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Effect of Conjugated Linoleic Acid on Cell Death in Adipose Tissue

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Summary

Mice fed conjugated linoleic acid (CLA) lose body fat. This loss of body fat is accompanied by an increase in DNA fragmentation, indicative of apoptosis or programmed cell death. Adipose apoptosis was observed in mice fed the trans-10,cis-12 isomer or a mixture of isomers but not the cis-9,trans-11 isomer. The trans-10,cis-12 isomer also induced DNA fragmentation in preadipocytes in vitro, *but not mature adipocytes. The cis-9,trans-11 CLA isomer, the predonimant isomer in ruminant-derived products, was reported to induce apoptosis of cancer cells. Determining the mechanism of action of CLA will improve our understanding of body fat regulation.*

Introduction

Conjugated linoleic acid (CLA) refers to a group of isomers of linoleic acid. One of these isomers, cis-9,trans-11, is produced naturally and found in the fat of ruminant animals. The cis-9,trans-11 isomer has anti-cancer properties while the other main commercially produced isomer, trans-10,cis-12, can induce a loss of body fat. One mechanism by which the cis-9,trans-11 isomer is beneficial in cancer is by inducing apoptosis, or programmed cell death, of malignant cells. It is possible that different isomers result in different effects through similar mechanisms. Moreover, before practices that will allow for alterations in fat deposition can be developed the regulation of body fat must be understood. Determining the mechanism by which CLA induces a loss of body fat may give insight into that regulation. The objective of these experiments was to determine if the loss of body fat induced by CLA was accompanied by an increase in apoptosis in the adipose tissue. Secondly, we determined if preadipocytes and mature adipocytes, both present in adipose tissue, were sensitive to CLA-induced apoptosis.

Procedure

Experiment 1

Seventy-two male mice (12 weeks old) were allotted to either a control (7% soy oil), mCLA (6% soy oil + 1% CLA mixture), CLA10/12 (6.5% soy oil + 0.5% trans-10,cis-12 CLA), or CLA9/11 (6.5% soy oil + 0.5% cis-9,trans-11 CLA) diet for 2 weeks. The CLA mixture contained 50% cis-9,trans-11 and 50% trans-10, cis-12 CLA. Mice were killed, body fat was determined with dual x-ray densitometry, and fat pads were weighed and collected. Apoptosis was determined in retroperitoneal fat pads by differentially precipitating fragmented and non-fragmented DNA and is expressed as a ratio of fragmented DNA:total DNA.

Experiment 2

3T3-L1 mouse fibroblasts were seeded into 12-well plates at a density of 4,000 — 5,000 cells per cm^2. For analysis of preadipocytes, the cells were allowed to attach to the plates overnight. Either 0, 50, 100, or 200 μM linoleic acid or trans-10,cis-12 CLA, complexed to albumin (6.6:1), or 50 nM staurosporine (a positive control for apoptosis) was then added to the basal medium (DMEM + 10% calf serum + 1% antibiotics). Cells were collected following 2 (proliferating) and 4 days (confluent). On day 6 of fatty acid treatment, cells were stimulated to differentiate with DMEM + 10% fetal bovine serum + 5 μg/ml insulin + 1 μM dexamethasone + 0.5 mM IBMX. Cells were also collected following 8, 10, and 12 (differentiating) days of fatty acid treatment. The experiment was replicated 3 times. Cell number was determined using a hemacytometer. Cellular triacylglycerol content was determined with a commercially available kit (Sigma). DNA fragmentation was determined as described in Experiment 1. For adipocytes, cells were grown to confluence, stimulated to differentiate, and allowed 7 — 9 days to accumulate lipid. Either 0, 50, 100, or 200 mM linoleic acid or trans-10,cis-12 CLA, complexed to albumin (6.6:1), or 50, 500, or 1000 nM staurosporine was then added to the medium (DMEM + 10% fetal bovine serum). Cells were collected

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following 2, 4, and 6 days of fatty acid treatment. The experiment was replicated 3 times. Cell number, cellular triacylglycerol and media glycerol content and DNA fragmentation were determined.

Results

Experiment 1

The CLA10/12 and mCLA diets caused a reduction ($P < 0.05$) in feed intake in both weeks. This reduction in feed intake, however, did not relate to a reduction in body weight. It is unlikely that the reduction in feed intake influenced either body fatness or DNA fragmentation. We previously reported (2002 Nebraska Beef Report, pp 92-93) mice fed a control diet at the intake level of CLA-fed mice did not lose body fat while the CLA-fed mice did. The addition of the trans-10,cis-12 isomer, either alone or in the CLA mixture, reduced ($P < 0.001$) the percentage body fat of the mice (Figure 1). Similar reductions ($P < 0.001$) in the weight of the retroperitoneal (74.4 and 51.5% for CLA10/12 and mCLA, respectively) and epididymal (57.6 and 29.7% for CLA10/12 and mCLA, respectively) fat pads compared to the control were observed. Paralleling the reduction in body fat was an increase ($P < 0.001$) in DNA fragmentation (Figure 2).

Experiment 2

In preadipocytes, both linoleic acid and trans-10,cis-12 CLA reduced ($P < 0.01$) the number of cells per well starting on day 4 for linoleic acid and every day for CLA. However, only 200 µM CLA caused an increase ($P < 0.05$) in DNA fragmentation. In the first replication of the experiment only cells that were not confluent or differentiating underwent apoptosis (Figure 3). In subsequent replications cells did not become fully confluent by day 4 and therefore DNA fragmentation was detected in later stages.

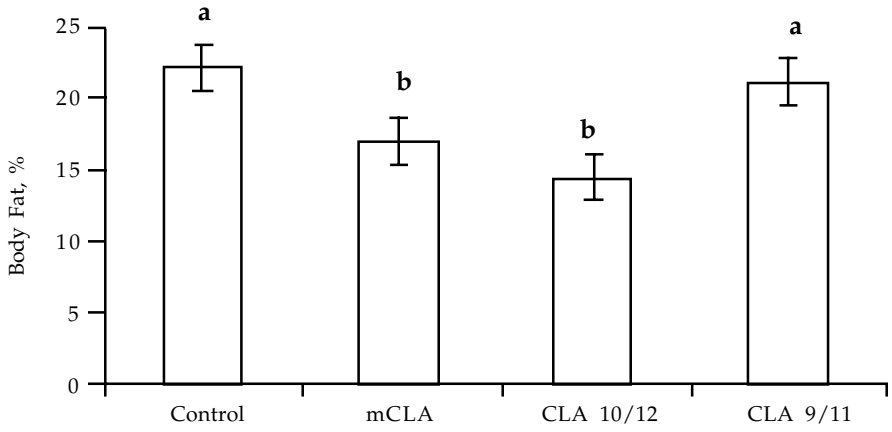

Figure 1. Effect of diets containing individual, or a mixture of, conjugated linoleic acid (CLA) isomers on body fat of mice, experiment 1. Control (7% soy oil), mCLA (6% soy oil + 1% CLA mixture), CLA10/12 (6.5% soy oil + 0.5% trans-10,cis-12 CLA), and CLA9/11 (6.5% soy oil + 0.5% cis-9,trans-11 CLA). [ab]Letters indicate differences, $P < 0.001$.

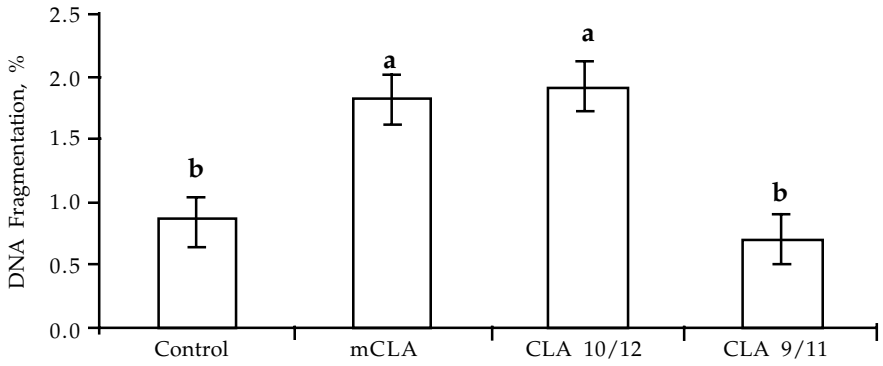

Figure 2. Effect of diets containing individual, or a mixture of, conjugated linoleic acid (CLA) isomers on DNA fragmentation in adipose tissue of mice, experiment 1. Control (7% soy oil), mCLA (6% soy oil + 1% CLA mixture), CLA10/12 (6.5% soy oil + 0.5% trans-10,cis-12 CLA), and CLA9/11 (6.5% soy oil + 0.5% cis-9,trans-11 CLA). [ab]Letters indicate differences, $P < 0.001$.

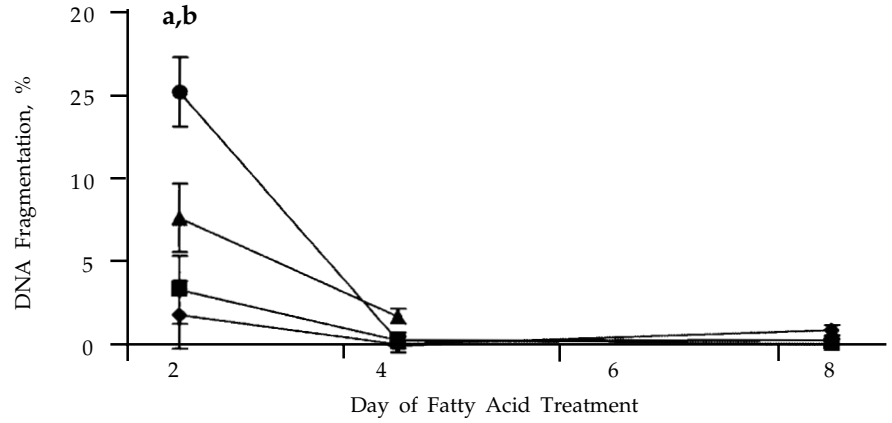

Figure 3. Effect of linoleic acid or trans-10,cis-12-conjugated linoleic acid (CLA) on DNA fragmentation of preadipocytes in culture. Circle — CLA (200 mM), triangle — staurosporine (50 nM), square — linoleic acid (200 mM), and diamond — control. [a]CLA differs from control, $P < 0.05$. [b]CLA differs from linoleic acid, $P < 0.05$.

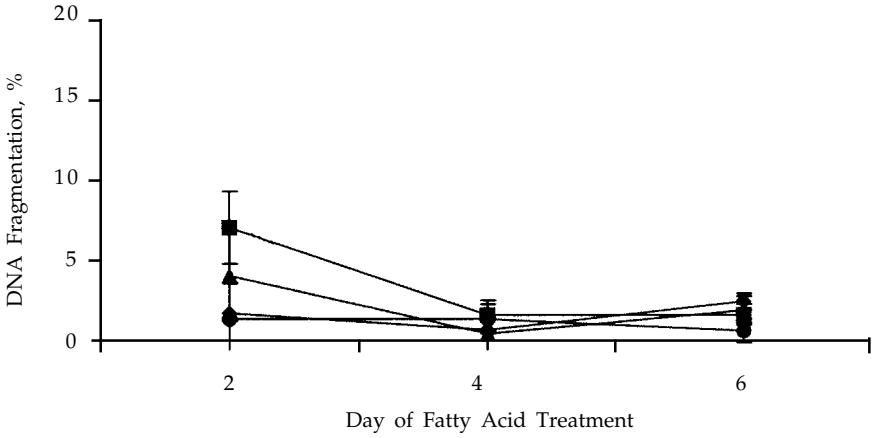

Figure 4. Effect of linoleic acid or trans-10,cis-12-conjugated linoleic acid (CLA) on DNA fragmentation of adipocytes in culture. Circle — CLA (200 mM) , triangle — staurosporine (1000 nM), square — linoleic acid (200 mM), and diamond — control.

In adipocytes, 50 and 100 µM CLA, but not linoleic acid, reduced ($P < 0.05$) cell number on day 2 of fatty acid treatment. On no day was there an effect of treatment on DNA fragmentation in adipocytes (Figure 4). Additionally, CLA had no effect on either cellular triacylglycerol content, or the content of glycerol in the media. These data indicate that the CLA supplementation did not alter lipid filling or lipolysis.

In conclusion, the trans-10,cis-12 isomer of CLA can induce apoptosis, as measured by DNA fragmentation, in adipose tissue of mice as well as cause a loss of body fat. The cis-9,trans-11 isomer appears to have no effect on either phenomenon. The increase in DNA fragmentation due to trans-10,cis-12 CLA supplementation was only observed in preadipocytes *in vitro* and it remains to be seen if the same holds true *in vivo*. Perhaps only cells that are dividing are susceptible to the apoptotic effect of CLA. Although both isomers of CLA can cause apoptosis, only the trans-10,cis-12 isomer does so in adipose tissue. These results may indicate that alterations in fat deposition need to be made prior to differentiation of preadipocytes.

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