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Dietary Conjugated Linoleic Acid (CLA) and Body Fat Changes

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Summary and Implications

Feeding mixed isomers of linoleic acid with conjugated double bonds (CLA) reduces body fat in several species, including pigs. To learn about the mechanism involved, we fed CLA to sexually mature mice at 0, 1 and 2% of the diet for 5, 12 and 14 days. Dietary CLA reduced body fat by nearly 50% but did not reduce body weight. Mice fed CLA also experienced programmed death (apoptosis) of fat cells. This implicates a new mechanism (fat cell death) by which CLA reduces body fat without reducing lean. The effectiveness of feeding CLA for as few as five days may indicate that benefits could be obtained in pigs by feeding CLA for a short duration at the conclusion of the finishing phase.

Introduction

Conjugated linoleic acids (CLA) are a group of polyunsaturated fatty acids that differ from linoleic acid only by position of double bonds. Some CLA are produced naturally by anaerobic ruminal metabolism and subsequent animal metabolism. Consequently, ruminant animal fats contain substantial CLA (~.5%). Inclusion of CLA in pig diets has consistently improved belly firmness, often reduced backfat and improved feed efficiency, and sometimes improved lean growth rate (*Nebraska and South Dakota Swine Nutrition Guide*, EC 95-273). The backfat reduction and growth rate responses to CLA have been variable. We believe that by understanding the mechanism by which CLA causes these benefits, it will be possible to control the variability in response.

Our hypothesis was that CLA can trigger a specific cellular mechanism which leads to an organized death of adipocytes (fat cells) known as programmed cell death, or apoptosis. The research reported here was designed to test this hypothesis in a mouse model. Several mouse experiments can be conducted with the resources required for one experiment using pigs. Subsequent mouse studies will aim to determine the mechanism by which CLA causes fat cell death. Our hope is that an understanding of this biology in mice will allow development of methods to reduce fatness in other mammals such as pigs, cattle, or humans. The specific objective of this study was to determine the effect of CLA on fat depots, feed intake, energy expenditure and apoptosis (programmed death), of fat cells.

Procedures

Diets

Conjugated linoleic acid was mixed into a purified base diet of corn starch, casein, soy oil, sucrose, cellulose, vitamins and minerals (AIN-93G). Soy oil was replaced (1:1) with CLA to create diets containing 0, 1 and 2% CLA. All diets were equal in fat content. This CLA was a mixture of isomers with approximately 44% being the type (cis-9/trans-11) that predominates in ruminant fats, and 41% was trans-10/cis-12 which is a major component of commercially synthesized CLA. The remainder of the added CLA was linoleic acid and several additional conjugated isomers.

Experiment 1

Ninety 10- to 12-week-old male mice were housed individually at 22° C and randomly assigned to one of the three experimental diets (0, 1 and 2% CLA). Feed disappearance and body weight were measured daily. Direct calorimetry was used to measure heat loss during a 4-hour period beginning at 1700 h (5 p.m.) on day nine. On the day of calorimetry, feed was unavailable from 1200 until 1900 h. Thus, heat loss was determined in the fasted and in the refed state for each animal. Heat loss was determined at one-minute intervals and collected every 30 minutes for two hours in each state. Water was not available in the calorimeter chambers. Three days after calorimetry, between 0800 and 1000, mice were sacrificed by CO_2 asphyxia. Brown, epididymal and retroperitoneal fat pads, and livers were removed and weighed. Twenty-one retroperitoneal fat pads were analyzed for apoptosis (programmed cell death).

Experiment 2

Twenty obese 26- to 30-week-old male mice were randomly assigned to one of three CLA diets: 1) 0% for 12 days; 2) 2% for 14 days; and 3) 0% for nine days followed by 2% for five days. Body fat, body weight, and apoptosis were assayed as in Experiment 1.

Results

Experiment 1

Feed intake (g/day) by mice fed 0, 1 and 2% CLA for 12 days was 5.0, 4.7, and 4.4 (SE=.11; P<.05), respectively. Despite consuming less feed, the mice fed 1 and 2% CLA expended as much energy as the controls; heat loss was

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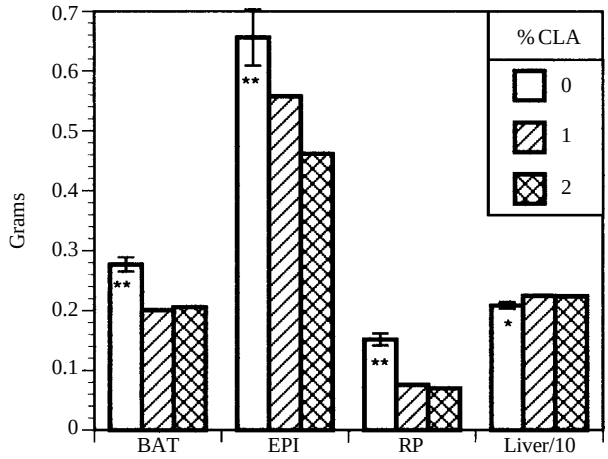

Figure 1. Experiment 1 weight in grams of brown (BAT), epididymal (EPI), and retroperitoneal (RP) fat pads, and of liver (scaled to 10% of actual weight). **CLA effect (P<.01). *CLA effect (P<.10). Error bars represent SEM; N=30 per diet group. Body weight was not influenced by CLA (not shown).

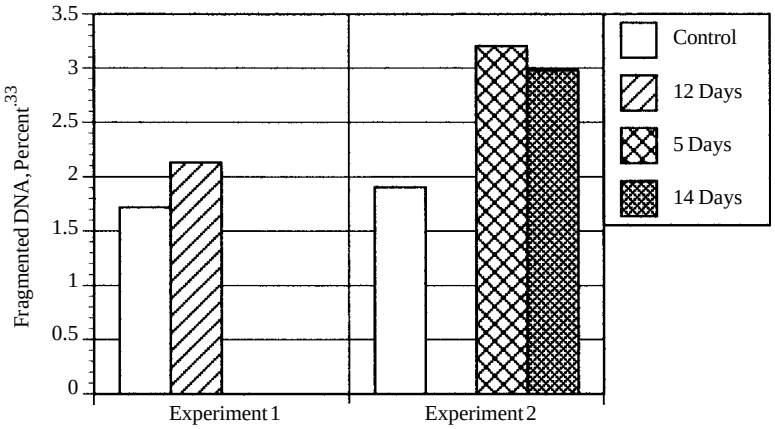

Figure 2. Apoptosis in retroperitoneal fat pads. Fragmentation of DNA is a hallmark of apoptosis. Pooled effect of CLA (P<.01). From left to right, bars represent data from 9, 12, 7, 6 and 7 animals. Pooled SEM is .19.

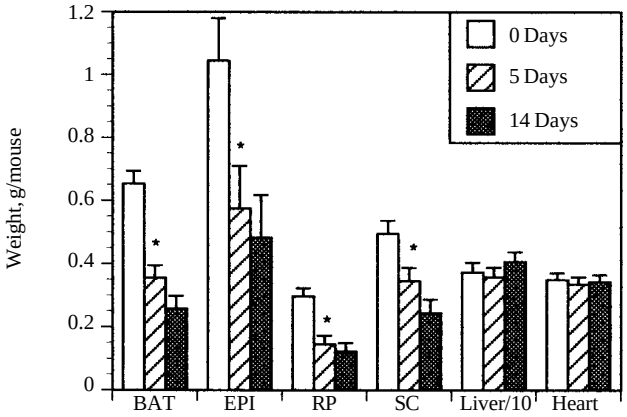

Figure 3. Experiment 2 weight of subcutaneous (SC), epididymal (EPI), retroperitoneal (RP), and brown (BAT) fat pads, liver (scaled to 10% of actual weight) and heart. **CLA effect (P<.01). Error bars represent SEM; N=7 per diet group.

317, 320, and 308 kcal/kg/day for 0, 1 and 2% CLA, respectively (SEM=12.5; P>.5). After consuming CLA for 12 days, mice had up to 50% less body fat than contemporaries which were not fed CLA (Figure 1). The fat cells from mice fed 2% CLA presented more apoptosis as indicated by DNA fragmentation than cells from control mice (Figure 2).

Experiment 2

Consumption of 2% CLA diet for either five or 14 days caused a significant loss of body fat in all of the depots measured (Figure 3; P<.01); however, total body weight increased in animals fed CLA versus control (P<.05). Consistent with the results of Experiment 1, analysis of retroperitoneal fat pads indicated that dietary CLA caused programmed cell death in fat cells (Figure 2).

Discussion

Feeding mixed isomers of conjugated linoleic acid to mice caused a rapid (within five days) loss of body fat, no loss of body weight, and apoptosis of cells in adipose tissue. Perhaps this apoptosis mediates the specific loss of body fat caused by CLA consumption. We speculate that the apoptosis may be mediated by a specific isomer of CLA which has been shown by others to activate a nuclear receptor that regulates gene expression. Perhaps the responses to CLA depend on the amount of this isomer in various sources of CLA. Further work with mice will be directed at identifying which isomer(s) of CLA cause the greatest fat cell apoptosis and reduction of body fat. This should allow more efficient design of treatments to influence composition of growth in swine.

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[1]Chris Cederberg was an undergraduate student at UNL. Some of the information in this paper is from his honors research thesis. Xiaoli Chen is a graduate research assistant at the University of Georgia. Clifton Baile is a faculty member at the University of Georgia. Merlyn Nielsen and Jess Miner are faculty of the UNL animal science department.