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[1]

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ABSTRACT Nearly half of the U.S. adult population is overweight or obese, which may be related to increased energy intake combined with lack of physical activity. Obesity increases the risk of several chronic diseases including diabetes, coronary heart disease, hypertension, and stroke. Conjugated linoleic acids (CLA) were shown to decrease fat and increase lean mass in several animal studies. However, the effects of CLA in combination with exercise (Ex) on body composition have not been studied in an animal model. We examined the effect of a low concentration of either safflower oil as control (0.5%) or mixed isomers of CLA (0.4%) along with treadmill exercise on body composition in male Balb/C mice fed a high-fat diet (20% corn oil) in a 2 × 2 factorial design. CLA consumption lowered change in fat mass ($P < 0.001$) confirming the results of other studies, and change in fat mass decreased further ($P < 0.001$) with CLA and exercise. Change in lean mass did not increase with exercise alone; it increased, although not significantly, with CLA alone and increased significantly ($P < 0.05$) due to the combination of CLA and exercise. This effect was accompanied by decreased serum leptin levels and lower leptin mRNA expression in peritoneal fat ($P < 0.001$). Serum insulin, glucose, tumor necrosis factor (TNF)-α, and interleukin-6 were lower in CLA-fed mice than in controls ($P < 0.05$), whereas serum TNF-α was increased by exercise ($P < 0.05$). Exercise increased oxygen consumption and energy expenditure when measured under resting conditions ($P < 0.05$). In summary, the combination of dietary CLA and exercise decreased fat mass and increased lean mass in mice fed a high-fat diet, and these effects may be related in part to decreased serum leptin and exercise-induced increases in oxygen consumption and energy expenditure. J. Nutr. 135: 1124–1130, 2005.

KEY WORDS: • *conjugated linoleic acid* • *exercise* • *body fat* • *leptin*

The recent increase in the worldwide prevalence of obesity has been influenced by both environmental and behavioral factors. Increased energy intake in the form of dietary fat together with lack of physical activity are crucial environmental factors imposed by the Western lifestyle; these factors comprise a major public health problem and reinforce the need to develop new and effective treatment strategies (1–3). Obesity is usually accompanied by abnormalities in leptin and insulin secretion and their action, together with defects in lipid and carbohydrate metabolism (4,5).

Recently, there has been increased interest in conjugated linoleic acid (CLA)[4] as a possible dietary supplement to reduce obesity. CLA refers to a group of PUFA that exist as positional and stereoisomers of octadecadienoic acid [linoleic acid, LA, 18:2(n-6), double bonds at position 9 and 12]. There is no methylene group separating the double bonds of CLA as there is in LA. They are found naturally in ruminant food products such as beef, lamb, and dairy products (6,7). The major isomer of CLA in natural foods is the *c*9, *t*11 isomer (8). CLA isomers can also be prepared commercially by heating linoleic acid under alkaline conditions or by partial hydrogenation of linoleic acid (9,10). Health benefits of CLA have been attributed to 2 of its isomers, i.e., *c*9, *t*11 and *t*10, *c*12.

Interest in CLA products has increased dramatically in recent years because of numerous health benefits associated with its consumption in animal models. CLA (*c*9, *t*11 and/or *t*10, *c*12) were shown to have significant anticarcinogenic and antitumorigenic activity in murine models (11–13). Either *t*10, *c*12 or a mixture of *c*9, *t*11 and *t*10, *c*12 CLA was also reported to reduce the risk of atherosclerosis, hypertension, and diabetes, and to improve feed efficiency, promote energy metabolism, and have a positive effect on immune function (14–21). However, one of the most interesting health benefits is that diets containing either *t*10,

[1] Supported by a grant from Loders-Croklaan, Inc. and National Institutes of Health grants AG020239 and AG23648.

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[4] Abbreviations used: BFM, body fat mass; CLA, conjugated linoleic acid; CO, corn oil; DEXA, dual energy X-ray absorptiometry; Ex, exercise; IL, interleukin; LA, linoleic acid; LBM, lean body mass; Sed, sedentary; SFO, safflower oil; TNF, tumor necrosis factor.

Manuscript received 1 December 2004. Initial review completed 7 January 2005. Revision accepted 21 January 2005.

*c*12 or a mixture of *c*9, *t*11 and *t*10, *c*12 CLA significantly reduce body fat mass (BFM) and increase lean body mass (LBM) in different strains of mice and rats (22,23). In addition, many human trials have now demonstrated similar beneficial effects. In recent clinical trials corroborating the findings obtained in animal studies, a mixture of *c*9, *t*11 and *t*10, *c*12 CLA reduced BFM (24–27). These results generated a great deal of interest among health professionals and athletes. Clinical studies indicated that one of the beneficial effects of exercise is maintaining lower body fat without significantly altering LBM (25,28,29). The rodent treadmill exercise model is a method of exercise in which duration of exercise and its intensity can be controlled (30) in contrast to voluntary wheel exercise (31).

Most of the animal studies with CLA reported to date involved measurements made after the animals were killed; thus, longitudinal effects of CLA supplementation with and without exercise on body composition have not been clearly investigated and established. Longitudinal studies were recently made possible by dual-energy X-ray absorptiometry (DEXA), which can be used to analyze BFM, LBM, and bone mineral density of the whole animal. To the best of our knowledge, only one previous study, conducted in pigs, evaluated the effect of a mixture of *c*9, *t*11 and *t*10, *c*12 CLA on body composition using DEXA (32). Human studies have also utilized DEXA to measure changes in body composition after intake of a mixture of *c*9, *t*11 and *t*10, *c*12 CLA (27). Because both CLA and exercise have beneficial effects on body composition, we wanted to determine whether a low dose of mixed isomers of CLA combined with moderate treadmill exercise would have a synergistic effect on body composition in male Balb/c mice fed a high-fat diet (20 g/100 g) using DEXA technology. We also analyzed serum insulin, glucose, leptin, adiponectin, the proinflammatory cytokines, interleukin (IL)-6 and tumor necrosis factor (TNF)-α, and the expression of mRNA for leptin, adiponectin, and TNF-α in peritoneal fat in mice fed diets containing CLA or safflower oil (SFO) as the control.

MATERIALS AND METHODS

Animals and experimental diets. Male Balb/c mice, 6 wk old, were obtained from Harlan. Weight-matched mice were housed in laboratory animal care facility in cages (5 mice/cage) and fed a standard diet (Harlan Teklad LM-485). At 8 wk of age, mice were divided into 2 dietary groups and fed a semipurified diet as described (33) with AIN-93M vitamin and mineral mixes (34) containing either 0.5% SFO or 0.4% CLA (Clarinol powder, Loders Croklaan) The diets contained 20% corn oil (CO) by weight (~35% energy from fat). The composition of the semipurified diets/kg diet was as follows: casein, 248 g; AIN93 mineral mix, 64 g; AIN93 vitamin mix, 17 g; cellulose, 55 g; cerelose, 405.5 g; choline bitartrate, 21.5 g; DL-methionine, 3 g; corn oil, 200 g; and either 5 g SFO or 6.76 g clarinol powder. The composition of the oil extracted from the Clarinol powder was: *c*9, *t*11, 36.9%; *t*10, *c*12, 37.4%; total *trans*, 2.7%. The total CLA content of the oil was 80.9% with the main isomers accounting for 74.4%. The oil in the powder was 75%, resulting in 60.7% total CLA in the powder added to the diet. Like CO, SFO contains 18:2(n-6) as the major fatty acid. Fresh diet was prepared weekly, stored in aliquots at −20°C, and provided daily (4 g/mouse) Body weight gain was monitored weekly and food consumption based on residual food was monitored daily (**Table 1**). Each dietary group was divided into sedentary (Sed) and exercised (Ex) groups of 10 mice each. Mice were maintained on a 12-h light:dark cycle in an ambient temperature of 22–25°C at 45% humidity. NIH guidelines were strictly followed and all studies were approved by the Institutional Laboratory Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

Exercise protocol. Mice were trained to run on a treadmill (Quinton Instruments) containing 10 running tracks with rubber belts driven at a controlled speed as described (35). Mice were exercised 5 d a week for 14 wk, gradually increasing the speed and duration. Mice in the Ex groups were trained to recognize 2 signals when they stopped running: *1*) a noise initiated by breaking a light beam at the middle of the track, and *2*) a mild stream of air before the end of the track. If they ignored these 2 signals and slipped further down they were gently nudged (by a small soft brush) to continue to run. After 2 wk of training at 160, 320, and 640 m/h, mice ran 40 min/d, 5 d/wk at 1000 m/h for the remainder of the study. Sedentary mice were placed on the stationery rodent treadmill twice a week for about the same period of time as the exercised groups.

TABLE 1

Body composition and food intake in Sed and Ex male Balb/C mice fed control (SFO) or CLA diets for 14 wk[1]

					P-values[2]		
	SFO/Sed	SFO/Ex	CLA/Sed	CLA/Ex	CLA	Ex	CLA × Ex
Body weight, *g*							
Baseline	25.64 ± 0.37	26.99 ± 0.37	24.67 ± 0.37	27.63 ± 0.37			
14 wk	34.67 ± 0.37[a]	33.00 ± 0.42[a]	30.10 ± 0.37[b]	33.20 ± 0.37[a]	0.045	0.016	0.008
Change	9.03 ± 0.93[a]	5.86 ± 0.63[b]	5.54 ± 0.46[b]	5.57 ± 0.42[b]			
Fat mass, *g*							
Baseline	3.07 ± 0.31	3.51 ± 0.31	2.48 ± 0.31	3.92 ± 0.31			
14 wk	8.08 ± 0.31[a]	5.18 ± 0.34[b]	3.65 ± 0.31[b]	3.61 ± 0.31[b]	<0.001	0.340	0.001
Change	5.02 ± 0.65[a]	1.66 ± 0.44[b]	1.23 ± 0.33[b]	−0.31 ± 0.27[c]			
% Fat							
Baseline	15.76 ± 0.99	16.76 ± 0.99	13.25 ± 0.99	18.13 ± 0.99			
14 wk	28.98 ± 0.99[a]	20.02 ± 1.11[b]	15.09 ± 0.99[c]	13.82 ± 0.99[c]	<0.001	0.207	0.002
Change	13.27 ± 1.55[a]	3.32 ± 1.61[b]	2.03 ± 1.08[b]	−4.30 ± 1.01[c]			
Lean mass, *g*							
Baseline	16.37 ± 0.26	17.37 ± 0.26	16.25 ± 0.26	17.64 ± 0.26			
14 wk	19.39 ± 0.26[b]	20.55 ± 0.26[b]	20.36 ± 0.26[b]	22.59 ± 0.26[a]	0.017	<0.001	0.254
Change	3.01 ± 0.53[b]	3.06 ± 0.32[b]	4.09 ± 0.31[ab]	4.94 ± 0.43[a]			
Food intake, *g/d*	3.19 ± 0.05[b]	3.86 ± 0.03[a]	3.03 ± 0.05[b]	3.84 ± 0.05[a]	0.059	<0.001	0.115

[1] Values are means ± SEM, $n = 10$. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] From 2-way ANOVA with repeated measures at 14 wk.

Collection of blood serum and peritoneal fat. Blood was collected by retro-orbital bleeding from anesthetized mice that had been deprived of food overnight, and serum was obtained by centrifugation at 300 × *g* for 15 min at 4°C for the measurement of glucose and insulin. At the termination of the study, mice were killed by cervical dislocation, blood was collected, and serum was separated for measurement of other metabolites. Peritoneal fat samples were frozen at −80°C until used.

Measurement of BFM and LBM. LBM and BFM were measured by DEXA using Lunar PIXImus mouse bone densitometer (GE), and data were analyzed with PIXImus software (36). The percentage of fat was calculated using the formula BFM/(LBM + BFM) × 100. Scanning was performed first at baseline (before starting the purified diet and initiating exercise) and at 6, 10, and 14 wk thereafter.

Serum metabolites. Serum TNF-α and IL-6 levels were measured by standard ELISA techniques as described previously (37). Insulin was analyzed using a rat/mouse insulin ELISA kit from Linco Research. Glucose was analyzed spectrophotometrically using Sigma Diagnostics Glucose (Trinder) reagent. Adiponectin was assayed using mouse adiponectin Quantikine immunoassay kit from R&D Systems. Leptin was assayed using an active murine leptin kit from Diagnostic Systems Laboratories.

Metabolic rate. Metabolic rate was measured indirectly by drawing air through sealed rodent cages and monitoring the partial pressures of oxygen and carbon dioxide of the air entering and leaving the cage, as described in several earlier studies (38). In brief, gas composition was measured using a zirconia cell O_2 detector and an infrared CO_2 analyzer (Applied Electrochemistry models S-3A and CD-3A, respectively), with flow regulated by a mass flow controller (Linde model FM4570). Gas pressures, airflow, cage temperature, ambient air temperature, pressure, and humidity were digitized, recorded every 10 s, and averaged on an hourly basis. Each mouse was monitored between 0800 and 1200 h for 3 h, with an interval of at least 24 h after the last bout of exercise in the case of Ex mice. Mice were acclimated to the flow-through system for 24 h before measurement of metabolic rate. During the time of measurement, air was also sampled from an empty cage situated adjacent to the experimental cage, as a control. The rate of O_2 consumption was calculated using the equations of Consolazio et al. (39). The metabolic rate was then calculated in kJ/min using these values and a energy equivalent of 1 L of oxygen per 4.76 kJ. This assumes that the fuel combusted in vivo is the same as that of the food ingested; only small errors are introduced if the fuel mix metabolized at a given time deviates from this value.

RT-PCR analysis of peritoneal fat. Total RNA from pooled peritoneal fat pads was extracted using TRIZOL according to manufacturer's instructions and RT-PCR analysis was performed. Briefly, RNA was reverse transcribed into complementary DNA (cDNA). The cDNA was subjected to enzymatic amplification in a DNA thermal cycler using specific primers. Specific primers used were as follows: leptin: 5′-TCC AGA AAG TCC AGG ATG ACA C and 5′-CAC ATT TTG GGA AGG CAG G; adiponectin: 5′-AAG GAC AAG GCC GTT CTC T and 5′-TAT GGG TAG TTG CAG TCA GTT GG; TNF-α: 5′-TTC TGT CTA CTG AAC TTC GGG GTG ATC GGT CC and 5′-GTA TGA GAT AGC AAA TCG GCT GAC GGT GTG GG; glyceraldehyde 3-phosphate dehydrogenase: 5′-GAT CGT GGA AGG GCT AAT GA AND 5′-GAC TTT GCC TAC AGC CTT GG. The amplified PCR products were subjected to 1.5% agarose gel electrophoresis and visualized by UV fluorescence after staining with ethidium bromide.

Statistics. Results are expressed as means ± SEM. Data were analyzed statistically by 2-way ANOVA or repeated-measures ANOVA using NCSS or Graphpad Prism 4 software. The Newman-Keuls multiple comparison test was used to test the differences among groups. Differences were considered significant at $P < 0.05$.

RESULTS

Food intake, body weight, and lean and fat mass. After determining maximum food consumption, mice were provided equal amounts of food each day. Food consumption did not differ between SFO- and CLA-fed mice. However, Ex mice in both dietary groups consumed more food than Sed mice (Table 1). All of the groups gained weight during the first 6 wk of the study. A significant effect of diet and exercise occurred, however, between 10 and 14 wk of age. At the end of the study, body weight gain was higher in the SFO/Sed group than in the other 3 groups. Exercise decreased weight gain in the SFO group. Exercised and sedentary CLA-fed mice gained less weight ($P < 0.05$) than the SFO/Sed group. The effect of CLA was comparable to exercise alone, but there was no additional effect on weight gain of exercise in combination with CLA (Table 1).

CLA/Ex but not CLA/Sed mice had greater gain in LBM than SFO/Sed and SFO/Ex mice (Table 1). BFM gain was greater in SFO/Sed mice than in the other 3 groups. CLA/Sed mice gained 76% less BFM than SFO/Sed mice. Exercise lowered change in BFM and the percentage of fat significantly in both dietary groups with levels less than baseline in the CLA/Ex group.

Serum biochemistry. Glucose concentrations were 15% lower in CLA/Sed and SFO/Ex than in SFO/Sed mice and were decreased even further in the CLA/Ex group ($P < 0.05$; **Table 2**). Serum insulin levels were significantly lower in CLA-fed mice than in SFO-fed mice, whereas exercise had no effect.

Serum adiponectin levels were not affected by diet or exercise alone, although the overall effect of CLA in combination with exercise was significant (Table 2). CLA/Sed mice had significantly lower leptin levels than SFO/Sed mice. Exercise decreased leptin levels but only in the CLA-fed mice.

Serum proinflammatory cytokines, TNF-α and IL-6, were decreased significantly in CLA/Sed mice compared with SFO/

TABLE 2

Serum concentrations of glucose, insulin, leptin, and adiponectin and proinflammatory cytokine production in Sed and Ex male Balb/C mice fed control (SFO) or CLA diets for 14 wk[1]

					P-values[2]		
	SFO/Sed	SFO/Ex	CLA/Sed	CLA/Ex	CLA	Ex	CLA × Ex
Glucose, *mmol/L*	8.95 ± 0.34[a]	7.49 ± 0.27[b]	7.60 ± 0.25[b]	6.49 ± 0.18[c]	<0.001	<0.001	0.526
Insulin, *pmol/L*	13.96 ± 1.41[a]	17.64 ± 0.73[a]	5.28 ± 0.22[b]	5.47 ± 0.61[b]	<0.001	0.189	0.610
Adiponectin, *mg/L*	3.53 ± 0.32	3.97 ± 0.16	3.88 ± 0.11	3.46 ± 0.13	0.693	0.945	0.045
Leptin, μ*g/L*	2.32 ± 0.11[a]	2.06 ± 0.11[a]	1.56 ± 0.07[b]	1.13 ± 0.08[c]	<0.001	0.002	0.365
TNF-α, *ng/L*	266 ± 48[b]	484 ± 1[a]	136 ± 4[c]	308 ± 11[b]	0.005	0.006	0.958
IL-6, *ng/L*	63.6 ± 6.6[a]	46.5 ± 1.8[b]	38.7 ± 0.9[b]	47.4 ± 3.4[b]	0.007	0.292	0.004

[1] Values are means ± SEM, $n = 5$ Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] From 2-way ANOVA.

TABLE 3

Oxygen consumption and energy expenditure in Sed and Ex male Balb/C mice fed control (SFO) or CLA diets for 14 wk[1]

					P-values[2]		
	SFO/Sed	SFO/Ex	CLA/Sed	CLA/Ex	CLA	Ex	CLA × Ex
O_2 consumption, *mL/h*	106 ± 3^{ab}	111 ± 6^{ab}	94.4 ± 6.5^{b}	119 ± 5^{a}	0.762	0.013	0.068
Energy expenditure, *kJ/h*	1.67 ± 0.05^{ab}	1.73 ± 0.09^{ab}	1.48 ± 0.10^{b}	1.86 ± 0.08^{a}	0.726	0.015	0.311

[1] Values are means ± SEM, $n = 5$. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] From 2-way ANOVA.

Sed mice. Exercise increased TNF-α in both dietary groups, but it remained lower in CLA/Ex mice than in SFO/Ex mice. IL-6 decreased by about the same amount in SFO/Ex and CLA/Sed mice compared with SFO/Sed mice but was not further decreased by exercise in CLA fed mice (Table 2).

Oxygen consumption and energy metabolism. Oxygen consumption and energy metabolism did not differ between SFO- and CLA-fed mice (**Table 3**). VO_2 (oxygen consumption) and energy expenditure were greater ($P < 0.05$) in CLA/Ex mice than in CLA/Sed mice, indicating that exercise had a positive effect in this group of mice, but not in SFO-fed mice. The SFO-fed groups did not differ, indicating that exercise did not have a beneficial effect. Values for CLA-fed exercised mice remained comparable to SFO/Sed and SFO/Ex mice.

Leptin, adiponectin and TNF-α mRNA expression in intraperitoneal fat. Peritoneal fat mRNA expression of leptin was lower in CLA/Sed mice than in SFO/Sed mice and it was decreased further in CLA/Ex mice (**Fig. 1**, **Table 4**). Leptin expression was also decreased by exercise in SFO-fed mice but the decrease was much greater in those fed CLA. Adiponectin expression was not affected by diet and/or exercise. Expression of TNF-α mRNA did not differ between SFO/Sed and CLA/Sed mice. However, expression was increased by exercise in mice fed SFO but not in those fed CLA.

DISCUSSION

The present study suggests that consumption of a low amount of CLA in the diet combined with moderate treadmill exercise can decrease gain in total body fat and increase change in LBM more than exercise alone in mice fed a high-fat diet. The present study also confirms that CLA supplementation reduces the overall fat deposition regardless of dietary fat concentration (23) and, in this case, also better than exercise alone. Although, to date, both animal and human studies reported fat-lowering effects of CLA, the combined effect of CLA and exercise has not been explored in detail either in humans or in animal models (25,40).

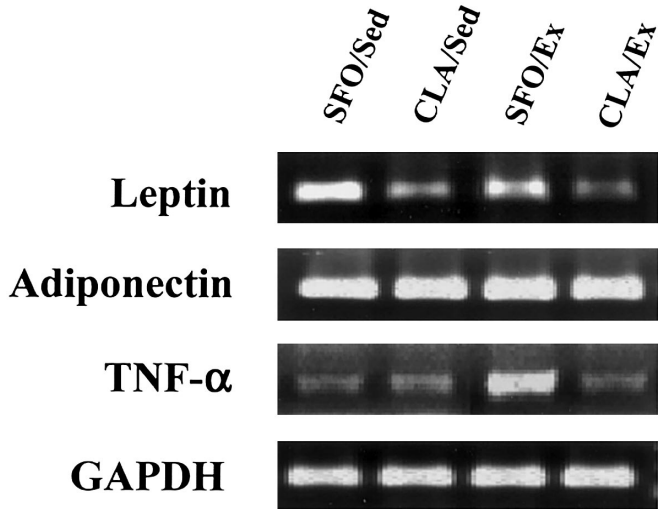

FIGURE 1 Leptin, adiponectin, and TNF-α mRNA expression in peritoneal fat in Sed and Ex male Balb/C mice fed control (SFO) or CLA diets for 14 wk. Representative results from 3 separate experiments.

CLA feeding did not affect food intake in this study. This result was not consistent with previous reports, which suggest that a mixture of *c*9, *t*11 and *t*10, *c*12 CLA isomers or *t*10, *c*12 CLA alone reduces feed intake in mice (41–43) A recent study showed that CLA reduced food intake, body weight, and body fat gain in mice fed a diet deficient in essential fatty acids (44). In contrast, when adequate 18:2(n-6) fatty acids were fed, although CLA reduced gain in fat mass, there was no effect on feed intake. We used a very high level of essential fatty acids in the form of corn oil in our diets, which may have decreased the effect of CLA on food intake.

Most of the animal studies showing beneficial effects of CLA on adiposity used a mixture of *c*9, *t*11 and *t*10, *c*12 isomers (45). However, ICR mice fed a CLA mixture containing a very low concentration of *t*10, *c*12 isomer (3%) did not show a reduction in fat mass, indicating that the CLA primarily responsible for the reduction in body weight, lipid accretion, and storage in these mice was the *t*10, *c*12 isomer (42,46,47). Although *t*10, *c*12 is the primary isomer involved in the CLA-induced fat lowering effect, the balance between the 2 CLA isomers seems to be a crucial factor in their fat-lowering effect as well as in increasing lean mass. In this study, we used equal amounts of the *c*9, *t*11 and *t*10, *c*12 isomers in our CLA test diet for 3 mo. The question of CLA's ability to provide long-term benefits in reducing adiposity was also raised. It was shown previously that mice fed a high-fat diet with 1% CLA (*c*9, *t*11 + *t*10, *c*12) had reduced body weight initially but the difference between mice fed CLA and those fed the control diet gradually diminished by 12 wk (48). On the other hand, we noticed a maximum effect of CLA and exercise between 10 and 14 wk of dietary treatment, suggesting that CLA in conjugation with regular exercise may have long-term benefits in preventing weight gain, reducing and maintaining lower body weight and fat mass, and increasing lean mass.

Adipose tissue not only stores excess energy in the form of fat but also releases physiologically active mediators known as adipo-cytokines such as leptin and adiponectin (49). Leptin, a product of the obese gene, is secreted primarily by adipocytes and plays an important role in regulating energy balance via its action on food intake and energy expenditure (50). Circulating leptin levels are highly correlated with adipose tissue mass (50). Leptin has a critical role in regulating energy homeosta-

TABLE 4

Densitometric analysis of leptin, adiponectin and TNF-α mRNA expression in peritoneal fat from Sed and Ex male Balb/C mice fed control (SFO) or CLA diets for 14 wk[1]

					P-values[2]		
	SFO/Sed	CLA/Sed	SFO/Ex	CLA/Ex	CLA	Ex	CLA × Ex
Leptin[3]	100 ± 3[a]	44.1 ± 1.7[c]	74.6 ± 3.1[b]	34.2 ± 2.6[d]	<0.001	<0.001	0.023
Adiponectin[3]	100 ± 1	95.9 ± 2.3	98.1 ± 1.9	94.9 ± 1.9	0.083	0.470	0.798
TNF-α[3]	100 ± 2[b]	98.0 ± 1.5[b]	179 ± 13[a]	99.6 ± 10.9[b]	0.002	0.001	0.002

[1] Values are means ± SEM, $n = 3$. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] From 2-way ANOVA.
[3] Normalized to glyceraldehyde 3-phosphate dehydrogenase.

sis and is affected by dietary micronutrients and exercise (50). Obese subjects were shown to have higher plasma leptin levels (51). Adiponectin enhances insulin action, suggesting that it protects against insulin resistance (19). Adiponectin levels in plasma were found to be reduced in obese subjects compared with lean subjects in a recent human study (52). We found that CLA reduced serum leptin levels in Sed mice, which decreased further with exercise. Leptin levels decreased in parallel with the decrease in fat mass in CLA/Sed and CLA/Ex mice. Both CLA (*c*9, *t*11 + *t*10, *c*12) and exercise were previously reported to decrease serum leptin levels in mice and humans (53,54). Our results suggest that CLA and exercise in combination may reduce serum leptin and peritoneal fat mRNA expression, which may explain in part the lowest change in fat mass in CLA/Ex mice. Surprisingly, CLA and exercise individually had no effect on serum adiponectin levels in our study. CLA (*c*9, *t*11 + *t*10, *c*12) was shown previously to increase adiponectin levels in diabetic rats (19) but not in humans consuming *t*10, *c*12 CLA (55). The effect of exercise on adiponectin was variable in human studies, with some indicating no change and others reporting increased activity (56,57). A recent study also suggested an inverse relation between adipose tissue-derived TNF-α and IL-6 and plasma levels of adiponectin (52). The role of CLA and exercise in adiponectin metabolism has to be clearly established. Insulin stimulates leptin secretion, increasing adipocyte glucose uptake and metabolism (58). CLA (*c*9, *t*11 + *t*10, *c*12) was shown to reduce insulin levels in animal models (19). Reduction of insulin in CLA-fed mice in our study may suggest a much desired improvement in insulin resistance. The significant decrease of glucose in both CLA/Sed and CLA/Ex mice compared with SFO/Sed and SFO/Ex mice suggests that improved insulin sensitivity may have led to increased glucose utilization in mice fed CLA. However, this has to be established in future studies.

In this study, TNF-α was significantly lower in serum from CLA/Sed mice compared with SFO/Sed mice. It was reported previously that CLA reduces serum TNF-α, irrespective of the fat content in the diet (53). Exercise increased the serum TNF-α level in both dietary groups but it was still significantly lower in the CLA-fed mice than in those fed SFO. CLA decreased serum IL-6 concentration in this study and it was shown to decrease IL-6 production in mouse macrophage cells (59). Although increased serum TNF-α and IL-6 were reported after moderate-to-severe exercise in human studies (60,61), in our study, IL-6 did not increase with exercise in either of the dietary groups. The precise mechanism involved in the increase of proinflammatory cytokines with exercise has not been clearly established. It was reported recently that an exercise-induced increase in TNF-α and IL-6 was attenuated by antioxidants, suggesting a possible role of reactive oxygen species in the proinflammatory activity (62). In this study, TNF-α was lower in serum and peritoneal fat of CLA fed-Ex mice compared with SFO fed-Ex mice, suggesting that CLA may have a role in ameliorating the adverse effects of exercise on the immune response.

We did not find any increase in O_2 consumption and energy expenditure in CLA/Sed mice compared with SFO/Sed mice. However, CLA/Ex mice had higher O_2 consumption and energy expenditure than CLA/Sed mice but not SFO/Sed and Ex mice, suggesting that exercise may have contributed to enhanced fat oxidation in the CLA-fed mice. To our knowledge, the effect of CLA and exercise on energy metabolism has not been studied previously in mice fed a high-fat diet. Both CLA (*c*9, *t*11 + *t*10, *c*12 and *t*10, *c*12 alone) and exercise individually were shown previously to increase oxygen consumption and energy expenditure in mice and rats (20,63,64). Recently, it was found that the effect of CLA on energy expenditure was due mainly to the *t*10, *c*12 isomer and not the *c*9, *t*11 isomer (20), further confirming the role of the *t*10, *c*12 isomer in fat reduction. The mechanism by which CLA increases energy expenditure has not been established, but an increase in catecholamines, an increase in glucocorticoids, and/or an enhancement of sympathetic nervous system activity were reported to trigger this increase (20). Our results suggest that fat reduction in CLA/Sed mice may be independent of increased oxygen consumption and energy expenditure but increased energy expenditure could have contributed to fat reduction in CLA/Ex mice.

In conclusion, the results of this study suggest that CLA and exercise can combine to lower gain in BFM and enhance change in LBM, which may be related to reduced leptin levels and increased energy expenditure by exercise. Decreased glucose levels may be due to improved insulin sensitivity in CLA-fed mice. CLA alone decreased fat gain, which was comparable to exercise alone. In the exercised groups, CLA increased LBM and ameliorated the inflammatory effect of exercise. Further short and long-term CLA supplement studies in athletes would determine whether these effects can be applied to optimize sports nutrition strategies and also possibly reduce the growing incidence of obesity in adolescents by increasing physical activity.

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