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Abstract

HARGRAVE, KIMBERLY M., MICHAEL J. AZAIN, STEPHEN D. KACHMAN, AND JESS L. MINER. Conjugated linoleic acid does not improve insulin tolerance in mice. *Obes Res.* 2003;11:1104–1115.

Objective: To determine if the addition or removal of dietary conjugated linoleic acid (CLA) would alter insulin tolerances in mice from two genetic lines.

Research Methods and Procedures: High metabolic rate (MH) and low metabolic rate (ML) mice were assigned to consume 1) a control diet ad libitum, 2) a control diet at a restricted intake, or 3) a diet containing 1% CLA ad libitum. After 9 weeks, an insulin tolerance test was conducted, and a portion of the mice were killed. All remaining mice consumed the control diet ad libitum. Insulin tolerance tests were conducted 11 and 32 days after the diet change, and mice were killed 3 days after each test. Body fatness, fat pad weights, and serum insulin concentrations of mice were determined at each time-point. Two follow-up experiments were also conducted.

Results: Restricted mice had insulin sensitivities not different than control mice. CLA-fed MH mice in experiment 1 were resistant ($p < 0.001$) to insulin on each day measured. CLA-fed ML mice were slightly resistant ($p = 0.08$) to exogenous insulin on day 0 of recovery and not different from control mice on day 11 or 32. Glucose response to insulin in MH mice fed CLA in experiments 2 or 3 did not differ from control mice.

Discussion: Mice fed CLA did not have improved insulin tolerances compared with control mice. In some cases, dietary CLA may cause insulin resistance. MH mice seem more sensitive to CLA than ML mice.

Key words: mice, insulin tolerance, body fat, conjugated linoleic acid

Introduction

Conjugated linoleic acid (CLA)[1] refers to linoleic acid isomers with conjugated double bonds. Some are formed naturally through biohydrogenation of linoleic acid by microorganisms in the stomach of ruminant animals. CLA is therefore found in the fat associated with the meat and milk of such animals. Dietary CLA may benefit human health because of anticancer (1–3), antiobesity (4,5), anti-atherosclerosis (6,7), and antidiabetes effects (8,9).

CLA is a ligand for the peroxisome proliferator-activated receptors (PPARs) (10,11). PPAR subtypes α and γ are transcription factors that regulate genes involved in glucose and lipid metabolism. CLA's proposed antidiabetic effect has been attributed to activation of PPARγ. Zucker diabetic fatty (ZDF) *fa/fa* rats fed CLA exhibited improved insulin action as measured by a smaller increase in blood glucose after administration of exogenous glucose (8). Dietary CLA also reduced plasma insulin, free fatty acid, and glucose concentrations, and increased PPARγ activity and *aP2* (a PPARγ-responsive gene) mRNA abundance. Similar effects were observed in rats fed the synthetic PPARγ ligand, troglitazone, a known insulin-sensitizing drug (8). Ryder et al. (9) confirmed the above results, again in ZDF rats, and implied that the *trans*-10,*cis*-12 CLA isomer is the isomer responsible for the apparent improved insulin sensitivity. However, these antidiabetic effects have not been demonstrated in other species or other rat strains. In contrast, consumption of CLA by mice increased plasma insulin concentrations and impaired insulin tolerance (12,13). Dietary CLA did not influence basal insulin concentrations or glucose use in lactating dairy cows (14) or OLETF rats (15), and the *trans*-10,*cis*-12 isomer reduced glucose incorpora-

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[1] Nonstandard abbreviations: CLA, conjugated linoleic acid; PPAR, peroxisome proliferator-activated receptor; ZDF, Zucker diabetic fatty; MH, high metabolic rate; ML, low metabolic rate.

Table 1. Fatty acid profile of dietary fat sources

Fatty acid*	Soy oil	CLA
C12:0	0.04	0.00
C14:0	0.11	0.08
C16:0	10.79	6.42
C16:1	0.12	0.06
C18:0	3.30	4.42
C18:1	21.59	24.01
C18:2	56.05	0.29
CLA *cis*-9, *trans*-11	0.00	27.83
CLA *trans*-10, *cis*-12	0.00	26.93
CLA other isomers	0.00	6.92
C18:3	8.01	0.00

* Fatty acid concentrations reported as percent of total fatty acids.

tion into total lipid in cultured human preadipocytes (16). Furthermore, in humans, supplementation with a mixture of CLA isomers has not altered plasma glucose or insulin concentrations (17) in abdominally obese men. The *trans*-10,*cis*-12 isomer, however, has reduced insulin sensitivity in nondiabetic men at risk for cardiovascular disease (18). Given these reports, the potential antidiabetic effects of dietary CLA are questionable.

Our hypotheses were that, although insulin tolerance would not be improved by CLA consumption, per se, the reduction in body fat caused by CLA would enhance insulin tolerance once CLA was removed from the diet.

Research Methods and Procedures

Mouse Lines and Diets

We used mouse lines that were developed by divergent genetic selection on metabolic rate: high metabolic rate (MH) and low metabolic rate (ML) mice. MH1, MH2, and MH3 exhibit basal energy expenditure that is 50% greater than that of ML1, ML2, and ML3 (19). MH mice typically consume more feed and are leaner than the ML mice but do not differ in body weight (20,21). MH or ML mice do not exhibit diabetic tendencies, nor do the lines differ in blood glucose or plasma insulin levels (unpublished observations). Dietary CLA causes a similar body fat loss in both MH and ML mice (22,23).

Mice were fed a purified base diet (AIN-93G) containing (g/kg) isolated soy protein 200, cornstarch 395.42, dextronized cornstarch 132, sucrose 100, cellulose 50, soy oil 70, AIN-93G mineral mix 35, AIN-93G vitamin mix 10, L-cystine 2.54, L-methionine 2.54, and choline bitartrate 2.5 (Dyets, Inc., Bethlehem, PA). Soy protein was used instead of casein to preclude presence of CLA in the base diet. The CLA mixture contained approximately equal concentrations each of the *cis*-9,*trans*-11 and the *trans*-10,*cis*-12 isomers and was provided by BASF (Offenbach a.d. Queich, Germany). CLA was included in the diets to provide 1% of the diet as CLA isomers and replace soy oil (w/w). The fatty acid profiles of the oil sources (soy and CLA) are provided in Table 1.

Animal Protocol

Experimental procedures were approved by the University of Nebraska Institutional Animal Care and Use Committee. Mice were housed either three per cage (experiment 1, weeks 1 to 6) or individually (experiment 1, weeks 7 to

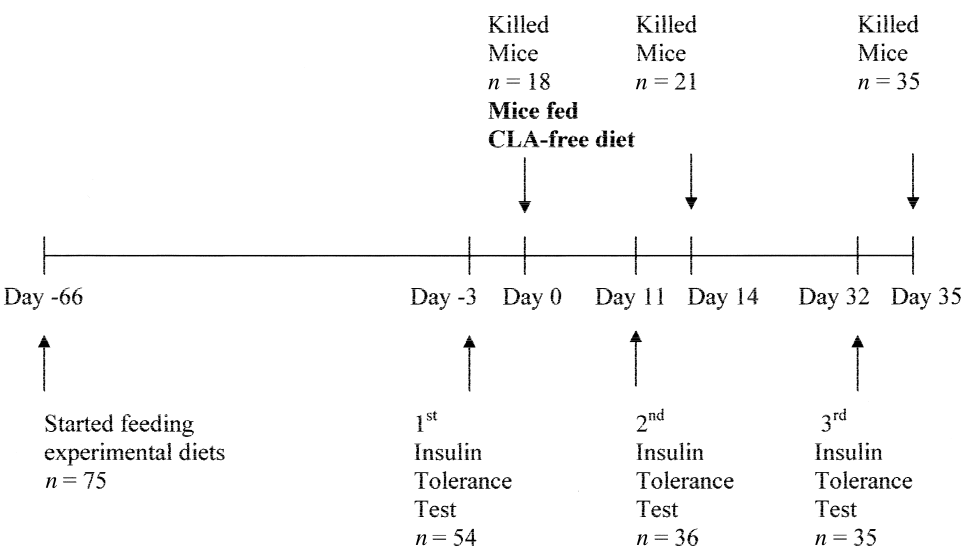

Figure 1: Timeline of events in animal procedure, experiment 1. From day 0 onward, all mice consumed the basal diet ad libitum.

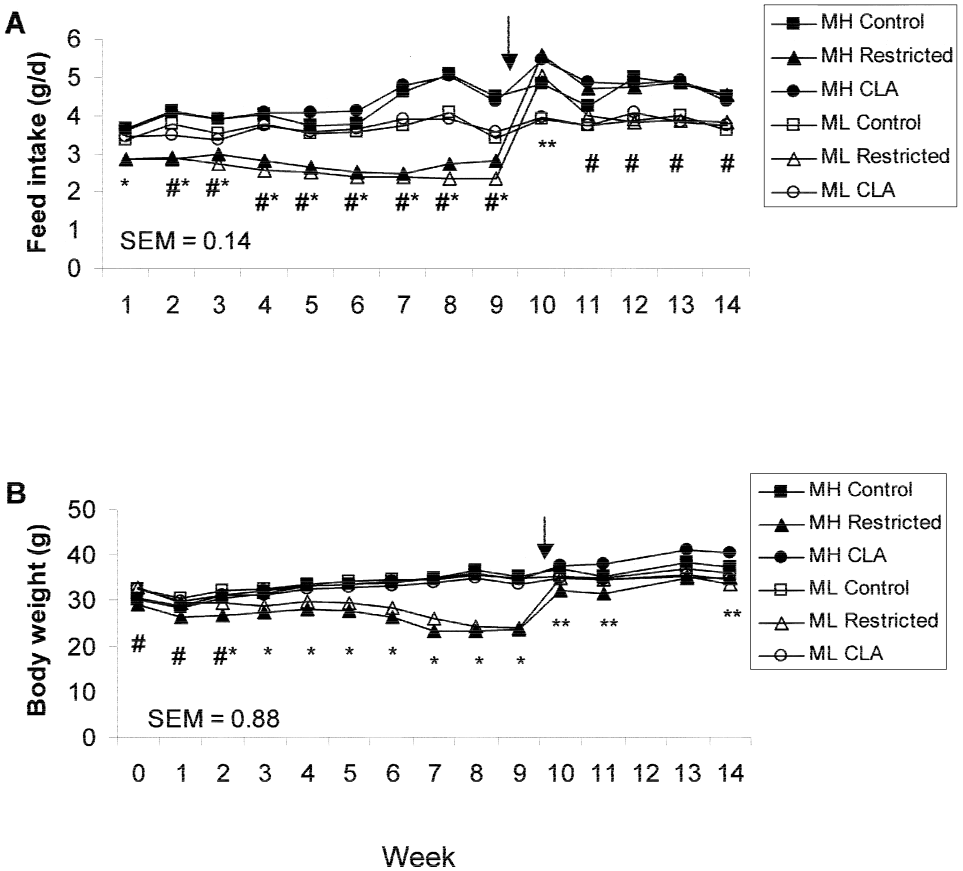

Figure 2: Effect of diet on feed intake and body weight, experiment 1. MH, MH1 (mouse line selected for high metabolic rate); ML, ML1 (mouse line selected for low metabolic rate); control, 7% soy oil diet; restricted, control diet at 65% to 70% intake of control mice; CLA, 6% soy oil + 1% CLA diet. Arrow indicates switch to all mice consuming control diet ad libitum. The experiment started with $n = 10$ to 14 mice per line per diet. (A) Feed intake, grams per day. #Main effect of line, $p < 0.05$. *Main effect of diet, $p < 0.05$. **Line × diet interaction, $p < 0.05$. (B) Body weight, grams. #Main effect of line, $p < 0.05$. *Main effect of diet, $p < 0.05$. **Line × diet interaction, $p < 0.05$.

14; experiment 2; and experiment 3) at 22 °C, under a photoperiod of 12 hours light, 12 hours dark.

Experiment 1. See Figure 1 for the timeline of events in this experiment. Seventy-five male mice (33 MH1, 42 ML1; 9 weeks of age) were blocked by litter and randomly assigned to three experimental treatments: control, base diet offered ad libitum; restricted, base diet offered at 65% to 70% of the intake of the control mice; CLA, base diet + 1% CLA isomers offered ad libitum. The restricted group was targeted to attain body fatness equal to the CLA group. This would allow, during the recovery phase, an insulin tolerance comparison between mice previously fed CLA and not fed CLA but at similar body fatness.

Feed intake and body weight were measured weekly. Feed intake was measured as disappearance from glass jars equipped with a cylindrical stainless steel screen attached to a small-holed lid. No feed spillage was observed. After 9 weeks, 54 mice underwent an insulin tolerance test. Three days later, 18 of them were killed; blood glucose was measured, and serum, epididymal fat pads, and livers were collected. A body fat index was calculated: body fat index = [(total collected fat pad weight + total ether extract of the remaining carcass)/total body weight] × 100. All remaining mice were offered the basal diet ad libitum from this time forward. Eleven days after the diet change, 36 mice underwent a second insulin tolerance test, and 3 days later, 21 mice were killed, and tissues were collected as before. Thirty-two days after the diet change, the remaining mice underwent a third insulin tolerance test and were killed 3 days later. Tissues from these mice were also collected as described above.

Experiment 2. Because the response to CLA in experiment 1 differed between MH1 and ML1, we again tested the effect of dietary CLA on insulin tolerance in experiment 2. In this experiment, we used a different line of MH mice, MH3. Twenty-four male MH3 mice (12 weeks of age) were blocked by weight and randomly assigned to treatment: control, base diet offered ad libitum; CLA, base diet + 1%

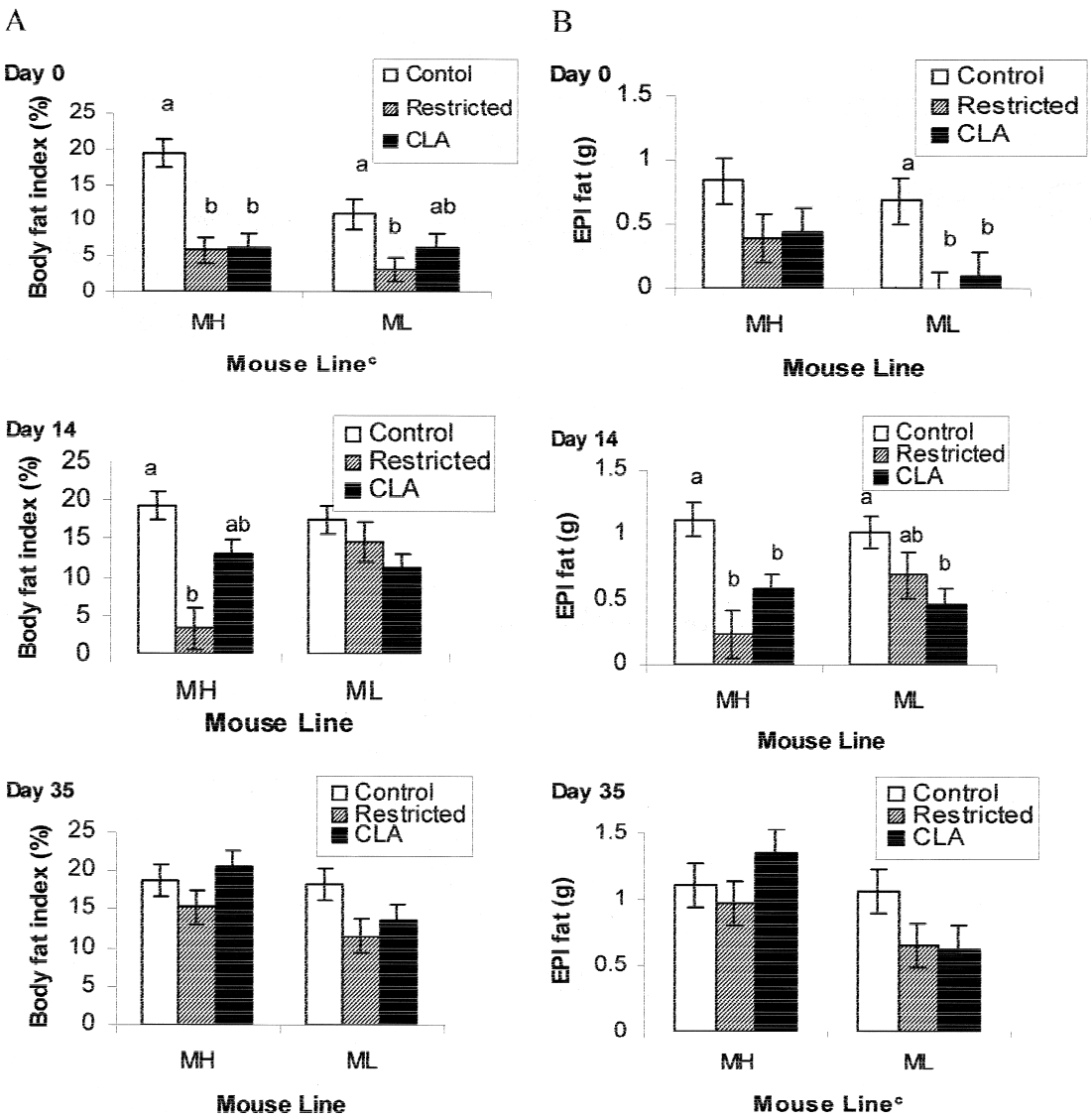

Figure 3: Effect of diet on body fatness, as measured by body fat index (A) and epididymal (EPI) fat pad weight (B) of mice fed control or CLA-containing diets for 9 weeks, followed by a control diet for 0, 14, and 35 days, experiment 1. Body fat index is calculated as total ether extract + fat pad weights, divided by body weight, multiplied by 100. MH, MH1 (mouse line selected for high metabolic rate); ML, ML1 (mouse line selected for low metabolic rate); control, 7% soy oil diet; restricted, control diet at 65% to 70% intake of control mice; CLA, 6% soy oil + 1% CLA diet. Day 0, $n = 3$ mice per line per diet; day 14, $n = 1$ to 5 mice per line per diet; day 35, $n = 5$ to 6 mice per line per diet. Error bars represent SEM. [ab]Different letters represent differences within line, $p < 0.05$. [c]Main effect of line, $p < 0.05$.

CLA isomers offered ad libitum. Feed intake and body weight were measured weekly, and after 6 weeks, the mice underwent an insulin tolerance test. All mice were then offered the control diet ad libitum for 24 days, and a second insulin tolerance test was conducted. No tissues were collected from mice in this experiment.

Experiment 3. Twenty-four male MH1 mice (12 weeks of age) were blocked by weight and randomly assigned to treatment: control, base diet offered ad libitum; CLA, base diet + 1% CLA isomers offered ad libitum. Feed intake and body weight were measured weekly, and after 6 weeks, the mice underwent an insulin tolerance test. The following day, the mice were killed, and the retroperitoneal and epididymal fat pads were weighed and collected.

Insulin Tolerance Tests

Mice were fasted for 3 hours and then bled from the tail tip to obtain a preinjection glucose concentration. Glucose was measured immediately using the SureStep Glucose Monitor (Lifescan, Milpitas, CA). Insulin (0.5 mU/kg body weight diluted in 0.9% saline) was injected intraperitoneally, subsequent blood samples were taken from the tail tip,

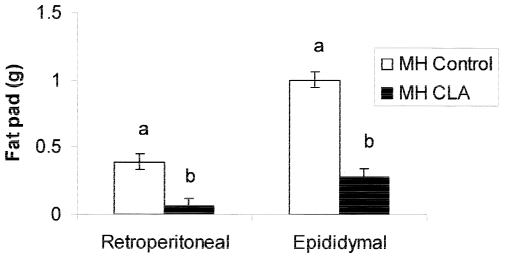

Figure 4: Effect of diet on fat pad weight of mice fed control or CLA-containing diets for 6 weeks, experiment 3. MH, MH1 (mouse line selected for high metabolic rate); control, 7% soy oil diet; CLA, 6% soy oil + 1% CLA diet. $N = 12$ mice per diet. Error bars represent SEM. [ab]Different letters within fat pad represent differences, $p < 0.001$.

and glucose was measured at 30, 60, 90, and 120 minutes postinjection in experiment 1. Experience gained in experiment 1 indicated that it would be possible to collect blood at closer intervals; therefore, in experiments 2 and 3, blood was collected at 10, 20, 30, 60, and 120 minutes after injection. Preinjection glucose concentration and the change in glucose from preinjection to 30 minutes postinjection were subjected to tests of treatment effect as described below.

Fatty Acid Analysis

Total fatty acids from epididymal fat pads and livers from mice in experiment 1 were extracted and methylated according to the Park and Goins method (24), and fatty acid methyl esters were analyzed by gas chromatography as previously reported (25).

Basal Insulin Analysis

Insulin was measured in serum samples from mice in experiment 1 using the Linco Rat Insulin Radioimmunoassay and Sensitive Rat Insulin RIA Kits (Linco Research, Inc., St. Charles, MO).

Data Analyses

Data were analyzed by day using a mixed model with line, diet, and line × diet as fixed effects and litter nested within line as a random effect. *F* tests, least-squares means, and SEMs were calculated using the mixed procedure of SAS (SAS Institute Inc., Cary, NC).

Results

Feed Intake and Body Weight

As expected, ML1 mice consumed less feed ad libitum than did MH1 mice ($p < 0.05$; Figure 2A). There was no effect of CLA on feed intake in experiment 1. However, feed intake was reduced ($p < 0.05$) in CLA-fed mice at week 1 (3.87 vs. 3.54 g/d) in experiment 2 and at weeks 5 and 6 (4.04 vs. 3.71 and 4.13 vs. 3.68 g/d, respectively) in experiment 3 (data not shown). The only dietary effect on body weight was that feed restriction reduced body weight ($p < 0.05$; Figure 2B).

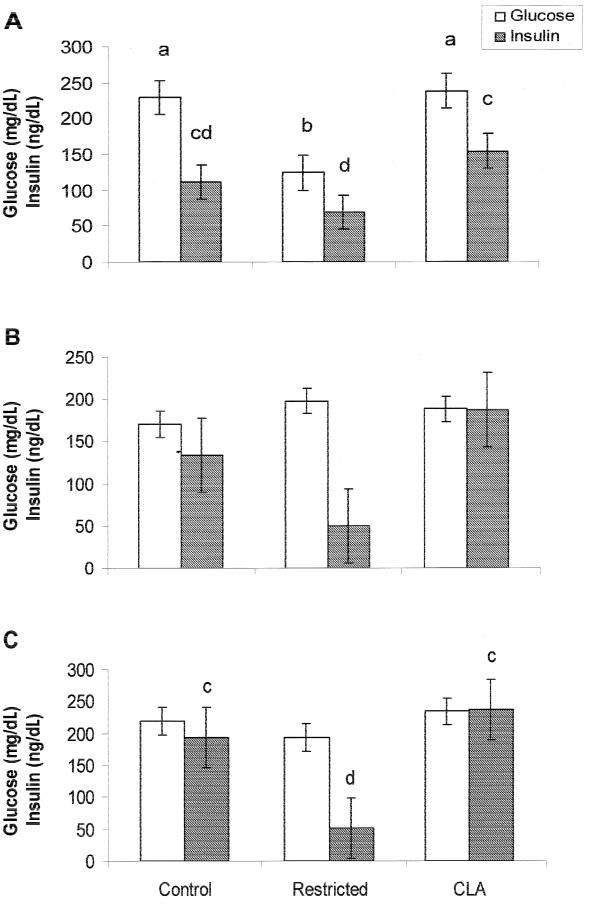

Figure 5: Effect of diet on basal blood glucose and serum insulin concentrations at death of mice fed control or CLA-containing diets for 9 weeks, followed by control diet for 0, 14, and 35 days, experiment 1. Line × diet = not significant; therefore, bars represent least squares means of glucose and insulin in mice from both genetic lines (MH1 and ML1). Control, 7% soy oil diet; restricted, control diet at 65% to 70% intake of control mice; CLA, 6% soy oil + 1% CLA diet. (A) Day 0 of recovery. (B) Day 14 of recovery. (C) Day 35 of recovery. Day 0, $n = 6$ mice per diet; day 14, $n = 3$ to 6 mice per diet; day 35, $n = 7$ to 10 mice per diet. Error bars represent SEM. [ab]Different letters represent differences in glucose, $p < 0.05$. [cd]Different letters represent differences in insulin, $p < 0.05$.

Tissue Weights and Body Composition

In experiment 1, mice that were feed restricted or fed CLA were leaner ($p < 0.05$) than control mice, as measured by epididymal fat pad weight and the body fat index, regardless of genetic line (Figure 3). After 14 days of recovery from CLA or restriction, MH1 mice previously

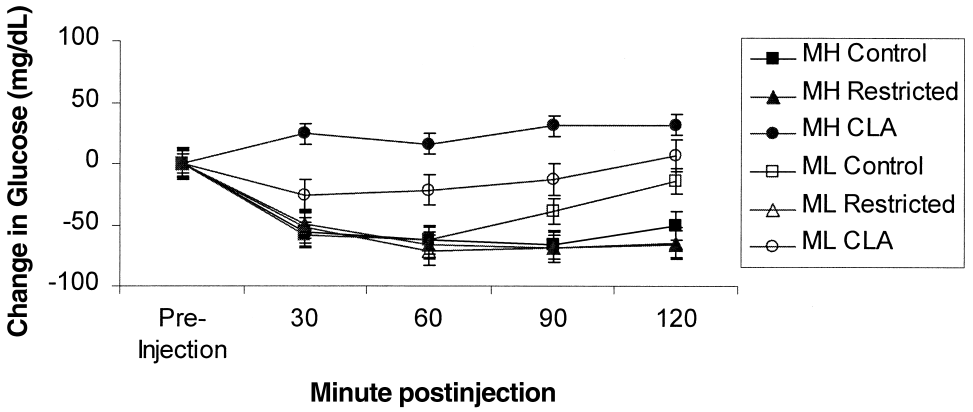

Figure 6: Change in glucose after insulin injection in mice on day 0 ($n = 9$ per line per diet), experiment 1. Error bars represent SEM.

restricted or fed CLA remained leaner ($p < 0.05$) than controls. ML1 mice previously fed CLA had lighter ($p < 0.05$) epididymal fat pads than control mice, but there were no differences ($p > 0.05$) in body fat index. After 35 days of recovery, all mice within a line were similar in body fatness. MH1 mice had higher ($p < 0.05$) body fat indexes on day 0 and heavier ($p < 0.05$) epididymal fat pads on day 35 than ML1 mice. In experiment 3, CLA-fed mice had lighter ($p < 0.001$) retroperitoneal and epididymal fat pads than control-fed mice (Figure 4).

Basal Glucose and Insulin

The line × diet interaction was not significant on any day for basal glucose or insulin. Therefore, the data are reported as the least-squares means for diet, averaged over the two lines (Figure 5). In experiment 1, the concentration of glucose in blood collected by cardiac puncture at death on day 0 was less in feed-restricted mice ($p < 0.05$) than in control or CLA-fed mice. There were no differences in cardiac blood glucose concentration at death on days 14 or 35.

Seventeen serum samples (evenly distributed between treatments) were not analyzed for insulin because, at the lowest dilution recommended by the manufacturer, the radioactive counts were below the level of detection for this assay. All remaining samples were assayed according to the manufacturer's directions. On day 0, CLA-fed mice exhibited a greater ($p < 0.05$) concentration of serum insulin than did restricted mice (Figure 5). On day 14, the ranking of CLA > control > restricted was not significant. On day 35, previously restricted mice again had the lowest ($p < 0.05$) serum insulin concentration, and there was no difference between CLA and control mice.

Blood Glucose Response to Insulin

A graph showing the change in blood glucose after an insulin injection in mice on day 0 in experiment 1 is shown in Figure 6. The 30-minute postinjection glucose measurement was chosen as the time-point to use for statistical analysis of the change in glucose, because at this time the blood glucose concentration was nearing the maximal drop but had not yet begun to rebound. The effect of CLA on serum glucose concentration at the 30-minute sampling is representative of its effect at the other sampling times.

Similar to cardiac blood, the basal glucose concentrations in tail blood differed on day 0 in experiment 1 (Figure 7). MH1 mice on the control treatment had a greater ($p < 0.05$) concentration of glucose than did CLA-fed mice, which had a greater ($p < 0.05$) concentration of glucose than restricted mice (Figure 7). ML1 mice on the control and CLA treatments had similar glucose concentrations, which were greater ($p < 0.05$) than those found in restricted mice. There were no differences in preinjection glucose values on days 11 or 32. In experiments 2 and 3, there were no differences ($p > 0.05$) in preinjection glucose concentration (Figure 8).

In experiment 1, in no instance was there a difference in the change in glucose at 30 minutes between control and restricted mice. MH1 mice fed CLA exhibited an unexpected pattern. Their blood glucose concentration was increased at 30 minutes after the insulin injection on each day (Figure 7). This pattern was not observed in the ML1 mice, although on day 0, there was a trend ($p = 0.08$) for the CLA-fed mice to have a smaller drop in blood glucose than the control or restricted mice. The MH3 and MH1 mice used in experiments 2 and 3, respectively, did not respond in the same manner as those used in experiment 1, because there were no differences ($p > 0.05$) in the change in glucose at 30 minutes in control or CLA-fed mice in either experiment (Figure 8).

Fatty Acid Profile

The fatty acid profiles of epididymal fat pads from experiment 1 were analyzed by gas chromatography. On day 0 of recovery, restricted mice had an increased ($p < 0.05$) percentage of fatty acids that were saturated (16:0 and 18:0)

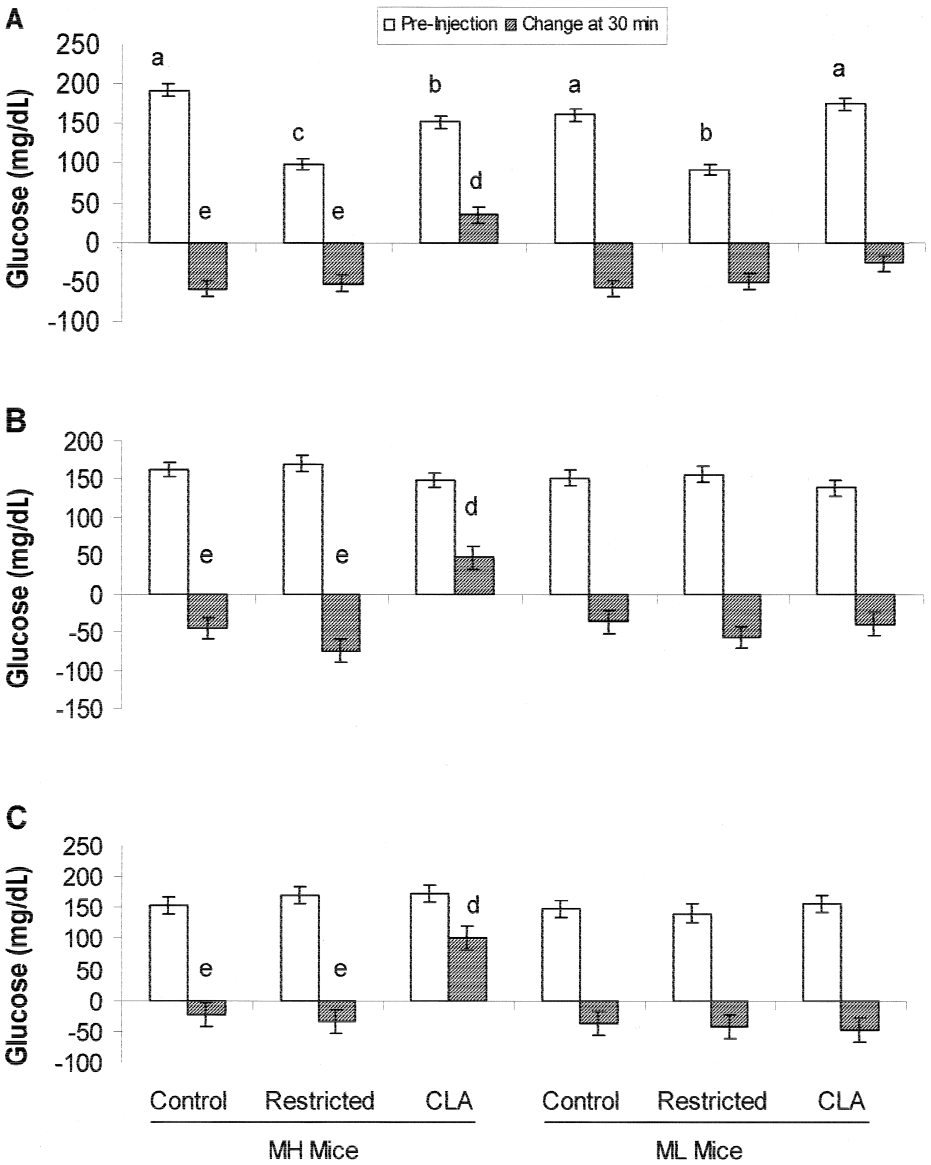

Figure 7: Effect of diet on preinjection and 30-minute postinsulin injection blood glucose concentrations of mice fed control or CLA-containing diets for 9 weeks, followed by control diet for 0, 11, and 32 days, experiment 1. MH, MH1 (mouse line selected for high metabolic rate); ML, ML1 (mouse line selected for low metabolic rate); control, 7% soy oil diet; restricted, control diet at 65% to 70% intake of control mice; CLA, 6% soy oil + 1% CLA diet. (A) Day 0 of recovery. (B) Day 11 of recovery. (C) Day 32 of recovery. Day 0, n = 9 mice per line per diet; day 11, n = 6 mice per line per diet; day 32, n = 5 to 6 mice per line per diet. [abc]Different letters represent differences in baseline glucose, within mouse line, $p < 0.05$. [de]Different letters represent differences in the change in glucose at 30 minutes, within mouse line, $p < 0.05$.

and a decreased ($p < 0.05$) concentration of linoleic (18:2) and linolenic acids (18:3; Table 2). These changes in the relative concentration of the fatty acids caused by feed restriction were maintained at least 35 days after the end of the restricted period. Mice fed CLA also showed changes in the fatty acid profile of their fat pads. CLA addition to the diet decreased ($p < 0.05$) the concentration of the monounsaturated palmitoleic acid (16:1) and the essential polyunsaturated linolenic acid (18:3), whereas it increased ($p < 0.05$) the concentration of both the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA isomers on day 0. After 14 d of receiving a diet lacking CLA, mice previously fed CLA exhibited only a trend ($p < 0.10$) for increased CLA isomers, and by day 35, there were no differences in the fatty acid concentrations between control and CLA-fed mice.

Livers from mice in experiment 1 also were analyzed for fatty acids. Similar to the results observed in adipose tissue, feed restriction caused a decrease ($p < 0.05$) in the essential

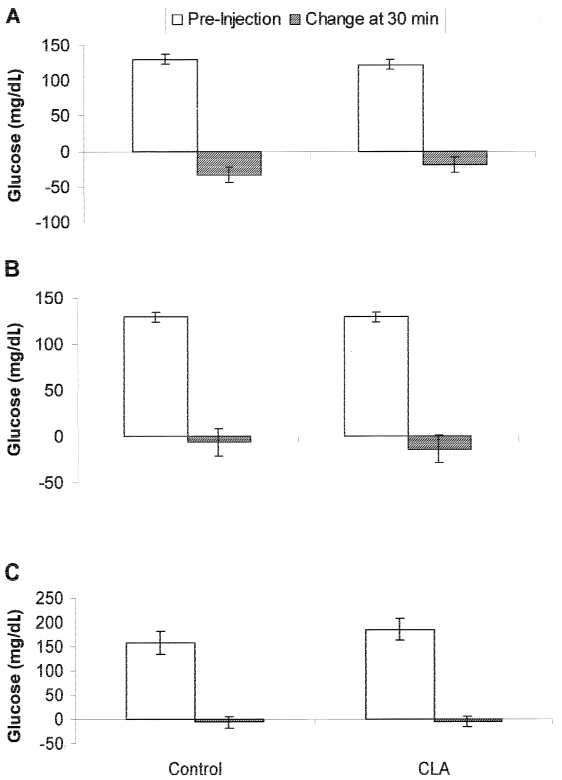

Figure 8: Preinjection and 30-minute postinsulin injection blood glucose concentrations of mice fed control or CLA-containing diets for 6 weeks, followed by control diet for 0 or 24 days, experiments 2 and 3. Control, 7% soy oil diet; CLA, 6% soy oil + 1% CLA diet. $N = 12$ mice per diet in each experiment. (A) Day 0 of recovery, mouse line MH3, experiment 2. (B) Day 24 of recovery, mouse line MH3, experiment 2. (C) Day 0 of recovery, mouse line MH1, experiment 3.

polyunsaturated fatty acids, linoleic acid (day 0) and linolenic acid (day 14; Table 3). However, these results were not maintained through day 35 in the liver. CLA supplementation caused a reduction ($p < 0.05$) in linolenic acid on day 14. Also, on day 35, MH1 mice that had previously been fed CLA had reduced ($p < 0.05$) stearic acid (C18:0) and increased ($p < 0.05$) oleic acid (C18:1) concentrations. Unlike adipose tissue, on no day was the concentration of either CLA isomer increased in the liver compared with control mice.

Discussion

Dietary CLA has been touted as having antidiabetic properties based on data collected using ZDF *fa/fa* rats (8,9), but the same effects have not been observed in other species, including humans (12–14,18). Nondiabetic Zucker rats have previously not responded to dietary CLA in a typical manner; lean rats fed CLA lost body fat, whereas obese rats fed CLA had heavier fat pads than non–CLA-fed rats (26). Therefore, ZDF rats may not be an adequate model for the effect of CLA for other species or genetic lines of rats.

Genetic lines of mice that have been selected for differing metabolic rates and energy expenditure were used in this study. Terpstra (27) stated that mice may be more responsive to CLA-induced body fat loss than humans because of a greater relative effect on energy expenditure. We therefore used the MH and ML mouse lines to determine if differing metabolic rates would influence the response to CLA. The effect of CLA on insulin tolerance seemed to be greater in the MH mice. The CLA-induced effect was not consistent with that observed in the Zucker rats (8,9). In the present study, mice fed CLA were either equally responsive or were less responsive to exogenous insulin. Mice in this study were supplemented with CLA for 9 weeks, whereas the Zucker rats in the previously cited literature were fed CLA for 2 weeks. The difference in length of CLA feeding is not likely to explain the opposing observations; however, DeLany et al. (12) observed numeric increases in plasma insulin concentration in CLA-supplemented mice in as little as 2 weeks and significant increases in 39 days. We also determined that reduced body fatness alone did not induce the changes in insulin tolerance seen in CLA-fed mice.

Dietary supplementation of 1% CLA in this study resulted in a significant loss of body fat in both genetic lines of mice, as has previously been shown (23,28). Unlike results reported previously (12,13), dietary CLA did not increase the serum insulin concentration in 3-hour fasted mice. The effect of CLA on insulin concentration seems to be greater in fed mice than fasted mice (13), and the mice being fasted in the current study may account for the lack of effect of CLA on serum insulin concentration.

Tsuboyama-Kasaoka et al. (13) observed an increased preinjection concentration of glucose and a reduced drop in glucose after insulin injection in mice fed CLA for 9 weeks. We report here no increase in blood glucose before the insulin injection after 9 weeks of CLA supplementation; CLA-fed MH mice actually had reduced blood glucose preinjection compared with control mice. ML mice fed CLA did exhibit a reduced drop in glucose compared with the control or restricted mice, similar to that observed by Tsuboyama-Kasaoka et al. (13). The MH mice that were fed CLA exhibited even less sensitivity to the exogenous insulin, resulting in an increase in blood glucose at 30-minutes postinjection. The MH mice typically have greater locomotor activity than ML mice, which contributes to the differences in body fatness in these lines (29,30). It is possible that the MH mice are more prone to react to the stress of the insulin tolerance test, and therefore, release a larger amount of epinephrine, which would increase blood glucose and amplify the CLA-induced insulin resistance.

It is interesting to note that the increases in body fat in the mice from days 0 to 35 correspond with the clearance of the CLA isomers from the epididymal fat pad. CLA isomers

Table 2. Effect of diet on fatty acid profile of epididymal fat, experiment 1*

	MH			ML			
Fatty acid†	**Con**	**Res**	**CLA**	**Con**	**Res**	**CLA**	**SEM**
Day 0‡							
C14:0	0.88^{b}	1.42^{a}	0.85^{b}	0.87^{b}	1.43^{a}	0.74^{b}	0.06
C16:0	18.14^{c}	24.79^{a}	19.80^{bc}	17.86^{c}	23.91^{ab}	18.67^{c}	0.93
C16:1	4.62^{a}	5.64^{a}	3.05^{bc}	4.28^{ab}	4.08^{abc}	2.59^{c}	0.35
C18:0	2.15^{b}	4.07^{ab}	1.97^{b}	2.20^{b}	5.78^{a}	1.86^{b}	0.57
C18:1	31.13	32.65	34.33	33.01	35.11	36.04	0.96
C18:2	39.83^{a}	29.07^{b}	37.32^{a}	39.41^{a}	28.01^{b}	37.04^{a}	1.06
CLA, c9,t11	0.45^{bc}	0.55^{b}	0.97^{a}	0.16^{c}	0.02^{d}	1.02^{a}	0.07
CLA,t10,c12	0.03^{b}	0.02^{b}	0.45^{a}	0.05^{b}	0.04^{b}	0.44^{a}	0.04
C18:3	2.53^{a}	1.88^{bc}	1.56^{de}	2.32^{ab}	1.69^{cd}	1.11^{c}	0.20
C20:4	0.11^{b}	0.04^{b}	0.01^{b}	0.00^{b}	0.32^{a}	0.03^{b}	0.03
Day 14‡							
C14:0	0.88^{b}	1.25^{a}	0.80^{b}	0.83^{b}	1.19^{a}	0.82^{b}	0.06
C16:0	15.21^{c}	21.06^{a}	17.07^{bc}	19.18^{b}	24.09^{a}	18.34^{b}	0.82
C16:1	5.86^{ab}	6.28^{ab}	4.20^{b}	4.71^{b}	6.83^{a}	5.00^{ab}	0.53
C18:0	1.51^{d}	4.37^{a}	1.66^{cd}	2.04^{c}	2.65^{b}	1.52^{d}	0.15
C18:1	32.18	37.50	32.11	32.10	30.44	32.09	1.14
C18:2	41.33^{a}	27.78^{b}	40.64^{a}	38.61^{a}	31.95^{b}	38.02^{a}	1.55
CLA,c9,t11	0.19	0.00	0.52	0.32	0.16	0.49	0.12
CLA,t10,c12	0.00	0.00	0.12	0.00	0.00	0.08	0.03
C18:3	2.60	1.76	2.77	2.38	2.78	2.58	0.23
C20:4	0.00	0.00	0.12	0.04	0.13	0.20	0.05
Day 35‡							
C14:0	0.84^{b}	1.04^{a}	0.82^{b}	0.75^{b}	1.03^{a}	0.80^{b}	0.04
C16:0	16.35^{c}	19.99^{ab}	18.29^{bc}	17.78^{c}	21.49^{a}	18.02^{bc}	0.66
C16:1	4.85^{b}	7.14^{a}	4.88^{b}	4.22^{b}	6.78^{a}	4.96^{b}	0.37
C18:0	1.75^{b}	2.59^{a}	1.87^{b}	2.16^{ab}	2.52^{a}	1.75^{b}	0.18
C18:1	31.97	31.26	31.32	32.28	30.91	31.25	0.79
C18:2	41.02^{a}	35.03^{c}	40.16^{ab}	40.49^{ab}	32.18^{d}	38.31^{b}	0.82
CLA,c9,t11	0.39^{a}	0.22^{ab}	0.36^{ab}	0.17^{b}	0.11^{b}	0.32^{ab}	0.07
CLA,t10,c12	0.00	0.00	0.00	0.00	0.00	0.03	0.02
C18:3	2.50	2.83	2.87	2.39	4.53	4.39	1.08
C20:4	0.03^{c}	0.13^{ab}	0.19^{a}	0.07^{bc}	0.09^{abc}	0.09^{bc}	0.03

* Different letters within a row represent differences ($p < 0.05$).
† Fatty acids were analyzed by gas chromatography and expressed as a percent of total fatty acids.
‡ Day, day of recovery.

have been reported to be cleared from adipose tissue within 4 weeks of CLA removal from the diet (31). This is consistent with the results from this study, because at 14 days of recovery, there were still small amounts of CLA present in the fat pad, but by day 35, the level of the isomers was not different from those found in the control mice. However, the changes in insulin tolerance seem to not be dependent on the isomers' presence in the fat pad or liver. By 11 days of recovery, the ML mice previously fed CLA had glucose responses to insulin not different from controls, even though

Table 3. Effect of diet on fatty acid profile of liver, experiment 1*

	MH			ML			
Fatty acid†	**Con**	**Res**	**CLA**	**Con**	**Res**	**CLA**	**SEM**
Day 0‡							
C16:0	23.95	23.62	23.26	23.67	25.64	23.24	1.028
C16:1	4.16	2.15	1.77	1.25	1.28	1.28	0.832
C18:0	9.95	11.95	12.61	13.35	12.24	12.82	1.252
C18:1	20.95	18.40	16.09	14.03	16.48	13.66	2.490
C18:2	27.10^{a}	22.09^{b}	24.71^{ab}	27.11^{a}	25.09^{ab}	23.55^{ab}	1.307
CLA,c9,t11	0.17	0.37	0.44	0.11	0.13	0.58	0.155
CLA,t10,c12	0.03	0.00	0.00	0.00	0.00	0.07	0.033
C18:3	0.95	0.63	0.67	0.75	0.66	0.52	0.125
Day 14‡							
C16:0	21.30	20.77	22.23	23.53	22.81	23.22	0.971
C16:1	2.12^{ab}	1.14^{b}	2.74^{a}	2.43^{ab}	2.31^{ab}	3.03^{a}	0.383
C18:0	10.11^{bc}	14.75^{a}	10.96^{bc}	9.63^{bc}	11.11^{b}	8.95^{c}	0.870
C18:1	15.80	13.31	19.29	18.88	18.62	20.40	2.069
C18:2	30.13	25.85	26.62	26.95	24.77	25.71	1.513
CLA,c9,t11	0.53	0.43	0.37	0.53	0.51	0.55	0.097
CLA,t10,c12	0.00	0.00	0.00	0.05	0.04	0.09	0.054
C18:3	1.40^{a}	0.57^{b}	0.94^{b}	1.10^{ab}	0.91^{b}	1.03^{b}	0.138
Day 35‡							
C16:0	24.13	23.91	25.14	24.04	25.07	24.70	0.673
C16:1	2.29^{ab}	2.54^{ab}	3.20^{a}	2.21^{b}	1.71^{b}	2.13^{b}	0.333
C18:0	11.72^{a}	11.47^{ab}	9.43^{b}	12.12^{a}	12.75^{a}	12.12^{a}	0.760
C18:1	15.62^{b}	16.22^{b}	21.59^{a}	16.24^{b}	15.46^{b}	15.75^{b}	1.301
C18:2	30.34	28.02	26.62	28.61	28.23	27.24	1.083
CLA,c9,t11	0.00	0.14	0.00	0.11	0.00	0.11	0.066
CLA,t10,c12	0.00	0.08	0.00	0.00	0.00	0.03	0.035
C18:3	1.09^{a}	1.16^{a}	1.14^{a}	0.63^{b}	0.89^{ab}	0.91^{ab}	0.137

* Different letters within a row represent differences ($p < 0.05$).
† Fatty acids were analyzed by gas chromatography and expressed as a percent of total fatty acids.
‡ Day, day of recovery.

their fat pad still contained some CLA. Conversely, MH mice remained insulin resistant after 35 d of recovery even though there were no differences in the percentage of fatty acids that were CLA isomers compared with the control mice. Accumulation of CLA in liver tissue was not significant in this study, indicating that CLA stores in the liver are not necessary for either a reduction in body fat or altered insulin tolerance. It is possible that CLA stores in other tissues contributed to the insulin resistance observed in the MH mice. Park et al. (31) reported that the level of CLA isomers in muscle did not return to control levels until 8 weeks after CLA removal.

CLA reduces the mRNA abundance and protein activity of hepatic stearyl-CoA desaturase (32–34). Stearyl-CoA desaturase is responsible for the conversion of stearic acid (C18:0) to oleic acid (C18:1). In this study, we did not detect significant differences in these fatty acids on day 0. There is, however, a numeric increase in stearic acid and reduction in oleic acid in MH1 mice fed CLA compared with controls. By day 35, this has reversed so that there is significantly less stearic acid and more oleic acid in the liver of mice that had been supplemented with CLA. This trend is not observed in the ML1 mice and may explain part of the different responses to CLA observed in the two lines.

No differences in insulin tolerance were observed between the control or CLA-fed MH mice in experiments 2 or 3. CLA-fed mice from experiment 3 did have reduced fat pad weights, indicating that CLA was present in the diet and able to induce a loss of body fat. It is possible that the feeding period of CLA was not long enough in these experiments to induce a change in insulin tolerance. The length of the treatment period was reduced from 9 weeks in experiment 1 to 6 weeks in experiments 2 and 3 to maintain the same age of the mice at the first insulin tolerance test. Increases in plasma insulin concentrations have been observed after 6 to 8 weeks of CLA supplementation (12). Additionally, mice from experiment 2 were from the MH3 line, unlike the mice from experiment 1, which were from MH1. Because the lines were selected independently, there may be differences in the mechanism behind the increased metabolic rate in the different lines that affects response to dietary CLA.

Previously, rats pair-fed to the intake of CLA-fed rats exhibited reduced blood glucose and plasma insulin concentrations and an intermediate improvement in whole-body glucose tolerance similar to the CLA-fed rats (9). In this study, the restricted mice exhibited reduced blood glucose and serum insulin concentrations compared with either the control or CLA mice; however, this did not result in a change in glucose response to insulin.

In conclusion, mice fed CLA did not exhibit improved insulin tolerances; they exhibited either no change in insulin tolerance or developed a resistance to insulin after dietary supplementation with CLA. It also seems that mice with a higher metabolic rate may be more sensitive to CLA-induced changes in insulin sensitivity. Furthermore, reducing the feed intake, and consequently the body weight and body fatness, of mice did not alter the insulin tolerance, as measured by the change in blood glucose after an insulin injection. It is unclear why the apparent species or genetic differences in CLA-induced changes in glucose metabolism exist, and until the effects of CLA on insulin sensitivity in humans is more completely studied, CLA should not be recommended as an antidiabetic food supplement.

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