

**ADIPOGENESIS IN POST-WEANLING  
PIGS FED CONJUGATED LINOLEIC ACID**

A Thesis

by

VANESSA LYNN ADAMS

Submitted to the Office of Graduate Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of  
**MASTER OF SCIENCE**

August 2004

Major Subject: Nutrition

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August 2004

Major Subject: Nutrition

**ABSTRACT**

Adipogenesis in Post-Weanling Pigs Fed

Conjugated Linoleic Acid. (August 2004)

Vanessa Lynn Adams, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Stephen B. Smith

The effects of conjugated linoleic acid (CLA) on lipogenesis and preadipocyte proliferation in young pigs were evaluated in two separate experiments. The first compared dietary effects of linoleic acid, beef tallow, and CLA on composition, lipogenesis, and DNA synthesis. Eighteen pigs weaned at 17 d of age were allotted randomly to corn-based diets supplemented with 1.5% corn oil, 1.5% tallow, or 1.5% CLA. The second experiment evaluated the effects of CLA included with diets high in polyunsaturated fat or beef tallow. Twenty-four pigs weaned at 17 d of age were allotted randomly to one of four corn-based diets supplemented with: 15% corn oil, 12% corn oil + 3% CLA, 15% tallow, and 12% tallow + 3% CLA. The piglets in both trials were fed a basal diet for 7 d and their respective diet for 35 d. [U-<sup>14</sup>C]Glucose incorporation into total lipids was (experiment 1): 10.64, 11.04, 13.64; (experiment 2): 21.15, 17.54, 21.34, and 19.52 nmol/(10<sup>5</sup> cells per h) for subcutaneous (s.c.) adipose tissue from corn oil, tallow, CLA; corn oil, corn oil + CLA, tallow, and tallow + CLA-fed piglets, respectively. Tritiated thymidine incorporation into DNA was not different in s.c. adipocytes across treatment groups, but was 5,581, 2,794, 6,573, and 3,760 dpm/(10<sup>5</sup> cells per h) in s.c. stromal vascular cells from corn oil, corn oil + CLA, tallow, and tallow + CLA-fed piglets, respectively (CLA main effect p<0.034). Additionally, there

was a greater proportion of s.c. adipocytes in the smaller, 180-pL cell fraction from the corn oil + CLA-fed pigs ( $p < 0.0074$ ). CLA in the diet increased the s.c. adipose tissue concentration of 18:0 and decreased 16:1 and 18:1 ( $p < 0.05$ ), suggesting depression of stearoyl-coenzyme A desaturase (SCD) enzyme activity in the CLA-fed pigs. The concentration of CLA isomers was raised only slightly in s.c. adipose tissue with the addition of CLA to the diets even though the CLA oil contained 62% CLA isomers. No effects on the growth of young pigs were observed. However, CLA caused a more saturated fatty acid composition and may suppress preadipocyte proliferation, apparent SCD activity, and lipid filling of smaller cells.

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## INTRODUCTION

The prevalence of obesity has risen in America and the rest of the world (1-3). This is an important issue because obesity is not only a large portion of the health care burden (4-6), it is also a known risk factor for various diseases, including hypertension, type 2 diabetes, coronary heart disease and cancer (7-14). Furthermore, various reports confirm the link between dietary saturated fat intake and undesirable body fat mass (15), while others the link between a lack of exercise and unhealthy blood profiles (16-17). Both the general public and scientific communities have noticed and have taken steps to battle this epidemic, and to improve the overall quality of health. Now more than ever we are equipped with the knowledge and the tools to succeed, but reducing the incidence of obesity has proven to be difficult. Busier lifestyles usually mean longer workdays and less time for voluntary exercise. This busy routine also allows less time for quality meal preparation and leaves readily accessible high-calorie fast foods as an attractive option. Hence, it is hardly a surprise to see why the energy balance equation is tipped to the side of excess.

Solving this problem is frequently through surgical techniques and/or (prescription/dietary) drugs (6, 12, 18-20), both of which carry with them an inherent risk and which may and often do have adverse effects on other systems of the body (21-30). Safer, non-invasive or non-surgical methods to resume energy balance (31) can only be accomplished by an increase in physical activity or by a decrease in energy

intake, or a combination of the two. The effects of the partnership of diet and exercise are well documented. Modifying current dietary patterns and physical activity levels can lead to an improvement in body composition and overall health (31-33). In fact, lowering body fat levels while maintaining or increasing lean body mass and increasing physical activity are effective in reducing risks of diet-related health disorders, including improving blood cholesterol profiles, and lowering blood pressure (15-17).

Undoubtedly, some of the effects should be the result of changes in cellular events mediated by dietary components. A group with the potential to elicit such changes is fat.

Dietary fat can be classified into three groups: saturated, monounsaturated and polyunsaturated. Fat is capable of regulating many cellular aspects, including the composition of membranes and gene expression (34). In particular, polyunsaturated fatty acids (PUFAs) play important roles in membrane structure, metabolism, and signal transduction (35). Apart from these functions, PUFAs also have been demonstrated to affect adipogenesis through the genetic regulation of the expression of lipid and carbohydrate metabolic enzymes (35-37).

One PUFA under investigation is conjugated linoleic acid (CLA), an isomer of linoleic acid (18:2n-6). Conjugated linoleic acid is a collective term describing the various positional and geometric isomers of octadecadienoic (linoleic) acid. The double bonds of CLA are separated by a single carbon-carbon bond and can occur at various locations along the carbon chain. The two primary isomers are *cis*-9,*trans*-11 and *trans*-10,*cis*-12 (38). CLA occurs naturally in human plasma phospholipids, dairy products

and meats. It is produced in ruminant animals via biohydrogenation of PUFAs and during the mechanical processing of dairy products.

The effects of CLA have been reported in different cell lines and animal models. 3T3-L1 is a murine preadipocyte secondary cell line committed to the adipocyte lineage and is used extensively in adipose studies (37,39-40). Under appropriate differentiation conditions, these cells undergo morphological and biochemical conversion to mature (white) adipocytes. Analysis of the 3T3-L1 differentiation program shows that a series of genetic events occurs before the adipogenic program is triggered (40). Induction of differentiation involves the cooperative interaction between the peroxisome proliferator-activated receptor (PPAR) and CCAAT/enhancer-binding protein (C/EBP) transcription families. Activation of adipogenesis begins with transcription of C/EBP $\beta$  (41). This is followed by transcription of C/EBP $\alpha$  and PPAR $\gamma$ , which then act together to trigger the adipogenic program and reciprocally activate transcription of one another (37,40). This is accompanied by increased mRNA, protein expression and activity of key enzymes relevant to lipid and carbohydrate metabolism, including lipoprotein lipase (LPL), carnitine palmitoyl transferase (CPT), stearoyl-CoA desaturase (SCD), fatty acid transporters (such as fatty acid binding protein) and glucose transporters (such as GLUT4) (35,37,40).

In 3T3-L1 preadipocyte cell systems, CLA was found to inhibit proliferation of pre-confluent cells (42-43), and decrease mRNA transcription of PPAR $\gamma$  and C/EBP $\alpha$  (43). Additionally, CLA was found to increase lipid filling in 3T3-L1 cells (42). However, treatment with only the *trans*-10,*cis*-12 isomer of CLA resulted in smaller

lipid droplets (44). In 3T3-L1 adipocytes, CLA decreased LPL activity (45) and hormone-releasable LPL activity (46). Lipoprotein lipase is produced by adipose and muscle tissue and is found on capillary endothelial cells. It hydrolyzes fatty acids from circulating very low-density lipoproteins (VLDL) and chylomicrons, both rich in triglycerides. The products include low-density lipoproteins, chylomicron remnants and free fatty acids. The hydrolyzed fatty acids are taken up by adipose tissue and muscle where they are either stored (as re-esterified triacylglycerols) or used for energy. Park et al. (45) also reported decreased intracellular concentrations of triacylglycerol and glycerol and increased free glycerol in the culture medium of cells treated with CLA compared to control treatment. These results suggest CLA might work by reducing lipid deposition in adipocytes via reduced LPL activity and increased lipolytic rates.

CLA modifies expression and/or activity of other metabolic enzymes, including SCD, and CPT. SCD converts the saturated fatty acids (SFA) 16:0 (palmitic acid) and 18:0 (stearic acid) to their respective  $\Delta^9$  monounsaturated fatty acids (MUFA) 16:1n-7 (palmitoleic acid) and 18:1n-9 (oleic acid). Interestingly, 18:1n-9 has been shown to affect fat metabolism. For instance, dietary 18:1n-9 increased the activity of fatty acid-binding protein (FABP) in liver and adipose tissue of pigs (47). FABP is responsible for fatty acid transport and/or storage and is usually found in or near tissues that synthesize lipids, such as adipose, or tissues that participate in  $\beta$ -oxidation, such as muscle. A separate swine study reported that adipose tissue from pigs fed 18:1n-9 was found to have less cell density, i.e. fewer cells per gram, than control pigs (48), suggesting that 18:1n-9 increased adipocyte size. Furthermore, obese rats were found to have much

higher levels of SCD1 mRNA than normal rats (49). CLA has been shown to decrease SCD mRNA, thus reducing the amounts of MUFA, in mouse liver and H2.35 mouse liver cells (50). Similar effects have been documented in differentiating 3T3-L1 (44) and human preadipocytes (51). Separate studies have found that CLA decreases SCD activity in HepG2 cells (52), and MCF-7 cells (53). Additionally, we previously reported an apparent depression of SCD activity in adipose tissue from pigs fed CLA (54-55).

In mice, CLA has also been shown to increase the activity of carnitine palmitoyltransferase (45). Carnitine palmitoyltransferase is a mitochondrial fatty acid transporter for  $\beta$ -oxidation in adipose and skeletal muscle. In addition, West et al. (56) reported that CLA-supplemented mice had a decreased nighttime respiratory quotient, indicating a higher rate of fat metabolism.

Other studies demonstrate the multiple effects of CLA. Looor & Herbein (57) suggested that the observed reduced bovine milk fat concentration and yield in cows fed CLA was due to inhibition of *de novo* fatty acid synthesis. Dugan et al. (58) showed that adult pigs fed CLA deposited less subcutaneous fat while gaining more lean mass throughout the course of the feeding trial, leading to improved body composition. Similar effects have been documented in mice (45).

In response to these observed positive effects, as well as reported anticarcinogenic and antioxidant properties (59-61), it is clear why CLA has become a subject of interest as a supplement for human nutrition. The question remains, however, as to whether these results are relevant in humans. To address this, dietary effects of

CLA were evaluated in a domesticated pig (swine) model. The swine model is appropriate because swine and humans have comparable coronary arterial systems. Moreover, both are affected by dietary lipids and fatty acids in a similar manner and also exhibit similar biochemical mechanisms (62). To illustrate, the genetic tendency of swine to develop atherosclerosis in response to dietary stimuli has allowed for numerous investigations involving nutrition and the development and onset of cardiovascular disease (63).

The present research involved measures of adipocyte proliferation and differentiation in weanling pigs. There were two experiments. The first compared individual dietary effects of linoleic acid, monounsaturated/saturated fatty acids, and CLA. The second feeding trial evaluated CLA effects when added to diets high in polyunsaturated fat or monounsaturated/saturated fat.

There have been no reports of the effects of CLA on *de novo* lipogenesis in animals fed CLA. Satory & Smith (43) reported that CLA stimulated the rate of  $^{14}\text{C}$ -glucose incorporation into total lipids in post-confluent 3T3-L1 preadipocytes. This was in contrast to expectations in animal models. Therefore, we investigated the effects of CLA on *de novo* lipogenesis in s.c. adipose tissue in weanling pigs, a time during which extensive preadipocyte proliferation and *de novo* fatty acid biosynthesis are expected (62).

The positive implications for human health target one central theme, improving body composition. Because CLA is a potential promoter of leanness, cardiovascular health will be improved and thus contribute to a lowered risk of developing diet-related

disorders. Based on the research with 3T3-L1 cells, we predicted that young, early weaned pigs fed CLA would exhibit decreased rates of lipogenesis from glucose precursors and decreased adipose cell numbers and size. In addition, we expected depressed preadipocyte proliferation and possibly increased differentiation in stromal vascular tissue. Therefore, we also measured [3-<sup>3</sup>H]thymidine incorporation into DNA in explant cultures of adipose tissue from pigs fed diets with and without CLA. To our knowledge, this work represents the first attempt to demonstrate depressed DNA synthesis in tissues of animals fed CLA.

## MATERIALS AND METHODS

**Animals and diets.** Forty-two pigs, weaned at 17 d of age, were purchased from the Texas Department of Criminal Justice, Huntsville, TX and transferred to the USDA/ARS Children's Nutrition Research Center, Houston, TX. Pigs were acclimatized for 1 wk on a basal corn-based diet which provided approximately 3% fat and 20% protein. After the adjustment period, animals were assigned to different treatments.

**TABLE 1**  
*Composition of the experimental diet fed to weanling pigs*

Ingredient	experiment 1 g/100 g diet	experiment 2 g/100 g diet
Ground corn	37.42	30.32
Soybean meal	33.71	27.31
Fat and cholesterol supplement <sup>a</sup>	1.5	15.0
Dry whey	11.69	11.69
Bakery product	9.06	9.06
Fishmeal	2.13	2.13
Blood products	1.75	1.75
Antimicrobial	0.85	0.85
Ground lime	0.70	0.70
Monocalcium phosphate	0.65	0.65
D,L-Methionine	0.15	0.15
Vitamin premix <sup>b</sup>	0.09	0.09
Mineral premix <sup>c</sup>	0.04	0.04
Other	0.26	0.26

<sup>a</sup>Experiment 1: Corn oil diets included 1.5 g corn oil/100 g diet, tallow diets included 1.5 g tallow/100 g diet, and CLA diets included 1.5 g CLA/100 g diet. Experiment 2: Corn oil diets included (15 g corn oil + 0.016 g cholesterol)/100 g diet, tallow diets included 15 g tallow/100 g diet, corn oil + CLA diets included (12 g corn oil + 3 g CLA + 0.016 g cholesterol)/100 g diet, and tallow + CLA diets included (12 g tallow + 3 g CLA + 0.003 g cholesterol)/100 g diet.

<sup>b</sup>Contributed the following per kilogram of diet: 8.2 mg of all-*trans* retinal, 13.8 µg of cholecalciferol, 44.1 mg of RRR- $\alpha$ -tocopherol, 18 µg cyanocobalamin, 4.5 mg of riboflavin, 16.4 mg of D-pantothenic acid, 30.1 mg of niacin, 3.3 mg of pyridoxine, 2.1 mg of thiamine, 100.5 mg of biotin, 726 g of folic acid.

<sup>c</sup>Contributed the following per kilogram of diet: 176 mg of Zn, 238 mg of Fe, 212 mg of Cu, 57 mg of Mn, 534 µg of I, 167 mg of Mg, and 402 mg of Se.



**Experiment 1.** Eighteen piglets were randomly assigned to one of three treatment groups. The diets were supplemented with either 1.5% corn oil, 1.5% tallow, or 1.5% conjugated linoleic acid (CLA).

**Experiment 2.** Twenty-four piglets were assigned to diets supplemented with either 15% corn oil, 12% corn oil + 3% CLA, 15% tallow, or 12% tallow + 3% CLA.

Composition of the diets is shown in **Table 1**. The CLA source for the treatments was CLA-60 from Conlinco (Detroit Lakes, MN). As per manufacturer's specifications, CLA-60 contained 62% total CLA isomers. Analysis and composition of the supplemental lipids for the two experiments are shown in **Tables 2 and 3**. Pigs were

**TABLE 2**  
*Fatty acid composition of the diet for experiment 1<sup>1</sup>*

Diet:	Corn	Tallow	CLA
Fatty acid	g/100 g fatty acids		
16:0	14.2	19.7	13.2
16:1n-7	nd <sup>2</sup>	2.25	nd
18:0	4.3	7.58	5.26
18:1n-9	27.65	32.05	27.11
18:2n-6	51.7	36.2	33.7
CLA <i>cis</i> -9, <i>trans</i> -11 <sup>3</sup>	nd	nd	6.86
CLA <i>trans</i> -10, <i>cis</i> -12	nd	nd	6.49
18:3n-3	2.04	1.99	1.62
Total MUFA	27.65	34.3	27.11
Total PUFA	53.74	38.19	48.67
Total SFA	18.5	27.28	18.46
MUFA:SFA	1.49	1.26	1.47
PUFA:SFA	2.90	1.40	2.64

<sup>1</sup>Values are based on identifiable peaks.

<sup>2</sup>not detectable.

<sup>3</sup>The values for CLA *cis*-9,*trans*-11 also included trace amounts of CLA *trans*-9,*cis*-11, whereas the CLA *trans*-10,*cis*-12 contained trace amounts of CLA *cis*-10,*trans*-12.

fed at the USDA/CNRC in Houston, TX, for 35 d. At the end of the feeding period, pigs were transported in an air-conditioned van to Texas A&M University Rosenthal Meat

Science and Technology Center, where they were killed humanely using standard industry techniques. Samples of subcutaneous adipose tissue from the dorsal neck region were collected immediately following decapitation for the measurements of DNA synthesis in explant cultures, *de novo* lipogenesis from glucose, and cellularity/adiposity.

**TABLE 3**  
*Fatty acid composition of the CLA source and diet for experiment 2<sup>1</sup>*

Diet:	Conlinco CLA oil	Corn	Corn + CLA	Tallow	Tallow + CLA
Fatty acid	g/100 g fatty acids				
12:0	0.04	0.03	0.03	0.08	0.07
14:0	0.09	0.17	0.16	1.57	1.32
14:1n-5	0.01	0.03	0.03	0.30	0.15
16:0	5.48	11.2	10.3	22.2	19.4
16:1n-7	0.09	0.22	0.20	4.65	3.89
18:0	3.02	2.38	2.49	9.42	8.35
18:1n-9	20.3	25.8	24.9	33.1	31.0
18:2n-6	6.74	54.5	46.6	16.4	14.8
CLA <i>cis</i> -9, <i>trans</i> -11 <sup>2</sup>	21.1	0.02	3.53	0.17	3.65
CLA <i>cis</i> -11, <i>trans</i> -13	12.0	0	2.02	0.03	2.03
CLA <i>trans</i> -10, <i>cis</i> -12	15.1	0	2.51	0.03	2.54
18:3n-3	0.15	0.98	0.84	0.76	0.66
20:4n-6	0	0.02	0.02	0.28	0.23
Other	15.9	4.35	5.87	10.9	12.4
Total MUFA <sup>3</sup>	20.4	26.3	25.3	38.3	35.4
Total PUFA	7.38	55.8	47.7	17.6	15.9
Total SFA	8.81	14.2	13.3	33.4	29.3
MUFA:SFA	2.32	1.85	1.93	1.15	1.35
PUFA:SFA	6.30	3.92	3.59	0.53	0.54

<sup>1</sup>Values are based on identifiable peaks.

<sup>2</sup>The values for CLA *cis*-9,*trans*-11 also included trace amounts of CLA *trans*-9,*cis*-11, whereas the CLA *trans*-10,*cis*-12 contained trace amounts of CLA *cis*-10,*trans*-12.

<sup>3</sup>MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids.

**Analysis of cellular lipids.** Total lipid was extracted by the method of Folch et al. (64). After methylation (65), the fatty acid methyl esters (FAME) were analyzed using a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler,

Varian Inc., Walnut Creek, CA) by the method of Sturdivant et al. (66). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 [100 m x 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands). Helium was the carrier gas. After 32 min at 180°C, oven temperature was increased at 20°C/min to 225°C and held for 13.75 min. Total run time was 48 min. Injector and detector temperatures were at 270 and 300°C, respectively. Individual FAME were quantified as a percentage of total FAME analyzed. An index of SCD enzyme activity, the  $\Delta^9$  desaturase index, was calculated as MUFA/(SFA + MUFA) (67).

**Cellularity/adiposity.** Adipose tissue cellularity (size and number of cells/g) was determined by procedures outlined in May et al. (68). Subcutaneous adipose tissue samples were frozen at 25°C and sliced in 1-mm thick sections to facilitate tissue fixation. Tissue was rinsed with 37°C 0.154 mol/L NaCl to remove free lipid. The rinsed adipose slices were then placed in 20-mL scintillation vials with 1.5 mL of 50 mmol/L collidine-HCl buffer (pH 7.4). Then, 2.5 mL of 3% osmium tetroxide in collidine was added and samples incubated for 72 to 96 h at 37°C. The osmium solution was removed and tissue rinsed with 0.154 mol/L NaCl until clear. To solubilize the connective tissue, samples were incubated in 10 mL of 8 mol/L urea at 22°C for 72 to 96 h. The fixed cells were filtered through 240- $\mu$ m, 64- $\mu$ m, and 20- $\mu$ m nylon mesh screens with 1 g/L Triton in 0.154 mol/L NaCl. The cells were collected to determine cell size, volume, and cells per g tissue with a Coulter counter (model ZM) equipped with a channelizer (model Z56, Coulter Electronics, Hialeah, FL).

**DNA synthesis.** Incorporation of  $^3\text{H}$ -thymidine into DNA was measured in cultured adipose tissue slices as described previously (68). Fresh explants of subcutaneous adipose tissue (two to three pieces weighing approximately 100 mg total) were transferred to 5-mL culture plates, 5 mL culture media was added, and plates were incubated for 24 h at  $37^\circ\text{C}$  in a humidified, 5%  $\text{CO}_2$  atmosphere. The culture media consisted of Dulbecco's modified Eagle's medium (25 mmol/L glucose), 100 g/L fetal bovine serum, 0.584 g/L L-glutamine, 1.7  $\mu\text{mol/L}$  insulin, 1.0  $\mu\text{mol/L}$  dexamethasone, 0.5  $\mu\text{mol/L}$  3-isobutyl-1-methylxanthine, and 1  $\mu\text{Ci/mL}$  [ $^3\text{H}$ ]thymidine (Amersham, Arlington Heights, IL). Media was refreshed at 12 h.

After  $^3\text{H}$ -thymidine incubations, the tissue was collagenase-treated using a modification of procedures described by May et al. (68). The tissue was rinsed with 150 mmol/L NaCl and 1 mmol/L HEPES buffer. Samples were placed in vials with 2.5 mL incubation media containing Krebs-Henseleit buffer (KHB), 10 mmol/L HEPES, 5 mmol/L glucose, 3% bovine serum albumin, (fatty acid-free), 1 mmol/L  $\text{CaCl}_2$ , 1.67 mg/mL collagenase, 0.3 mg/mL elastase, and 0.5 mg/mL hyaluronidase. Samples were incubated for 1 h in a shaking water bath at  $37^\circ\text{C}$ . At the end of the incubation period, vial contents were transferred to centrifuge tubes and centrifuged at  $21,000 \times g$  for 5 min. The top layers containing the fat cells were transferred to new tubes, leaving the stromal-vascular fraction behind. To lyse the cells, 0.5 mL of 20% TCA was added to both fractions, the samples centrifuged at  $21,000 \times g$  for 5 min, and the top layer aspirated. The resulting pellet was redissolved in 1 mL of 0.5 mol/L NaOH and 0.25 mL was transferred to a clean scintillation vial. To this, 0.1 mL of 5 N HCl and 10 mL

scintillation cocktail was added and radioactivity counted on a liquid scintillation counter.

**Lipogenesis.** Immediately postmortem, adipose tissue samples were transported to the laboratory in oxygenated 37°C KHB, containing 5 mmol/L glucose. Two-hour *in vitro* incubations were performed with approximately 100 mg tissue slices, as described previously (48). Explants were placed in 3 mL of a KHB system (pH 7.4) containing 10 mmol/L glucose, 10 mmol/L Hepes buffer, and 0.5  $\mu\text{Ci}$  [ $U\text{-}^{14}\text{C}$ ]glucose (Amersham, Arlington Heights, IL). Vials were gassed for 1 min with 95%  $\text{O}_2$ :5%  $\text{CO}_2$ , capped, and incubated for 2 h in a shaking water bath at 37°C. After 2 h, reactions were stopped with addition of 3 mL 5% trichloroacetic acid (TCA). Samples were removed from the incubation media and rinsed with KHB and 0.154 mol/L NaCl to remove free lipid and unincorporated substrate.

The neutral lipids were extracted using a modification of the Folch et al. (64) procedure. Rinsed tissue was transferred to individual 50-mL screw cap centrifuge tubes containing 5 mL chloroform:methanol ( $\text{CHCl}_3$ : $\text{CH}_3\text{OH}$ , 2:1, vol/vol). Samples were homogenized using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) and rinsed with additional  $\text{CHCl}_3$ : $\text{CH}_3\text{OH}$  to a final volume of 15 mL. Next, 5 mL of 4%  $\text{Na}_2\text{CO}_3$  was added and samples were shaken at high speed for 20 min. Samples were centrifuged at 1,000 x g for 20 min, and the top layer containing  $\text{Na}_2\text{CO}_3$  and nonesterified fatty acids aspirated. The  $\text{CHCl}_3$ : $\text{CH}_3\text{OH}$  layer was extracted twice more. The remaining solvent was filtered through a Whatman volumetric filtration device (Fisher Scientific, Pittsburgh, PA) to clean 50-mL screw cap tubes. The solvent was

evaporated to a volume between 5 and 10 mL, and samples transferred to scintillation vials, where they were evaporated to dryness, resuspended in 10 mL of scintillation cocktail, and radioactivity counted on a liquid scintillation counter (Model LS3800, Beckman Instruments, Palo Alto, CA).

**Statistical analysis.** Data for the first trial was analyzed with the SuperAnova program (Abacus Concepts, Inc., Berkeley, CA) as a simple one-factor analysis of variance. Means were separated by Fishers LSD method with significance at  $p < 0.05$ . Data for the second trial was analyzed as a two-factor analysis of variance, with factor one being the type of supplemental dietary fat (corn oil vs. tallow) and factor two being the dose of CLA (0 vs. 3%). Main effects and the dietary fat x CLA dose interaction were tested. All effects at  $p < 0.05$  were considered significant.

**Source of chemicals.** All biochemicals, unless otherwise indicated, were purchased from Sigma Chemical Co. (St. Louis, MO) and Gibco BRL (Gaithersburg, MD).

## RESULTS

**Production.** For experiment 1, there were no effects of diet on final weight (**Fig. 1A**), and all animals gained comparable amounts of weight per feed consumption (**1B**).

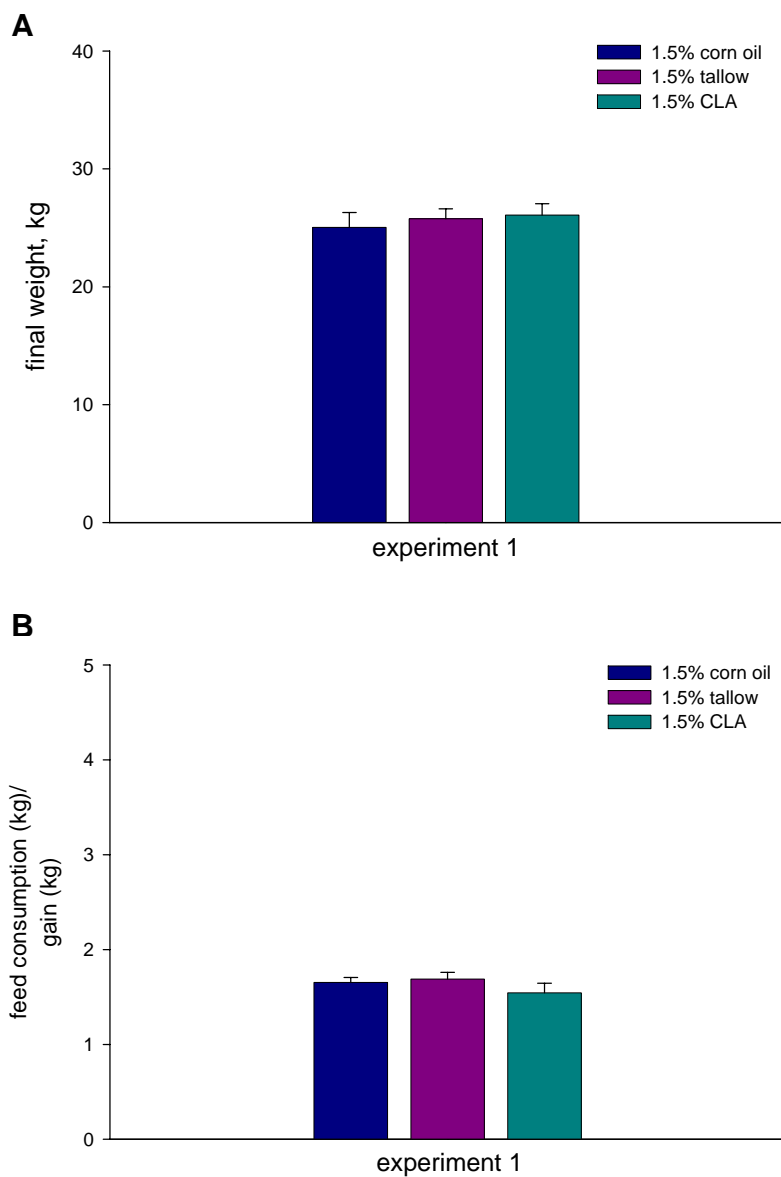


Figure 1. Production of pigs in experiment 1. (A) Animal body weights and (B) feed conversion. All groups displayed similar end weights and feed/gain ratios.

There were also no differences in start weight, daily gain or feed intake (data not shown). Similarly, animals in experiment 2 did not differ in final weight or other feed qualities (not shown).

**The effects of CLA on cellular lipids.** In experiment 1 (**Table 4**), animals fed CLA had less 18:1n-9 and total MUFA than corn oil- and tallow-fed pigs ( $p < 0.001$ ). CLA-fed pigs also had correspondingly more 16:0, 18:0 and total SFA (all  $p < 0.02$ ). As a result of these differences in MUFA and SFA, the desaturase index, a ratio of MUFA/(MUFA + SFA), of CLA-fed pigs was less than in corn oil- and tallow-fed pigs.

**TABLE 4**

*Fatty acid concentrations in subcutaneous adipose tissue for experiment 1*

Fatty acid	Corn	Tallow	CLA	SEM	p-value
14:0	1.91	2.00	3.63	0.23	0.001
14:1n-5	0.16	0.16	0.22	0.014	0.148
16:0	30.2	31.7	38.2	1.20	0.002
16:1n-7	2.60	2.69	1.98	0.138	0.062
18:0	11.7	10.8	15.4	0.76	0.020
18:1n-9	31.9	31.5	18.0	1.91	0.001
18:2n-6	13.8	9.2	7.76	0.81	0.003
CLA	0.73	0.75	1.95	0.186	0.001
18:3n-3	0.36	0.33	0.17	0.028	0.003
20:4n-6	0.09	0.10	0.02	0.026	0.390
Total MUFA	34.7	34.4	20.2	2.02	0.001
Total SFA	43.8	44.5	57.2	1.97	0.004
$\Delta^9$ Index	0.44	0.44	0.26	0.024	0.001

$\Delta^9$  Index = index of stearyl-coenzyme A desaturase activity; MUFA/(MUFA + SFA)

In experiment 2 (**Table 5**), there was a significant main effect of diet on all fatty acids except CLA isomers. This was a result of fatty acid composition of the diets, and demonstrates the tendency of lipid incorporation into tissues as they are consumed in the diet. Adding CLA to the diets lowered percentages of 16:1n-7, 18:1n-7, 18:1n-9 and



total MUFA (all  $p < 0.013$ ) and there was a diet x CLA interaction for these fatty acids; they were reduced more by CLA in tallow-fed pigs than in corn oil-fed pigs. Conversely, CLA reduced 18:2n-6 in corn oil-fed pigs, but not in tallow-fed pigs (interaction  $p < 0.007$ ).

**TABLE 5**  
*Fatty acid concentrations in subcutaneous adipose tissue for experiment 2*

Fatty acid	Corn +		Tallow +		SEM	p-values		
	Corn	CLA	Tallow	CLA		Diet	CLA	Diet x CLA
14:0	0.15	0.72	1.45	1.76	0.149	0.001	0.009	0.396
16:0	15.4	15.9	22.7	23.2	0.785	0.001	0.183	0.962
16:1n-7	1.61	1.28	4.31	3.55	0.269	0.001	0.001	0.013
18:0	6.23	9.37	11.2	14.4	0.636	0.001	0.001	0.995
18:1 t11	0	0.15	1.49	1.95	0.190	0.001	0.067	0.343
18:1 c11	1.26	1.08	2.79	2.17	0.147	0.001	0.001	0.003
18:1n-9	30.9	26.7	40.2	32.9	1.03	0.001	0.001	0.001
18:2n-6	42.5	39.8	14.8	14.5	2.77	0.001	0.001	0.007
CLA <i>cis</i> -9, <i>trans</i> -11	0	3.37	0	3.98	0.224	0.509	0.001	0.509
CLA <i>cis</i> -11, <i>trans</i> -13	0	2.00	0	2.16	0.089	0.243	0.003	0.243
CLA <i>trans</i> -10, <i>cis</i> -12	0	0.31	0	0.66	0.137	0.758	0.001	0.758
Total MUFA	33.8	29.2	48.85	40.6	1.458	0.001	0.001	0.001
Total SFA	21.8	26.0	35.4	39.4	1.499	0.001	0.001	0.797
$\Delta^9$ Index	0.61	0.53	0.58	0.51	0.53	0.54	0.001	0.211

$\Delta^9$  Index = index of stearoyl-coenzyme A desaturase activity; MUFA/(MUFA + SFA)

**The effects of CLA on adipocyte cellularity.** For each experiment, there were no effects of dietary fat, nor was there a fat x CLA interaction for adiposity of the subcutaneous adipose tissue overlying the neck (**Table 6**). However, pigs in experiment 2 had fewer cells/100 mg than pigs in experiment 1. This was the result of larger adipocytes (larger mean volume) in pigs in experiment 2. Tallow-fed pigs had a greater proportion of larger cells than corn oil- or CLA-fed pigs in experiment 1 (**Fig. 2A**). In experiment 2, there was a greater proportion of smaller 180-pL cells in the corn oil + CLA-fed pigs than in the corn oil-fed, tallow-fed, or CLA + tallow-fed pigs (**Fig. 2B**).

There was a significant CLA main effect for all cell volumes greater than 500 pL; adipocytes from CLA-fed pigs were smaller than those from pigs fed fat without CLA.

**TABLE 6**  
*Effects of diets on subcutaneous adipose tissue cellularity in weanling pigs<sup>1</sup>*

	cells/100mg, cells $\times 10^5$	mean volume, pL
Experiment 1		
corn	7.77	84.05
tallow	6.62	88.10
CLA	7.95	83.71
SEM	0.31	2.86
p-value	0.18	0.80
Experiment 2		
corn	5.14	130.23
corn + CLA	5.34	120.28
tallow	5.18	129.14
tallow + CLA	5.71	122.53
SEM	0.16	3.60
<i>p-values</i>		
<i>Fat</i> <sup>2</sup>	0.55	0.93
<i>CLA</i> <sup>2</sup>	0.30	0.28
<i>Fat x CLA</i>	0.63	0.82

<sup>1</sup>Values are means (n=6 per treatment group).

<sup>2</sup>Pigs were fed either 15 g corn oil or beef tallow/100 g diet, or 12 g corn oil or beef tallow + 3 g CLA/100 g diet. Main effects compared corn oil to beef tallow (Fat) or diets containing CLA to those without CLA (CLA).

### **The effects of CLA on proliferation of adipocytes and stromal vascular cells.**

There was no treatment effect on DNA synthesis in lipid-filled adipocytes for either experiment (**Fig. 3A**). However, DNA synthesis in stromal vascular (s.v.) cells was much less in tallow- and CLA-fed groups (1,800 and 1,602 dpm/10<sup>5</sup> cells, respectively) than in corn oil-fed pigs (3,402 dpm/10<sup>5</sup> cells) in experiment 1. This was also true for pigs in experiment 2 (**Fig. 3B**) when CLA was added to the diet (corn oil with and

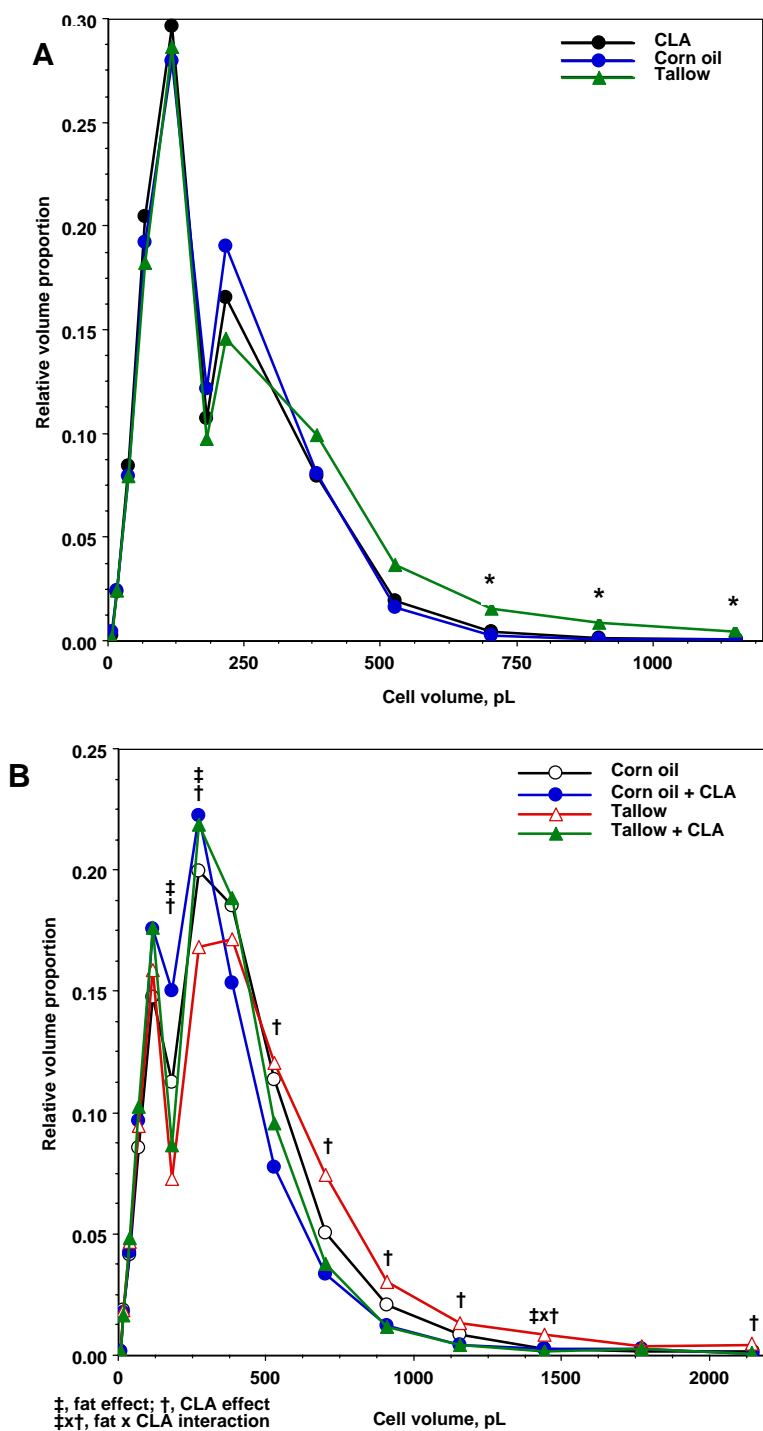


Figure 2. Volume proportion of adipocytes. Volume distribution of s.c. adipose tissue from pigs fed 1.5% corn oil, tallow, or CLA (A) and either 15% corn oil or tallow, or 12% corn oil or tallow + 3% CLA (B). Significant differences are noted. Pigs fed tallow had a larger proportion of cells with volumes of 750-pL or greater. Pigs fed corn oil + CLA had a smaller proportion of smaller (180-pL) cells than other groups.

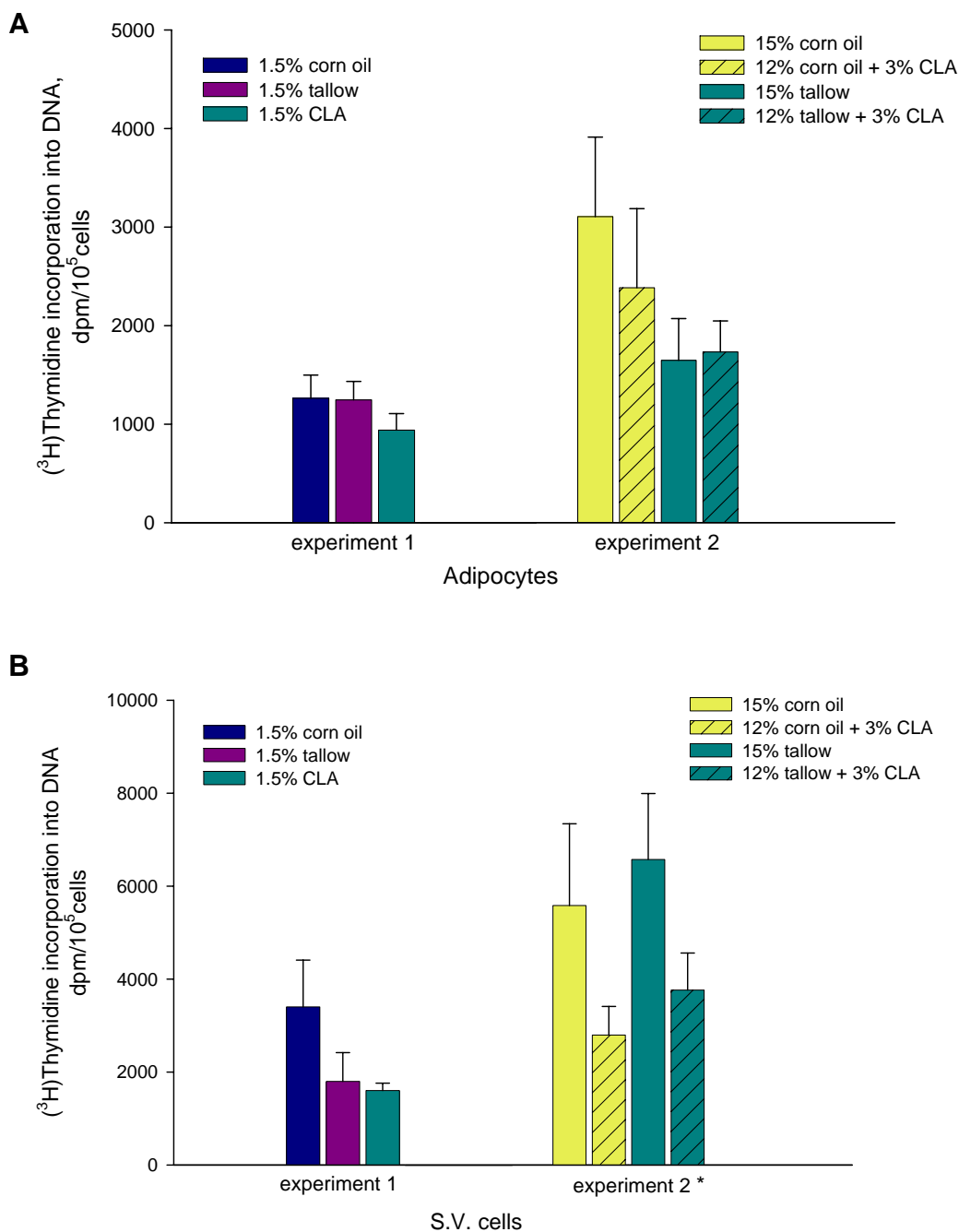


Figure 3. DNA synthesis.  $^3\text{H}$ Thymidine incorporation into DNA in adipocytes (A) and s.v. cells (B) in s.c. adipose tissue from pigs fed 1.5% corn oil, tallow, or CLA; or from pigs fed 15% corn oil, 12% corn oil + 3% CLA, 15% tallow, or 12% tallow + 3% CLA. Thymidine incorporation in s.v. cells is greatest in corn oil-fed pigs (B) in experiment 1 and is depressed by addition of CLA to corn oil and tallow diets in experiment 2. Asterisk denotes main effect of CLA ( $p < 0.034$ ).

without CLA: 5,581 and 2,794 dpm/ $10^5$  cells; tallow with and without CLA: 6,573 and 3,760 dpm/ $10^5$  cells) (CLA main effect  $p < 0.034$ ).

**The effects of CLA on *de novo* lipogenesis in adipocytes.** Lipogenesis from glucose precursors in experiment 1 was highest in CLA-fed pigs (**Fig. 4**), but was not statistically significant from the other treatment groups. Measures for lipogenesis were higher in experiment 2 than in experiment 1, but cannot be compared statistically since analyses were performed at different times. For the higher fat diets, addition of CLA to both corn oil- and tallow-fed groups had no effect on rates of lipogenesis (CLA main effect  $p < 0.48$ ).

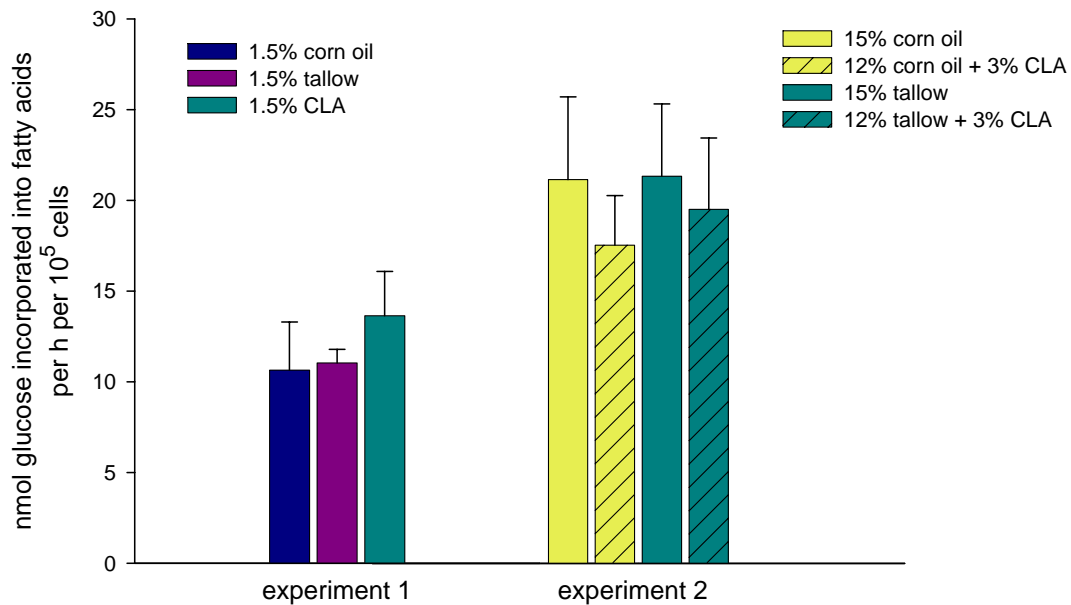


Figure 4. Lipogenesis from glucose precursors. Measures for both experiments were not affected by dietary treatments.

## CONCLUSION

This study was done to measure the effects of low-fat diets supplemented with corn oil (high in PUFA, especially 18:2n-6), beef tallow (high in 16:0 and 18:1n-9), and CLA (highest in 18:2 *cis*-9,*trans*-11, and 18:2 *trans*-10,*cis*-12). It also tested high-fat diets (corn oil or beef tallow) that included CLA. We had anticipated that CLA-fed pigs would display altered fatty acid compositions of subcutaneous adipose tissue, lowered  $\Delta^9$  desaturase indexes, inhibited proliferation (as measured by DNA synthesis), and lowered rates of *de novo* lipogenesis.

Fatty acids were different among the dietary groups of both experiments. For the low-fat diets (experiment 1), CLA supplementation resulted in significantly less MUFA (16:1n-7 and 18:1n-9) and significantly more SFA (16:0 and 18:0). Other reports using swine models indicated similar results from feeding different levels of CLA (69-71) and feeding for different time periods (70). Fatty acid composition of subcutaneous adipose tissue also was altered when CLA was added to a high-fat diet (experiment 2). As with experiment 1, CLA supplementation resulted in significantly lesser percentages of 16:1n-7 and 18:1n-9, and more 18:0. Also, in the corn oil + CLA groups, less 18:2n-6 (linoleic acid) was detected. This suggests that CLA competes with 18:2n-6 for incorporation into phospholipids. CLA incorporation into lipids of adipose tissues follows dietary intake, and is also enhanced when CLA is included in diets that have other supplemental fats (69). The same effects on fatty acid composition in mice (50) and different cell cultures (44,50-53) have been documented.

The mouse and cell studies indicated that CLA treatment significantly reduces the amount of detectable SCD mRNA (44,50-53). Reducing SCD activity results in less production of MUFA from their respective SFA precursors, altering the  $\Delta^9$  desaturase index. Our results show that CLA supplementation to the diet, either with or without supplemental fat (corn oil or tallow) reduces the  $\Delta^9$  desaturase index. We previously reported that CLA depressed SCD enzyme activity in subcutaneous adipose tissue of pigs in experiment 1 (54).

We expected to see changes in cellularity with CLA treatment. Previously, we reported that pigs fed diets high in 16:0 or 18:2n-6 as free fatty acids had significantly smaller mean diameters than pigs fed 18:0, 18:1n-9, or a combination of 14:1n-5 + 16:1n-7 free fatty acids (48). Furthermore, the fatty acid composition of the adipose tissue in the pigs mimicked their respective dietary treatments. Our results here show that adipose tissue of pigs fed CLA had a more saturated fatty acid composition. Cellularity data indicate more adipocytes per gram in the subcutaneous adipose overlying the neck in the animals whose diets included CLA. CLA treatment may delay the signal for differentiation (51), allowing a rise in the number of cells compared to other fatty acids. Also, the mean volume of these cells was less in CLA-fed groups in both experiments, suggesting that CLA reduced lipid filling. However, this observation is in direct contrast with our previous report (42) that mixed CLA isomers increased lipid filling in 3T3-L1 preadipocytes. The two predominant isomers of CLA, *cis*-9,*trans*-11 and *trans*-10,*cis*-12, are reported to have different effects on adipocyte gene expression (44,51). It has been reported that *trans*-10,*cis*-12 CLA reduced the

expression of SCD mRNA and the amount of stainable triacylglycerol in human preadipocytes (51), while *cis-9,trans-11* CLA increased lipid filling compared to control cultures. Others have detected similar results with CLA in 3T3-L1 (44-45) and HepG2 (52) cells. Therefore, the reduction in lipid filling we observed may have been due to the overriding effect of the *trans-10,cis-12* isomer.

<sup>3</sup>H-Thymidine incorporation, an indicator of DNA synthesis (proliferation) was not affected by dietary manipulations in lipid-filled adipocytes. However, CLA decreased <sup>3</sup>H-thymidine incorporation in the stromal vascular (s.v.) cells, which contain preadipocytes. This finding is consistent with other previous studies in MCF-7 cells (59,72-74) and 3T3-L1 cells (42-43), which reported that CLA inhibited proliferation. Our results show that after 5 wk of dietary treatment, DNA synthesis in s.v. cells was significantly less in tallow- and CLA-fed pigs than in the corn oil-fed pigs in experiment 1. We did not see a difference between tallow-fed and CLA-fed pigs. This may be due in part to the low-fat nature and the more similar fatty acid composition of these two diets. In experiment 2, addition of CLA to a high-fat diet reduced the amount of <sup>3</sup>H-thymidine incorporation by almost half, regardless of corn oil or tallow base. To our knowledge, this is the first in vivo report for pigs or any other species showing a reduction in DNA synthesis with dietary CLA.

We previously reported that lipogenesis was specifically depressed by different dietary fatty acids (48). Lipogenesis was lower in s.c. adipose from pigs fed diets enriched with 16:0 compared to those enriched with 18:2n-6. In the current study, this might be compared to the beef tallow diet versus the corn oil diet. These data, along



with our previous observations on SCD activity (55), led us to presume that tallow- and CLA-fed pigs, who would have ingested more SFA, might display significantly lowered rates of *de novo* lipogenesis. Instead, the rates of lipogenesis were not different among treatment groups in experiment 1. Our measures of lipogenesis were higher overall in experiment 2, but we again observed no differences between dietary treatments on *de novo* lipogenesis. In our previous study (48) we had also reported that rates of lipogenesis were not measurably different among pigs fed 18:1n-9 and 18:2n-6. Because the beef tallow diet was high not only in 16:0, but also 18:1n-9 and 18:2n-6, this may explain our results. Additionally, the fat supplement in the previous study was in the form of free fatty acids, and not as triacylglycerols.

In summary, feeding mixed CLA isomers to weanling pigs for a period of 5 wk did not cause differences in carcass measurements, but was able to affect events at the cellular level. We previously reported no difference in dissectable fat or muscle from the 7th through 9th thoracic rib section (54). These results are consistent with other published reports with CLA on final weight in pigs (69-71), rabbits (75), and mice (45). Nonetheless, dietary fatty acids are incorporated in the way they are consumed and cause modifications in fatty acid composition of adipose tissues. These changes play a role in cellular metabolism. One way in which CLA may elicit these effects is by reducing the  $\Delta^9$  desaturase index, subsequently reducing the amount of preferred fatty acids (MUFA) that contribute to the formation of intracellular triacylglycerols. Alternatively, CLA may affect uptake of other fatty acids. Brown et al. (51) showed that *trans*-10,*cis*-12 CLA significantly dose-dependently reduced *in vitro* s.v. uptake of oleic acid as compared to

*cis-9,trans-11* CLA and LA. This results in reduced lipid filling and can slow the process of preadipocyte differentiation (39) to a mature adipocyte. The data presented here (Table 6) indicate similar results although our study used mixed CLA isomers. The *trans-10,cis-12* CLA isomer of CLA also inhibited uptake of glucose and mRNA of the glucose transporter GLUT4 (51). These events over a prolonged period of time can not only affect *de novo* lipogenesis, but can also lead to insulin resistance and other symptoms of metabolic disease, such as hyperlipidemia. Further studies are warranted to elucidate the antiadipogenic effects seen with use of mixed and single isomer CLA doses.

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