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Adipose Depletion and Apoptosis Induced by Trans-10, Cis-12 Conjugated Linoleic Acid in Mice*

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Abstract

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Objective: To compare the effectiveness of a conjugated linoleic acid (CLA) isomer mixture (mCLA) with each main isomer [trans-10,cis-12 CLA (CLA10,12) and cis-9,trans-11 CLA (CLA9,11)] in causing body lipid loss and adipose tissue apoptosis.

Research Methods and Procedures: Mice selected over 16 generations for high (MH) or low (ML) energy expenditure and a control group (MC) were fed diets containing either soy oil or soy oil plus mCLA, CLA10,12, or CLA9,11 for 5 days in one study and 14 days in a second study.

Results: Mice fed mCLA or CLA10,12 had less body lipid ($p < 0.05$), smaller retroperitoneal fat pads ($p < 0.05$), and ate less ($p < 0.01$) than mice fed no CLA or CLA9,11 for 5 days. Mice consuming 1% mCLA or 0.5% CLA10,12 gained less weight ($p < 0.01$) and had less body lipid ($p < 0.05$) and smaller epididymal ($p < 0.05$) and retroperitoneal fat pads ($p < 0.01$) than mice consuming either control or 0.5% CLA9,11-containing diets for 14 days. Only mCLA and CLA10,12 increased apoptosis in retroperitoneal fat pads ($p < 0.01$). The effects of mCLA and CLA10,12 were independent of genetic line except for the effect on adipocyte apoptosis. Mice of the MH line were slightly less sensitive than MC or ML mice to CLA-induced adipose tissue apoptosis.

Discussion: CLA10,12, but not CLA9,11, can induce both body fat loss and adipose apoptosis. Although mice of a genotype with less body fat and greater metabolic rate and feed intake appear less sensitive, these CLA effects are robust for mice of varying metabolic background.

Key words: mouse, conjugated linoleic acid, apoptosis, body fat

Introduction

Conjugated linoleic acid (CLA), a group of positional and geometric isomers of linoleic acid, has received considerable attention recently because of its potential health benefits. In addition to anticarcinogenic (1,2), antiatherogenic (3,4), and antidiabetic effects (5,6), dietary CLA can induce body-fat loss in multiple species (7–10). Body-fat depletion is fairly specific in that body protein is spared or even increased (11). Although the mechanism for CLA's effect on body fat is not yet defined, we and others have shown that it is associated with apoptosis of adipocytes (12–14).

Commercial preparations of CLA are composed mainly of cis-9, trans-11 CLA (CLA9,11) and trans-10, cis-12 CLA (CLA10,12). Many of CLA's effects seem to be isomer specific. In cultured 3T3-L1 adipocytes, CLA10,12 decreased lipoprotein lipase activity and intracellular triacylglycerol and glycerol content and increased glycerol release. In contrast, CLA9,11 had no effect (15). Based on these findings, Park et al. (15) proposed that CLA10,12 was the isomer that alters body composition.

Our goal was to test this hypothesis directly by supplementing the diet of mice with either CLA10,12 or CLA9,11. In addition, we were interested in whether the effectiveness of CLA in altering body composition would depend on

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genetic background. To this end, we studied the responses to CLA of mice selected over 16 generations for high metabolic rate/heat loss (MH) or low metabolic rate (ML), and control mice from the same outbred background that were not developed by selection on metabolic rate (MC) (16).

Research Methods and Procedures

Animals

We used mice from lines that had been developed by genetic selection on energy expenditure (16). The original population had equal contributions of Harlan Sprague-Dawley ICR and National Institutes of Health strains, and Charles River CF-1 and CFW(Sw) strains. Lines were developed by applying selection pressure to males of the base population for high energy expenditure (MH), low energy expenditure (ML), or no selection (MC) over 16 generations. MH mice exhibit 50% greater energy expenditure, consume 23% more feed, and are 30% leaner than the ML mice (16,17). For the present study, mice from each line were housed individually at 22 °C under a photoperiod of 12 hours light and 12 hours dark. All mice were fed AIN-93G for 1 week before experiments. Procedures were approved by the University of Nebraska Institutional Animal Care and Use Committee.

Materials

Purified (99%) CLA10,12 and CLA9,11, and mixed CLA (mCLA) (18) were purchased from Nu-Check-Prep (Elysian, MN). The mCLA used in Trial 2 was kindly provided by BASF (BASF Nutrition Research; MEM/BD Neumuehle 13D-76,877 Offenbach). The CLA treatments were mixed into a purified base diet (AIN-93G) by Dyets, Inc. (Bethlehem, PA). Soy oil was replaced with CLA (1:1 wt/wt), depending on treatments listed below. All diets contained 7% total fat.

Trial 1: Effect of Dietary mCLA, CLA9,11, and CLA10,12 on Body Weight, Body Lipid, and Feed Intake

Male and female mice (12 weeks old) were blocked by sex and line, and within blocks were randomly assigned to five diets ($N = 72$):

1. control diet (AIN-93G) containing 7% soy oil ($n = 18$)
2. control diet pair-fed to treatment group 3 ($n = 18$)
3. diet containing 2% mCLA (1.8% conjugated isomers, 0.44% CLA10,12, 41% CLA9,11; $n = 12$)
4. diet containing 0.88% CLA10,12 (same concentration of CLA10,12 as diet 3; $n = 12$)
5. diet containing 0.82% CLA9,11 (same concentration of CLA9,11 as diet 3; $n = 12$)

Feed intake was measured as disappearance from glass jars equipped with a cylindrical stainless steel screen attached to a small-holed lid. Spillage of powdered feed occurs only rarely from these jars. Feed intake and body weights were measured daily for 5 days. On day 6, mice were killed by CO_2 asphyxia, and retroperitoneal fat pads were removed and weighed. The carcasses (less retroperitoneal fat) were freeze-dried and extracted with ether to determine percentage of body lipid.

Trial 2: Effect of Dietary mCLA, CLA9,11, and CLA10,12 on Body Weight, Body Lipid, Feed Intake, and Apoptosis in Adipose Tissue of Mice Selected for High and Low Metabolic Rate

Male mice (12 weeks old) were assigned to the following treatments ($n = 18$ per treatment):

1. control diet (AIN-93G) containing 7% soy oil
2. mCLA (1.0% total conjugated isomers)
3. 0.5% CLA10,12
4. 0.5% CLA9,11

Feed intake and body weights were measured weekly for 2 weeks. On day 15, mice were killed by CO_2 asphyxia. Blood was collected by cardiac puncture and immediately analyzed for glucose concentration by a SureStep glucose monitor (Lifescan, Milpitas, CA). Percentage of body lipid and lean mass were determined by DXA (PixiMus, Madison, WI). Liver and retroperitoneal and epididymal fat pads were removed and weighed. Retroperitoneal fat pads were frozen at −80 °C until assay.

Apoptosis Assay

Apoptosis was determined by assay of internucleosomal DNA degradation. Genomic DNA was isolated using DNAzol (Molecular Research Center, Inc., Cincinnati, OH) with minor modification to the manufacturer's protocol. Retroperitoneal fat (100 mg) was minced in 1 mL of DNAzol and 10 μL of polyacryl carrier (Molecular Research Center, Inc.). After digestion with 0.1 mg/mL proteinase K and centrifugation, the supernatant was transferred to a new tube and DNA was precipitated with ethanol. Tissue DNA (300 ng/500 μL TE buffer) was loaded onto a 100K Nanosep spin column (Gelman Laboratory, Ann Arbor, MI), centrifuged at 5000*g* for 10 minutes, and washed with 100 μL TE. Eluate and retained DNA were quantified using PicoGreen (Molecular Probes, Inc., Eugene, OR). Apoptosis was quantified as the ratio of eluate (fragmented DNA) to total DNA.

Data Analyses

For each trial, the General Linear Models procedure of SAS was used to compute ANOVA. In Trial 1, main effects of treatment, sex, and genetic line were included in analyses, and significance of interactions was determined. Analyses for Trial 2 were similar but included no sex effect. When main effects were significant, the least significant

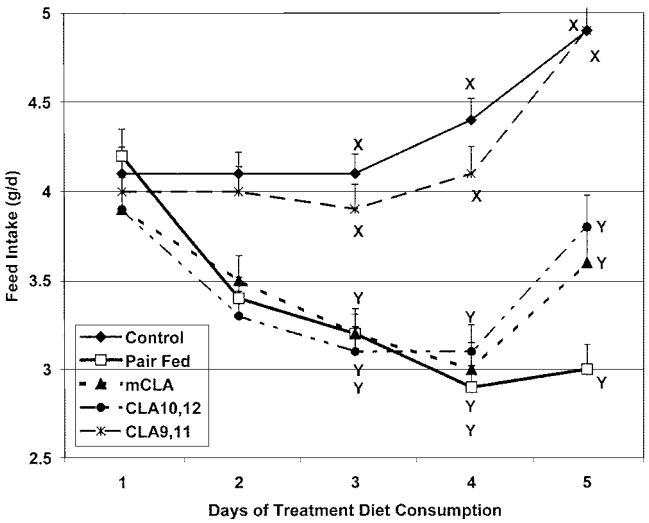

Figure 1: Feed intake of 12-week-old mice (n = 12 to 18/treatment) fed control diet (ad libitum), control diet (pair-fed to mCLA group), 2% mCLA, 0.88% CLA10,12, or 0.82% CLA9,11 for 5 days (Trial 1). Means with different letters differ ($p < 0.01$). Error bars represent SEM. CLA, conjugated linoleic acid.

Table 1. Effects of line and sex on feed intake and body weight in Trial 1

Line	Mean ± SE	Sex	Mean ± SE
Feed Intake (g/d)			
MH	4.4 ± 0.1^{x}	M	3.8 ± 0.1
MC	3.6 ± 0.1^{y}	F	3.6 ± 0.1
ML	3.2 ± 0.1^{z}		
Body Weight (g)			
MH	33.4 ± 0.7	M	36.8 ± 0.6^{x}
MC	34.0 ± 0.7	F	30.2 ± 0.6^{y}
ML	33.2 ± 0.7		

^{xyz} Means with different superscripts differ ($p < 0.01$).
MH, MC, and ML are lines developed by genetic selection for high, control, and low metabolic rate, respectively.
Two-way interactions involving line, diet, sex, or day were not significant.

difference test was used for mean separation. Probability <0.05 was considered significant.

Results

Trial 1: Feed Intake, Body Weight, Body Lipid, and Retroperitoneal Fat Pad Weight in Mice Fed mCLA, CLA9,11, or CLA10,12 for 5 Days

Feed intake was reduced by both mCLA and CLA10,12 consumption (Figure 1 ; $p < 0.01$). Feed intake by mice consuming CLA9,11 was essentially the same as control. The reduction in intake caused by mCLA and CLA10,12 was not observed after 1 full day of consumption, but was clearly established after 2 days of consumption (diet × day interaction, $p < 0.05$). What seems to be an increase in feed intake on day 5 is a consequence of this period being 28 hours rather than 24 hours. This technical factor did not influence intake of the pair-fed mice because their limited feed allowance was generally consumed in <24 hours.

As expected, mice of the high metabolic rate background (MH) consumed 22% more feed ($p < 0.01$; Table 1) than the control background (MC), which consumed 13% more ($p < 0.01$) than the low metabolic rate genotype (ML). Body weight at the experiment's conclusion did not differ between these genetic lines. Males were 22% heavier than females ($p < 0.01$), but did not consume more feed. We detected no significant interaction of either sex or line with treatment diet or day.

Compared with control, percentage of body lipid was reduced by 20% in mice fed mCLA and CLA10,12, but not in either pair-fed mice or those fed CLA9,11 ($p < 0.05$; Table 2). Similarly, retroperitoneal fat pad weight was less in the mCLA and CLA10,12 groups than in mice fed CLA9,11 ($p < 0.05$). Fat pad weight of mice fed CLA9,11 was similar to control. Although the genetic lines of mice differed ($p < 0.01$) in body lipid content and retroperitoneal fat pad weight, there were no interactions of line or sex with treatment (Table 2).

Trial 2: Feed Intake, Body Weight, Body Lipid, and Apoptosis in Adipose of Mice Fed mCLA, CLA9,11, or CLA10,12

Feed intake during the 2-week trial was 4.76, 4.17, 4.33, and 4.81 g/d, for mice fed the control, mCLA, CLA10,12, and CLA9,11 diets, respectively (SEM = 0.147). Consumption was less in both the mCLA and CLA10,12 treatments than in either the control or CLA9,11 treatments ($p < 0.05$). None of the treatments affected body weight (Table 3). However, body-weight gain was reduced by dietary CLA ($p < 0.05$). There was no genetic line × diet interaction for either feed intake or body weight.

The mCLA and CLA10,12 diets reduced percentage of body lipid compared with both control and CLA9,11 ($p < 0.05$; Table 3). Similarly, both epididymal and retroperitoneal fat pad weights were decreased ($p < 0.01$) by mCLA and CLA10,12 compared with control and CLA9,11 (Figure 2). Lean body mass was not affected by either diet or line. The effects of genetic line on body lipid were essentially the same as observed in Trial 1 (data not shown). There was no line × treatment interaction.

There was no significant treatment effect on serum glucose, but there was a significant line effect ($p < 0.01$). MH

Table 2. Main effects of line and sex on RP fat pad weight and percent body fat in mice fed control diet or diets containing CLA for 5 days (Trial 1)

Treatment	Mean ± SE	Line	Mean ± SE	Sex	Mean ± SE
RP fat pad weight (g)					
Control	0.24 ± 0.02^{abxy}	MH	0.17 ± 0.02^{a}	M	0.27 ± 0.02^{x}
Pair Fed	0.22 ± 0.02^{ax}	MC	0.28 ± 0.02^{b}	F	0.17 ± 0.02^{y}
mCLA	0.16 ± 0.03^{bx}	ML	0.21 ± 0.02^{a}		
CLA10,12	0.19 ± 0.03^{bxy}				
CLA9,11	0.28 ± 0.03^{ay}				
Percent body lipid					
Control	14.8 ± 0.9^{ab}	MH	11.6 ± 0.8^{ax}	M	12.4 ± 0.7^{x}
Pair Fed	14.4 ± 0.9^{a}	MC	14.2 ± 0.8^{bxy}	F	15.3 ± 0.7^{y}
mCLA	11.8 ± 1.0^{c}	ML	15.8 ± 0.8^{by}		
CLA10,12	12.0 ± 1.0^{bc}				
CLA9,11	16.1 ± 1.0^{a}				

[a,b,c] Means with different superscripts differ ($p < 0.05$).
[xy] Means with different superscripts differ ($p < 0.01$).
MH, MC, and ML are lines developed by genetic selection for high, control, and low metabolic rate, respectively.
Two-way interactions involving line, diet, or day were not significant.
RP, retroperitoneal; CLA, conjugated linoleic acid.

mice had greater ($p < 0.01$) serum glucose concentration (224 mg/dL; SEM = 12) compared with MC (173 mg/dL) and ML (142 mg/dL) mice.

Liver weight was affected by treatment ($p < 0.05$), but there was no difference between control (1.55 g; SEM = 0.05) and any of the CLA treatments (mCLA, 1.70 g; CLA10,12, 1.53 g; CLA9,11, 1.45 g). Liver weight of CLA9,11-fed mice was less ($p < 0.05$) than that of mCLA- and CLA10,12-fed mice.

There were significant treatment ($p < 0.01$), line ($p < 0.01$), and treatment × line ($p < 0.05$) effects on DNA fragmentation (apoptosis) in retroperitoneal fat pads (Figure 3). In MC and ML genetic lines, apoptosis was markedly increased by mCLA and CLA10,12 ($p < 0.01$), but not by CLA9,11. In MH mice, both mCLA and CLA10,12 increased apoptosis compared with CLA9,11 ($p < 0.05$), but the effects of mCLA and CLA10,12 compared with control did not meet the 5% standard of significance ($0.05 < p < 0.10$).

Table 3. Feed intake, gain, and percentage of body lipid (Mean ± SE) in mice consuming control diet or diets containing mCLA, CLA10,12 or CLA9,11 (Trial 2)

	Feed intake (g/d)		Weight gain (g/d)		Percentage of body lipid
Diet	Week 1	Week 2	Week 1	Week 2	
Control	4.89 ± 0.02^{a}	5.03 ± 0.25^{a}	0.21 ± 0.03^{ax}	0.19 ± 0.03^{x}	22.2 ± 0.9^{x}
1% mCLA	4.02 ± 0.25^{b}	4.08 ± 0.25^{b}	0.03 ± 0.03^{by}	0.003 ± 0.03^{y}	17 ± 0.9^{y}
0.5% CLA10,12	4.39 ± 0.25^{a}	4.37 ± 0.25^{a}	0.06 ± 0.03^{by}	0.02 ± 0.03^{y}	14.5 ± 0.9^{y}
0.5% CLA9,11	5.01 ± 0.25^{a}	4.96 ± 0.25^{a}	0.11 ± 0.03^{bx}	0.22 ± 0.03^{x}	21.3 ± 0.9^{x}

[a,b] Means with different superscripts differ ($p < 0.05$).
[xyz] Means with different superscripts differ ($p < 0.01$).
Diet × genetic line interaction was not significant.
CLA, conjugated linoleic acid.

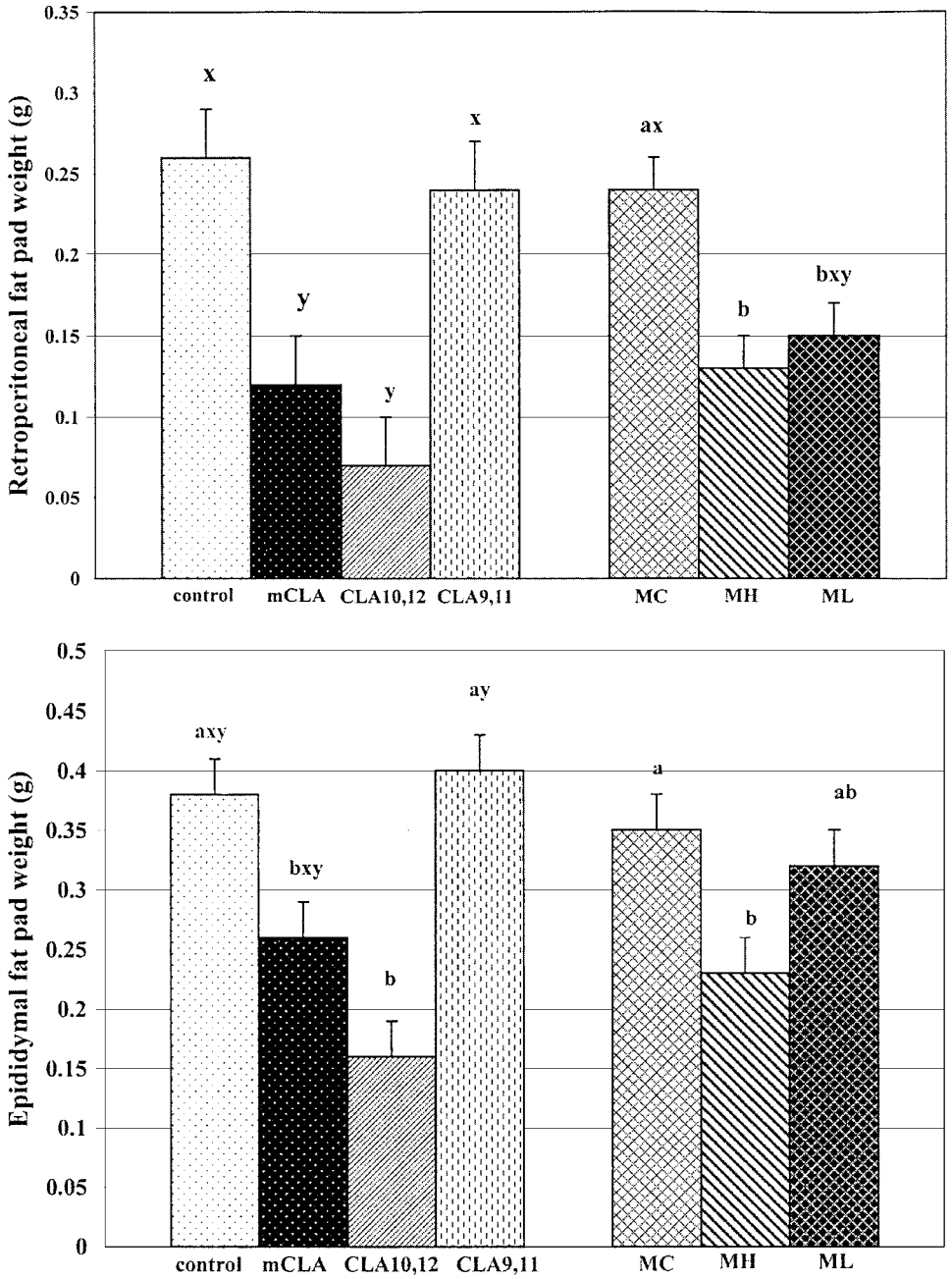

Figure 2: Effects of diet ($n = 18$/treatment) and genetic line ($n = 24$/line) on retroperitoneal fat pad (A) and epididymal fat pad (B) weights of 12-week-old male mice selected for high (MH) or low (ML) metabolic rate or unselected (MC) and fed a control diet, 1% mCLA, 0.5% CLA10,12, or 0.5% CLA9,11 for 14 days (Trial 2). [a,b]Means with different letters differ ($p < 0.05$). [x,y]Means with different letters differ ($p < 0.01$). Error bars represent SEM. CLA, conjugated linoleic acid.

Discussion

This work supports previous reports that dietary CLA can decrease body fat and cause adipocyte apoptosis. Three novel findings of this work are that: 1) the trans-10, cis-12 CLA isomer can produce these effects as well as the often-used CLA mixture, 2) the cis-9, trans-11 CLA isomer does not produce either of these effects, and 3) the body fat-depleting effects of CLA are essentially the same in three lines of mice, which differ significantly in baseline metabolic rate, voluntary feed intake, and body composition.

Consumption of CLA10,12 for as little as 5 days caused a 20% depletion of body fat. The mechanism of this effect probably is not overly dependent on reduced feed intake. Although CLA did cause mice to eat less, the pair-fed group

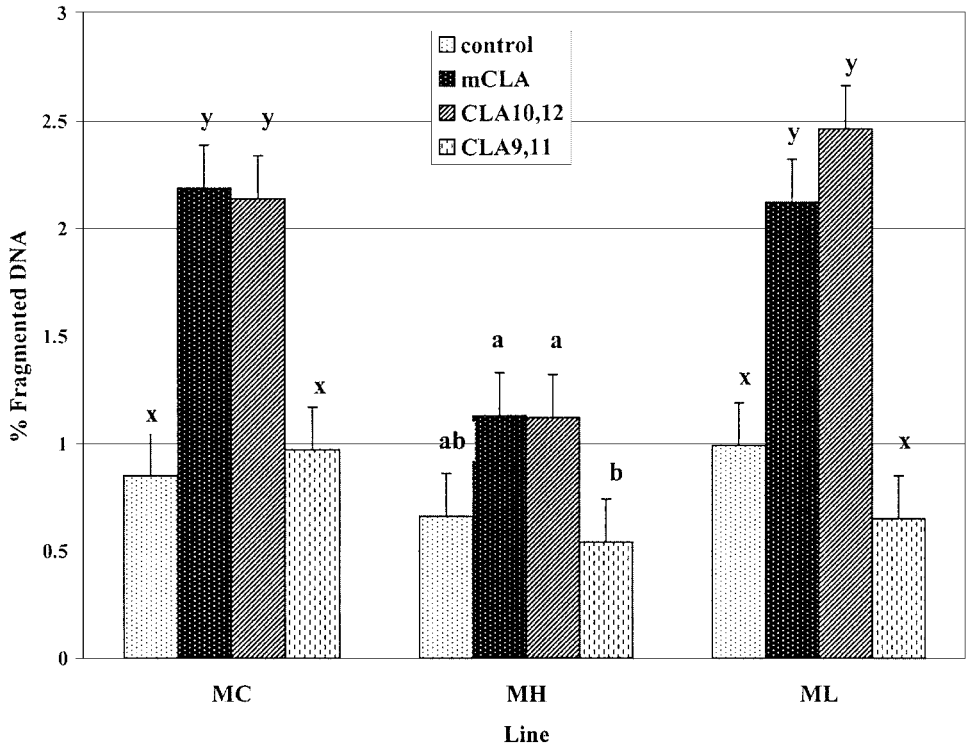

Figure 3: Trial 2: apoptosis in retroperitoneal fat pads of 12-week-old male mice ($n = 18$/treatment) fed a control diet, 1% mCLA, 0.5% CLA10,12, or 0.5% CLA9,11 for 14 days. [a,b]Means with different letters differ ($p < 0.05$). [x,y]Means with different letters differ ($p < 0.01$). MC, MH, and ML represent genetic lines that were unselected or selected for high or low metabolic rate, respectively. Error bars represent SEM. CLA, conjugated linoleic acid.

in Trial 1 did not experience a reduction in body fat relative to the ad libitum-fed control group. That the feed intake reduction caused by CLA10,12 did not occur until the second day of CLA consumption may be evidence that CLA does not affect feed taste but rather influences a mechanism that requires time for metabolism of this molecule or accumulation of some critical concentration.

There seems to be a number of mechanisms involved in CLA-induced reduction in body fat. CLA mixtures and CLA10,12 have been shown to inhibit proliferation and reduce triglyceride content in 3T3-L1 cells (12,19). CLA10,12 reduced uptake of lipid by inhibiting the activities of lipoprotein lipase (20) and stearoyl-CoA desaturase (21) in 3T3-L1 cells. A loss of adipocytes by apoptosis is also responsible for the decreased body-fat content induced by dietary CLA. CLA mixtures increased adipocyte apoptosis in mice (13,14), and CLA10,12 was shown to increase apoptosis of 3T3-L1 cells in vitro (12).

The potential mechanisms involved in CLA-induced adipocyte apoptosis have been investigated by Tsuboyama-Kasaoka et al. (13), who found increased levels of tumor necrosis factor alpha (TNFα) and uncoupling protein-2 mRNA in white adipose tissue of mice fed CLA-containing diets. TNFα has been shown to induce adipocyte apoptosis in vitro (22); however, the mechanism by which CLA causes increased TNFα levels is not known. CLA has been shown to alter the type and amount of cellular fatty acids in 3T3-L1 cells; in particular, arachidonic acid content was reduced more than 50% (23). Arachidonic acid is a precursor to prostaglandins that act as ligands for peroxisome proliferator activated receptor-gamma, which activates genes important in adipogenesis. In fact, Brodie et al. (24) found reduced levels of peroxisome proliferator activated receptor-gamma in 3T3-L1 cells treated with CLA, and Evans et al. (12) showed that CLA supplementation reduced growth of preconfluent 3T3-L1 cells. Thus, CLA may interfere with normal cellular mechanisms involved in preadipocyte maturation. Susceptibility to apoptosis is higher in immature adipocytes (25); thus, CLA may act by increasing the proportion of cells that are susceptible to other factors that trigger apoptosis.

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