

Conjugated linoleic acid depresses the Δ^9 desaturase index and stearoyl coenzyme A desaturase enzyme activity in porcine subcutaneous adipose tissue¹

S. B. Smith*², T. S. Hively*, G. M. Cortese†, J. J. Han*, K. Y. Chung*, P. Casteñada‡, C. D. Gilbert*, V. L. Adams*, and H. J. Mersmann§

*Department of Animal Science, †Department of Health and Kinesiology, ‡Department of Poultry Science, Texas Agricultural Experiment Station, Texas A&M University, College Station 77843 and §USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Pediatrics, Houston, Texas 77030-2600

ABSTRACT: Conjugated linoleic acid (CLA) has been shown to have an effect on subcutaneous fatty acid composition and has been reported to decrease stearoyl coenzyme A desaturase (SCD) activity by decreasing mRNA expression and/or catalytic activity in rodents and rodent cell lines. This investigation was designed to study the effects of CLA, corn oil, or beef tallow supplementation on s.c. adipose tissue fatty acid composition, adiposity, SCD enzyme activity, and the Δ^9 desaturase index in piglets. Eighteen crossbred barrows 16 to 18 d of age were adapted to diet for 1 wk and then assigned randomly to one of three treatments: 1.5% added CLA, 1.5% added corn oil, or 1.5% added beef tallow. Barrows were penned individually and fed the supplemental oils for 35 d (to 25.6 ± 0.6 kg BW). Subcutaneous adipose tissue samples were obtained after

slaughter. Fatty acid composition of the s.c. adipose tissue differed for each fatty acid measured due to diet with the exception of 18:3. The concentrations of CLA *trans*-10, *cis*-12 and *cis*-9, *trans*-11 were elevated from nondetectable to 1.62 and 2.52 g/100 g lipid, respectively ($P < 0.001$ for both isomers). Conjugated linoleic acid decreased the Δ^9 desaturase index ($P < 0.01$) and SCD enzyme activity, expressed as nanomoles of palmitate converted to palmitoleate/(7 min·g of tissue) ($P = 0.075$) and nanomoles of palmitate converted to palmitoleate/(7 min·10⁵ cells) ($P = 0.056$). Tallow-fed pigs had a greater proportion of large adipocytes (> 700 pL) and the greatest SCD activity. These data provide the first direct evidence that dietary CLA depresses SCD enzyme activity in porcine adipose tissue, which may in part be responsible for the depression of adiposity by CLA observed by others in market weight pigs.

Key Words: Acyl-CoA Desaturase, Conjugated Dienes, Fatty Acids, Piglets

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J. Anim. Sci. 2002. 80:2110–2115

Introduction

Numerous beneficial physiological effects have been attributed to conjugated linoleic acid (CLA), including enhancing immune response, reducing atherosclerosis

in rabbits and hamsters, and reducing body fat gain in mice, rats, hamsters, pigs, dogs, and humans (Pariza et al., 1997; 2001; Wiegand et al., 2001). The *trans*-10, *cis*-12 isomer of CLA is associated with changes in body composition and apparently works by reducing adipocyte hypertrophy rather than hyperplasia in rodents (Azain et al., 2000; Poulos et al., 2001; Sisk et al., 2001). The exact mechanism of action is unknown, although CLA *trans*-10, *cis*-12 isomer may reduce the uptake of lipid by adipocytes by inhibiting lipoprotein lipase and stearoyl-CoA desaturase (SCD) enzyme activities in murine cell lines (Pariza et al., 2001).

Previous research has indicated that fatty acid composition of the diet is sufficient to alter SCD enzyme activity of porcine s.c. adipose tissue (Klingenberg et al., 1995). Fatty acid composition of the diet has also been shown to affect the amount of CLA in beef tallow (Pariza et al., 2001) and lamb s.c. adipose tissue (Mir

¹Supported by USDA/CSREES Competitive Grant 98-35206-6286 and the Texas Agricultural Experiment Station. The authors wish to express gratitude to Conlinco, Inc., of Detroit Lakes, MN, for providing the conjugated linoleic acid. This project has been funded in part with federal funds from the USDA, ARS, under Cooperative Agreement no. 58-6250-1-003. The contents of this publication do not necessarily reflect the views or policies of the USDA, nor does mention of trade names, commercial products, or organizations imply endorsement from the U.S. Government.

²Correspondence: 2471 TAMU (phone: 979-845-3939; fax: 979-845-9454; E-mail: sbsmith@tamu.edu).

Received March 20, 2002.

Accepted April 24, 2002.

et al., 2000), and pork intramuscular lipids (Joo et al., 2002).

To date, there are no reports of the effects of dietary CLA on actual SCD enzyme activity in porcine adipose tissue. We previously demonstrated marked increases in SCD gene expression in postweaning pigs during a period of adipocyte hypertrophy (Smith et al., 1999) and, therefore, wished to document the potential relationship between apparent SCD enzyme activity and adiposity in pigs fed CLA during the postweaning period. A final objective was to compare actual SCD enzyme activity with a calculated estimate of desaturase activity.

Materials and Methods

Animals and Diets. The Baylor College of Medicine Animal Care and Use Committee approved the animal protocol. Crossbred barrows ($n = 18$) between 16 and 18 d of age were purchased from the Texas Department of Criminal Justice, Huntsville, TX, and transported to the USDA/ARS Children's Nutrition Research Center, Houston, TX. Barrows were housed in individual stainless steel cages (initially 0.6×0.6 m and then expanded to 0.6×1.2 m as the barrows grew) and had ad libitum access to a corn-soybean-meal-based diet containing approximately 20% crude protein. After a 1-wk diet adaptation period, barrows were then assigned to one of three treatments: 1.5% beef tallow, 1.5% corn oil, or 1.5% CLA top-dressed on the diet. The CLA source was CLA-60 provided by Conlinco (Detroit Lakes, MN). According to manufacturer's specifications, CLA-60 contained 62% total CLA isomers. Cholesterol was added to both the corn oil and CLA diets to equal the cholesterol content of the tallow diet. Barrows were fed twice daily for 5 wk and then transported to the Texas A&M University Rosenthal Meat Science and Technology Center, where they were slaughtered according to current commercial practices. Subcutaneous adipose tissue samples were obtained at slaughter, frozen, and stored at -80°C until analyzed. The initial BW of the pigs was 5.6 ± 0.2 kg, and final BW was 25.6 ± 0.6 kg after 35 d of treatment. There was no difference ($P = 0.76$) in final BW.

Fatty Acid Composition. Total lipid was extracted by the method of Folch et al. (1957). After methylation (Morrison and Smith, 1964), 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. (1992). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 [$100 \text{ m} \times 0.25 \text{ mm}$ (i.d.)] (Chrompack Inc., Middleburg, The Netherlands). Helium was the carrier gas. After 32 min at 180°C , oven temperature was increased at $20^{\circ}\text{C}/\text{min}$ 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.

The Δ^9 desaturase index was calculated as $(16:1 + 18:1 \text{ cis-9} + 18:1 \text{ cis-11}) / (14:0 + 16:0 + 18:0 + 16:1 + 18:1 \text{ cis-9} + 18:1 \text{ cis-11})$. This index has been used as an estimator of SCD enzyme activity in dairy cows (Corl et al., 2001).

Microsome Extraction and Stearoyl-CoA Desaturase Assay. Microsomal fractions were prepared from subcutaneous adipose tissue homogenized in a 3:1 dilution (1.5 g tissue in 4.5 mL of 0.1 M KPO_4 buffer, pH 7.4) for 1 min in a Polytron homogenizer (Kinematica, Luzern, Switzerland). Homogenates were then centrifuged at $5,000 \times g$ for 15 min. The supernatant fractions were centrifuged at $17,300 \times g$ for 30 min. Five milligrams of supernatant proteins were added to 1.5 mL of a solution containing 100 mM Tris-HCl (pH 7.4), 2 mM NADPH and $0.025 \mu\text{Ci}$ [$1\text{-}^{14}\text{C}$]palmitoyl-CoA essentially as described by St. John et al. (1991). The resultant solutions were incubated in a 37°C water bath for 7 min. Incubations were terminated by addition of 3 mL of 10% KOH in MeOH in a 37°C water bath for 30 min and then acidified by addition of 9 mL of 3 N HCl. Fatty acids were extracted by three washes with 9 mL of n-pentane, and combined extracts were washed with 9 mL of acidic water (pH 3.0). The pentane phases were evaporated under nitrogen and methylated with the addition of 14% boron trifluoride in MeOH. Methyl esters were separated by thin layer chromatography on a 10% AgNO_3 impregnated silica gel plate in a petroleum ether:diethylether solvent system (97:3). After separation, the plate was sprayed with a 0.2% dichlorofluorescein in ethanol. Spots were scraped and counted using a Beckman liquid scintillation spectrometer (Beckman, Palo Alto, CA).

Cellularity. Adipocyte size and volume were determined as described by Etherton et al. (1977) with modification by Prior (1983). Frozen adipose tissue from the two sites (medial and lateral subcutaneous) were sliced into sections 1-mm thick and placed in 20-mL scintillation vials. Tissues were rinsed three times with 37°C 0.154 M NaCl at 1-h intervals to remove free lipid. Following the last rinse, 0.6 mL of 50 mM collidine-HCl buffer (pH 7.4) was added to each sample, followed by 1.0 mL of 3% osmium tetroxide in collidine. After incubating for 96 h at 37°C , the osmium solution was removed, and the tissue rinsed three times with 0.154 M NaCl until clear. Samples were incubated in 10 mL of 8 M urea at 25°C for 96 h. After filtering the fixed cells through 240- μm , 64- μm , and 20- μm nylon mesh screens with 0.01% Triton in 0.154 M NaCl, cells collected from the 64- and 20- μm screens were used for determination of cell size, volume, and cells per g tissue, using a Model ZM Coulter Counter equipped with a Model 256 channelizer (Coulter Electronics, Hialeah, FL). Total subcutaneous adipocytes were estimated for each rib section using the average cells per g from this analysis and total adipose tissue weight.

Statistical Analysis. Data were analyzed using SAS (SAS Inst. Inc., Cary, NC). The level of probability for all analyses was predetermined ($P < 0.05$). To determine

Table 1. Fatty acid composition of diets containing 1.5% corn oil, 1.5% beef tallow, or 1.5% conjugated linoleic acid (CLA)

Fatty acid	Diet		
	Corn oil	Tallow	CLA
16:0	14.2	19.7	13.2
16:1	nd ^a	2.25	nd
18:0	4.30	7.58	5.26
18:1 <i>trans</i> -9	1.35	1.95	2.11
18:1 <i>cis</i> -9	26.3	30.1	25.0
18:1 <i>cis</i> -11	nd	nd	0.83
18:2 <i>n</i> -6	51.7	36.2	33.7
18:3 <i>n</i> -3	2.04	1.99	1.62
CLA <i>cis</i> -9, <i>trans</i> -11 ^b	nd	nd	6.86
CLA <i>tran</i> -10, <i>cis</i> -12 ^c	nd	nd	6.49

^and = not detectable.

^bContains trace amounts of *trans*-9, *cis*-11, *cis*-8, *trans*-10, and *trans*-8, *cis*-10 CLA isomers.

^cContains trace amounts of *cis*-10, *trans*-12 CLA.

differences among diets, Proc GLM was used when the model included all dependent variables (s.c. adipose tissue fatty acids, adipocyte cells/100 mg, mean diameter and volume, adipocyte relative proportions at each volume, the Δ^9 desaturase index, and SCD enzyme activity). Means were separated using the lsmeans procedure.

Results and Discussion

Fatty acid compositions of the three diets are shown in Table 1. The basal diet contained approximately 3% lipid, to which an additional 1.5% of each lipid was top-dressed, for an approximate total of 4.5% lipid in each diet. The high percentage of 18:2 *n*-6 present in the diets resulted from the corn-soybean meal used for the basal diet. The CLA diet contained approximately 7% CLA *cis*-9, *trans*-11 and 6.5% CLA *trans*-10, *cis*-12.

Dietary CLA increased s.c. adipose tissue CLA concentrations from undetectable to 2.54% for the *cis*-9, *trans*-11 isomer and 1.62% for the *trans*-10, *cis*-12 CLA isomer (Table 2). All of the saturated fatty acids were more abundant in adipose tissue from CLA-fed pigs than in adipose tissue from corn oil- or tallow-fed pigs, suggesting depression of SCD activity. Stearic (18:0) and oleic acid (18:1*cis*-9) concentrations in adipose tissue of corn oil- and tallow-fed pigs were not different (Table 2), and therefore were independent of their concentrations in the diet (Table 1). Conjugated linoleic acid significantly decreased palmitoleic acid (16:1), 18:1*cis*-9, and 18:1*cis*-11 relative to concentrations seen in pigs fed the tallow diet (Table 2), consistent with the putative depression of SCD activity by CLA. Concentrations of 16:1 and 18:1*cis*-11 were greater ($P < 0.05$) in adipose tissue lipids from tallow-fed pigs than in lipids from corn oil- or CLA-fed pigs. Palmitoleic acid was lower in the CLA diet than in the beef tallow diet (Table 1), suggesting that CLA did not affect concentrations

of 16:1 via SCD activity. However, 18:1*cis*-11 (also a product of SCD) was not detectable in the corn oil or beef tallow diets, so that the lesser concentration of 18:1*cis*-11 in adipose tissue of corn oil-fed pigs relative to tallow-fed pigs suggests lower SCD activity in response to feeding of corn oil.

Feeding CLA to postweanling lambs significantly increased CLA content and lowered 18:1*cis*-9 and 18:3*n*-3 content of s.c. adipose tissue (Mir et al., 2000). Variable results have been observed in pig s.c. adipose tissue. Joo et al. (2002) fed a preparation of CLA containing 91% CLA isomers to 5-mo-old gilts for 4 wk. Even at 5% of the diet, the CLA mixture caused only small decreases in 18:1*cis*-9 (from 40.75 to 38.13%). However, our results are similar to those of Ramsay et al. (2001), who fed up to 2% CLA (in place of corn oil) to crossbred barrows and gilts from 20 to 55 kg BW, and observed marked reductions in 18:1*cis*-9.

This investigation is the first to report the effects of CLA on SCD enzyme activity in porcine adipose tissue (Table 3). Conjugated linoleic acid tended to depress SCD activity when rates were expressed per gram of adipose tissue ($P = 0.075$) or per 10^6 cells ($P = 0.056$), relative to SCD activity in s.c. adipose tissue from pigs fed beef tallow. Rates in adipose tissue from pigs fed corn oil were intermediate and tended ($P = 0.09$) to be different from rates in pigs fed beef tallow. The depression in desaturase activity by CLA, relative to that in tallow-fed pigs, was over 75% (on a per cell basis), and we interpret this to be a significant effect of CLA.

Stearoyl-CoA desaturase is thought to be the rate limiting step in fatty acid desaturation, and has been shown to be the rate-limiting enzyme in the conversion of palmitate to oleate in bovine s.c. adipose tissue (St. John et al., 1991). Stearoyl-CoA desaturase gene expression is important in the differentiation of 3T3-L1 preadipocytes (Ntambi et al., 1988) and increases dramatically during periods of adipocyte hypertrophy in s.c. adipose tissue of postweanling piglets (Smith et al., 1999) and calves (Martin et al., 1999).

We previously reported that CLA increased lipid filling and monounsaturated fatty acid content of 3T3-L1 preadipocytes (Satory and Smith, 1999). However, most investigators have found the opposite in cell lines, which has been more consistent with observations in animal studies. Lee et al. (1998) fed mice CLA and observed decreases in hepatic SCD mRNA levels. Similarly, conjugated linoleic acid decreased SCD gene expression and enzyme activity in 3T3-L1 preadipocytes (Choi et al., 2000; Park et al., 2000). Choi et al. (2001) reported that treating cultured human hepG2 cells with CLA caused no changes in SCD mRNA levels but decreased SCD enzyme activity and concentrations of monounsaturated fatty acids. Park et al. (2000) had previously reported that adding CLA directly to mice liver microsomal fractions directly inhibited SCD catalytic activity. We did not measure SCD mRNA in the current investigation, so we cannot distinguish between direct

Table 2. Fatty acid composition of s.c. adipose tissue from pigs fed diets containing 1.5% corn oil, 1.5% beef tallow, or 1.5% conjugated linoleic acid (CLA)

Fatty acid	Diet			SE ^x	P-value
	Corn	Tallow	CLA		
	g/100 g total lipid				
14:0	1.16 ^b	1.46 ^b	2.43 ^a	0.19	0.001
16:0	23.8 ^b	24.2 ^b	28.6 ^a	2.05	0.016
16:1	2.80 ^b	3.40 ^a	2.46 ^b	0.23	0.049
18:0	13.5 ^b	12.8 ^b	17.7 ^a	0.70	< 0.001
18:1 <i>cis</i> -9	35.9 ^a	35.7 ^a	21.2 ^b	1.36	< 0.001
18:1 <i>cis</i> -11	3.06 ^b	5.15 ^a	2.79 ^b	0.48	0.009
18:2 <i>n</i> -6	17.0 ^a	13.1 ^b	14.6 ^{ab}	0.69	0.002
18:3 <i>n</i> -3	1.04	1.35	1.04	0.14	0.259
CLA <i>cis</i> -9, <i>trans</i> -11	nd ^y	nd ^y	2.54 ^a	0.03	< 0.001
CLA <i>trans</i> -10, <i>cis</i> -12	nd ^y	nd ^y	1.62 ^a	0.02	< 0.001

^{ab}Values with different superscripts within a row are different ($P < 0.05$).

^xStandard error (n = 6 animals per diet).

^ynd = not detectable. Values were considered as zeros for statistical analysis.

effects of CLA on SCD enzyme activity or gene expression in porcine adipose tissue.

The *trans*-10, *cis*-12 isomer of CLA has a strong inhibitory effect on SCD activity compared to other isomers of CLA (Park et al., 2000), indicating that a *cis*-12 bond is the key structural feature for inhibition of SCD activity. The *cis*-9, *trans*-11 isomer of CLA had no effect on SCD activity (Park et al., 2000). Given these results, decreases in SCD activity in the present study likely were due to the presence of CLA *trans*-10, *cis*-12 in the diet and deposited in the adipose tissue.

Previous investigators have reported reductions in carcass adiposity of pigs fed CLA (Pariza et al., 1997; 2001; Wiegand et al., 2001). We did not measure carcass s.c. fat thickness, but did measure s.c. adipose tissue cellularity. There was a greater proportion of larger adipocytes (> 700 pL) in samples from the tallow-fed pigs (Figure 1), but we observed no differences in mean diameter, mean volume, or cells/100 mg adipose tissue (Table 3). This is in contrast to studies by Azain and coworkers (Azain et al., 2000; Poulos et al., 2001; Sisk

et al., 2001) using rodent models, in which CLA consistently reduced adipocyte volume. Wiegand et al. (2002) demonstrated that the timing of CLA administration influenced the effectiveness of CLA on carcass adiposity, such that CLA was more effective in heavier-weight pigs. Thus, feeding CLA to early-weaned pigs from 5.6 to 25.6 kg BW in this study may have been too early in their growth to elicit an effect on mean adipocyte volume. The lesser proportion of larger adipocytes in pigs fed either corn oil or CLA, relative to those fed tallow, was consistent with the lesser SCD enzyme activity in adipose tissue from corn oil- and CLA-fed pigs. These findings thus confirm our earlier observations that elevated SCD gene expression is associated with adipocyte hypertrophy (Martin et al., 1999; Smith et al., 1999).

Finally, one goal of this investigation was to compare actual SCD enzyme activity and the Δ^9 desaturase index (Table 3). The Δ^9 desaturase index has been proposed as an index of SCD enzyme activity in studies with dairy cows (e.g., Corl et al., 2001). The depression

Table 3. Least squares means for adipose tissue cellularity, stearoyl-CoA desaturase enzyme activity, and the Δ^9 desaturase index in s.c. adipose tissue of pigs fed diets containing 1.5% corn oil, 1.5% beef tallow, or 1.5% conjugated linoleic acid (CLA)

Measure	Corn	Tallow	CLA	SE ^x	P-value
Cells/100 mg, $\times 10^{-5}$	7.76	6.61	7.95	0.32	0.186
Mean diameter, μm	48.83	49.59	49.36	0.55	0.863
Mean volume, pL	340.0	371.0	318.8	32.6	0.824
Stearoyl-CoA desaturase activity ^y					
nmol/(7 min·g of tissue)	1.94 ^{cd}	3.62 ^c	0.90 ^d	0.50	0.075
nmol/(7 min·10 ⁶ cells)	0.66 ^{cd}	1.49 ^c	0.30 ^d	0.21	0.056
Δ^9 desaturase index ^z	0.53 ^a	0.54 ^a	0.36 ^b	0.02	< 0.001

^xStandard error (n = 6 animals per diet).

^yPalmitate (nmol) converted to palmitoleate/7 min of incubation either per gram of tissue or per 10⁶ adipocytes.

^zRatio of monounsaturated fatty acids/(saturated plus monounsaturated fatty acids).

^{a,b}Values with different superscripts within a row are different ($P < 0.05$).

^{c,d}Values with different superscripts within a row tend to be different ($P < 0.10$).

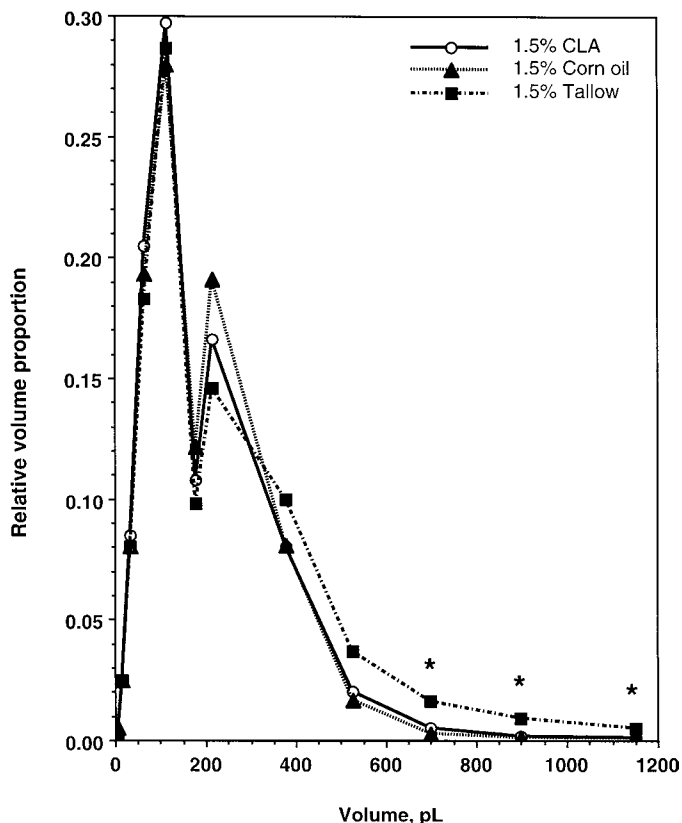


Figure 1. Relative volume distribution of adipocytes in s.c. adipose tissue of pigs fed diets containing 1.5% corn oil, 1.5% beef tallow, or 1.5% conjugated linoleic acid (CLA). Asterisks indicate significant differences ($P < 0.03$) in proportions of cells at the indicated volumes between tallow-fed pigs and the other treatment groups.

of the Δ^9 desaturase index was highly significant ($P < 0.001$), and the index was lower in adipose tissue of CLA-fed pigs than in adipose tissue from either the corn oil- or tallow-fed pigs (Table 3). We previously demonstrated that high oleic sunflower oil depressed s.c. adipose tissue SCD enzyme activity relative to activity in adipose tissue of pigs fed beef tallow (Klingenberg et al., 1995). In that study, we concluded that the higher polyunsaturated fatty acid composition of the sunflower oil was responsible for the lesser SCD enzyme activity (Garg et al., 1988; Periago et al., 1989). Actual SCD enzyme activity in the current study tended to be lower ($P = 0.09$) in adipose tissue from the corn oil-fed pigs than in the tallow-fed pigs. If the difference in enzyme activity between the corn oil- and tallow-fed groups was real, then SCD activity was not sufficiently depressed in the corn oil-fed pigs to affect monounsaturated fatty acid composition or the Δ^9 desaturase index. We conclude that, whereas the Δ^9 desaturase index was not an indicator of absolute enzyme activity, it reliably predicted the depression of SCD enzyme activity in response to dietary CLA.

Implications

The mechanisms by which the *trans*-10, *cis*-12 isomer of conjugated linoleic acid reduces adiposity in pigs remain unknown. Results of this study clearly demonstrated that stearoyl CoA desaturase enzyme activity was greatest in s.c. adipose tissue of pigs fed beef tallow, and those samples exhibited the greatest proportion of large adipocytes. This implies that 1) maximal desaturase activity may be required for attainment of larger adipocyte volumes and 2) a significant effect of conjugated linoleic acid on mean adipocyte volume, hence carcass adiposity, may have been observed had the pigs been grown to a typical slaughter weight.

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