The Decrease in Body Fat in Mice Fed Conjugated Linoleic Acid Is Due to Increases in Energy Expenditure and Energy Loss in the Excreta[1]

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ABSTRACT We carried out energy balance studies in four groups of young, growing, 5-wk-old Balb-C mice (n = 12/group) that were either food restricted or nonrestricted and fed high fat diets (38 energy%) with or without 0.93 g/100 g conjugated linoleic acid (CLA) for 39 d. The energy in carcasses, excreta and food was measured in a bomb calorimeter. CLA lowered the percentage of the energy intake that was stored in the body from 1.9 ± 0.8 to −2.3 ± 0.7% (mean ± SD, $P < 0.05$) in the nonrestricted mice and from 1.4 ± 1.3 to −2.9 ± 0.7% ($P < 0.05$) in the restricted mice. Thus, the CLA-treated mice had a net loss of body energy. The percentage of the energy intake eliminated in the excreta increased from 7.6 ± 0.9% in controls to 8.7 ± 1.0% ($P < 0.05$) in the CLA-treated mice that were nonrestricted and from 7.3 ± 0.8 to 8.4 ± 0.6 ($P < 0.05$) in the restricted mice. The amount of energy ingested minus the amount retained in carcasses and excreta equals the energy expenditure. The percentage of the energy intake that was expended as heat increased from 90.5 ± 1.2 in controls to 93.6 ± 1.5% ($P < 0.05$) in the CLA-treated nonrestricted mice and from 91.3 ± 1.5 to 94.5 ± 1.0% ($P < 0.05$) in the restricted mice. The lower energy storage in the CLA-fed mice was accounted for by an increase in the energy expenditure (74%) and by an increase in energy lost in the excreta (26%). Feeding CLA also increased liver weight, which may warrant further studies on the safety of CLA. J. Nutr. 132: 940–945, 2002.

KEY WORDS: • *mice* • *conjugated linoleic acid (CLA)* • *energy balance* • *energy expenditure*

Conjugated linoleic acid (CLA) has attracted considerable interest because of its body fat–lowering properties and its potential to promote weight loss in humans and to produce leaner meat in livestock. Studies in mice have indicated that incorporation of ≤1% CLA in diets can substantially reduce the proportion of body fat (1–6). Similar results have been reported in studies with rats (7–9), chickens (7) and pigs (10,11). Some studies suggest that CLA also decreases body fat in humans (12–15), but these effects are much less striking than in mice (16). CLA not only lowers the amount of body fat but also appears to increase the lean body mass and the amount of protein in the body (9–12,14,17).

CLA occurs naturally in dairy and ruminant fats, and this source of CLA consists essentially of *cis*-9, *trans*-11 octadecadienoic acid (18–22). CLA is also produced commercially by isomerization of linoleic acid, which generates a mixture of several geometrical and positional CLA isomers, predominantly *trans*-10,*cis*-12 and *cis*-9, *trans*-11 octadecadienoic acids in a 1:1 ratio (23). Studies in hamsters (24,25) and mice (2) indicated that the *trans*-10,*cis*-12 CLA isomer is the active isomer in affecting lipid and energy metabolism.

Energy in food can be retained in the body, eliminated in the excreta or expended as heat. Other studies in mice have indicated that CLA reduces body fat and energy retention (1–6) and increases energy expenditure as measured in a metabolic chamber (3,4). There are, however, no data available that clearly show whether the increased energy expenditure accounts completely for the lower energy retention or whether changes in fecal excretion of energy take place. The objective of our study was to address whether CLA changes the energy lost in excreta. Therefore, we carried out energy balance studies in mice fed CLA to completely account for the food energy. The energy balance is represented by the following formula:

$$\text{Energy in food} = [1]\ \text{energy stored in body} + [2]\ \text{energy in excreta} + [3]\ \text{energy expended or lost as heat}$$

Some studies, although not all, indicate that dietary CLA lowers food intake (1,3). To exclude any nonspecific effect of CLA on food intake, we equalized intake of CLA and control diets by restricting food intake.

MATERIALS AND METHODS

The experimental protocol was approved by the animal experiments committee of the Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.

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Animals and diets. Young growing male mice ($n = 60$; 5 wk old; Balb-C/Utrecht) were obtained from the breeding facilities of Utrecht University (Central Laboratory Animal Institute, Utrecht, The Netherlands) and housed in a temperature-controlled (21°C) animal room with a 12-h light:dark cycle (lights on 0600–1800 h). On arrival, the mice were placed in individual polycarbonate cages with a wire-mesh bottom. A polyethylene pipe with a diameter of 5 cm and a length of 14 cm was added to the cages as environmental enrichment. The mice were fed a commercial rodent diet (Hope Farms, 3440 AB Woerden, The Netherlands) for 2 d and then the semipurified control diet (**Table 1**) for 8 d. Then, the mice were divided into 5 groups of 12, balanced for body weights. One group was killed to collect preexperimental values on body composition and energy; the remaining four groups were used for the 39-d feeding trial. Mice that had been spilling food during the 8-d preexperimental period of consuming the semipurified diets were allocated to the group that was killed at the beginning of the study. Thus, the experimental groups comprised only mice that did not spill any food.

We used high fat semipurified diets (Table 1) containing either 1.5 g/100 g of a conjugated linoleic acid preparation (Clarinol, donated by Loders Croklaan B.V., Hogeweg 1, 1521 AZ Wormerveer, The Netherlands) or 1.5 g/100 g hydrolyzed sunflower oil (control diet) that had been used to prepare the conjugated linoleic acid mixture (**Table 2**). Two groups of mice consumed the control or experimental diet ad libitum (nonrestricted) and two groups were food restricted. The restricted groups were provided ~80% of the amount of food that had been consumed during the pre-experimental period at the beginning of the study. When the food intake of the nonrestricted mice increased during the experimental period, we also increased the amount of food offered to the restricted mice as described below.

The air-dried semipurified diets containing the CLA and hydrolyzed sunflower preparations were stored at 4°C; every other day, one part of air-dried diet was mixed with one part of water in a KitchenAid kitchen machine (Model K5SS/PKM5, KitchenAid Europe, Brussels, Belgium). A dough-like mixture was obtained by using $KHCO_3$, which prevented the mice from spilling the food. The freshly prepared diets were fed to the mice in heavy glass containers that could not be tipped. In this way, food consumption could be measured accurately and cumulative food intake over the whole experimental period could be calculated. The restricted mice started with 12 g of wet food/2 d; this amount was later increased to 13 g. The restricted mice consumed 100% of the food that was provided every other day. None of the mice spilled any food. All excreta were collected throughout the 39-d experiment. The excreta comprised the feces together with the dried urine that was scraped from the bottom of the cages.

TABLE 1

Composition of semipurified diets[1]

Ingredient	*g/100 g*	*Metabolizable energy %*
Casein	20.00	16.97
Total Fat	20.00	38.18
Oil	18.51	
Corn oil	3.24	
Coconut oil	3.91	
Olive oil	5.68	
Palm oil	5.68	
CLA preparation[2]	1.49	
Total carbohydrates	50.72	43.03
Cornstarch	25.36	
Dextrose	25.36	
Cellulose	3.36	
$CaCO_3$	1.24	
$NaH_2PO_4 \cdot 2H_2O$	1.51	
$MgCO_3$	0.14	
KCl	0.11	
$KHCO_3$	0.72	
Mineral premix[3]	1.00	0.82
Vitamin premix[3]	1.20	1.00
Total	100.00	100.00

[1] The diets contained a calculated amount of 19.73 kJ metabolizable energy per gram and 38 energy% fat. The calculated polyunsaturated/monounsaturated/saturated (P/M/S) fatty acid ratio of the oil mixture was 16.8:41.3:41.9 and the calculated polyunsaturated/saturated fatty acid (P/S) ratio was 0.40 [data obtained from (26)].

[2] The control group was fed a diet containing 1.49% of the hydrolyzed sunflower oil that was used for the preparation of the CLA. The diets contained 228 mg trans-10, cis-12 octadecadienoic acid/1000 kJ metabolizable energy.

[3] Composition of the vitamin and mineral mixture has been described previously (27).

TABLE 2

Composition of the hydrolyzed sunflower oil and conjugated linoleic acid preparations

	Hydrolyzed sunflower oil[1]	Conjugated linoleic acid[1]
	g/100 g fatty acid methyl esters	
Free fatty acids	99.2	99.98
16:0 (Palmitic acid)	4.4	4.1
18:0 (Stearic acid)	1.3	1.4
18:1 (Oleic acid)	24.6	25.6
18:2 (Linoleic acid)	68.3	3
cis-9, trans-11 CLA		29.6
trans-10, cis-12 CLA		30.1
trans-9, trans-11 CLA and trans-10, trans-12 CLA		2.4
Total CLA		62.1
Oxidized CLA		0.2
Saturated fatty acids		6.3
Other	1.4	3.6
Total	100	100
Peroxide number (mEq O_2/kg)	4.7	0.4

[1] Data as provided by the manufacturer (Loders Croklaan, Hogeweg 1, 1521 AZ Wormerveer, The Netherlands).

Carcass analysis. At the end of the study, the mice were killed by cervical dislocation, the livers were removed and weighed and the carcasses were cut into pieces. Liver and carcasses were dried in a forced-hot air oven at 60°C for 3 d. The dried carcasses were weighed to calculate the percentage of water, homogenized in a coffee grinder and stored in air-tight glass containers. Excreta were dried, homogenized, and stored in the same way.

Total lipids in the dried, homogenized carcasses and feces were extracted according to the Official Methods of Analysis of the Association of Official Analytical Chemists. Dried material (~1 g) was added to a Majonnier flask; 2 mL of ethanol was added to wet the material. Subsequently, 10 mL of HCl (8 mol/L) was added, the contents were gently mixed and the flask was placed in a waterbath of 80°C for 30–40 min. The tubes were cooled, 10 mL of ethanol (96%) and 25 mL of diethyl ether were added and the tube was vigorously shaken for 1 min. Then, 25 mL of petroleum ether (40–60°C) was added and the tube was again vigorously shaken for another minute. The fat-containing upper layer was decanted into a 150-mL round bottom flask. The extraction procedure was repeated twice with 15 mL of diethyl ether and 15 mL of petroleum ether and the lipid extract was evaporated completely under nitrogen in a water bath at 40°C. The round bottom flask containing the lipids was dried overnight at 60°C and the total lipids were measured gravimetrically.

For the determination of the ash content, ~1 g of dried, homogenized carcass was added to a small porcelain container and put in an oven that was programmed as follows: 1 h at 200°C, 2 h at 300°C, 3 h at 400°C and 10 h at 500°C. The protein content of the dried carcasses was determined with the macro-Kjeldahl method.

Bomb calorimetry. The gross energy content in the dried, homogenized carcasses, excreta and diet was determined with a bomb calorimeter (IKA Calorimeter C4000 Adiabatic, IKA Analysetechnik, Heitersheim, Germany). The total amount of energy that was lost as heat (heat production or energy expenditure) was calculated by the formula:

$$\text{Energy in food} = \text{energy stored in body} + \text{energy in excreta} + \text{energy lost as heat}$$

Energy stored in the body was determined as total energy at the end of the 39-d feeding period minus the energy in the body at the beginning of the 39-d feeding period. Total body energy in the mice at the beginning of the experiment was calculated from a regression line describing the correlation between body weight and total body energy in the 12 mice that had been killed at the beginning of the study. The same procedure was used to calculate the water, protein, fat and ash retentions.

Statistical methods. The data were statistically analyzed with a two-way ANOVA with diet (control diet and CLA diet) and feeding regimen (nonrestricted and restricted) as independent variables. When ANOVA indicated a significant effect, the following groups were compared pairwise with correction for multiple comparisons (*t* test with the Bonferroni adaptation): *1)* control diet vs. CLA diet within each dietary regimen and *2)* nonrestricted and restricted within each diet; thus, each group was used for two comparisons, and therefore, the level of significance for these multiple comparisons was preset at $P < 0.025$ (=0.05/2). The SigmaStat statistical software package (Version 2.0, Jandel Corporation, San Rafael, CA) was used for all the statistical analyses.

RESULTS

Food and water consumption. The food intake of the nonrestricted CLA-fed mice was not different from that of the control mice (**Table 3**). Drinking water consumption during the 39-d experiment was greater in the CLA-fed mice, although the difference was not significant in the restricted mice ($P = 0.036$, *t* test with the Bonferroni adaptation). The greater water intake may have reflected a greater need for water because of increased heat production.

Body weights and composition. Feeding CLA significantly slowed the increase of body weight over the 39-d treatment period (Table 3). This effect was found for both feeding protocols, but it was significantly greater in restricted than in nonrestricted mice. The nonrestricted mice fed the CLA diet had 3.5% lower final body weights than controls, whereas the mice fed a restricted amount of the CLA diet had 9.8% lower body weights. CLA also significantly reduced the amount (Table 3, **Fig. 1**) and proportion (Fig. 1) of body fat. CLA lowered the proportion of body fat by 66% in the nonrestricted mice and by 63% in the restricted mice. Protein retention was greater than in controls in the nonrestricted mice fed CLA but was lower in the restricted mice fed CLA. Ash retention was significantly higher in the nonrestricted fed mice fed CLA but not in the restricted mice. CLA increased liver weights by 30% in the nonrestricted mice and by 49% in the restricted mice.

Energy balance. The percentage of energy in the food that was stored in the body decreased from 1.9% in the control to −2.3% in the CLA group when the mice were nonrestricted (Table 3). This percentage decreased from 1.4% in the restricted control group to −2.9% in the restricted CLA group. The negative values for the CLA-fed mice indicate that no energy was stored but that a loss of body energy occurred.

The percentage of energy in the food that was lost in the excreta increased from 7.6% in the control group to 8.7% in the CLA group when the mice were nonrestricted. This percentage had increased from 7.3% in the restricted control group to 8.4% in the restricted CLA group. The apparent gross energy digestibility was decreased by CLA in both feeding groups. The apparent fat digestibility was also significantly lower than controls in the restricted mice fed CLA but not in the nonrestricted mice. The apparent digestibility is the percentage of energy or fat that is apparently absorbed from the food and was calculated from the difference between energy or fat in the diet and energy or fat in the excreta; fat and energy in the excreta can also have an endogenous origin. Further, we calculated that 27% of the increase in fecal energy excretion was accounted for by an increase in fecal fat excretion in the nonrestricted mice and 36% of the increase in the restricted mice.

The percentage of energy in the food that was expended as heat increased from 90.5% in the control group to 93.6% in the CLA group in the nonrestricted mice. This percentage increased from 91.3% in the control group to 94.5% in the CLA group in the restricted mice.

Thus, the control mice had a positive energy balance, i.e., their body energy increased. The CLA-fed mice, on the other hand, were in negative energy balance, i.e., they lost body energy. We calculated that this lower energy storage in the CLA-fed mice was accounted for by an increase in energy expenditure (74%) and by an increase in energy lost in the excreta (26%). This was true for both the nonrestricted and food-restricted mice. Thus, the increased energy expenditure in the mice fed CLA did not completely account for their lower energy retention.

DISCUSSION

Our study confirms the results of other studies in mice showing that CLA lowers body fat and energy retention (1–6) and increases energy expenditure (3,4). In addition, we carried out a complete energy balance study over a period of 39 d and also examined possible effects of CLA on energy losses in the excreta. The results of our study indicated that 74% of the lower energy retention in the CLA-fed mice was accounted for by an increase in energy expenditure and 26% by an increase of energy loss in the excreta. West et al. (4) concluded that the decrease in body fat and energy in CLA-treated mice likely was the result of an increase in energy expenditure. Their conclusion, however, was based on an estimate of the increase in energy expenditure due to feeding CLA and an estimate of the loss of body energy. In our study, these energy balance variables were actually measured.

We found that feeding CLA also increased the excretion of energy in the feces and significantly lowered the apparent gross energy digestibility. The apparent fat digestibility in the restricted mice fed CLA was also significantly lower. We estimated that 27% of the increase in fecal energy excretion was accounted for by an increase in fecal fat excretion in the nonrestricted mice and 36% of the increase in the restricted mice. Thus, the increase in energy excretion in the feces in the CLA-fed mice was only partly attributable to an increase in fecal fat excretion. These calculations suggest that the absorption of energy from components other than fat, such as proteins or carbohydrates, was also affected by CLA.

Our studies do not give any information on the mechanism of the increased energy excretion in the mice fed CLA and how CLA may affect the absorption of dietary fat and other dietary components such as proteins or carbohydrates. Sugano et al. (29) reported that the apparent lymphatic recovery of CLA in lymph-cannulated rats was considerably lower than the recovery of linoleic acid (55 vs. 80%). This is consistent with a lower apparent fat absorption in the mice fed CLA diets

TABLE 3

Body composition and energy balance in food-restricted and non-restricted mice fed semipurified diets containing either a CLA preparation or hydrolyzed sunflower oil (control) for 39 d[1]

	Nonrestricted		Restricted		Two-way ANOVA[2]
	Control	CLA	Control	CLA	
Total drinking water intake, *mL*	53.5 ± 8.0	70.1 ± 16.6*	60.5 ± 11.8	74.7 ± 18.6	D
Total food intake,[3] *g*	138.1 ± 7.3	135.0 ± 7.1	124.4	124.4	
Initial body weight, *g*	23.3 ± 1.5	23.3 ± 1.9	22.8 ± 1.6	23.1 ± 1.7	
Final body weight, *g*	27.7 ± 1.4	26.7 ± 1.4*	27.1 ± 2.6	24.4 ± 1.1*†	D, R, D × R
Liver weight, *g*	1.56 ± 0.12	2.03 ± 0.23*	1.52 ± 0.21	2.26 ± 0.33*	D
Liver weight, *g/100 g body*	5.6 ± 0.3	7.6 ± 0.8*	5.6 ± 0.6	9.3 ± 1.3*†	D, R, D × R
Apparent fat digestibility, %	96.6 ± 0.7	96.4 ± 1.1	97.5 ± 0.4	96.7 ± 0.5*	D
Apparent gross energy digestibility, %	92.3 ± 0.9	91.3 ± 1.0*	92.7 ± 0.8	91.2 ± 0.6*	D
Body composition, *g*					
Fat	4.19 ± 0.63	1.38 ± 0.18*	3.91 ± 0.90	1.27 ± 0.07*	D
Water	16.81 ± 0.71	18.41 ± 0.90*	16.76 ± 1.50	16.67 ± 0.75†	D, R, D × R
Protein	5.32 ± 0.27	5.49 ± 0.30	5.13 ± 0.44	4.93 ± 0.27†	R
Ash	0.94 ± 0.05	0.98 ± 0.04	0.92 ± 0.07	0.94 ± 0.06	
Recovery, (%)	98.50 ± 0.43	98.38 ± 0.46	98.71 ± 0.62	97.64 ± 0.50	
Change in body composition, *g/39 d*					
Body weight gain	4.42 ± 0.86	3.41 ± 0.99*	4.30 ± 1.49	1.33 ± 1.12*†	D, R, D × R
Fat retention	0.68 ± 0.53	−2.18 ± 0.38*	0.50 ± 0.73	−2.22 ± 0.38*	D
Water retention	2.78 ± 0.53	4.42 ± 0.56*	2.94 ± 1.01	2.88 ± 0.65*†	D, R, D × R
Protein retention	0.81 ± 0.20	0.99 ± 0.22*	0.72 ± 0.18	0.51 ± 0.21*	R, D × R
Ash retention	0.15 ± 0.03	0.19 ± 0.05*	0.15 ± 0.04	0.16 ± 0.04	D
Energy balance, *kJ*					
Intake	2779 ± 147	2717 ± 142	2502.79	2502.79	
Storage	47 ± 21	−61 ± 18*	34 ± 31	−71 ± 18*	D, R
Expenditure	2519 ± 121	2542 ± 126	2286 ± 38†	2364.39 ± 23.73*†	R
In excreta	214 ± 33	237 ± 32	182 ± 19†	210 ± 15*†	D, R, D × R
In excreta as fat[4]	35 ± 8	40 ± 14	25 ± 4†	34 ± 5*	D
Energy in whole body, *kJ*					
Measured[5]	293 ± 29	185 ± 13*	273 ± 43	172 ± 9*†	D, R
Calculated[6]	293 ± 29	185 ± 12*	277 ± 45	168 ± 8*†	D, R
% of energy intake					
Stored in the body	1.9 ± 0.8	−2.3 ± 0.7*	1.4 ± 1.3	−2.9 ± 0.7*	D
Expended as heat	90.5 ± 1.2	93.6 ± 1.5*	91.3 ± 1.5	94.5 ± 1.0*	D, R
Lost in excreta	7.6 ± 0.9	8.7 ± 1.0*	7.3 ± 0.8	8.4 ± 0.6*	D
Lost in excreta as fat	1.2 ± 0.3	1.5 ± 0.5	1.0 ± 0.2†	1.4 ± 0.2*	D

[1] Values are means ± SD, $n = 12$.

[2] The data were analyzed with a two-way (diet and feeding regimen as factors) (ANOVA) and the significance level was preset at $P < 0.05$. Subsequently, multiple comparisons were made with *t* tests, and the level of significance was preset at $P < 0.025$ according to the Bonferroni adaptation. Abbreviations: D, diet effect; R, feeding regimen effect; D × R, interaction between diet and feeding regimen. * Significant effect of diet within feeding regimen; † significant effect of feeding regimen within diet.

[3] Air-dried food intake.

[4] Energy in fecal fat was calculated on the basis of the amount of measured fat in the feces, given 1 g of fat has a gross energy of 39.8 kJ (28).

[5] Measured with a bomb calorimeter.

[6] Calculated on basis of the body composition, given 1 g of animal fat has a gross energy of 39.8 kJ and 1 g of protein has a gross energy of 23.7 kJ (28).

in our studies. It is not clear, however, how CLA affects the absorption of energy from other dietary components such as proteins or carbohydrates.

We collected the feces and the dried urine together because it was not feasible to collect them separately. It is possible that urea in the urine had been decomposed by urease activity and that theNH_3 had evaporated. As a consequence, energy in the excreta in the form of urea may have been lost and the amount of energy in the feces may have been underestimated. Because energy expenditure was calculated as the difference between the energy in food and the energy in the feces and retained in the body, energy expenditure may have been somewhat overestimated. We calculated that energy expenditure would have been overestimated by ~5% if all of the energy excreted in the form of urea had been lost. Further, we estimated that 74% of the lower energy storage in the CLA-fed mice was due to an increase in the energy expenditure and 26% due to an increase in energy lost in the excreta. However, a loss of energy excreted in the form of urea would mean that the energy expenditure would in fact be lower, and the excretion of energy in the excreta higher than our values. We calculated that 71% (instead of 74%) of the lower energy storage in the CLA-fed mice would be due to an increase in energy expenditure and 29% (instead of 26%) due to an increase of energy loss in the excreta if all of the energy excreted in the form of urea had indeed been lost. It seems reasonable, however, to assume that the proportion of energy lost by the decomposition of urea was the same in the control and the CLA-fed mice so that the energy balance results likely were similarly affected in all dietary groups.

We calculated that CLA increased energy expenditure by 3.3%. West et al. (3) also measured energy expenditure of mice fed high fat control and CLA diets. After 42 d of CLA feeding, they placed mice in a metabolic chamber for a 24-h

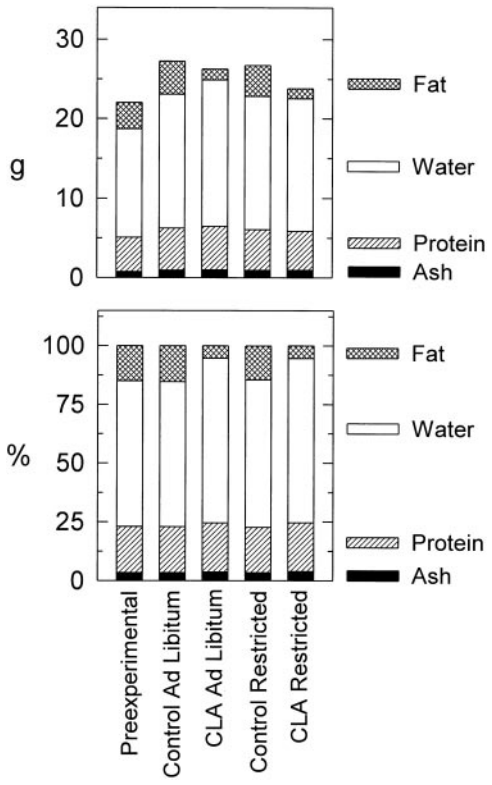

FIGURE 1 Absolute (*upper panel*) and relative (*lower panel*) body compositions of mice that consumed ad libitum or a restricted amount (~80% of the food intake of the nonrestricted mice) of semipurified diets containing conjugated linoleic acid (CLA) or hydrolyzed sunflower oil (control) for 39 d. The body composition of the mice that were killed at the beginning of the experiment (preexperimental group) is also given. Values are means, $n = 12$. The results of the statistical analyses of the data in the *upper panel* are given in Table 3. *Lower panel*: there was a significant ($P < 0.05$) effect of conjugated linoleic acid on all variables. Subsequent pairwise comparison (*t* test with the Bonferroni adaptation) of the CLA and control groups within each dietary regimen (nonrestricted and restricted feeding regimen) also indicated that there was a significant effect of conjugated linoleic acid ($P < 0.025$) on all the body composition variables.

period and found a significant 16% increase in energy expenditure. In another study, West et al. (4) fed CLA to mice for 5 wk. Energy expenditure was measured every week in a metabolic chamber for 24 h and they observed a significant 7.7% average increase in energy expenditure. These 16 and 7.7% increases in energy expenditure are substantially higher than the 3.3% increase in our study. The dose of the *trans*-10,*cis*-12 CLA isomer that is responsible for the body fat–lowering properties (2) was similar, i.e., 228 mg/1000 kJ metabolizable energy in our study and ~235 mg/1000 kJ in the studies of West et al. (3,4). We have no clear explanation for these differences but the methods used may play a role. We measured energy expenditure over the entire 39-d period with an energy balance study, whereas West et al. (3,4) measured energy expenditure for 24-h periods in a metabolic chamber. Another possible explanation may be the difference in strain of mice used. We used Balb-C mice, whereas West et al. (3,4) used AKR/J mice.

Studies of Tsuboyama-Kasaoka et al. (6) suggested that the body fat–lowering effect of CLA in mice was due mainly to apoptosis and resulted in a situation resembling lipoatrophic diabetes, i.e., ablation of brown adipose tissue, a marked reduction of white adipose tissue, hepatomegaly (250% increase) and a considerable increase in plasma insulin levels. Further, plasma levels of the cytokine tumor necrosis factor-α (TNF-α) were increased and those of the cytokine leptin were decreased. Infusion of leptin in CLA-fed mice reversed hyperinsulinemia and fat accumulation in the liver (6). Other studies have also reported an increase in insulin levels (4,5) and a decrease in plasma leptin (5) in mice fed CLA. As discussed by Tsuboyama-Kasaoka et al. (6), there are studies that suggest that TNF-α can induce apoptosis and be a mediator of non-insulin-dependent diabetes mellitus. We also found in our study a considerable increase in liver weight (30 and 50%); similar results were reported by West et al. (3) and Delany et al. (5) in mice (20–30% increase) and by deDeckere et al. (24) in hamsters (25% increase). Moreover, CLA increases total lipids per gram liver in mice (30). Because of these adverse side effects of CLA in animal models, it is important that safety studies be done in animal models and humans before CLA is used routinely for weight and fat reduction in humans.

In conclusion, our study confirms that CLA lowers body fat and increases energy expenditure. In addition, we found that the decrease in energy retention could not be explained completely by increased energy expenditure but that CLA also increases energy loss in the excreta.

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