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Stearoyl Coenzyme A Desaturase Enzyme Activity and mRNA Levels Are Not Different in Subcutaneous Adipose Tissue from Angus and American Wagyu Steers[1]

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ABSTRACT: We proposed that greater stearoyl coenzyme A (CoA) desaturase enzyme activity caused the elevated monounsaturated fatty acids observed in American Wagyu adipose tissue. Stearoyl CoA desaturase mRNA concentrations and enzyme activities were measured in subcutaneous adipose samples from Angus (n = 5) and American Wagyu (n = 5), fed to the Japanese market end point. A rat liver stearoyl CoA desaturase cDNA clone was used to measure the relative amounts of stearoyl CoA desaturase mRNA. Enzyme activities and mRNA concentrations, as measured by laser densitometry of slot-blot autoradiograms, were not significantly different between the two breeds at this stage of growth. This investigation has demonstrated that, at this stage of maturity, differences in fatty acid composition between Angus and American Wagyu steers cannot be attributed to differences in stearoyl CoA desaturase enzyme activity.

Key Words: Acyl-CoA Desaturase, RNA, Cattle

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Introduction

Previous research has indicated that Japanese Black and their North American counterparts, American Wagyu, deposit a greater amount of intramuscular adipose tissue than do Angus cattle (Yamazaki, 1981; Lunt et al., 1993). Japanese Black and American Wagyu cattle also contain a higher amount and concentration of monounsaturated fatty acids in their subcutaneous adipose tissue in comparison to Angus steers (Sturdivant et al., 1992; May et al., 1993). These results suggest that the variability between breeds in ability to deposit monounsaturated fatty acids may have a genetic basis (Sturdivant et al., 1992). Stearoyl coenzyme A (**CoA**) desaturase is responsible for the conversion of saturated fatty acids to monounsaturated fatty acids, and differences in the expression of the stearoyl CoA desaturase gene between Angus and Wagyu cattle may be responsible for their differences in adipose tissue fatty acid composition. As the public becomes more health-conscious, determination of the physiological basis for these differences could prove to be useful for cattle producers. In this experiment, the mRNA concentrations and enzyme activities corresponding to stearoyl CoA desaturase were compared in subcutaneous adipose tissue of Angus and Wagyu steers that had been raised to the Japanese market end point.

Materials and Methods

Sample Collection

Angus (n = 10) and crossbred (3/4 and 7/8) Wagyu (n = 10) steers were fed by typical Japanese standards for 552 d. The diet, containing 25% roughage, was started when the Angus and Wagyu steers had mean initial weights of 220 and 260 kg, respectively (Lunt et al., 1993). The 20 steers were shipped to the Rosenthal Meat Science and Technology Center at Texas A&M University for slaughter. Immediately after exsanguination, subcutaneous adipose tissue samples were obtained by cutting through the hide at the sixth and seventh ribs. Samples of subcutaneous adipose tissue were frozen in liquid nitrogen for RNA extraction at a later time. Microsomes were extracted immediately for analysis of enzyme activity.

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RNA Extraction

Subcutaneous adipose tissue samples from a subset of five Angus and five Wagyu were selected randomly and homogenized in a solution containing 6.05 *M* guanidinium isothiocyanate, 1 m*M* sodium citrate, 26 m*M* sarkosyl, and .72% (vol/vol) β-mercaptoethanol (Chomczynski and Sacchi, 1987). The homogenate was washed twice with chloroform to remove the lipid followed by centrifugation at 2,500 × *g* for 10 min at 4°C. Sodium acetate was added to the aqueous, upper layer and mixed thoroughly. An equal volume of water-saturated phenol was added and mixed thoroughly. Chloroform/isoamyl alcohol (49:1) was added and the mixture was shaken vigorously for 10 s. The solution was incubated on ice for 15 min and centrifuged at 10,000 × *g* for 20 min at 4 °C. The upper aqueous phase was precipitated twice with equal volumes of isopropanol at −70°C for at least 1 h, followed by centrifugation at 10,000 × *g* for 20 min at 4°C. The RNA pellets were resuspended in 100 μL of sterile water. The RNA was quantified by spectrophotometric analysis at A_{260}/A_{280} with a Beckman DU-7 Spectrophotometer (Palo Alto, CA). The integrity of the RNA was confirmed by electrophoresis using a 1% denaturing formaldehyde/agarose gel (Current Protocols in Molecular Biology, 1991).

Hybridization of the rat liver stearoyl coenzyme A cDNA probe (**SCD1**; donated by R. MacDonald, Baylor College of Medicine) to the bovine RNA was confirmed by Northern transfer of 50 ng of the recovered RNA to Nytran® nylon membrane. Northern hybridization was carried out overnight at 42°C in prehybridization solution containing dextran sulfate and 2.3×10^7 cpm ^{32}P-dCTP-labeled SCD1 cDNA probe. The membrane was washed twice with 1 × SSC, .1% SDS for 15 min at 45°C, dried briefly, and exposed to x-ray film for 3 d at −70°C. Autoradiographs displayed a band at approximately 4.9 kb (Figure 1). The SCD1 cDNA clone hybridized to the same size transcript from rat liver (not shown). Only a light band of hybridization was observed for muscle in this preparation, although in other preparations the rat liver SCD1 cDNA bound strongly to muscle RNA. There was no evidence of binding to liver RNA. Adipose tissue RNA exhibited a diffuse band of hybridization (Figure 1).

One and 2 μg of RNA from Angus subcutaneous adipose tissue, liver, and muscle, and from Wagyu subcutaneous adipose tissue, liver, and muscle were used to determine the tissue distribution of stearoyl CoA desaturase mRNA. Isolated RNA was added to an equal volume of 10× SSC, 5.7 *M* formaldehyde and loaded onto nylon membrane using a Schleicher & Schuell Minifold II Slot-Blot Apparatus (Keene, NH). After drying the membrane for 7 min under a heat lamp, and for 2 h at 80°C in a vacuum oven, the membrane was prehybridized for 2 h at 42°C in a solution containing 25 m*M* potassium phosphate (pH 7.4), 5× SSC, 50% formamide, 5× Denhardt's solution, and 50 μg/mL salmon sperm DNA. The rat liver SCD1 cDNA was used as a probe to measure the hybridization signal for stearoyl CoA desaturase mRNA. Rat liver RNA was used as a positive control and bovine liver RNA was used as a negative control. The resultant autoradiograph was analyzed by laser densitometry. Results were analyzed per gram of tissue and per cell × 10^5.

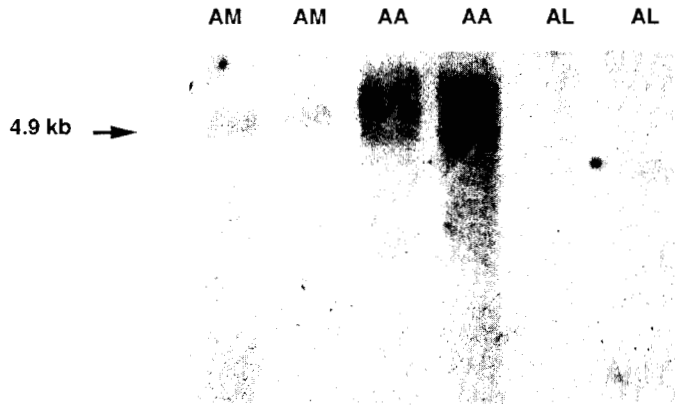

Figure 1. Northern analysis of 50 ng of RNA from Angus longissimus muscle (AM), Angus subcutaneous adipose tissue (AA), and Angus liver (AL) indicated hybridization of the rat liver SCD1 cDNA clone to an RNA band approximately 4.9 kb in size.

Enzyme Activity

Subcutaneous adipose tissue samples were homogenized immediately after slaughter in three volumes of .25 *M* sucrose, .01 *M* potassium phosphate (pH 7.4), 1 m*M* EDTA, and 1 m*M* DTT. The homogenate was centrifuged at 5,000 × *g* for 15 min. The supernate was centrifuged for 30 min at 17,300 × *g*. The microsomal fraction was isolated by centrifugation of the resultant supernate at 104,000 × *g* for 60 min. The microsomal pellets were resuspended in 3 mL of the homogenization buffer, frozen in liquid nitrogen, and stored at −70°C. Previous research has indicated that rat liver microsomal fractions that are frozen in liquid nitrogen and thawed rapidly (at 37°C) prior to analysis have desaturase activity comparable to that of fresh rat liver microsomal fractions (St. John et al., 1991).

The desaturation assay used a solution of 100 m*M* Tris-HCl (pH 7.4), 2 m*M* NADPH, 50 μ*M* [1-^{14}C]-stearate, 5 m*M* ATP, and 1 m*M* CoA, which had been allowed to equilibrate for 5 min. Three milligrams of microsomal protein was added and incubated for 7 min at 37°C in a shaking water bath (St. John et al., 1991). Three milliliters of 10% KOH was added and the incubations were heated at 70°C for 30 min. The solution was acidified using 9 mL of 3 *N* HCl. Fatty acids were extracted with three washes using 9 mL of *N*-pentane and one wash with 9 mL of acidic

water (pH 3.0). The pentane phase was evaporated under nitrogen and methylated with 14% BF_3 in methanol. The stearate and oleate methyl esters were separated using 10% $AgNO_3$ plates in a hexane: diethylether solvent system (9:1), and sprayed with .2% dichlorofluoroscein in ethanol. The spots were scraped and counted using a Beckman LS-3800 liquid scintillation spectrometer (Beckman, Houston, TX). The specific activity of the labeled stearate was used to calculate the rates of oleate formation. Protein content was measured by the bicinchoninic acid method (Pierce Chemical, Rockford, IL).

All data pertaining to total RNA, SCD1 mRNA levels, and desaturase activity were analyzed as a one-way ANOVA procedure (SAS, 1986). Means were generated for each breed using the Student-Newman-Keul procedure (Ott, 1984). A more detailed description of the fatty acid composition of the subcutaneous adipose tissues of the experimental animals is reported by May et al. (1993).

Adipose Tissue Cellularity

Procedures outlined by Etherton et al. (1977) as modified by Prior (1983) were used to determine adipocyte cellularity. Adipose tissue samples (100 to 150 mg) were stored at −40°C in screw-cap vials for the determination of adipose tissue cellularity. The samples were removed from the vials while they were still frozen and sliced to a 1-mm thickness, returned to the vials, and then fixed with osmium tetroxide. Subcutaneous and i.m. adipose tissue samples were frozen at −25°C and sliced in 1-mm-thick sections to facilitate tissue fixation. Fixed cells were filtered through 240-μm, 64-μm, and 20-μm nylon mesh screens using .01% Triton in .154 *M* NaCl. Cell fractions were collected from the 64-μm and 20-μm mesh screen for cell size and number determination using a Coulter Counter, Model ZM equipped with a channelizer, Model 256.

Results and Discussion

The Angus and Wagyu steers did not have significantly different amounts of recovered RNA when measured per gram of tissue. Because there was no difference in cellularity of the adipose tissues between breed types, RNA content also was not different when expressed per 10^5 cells (Table 1). The RNA yield in the subcutaneous adipocytes ranged rom 25 to 127 pg per cell. This is slightly higher than reported values of 26 pg for mouse fibroblast tissue culture cells (Brandhorst and McConkey, 1974).

Stearoyl CoA desaturase is the terminal step in the desaturation of fatty acids. It is responsible for the conversion of myristate, palmitate, and stearate into the Δ-9 monounsaturated fatty acids myristoleate, palmitoleate, and oleate, respectively. The terminal desaturase of the stearoyl CoA desaturase enzyme system is thought to be rate-limiting to fatty acid desaturation (Bernert and Sprechner, 1977). Because the desaturase enzyme has a lower maximal activity than fatty acid elongase (Bernert and Sprechner, 1975; St. John et al., 1991), stearoyl CoA desaturase also is the rate-limiting enzyme for the conversion of palmitate to oleate (St. John et al., 1991; Change et al., 1992).

Previous research in our laboratory has shown that, in cattle, stearoyl CoA desaturase enzyme activity is highest in adipose tissue and detectable in longissimus muscle and intestinal mucosa, but only rarely is measurable in bovine liver (St. John et al., 1991; Chang et al., 1992). This corresponds to the intensity of the hybridization signal seen when liver, muscle, and adipose tissue RNA were analyzed using a rat liver SCD1 cDNA clone in this study (Figure 1).

Slot blot analysis was used to compare stearoyl CoA desaturase mRNA levels across tissues, with rat liver RNA as a positive control. The resulting autoradiograph revealed that the bovine muscle and adipose

Table 1. RNA content, SCD1 mRNA content, and desaturase activity in subcutaneous adipose tissue of Angus and Wagyu steers

Measure	Angus	Wagyu	SEM[a]
Cells/g of tissue, × 10^5	7.957	7.958	.680
Desaturase activity[b]			
nmol/(7 min·g of tissue)	1.751	1.566	.540
nmol/(7 min·10^5 cells)	.228	.201	.72
nmol/(7 min·mg of protein)	1.359	1.110	.536
SCD1 mRNA[c]			
Units/g of tissue	116.56	124.77	30.11
Units/10^5 cells	14.204	15.251	3.215
Units/μg of RNA	2.260	3.277	.978
Total RNA			
μg/g of tissue	68.105	43.925	11.825
μg/10^5 cells	8.452	5.712	1.437

[a]SEM: standard error of the mean (n = 5 per breed type).
[b]Stearate (nmol) converted to oleate/7 min of incubation.
[c]Arbitrary hybridization units determined by laser densitometry.

tissue RNAs bound strongly to the rat liver SCD1 cDNA (Figure 2). There was no hybridization to bovine liver RNA, as seen in Figure 1. Although Figure 2 suggests a difference between breed types for adipose tissue stearoyl-CoA desaturase mRNA, there was no difference in mean stearoyl CoA desaturase mRNA concentrations between Angus and Wagyu subcutaneous adipose tissue samples (Table 1).

Tanaka (1985) and Sturdivant et al. (1992) documented an elevation of all monounsaturated fatty acids in subcutaneous adipose tissue of Japanese Black cattle, implying greater stearoyl CoA desaturase enzyme activity relative to other breed types. Sturdivant et al. (1992) and May et al. (1993) provided evidence that the elevated monounsaturated fatty acids in Japanese Black or American Wagyu cattle were due to genetic differences in stearoyl CoA desaturase gene transcription and(or) stearoyl CoA desaturase enzyme activity between the Angus and the Wagyu breeds. We recently reported a *Taq*I restriction fragment length polymorphism for the bovine stearoyl CoA desaturase gene in purebred Japanese Black cattle (Wilson et al., 1993). We observed alleles of of 7.4 and 19.7 kb, with frequencies of .69 and .31, respectively. Frequencies for the 7.4 kb and 19.7-kb alleles in 29 Angus, Hereford, and Brahman cattle were .83 and .17, respectively (J. J. Wilson and S. B. Smith, unpublished observations). Thus, there exists the possibility of differences in allelic frequency for the stearoyl CoA desaturase gene between Japanese Black (and American Wagyu) and other North American breeds of cattle, which may have functional significance.

However, in spite of potential differences in allelic frequency, and significant differences in monounsaturated fatty acids (May et al., 1993) between these breed groups, the concentration of stearoyl CoA desaturase mRNA was not significantly different between the two breeds when measured per gram of tissue, per microgram of RNA, or per 10^5 cells. The stearoyl CoA desaturase enzyme activity in subcutaneous adipose tissue also was not different ($P > .05$) between breeds (Table 1).

This experiment indicates that, although previous research has pointed to stearoyl CoA desaturase as the rate-limiting enzyme in the synthesis of monounsaturated fatty acids in bovine tissues (St. John et al., 1991; Chang et al., 1992), desaturase mRNA amounts and enzymatic activities could not explain differences in fatty acid composition between Angus and Waygu breed types. It is possible that the original hypothesis was incorrect; stearoyl CoA desaturase mRNA concentrations or enzyme activities were not related to monounsaturated fatty acid content of subcutaneous adipose tissue. Another possibility is that the animals were tested at the wrong stage of maturity. The Angus and Wagyu cattle were fed for 552 d and had fat thicknesses over the 12th rib exceeding 3 cm (Lunt et al., 1993). It is likely that the fatty acid composition of adipose tissue is determined by biochemical events that occur substantially earlier in the development of the adipose tissue.

It also is possible that differences in chronological age of the Wagyu and Angus steers was responsible for differences in fatty acid composition between these breed groups. Although the Angus and Wagyu steers were the same physiological maturity at slaughter (A^{91} and A^{87}, respectively), they were not the same age at slaughter (25.9 and 34.2 months, respectively) (Lunt et al., 1993). Monounsaturated fatty acids increase in bovine adipose tissue with age (Waldman et al., 1968), although the mechanism for this is unknown. Clearly, further experimentation using such techniques as nuclear run-on transcription rate studies, particularly with tissues earlier in development, should be used to clarify the contribution of

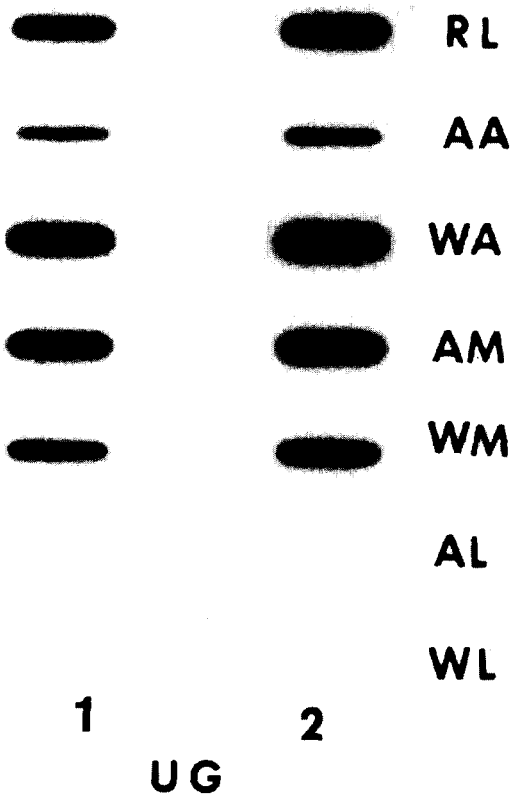

Figure 2. Slot-blot analysis of RNA from rat liver (RL), Angus and Wagyu subcutaneous adipose tissue (AA and WA), Angus and Wagyu longissimus muscle (AM and WM), and Angus and Wagyu liver (AL and WL). One and 2 μg of RNA were transferred to nylon membrane, and the rat liver SCD1 cDNA clone was hybridized to the RNA as described in the text. As indicated in Figure 1, there was virtually no binding of the cDNA clone to bovine liver RNA.

stearoyl CoA desaturase enzyme activity to the establishment of adipose tissue fatty acid composition.

Implications

This investigation was unsuccessful in demonstrating an enzymatic basis for differences in fatty acid composition between Angus and Wagyu steers. This suggests that events occurring earlier in the development of their adipose tissue were responsible for differences in fatty acid composition. To document this, animals must be sampled earlier in the development of their adipose tissue depots.

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