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Differential effects of conjugated linoleic acid isomers in insulin-resistant female C57Bl/6J mice☆

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Abstract

Obesity is associated with a high risk of developing diabetes and cardiovascular disease. Therefore, management of body weight to prevent obesity remains as an important priority. The present investigation addresses the effects of conjugated linoleic acid (CLA) isomers on body weight and composition of body fat in female C57Bl/6J mice. To investigate the differential effects of individual CLA isomers and their mixture on changes in lean mass, fat mass, glucose and insulin, 6-month-old female C57BL/6J mice were fed with 10% corn oil (CO) as a dietary fat source and either supplemented with purified *cis* 9,*trans* 11 (c9t11) CLA (0.5%) or *trans* 10,*cis* 12 (t10c12) CLA (0.5%) and/or their mixture (50:50) for 6 months. As a result of 6 months' dietary intervention, both the t10c12-CLA and CLA mix showed increased lean mass and reduced fat mass compared to the CO and c9t11-CLA groups. Insulin resistance was, however, increased in t10c12-CLA and CLA mix-fed groups based on the results of homeostasis model assessment (HOMA), the revised quantitative insulin-sensitivity check index (R-QUICKI) and also with intravenous glucose tolerance test (IVGTT). In conclusion, long-term feeding of the major CLA isomers in 12-month-old C57Bl/6J mice revealed a contrasting effect on fat mass, glucose and insulin metabolism. The t10c12 isomer is found to reduce the fat mass and increase the lean mass but significantly contributed to increase insulin resistance and liver steatosis, whereas c9t11 isomer prevented the insulin resistance.

Keywords: Conjugated linoleic acid; Fat mass; Glucose; Insulin; Lean mass; Obesity

1. Introduction

Obesity is characterized by excess adipose tissue; it has reached epidemic proportions globally, with more than 1 billion adults being overweight and at least 300 million among them clinically obese, which is a major contributor to the global burden of chronic disease and disability [1]. Overweight as well as obesity is a complex condition, with serious social and psychological dimensions, affecting virtually all ages and socioeconomic groups. It is a major risk for chronic diseases, including type 2 diabetes, cardiovascular disease, hypertension, stroke and certain forms of cancer. During the past 20 years, there has been a dramatic increase in childhood and adult obesity in the United States [2].

CLA refers to the positional and geometric conjugated dienoic isomers of linoleic acid (C18:2 n-6) and is a natural food component found in the lipid fraction of meat, milk and other dairy products. CLA feeding has been shown to modify the size of adipose tissue, the main store of fat in the body [3,4]. CLA has attracted considerable attention because of its antidiabetic [5], antiatherosclerosis [6] and anti-inflammatory [7] properties, but the mechanisms for these effects are not yet fully understood. The body fat-lowering effect of CLA has been reported in different rodent species and pigs [8–10]; however, differences in terms of responsiveness to CLA have been observed among species. In mice, several authors have found an intense reduction in fat accumulation (lipodistrophy) after CLA feeding. On the other hand, body fat reductions in hamsters and rats were moderate. CLA primarily influences fat metabolism in adipose tissue and also in the liver. These effects have been shown to be species specific. In mice, CLA induces high fat accumulation in liver leading to steatosis [9,11,12]; on the other hand, no changes [13,14] or even a reduction in the size of the liver has been observed in rats and hamsters [15].

CLA reduces body fat mass (BFM) not only in mice models but also in overweight humans [3,4,16,17]. The double bonds of CLA can be in Position 7,9; 8,10; 9,11; 10,12; or 11,13 with multiple combination of *cis* or *trans* configurations. *Cis*9,*trans*11-CLA (c9t11) and *trans*10,*cis*12-CLA (t10c12) are the major CLA isomers in the commercial preparations, but that t10c12 isomer content in natural resources is very low [18,19]. There is growing evidence that individual isomers of CLA have specific physiological functions, but these changes have still not been studied yet in detail. Some paradoxical findings using CLA may arise from the differential effects of c9t11-CLA and t10c12-CLA, different ratio of two isomers, the different levels or doses of CLA used or species variation, and metabolic status of the experimental animal [20–22].

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The primary objective of the present investigation is to unravel the isomer-specific effects of CLA in insulin-resistant female C57Bl/6J mice. As an animal model, we used C57Bl/6J mice and fed AIN93 diet containing 10% corn oil (CO) as a dietary fat source which promotes obesity and induces impaired glucose tolerance and insulin resistance, and thus represents as a useful model for studying the early stages in the development of obesity and type 2 diabetes [23,24]. This is a first kind of study feeding CLA isomers with a high fat diet to 6-month-old C57Bl/6J mice for a duration of 6 more months primarily to prevent muscle loss with age. We noted ~10% muscle loss in mice fed a diet enriched with CO, which can be used as an animal model to study age-associated sarcopenia.

2. Materials and methods

2.1. Animal

Five-month-old female C57Bl6/J mice, weighing 21–22 g, were purchased from Jackson Laboratories (Bar Harbor, Maine, USA). The age- and weight-matched animals were housed in a standard controlled animal care facility in cages (five mice/cage) and fed a standard diet (Harlan Teklad LM-485) for 1 month. The animals were maintained in a temperature-controlled room (22–25°C, 45% humidity) on a 12:12-h dark–light cycle. National Institutes of Health guidelines were strictly followed, and all the studies were approved by the Institutional Laboratory Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio (San Antonio, TX). At completion of 6 months of age, the mice were divided into four groups containing 10 in each. The mice were fed the AIN93 diet containing CLA isomer and CO *ad libitum* for 6 more months; body weight was measured weekly.

2.2. Diet preparation

The diets were supplemented with 10% CO as a dietary fat source, 0.5% *cis* 9,*trans* 11 CLA (c9-CLA), 0.5% *trans* 10,*cis* 12 CLA (t10-CLA) and a mixture of *cis* 9,*trans* 11 CLA and *trans* 10,*cis* 12 CLA (CLA mix). The mixture of c9-CLA and t10-CLA isomers is in equal amounts i.e., 0.25:0.25%. The CLA isomers were obtained as a free gift from Lipid Nutrition (Channahon, IL, USA). The c9-CLA-enriched diet contained approximately 61% of c9t11-CLA isomer and the t10-CLA diet contained 71% of t10c12-CLA. The mice were provided with an equal amount of fresh food everyday, in the afternoon, and leftover food was measured each day (between 1:00 and 2:00 p.m.). Diets were prepared each week, purged with nitrogen and frozen in daily portions in sealed polythene bags to minimize the oxidation of fatty acids. The composition of semipurified diets is presented in Table 1.

Food intake and body weight were measured weekly. The intravenous glucose tolerance test (IVGTT) was performed after 20 weeks using five mice from each dietary group. Before the start of the AIN93 diet and after 6 months on the diets, the body composition was determined with dual-energy X-ray absorptiometry (DXA) using the Lunar PIXImus (GE, Madison, WI, USA). One week prior to sacrifice, blood samples were taken from the intraorbital, retrobulbar plexus from anesthetized mice to measure fasting glucose, insulin and nonesterified fatty acid in serum. Finally, after 6 months the mice were sacrificed; the organs, like liver, muscle, spleen and adipose tissue, were harvested, weighed and preserved at −80°C until further processing.

Table 1
Composition of semipurified experimental diets

Ingredients[a]	Percent
Casein	14.00
Corn starch	42.43
Dextronized corn starch	14.50
Sucrose	9.00
Cellulose	5.00
AIN-93 mineral mix	3.50
AIN-93 vitamin mix	1.00
L-Cystine	0.18
Choline bitartrate	0.25
TBHQ	0.10
Vitamin E	0.04
CO or CO+CLA[b,c,d]	10.00

[a] All diet ingredients were purchased from MP Biomedicals (Irvine, CA, USA).
[b,c,d] Diets consisted of 10% CO or 9.5% CO+0.5% c9-CLA, 0.5% t10-CLA and a mixture of 0.25% c9-CLA and 0.25% t10-CLA, respectively.

2.3. Measurement of fat mass and lean mass by DXA

Body fat mass, abdominal fat mass (AbFM) and body lean mass (BLM) were measured by DXA, using the Lunar PIXImus mouse bone densitometer (GE, Madison, WI), and data were analyzed with PIXImus software [22,25,26]. Before scanning was performed, the mice were anesthetized with an intramuscular injection of 0.1 ml/100 g body weight of mouse cocktail containing ketamine/SA rompun/NaCl (3:2:5, by volume). BFM and BLM were obtained for the entire body, excluding the head. Abdominal fat or visceral fat mass (VFM), hind leg lean mass (HLLM) and fat mass (HLFM) were determined manually using DXA PIXImus software. Scanning was performed first at baseline and again at the end of 6 months on experimental diet.

2.4. Intravenous glucose tolerance test

For the IVGTT, 4 h-fasting mice were anesthetized with an intramuscular injection of 0.1 ml/100 g body weight of mouse cocktail containing ketamine/SA rompun/NaCl (3:2:5, by volume). A blood sample was drawn from the retrobulbar, intraorbital and capillary plexus, and D-glucose (1 g/kg) was injected intravenously in a tail vein (volume load 10 μl/g). Additional blood samples were collected 5, 10, 20, 50 and 75 min after the glucose injection. After immediate centrifugation at 4°C, serum was collected and stored at −80°C.

2.5. Serum metabolites

Glucose was analyzed spectrophotometrically using Glucose Colorimetric Assay Kit (QuantiChrom, Hayward, CA, USA). Insulin was analyzed using a rat/mouse ultrasensitive rat insulin ELISA kit (Crystal Chem, Inc., Research, Downers Grove, IL, USA). IGF-1 and adiponectin were assayed using the mouse adiponectin Quantikine immunoassay kit (R&D Systems, Minneapolis, MN, USA). Leptin was assayed using an active murine leptin kit (Diagnostic Systems Laboratories, Webster, TX, USA). Triglycerides were analyzed spectrophotometrically using the Triglycerides Colorimetric Assay Kit (Cayman Chemical Company, Michigan, USA).

2.6. Tissue collection for biochemical analysis

After 6 months on the experimental diet, the mice were anesthetized and blood was obtained by intraorbital capillary plexus. Serum was collected and stored at −80°C. Liver, spleen, gastrocnemius and quadriceps muscles were collected, weighed and frozen in liquid nitrogen and stored at −80°C.

2.7. HOMA and R-QUICKI

HOMA was calculated by the following formula: [fasting serum insulin (ng/ml)×fasting serum glucose (mM)]/22.5. A high HOMA index denotes low insulin sensitivity [27], although it should be acknowledged that the HOMA model has not been validated for use in animal models [28]. To assess insulin sensitivity, another derived index of insulin resistance was suggested, i.e., the revised quantitative insulin sensitivity check index (R-QUICKI) [1/log insulin (mU/ml)+log glucose (mg/dl)+log NEFA (mmol/l)] [29].

2.8. Serum proinflammatory cytokines

TNF-α and IL-6 were measured by using ELISA kits (eBiosciences, San Diego, CA, USA). Sensitivity of the assays was approximately 8 pg/ml. In brief, each well of flatbottom 96-well microtiter plates was coated with 100 μl of purified anti-TNF-α and anti-IL-6 antibodies (4 μg/ml in binding solution) overnight at 4°C. The plates were rinsed five times with washing buffer, and diluted serum was added, followed by incubation for 2 h at room temperature. The plates were again washed five times with washing buffer, followed by the addition of biotinylated anticytokine antibodies. The plates were incubated in room temperature for 1 h and then washed five times with washing buffer. Avidin-HRP conjugate was added, and the plates were incubated for 30 min at room temperature. The plates were again washed five times with washing buffer, and the chromogen substrate was added. This was followed by incubating the plate at room temperature to achieve the desired maximum absorbance and was read at 450 nm in an ELISA reader (Dynex Technologies, Worthington, West Sussex, UK).

2.9. Statistical analysis

Data are presented as mean values±S.E.M. Statistical significance of experimental observations was determined by one-way analysis of variance (ANOVA) followed by Newman–Keuls test with the level of significance set at $P<.05$. The analyses were performed with GraphPad Prism for Windows (La Jolla, CA, USA).

3. Results

3.1. Body weights and organ weights

The initial body weights for C57Bl/6J mice in 10% CO group, the c9-CLA group, the t10-CLA group and the CLA mix group were 21.58 ±0.54, 21.39±0.43, 22.10±0.51 and 21.51±0.50 g, respectively. As shown in Fig. 1A, the chronic 6 months' administration of CLA mix caused a significant decrease ($P<.05$) in body weight gain (37%) when compared to CO (49%). The t10-CLA group showed highly pronounced difference in weight gain (9%) compared to the CO (49%) group. In contrast, there was no change in body weight gain (48%) in the c9-CLA-fed group relative to the CO (49%) group.

The chronic treatment of t10-CLA and CLA mix showed a significant increase in liver weight compared to the CO group. The CO group liver weights were 1.52±0.08 g; however, in t10-CLA and CLA mix-fed mice, livers weighed 2.21±0.22 and 2.09±0.14 g, respectively, which is 45.39% more in t10-CLA and 37.09% in CLA mix-fed mice as shown in Fig. 1B. Interestingly, the skeletal muscle quadriceps and gastrocnemius weights were moderately increased (not significant) in t10-CLA and CLA mix-fed mice compared to CO-fed mice as shown in Table 3.

The total fat content in the peritoneal abdominal cavity was also assessed. The CO-fed group showed abdominal fat (2.64±0.25 g) that is significantly higher than that in the t10-CLA (0.98±0.15 g) and CLA mix (1.17±0.16 g) group; however, the total abdominal fat was not changed in c9-CLA (2.15±0.19 g) compared to the CO group. These results are supported by DXA which was performed before starting the diet and finally at the end of the study. The percent abdominal fat gain in t10-CLA (32.59±9.12%) and CLA mix (73.42±8.80%) was significantly lower when compared to the CO group (156±7.08%); however, the percent fat in the c9-CLA group (145.4±13.14%) remained much closer to that in the CO control group.

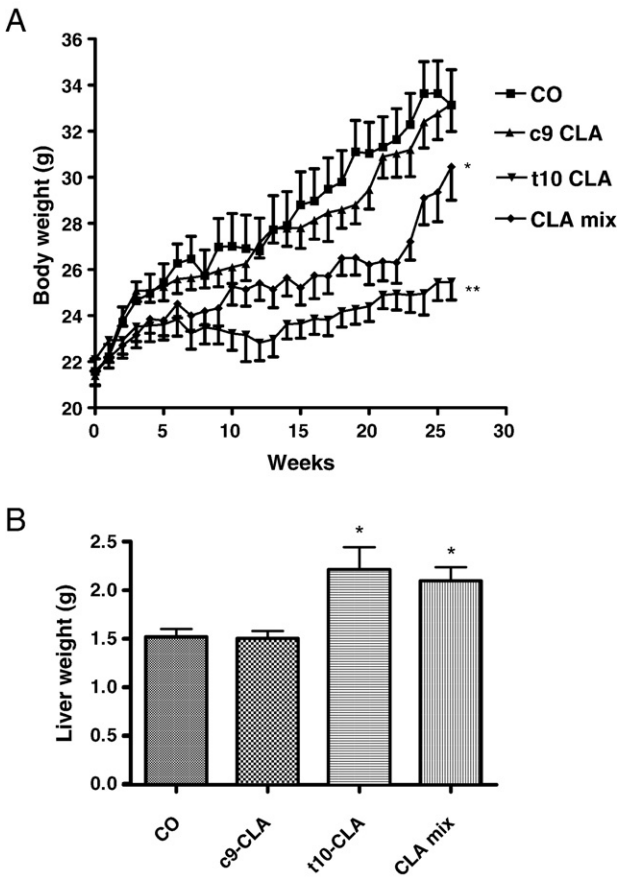

Fig. 1. Effect of CLA isomers on body weight and liver weight in C57Bl/6J mice. (A) Body weight in mice fed with the 10% CO diet or diet supplemented with 0.5% of c9-CLA and t10-CLA isomer and 0.5% CLA mix. **$P<.01$, CO vs. t10-CLA; *$P<.05$, CO vs. CLA mix. (B) Liver weight in mice fed with 10% CO, CLA isomers and CLA mix. *$P<.05$, CO vs. t10-CLA and CLA mix. Data are means±S.E.M. (n=8–10 mice/group); statistically significant differences between groups were determined by Newman–Keuls one-way ANOVA ($P<.05$).

3.2. Total lean, fat and AbFM by DXA

As shown in Table 2, t10-CLA and CLA mix diet-fed mice showed significantly increased total BLM compared to the CO group, whereas it was decreased (9%) in the CO-fed group. In c9-CLA-fed mice, there was no significant change noted in total BLM. The total body fat mass (BFM) gain was greater in CO mice (280.6±16.90%) than in the remaining three groups on CLA diet. The t10-CLA (48.56±12.85%) and CLA mix (132.5±15.4%)-fed mice showed less gain in total BFM than the CO mice (280.6±16.90%). Interestingly, AbFM was significantly reduced in t10-CLA and CLA mix-fed mice ($P<.001$) compared to the CO group. HLLM was increased significantly in the t10-CLA and CLA mix-fed group ($P<.01$) compared to the CO group. Also, HLFM was significantly decreased in the t10-CLA and CLA mix-fed mice compared to the CO-fed mice.

3.3. Serum triglycerides, NEFA, fasting glucose and insulin determination

Fasting serum NEFA was decreased 14% in c9-CLA-fed mice compared to the CO group. However, in t10-CLA and CLA mix-fed mice NEFA was increased by 16% and 10%, respectively. The fasting serum glucose concentration was also found increased significantly in t10-CLA and CLA mix-fed mice compared to the CO group as shown in Table 3. The fasting serum insulin concentration was also significantly increased after 6 months of chronic dietary feeding with t10-CLA demonstrating hyperinsulinemia compared to CO, but in c9-CLA, the insulin levels were unchanged. The CLA mix diet has shown moderate increase in insulin levels (not significant). The serum triglycerides were significantly ($P<.01$) decreased in t10-CLA and CLA mix group compared to the CO and c9-CLA groups as shown in Table 3.

3.4. Intravenous glucose tolerance test

The IVGTT was performed at 20 weeks after the start of the CLA isomers and CO supplementation. At 5, 10 and 20 min, the c9-CLA-fed mice eliminated glucose faster than the CO and t10-CLA mice. In contrast, the t10-CLA and CLA mix-fed mice had decreased glucose clearance at both 50- and 75-min time points. Thus, taken together, the basal levels of glucose and insulin results from the glucose challenges demonstrated that t10-CLA- and CLA-treated mice display a more extensive impairment of β-cell function and, consequently, may exaggerate diabetes compared with c9-CLA- and CO-fed mice. The 5-min insulin response to intravenous glucose challenge was increased rapidly to 0.14±0.03 (ng/ml) in the CO group, whereas in the t10-CLA and CLA mix group it was 0.95±15 and 0.54±0.13 (ng/ml), respectively, but it was apparently insufficient to maintain normal glucose tolerance. More pronounced increase in insulin secretion observed by glucose challenge in the t10-CLA group is shown in Fig. 2.

3.5. Serum proinflammatory cytokines

As shown in Table 3, the serum TNF-α level was decreased in t10-CLA and CLA-fed mice compared with the CO-fed mice ($P<.05$). One-way ANOVA revealed statistically significant difference in t10-CLA and

Table 2
Lean and fat mass in C57Bl/6J mice fed with CLA isomers for 6 months

Parameter		Diet			
		CO	c9-CLA	t10-CLA	CLA mix
No. of mice		9	10	8	10
Body weight (g)	Baseline	21.58±0.543	21.39±0.43	21.70±0.51	21.51±0.50
	Final	33.13±1.52	33.17±1.1	25.45±0.83#	30.45±1.44
	% Difference	49.15±5.70	48.52±5.22	9.709±3.23#	37.44±6.32
Total BLM (g)	Baseline	16.89±0.28	16.57±0.18	17.27±0.38	16.36±0.35
	Final	15.36±0.50	16.62±0.20	17.83±0.47*	17.83±0.35*
	% Difference	−9.00±2.78	0.46±1.95	3.19±1.17*	9.29±2.45*
Total BFM (g)	Baseline	4.21±0.20	3.89±0.25	3.91±0.32	4.15±0.28
	Final	16.13±1.25	13.87±0.90	5.74±0.59#	9.50±0.63#
	% Difference	280.6±16.90	266.7±31.00	48.56±12.85#	132.5±15.46#
AbFM (g)	Baseline	0.96±0.08	0.80±0.08	0.80±0.11	0.90±0.07
	Final	6.84±0.60	5.76±0.48	1.88±0.26#	3.6±0.30#
	% Difference	618.2±32.77	618.3±69.81	147.8±28.20#	311.9±35.53#
HLLM (g)	Baseline	0.74±0.017	0.74±0.01	0.74±0.02	0.76±0.01
	Final	0.65±0.01	0.74±0.01	0.84±0.03*	0.78±0.03*
	% Difference	−11.51±3.28	0.357±2.82	13.52±4.15*	6.54±5.29*
HLFM (g)	Baseline	0.26±0.016	0.21±0.02	0.17±0.03	0.23±0.01
	Final	0.67±0.03	0.55±0.03	0.35±0.029*	0.41±0.02*
	% Difference	157.4±9.25	195.0±37.9	171.4±56.54*	79.63±8.68*

Effect of CLA isomers on lean and fat mass in C57Bl/6J mice fed with CLA isomers and CLA for 6 months. Data are means±S.E.M. (n=8–10 mice/group). Superscript indicates statistically significant differences compared to CO and c9-CLA groups using Newman–Keuls one-way ANOVA (#P<.001, *P<.05).

# P<.001.
* P<.05.

CLA-fed group for TNF-α levels. Serum IL-6 was also decreased in t10-CLA and CLA-fed mice compared to the CO-fed mice. C9-CLA-fed mice, however, showed no effect on IL-6 and TNF-α cytokines.

4. Discussion

Obesity-induced insulin resistance is the principal etiological factor of the metabolic syndrome and type 2 diabetes. Several lines of evidence suggest that low-grade inflammation in adipose tissue predisposes to developing and augmenting the severity of whole-body insulin resistance. Given the increasing prevalence of obesity, it would be advantageous to identify potential therapeutic nutrients/functional foods to improve glucose and lipid metabolism within the context of obesity. In recent years, use of CLA as a dietary supplement has been receiving much attention [20,30]. Indeed, Clarinol, which has CLA as an active ingredient, produced by Lipid Nutrition has received FDA approval as GRAS (generally recognized as safe) for its use in various food supplements [31]. In this paper, we have differentiated the long-term dietary effect of CLA isomers in 12-month-old C57Bl/6J mice on lean mass as well as fat mass, glucose, triglycerides, NEFA and insulin metabolism. We observed ~10% muscle loss in CO (10%)-fed mice, demonstrating the use of C57Bl/6J mice as an ideal animal model to study the development of old age sarcopenia.

Table 3
Metabolic and serum parameters in C57Bl/6J mice fed with CLA isomers and CLA mix for 6 months

Parameter	CO	C9t11	t10c12	CLA Mix
Serum metabolites				
Glucose (mg/dl)	259.6±17.89	228.7±10.98	303.8±20.91*	293.6±18.07*
Insulin (ng/ml)	0.37±0.06	0.43±0.08	0.85±0.14*	0.64±0.14*
Triglycerides (mg/dl)	68.38±3.15	65.67±2.88	43.73±7.11*	37.40±4.09*
NEFA (mEq/L)	0.84±0.06	0.72±0.06*	0.98±0.03	0.92±0.03
HOMA-IR	4.13±0.64	4.51±0.93	10.49±2.35*	7.82±1.10*
R-QUICKI	0.50±0.23	0.54±0.17	0.41±0.37*	0.44±0.26*
Serum hormones and adipocyte cytokines				
Leptin (μg/ml)	6.65±0.87	5.23±0.70	1.65±0.52*	2.30±0.98*
Adiponectin (μg/ml)	2.91±0.06	2.86±0.07	3.16±0.04	2.927±0.05
TNF-α (pg/ml)	50.72±5.16	41.32±3.62	36.51±1.56*	36.83±1.59*
IL-6 (pg/ml)	112.9±8.80	121.0±4.81	91.01±8.92*	54.29±2.49*
IGF-1 (pg/ml)	18.55±0.45	22.90±0.13	24.47±0.15	25.50±0.20*
Organ weights				
Spleen (g)	0.09±0.00	0.11±0.01	0.12±0.00	0.09±0.00
Adipose tissue (g)	2.64±0.25	2.15±0.19	0.98±0.15*	1.17±0.16*
Quadriceps (g)	0.14±0.01	0.16±0.01	0.17±0.01	0.16±0.01
Gastrocnemius (g)	0.15±0.02	0.16±0.02	0.16±0.00	0.18±0.01

Effect of CLA isomers on serum metabolites, hormones and organ weights in C57Bl/6J mice fed with CLA isomers and CLA for 6 months. Data are means±S.E.M. (n=8–10 mice/group). Asterisk denotes statistically significant differences compared to CO and c9-CLA groups using Newman–Keuls one-way ANOVA (*P<.05).

In this CLA isomer-specific study, t10-CLA and CLA mix-fed mice showed increased lean mass, decreased body weight, BFM and VFM compared to CO as a control group. Previous studies in animals fed CLA or the t10c12 isomer showed significantly decreased body weight and fat mass compared with control animals [32–35]. Dietary supplementation with CLA isomers induces an intricate response in the mouse, including marked changes in adipose tissue and liver weight and profound alterations in several endocrine blood parameters. In the present CLA isomers study, increased glucose and insulin levels were found only in t10-CLA and CLA mix group demonstrating the hyperinsulinemia followed by glucose intolerance. Furthermore, t10-CLA isomer increased NEFA and induced the prodiabetic state, whereas the beneficial effect of c9-CLA isomer on glucose, insulin and NEFA levels was observed. Similary, CLA mediated increases in plasma insulin levels and insulin resistance have also been reported in humans [36,37]. Moloney et al. [38] demonstrated that intervention with a c9-CLA-enriched diet significantly reduced plasma insulin and glucose concentrations as well as decreased the HOMA-IR index of insulin resistance and improved the revised QUICKI indicator of insulin sensitivity in the well-characterized *ob*/*ob* mouse model that displays an obese insulin-resistant phenotype. Furthermore, we also noted that feeding c9-CLA isomer showed decreased glucose, HOMA-IR and improved QUICKI compared to CO-fed C57Bl/6J mice.

There are a number of evidences showing that increased abdominal, particularly visceral, obesity is associated with insulin resistance (the so-called abdominal or central obesity syndrome) [39,40]. Rodents fed a high-fat diet are also rapidly known to develop an increase in visceral fat. In the present study, mice fed with CO diet exhibited increased visceral fat weight (retroperitoneal, mesenteric and epididymal fat depots) which is significantly higher than that of

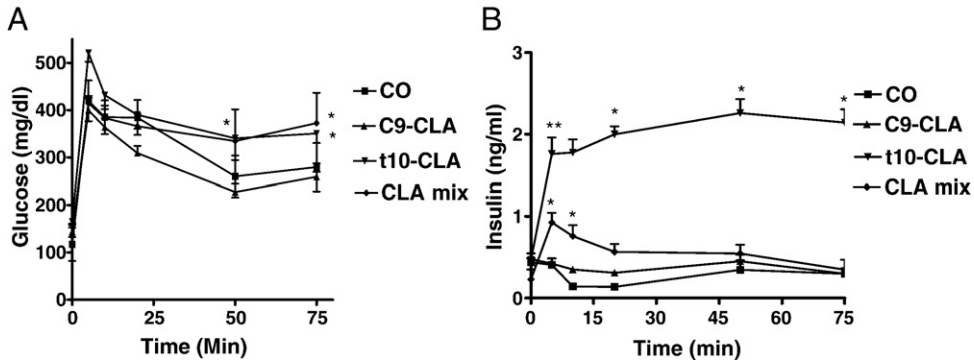

Fig. 2. IVGTT result for C57Bl/6J mice fed with CO, CLA isomers and their mixture. (A) Fasting serum levels of glucose after intravenous glucose administration. (B) Fasting serum levels of insulin after intravenous glucose administration. One gram per kilogram of glucose was injected into the tail vein of mice fed with CO, c9-CLA, t10-CLA and CLA mix. The IVGTT was performed 5 months after starting the diets. Data are means±S.E.M. from five independent experiments. Values with asterisk denote significant difference compared to t10-CLA using Newman–Keuls one-way ANOVA ($P<.05$).

t10-CLA and CLA mix-fed mice after 6 months. In this context, it seemed possible that the insulin resistance induced in these mice could be mediated either by increased liver weight or a possible membrane composition-related changes in receptor function [41,42].

Leptin, a product of the obese gene, is secreted primarily by adipocytes and plays an important role in food intake and regulating energy balance. Circulating leptin is highly correlated with general adiposity in obese rodents [39], and individuals exhibiting higher plasma leptin levels were indeed found to be more obese [43,44]. Decreased leptin levels in CLA-fed mice were also correlated with reduced fat mass in the present study, suggesting that this may be one of the possible mechanisms involved in causing insulin resistance in the present observation. Previously, we have shown that CLA decreases leptin mRNA expression in peritoneal fat tissues, and it also decreases circulating leptin levels in high-fat diet-fed mice [22]. Adiponectin is an adipokine exclusively produced by adipocytes, which decreases the hepatic lipogenesis and increases the FFA oxidation and hepatic insulin sensitivity in mice [45]. However, we did not find any significant change in adiponectin levels between the CLA isomers. Interestingly, t10-CLA and CLA-fed mice also showed fatty livers, similar to other's observation [46], which therefore may act as one of the possible causes of insulin resistance we noted in these mice. The chronic 6-month feeding of CLA isomers to 12-month-old mice revealed that the adverse effect of t10-CLA and CLA mix is not transient but is also observed in a long-term feeding trial.

In the present study, we also noted decreased TNF-α and IL-6 production in t10-CLA and CLA mix-fed mice when compared to CO-fed mice. Our previous study particularly in peritoneal fat derived from CLA-fed, high-fat diet-fed mice showed a significant decrease in TNF-α mRNA expression, which suggests that inhibition of TNF-α may occur at the adipose tissue level [22]. CLA has been previously shown to decrease TNF-α and IL-6 in serum and macrophages derived from rats and mice [7,47–49], whereas other studies have reported either no effect or a significant elevation of these cytokines in cultured cells obtained from mice and humans [50,51]. The present data also suggests that t10-CLA and CLA have positive effects in decreasing fat mass, TNF-α and IL-6, thus maintaining lean body mass in high-fat diet-fed mice, which, in part, could explain the anti-inflammatory properties of CLA in mice. Our study further confirms that only the t10-CLA isomer contributes to a significant change of body composition in C57Bl/6J mice. The anti-obesity effect of CLA mix has been ascribed to reduced adipocyte size [52], reduced adipocyte proliferation [53], increased adipocyte lipolysis [3] and apoptosis [9], as well as to enhanced fatty acid oxidation and energy expenditure [35], which is particularly found to be higher in t110-CLA-fed mice.

In conclusion, the present study suggests for the first time that long-term feeding of individual CLA isomers to older mice has different specific effects on lean mass, fat mass, glucose and insulin levels. Although t10-CLA and the combination of t10-CLA and c9-CLA significantly reduced fat mass, they were also found to cause the induction of liver steatosis and insulin resistance which are of major concern. Thus, considering the recent efficacy and safety of n-3 fatty acids to reduce hypertriglycerdemia, the use of n-3 fatty acids along with CLA may improve insulin sensitivity and reduce liver steatosis [45,54]. Additional studies in different strains of mice and also in other species are still needed before CLA isomers can be used in humans as a dietary supplement to reduce obesity.

References

[1] Wasan KM, Looije NA. Emerging pharmacological approaches to the treatment of obesity. J Pharm Pharm Sci 2005;8:259–71.
[2] Racette SB, Deusinger SS, Deusinger RH. Obesity: overview of prevalence, etiology, and treatment. Phys Ther 2003;83:276–88.
[3] DeLany JP, Blohm F, Truett AA, Scimeca JA, West DB. Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. Am J Physiol 1999;276:R1172–9.
[4] Smedman A, Vessby B. Conjugated linoleic acid supplementation in humans—metabolic effects. Lipids 2001;36:773–81.
[5] Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY, et al. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat. Biochem Biophys Res Commun 1998;244:678–82.
[6] Rudel LL. Atherosclerosis and conjugated linoleic acid. Br J Nutr 1999;81:177–9.
[7] Yu Y, Correll PH, Vanden Heuvel JP. Conjugated linoleic acid decreases production of pro-inflammatory products in macrophages: evidence for a PPAR gamma-dependent mechanism. Biochim Biophys Acta 2002;1581:89–99.
[8] Tsuboyama-Kasaoka N, Miyazaki H, Kasaoka S, Ezaki O. Increasing the amount of fat in a conjugated linoleic acid-supplemented diet reduces lipodystrophy in mice. J Nutr 2003;133:1793–9.
[9] Tsuboyama-Kasaoka N, Takahashi M, Tanemura K, et al. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. Diabetes 2000;49:1534–42.
[10] Poirier H, Rouault C, Clement L, et al. Hyperinsulinaemia triggered by dietary conjugated linoleic acid is associated with a decrease in leptin and adiponectin plasma levels and pancreatic beta cell hyperplasia in the mouse. Diabetologia 2005;48:1059–65.
[11] Clement L, Poirier H, Niot I, et al. Dietary *trans*-10,*cis*-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse. J Lipid Res 2002;43:1400–9.
[12] Degrace P, Demizieux L, Gresti J, Chardigny JM, Sebedio JL, Clouet P. Hepatic steatosis is not due to impaired fatty acid oxidation capacities in C57BL/6J mice fed the conjugated *trans*-10,*cis*-12-isomer of linoleic acid. J Nutr 2004;134:861–7.
[13] Rahman SM, Wang Y, Yotsumoto H, et al. Effects of conjugated linoleic acid on serum leptin concentration, body-fat accumulation, and beta-oxidation of fatty acid in OLETF rats. Nutrition 2001;17:385–90.

[14] Sakono M, Yuji K, Miyanaga F, et al. Combined effects of dietary conjugated linoleic acid and sesamin triacylglycerol and ketone body production in rat liver. J Nutr Sci Vitaminol (Tokyo) 2002;48:405–9.
[15] Nagao K, Inoue N, Wang YM, Yanagita T. Conjugated linoleic acid enhances plasma adiponectin level and alleviates hyperinsulinemia and hypertension in Zucker diabetic fatty (fa/fa) rats. Biochem Biophys Res Commun 2003;310:562–6.
[16] Park Y, Storkson JM, Albright KJ, Liu W, Pariza MW. Evidence that the *trans*-10,*cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. Lipids 1999;34:235–41.
[17] Thom E, Wadstein J, Gudmundsen O. Conjugated linoleic acid reduces body fat in healthy exercising humans. J Int Med Res 2001;29:392–6.
[18] Bauman DE, Barbano DM, Dwyer DA, Griinari JM. Technical note: production of butter with enhanced conjugated linoleic acid for use in biomedical studies with animal models. J Dairy Sci 2000;83:2422–5.
[19] Eulitz K, Yurawecz MP, Sehat N, et al. Preparation, separation, and confirmation of the eight geometrical *cis*/*trans* conjugated linoleic acid isomers 8,10- through 11,13-18:2. Lipids 1999;34:873–7.
[20] Bhattacharya A, Banu J, Rahman M, Causey J, Fernandes G. Biological effects of conjugated linoleic acids in health and disease. J Nutr Biochem 2006;17:789–810.
[21] Bhattacharya A, Rahman MM, McCarter R, O'Shea M, Fernandes G. Conjugated linoleic acid and chromium lower body weight and visceral fat mass in high-fat-diet-fed mice. Lipids 2006;41:437–44.
[22] Bhattacharya A, Rahman MM, Sun D, et al. The combination of dietary conjugated linoleic acid and treadmill exercise lowers gain in body fat mass and enhances lean body mass in high fat-fed male Balb/C mice. J Nutr 2005;135:1124–30.
[23] Winzell MS, Ahren B. The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. Diabetes) 2004;53(Suppl 3):S215–9.
[24] Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN. Diet-induced type II diabetes in C57BL/6J mice. Diabetes 1988;37:1163–7.
[25] Sun D, Krishnan A, Zaman K, Lawrence R, Bhattacharya A, Fernandes G. Dietary n-3 fatty acids decrease osteoclastogenesis and loss of bone mass in ovariectomized mice. J Bone Miner Res 2003;18:1206–16.
[26] Bhattacharya A, Rahman M, Banu J, et al. Inhibition of osteoporosis in autoimmune disease prone MRL/Mpj-Fas(lpr) mice by N-3 fatty acids. J Am Coll Nutr 2005;24:200–9.
[27] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[28] Wallace TM, Levy JC, Matthews DR. An increase in insulin sensitivity and basal beta-cell function in diabetic subjects treated with pioglitazone in a placebo-controlled randomized study. Diabet Med 2004;21:568–76.
[29] Perseghin G, Caumo A, Caloni M, Testolin G, Luzi L. Incorporation of the fasting plasma FFA concentration into QUICKI improves its association with insulin sensitivity in nonobese individuals. J Clin Endocrinol Metab 2001;86:4776–81.
[30] Silveira MB, Carraro R, Monereo S, Tebar J. Conjugated linoleic acid (CLA) and obesity. Public Health Nutr 2007;10:1181–6.
[31] http://www.nutraingredients-usa.com/Industry/CLA-achieves-US-approval-for-use-in-foods.
[32] Ryder JW, Portocarrero CP, Song XM, et al. Isomer-specific antidiabetic properties of conjugated linoleic acid. Improved glucose tolerance, skeletal muscle insulin action, and UCP-2 gene expression. Diabetes 2001;50:1149–57.
[33] Park Y, Albright KJ, Liu W, Storkson JM, Cook ME, Pariza MW. Effect of conjugated linoleic acid on body composition in mice. Lipids 1997;32:853–8.
[34] Park Y, Albright KJ, Storkson JM, Liu W, Cook ME, Pariza MW. Changes in body composition in mice during feeding and withdrawal of conjugated linoleic acid. Lipids 1999;34:243–8.
[35] West DB, Delany JP, Camet PM, Blohm F, Truett AA, Scimeca J. Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. Am J Physiol 1998;275:R667–72.
[36] Riserus U, Arner P, Brismar K, Vessby B. Treatment with dietary *trans*10*cis*12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. Diabetes Care 2002;25:1516–21.
[37] Medina EA, Horn WF, Keim NL, et al. Conjugated linoleic acid supplementation in humans: effects on circulating leptin concentrations and appetite. Lipids 2000;35:783–8.
[38] Moloney F, Toomey S, Noone E, et al. Antidiabetic effects of cis-9, trans-11-conjugated linoleic acid may be mediated via antiinflammatory effects in white adipose tissue. Diabetes 2007;56:574–82.
[39] Despres JP, Nadeau A, Tremblay A, et al. Role of deep abdominal fat in the association between regional adipose tissue distribution and glucose tolerance in obese women. Diabetes 1989;38:304–9.
[40] Kissebah AH. Insulin resistance in visceral obesity. Int J Obes) 1991;15(Suppl 2):109–15.
[41] Field CJ, Ryan EA, Thomson AB, Clandinin MT. Diet fat composition alters membrane phospholipid composition, insulin binding, and glucose metabolism in adipocytes from control and diabetic animals. J Biol Chem 1990;265:11143–50.
[42] Liu S, Baracos VE, Quinney HA, Clandinin MT. Dietary omega-3 and polyunsaturated fatty acids modify fatty acyl composition and insulin binding in skeletal-muscle sarcolemma. Biochem J) 1994;299(Pt 3):831–7.
[43] Staiger H, Haring HU. Adipocytokines: fat-derived humoral mediators of metabolic homeostasis. Exp Clin Endocrinol Diabetes 2005;113:67–79.
[44] Maffei M, Halaas J, Ravussin E, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1995;1:1155–61.
[45] Yamauchi T, Kamon J, Waki H, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001;7:941–6.
[46] Yanagita T, Wang YM, Nagao K, Ujino Y, Inoue N. Conjugated linoleic acid-induced fatty liver can be attenuated by combination with docosahexaenoic acid in C57BL/6N mice. J Agric Food Chem 2005;53:9629–33.
[47] Yamasaki M, Ikeda A, Oji M, et al. Modulation of body fat and serum leptin levels by dietary conjugated linoleic acid in Sprague-Dawley rats fed various fat-level diets. Nutrition 2003;19:30–5.
[48] Inoue N, Nagao K, Hirata J, Wang YM, Yanagita T. Conjugated linoleic acid prevents the development of essential hypertension in spontaneously hypertensive rats. Biochem Biophys Res Commun 2004;323:679–84.
[49] Akahoshi A, Goto Y, Murao K, et al. Conjugated linoleic acid reduces body fats and cytokine levels of mice. Biosci Biotechnol Biochem 2002;66:916–20.
[50] Kelley DS, Warren JM, Simon VA, Bartolini G, Mackey BE, Erickson KL. Similar effects of c9,t11-CLA and t10,c12-CLA on immune cell functions in mice. Lipids 2002;37:725–8.
[51] Kelley DS, Simon VA, Taylor PC, et al. Dietary supplementation with conjugated linoleic acid increased its concentration in human peripheral blood mononuclear cells, but did not alter their function. Lipids 2001;36:669–74.
[52] Azain MJ, Hausman DB, Sisk MB, Flatt WP, Jewell DE. Dietary conjugated linoleic acid reduces rat adipose tissue cell size rather than cell number. J Nutr 2000;130:1548–54.
[53] Brodie AE, Manning VA, Ferguson KR, Jewell DE, Hu CY. Conjugated linoleic acid inhibits differentiation of pre- and post- confluent 3T3-L1 preadipocytes but inhibits cell proliferation only in preconfluent cells. J Nutr 1999;129:602–6.
[54] Ide T. Interaction of fish oil and conjugated linoleic acid in affecting hepatic activity of lipogenic enzymes and gene expression in liver and adipose tissue. Diabetes 2005;54:412–23.