Published December 8, 2014

Lipogenesis and stearoyl-CoA desaturase gene expression and enzyme activity in adipose tissue of short- and long-fed Angus and Wagyu steers fed corn- or hay-based diets

K. Y. Chung,* D. K. Lunt,‡ H. Kawachi,† H. Yano,† and S. B. Smith*[1]

*Department of Animal Science, Texas A&M University, College Station 77843;
†Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan; and
‡Texas A&M University McGregor Research Center, McGregor 76657

ABSTRACT: Angus and Wagyu steers consuming high-roughage diets exhibit large differences in adipose tissue fatty acid composition, but there are no differences in terminal measures of stearoyl-CoA desaturase (SCD) activity or gene expression. Also, adipose tissue lipids of cattle fed corn-based diets have greater MUFA:SFA ratios than cattle fed hay-based diets. We hypothesized that any changes in SCD gene expression and activity would precede similar changes in adipose tissue lipogenesis between short- and long-fed endpoints. Furthermore, changes in SCD activity and gene expression between production endpoints would differ between corn- and hay-fed steers and between Wagyu and Angus steers. Angus (n = 8) and Wagyu (n = 8) steers were fed a corn-based diet for 8 mo (short-fed; 16 mo of age) or 16 mo (long-fed; 24 mo of age), whereas another group of Angus (n = 8) and Wagyu (n = 8) steers was fed a hay-based diet for 12 mo (short-fed; 20 mo of age) or 20 mo (long-fed; 28 mo of age) to match the end point BW of the corn-fed steers. Acetate incorporation into lipids in vitro was greater ($P < 0.01$) in corn-fed steers than in hay-fed steers and tended ($P = 0.06$) to be greater in Wagyu than in Angus s.c. adipose tissue because the rate in Wagyu was twice that of Angus adipose tissue in the corn-fed, short-fed steers. There were diet × end point interactions for lipogenesis in i.m. and s.c. adipose tissues (both $P < 0.01$) because lipogenesis was 60 to 90% lower in the long-fed cattle than in short-fed cattle fed the corn-based diet. The greatest SCD enzyme activity in Angus s.c. adipose tissue was observed at 24 mo of age (corn-based diet), but activity in Wagyu adipose tissue was greatest at 28 mo of age (hay-based diet; breed × diet × end point interaction, $P = 0.08$). For short- vs. long-fed endpoints in Angus, s.c. adipose tissue SCD activity was less (hay diet) or the same (corn diet). Conversely, SCD gene expression was greatest in long-fed Wagyu steers fed the hay- or corn-based diets (breed × end point interaction; $P < 0.01$). Contrary to our hypotheses, SCD activity increased over time, whereas lipogenesis from acetate decreased. However, the developmental pattern of SCD gene expression and activity differed markedly between hay-fed Angus and Wagyu adipose tissues, which may explain the differences in the MUFA:SFA ratios observed in adipose tissues from these cattle.

Key words: adipose tissue, bovine, lipogenesis, stearoyl-CoA desaturase

J. Anim. Sci. 2007. 85:380–387
doi:10.2527/jas.2006-087

INTRODUCTION

We previously demonstrated that s.c. and i.m. adipose tissues long-fed Japanese Black and their US counterparts, American Wagyu, accumulated more MUFA and fewer SFA than Angus steers (Sturdivant et al., 1992; May et al., 1993; Chung et al., 2006). In steers fed identical, high-roughage diets to the same physiological maturity, Wagyu s.c. adipose tissue lipids had a greater MUFA:SFA ratio than Angus adipose tissue lipids (May et al., 1993). However, stearoyl-CoA desaturase (**SCD**) enzyme activity and gene expression were similar in the s.c. adipose tissues of the Wagyu and Angus steers (Cameron et al., 1994). Therefore, the greater MUFA:SFA ratio the Wagyu steers may have been caused by greater SCD activity in Wagyu adipose tissue at some point earlier in production. To match physiological maturity, the Wagyu steers of May et al. (1993) were approximately 8 mo older than the Angus steers (Lunt et al., 1993), so the differences in the MUFA:SFA ratio reported by May et al. (1993) may have been

[1]Corresponding author: sbsmith@tamu.edu
Received February 15, 2006.
Accepted July 18, 2006.

caused by disparities in age rather than genetic differences between the Wagyu and Angus steers.

Expression of the SCD gene tripled from 5 to 12 mo of age in s.c. adipose tissue of Angus steer calves (Martin et al., 1999). From 12 to 18 mo of age, while the steers were fed a corn-based finishing diet, de novo lipogenesis increased nearly 3-fold, but SCD mRNA levels declined somewhat. Lipogenesis in s.c. adipose tissue declined in British-cross cattle after 19 mo of age (Smith et al., 1984). Therefore, we hypothesized that any changes in SCD gene expression and activity would precede similar changes in adipose tissue lipogenesis between short- and long-fed endpoints. Furthermore, changes in SCD activity and gene expression between production endpoints would differ between corn- and hay-fed steers and between Wagyu and Angus steers.

MATERIALS AND METHODS

Animals and Diets

Details of the production of these cattle were reported previously (Lunt et al., 2005) and are summarized briefly here. Care, handling, and sampling of the steers were approved by the Texas A&M University Institutional Animal Care and Use Committee. Sixteen Angus and 16 American Wagyu steers were purchased as weanling calves (approximately 8 mo of age) and assigned to 1 of 2 dietary treatments: a high-energy, corn-based finishing diet designed to support 1.36 kg of ADG/d; and a medium-energy, coastal Bermudagrass hay-based diet designed to support 0.9 kg of ADG/d (Table 1). One Angus steer from the corn-fed group escaped the holding pen before slaughter and had to be removed from the investigation.

Corn-fed steers were fed for 8 mo (short-fed) or 16 mo (long-fed), and the hay-fed steers were fed for 12 mo (short-fed) or 20 mo (long-fed) after weaning (n = 4 per breed and time on feed). Because age at weaning was similar between breed types, age at each sampling period was similar between breed types. Short-fed steers fed the corn- or hay-based diets were 16 and 20 mo of age, respectively, and long-fed steers fed the corn- or hay-based diets were 24 and 28 mo of age, respectively.

For the corn-fed steers, actual ADG for the Angus and Wagyu steers were 1.25 and 1.03 kg, respectively (short-fed), and 0.93 and 0.84 kg, respectively (long-fed). For the hay-fed steers, actual ADG for the Angus and Wagyu steers were 0.88 and 0.84 kg, respectively (short-fed) and 0.78 and 0.73 kg, respectively (long-fed; Lunt et al., 2005). The average initial BW for Angus and Wagyu steers were 210 and 174 kg, respectively, and the diets were designed so that hay-fed and corn-fed steers within a breed type achieved a similar BW at sampling. The short-fed Angus and Wagyu steers weighed 527 and 454 kg, respectively, at sampling. The long-fed Angus and Wagyu steers weighed 663 and 588 kg, respectively, at sampling (Lunt et al., 2005).

Table 1. Ingredients and chemical composition of the high-corn diet at each time-on-feed interval

	Diet at each interval			
Item[1]	1 mo	2 mo	3 mo	4 mo
Ground milo	20.00	20.00	20.00	20.00
Ground corn	21.80	40.55	47.55	48.05
Cottonseed meal	10.00	8.00	6.50	6.00
Cottonseed hulls	35.00	20.00	15.00	15.00
Molasses	10.00	8.00	7.50	7.50
Limestone	0.96	0.96	0.96	0.96
Trace mineralized salt[2]	0.56	0.56	0.56	0.56
Dicalcium phosphate	0.23	0.23	0.23	0.23
Potassium chloride	0.16	0.16	0.16	0.16
Zinc oxide	0.01	0.01	0.01	0.01
Ammonium sulphate	0.00	0.25	0.25	0.25
Vitamin premix[3]	0.08	0.08	0.08	0.08
R-1500[4]	1.20	1.20	1.20	1.20
Total percentage	100.00	100.00	100.00	100.00
Nutritional composition[5]				
Dry matter, %	88.80	89.08	89.13	89.13
CP, %	11.41	11.58	11.34	11.16
NE_m, Mcal/kg	1.48	1.72	1.81	1.81
NE_g, Mcal/kg	0.88	1.11	1.19	1.19
ADF, %	27.04	17.50	14.19	14.12
Calcium, %	0.58	0.54	0.52	0.52
Phosphorus, %	0.34	0.36	0.36	0.36

[1]Percentage of total diet (as-fed basis).
[2]Trace mineralized salt: NaCl, 98%; Zn, 0.35%; Mn, 0.28%; Fe, 0.175%; Cu, 0.035%; I, 0.007%; and Co, 0.0007%.
[3]Vitamin premix: vitamin A, 2.2×10^6 IU/kg; vitamin D, 1.1×10^6 IU/kg; and vitamin E, 2.2×10^3 IU/kg.
[4]R-1500 = 1.65 g of monensin sodium (Rumensin)/kg.
[5]Percentage of DM (NRC, 1996).

After being fed for their respective time periods, the steers in each group were slaughtered on 2 consecutive days. All steers were slaughtered at the Rosenthal Meat Science and Technology Center on the Texas A&M University campus. Immediately postexsanguination, a portion of the LM from the fifth to eighth thoracic vertebrae region (with adhering s.c. adipose tissue) was removed for sampling of adipose tissue for lipogenesis and cellularity (s.c. and i.m.), or SCD enzyme activity and gene expression (s.c. only).

In Vitro Lipogenesis

Two-hour in vitro incubations were performed with fresh i.m. and s.c. adipose tissues as described previously (May et al., 1994). Acetate incorporation into neutral lipids was measured by incubating adipose tissue (50 to 100 mg) in Krebs-Henseleit bicarbonate buffer (pH 7.4), 5 m*M* sodium acetate, 5 m*M* glucose, 10 m*M* hepes buffer, and 1 μCi [U-^{14}C]acetate (Amersham, Arlington Heights, IL) in 3 mL of total volume. The vials were gassed for 1 min with 95% O_2:5% CO_2, capped, and incubated in a shaking water bath (37°C) for 2 h. The reactions were terminated by adding 3 mL of 5% trichloroacetic acid to each vial.

Lipids were extracted according to published procedures (Folch et al., 1957; May et al., 1994). Lipids were

evaporated to dryness with a MultiVap evaporator (Associates Inc., South Berlin, MA) in a 40°C water bath. The lipids were resuspended with 10 mL of Econo-Safe scintillation fluid (Research Products International Corp., Mount Prospect, IL) and counted on a Beckman LS-3800 scintillation counter (Beckman Instruments, Palo Alto, CA). The incorporation rate of acetate into neutral lipid is expressed as nmol·2 h^{-1}·10^5 $cells^{-1}$.

Cellularity

The procedure of Etherton et al. (1977), as modified by Prior (1983), was used to estimate the number of adipocytes per gram of adipose tissue. Frozen i.m. and s.c. adipose tissues were sectioned (1-mm thick), fixed with 3% osmium tetraoxide, and digested with 8 *M* urea. The fixed cells were filtered through 240-, 64-, and 20-μm nylon mesh screens with 0.01% Triton in 0.154 *M* NaCl. Cell fractions collected from the 64 and 20-μm mesh screens were used to determine cells per gram of adipose tissue with a Coulter Counter [Model ZM equipped with a channelizer (Model Z56), Coulter Electronics, Hialeah, GA].

Microsome Extraction and SCD Enzyme Activity

Subcutaneous adipose tissue was homogenized in 3 volumes (wt/vol) of buffer with a Polytron (The Virtis Company Inc., Gardiner, NY) for 60 s. The buffer (pH = 7.4) was composed of 0.25 *M* sucrose, 0.01 *M* potassium phosphate, 1 m*M* EDTA, and 1 m*M* dithioerythritol. The homogenate was centrifuged at 5,000 × *g* for 15 min. The supernate was decanted into another tube, and the pellet and fat cake were discarded. The supernate was centrifuged at 17,300 × *g* for 30 min and decanted into another tube, which was centrifuged at 104,000 × *g* for 2 h. The supernate was discarded, and the microsomes were resuspended in 100 m*M* Tris-HCl buffer (pH = 7.4), snap-frozen in liquid nitrogen, and stored at –80°C until further analysis.

Stearoyl-CoA desaturase enzyme activity was determined as described by St. John et al. (1991) and as modified by Archibeque et al. (2005). The assay system was composed of 100 m*M* Tris-HCl (pH 7.4), 2 m*M* NADPH, 25 μ*M* palmitoyl-CoA, and 0.025 μCi of [1-^{14}C]palmitoyl-CoA in 1.5 mL of total volume. The reactions were begun by addition of 0.1 mg of protein and were incubated in a 37°C water bath for 7 min. The reaction was stopped by adding 1 mL of 12% KOH in ethanol, followed by heating for 1 h at 80°C. After acidification by addition of 9 mL of 3 *N* HCl, fatty acids were washed with 9 mL of n-pentane. The pentane phases were evaporated under nitrogen and methylated by the addition of 14% BF_3 in methanol. Methyl esters were separated by thin layer chromatography on a 10% $AgNO_3$-impregnated, silica gel plate in a petroleum ether:diethylether (97:3) solvent system. Plates were sprayed with 0.2% dichlorofluoroscein in ethanol, and spots containing palmitate methyl ester and palmitoleate methyl ester were scraped and counted using a liquid scintillation spectrometer. The ratio of palmitate methyl ester:palmitoleate methyl ester was used to quantify nanomoles of palmitic acid converted to palmitoleic acid·7 min^{-1}·mg of microsomal $protein^{-1}$.

Northern Blot Analysis

The cDNA fragment of the bovine SCD gene (662 bp, GenBank accession number AB075020) was cloned into the vector of pCRII-TOPO (Invitrogen, Carlsbad, CA) using reverse transcription-PCR (**RT-PCR**) and conventional recombinant DNA techniques. The primers used for RT-PCR were: forward primer, 5′-CCTGTGGAGTCACCGAACC-3′, and reverse primer, 5′-CCTTGGATACTTTCTTCCGGTC-3′. The cDNA insert was confirmed by sequencing. The digoxigenin (**DIG**)-labeled anti-sense RNA probe was generated with DIG RNA Labeling Kit (SP6/T7, Roche Diagnostics, Mannheim, Germany).

Total RNA samples (2 μg) were separated with a 1% agarose gels containing 6.7% formaldehyde and transferred to a Hybond N+ membrane (Amersham). The membrane was hybridized with the DIG-labeled RNA probes. The bands corresponding to SCD mRNA were detected using DIG Luminescent Detection Kit for Nucleic Acids (Roche Diagnostics). Northern blotting was analyzed by ImageQuant, version 5.2 software (Molecular Dynamics, GE Healthcare Bio-Sciences Corp., Piscataway, NJ). The SCD bands were normalized to the 28S ribosomal RNA bands.

Statistical Analyses

Data were analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC) as a 3-factor design. Data for cellularity, lipogenesis, SCD enzyme activity, and SCD gene expression were evaluated for the main effects of breed type, diet, and end point (short- vs. long-fed), and the model tested all 2- and 3-way interactions against their appropriate error terms in the *F*-test. Interaction means were separated using the probability statement of GLM ($P < 0.05$).

RESULTS

Cellularity and Lipid Synthesis

Intramuscular adipose tissue contained more adipocytes/100 mg of adipose tissue of Angus steers than in Wagyu steers ($P < 0.01$). There was a breed type × end point interaction ($P = 0.01$); short-fed Angus steers had fewer i.m. adipocytes/100 mg of adipose tissue than long-fed Angus steers. There also was a diet × end point interaction for i.m. adipose tissue ($P = 0.05$), in that adipocytes/100 mg of adipose tissue was greater in long-fed steers than in short-fed steers consuming corn but did not differ between production endpoints in hay-fed steers. The number of adipocytes/100 mg of adipose

Table 2. Adipose tissue cellularity and acetate incorporation into lipids in s.c. and i.m. adipose tissues of short-fed and long-fed Angus and Wagyu steers

	Tissue and measure			
	Intramuscular		Subcutaneous	
Group, diet, and breed	Cells[1]/100 mg	Lipogenesis[2]	Cells[1]/100 mg	Lipogenesis[2]
Short-fed steers				
Corn (16 mo of age)				
Angus	0.171	18.5	0.177	47.8
Wagyu	0.161	31.1	0.234	109.2
Hay (20 mo of age)				
Angus	0.210	3.8	0.109	11.5
Wagyu	0.194	3.1	0.161	14.9
Long-fed steers				
Corn (24 mo of age)				
Angus	0.336	1.2	0.218	15.4
Wagyu	0.171	5.7	0.120	35.6
Hay (28 mo of age)				
Angus	0.240	7.4	0.195	27.9
Wagyu	0.138	9.9	0.137	25.3
SE	0.015	2.6	0.015	7.8
P-value				
Breed	<0.01	0.29	0.69	0.05
Diet	0.61	0.08	0.20	<0.01
End point	0.10	0.08	0.93	0.06
Breed × diet	0.62	0.40	0.75	0.06
Breed × end point	0.01	0.79	0.03	0.25
Diet × end point	0.05	<0.01	0.24	<0.01
Breed × diet × end point	0.52	0.53	0.68	0.39

[1]Adipocytes/100 mg of adipose tissue, millions.
[2]Nanomoles of acetate incorporated·2 h^{-1}·10^5 $cells^{-1}$.

tissue was similar across breed type ($P = 0.68$), diet ($P = 0.20$), and end point ($P = 0.92$) in s.c. adipose tissue (Table 2). There was a breed type × end point interaction ($P = 0.03$); short-fed Angus steers had fewer adipocytes/100 mg of s.c. adipose tissue than long-fed Angus steers, whereas the reverse was true for Wagyu steers.

There was a diet × end point interaction for acetate incorporation into fatty acids in i.m. and s.c. adipose tissues (both $P < 0.01$; Table 2). The greatest lipogenic activity was observed in adipose tissues of short-fed cattle and was over 90% less than in long-fed cattle consuming the corn-based diet. Conversely, lipogenesis in i.m. and s.c. adipose tissues was greater in long-fed steers than in short-fed steers consuming the hay-based diet. Lipogenesis tended to be greater ($P = 0.06$) in Wagyu s.c. adipose tissue than in Angus s.c. adipose tissue, primarily because of the greater rates in samples from the short-fed Wagyu steers consuming the corn-based diet. Lipogenesis was greater ($P < 0.01$) in s.c. adipose tissue of corn-fed steers than in hay-fed steers.

Stearoyl-CoA Desaturase Enzyme Activity and Gene Expression

There was no effect of breed type ($P = 0.99$) or diet ($P = 0.40$) on SCD enzyme activity (Figure 2). There was greater SCD activity in s.c. adipose tissue from long-fed steers than in short-fed steers ($P < 0.01$), and there tended to be diet × end point ($P = 0.08$) and breed × diet × end point ($P = 0.08$) interactions. The diet × end point interaction was caused by a greater increase in SCD activity over time in corn-fed steers than in hay-fed steers (Figure 1). The 3-way interaction indicated that SCD activity was greater in long-fed than in short-fed Angus steers consuming the corn-based diet; SCD activity was similar in short- and long-fed Angus steers consuming the hay-based diet, and the increase in SCD activity over time was similar in corn- and hay-fed Wagyu steers.

Stearoyl-CoA desaturase mRNA was approximately 4.9 kb in size (Figure 2). The northern blot indicated that SCD gene expression was very low in adipose tissue from the short-fed steers fed the corn-based diet. For this reason, there was a diet × end point interaction ($P = 0.05$), in that SCD gene expression was less in corn-fed, short-fed steers than in the any of the other production groups.

Expression of the SCD gene tended ($P = 0.06$) to be greater in hay-fed steers than in corn-fed steers and tended ($P = 0.06$) to be greater in long-fed cattle than in short-fed cattle (Figure 2). The northern blots indicated that the greatest degree of SCD hybridization was observed in Wagyu adipose tissue, especially in samples from long-fed Wagyu steers fed the corn-based diet. There was no main effect of breed type on SCD gene expression ($P = 0.34$), but there was a breed × end point

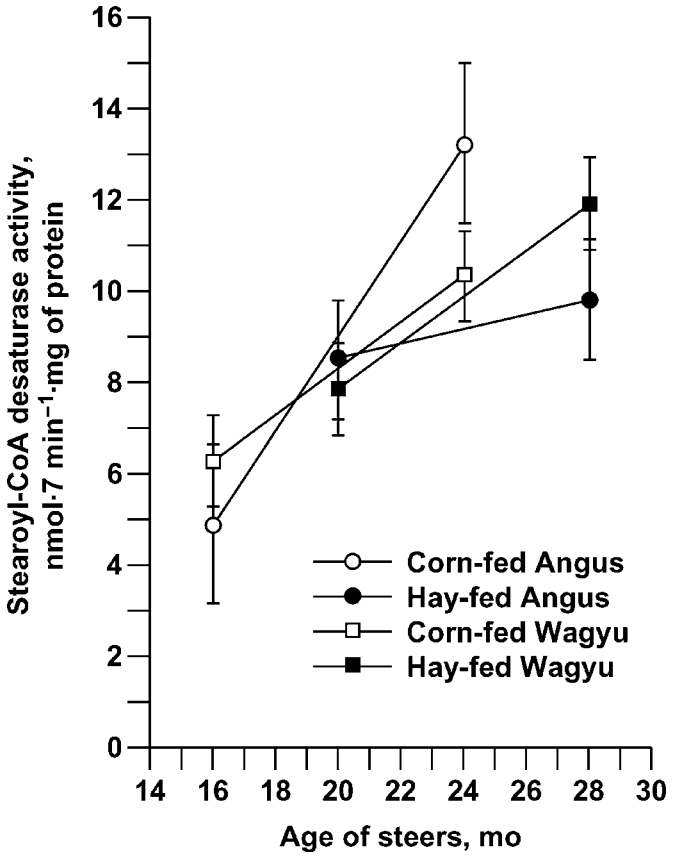

Figure 1. Stearoyl-CoA desaturase (SCD) enzyme activity in s.c. adipose tissue of short-fed and long-fed Angus and Wagyu steers fed corn- or hay-based diets. Values are for 3 short-fed, corn-fed Angus steers and for 4 steers at all other sampling periods. The SEM for the diet × end point interactions are affixed to the symbols. *P*-values for Breed (B), Diet (D), End point (E), B × D, D × E, B × E, and B × D × E are 0.99, 0.40, <0.01, 0.45, 0.08, 0.70, and 0.08, respectively.

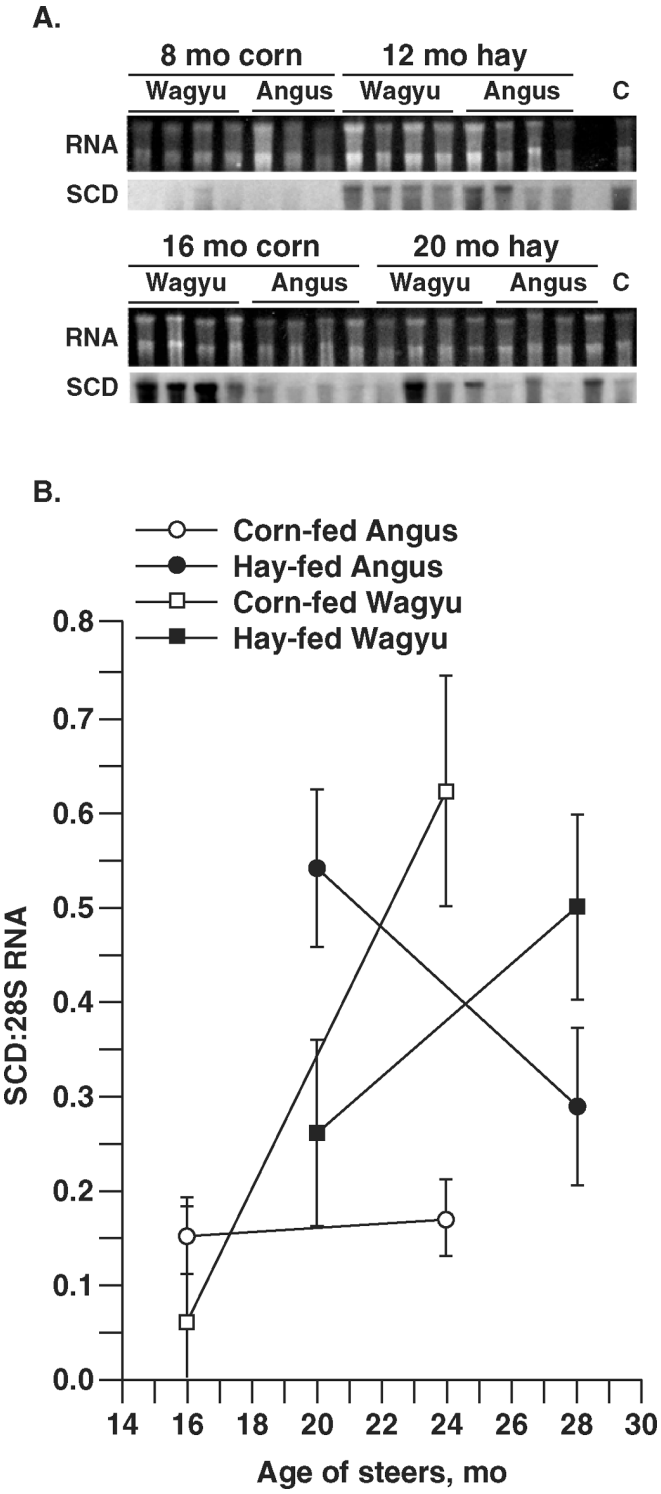

Figure 2. Stearoyl-CoA desaturase (SCD) gene expression in s.c. adipose tissue of short-fed and long-fed Angus and Wagyu steers fed corn- or hay-based diets. A) ethidium-stained gels and northern blots. Steers were fed the corn-based diet for 8 or 16 mo (16 and 24 mo of age) or the hay-based diet for 12 or 20 mo (20 and 28 mo of age). C = internal control. B) mean values for the SCD:28S ratios. The SEM for the diet × end point interactions are affixed to the symbols. *P*-values for Breed (B), Diet (D), End point (E), B × D, D × E, B × E, and B × D × E are 0.34, 0.06, 0.06, 0.15, 0.05, <0.01, and 0.87, respectively.

interaction ($P < 0.01$); SCD gene expression was less in adipose tissue of short-fed than in long-fed Wagyu steers but was greater in adipose tissue of short-fed than in long-fed Angus steers.

There was a significant relationship between the 16:1n-7/18:0 ratio and SCD activity ($R^2 = 0.37$; $P = 0.02$; Figure 3A) and between the 16:1n-7/18:0 ratio and SCD gene expression ($R^2 = 0.27$; $P = 0.05$; Figure 3B), but only for the corn-fed steers. There was no relationship between the 16:1n-7/18:0 ratio and SCD activity or gene expression for the hay-fed steers ($R^2 = 0.05$, $P = 0.43$; and $R^2 = 0.05$, $P = 0.42$, respectively).

DISCUSSION

Carcass data for this study were reported previously (Lunt et al., 2005) and are summarized briefly in Table 3. Angus steers had greater carcass weights, adjusted fat thickness, and yield grades than Wagyu steers. Long-fed steers had greater carcass weights, marbling

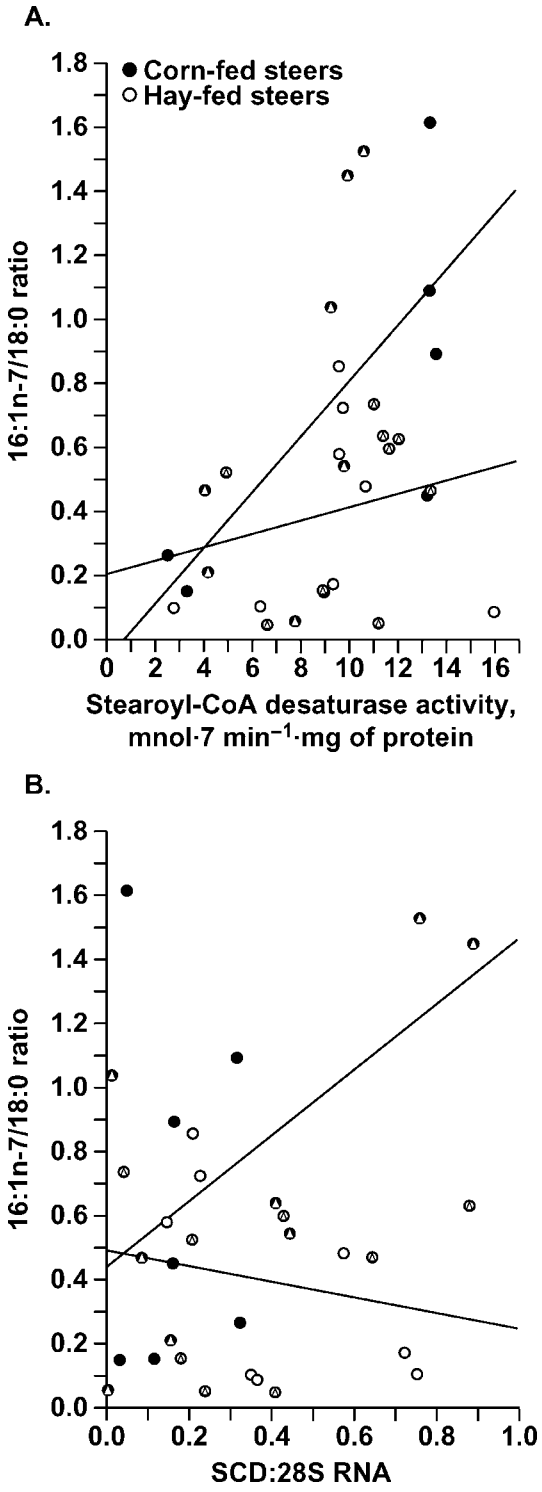

Figure 3. Relationship between the 16:1n-7/18:0 ratio and (A) stearoyl-CoA desaturase (SCD) activity or (B) SCD RNA in s.c. adipose tissue of short- and long-fed Wagyu and Angus steers fed corn- or hay-based diets. (A) For 16:1n-7/18:0 vs. stearoyl-CoA desaturase activity: corn-fed steers, y = 0.08x – 0.05, R^2 = 0.37, *P* = 0.02; hay-fed steers, y = 0.02x + 0.22, R^2 = 0.05, *P* = 0.43. (B) For 16:1n-7/18:0 vs. SCD RNA: corn-fed steers, y = 1.02x + 0.44, R^2 = 0.27, *P* = 0.05; hay-fed steers, y = –0.25x + 0.49, R^2 = 0.05, *P* = 0.42. Symbols for Wagyu steers contain open triangles in both panels.

Table 3. Selected carcass traits of short-fed and long-fed Angus and Wagyu steers fed corn- or hay-based diets[1]

	Item			
Group, diet, and breed	Carcass wt, kg	Marbling score[2]	AFT,[3] cm	Yield grade[2]
Short-fed steers				
Corn (16 mo of age)				
Angus	323.4	673.3	1.44	3.33
Wagyu	252.3	612.5	0.95	2.75
Hay (20 mo of age)				
Angus	307.7	580.0	1.30	3.33
Wagyu	283.0	572.5	1.05	3.08
Long-fed steers				
Corn (24 mo of age)				
Angus	407.8	802.5	2.51	5.17
Wagyu	357.2	897.5	1.53	3.27
Hay (28 mo of age)				
Angus	403.0	672.5	1.90	4.04
Wagyu	353.1	762.5	1.30	3.29
SE	11.2	29.2	0.11	0.16
P-value				
Breed	<0.01	0.56	<0.01	<0.01
Diet	0.90	0.05	0.19	0.33
End point	<0.01	<0.01	<0.01	<0.01
Breed × diet	0.40	0.81	0.35	0.08
Breed × end point	0.92	0.21	0.20	0.03
Diet × end point	0.66	0.51	0.24	0.08
Breed × diet × end point	0.40	0.77	0.82	0.31

[1]Data are from Lunt et al. (2005), modified to include *P*-values for all possible interactions.
[2]Modest = 500; Moderate = 600; Slightly Abundant = 700; Moderately Abundant = 800.
[3]AFT = adjusted fat thickness.

scores, adjusted fat thickness, and yield grades than short-fed steers. Corn-fed steers had greater marbling scores than hay-fed steers. There was a much greater increase in yield grades between the short- and long-fed endpoints in Angus steers than in Wagyu steers.

Adipocyte volume was not measured for these adipose tissue samples. However, in short-fed Angus and Wagyu steers, there was no difference in the number of adipocytes/100 mg of adipose tissue (i.e., adipocyte density), suggesting that i.m. and s.c. adipocytes volumes were similar at this production end point. As expected, adipocyte density was less in long-fed than in short-fed Wagyu steers, consistent with a putative increase in adipocyte volume. Conversely, adipocyte density was greater in long-fed than in short-fed Angus steers, which was contrary to expectations. Greater adipocyte density in the long-fed Angus steers suggests an emergence of a new, smaller population of adipocytes in these steers, but we do not have the necessary data to confirm this.

We previously demonstrated that acetate incorporation into fatty acids in s.c. adipose tissue of Angus × Hereford and Red Poll steers increased approximately 9-fold between 10 and 16 mo of age and then decreased by 50% with additional time on feed (to 19 mo of age; Smith et al., 1984). A similar pattern was observed for activities of the lipogenic enzymes acetyl-CoA carboxyl-

ase, fatty acid synthetase, ATP-citrate lyase, and NADP-malate dehydrogenase (Smith et al., 1984). In the current study, the depressed rate of lipogenesis in adipose tissues of long-fed steers fed the corn-based diet was similar to the loss of lipogenic capacity reported in our early study. Expression of the SCD gene tripled from 5 to 12 mo of age in s.c. adipose tissue of Angus steer calves (Martin et al., 1999). From 12 mo of age, the calves were fed a corn-based finishing diet to 18 mo of age. During this latter period, the rate of de novo lipogenesis nearly tripled, but SCD mRNA levels declined somewhat. Taken together, the results of our previous experiments suggested that increases in SCD gene expression preceded proportional increases in lipogenesis in s.c. adipose tissue. Therefore, we hypothesized that any changes in SCD activity that occurred between production endpoints would be reflected in parallel changes in lipogenesis. This was true for hay-fed steers of the current study, which exhibited slightly greater rates of SCD activity and lipogenesis in long-fed cattle than in short-fed cattle. In corn-fed steers, however, there was over a 2-fold increase in SCD activity between endpoints, whereas lipogenesis declined sharply at some time between endpoints. This suggests that SCD gene expression and the expression of genes contributing to de novo fatty acid biosynthesis were regulated independently in s.c. adipose tissue of the corn-fed cattle.

The results of this study confirmed our final hypothesis that the patterns of change over time for SCD activity and gene expression would differ between breed types. The long-fed, hay-fed Angus and Wagyu steers represented the group most similar to the steers of our previous reports (Lunt et al., 1993; May et al., 1993; Cameron et al., 1994). In the current study, SCD activity was 20% greater, and SCD mRNA was 70% greater, in Wagyu than in Angus adipose tissue. Cameron et al. (1994) reported that SCD activity was 20% less (not greater) and SCD mRNA was 45% greater in Wagyu than in Angus adipose tissue (n = 5 per breed type), although neither difference was significant. The Wagyu steers of Cameron et al. (1994) were over 32 mo of age, whereas the Angus steers were 26 mo of age; and SCD gene expression and catalytic activity may have been declining in the Wagyu steers by the time the cattle were sampled.

Lee et al. (2005) reported a nearly 3-fold increase in SCD gene expression from 6 to 12 mo of age in LM of Korean Hanwoo steers; there was a slight decline in SCD mRNA levels between 18 and 30 mo of age. The Hanwoo steers of Lee et al. (2005) were fed a commercial concentrate to 12 mo of age, followed by free choice access to concentrates to 30 mo of age. Details of the diets were not provided by Lee et al. (2005), but probably the diet was intermediate in concentrates between our corn- and hay-based diets. The small decline in SCD mRNA levels between 18 and 30 mo of age reported by Lee et al. (2005) was similar to the results observed for the short- and long-fed Angus steers of this study. Therefore, the pattern of SCD gene expression over time in the Hanwoo steers more closely resembled that of the Angus steers than the Wagyu steers.

Lee et al. (2005) also demonstrated that the increase in SCD gene expression in Hanwoo adipose tissue preceded an increase in a Δ9 desaturase index (the ratio of 18:1n-9/18:0). To confirm the relationship between SCD activity and gene expression and adipose tissue fatty acid composition in the current study, s.c. adipose tissue 16:1n-7/18:0 ratios were plotted as a function of SCD activity and gene expression. Although there was a significant relationship between the 16:1n-7/18:0 ratio and SCD activity and between the 16:1n-7/18:0 ratio and SCD gene expression, this was only for the corn-fed steers. We have argued against the use of fatty acid ratios to predict SCD activity (Archibeque et al., 2005). The 16:1n-7/18:0 ratio may be predictive of SCD activity under some conditions, such as the corn-fed steers of this study, but certainly failed to predict SCD activity in the hay-fed steers.

There is a remarkably consistent relationship between the concentrations 16:1n-7 and 18:0 in adipose tissues across studies. For adipose tissue lipids from these steers, the relationship between 16:1n-7 and 18:0 was $y = 0.015x^2 - 0.796x + 12.32$; $R^2 = 0.93$ (Chung et al., 2006). The relationship across 4 independent studies from our laboratory was $0.016x^2 - 0.835x + 12.04$; $R^2 = 0.91$ (reviewed in Smith et al., 2004). Unlike 18:1n-9, there is little 16:1n-7 present in the diet, so any 16:1n-7 occurring in adipose must arise from desaturation of 16:0. The high correlation between 16:1n-7 and 18:0 further indicates that, as 16:1n-7 concentrations increase, 18:0 concentrations decrease, arguing strongly that SCD activity regulates the 16:1n-7/18:0 ratio. However, the lack of correlation between the 16:1n-7/18:0 ratio and SCD activity of the hay-fed steers indicates that SCD activity did not solely dictate the concentrations of these fatty acids.

The plasma 16:1n-7/18:0 ratio was over 50% less in hay-fed steers than in corn-fed steers (Chung et al., 2006). We previously reported SCD activity in bovine liver and duodenal mucosal cells (Chang et al., 1992; Archibeque et al., 2005) and suggested that some portion of the MUFA in adipose tissues may arise from hepatic and mucosal desaturation of dietary SFA. The lesser 16:1n-7/18:0 ratio in plasma of hay-fed steers suggests depressed SCD activity in liver cells, intestinal mucosal cells, or both, relative to corn-fed steers. Thus, the uptake and deposition of plasma fatty acids may strongly influence adipose tissue fatty acid composition, especially under conditions in which endogenous rates of fatty acid biosynthesis are depressed (i.e., as in the adipose tissues of the hay-fed steers).

Chung et al. (2006) confirmed our previous observation (May et al., 1993) that Wagyu adipose tissue has a greater MUFA:SFA ratio than Angus adipose tissue and that this is true even when cattle are sampled at the same age. Previous studies reported contrasts in SCD activity between breed types (Cameron et al.,

1994; Siebert et al., 2003), whereas others reported developmental changes in SCD gene expression (Martin et al., 1999; Lee et al., 2005). This is the first study to demonstrate the interactions between breed type, age, and diet on adipose tissue lipogenesis and SCD activity and gene expression.

LITERATURE CITED

Archibeque, S. L., D. K. Lunt, R. K. Tume, and S. B. Smith. 2005. Fatty acid indices of stearoyl Co-A desaturase activity do not reflect actual stearoyl Co-A desaturase enzyme activity in adipose tissues of beef steers finished with corn-, flaxseed-, or sorghum-based diets. J. Anim. Sci. 83:1153–1166.

Cameron, P. J., M. Rogers, J. Oman, S. G. May, D. K. Lunt, and S. B. Smith. 1994. Stearoyl coenzyme A desaturase enzyme activity and mRNA levels are not different in subcutaneous adipose tissue from Angus and American Wagyu steers. J. Anim. Sci. 72:2624–2628.

Chang, J. H. P., D. K. Lunt, and S. B. Smith. 1992. Fatty acid composition and fatty acid elongase and stearoyl-CoA desaturase activities in tissues of steers fed high oleate sunflower seed. J. Nutr. 122:2074–2080.

Chung, K. Y., D. K. Lunt, C. B. Choi, S. H. Chae, R. D. Rhoades, T. L. Adams, B. Booren, and S. B. Smith. 2006. Lipid characteristics of subcutaneous adipose tissue and M. longissimus thoracis of Angus and Wagyu steers fed to U.S. and Japanese endpoints. Meat Sci. 73:432–441.

Etherton, T. D., E. H. Thompson, and C. E. Allen. 1977. Improved techniques for studies of adipocyte cellularity and metabolism. J. Lipid Res. 18:552–557.

Folch, J., M. Lees, and G. H. Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226:497–509.

Lee, S. H., D. H. Yoon, N. J. Choi, S. H. Hwang, E. Y. Cheong, S. J. Oh, I. C. Cheong, and C. S. Lee. 2005. Developmental relationship of unsaturated fatty acid composition and stearoyl-CoA desaturase mRNA level in Hanwoo steers' muscle. Asian-australas. J. Anim. Sci. 18:562–566.

Lunt, D. K., K. Y. Chung, C. B. Choi, and S. B. Smith. 2005. Production characteristics and carcass quality of Angus and Wagyu steers fed to U.S. and Japanese endpoints. J. Anim. Vet. Adv. 4:949–953.

Lunt, D. K., R. R. Riley, and S. B. Smith. 1993. Growth and carcass characteristics of Angus and American Wagyu steers. Meat Sci. 34:327–334.

Martin, G. S., D. K. Lunt, K. G. Britain, and S. B. Smith. 1999. Postnatal development of stearoyl coenzyme A desaturase gene expression and adiposity in bovine subcutaneous adipose tissue. J. Anim. Sci. 77:630–636.

May, S. G., J. W. Savell, D. K. Lunt, J. J. Wilson, J. C. Laurenz, and S. B. Smith. 1994. Evidence for preadipocyte proliferation during culture of subcutaneous and intramuscular adipose tissues from Angus and Wagyu crossbred steers. J. Anim. Sci. 72:3110–3117.

May, S. G., C. A. Sturdivant, D. K. Lunt, R. K. Miller, and S. B. Smith. 1993. Comparison of sensory characteristics and fatty acid composition between Wagyu crossbred and Angus steers. Meat Sci. 35:389–398.

NRC. 1996. Nutrient Requirements of Beef Cattle. 7th ed. Natl. Acad. Press, Washington, DC.

Prior, R. L. 1983. Lipogenesis and adipose tissue cellularity in steers switched from alfalfa hay to high concentrate diets. J. Anim. Sci. 56:483–492.

Siebert, B. D., W. S. Pitchford, Z. A. Kruk, H. Kuchel, M. P. Deland, and C. D. Bottema. 2003. Differences in Δ9 desaturase activity between Jersey- and Limousin-sired cattle. Lipids 38:539–543.

Smith, S. B., R. L. Prior, C. L. Ferrell, and H. J. Mersmann. 1984. Interrelationships among diet, age, fat deposition and lipid metabolism in growing steers. J. Nutr. 114:153–162.

Smith, S. B., D. R. Smith, and D. K. Lunt. 2004. Chemical and physical characteristics of meat: Adipose tissue. Pages 225–238 in Encyclopedia of Meat Sciences. W. Jensen, C. Devine, and M. Dikemann, ed. Elsevier Science Publishers, Oxford, UK.

St. John, L. C., D. K. Lunt, and S. B. Smith. 1991. Fatty acid elongation and desaturation enzyme activities of bovine liver and subcutaneous adipose tissue microsomes. J. Anim. Sci. 69:1064–1073.

Sturdivant, C. A., D. K. Lunt, G. C. Smith, and S. B. Smith. 1992. Fatty acid composition of subcutaneous and intramuscular adipose tissues and M. longissimus dorsi of Wagyu cattle. Meat Sci. 32:449–458.