluate safety of CLA in obese subjects. After 3 phases, diets enriched with a CLA isomer mixture (50% *c*9, *t*11; 50% *t*10, *c*12) led to an elevated triglyceride level and a decreased HDL-cholesterol level in these obese subjects. Most of the studies either showed no effect or an unfavorable alteration in human plasma lipid profile. The dispersing results between human trials could due to the isomer mixture or dosages. By using the mixed isomers, the effect of one isomer may negate by the other; also the doses used in human trials were lower than those used in animals (Brown and McIntosh 2001).

VI Safety of CLA Consumption

Even though CLA may favorably alter body composition or potentially inhibit body weight gain, safety concerns have been raised about CLA consumption since it may induce liver hypertrophy, increase liver weight and accelerate fatty liver formation (DeLany *et al.*, 1999; Clement *et al.*, 2002; Miranda *et al.*, 2009). Macarulla, *et al*. (2005) examined CLA effects on liver composition and fatty acid oxidation in hamsters. In this study, atherogenic diets supplemented with 0.5% of *c*9, *t*11or *t*10, *c*12 CLA were used as the treatment diet. After 6 weeks of feeding, significantly increased liver weight was observed in animals fed the *t*10, *c*12 CLA isomer. Delany *et al*. (1999) also reported significantly increased liver weight in AKR/J mice after feeding 1% CLA isomer mixture enriched diet for 39 days. Such results were also reported by Javadi, *et al*. (2001) using a treatment diet containing 0.5% *c*9, *t*11and *t*10, *c*12 (50:50) CLA mixtures over 12 weeks. Similar results were also obtained from another study, by feeding 0.5% of either *c*9, *t*11 or *t*10, *c*12 CLA isomers or their mixture to 12-months-old female C57Bl/6J mice for 6

months; liver hypertrophy was observed in animals fed *t10, c*12 CLA isomer (Halade *et al.*, 2009).

In humans Blackson *et al*. (2000) investigated CLA daily consumption from 1.7g/day to 6.8 g/day in overweight or obese subjects and found no significant differences between the treatment group and the control regarding the frequency of side effects. Another study conducted by Iwata *et al*.2007 reported after 12 weeks of supplementation of CLA (50% *t*10, *c*12; 50% *c*9, *t*11) at dosages of 3.4-6.8g, more adverse effect was reported from the CLA treatment groups. Such adverse effects include diarrhea, cough, headache, fever, nasal inflammation and abdominal distention. Elevated serum AST and ALT activities in the high CLA group (6.8g) was also observed at 12 weeks. In addition to its potential detrimental effect in the liver, CLA consumption also has adverse effect on insulin sensitivity. Brown & McIntosh (2004) reported that in obese men, *t*10, *c*12 CLA induces hyperproinsulinemia which is related to the impaired insulin sensitivity. Future studies are required to determine whether the effect of CLA consumption is safe without causing health concernsn humans.

V Possible Mechanism of CLA Antiobesity Effect

5.1 CLA regulation of energy intake and energy expenditure

CLA supplementation has been reported to lower energy intake and increase energy expenditure. Previous data suggested that mice supplemented with a CLA mixture or a *t*10, *c*12 CLA enriched diet had reduced energy intake after a 4 week experimental period (Park *et al.*, 1997). Similar results were also demonstrated by other

authors (Miner *et al.*, 2001; Takahashi *et al.*, 2002; So *et al.*, 2009). Data from an animal study conducted by So *et al*. (2009) suggested that lowered food intake observed in mice fed with a low-fat diet containing CLA was cause by *t*10, *c*12 CLA induced down-regulation of hypothalamic appetite regulating genes. More supportive data pursuing this hypothesis have been demonstrated by Cao *et al*. (2007). By injecting the mixed CLA isomers into rat hypothalamus led to a decreased expression of neuropeptide Y and agouti-related protein neuropeptides. Both of these neuropeptides are responsible for increasing food intake. Other evidence has suggested reduced body fat mass is not necessary associated with less energy intake following CLA administration (Azain *et al*. 2000; West *et al*. 2000; Terpstra, *et al*. 2002). Thus, although several studies have suggested CLA consumption may lead to lowered energy intakes, however, previous data also support a CLA effect on lowering body fat mass, which can be independent from energy intake.

Other than its effect on energy intake, previous data have led to the suggestion that CLA has a potential effect on up regulating energy expenditure in animals. CLA has been proposed to reduce adipose tissue accumulation by elevating the energy expenditure through up regulation of basal metabolic rate, thermogenesis and lipid oxidation in animals (West *et al.*, 2000; Miner *et al.*, 2001; Ohnuki *et al.*, 2001; Terpstra *et al.*, 2002). Terpstra *et al*. (2002) observed lowered body fat mass and increased basal metabolic rate in BALB/c male mice after consumption of CLA mixture for 6 weeks. The effect of CLA on enhancing theremogenesis maybe associated with its up regulation of uncoupling proteins which can divert the energy from energy synthesis to heat production (Kennedy *et al.*, 2009).

5.2 CLA regulation of adipogenesis and lipogenesis

The preoxisome proliferator activated receptor-ɤ (PPAR ɤ) and CAAT/enchancer binding protein (C/EBP) are two key factors that are responsive for preadipocytes and adipocytes conversion. Previous data indicate that CLA suppresses the adipocyte differentiation in both animals and humans. Data from animal studies indicated that *t*10, *c*12 CLA attenuates PPAR ɤ and its target gene expression (LaRosa *et al.*, 2006; Poirier *et al.*, 2006). In *vitro*, in primary human adipocyte and mature human 3T3-L1 adipocytes, a *t*10, *c*12 CLA treatment induces a decreased expression and activity of PPAR ɤ and its target genes (Kennedy *et al.*, 2008; Miller *et al.*, 2008). The exact mechanism of how CLA regulates PPAR ɤ still remains unknown. CLA also regulates proteins involved in lipogenesis through in similar path. Lipoprotein lipase, acetyl-CoA carboxylase, fatty acid synthase and steroyl CoA desaturase were all reduced by either *t*10, *c*12 CLA or CLA mixture (Kennedy *et al.*, 2009).

RATIONALE

During the past two decades, a great deal of work has been performed to investigate beneficial effects of CLA. The *c*9, *t*11 and *t*10, *c*12 are two major CLA isomers that have been studied the most. In particular *t*10, *c*12 CLA isomer shows promising results on altering body composition which has attracted considerable attention. Such results include modulating body weight, lowering body fat deposition, and increasing lean body mass. Due to these exciting discoveries with CLA, more and more commercial opportunities are available; products containing CLA isomers are sold as

sliming agents. However, such results fail to remain consistent in human studies. Moreover, some health related issues are revealed due to CLA consumption. A study done by Tarling *et al*. (2008) reported that consumption of 0.25% *t*10, *c*12 CLA enriched diet caused increased liver weights in hamsters. Recently a case of suspected CLA supplementation induced hepatoxicity has been reported in Portugal (Ramos *et al.*, 2009).

The present study is designed to evaluate the efficacy of two isomers of CLA on body composition changes, circulation lipid profile as well as safety of CLA consumption in adult hamster model. In most previous studies relatively low dosage of CLA isomer mixture or independent CLA isomer has been investigated. The current study will focus on *c*9, *t*11 and *t*10, *c*12 CLA isomer solely at high level consumption in hamsters. Furthermore, there is limited information available of dose ranging effect of CLA since most studies examined the single dose of either *t*10, *c*12, or *c*9, *t*11 or their mixtures. Present study is designing to evaluate three dosages (1%, 2%, 3%) of both *c*9, *t*11 and *t*10, *c*12 CLA isomers on body composition, serum lipid profile, liver biomarkers and cholesterol synthesis status in a hamster model. As well aware of that hamster is a suitable model for studying lipid profile changes due to its lipoprotein metabolism and profile are similar to human (Spady & Dietschy, 1983). Also the 28 day dietary intervention has been proved as a sufficient time frame in order to observe the physiological changes (Spady & Dietschy, 1983).

In the present study, hamster cholesterol synthesis is measured by isotope labeling technique. In general, the cholesterol synthesis can be described as following: liver is the one of the major sites of cholesterol synthesis. About twenty percent of endogenous cholesterol is synthesized by liver. The rest can be synthesized from extrahepatic tissue

like intestine (Gropper *et al*. 2005). Cholesterol synthesis process starts from converting the Acetyl CoA to Acetoacetyl CoA., follow by the conversion to 3-hydroxy-3-methyglutaryl (HMG) CoA (Gropper *et al*. 2005). The HMG CoA is then reduced by HMG CoA reductase formed mevalonate. The final step of cholesterol synthesis includes formation of cholesterol from sequlene which derived from mevalonate (Gropper *et al*. 2005).

From this study, an overall evaluation on safety, efficacy and dose ranging effect of *c*9, *t*11 and *t*10, *c*12 CLA isomers will be provided. The CLA dosages selected from the present study is very similar to the EFSA recommendation 3.6-4.5g/day (*c*9, *t*11: *t*10, *c*12, 50:50). The current study will also provide evidential suggestion of whether CLA is a suitable candidate for commercialization.

We calculate the equivalent dosage to human daily consumption by followings:

In our experimental diet: every 1000g of diet contains 4011.2kcal. On average, 6g of food was consumed per hamster.

Daily energy intake: (4011.2kcal/1000g) x 6g = 24.0672 kcal/day.

Also, 5% fat was included in the diet: 6g x 5% = 0.3g fat was consumed per day, 0.3g x 9kcal/g fat = 2.7 kcal. In this 5% fat, 60% fat was replaced by CLA at 3% CLA enriched diet: 2.7 kcal x 60% = 1.62 kcal.

As such, 1.62 kcal/24.1kcal = 0.067 or 6.7% energy from CLA was consumed daily by hamster.

By comparison to the human diet, if 3000kcal is taken daily:

3000 kcal /day x 30% fat = 900 kcal.

If assuming the same percent of energy provide by CLA as there is 6.7% of daily energy is from CLA. Hence for human, from 900kcal x 6.7% = 60.3kcal from CLA is equivalent to what we have observed in hamster.

60.3 kcal/9kcal/g = 6.7 g CLA

For 3% *t*10, *c*12 CLA FFA (contained 70.1% *t*10, *c*12 and 13.16% *c*9, *t*11)

6.7 x 70.1% = 4.6g *t*10, *c*12

For 3% *c*9, *t*11 CLA FFA (contained 57.54% *c*9, *t*11 and 10.65% *t*10, *c*12)

6.7 x 57.54% = 3.8g *c*9, *t*11

Following the same calculation:

For the 2% *t*10, *c*12 diet when convert to human diet equivalent 3.1g from daily intake is obtained

For the 1% *t*10, *c*12 diet when convert to human diet equivalent, 1.5g from daily intake is obtained

For the 2% *c*9, *t*11 diet when convert to human diet equivalent, 2.6g from daily intake is obtained

For the 1% *c*9, *t*11 diet when convert to human diet equivalent, 1.3g from daily intake is obtained

NULL HYPOTHESES

- Dosage and different CLA isomers have no effect on body composition
- Dosage and different CLA isomers have no effect on serum lipid profile
- CLA does not cause safety concerns

MATERIALS AND METHODS

Animals and study design

A hundred and five Male golden Syrian hamsters (Charles River Laboratories, Montreal, Quebec) weighing between 90-100g were housed individually in plastic cages and subjected to a 12-h light/dark cycle at constant room temperature of 25 ℃. Upon arrival, hamsters were provided with free access to rodent chow diet (Nestle, Purina, USA) and water for 3 week, 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 *c*9, *t*11 CLA as well as 1, 2, 3% of *t*10, *c*12 CLA, each provided for 4 weeks. CLA isomers were provided in the free fatty acid form, supplied by Lipid Nutrition (Wormerveer, Netherlands). **Table 3** 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 hydroxtoluene (Sigma-Aldrich, Inc. ON, Canada) was added as an

antioxidant. Food intake was measured every two days, and body weight 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 in -80 ℃ freezer for further analysis. Animal care was approved by the University of Manitoba Animal Care Protocol Review Committee, and for in accordance with the guideline of the Canadian Council on Animal Care (1993).

Table 3: Experimental diet composition (w/1000g)

	Control	**1% *c*9,*t*11[a]**	**2% *c*9,*t*11[a]**	**3% *c*9,*t*11[a]**	**1% *t*10,*c*12[b]**	**2% *t*10,*c*12[b]**	**3% *t*10,*c*12[b]**
Casein	200	200	200	200	200	200	200
Cornstarch	280	280	280	280	280	280	280
Surcose	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 Bitarate	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	25	20	15	10	20	15	10
Safflower	25	20	15	10	20	15	10
c9,t11 CLA[a]	***0***	***10***	***20***	***30***	***0***	***0***	***0***
t10, c12 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*

Body composition measurement

Body composition was measured by DEXA from Lunar Prodigy advance. Tissue analysis was conducted by software enCORE version 9.30.044. Percentage body fat, body fat mass and lean body mass were identified.

Lipid profile and hepatic biomarker measurement:

Blood samples were thawed at room temperature then centrifuged at 3500 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. Analysis was conducted by the Vitro Chemistry System 350 (Ortho-Clinical Diagnostics, Inc. Rochester, NY, USA). Triglycerides, total cholesterol, HDL-cholesterol were measured. Non HDL-cholesterol was calculated by subtracting HDL-cholesterol from total cholesterol content. Serum concentration of liver enzymes AST, ALT and GGT were also measured in the present study.

Cholesterol synthesis measurement:

Hamsters received 0.5ml of deuterium (D_2O, 99.9%, Cambridge Isotope Laboratories, Inc. MA, USA) by intraperitoneal injection 2 hr prior to sacrifice to assess cholesterol synthesis rate. Deuterium enriched cholesterol was used as an indication of cholesterol synthesis (Jones *et al.*, 2000). Isotope ratios of DH/H_2 of cholesterol peak 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 an on-line GC/pyrolysis/isotope 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). Deuterium enrichments were measured both in plasma water and RBC cholesterol. Values of cholesterol FSR were derived using the equation (Jones et al., 2000):

$$\text{CS-FSR (pools. d-1)} = (\delta \text{ CS } ‰ \times 24 \text{ (hr)/interval period (hr)}) / (\delta \text{ PW } ‰ \times 0.478)$$

Where, δ CS and δ PW represent D enrichments in cholesterol and plasma water, respectively, over the interval period between injection and sacrifice. The multiplication factor of 0.478 accounts for the fraction of D atoms obtained from body water during cholesterogenesis.

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 hr per animal.

Hepatic lipid content analysis

Liver lipid content was extracted according to the Folch method (Folch *et al.*, 1957) 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, Quebec, 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. The ANOVA was conducted between control group and those given either 1% 2% or 3% of *c*9, *t*11 or 1% 2% or 3% of *t*10, *c*12 CLA isomers followed by Tukey's tests between the control group and 1% 2% and 3% of *c*9, *t*11 or 1% 2% and 3% of *t*10, *c*12 CLA isomers, as pair wise comparisons.Results were expressed as mean ± SEM (standard error mean). Pearson correlation coefficient was performed as regression analysis between food intake and other parameters. 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 1% and 3% *t*10, *c*12 CLA enriched diets showed a lower food intake in comparison to control and other groups ($p<0.05$, **Table 4**). No differences were noticed in average daily food intake between animals in control group and *c*9, *t*11 treatment groups. At the end of the 28 day experimental period, hamsters in all seven treatment groups exhibited similar body weight. Results on body composition are presented on **Table 4**. DEXA analysis indicates that hamsters fed with1%, 2%, and 3% *t*10, *c*12 CLA enriched diets had 27%, 30% and 23% less body fat mass compared to groups control and *c*9, *t*11 CLA fed animal, respectively ($p<0.0001$). Additionally, increased lean body mass was found in groups fed with all three dosages of *t*10, *c*12 CLA

supplemented diets. Similarly, such effect were also observed in animals fed with 3% *c*9, *t*11 CLA compared to control (p=0.0002).

Table 4: Food intake, body weight and body composition of hamster fed diets enriched with 1, 2, 3% *c*9, *t*11 CLA or 1, 2, 3% *t*10, *c*12 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 ±0.2	0.8 ±0.1	145.4 ±3.6	58.2 ±2.3	65.5 ±2.8
*c*9,*t*11 1%	6.5 ±0.2	0.8 ±0.0	144.8 ±2.6	57.3 ±2.3	64.9 ±1.8
*c*9,*t*11 2%	6.1 ±0.1	0.8 ±0.1	141.9 ±2.9	51.0 ±1.7	68.9 ±1.6
*c*9,*t*11 3%	6.3 ±0.3	0.8 ±0.1	149.9 ±3.3	53.4 ±2.6	73.8 ±2.8*
*t*10,*c*12 1%	5.7 ±0.1*	0.9 ±0.1	139.5 ±3.1	42.5 ±2.6*	74.8 ±2.6*
*t*10,*c*12 2%	5.9 ±0.2	0.9 ±0.1	141.9 ±2.7	41.0 ±1.7*	77.7 ±1.5*
*t*10,*c*12 3%	5.7 ±0.2*	0.9 ±0.1	139.5 ±2.9	44.5 ±2.1*	71.7 ±2.0*

*p < 0.05 vs. Control

Values are expressed as Mean ± SEM

Effect of CLA consumption on lipid profiles

Serum lipid content was affected by *t*10, *c*12 CLA isomers in hamsters (**Table 5**). Neither the serum HDL-cholesterol concentration nor the triglyceride concentration differed across treatment groups. However, animals consuming 3% of *t*10, *c*12 CLA enriched diet displayed the highest total cholesterol and non-HDL cholesterol levels in contrast to the rest of the treatment groups after 28 days dietary intervention ($p<0.05$).

Table 5: Serum cholesterol, HDL cholesterol and LDL cholesterol level of hamster fed with diets enriched with 1, 2, 3% *c*9, *t*11 CLA or 1, 2, 3% *t*10, *c*12 CLA for 28 days

Diets	Cholesterol (mmol/L)	HDL cholesterol (mmol/L)	non-HDL cholesterol (mmol/L)	Triglyceride (mmol/L)
Control	5.9 ±0.3	3.3 ±0.1	2.6 ±0.2	4.4 ±0.4
*c*9,*t*11-1%	6.0 ±0.3	3.3 ±0.1	2.6 ±0.1	4.3 ±0.3
*c*9,*t*11-2%	5.8 ±0.3	3.1 ±0.1	2.5 ±0.1	4.1 ±0.2
*c*9,*t*11-3%	5.9 ±0.2	3.3 ±0.1	2.9 ±0.2	4.4 ±0.3
*t*10,*c*12-1%	5.5 ±0.2	3.1 ±0.1	2.3 ±0.2	4.3 ±0.4
*t*10,*c*12-2%	6.4 ±0.3	3.2 ±0.1	3.3 ±0.2	4.6 ±0.3
*t*10,*c*12-3%	7.5 ±0.5*	3.4 ±0.1	3.9 ±0.2*	4.0 ±0.3

*$p < 0.05$ vs. Control

Values are expressed as Mean ± SEM

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 6.** Hamsters in the 2% and 3% *t*10, *c*12 CLA diet groups exhibited increased liver weight (p=0.0008).With regard to hepatic lipid content, neither liver cholesterol nor the triglyceride concentration differed between groups after 28 day consumption of CLA enriched diet. No differences were observed between dosages.

Table 6: Liver cholesterol concentration and triglyceride content fed diets enriched with 1, 2, 3% *c*9, *t*11 CLA or 1, 2, 3% *t*10, *c*12 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
*c*9,*t*11-1%	8.3 ±0.2	111.6 ±10.7	6.0 ±0.5
*c*9,*t*11-2%	8.0 ±0.2	156.6 ±22.7	4.9 ±0.4
*c*9,*t*11-3%	8.9 ±0.3	146.2 ±30.0	5.8 ±0.7
*t*10,*c*12-1%	8.5 ±0.3	124.3 ±19.8	4.7 ±0.4
*t*10,*c*12-2%	9.3 ±0.4*	105.3 ±19.0	4.8 ±0.3
*t*10,*c*12-3%	9.4 ±0.4*	82.3 ±13.4	5.6 ±0.5

*p < 0.05 vs. Control

Values are expressed as Mean ± SEM

Effect of CLA consumption on hamster liver enzyme level

After the four 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 the rest of treatment groups. No differences were noted in hepatic AST or GGT levels across the seven experimental diets (**Table 7**).

Table 7: Serum concentration of liver enzymes of hamster fed diets enriched with 1, 2, 3% *c*9, *t*11 CLA or 1, 2, 3% *t*10, *c*12 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
*c*9,*t*11-1%	127.7 ±18.2	131.9 ±20.4	5.8 ±0.3
*c*9,*t*11-2%	128.6 ±21.2	148.7 ±23.5	5.6 ±0.2
*c*9,*t*11-3%	105.0 ±9.2	123.0 ±20.5	5.9 ±0.4
*t*10,*c*12-1%	129.3 ±14.8	126.7 ±28.9	5.9 ±0.2
*t*10,*c*12-2%	197.8 ±30.7*	194.9 ±21.1	6.0 ±0.3
*t*10,*c*12-3%	306.2 ±50.9**	182.1 ±35.7	5.4 ±0.2

*$p < 0.05$ vs. Control

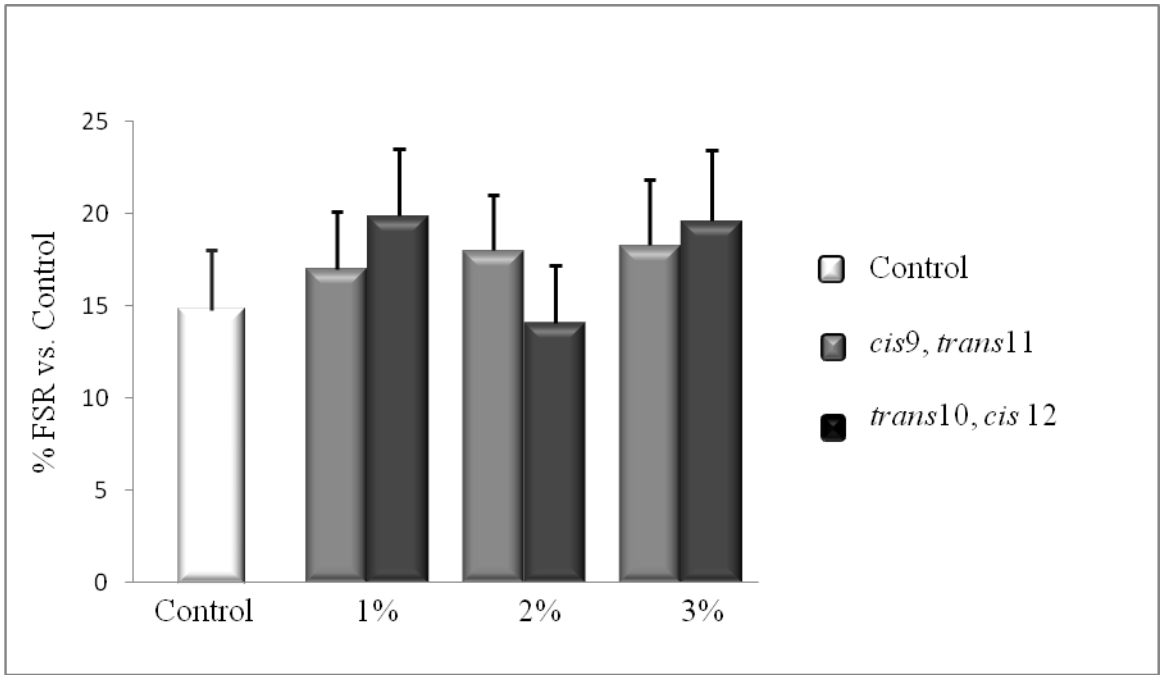
**$p < 0.0001$ vs. Control

Values are expressed as Mean ± SEM

Effect of CLA consumption on cholesterol synthesis:

Hamsters displayed similar cholesterol synthesis rates regardless of CLA intervention after 28 days (**Figure 2**).Regression analysis suggested that the elevated serum cholesterol level of 2% and 3% *t*10, *c*12 CLA treatment groups occurred independently of the individual rates of cholesterol synthesis across animals.

Figure 2: Cholesterol synthesis rate in hamsters fed diets enriched with 1, 2, 3% *c*9, *t*11 CLA or 1, 2, 3% *t*10, *c*12 CLA for 28 days n=14-15



DISCUSSION:

The primary finding of the present study was that low dosage of *t*10, *c*12 CLA exists as an effective agent for body composition alteration without exhibiting potential adverse effects. Similar body composition changes were also observed at the higher dosages of *t*10, *c*12 CLA, however, data from both serum lipid profile and hepatic biomarkers suggest that such changes occur at the expense of safety at higher dosages of *t*10, *c*12 CLA.

As secondary effect of CLA, data from current study indicate that *t*10, *c*12 CLA decrease energy intake in hamsters. These data are in line with results reported by others (West *et al.*, 1998; Park *et al.*, 1999; Takahashi *et al.*, 2002). Present study data show in this animal model that *t*10, *c*12 CLA treatment groups had lowered fat body mass which was in accordance with previous observations (West *et al.*, 1998; Azain *et al.*, 2000; DeLany & West, 2000; West *et al.*, 2000). In previous studies, *t*10, *c*12 CLA isomers have been identified as being responsible for the biological effects on body composition (De Deckere *et al.*, 1999). 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 *t*10, *c*12 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, PPARɤ and PPARɤ target gene (Brown *et al.*, 2003; Kang *et al.*, 2003; Granlund *et al.*, 2005; LaRosa *et al.*, 2006) There has also been evidence supporting a *t*10,*c*12 CLA-induced down regulation of the hypothalamic

appetite regulating gene expression which suppresses appetite leading to reduced energy intakes(Cao *et al.*, 2007; So *et al.*, 2009). In the present work, the data indicate reductions in fat mass following three doses of *t*10, *c*12 CLA, attributable to decreased energy intake as well as a tendency towards increased energy expenditure ($p=0.0641$).

Notably, animals fed with 3% *c*9, *t*11 CLA had increased lean body mass; the same observation was also noticed in animals fed all three dosages of *t*10, *c*12 CLA, ($p<0.05$). To our knowledge, the current study is the first evidence supporting an effect on hamster body composition by *c*9, *t*11 CLA isomer solely. There is evidence to suggest that CLA mixture that included 25% *c*9, *t*11 CLA induced increased carcass lean tissue in growing pig (Ostrowska *et al.*, 1999). Previous studies also reported that CLA increases lean body mass in several species (Wang & Jones, 2004). The lack of evidence of such effect on *c*9, *t*11 CLA isomer may be explained by the relative low dose of *c*9, *t*11 CLA isomer or *c*9, *t*11 contained mixture used in previous studies. Recently, Nall *et al*. 2009 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 is not fully understood yet; and requires further investigation.

The effect of *c*9, *t*11 and *t*10, *c*12 CLA on serum lipid profiles varies between animals and humans (Salas-Salvado *et al.*, 2006; Mitchell & McLeod, 2008). In the present study, animals fed 3% *t*10, *c*12 enriched diet exhibited the highest total cholesterol as well as non-HDL cholesterol levels, in comparison with the rest of 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 *t*10, *c*12 isomer. Bissonauth *et al*. (2006) reported an increase in LDL-cholesterol induced by *t*10, *c*12 CLA. Kritchevsky *et*

al. (2002) demonstrated elevated total cholesterol levels induced by CLA mixtures in rabbits. In contrast, other studies have reported either decreasing or no effect in total cholesterol and non-HDL cholesterol levels by either pure or mixed CLA consumption (Lee *et al.*, 1994; Nicolosi *et al.*, 1997; LeDoux *et al.*, 2007). Current data fail to provide any further evidence of CLA improving serum lipid profile as suggested in previous studies. Most of the human studies reported no effect of supplementation with CLA mixture on lipid profile (Salas-Salvado *et al.*, 2006). Tricon *et al*. 2004 reported serum concentration of total cholesterol, LDL cholesterol, triglyceride and the ratio of total to HDL cholesterol were elevated by *t*10, *c*12 CLA supplementation in healthy subjects. Moloney *et al*. 2004 also demonstrated an increased LDL to HDL ratio in subjects with type 2 diabetes. Moreover, one of the most recent reviews has suggested that all fatty acids with a double bond in *trans* configuration raise the LDL to HDL ratio (Brouwer *et al.*, 2010). Data from present work partially reflects this adverse effect of high dose *t*10, *c*12 CLA isomer on serum lipid content. The isotope work indicates that the increased total serum cholesterol is not the result of augmented endogenous cholesterol synthesis. Navarro *et al*. 2007 found *t*10, *c*12 CLA significantly reduced the LDL-receptor number when expressed as an arbitrary value of per milligram of protein in hamster liver. One possible theory offered by these authors was that *t*10, *c*12 CLA induced free cholesterol pool size increasing may related to down regulation of LDL receptor. In comparison with the aforementioned study, the high dosage of *t*10, *c*12 CLA used in current study, significantly increased serum total and non-HDL cholesterol levels alone without changing hepatica lipid content; this may suggest a similar direction. Taken together, there is growing evidence suggest *t*10, *c*12 CLA does not favorably change the serum

lipid. However, future investigations should address the molecular mechanisms of *t*10, *c*12 CLA's effect on liver and serum lipid content.

Recently, the safety of CLA consumption has become a concern. In the present study, the high dosages of *t*10, *c*12 CLA (2% and 3%) significantly increased hamster liver weight compared with the control and the *c*9, *t*11 CLA treatments. This is consistent with a number of other studies (West *et al.*, 1998; De Deckere *et al.*, 1999; Navarro *et al.*, 2003). Previous data have suggested *t*10, *c*12 CLA induces elevated liver weight, associated with increased hepatocytes rather than hepatic steatosis which is mainly caused by the triglyceride accumulation in liver (De Deckere *et al.*, 1999; Macarulla *et al.*, 2005). Along with this observation, our study has shown that CLA supplementation did not affect lipogensis in liver since the triglyceride concentration did not differ across treatment groups.

It is well known that serum liver enzyme activity exist as indicators for liver function. In our study, elevated ALT concentration in the liver was observed in animals fed with high doses of *t*10, *c*12 CLA. Such results suggested high doses *t*10, *c*12 may lead to liver malfunction, since ALT is served as a biomarker for hepatocellular necrosis (Meeks *et al.*, 1991). In contrast, an animal study conducted by Macarulla *et al*. (2005) reported no changes in hepatic ALT concentration after 6-wk consumption of *t*10, *c*12 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*. (2007) reported a slight increase in ALT activities in high dose CLA (6.8/d) group in after 12-wk intervention in healthy overweight Japanese subjects. Taken together, *c*9, *t*11 CLA and low dosage *t*10, *c*12 CLA did not exert any adverse effect on

liver health in the hamster model. Present data show clearly that the impact of CLA supplementation on liver health is isomer dependent. Diet enriched with high dosages (2% and 3%) of *t*10, *c*12 CLA has adverse effect on liver health in hamsters. Iwata *et al*. (2007) reported a mild to moderate adverse effect was observed in over weight male Japanese subjects where diets were supplemented with either 3.4g or 6.8g CLA (50:50, *c*9, *t*11: *t*10,*c*12). There was a slight increase in AST and ALT activity levels at 12 wk (Iwata *et al.*, 2007). The author indicated such elevation was small and within the normal range. Moreover, one of the most recent reviews conducted by Brouwer and colleagues suggested that CLA has a negative effect on circulation lipid profile (Brouwer *et al.*, 2010). As a summary, it is still too soon to conclude that CLA consumption is safe. More researches in this field are required.

CONCLUSTION

The present study provided a systematic comparison between the two major CLA isomers. In the current study effects of *c*9, *t*11 CLA and *t*10, *c*12 CLA on body composition changes, serum lipid profile and safety has been evaluated in a hamster model. In conclusion, the present study suggests that low dose intake of *t*10, *c*12 CLA (1% w:w) effectively lowered body fat mass and increased lean body mass without posing unfavorable changes in serum lipid profile after 28 days of dietary intervention. High dosages (2%, 3%) of *t*10, *c*12 CLA supplementation produce adverse effects in liver function and serum lipid content. Also, the current study is, by our knowledge, the first demonstrated effect of *c*9, *t*11 CLA on increasing lean body mass. Future investigations

are required in order to address the safety of long term CLA consumption. In the present study, only the endpoint hepatic biomarker assessment has been evaluated, hence there is not sufficient evidence to provide a conclusive observation of these physiological changes. Moreover, future research may focus on determining the optimal dosage of CLA by establishing the balance point between the maximum beneficial effects with little or no adverse effect. European food safety authority (EFSA) has recently published a positive opinion on the safety of two CLA containing products in Europe. Even though the final decision has not been made, it seems CLA is one step closer to getting the green light of safety from EFSA. If this motion is approved, CLA can be added into food and beverage as a safe ingredient in Europe. EFSA suggested that CLA is safe for consuming on a daily base of 3.5- 4.5g for up to six month. The current study did not provide more evidence about safe consumption of CLA. Even though relatively high dosages of CLA has been tested in the present study compared to the EFSA recommendation for daily intake, the experimental period of the present study was much shorter than the six months recommended by EFSA.

CLA has been promoted as a sliming agent globally for years. However, the adverse effect of *t*10, *c*12 CLA on lipid profile should not be ignored. The upper limit of daily consumption of CLA should be investigated and established in the near future. Evidence from the present study did not support CLA being a suitable candidate as a safe food ingredient.

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APPENDICES

Trans-8, *cis*-10 + *cis*-9, *trans*-11 conjugated linoleic acid mixture alters body composition in Syrian Golden hamsters fed a hypercholesterolemic diet

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Pearson correlation coefficient regression analysis on CLA intake vs body weight, body composition and lipid profile

*c*9, *t*11 intake vs body fat mass

Pearson Correlation Coefficients, N = 44
Prob > |r| under H0: Rho=0

	FM	CLA
FM	1.00000	-0.12703 0.4112
CLA	0.12703 0.4112	1.00000

*t*10, c12 intake vs body fat mass

Pearson Correlation Coefficients, N = 45
Prob > |r| under H0: Rho=0

	FM	CLA
FM	1.00000	0.53087 <.0001
CLA	0.53087 <.0001	1.00000

*c*9, *t*11 intake vs total body mass

Pearson Correlation Coefficients, N = 44
Prob > |r| under H0: Rho=0

	TBM	CLA
fTBM	1.00000	0.26683 0.0800
CLA	0.26683 0.0800	1.00000

*t*10, *c*12 intake vs total body mass

Pearson Correlation Coefficients, N = 45

Prob > |r| under H0: Rho=0

	TBM	CLA
TBM	1.00000	0.54823 <.0001
CLA	0.54823 <.0001	1.00000

*c*9, *t*11 intake vs lean body mass

Pearson Correlation Coefficients, N = 44
Prob > |r| under H0: Rho=0

	LM	CLA
LM	1.00000	0.22637 0.1348
CLA	0.22637 0.1348	1.00000

*t*10, *c*12 intake vs lean body mass

Pearson Correlation Coefficients, N = 45
Prob > |r| under H0: Rho=0

	LM	CLA
LM	1.00000	0.32892 0.0274
CLA	0.32892 0.0274	1.00000

*c*9, *t*11 intake vs HDL

Pearson Correlation Coefficients, N = 44
Prob > |r| under H0: Rho=0

	HDL	CLA
HDL	1.00000	-0.03240 0.8366
CLA	-0.03240 0.8366	1.00000

*t*10, *c*12 intake vs HDL

Pearson Correlation Coefficients, N = 45

Prob > |r| under H0: Rho=0

	HDL	CLA
HDL	1.00000	0.23644 0.1223
CLA	0.23644 0.1223	1.00000

*c*9, *t*11 intake vs LDL

Pearson Correlation Coefficients, N=44

Prob > |r| under H0: Rho=0

	LDL	CLA
LDL	1.00000	-0.16037 0.3043
CLA	-0.16037 0.3043	1.00000

*t*10, *c*12 intake vs LDL

Pearson Correlation Coefficients, N = 45
Prob > |r| under H0: Rho=0

	LDL	CLA
LDL	1.00000	0.51865 0.0003
CLA	0.51865 0.0003	1.00000

*c*9, *t*11 intake vs TC

Pearson Correlation Coefficients, N = 44
Prob > |r| under H0: Rho=0

	TC	CLA
TC	1.00000	-0.03456 0.8238
CLA	-0.03456 0.8238	1.00000

*t*10, *c*12 intake vs TC

Pearson Correlation Coefficients, N = 45
Prob > |r| under H0: Rho=0

	TC	CLA
TC	1.00000	0.49057 0.0006
CLA	0.49057 0.0006	1.00000

*c*9, *t*11 intake vs TRIG

Pearson Correlation Coefficients, N = 44
Prob > |r| under H0: Rho=0

	TRIG	CLA
TRIG	1.00000	0.03600 0.8165
CLA	0.03600 0.8165	1.00000

*t*10, *c*12 intake vs TRIG

Pearson Correlation Coefficients, N = 45
Prob > |r| under H0: Rho=0

	TRIG	CLA
TRIG	1.00000	-0.05766 0.7067
CLA	-0.05766 0.7067	1.00000