Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse

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West, David B., James P. Delany, Patricia M. Camet, Fawn Blohm, Alycia A. Truett, and Joseph Scimeca. Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. *Am. J. Physiol.* 275 (*Regulatory Integrative Comp. Physiol.* 44): R667–R672, 1998.—Conjugated linoleic acid (CLA) is a naturally occurring group of dienoic derivatives of linoleic acid found in the fat of beef and other ruminants. CLA is reported to have effects on both tumor development and body fat in animal models. To further characterize the metabolic effects of CLA, male AKR/J mice were fed a high-fat (45 kcal%) or low-fat (15 kcal%) diet with or without CLA (2.46 mg/kcal; 1.2 and 1.0% by weight in high- and low-fat diets, respectively) for 6 wk. CLA significantly reduced energy intake, growth rate, adipose depot weight, and carcass lipid and protein content independent of diet composition. Overall, the reduction of adipose depot weight ranged from 43 to 88%, with the retroperitoneal depot most sensitive to CLA. CLA significantly increased metabolic rate and decreased the nighttime respiratory quotient. These findings demonstrate that CLA reduces body fat by several mechanisms, including a reduced energy intake, increased metabolic rate, and a shift in the nocturnal fuel mix.

body composition; indirect calorimetry; obesity; fatty acids

CONJUGATED LINOLEIC ACID (CLA) is a naturally occurring group of dienoic derivatives of linoleic acid formed by rumen bacteria (3). The major dietary sources of CLA for humans are beef and dairy products; beef tallow contains ~0.5% of fatty acids as CLA (2). These derivatives of linoleic acid have been shown to have a variety of effects in animal models. For example, CLA is reported to have anticarcinogenic activity (7, 8), be protective against atherosclerosis in rabbits (11), and partially overcome the catabolic responses due to endotoxin injection (12). The purported active form of CLA is *cis*-9,*trans*-11-octadecadienoic acid (6).

CLA has recently been reported to reduce body fat content in mice when added as a dietary admixture (13, 14). Evaluation of the metabolic effects of CLA in both intact animals and adipocyte culture suggests that CLA directly affects key enzymes and processes involved in lipid mobilization and storage (14).

The purpose of the studies described here is to further characterize the metabolic effects of CLA in the mouse. We have extended the original studies in mice to show that CLA effectively reduces body fat in another mouse strain (AKR/J in addition to the ICN strain). Furthermore, we report here that CLA reduces body fat in animals fed both a low- and a high-fat diet, has regional adipose depot specificity, and acts by suppressing energy intake as well as increasing energy expenditure.

METHODS AND PROCEDURES

Male inbred AKR/J mice were obtained from the Jackson Laboratory (Bar Harbor, ME) at 5 wk of age and habituated to individual housing in hanging stainless steel cages in a room maintained at an ambient temperature of 25 ± 1°C. Initially, the mice were maintained on ad libitum Purina rodent chow (diet no. 5001; Ralston Purina, St. Louis, MO) and water. At 6 wk of age, the mice were placed on a high-fat (45 kcal%) or low-fat (15 kcal%) diet with or without CLA (2.46 mg CLA/kcal of diet; 1% by weight for low-fat diet and 1.2% by weight for high-fat diet) for 6 wk. The CLA dosage was based on previous reports using CLA in the mouse (13, 14). See Table 1 for the composition of these diets (17). Sample size was 8–10 mice per diet and treatment group. CLA was obtained from Nu Chek Prep (Elysian, MN), and the composition ascertained by HPLC was 39.1% *cis*-9,*trans*-11 and *trans*-9,*cis*-11 CLA; 40.7% *trans*-10,*cis*-12 CLA; 1.8% *cis*-9,*cis*-11 CLA; 1.3% *cis*-10,*cis*-12 CLA; 1.9% *trans*-9,*trans*-11 and *trans*-10,*trans*-12 CLA; 1.1% *cis*-9,*cis*-12 linoleic acid; and 14.1% remainder. The defined pelleted diets were placed onto the bottom of the cages and replaced with fresh diet on Monday, Wednesday, and Friday. The mice were weighed three times each week, and food intake (corrected for spillage and measured to 0.1 g) was also measured at these times.

Beginning 2 wk after starting the defined diets, we habituated the animals to metabolic chambers by placing each animal into a chamber for a 24-h period. This 24-h habituation was repeated for each animal during *week 4* after the start of the defined diet manipulation. During the period of habituation to the metabolic chambers, the animals had ad libitum access to water and the same diet as they were fed in their home cages. During the sixth week after the start of the defined diets, each animal was placed into a metabolic chamber for the measurement of 24-h CO_2 production and O_2 consumption by indirect calorimetry. The system used for the assessment of metabolic rate in the mouse has been previously described (18). It consists of four metabolic chambers, CO_2 and O_2 analyzers, electronic flow regulators, and a switching system to sample gas from each metabolic chamber. The metabolic chambers have a 205-cm^2 floor area and are 9.5 cm in height. Room air was pumped through the chambers at a rate of 0.3 l/min. Expired air was dried in a drierite column and then directed to an O_2 analyzer (model S-3A, Ametek, Pittsburgh, PA) and a CO_2 analyzer (model CD-3A, Ametek).

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Table 1. *Diet composition*

	High Fat		Low Fat	
Fat, kcal%	44.9		15.0	
Protein, kcal%	20.5		20.5	
Carbohydrate, kcal%	33.6		63.5	
Mineral mix, kcal%	1.0		1.0	
kcal/g	4.88		4.06	
	g	kcal	g	kcal
Fat				
Corn oil	44.5	400.5	14.8	133.2
Hydrogenated coconut oil	5.4	48.6	1.8	16.2
Protein				
Casein	50.4	201.6	50.4	201.6
DL-Methionine	0.8	3.2	0.8	3.2
Carbohydrate				
Sucrose	28.2	112.8	53.0	212.0
Maltodextrin 10	38.4	153.6	38.4	153.6
Corn starch	17.4	69.6	67.3	269.2
Cellulose fiber	7.7		7.7	
Mineral mix	9.0		9.0	
Vitamin mix	2.6	10.2	2.6	10.2
Choline bitartrate	0.5		0.5	
Total	204.9	1000.1	246.3	999.2

Diets were formulated by Research Diets (New Brunswick, NJ). Dietary fat content was designed such that fatty acid composition was ~1 part saturated and 3 parts unsaturated. For kilocalorie values, energy content was calculated using 4 kcal/g for protein and carbohydrate and 9 kcal/g for fat. Mineral and vitamin mix content conformed to minimum requirements as defined by American Institute of Nutrition 76A standard. In those diets containing conjugated linoleic acid (CLA), CLA was substituted for corn oil on a weight basis.

Air from each chamber was sampled for a 75-s period, and the last 45-s interval was used for the analysis. Therefore, each chamber was sampled one time every 5 min, and data were collected for 45 s. The output from the analyzers and switching system were dumped to a computer, and data were stored in a spreadsheet.

After 6 wk of the diet and CLA manipulations, the mice were killed by cervical dislocation and selected adipose depots and organs were removed and weighed. Liver, left and right kidney, left and right testis, and the spleen were removed and weighed to 0.0001 g. Similarly, the left and right inguinal adipose depot, left and right epididymal adipose depot, left and right retroperitoneal adipose depot, and the mesenteric adipose depot were removed and weighed. The carcass was eviscerated. The eviscerated carcass, including the organs and depots that had been removed, was weighed and then homogenized in 3× weight distilled water. The resulting homogenate was placed in a drying oven at 80°C and weighed daily until the sample weight was constant. The dried, homogenized carcass was then mixed thoroughly and further crushed using a mortar and pestle. This dried homogenate was analyzed for lipid using the Soxhlet method (modified to extract lipid using 2:1 chloroform-methanol) and a Tecatur Instruments Soxtec system HT no. 1043, analyzed for protein using a Perkin-Elmer (Norwalk, CT) series II nitrogen analyzer, and analyzed for ash content using a CEM MAS 7000 muffle furnace. Water content of the carcass was calculated by subtracting the dried carcass weight from the original eviscerated carcass weight.

The data are presented in Figs. 1–5, Tables 1–4, and in text as means ± SE; however, in some figures SEs are not plotted. Body weight, energy intake, organ and adipose depot weights, and carcass composition data were analyzed by ANOVA, and post hoc comparisons were made using a protected least-significant difference test in SAS release 6.12. Energy expenditure observations were modeled as repeated measures on each subject, with diet, CLA, chamber number, and day or night as explanatory variables arranged as a $2 \times 2 \times 4 \times 2$ factorial treatment structure (highest order interaction term was not included). Body weight was included as a covariate in the model for energy expenditure. A similar model was adopted for observations of respiratory quotient (RQ), but body weight was not included as a covariate in that model. Solutions were obtained using restricted maximum likelihood estimation as implemented in mixed procedure of SAS release 6.12. Observed significance levels for multiple comparisons of estimated treatment means (least squares means) were adjusted using the Tukey joint estimation procedure.

RESULTS

The CLA treatment significantly reduced the growth rate in both diet groups (Fig. 1). There were no differences in body weight among the groups before starting the diets; however, after 6 wk, the high-fat control group weighed 5.7 g more than the high-fat CLA treatment group ($P < 0.0001$). Similarly, after 6 wk, the low-fat control group weighed 3.2 g more than the low-fat CLA group ($P < 0.005$). The high-fat diet promoted significantly greater weight gain over the 6 wk of the study compared with the low-fat diet.

CLA supplementation also significantly reduced energy intake of both diet groups (Figs. 2 and 3). Initially, the high-fat diet induced a relative hyperphagia compared with the low-fat diet; this hyperphagia persisted during the entire 6-wk dietary manipulation, but the kilocalories per day diminished from ~26 to ~18 kcal/day in the high-fat control group (see Fig. 2). Over the course of the study, the high-fat control group consumed significantly more calories than the low-fat control group (766.2 ± 12.0 kcal vs. 624.7 ± 13.4 kcal; $P < 0.0001$). CLA treatment significantly reduced total energy intake independent of diet group (695.4 ± 9.0 kcal for control vs. 611.8 ± 8.4 kcal for CLA treatment; $P < 0.0001$). Most of the effect of CLA on energy intake occurred during the first 3 wk of the study (see Fig. 2).

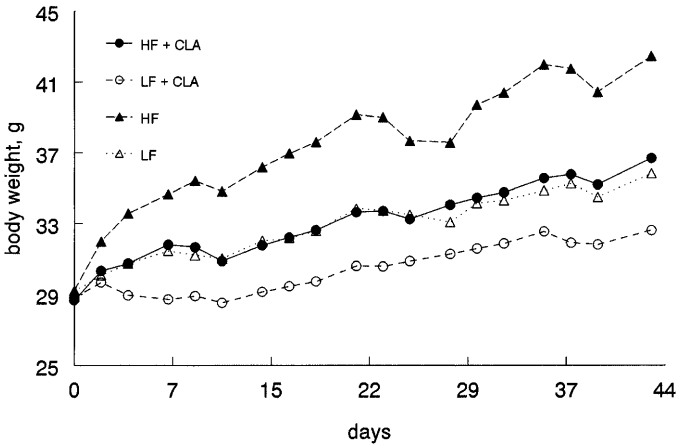

Fig. 1. Body weight curves for high-fat (HF) control (▲, $n = 10$), HF conjugated linoleic acid (CLA)-treated (●, $n = 10$), low-fat (LF) control (△, $n = 8$), and LF CLA-treated mice (○, $n = 10$). *Day 0* was the first day animals were placed on defined diets, and CLA treatment also began on *day 0* of experiment. CLA significantly suppressed body weight in both dietary groups (both $P < 0.001$).

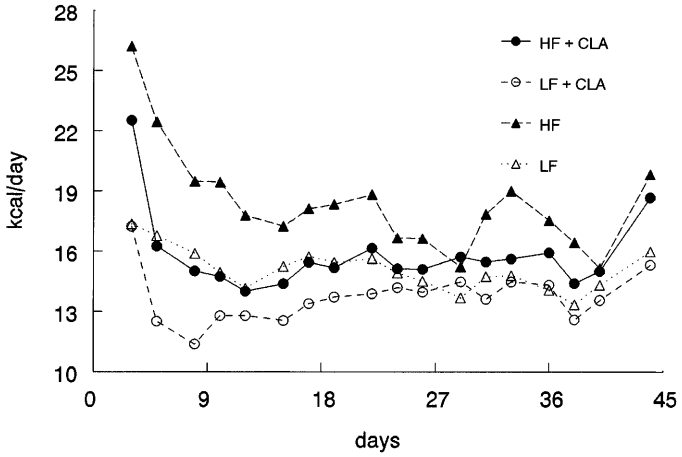

Fig. 2. Twenty-four-hour energy intake averaged over each preceding 2- to 3-day period during the 6 wk of study.

Overall, CLA reduced total cumulative energy intake in the high-fat diet group by ~14% and reduced cumulative energy intake in the low-fat diet group by ~9.6%.

Both diet and CLA treatment had significant effects on organ weights (Table 2). The high-fat diet was associated with significantly larger kidneys ($P < 0.001$), whereas diet had no effect on liver, testis, or spleen weight. However, this effect on kidney weight was eliminated when the data were adjusted for body weight. CLA significantly increased the weight of both the liver and the spleen independent of diet group and body weight ($P < 0.0001$ and $P < 0.014$ for liver and spleen, respectively).

Adipose depot weights were significantly affected by diet and CLA treatment (Fig. 4). The high-fat diet significantly increased body fat content. Relative to the control diet group, the high-fat diet increased inguinal, epididymal, retroperitoneal, and mesenteric depot weights by 87, 79, 93, and 85%, respectively ($P < 0.001$ for all comparisons). CLA treatment significantly reduced adipose depot weights relative to control diets for both diet groups ($P < 0.001$ for all comparisons). There

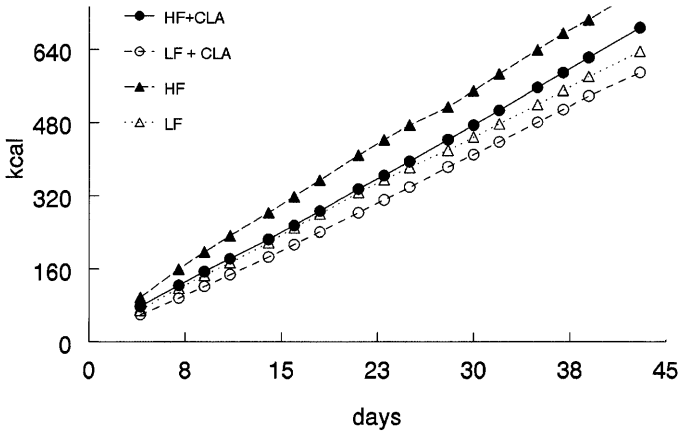

Fig. 3. Cumulative energy intake (kcal) for 6-wk feeding study is presented for same groups as described in Fig. 1. CLA and diet both had main effects on total cumulative intake (both $P < 0.0001$). CLA significantly reduced cumulative intake in both diet groups, and HF diet significantly increased total energy intake.

Table 2. *Organ weights*

	High Fat		Low Fat	
	Control	CLA	Control	CLA
Liver, g	1.658±0.025[a]	2.013±0.053[b]	1.591±0.030[a,c]	1.946±0.066[d]
Kidney, g	0.311±0.009[a]	0.312±0.006[a]	0.284±0.010[b]	0.279±0.006[b]
Testis, g	0.062±0.001	0.061±0.001	0.062±0.001	0.062±0.001
Spleen, g	0.063±0.002	0.068±0.002	0.060±0.002	0.067±0.003

Values are means ± SE. Kidney and testis values are average weight of left and right organs. Values in same row that do not share a superscripted letter are significantly different ($P < 0.001$). There was no main effect of diet except on kidney weight; a high-fat diet was associated with significantly larger kidneys. There was a main effect of CLA on liver ($P < 0.0001$) and spleen ($P < 0.014$) weight.

was no diet by CLA treatment interaction on adipose depot weights; CLA treatment reduced adipose depot weights by approximately the same percentage relative to same-diet controls in both the high-fat and low-fat diet groups. CLA treatment had the largest effect on the retroperitoneal adipose depot, reducing it by 78.2 and 87.7% in the high-fat and low-fat CLA treatment groups, respectively. In contrast, the effect of CLA was less marked on the epididymal adipose depot, in which depot weight was reduced by 43.3 and 61.1% in the high- and low-fat CLA groups, respectively.

The body composition data supported the data from adipose depot weights that CLA reduced body fat in both diet groups (Table 3). High-fat control animals had an average of 10.7 ± 0.7 g of carcass lipid, whereas high-fat CLA-treated animals had an average of 4.08 ± 0.27 g of fat ($P < 0.0001$). Similarly, low-fat control animals had an average of 6.28 ± 0.56 g of carcass lipid, whereas low-fat CLA-treated animals had 2.08 ± 0.11 g of fat ($P < 0.0001$). CLA-treated mice in both diet

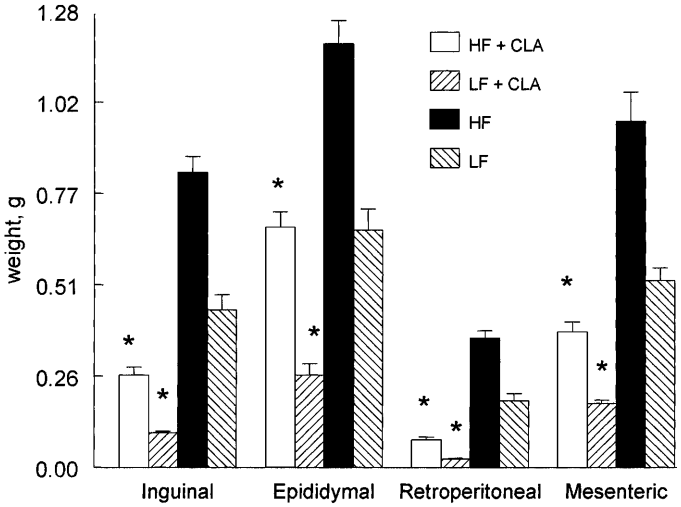

Fig. 4. Weights ± SE (in g) are depicted for inguinal, epididymal, retroperitoneal, and mesenteric adipose depots for HF control (n = 10), HF CLA-treated (n = 10), LF control (n = 8), and LF CLA-treated (n = 10) groups. For all depots except mesenteric depot, data are presented as average of left and right depot weights. CLA treatment significantly reduced adipose depot weight in both HF and LF diet groups. *Significant difference between control and CLA-treated within a diet group; all $P < 0.0001$. There was no diet by CLA treatment interaction; depot weights were reduced approximately the same amount by CLA in both diet groups.

Table 3. *Carcass composition*

	High Fat		Low Fat	
	Control	CLA	Control	CLA
Eviscerated carcass, g	34.10±0.89[a]	28.49±0.45[b]	28.48±0.77[b]	25.06±0.33[c]
Lipid, g	10.77±0.67[a]	4.08±0.26[b]	6.28±0.56[c]	2.08±0.11[d]
Protein, g	7.80±0.32[a]	6.19±0.11[b]	6.01±0.22[b]	5.46±0.07[c]
Ash, g	1.16±0.02[a]	1.07±0.04[a,b]	1.05±0.02[b,c]	0.96±0.05[c]
Water, g	15.93±0.19[a,e]	16.89±0.24[b]	15.41±0.26[a,c]	16.27±0.19[d,e]

Data are means ± SE. Values in same row not sharing a superscripted letter are significantly different ($P < 0.05$). There was an overall main effect of CLA on both lipid and protein content ($P < 0.001$).

groups had significantly less total carcass protein than same-diet control animals, whereas carcass ash was not affected by CLA treatment (Table 3). The high-fat diet produced significantly fatter animals with more carcass protein and ash compared with low-fat diet animals.

The energy expenditure data were adjusted for body weight differences using body weight as a covariate, and the data are presented in Table 4. During *week 6*, there was a difference in energy expenditure during the day and night when the diet groups were combined (Fig. 5*A*, $P < 0.0001$). This would be expected because mice are more physically active during the night. There was no diet effect on energy expenditure (Fig. 5*A*). This result is not consistent with the food intake data (Fig. 3), which showed that high-fat diet animals were still consuming more energy than the mice on the low-fat diet during *week 6*. However, during the actual period during which energy expenditure was measured (approximately *days 36-40*), the diet effects on energy intake were relatively small and may not have resulted in detectable differences in energy expenditure. There was a statistically significant increase in the total energy expenditure in CLA-treated animals when both diet groups were combined ($P < 0.0067$). This increased energy expenditure occurred despite a reduced total body mass and a marginally reduced energy intake in these animals compared with controls.

During *week 6*, there were significant effects of diet and light cycle on RQ (Table 4 and Fig. 5*B*). Combining CLA-treated and untreated groups, we found that the animals on the low-fat diet had a higher RQ compared with animals fed the high-fat diet. This would be

Table 4. *Energy expenditure and RQ*

	High Fat		Low Fat	
	Control	CLA	Control	CLA
Energy expenditure, kcal	9.98±0.55	11.58±0.31	10.98±0.39	12.32±0.49
RQ				
24-h	0.79±0.03[a]	0.80±0.03[a]	0.97±0.03[b]	0.90±0.03[b]
Day	0.78±0.03[a]	0.80±0.03[a]	0.94±0.03[b]	0.89±0.03[a,b]
Night	0.79±0.03[a]	0.80±0.03[a]	1.01±0.03[b]	0.91±0.03[b]

Data are means ± SE. RQ, respiratory quotient. Values in same row not sharing a superscripted letter are significantly different ($P < 0.05$).

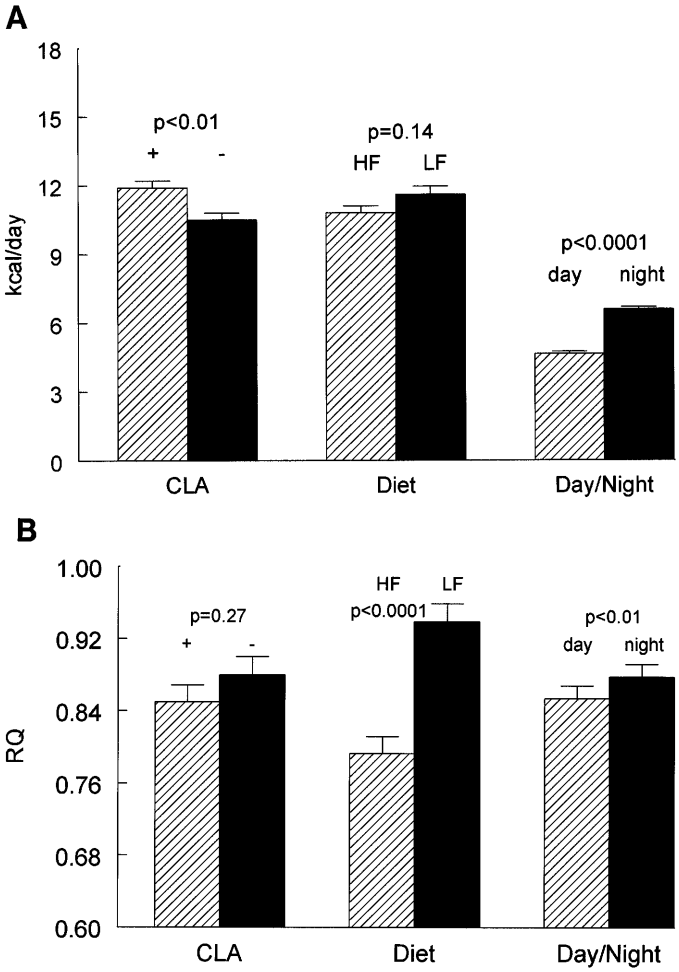

Fig. 5. *A*: energy expenditure ± SE (kcal/24 h) during *week 6* of study. Main effects of CLA treatment, diet, and day vs. night energy expenditure are shown. CLA significantly increased energy expenditure, and this was independent of diet ($P < 0.0067$). Diet had no significant effect on 24-h energy expenditure, whereas energy expenditure was significantly elevated during night. *B*: respiratory quotient (RQ) ± SE during *week 6* of study. Main effects of CLA treatment, diet, and diurnal rhythm are shown. CLA had no significant effect on RQ. HF diet significantly reduced RQ ($P < 0.0001$), whereas RQ was significantly reduced during day relative to night ($P < 0.0033$).

expected due to the higher carbohydrate content of the low-fat diet. Combining all groups and comparing day versus night RQ showed that the RQ was lower during the day (Fig. 5*B*). The lower daytime RQ is likely attributable to the mobilization of stored lipid during the day due to reduced feeding during the light hours. This effect was most apparent in the animals fed the low-fat diet. There was no overall effect of CLA on RQ measured over 24 h. However, CLA appeared to block the normal day-night difference in RQ (CLA by day-night interaction; $P = 0.06$). In animals not treated with CLA, the nighttime RQ was significantly higher than daytime RQ (night, 0.900 vs. day, 0.862; $P < 0.0077$), whereas in CLA-treated animals there was no significant day versus night RQ difference (night, 0.855 vs. day, 0.845; $P < 0.81$). This effect of CLA to block the normal diurnal RQ differences could be explained by

CLA promoting fat oxidation during the night (perhaps due to mobilization of stored lipid or impairment of incorporation of ingested lipid into adipocytes), resulting in a lowering of RQ to daytime levels. Most of this effect was observed in the animals fed the low-fat diet because the animals fed the high-fat diet already had significantly lower RQs, and therefore CLA had a minimal effect on the day versus night difference in RQ (see Table 4).

DISCUSSION

The observation that CLA fed as a dietary admixture reduces body fat content in mice is consistent with the reports of Pariza et al. (13) and Park et al. (14). We have now shown that CLA will reduce body fat content in the AKR/J mouse strain as well as in the ICN strain. We also report here that these effects are independent of dietary fat content; CLA reduced body fat content relative to appropriate control groups to approximately the same extent in animals fed a high-fat or low-fat diet. Of interest is the observation that different regional fat depots responded differentially to the effects of CLA. The retroperitoneal adipose depot weight was reduced the most by CLA treatment, whereas the epididymal depot was reduced in size to a lesser extent. This is consistent with significant regional differences in adipose tissue cell size, metabolism, and response to specific endocrine agents in the mouse (e.g., Ref. 15). However, the specific regional metabolic differences that explain the differential regional response to CLA treatment are unknown.

In a previous report, it was suggested that CLA actually results in a redistribution of carcass composition and both a decrease of body fat and increase of lean tissue (14). In this study, CLA reduced lipid but also reduced total carcass protein content while having no effect on carcass ash content. The discrepancy between these two studies in their effect on carcass protein is not clearly explainable. There were a number of differences in methodology between these two studies. Different concentrations of CLA were used (0.5 vs. ~1.0%), the animals were treated longer in this report (42 vs. 30 days), different mouse strains were studied (ICN vs. AKR/J), and different diets were used. However, it is not clear that any of these factors would be likely to modulate the reported effect of CLA to increase carcass protein content. This putative action of CLA to increase lean tissue mass certainly deserves further evaluation.

CLA significantly increased both liver weight and spleen weight. The increased liver weight associated with CLA treatment is likely due to liver lipid accumulation because this has been found in a variety of dietary manipulations, including rapid weight loss (1), as well as modifications of dietary protein and fat composition (e.g., Ref. 9). Similarly, because CLA has been reported to modulate immune function (12), perhaps through cytokines, it is also not surprising that spleen weight was affected. Although a metabolic and histological assessment of CLA effects on these organs should be completed to rule out CLA-induced pathogenic changes, such an assessment was outside the scope of the present study.

The reduced energy intake associated with CLA treatment was reported previously (14), and it was argued that the effects on carcass lipid content could not be explained by the observed reduction of food intake. This effect of CLA on energy intake is an important observation because it certainly explains some of the reduced carcass lipid content of CLA-treated animals in our study. However, we agree that this effect is unlikely to completely account for the reduction in carcass energy content.

CLA treatment increased energy expenditure significantly during the sixth week of treatment despite simultaneously causing a reduction of food intake and a loss of body lipid stores. Normally, a loss of body weight due to reduced energy intake is associated with a decreased metabolic rate (5). Therefore, the finding that CLA treatment was associated with an increased energy expenditure suggests that this effect was causally related to the loss of body fat. The mechanisms by which CLA could promote an increase of energy expenditure are numerous. It could be acting through stimulation of the autonomic nervous system, direct effects on metabolism in specific tissues or organs, or effects on energy substrate flux and availability. Little work has been done on the mechanisms by which CLA might affect energy metabolism. However, a recent report using isolated adipocytes suggests that CLA directly stimulates lipolysis and also decreases lipoprotein lipase (14). This could affect overall energy expenditure due to the increased energy needs for recycling of lipids or could shunt fatty acids into beta oxidation if storage was impaired. This latter explanation is supported by the increased activity of carnitine palmitoyltransferase in muscle of animals treated with CLA (14). Our finding that CLA blocks the normal diurnal differences in RQ is consistent with the conclusion that CLA is stimulating lipolysis during the night, providing more fatty acids for metabolism and thereby lowering the RQ.

It is clear that the reduced food intake associated with CLA treatment is not entirely responsible for the loss of body fat. There are a number of arguments that support this conclusion. First, the high-fat CLA-treated animals actually consumed more total energy over the 6-wk study than the low-fat control group (see Fig. 3), yet the low-fat control group has significantly larger adipose depots (Fig. 4) and more carcass lipid (Table 3). This suggests that energy intake alone was not the major factor in determining body lipid stores in this study and that CLA was having other effects on energy metabolism. Second, CLA reduced cumulative intake by 10 and 14% in the two diet groups, yet the adipose depot mass was reduced by 43–88%, depending on diet group and adipose depot examined. Usually with slight or moderate food restriction in rodents (up to a 15% reduction in calories), the effect on total body lipid stores is minimal due to adjustments in energy expenditure (e.g., Ref. 5). Third, as described, energy expenditure was actually stimulated by CLA despite a reduction of energy intake and a loss of body mass in the

CLA-treated animals. These factors together suggest that the reduced food intake alone was not responsible for the loss of body fat that occurred with CLA treatment.

The mechanisms by which CLA caused a reduction of food intake in the study reported here are unknown. It is possible that there were palatability or postingestive effects leading to malaise or aversion. Alternatively, the postingestive metabolic effects of CLA could have modulated appetite without having aversive effects on the animals. Additional studies would be necessary to determine the specific mechanisms for the observed effects on energy expenditure. However, in other work in our laboratory (not shown), a 1% CLA admixture in the same high-fat diet decreased body fat content while having no effect on energy intake. The concentration of CLA in the high-fat diet used in the study reported here was 1.2%. Therefore, a small reduction of CLA concentration eliminated the effects on energy intake but preserved the metabolic effects leading to a reduction in body fat in the mouse.

The reported observations that CLA reduced circulating cholesterol (11) and reduced tumor size and number (7, 8) could partly be attributed to the effects of CLA on energy intake and body fat. Supporting this conclusion are reports in rats that restriction of food intake and loss of body fat can have significant effects on lipoprotein metabolism and circulating cholesterol (4, 16). Similarly, because undernutrition reduces cancer risk in animal models (e.g., Ref. 10), the reported effects of CLA on cancer risk could be attributable to the reduced energy intake and body fat loss after CLA treatment. However, it is still not clear whether it is the reduction of energy intake or the loss of body fat content that is primarily responsible for the reduced cancer risk in these animal models.

It should be noted that the CLA used in these experiments was not pure and included a number of isomers in addition to the putative active form of the molecule (*cis*-9,*trans*-11 octadecadienoic acid). It is certainly possible that the different isomers have different metabolic effects. Further studies using pure reagents are clearly needed to fully understand the metabolic specificity of these different isomers of linoleic acid.

In conclusion, CLA at a concentration of 1–1.2% (wt/wt) in both low-fat and high-fat diets has profound metabolic effects in mice, resulting in an increased energy expenditure, a shift in the fuel mix burned, and a decrease of body fat content. This loss of body fat is only partially explained by the reduced energy intake associated with CLA treatment.

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