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Evidence for Preadipocyte Proliferation During Culture of Subcutaneous and Intramuscular Adipose Tissues from Angus and Wagyu Crossbred Steers[1]

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ABSTRACT: The primary objective of this study was to provide evidence for preadipocyte proliferation during culture of adipose tissue explants; a secondary objective was to compare the lipogenic activity and cellularity of adipose tissues from American Wagyu crossbred steers. Subcutaneous (s.c.) and intramuscular (i.m.) adipose tissues were obtained at slaughter from the 2nd to 6th lumbar region of the loin from Angus (n = 10) and Wagyu crossbred steers (n = 10) that had been fed for 552 d by typical Japanese production standards. Adipose tissue explants were incubated 36 h with [^{3}H]thymidine in the absence and presence of aphidicolin (a specific inhibitor of genomic DNA replication). Adipocytes were liberated by collagenase treatment and [^{3}H]thymidine incorporation into DNA was measured. Whereas there were no significant differences between adipose tissue depots, Wagyu s.c. and i.m. preadipocytes and stromal-vascular cells exhibited greater ($P < .05$) [^{3}H]thymidine incorporation into DNA than adipocytes from Angus steers. Intramuscular adipose tissue from both breeds exhibited lower ($P < .05$) rates of lipogenesis from acetate both before and after long-term (36-h) incubation than s.c. adipose tissue. Furthermore, i.m. adipocytes were smaller ($P < .05$) than s.c. adipocytes. The activities of fatty acid synthetase and glucose-6-phosphate dehydrogenase were greater ($P < .05$) in Wagyu s.c. adipose tissue and less in Wagyu i.m. adipose tissue than in corresponding Angus tissues. There were no differences between breed types ($P = .17$) in rates of lipogenesis from acetate, either before or after explant culture. These data suggest that lipid-filling cells that have the capacity to proliferate exist in bovine s.c. and i.m. adipose tissues. The smaller size and greater rates of DNA synthesis indicate that Wagyu s.c. and i.m. adipose tissues are less mature and more proliferatively active than Angus adipose tissues.

Key Words: Steers, Breeds, Lipogenesis, Adipocytes, DNA, Cellularity

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Introduction

Miller et al. (1991) compared the lipogenic activities of subcutaneous (s.c.) and intramuscular (i.m.) adipose tissues from Angus and Santa Gertrudis steers before and after long-term (48-h) tissue explant cultures. Unlike s.c. adipose tissue, which displayed a decrease in the incorporation of acetate into neutral lipids, lipogenesis in i.m. adipose tissue was unchanged during the culture period. In fact, lipogenesis from acetate in i.m. adipose tissue from the Santa Gertrudis steers increased nearly fourfold during culture. This increase in activity resulted from an increase in acetate incorporation into existing adipocytes (hypertrophy), through proliferation and subsequent lipid filling of new adipocytes, or through a combination of both processes. A primary objective of this study was to establish a means of documenting DNA synthesis (as an index of cell proliferation) in adipocytes during long-term explant culture.

At the time we were developing the procedure for measuring DNA synthesis in adipose tissue explants, biological samples from American Wagyu steers became available. The American Wagyu breed (derived from Japanese Black and Japanese Brown purebred

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cattle) has a unique fat deposition pattern characterized by deposition of large amounts of i.m. adipose tissue (Yamazaki, 1981; Zembayashi et al., 1988; Lunt et al., 1993). Because little is known about the metabolism of Wagyu adipose tissue in vitro, this investigation had the secondary objective of documenting aspects of lipogenesis, cellularity, and adipocyte DNA synthesis in acute and long-term cultures of Wagyu s.c. and i.m. adipose tissues.

Materials and Methods

Angus (n = 10) and crossbred (3/4- and 7/8-blood) Wagyu (n = 10) steers with mean initial weights of 220 and 260 kg, respectively ($P > .05$), were fed a diet according to typical Japanese standards for 552 d. All steers were fed a barley and corn-based diet to limit the rate of gain to approximately .9 kg/(animal·d). The diet was adjusted at 14-d intervals to regulate weight gain. The diet was varied through the course of the experiment according to feed grain availability, cost, and animal preference. The diet contained at least 25% roughage throughout the feeding period (Lunt et al., 1993).

American Wagyu is a synthetic breed developed through the crossbreeding of Japanese Black, Japanese Brown, Angus, and Hereford × Angus cattle. The American Wagyu steers used in this trial were selected from the foundation herd. Three Wagyu bulls were used to produce the Wagyu steers in this study. Angus steers were produced from purebred Angus cows and bulls at the Texas A&M University McGregor Research Center.

Steers were transported to the Rosenthal Meat Science and Technology Center at Texas A&M University. The steers were humanely slaughtered in compliance with the Humane Methods of Slaughter Act of 1978. Immediately after exsanguination, the 2nd to 6th lumbar region of the loin was removed and transported to the laboratory in Krebs-Henseleit bicarbonate buffer with 5 m*M* glucose (pH 7.4) at 37°C. Subcutaneous and i.m. adipose tissues for all subsequent assays were obtained from this section of the loin.

Adipose Tissue Cellularity. Procedures outlined by Etherton et al. (1977) as modified by Prior (1983) were used to determine adipocyte cellularity. 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- and 20-μm mesh screen for cell size and number determination using a Coulter Counter, Model ZM equipped with a channelizer, Model 256.

Adipose Tissue Explant Culture. Adipose tissue explant cultures were conducted according to procedures described by Etherton and Evock (1983), as modified by Miller et al. (1991). Tissue explants of s.c. and i.m. adipose tissue were incubated for 36 h at 37°C at pH 7.4 in a NuAire (Model NU1500) and maintained under a gas phase of 95% O_2:5% CO_2. Adipose tissue was sliced to obtain explants of 30 to 50 mg. A total of approximately 100 mg of adipose tissue was transferred to each well in 12-well culture plates with wells 17.6 mm × 22.1 mm in diameter containing 5 mL of culture media. All explants were in culture within 30 min postmortem. Explant medium was Medium 199 (with Hank's salts and L-glutamine) supplemented with .35 g/L $NaHCO_3$, 10% bovine calf serum, 20 m*M* HEPES buffer (pH 7.4), 5 m*M* glucose, 5 m*M* sodium acetate, and antibiotics (100 μg/mL of penicillin, 10 μg/mL of streptomycin, and 10 μg/mL of amphotericin B). To maintain proper pH and nutrient supply, media were changed every 6 h. Less frequent changes (12- or 24-h changes) resulted in a greater decline in lipogenic activity (S. B. Smith, unpublished observations). Culture was terminated after 36 h, and the appropriate assays were conducted.

DNA Synthesis. The incorporation of [^{3}H]thymidine into DNA was taken as an index of the proliferation of lipid filling cells during explant culture (Hollenberg and Vost, 1968). However, in addition to incorporation into genomic DNA during replication, [^{3}H]thymidine also is incorporated into DNA during repair and into mitochondrial DNA during the independent process of mitochondrial DNA replication (Spadari et al., 1982). Therefore, a method was developed to measure [^{3}H]thymidine incorporation specifically into genomic DNA during replication. Aphidicolin is an antibiotic produced from the mold *Cephalosporium aphidicola Petch* (Wist and Prydz, 1979). Aphidicolin is a unique inhibitor of DNA synthesis that does not bind directly to DNA. This inhibitor acts directly on replicative DNA polymerase, more specifically, on DNA polymerase alpha (Wist and Prydz, 1979; Spadari et al., 1982). DNA polymerase beta (repair) and DNA polymerase gamma (mitochondrial; Spadari et al., 1982) are not affected. In addition, the use of the aphidicolin aided in accounting for indiscriminate incorporation of the radioisotope (Maurer, 1981). The total amount of [^{3}H]thymidine incorporation into DNA (in the absence of aphidicolin) was corrected by [^{3}H]thymidine incorporation into DNA in the presence of aphidicolin to provide a more accurate measurement of genomic DNA replication.

In a preliminary experiment designed to establish the level of aphidicolin necessary to inhibit DNA synthesis in adipose tissue, the epididymal fat pad from a male Sprague-Dawley rat was incubated as described below for bovine adipose tissues. Aphidicolin was added to the incubation media at levels of .5 to 10 μg/mL. At the termination of the incubation period (48 h), [^{3}H]thymidine incorporation into DNA was measured. Based on the dose-response curve for rat

adipose tissue (Figure 1), aphidicolin at a concentration of 8 μg/mL was used to inhibit DNA synthesis in explants of bovine s.c. and i.m. adipose tissues.

Fresh explants of s.c. and i.m. adipose tissue (200 mg total of pieces weighing 35 to 50 mg) were transferred to each well in 12-well culture plates with wells 17.6 mm × 22.1 mm in diameter containing 5 mL of culture media. Four plates were used for each breed and depot combination. Culture media was prepared as described above with the addition of 2 μCi/mL [methyl-^{3}H]thymidine. Two plates for each breed and depot combination received 8 μg/mL of aphidicolin.

The goal of this research was to demonstrate genomic DNA synthesis in bovine preadipocytes (i.e., adipocyte precursor that already had accumulated sufficient lipid to float during the separation procedure). The minimum cell doubling time for rat adipocyte precursor cells is 25 h (Djian et al., 1983), and 2 to 5 d are needed for rat adipoblasts labeled with [^{3}H]thymidine to differentiate (fill will lipid) and be isolated (float) using the collagenase procedure (Hirsch and Han, 1969; Greenwood and Hirsch, 1974). Similar data are not available for bovine adipocytes. However, the 36-h incubation period should have been too brief for bovine adipocyte precursor cells that were devoid of lipid (i.e., adipoblasts) to incorporate [^{3}H]thymidine into DNA, divide, and subsequently fill with lipid sufficiently to float during the adipocyte liberation procedure.

Adipocyte Liberation. Hirsch and Han (1969) stated that approximately one-fourth of the DNA in intact rat adipose tissue is in the adipocyte fraction, with the majority of the DNA associated with cells of the supportive matrix. Therefore, adipocytes were collagenase-liberated to measure [^{3}H]thymidine incorporation in lipid-filled cells by a modification of the procedure of Rodbell (1964). After culture, the [^{3}H]thymidine-labeled adipose tissue was rinsed with 150 m*M* NaCl and 1 m*M* HEPES and placed in 20-mL scintillation vials containing 2.5 mL of incubation media. Incubation media contained Krebs-Henseleit buffer (pH 7.4), 10 m*M* HEPES, 5 m*M* glucose, 3% bovine serum albumin (fatty acid free), 1 m*M* $CaCl_2$, 1.67 mg/mL of collagenase, .3 mg/mL of elastase, and .5 mg/mL of hyaluronidase (Bjornthorp et al., 1979). The samples were incubated in a water bath at 120 oscillations per min at 37°C for 1 h. After the incubation period, cells were washed gently through a 1,000-μm nylon screen with 5-mL of Krebs-Henseleit buffer into a 15-mL plastic centrifuge tube and lipid-containing cells were allowed to float for 15 min at 37°C. The buffer/enzyme solution was removed by aspiration using plastic tubing and a 5-mL syringe. The cells were rinsed twice through a 250-μm screen and brought to 2 mL with Krebs-Henseleit buffer. Two .5-mL aliquots were removed for [^{3}H]thymidine determination and .5 mL was used for cell number determination.

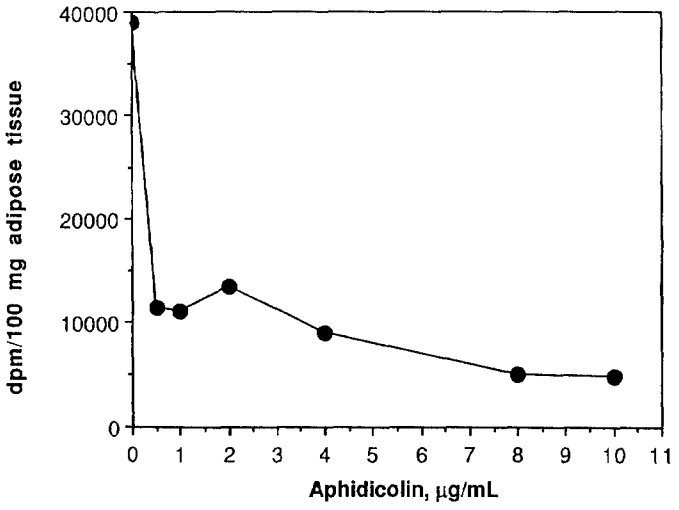

Figure 1. The incorporation of [^{3}H]-labeled thymidine incorporation into DNA as a function of aphidicolin concentration. Slices from the epididymal fat pad of a male Sprague-Dawley rat were incubated with 0 to 10 μg/mL aphidicolin under the conditions described in the text.

The cell suspension for [^{3}H]thymidine incorporation into DNA was placed in a 5-mL centrifuge tube. One-half milliliter of 20% trichloroacetic acid was added to the solution to lyse the cells. The suspension was centrifuged at 21,000 × *g* for 5 min, and after centrifugation, the top layer was aspirated. The pellet was redissolved in 1 mL of .5 *M* NaOH. Aliquots (.25 mL) were transferred to 10-mL scintillation vials, .1 mL of 5 *N* HCl, and 5 mL of scintillation cocktail were added, and samples were counted. Because the yield of adipocytes varied when using the collagenase liberation procedure, incorporation of ^{3}H-labeled thymidine into DNA was reported as disintegrations per minute per 10^4 cells. The differences between samples containing the aphidicolin and the aphidicolin-free samples was considered to be [^{3}H]thymidine incorporation into genomic DNA.

Lipogenic Enzyme Activities. Fresh adipose tissue samples (1 g s.c.; 100 mg i.m.) were homogenized on ice in 3 volumes (wt/vol) of .154 *M* KCl:.01 *M* phosphate buffer (pH 7.4, 25°C). The homogenate was centrifuged at 3,000 × g for 15 min at 4°C and decanted. The supernate was centrifuged at 15,000 × *g* for 30 min at 4°C. The resulting supernate was assayed for selected enzymes associated with fatty acid synthesis. Fatty acid synthetase activity was assayed according to procedures of Martin et al. (1961). The activity of NADP-malate dehydrogenase was assayed as described by Ochoa (1955). Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were determined as described by Bernt and Bergmeyer (1974). All enzyme assays were determined in triplicate using

spectrophotometric absorbance of solutions in cuvettes at 340 nm. Slopes of the linear rates of NADPH consumption (fatty acid synthetase) or production (all other enzymes) were used to calculate enzyme activities. All enzyme activities were linear to at least 10 times the amount of homogenate added and were assayed under substrate conditions that yielded maximal velocities.

Lipogenesis In Vitro. Two-hour in vitro incubations were performed with s.c. and i.m. adipose tissues immediately after slaughter and after the 36-h explant culture period. Adipose tissue explants (50 to 100 mg) were incubated in 3 mL of incubation media containing 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. Vials were gassed for 1 min with 95% O_2:5% CO_2, capped, and incubated for 2 h in a shaking water bath at 37°C. At the end of the incubation period, reactions were terminated by addition of 3 mL of 5% trichloroacetic acid. Explants were rinsed with .154 *M* NaCl and Krebs-Henseleit bicarbonate buffer to remove free lipid and unincorporated substrate. The neutral lipids were extracted using the Folch et al. (1957) procedure as modified by Mersmann (1987). Samples were evaporated to dryness and resuspended in 10 mL of scintillation cocktail and radioactivity was counted with a Beckman model LS-3800 scintillation counter. Incorporation of ^{14}C-labeled acetate into neutral lipids was calculated as acetate incorporated per 2-h incubation period per 10^5 cells.

Statistical Analyses. Statistical analyses used the GLM procedures of SAS (1985). The influence of sire was not significant and was removed from all models. For lipogenic enzyme activities, cellularity and [^{3}H]thymidine incorporation into DNA, the statistical model included breed and depot site as the main effects and the breed × depot site interaction. The model for ^{14}C-labeled acetate incorporation into lipid included breed, depot site, and culture period as the main effects and the breed × depot site, breed × culture period, depot site × culture period, and breed × depot site × culture period interactions. The residual mean square was used as the error term. If an interaction was not significant at $P < .05$, it was removed from the model and the new residual mean square was used as the error term. For significant *F*-tests ($P < .05$), mean separations were performed using Tukey's test (Steel and Torrie, 1980).

Results and Discussion

We reported previously the carcass data and growth characteristics for these cattle (Lunt et al., 1993). Age at slaughter was 34 ± .6 mo (mean ± SE) for the American Wagyu steers and 26 ± .3 mo for the Angus steers ($P < .05$). However, carcass maturity was the same (A^{96} for the Wagyu steers vs A^{91} for the Angus steers) (Lunt et al., 1993). Thus, these cattle were at the same point in their physiological maturity and development, as determined by these measures.

These cattle were excessively fat by U.S. standards, with adjusted fat thicknesses of 3.30 and 3.71 cm for the Angus and Wagyu steers, respectively ($P > .05$). Previous studies comparing Japanese Black to other breeds have shown that these cattle produce carcasses containing high levels of i.m. adipose tissue (Yamazaki, 1981; Zembayashi et al., 1988). Marbling scores were almost a full degree higher for American Wagyu steers (Slightly Abundant^{66} for the Angus steers vs Moderately Abundant^{47} for the Wagyu steers), but variation was high within breed types (SE = .35) (Lunt et al., 1993). At the 6th rib, where Japanese carcasses are graded, there was a significant difference in marbling scores (Japanese Marbling Score 3.4 for Angus steers vs 4.4 for Wagyu steers). Similarly, longissimus muscle from the carcasses of the Wagyu crossbred steers contained higher ($P < .05$) levels of ether extractable lipid than samples from Angus steers (18.93 vs 14.50%, respectively) (Lunt et al., 1993).

Cellularity. Regardless of breed type, i.m. adipose tissue contained more adipocytes per gram of tissue that had lesser mean cell diameter and correspondingly lesser mean cell volume than adipocytes in s.c. adipose tissue ($P < .05$; Table 1). These findings are in agreement with those of earlier studies (Hood and Allen, 1975; Smith and Crouse, 1984; Cianzio et al., 1985; Miller et al., 1991) and reflect the lesser maturity of i.m. adipose tissue relative to s.c. adipose tissue.

The values for cell diameter and volume in Table 1 are means combining two separate populations of

Table 1. Subcutaneous and intramuscular adipose tissue characteristics of Angus and Wagyu crossbred steers

	Subcutaneous		Intramuscular			
Item	Angus	Wagyu	Angus	Wagyu	SEM	Significance[a]
Cells per gram, × 10^{-5}	9.6	9.7	11.8	12.5	.8	D
Mean diameter, μm	62.9	56.3	60.5	53.3	2.5	B, D
Mean volume, pL	537	430	369	308	41	B, D

[a]D = depot effect ($P < .05$); B = breed effect ($P < .05$).

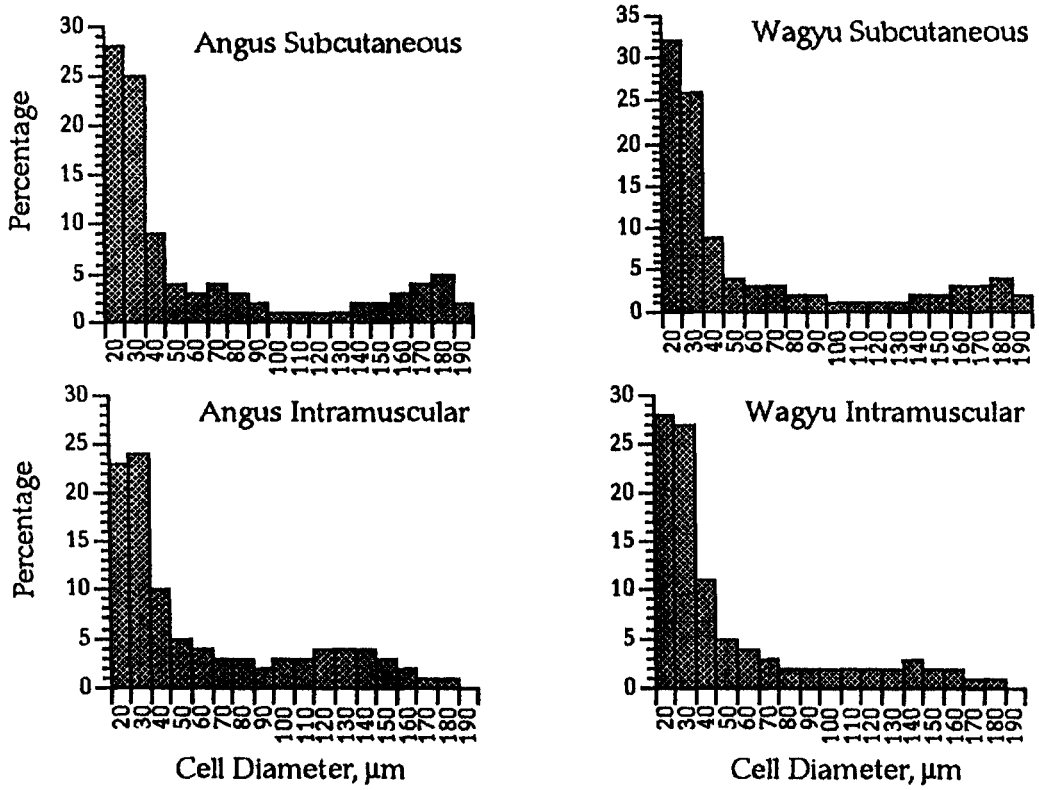

Figure 2. Cell diameter distribution for adipocytes from subcutaneous and intramuscular adipose tissues from Angus and Wagyu crossbred steers.

adipocytes (Figure 2). In s.c. adipose tissue, there was a discrete population less than 80 µm in diameter and a second population of adipocytes with a peak diameter (i.e., the diameter represented by the greatest proportion of cells within that population) of 180 µm. For i.m. adipose tissue, the peak of the larger population of adipocytes was approximately 140 µm. These peak diameters were nearly 40 µm greater than those observed in 18-mo-old Angus steers (Smith and Crouse, 1984), which is consistent with the greater age and adiposity of the Angus and Wagyu steers of the current study.

Mean cell diameter and volume were greater ($P < .05$) for i.m. and s.c. adipose tissues of Angus steers than for adipose tissues from Wagyu steers. Correspondingly, both adipose tissues from Angus steers had a lesser percentage of smaller cells, 20 to 50 µm in diameter, and a greater percentage of cells measuring over 100 µm in diameter than adipose tissues from Wagyu crossbred steers (Figure 2). These results suggest that Wagyu s.c. and i.m. adipose tissues were less mature than the corresponding Angus tissues, in contrast to the greater chronological age of the Wagyu steers.

The occurrence of a discrete population of small adipocytes in excessively fat cattle and pigs was demonstrated in earlier studies (Allen et al., 1974; Allen, 1976). Allen (1976) demonstrated a biphasic distribution for cell diameters for cattle with an excess of 5 cm of external fat and suggested that the occurrence of the small adipocytes was the result of reinitiation of hyperplasia or the result of differentiation of preadipocytes (recruitment).

DNA Synthesis in Explant Cultures. The difference between the [^{3}H]thymidine incorporation from tissue incubated without aphidicolin in culture and that treated with aphidicolin media represented the incorporation of [^{3}H]thymidine into genomic DNA during replication (Table 2). The incorporation of [^{3}H]thymidine into DNA in stromal-vascular cells from Wagyu adipose tissues was approximately twice the rate observed in Angus adipose tissue samples (Table 2). This suggests a greater degree of stromal-vascular cell proliferation in tissues from Wagyu steers. However, we cannot separate adipocyte precursor cells in the stromal-vascular fraction from other cell types (such as nondifferentiating fibroblasts) by our techniques. Thus, in spite of the potential importance of adipocyte precursor cell proliferation in stromal-vascular cells, nothing can be concluded about differences in rates of DNA synthesis in that population.

Approximately 30% of the total [^{3}H]thymidine incorporation into DNA was recovered in cells that contained enough lipid to float during the isolation process; the remainder of the radioactivity was

Table 2. Specific [^{3}H]thymidine incorporation into genomic DNA in collagenase-liberated bovine adipose tissue fractions

Item	Subcutaneous		Intramuscular		SEM	Significance[a]
	Angus	Wagyu	Angus	Wagyu		
	dpm/10^4 cells					
Incorporation into stromal-vascular cells	1,727	4,345	1,713	3,090	972	B
Incorporation into lipid-filling cells	400	1,091	387	887	482	B
Percentage incorporation into lipid-filling cells	23	35	23	29		—

[a]B = breed effect ($P < .05$). Incorporation rates for stromal-vascular and lipid-filling cells were corrected for nonspecific incorporation by subtracting [^{3}H]thymidine incorporation into DNA in the presence of aphidicolin from [^{3}H]thymidine incorporation into DNA in the absence of aphidicolin.

recovered in the pelleted fraction containing stromal-vascular cells. There was insufficient time during the 36-h culture period for adipoblasts to undergo DNA replication, cell division, and lipid filling (i.e., differentiation); this indicates that cells that had accumulated lipid prior to explant culture still retained the capacity to synthesize DNA. These data clearly demonstrate DNA replication had occurred in preadipocytes during explant culture.

Although it is accepted that mature, unilocular adipocytes do not proliferate, there is a question about the exact point at which cell proliferation stops and differentiation begins. Pilgrim (1971) pulse-labeled prenatal and early postnatal rats with [^{3}H]thymidine and demonstrated that the cell fraction with the highest labeling index was that containing newly differentiated cells (preadipocytes). Pilgrim (1971) concluded that proliferation and differentiation were occurring simultaneously (i.e., DNA synthesis did not terminate in partially differentiated adipocytes). Hausman et al. (1980) suggested that Pilgrim (1971) could not distinguish between vacuoles containing glycogen or lipid. However, based on [^{14}C]thymidine incorporation into DNA, Gaven-Cogneville and Swierczewski (1979) demonstrated that in young rats (3 to 10 d of age) some adipocytes may synthesize DNA for cell division after the initiation of lipid mobilization. In a summary of work conducted by Bjorntorp et al. (1978), Cryer (1980) concluded that, based on a study in which [^{3}H]thymidine-labeled cells were isolated by collagenase digestion, a subpopulation of cells within the stromal fraction was partially differentiated but possessed the ability to proliferate. Cryer (1980) stated that for these adipocyte precursors, cell proliferation and differentiation were not mutually exclusive; this is supported by the findings of the current investigation.

There was a significant difference ($P < .05$) between breed types for DNA synthesis in lipid-filling cells (Table 2). These results coincide with the occurrence of more adipocytes with proportionally smaller diameters in adipose tissues from Wagyu steers than in adipose tissues from Angus steers (Figure 2). As stated above, there was a large proportion of smaller (20 to 50 μm in diameter) adipocytes in s.c. and i.m. adipose tissues from both breed types. We propose that the DNA synthesis we measured in the lipid-containing cells occurred in that population of smaller adipocytes, which putatively represents newly differentiated preadipocytes. The data further suggest that the adipose tissues from Wagyu steers possessed a greater capacity for cell proliferation than adipose tissues from Angus steers. This is consistent with the capacities of these breed types to accumulate carcass (and especially marbling) fat.

The occurrence of DNA synthesis in preadipocytes at best provides indirect evidence for proliferation in that cell population. In a separate experiment, we measured both [^{3}H]thymidine incorporation into DNA and cell number (measured with a Coulter Counter, Model ZBI) in cultured L-6 myoblasts after exposure to 10% fetal bovine serum [J. C. Laurenz and S. B. Smith, unpublished observations. See also Laurenz and Smith (1990)]. We demonstrated that a high rate of [^{3}H]thymidine incorporation into DNA of L-6 myoblasts occurred during the period of exponential cell growth. As the rate of cell proliferation declined, [^{3}H]thymidine into DNA decreased commensurably, indicating a strong correlation between [^{3}H]thymidine incorporation into DNA and cell proliferation. Because it is not possible to obtain an accurate measurement of the change in cell number during culture in bovine adipose tissue explants, we cannot perform the same experiments in the adipose tissue explant system. However, our experiment with the myoblast cell line provides evidence that [^{3}H]thymidine incorporation into DNA is an index of cell proliferation. The data therefore provide evidence for preadipocyte proliferation under our culture conditions.

Enzyme Activities. Chakrabarty and Romans (1972) compared beef and dairy breed types and found higher lipogenic enzymatic activities in s.c. adipose tissue than in i.m. adipose tissue regardless of

breed type. Similarly, we previously reported higher enzymatic activities for the pentose cycle reductases in s.c. adipose tissue than in i.m. adipose tissue of Angus and Santa Gertrudis steers (Smith and Crouse, 1984; Miller et al., 1991). In the current study, NADP-malate dehydrogenase and 6-phosphogluconate dehydrogenase were higher ($P < .05$) in s.c. adipose tissue than in i.m. adipose tissue (Table 3). The activity of fatty acid synthetase, which may be rate-limiting to lipogenesis in bovine adipose tissue (Smith and Prior, 1986), was markedly lower in i.m. adipose tissue from the Wagyu steers. Additionally, there was a significant breed × depot interaction for fatty acid synthetase and glucose-6-phosphate dehydrogenase. Activities of both enzymes were higher in Wagyu s.c. adipose tissue, but lower in Wagyu i.m. adipose tissue, relative to activities in adipose tissues from Angus steers.

Lipogenesis in Acute and Long-Term Explant Cultures. In spite of significant breed × depot interactions for lipogenic enzyme activities, acetate incorporation into neutral lipids was not different ($P > .05$) between breed types for acute incubations or after 36 h of explant culture (Table 3). In a comparison of lipogenic activity of adipose tissue from large- and small-framed cattle with a similar physiological maturity, Scott and Prior (1980) found differences between i.m. and s.c. adipose tissues but few differences between biological types. The Angus and Wagyu cattle of this study were of similar physiological maturity, which may have obviated any differences in lipogenesis between breed types.

There was a significant depot × culture time interaction for acetate incorporation into neutral lipids (Table 3). Lipogenesis in vitro decreased during explant culture by approximately 50% in both s.c. and i.m. adipose tissue, but the magnitude of the decrease was greater for s.c. adipose tissue. Miller et al. (1991) reported that lipogenesis in i.m. adipose tissue of Santa Gertrudis increased markedly after extended explant culture, whereas lipogenesis in i.m. from Angus steers was unchanged. We proposed that the increase in lipogenesis in vitro in i.m. adipose tissue of Santa Gertrudis steers was related to the relative immaturity of that depot. Tissues with initially low rates of lipogenesis and small adipocytes, and especially those that exhibit relatively high rates of preadipocyte DNA synthesis, were expected to flourish in culture. The smaller cell sizes and greater rates of DNA synthesis of Wagyu s.c. and i.m. adipose tissues suggested that Wagyu adipose tissues were less mature and more actively proliferating than Angus adipose tissues. Thus, we anticipated that differences in cellularity and DNA synthesis between Angus and Wagyu steers of the present study would be reflected in their response to long-term explant culture. This was not the case. The lack of a significant breed × culture time interaction indicated that, in spite of differences in cellularity and putative preadipocyte proliferation, adipose tissues from Angus and Wagyu steers responded similarly to explant culture.

Implications

These studies provide additional evidence that intramuscular adipose tissue is a later-developing depot than subcutaneous adipose tissue. Additionally, adipocytes of Wagyu steers mature at slower rates or at smaller diameters than those of Angus steers. Intramuscular and subcutaneous adipose tissues from both breed types exhibited DNA replication within lipid-containing adipocytes, suggesting that preadipocyte proliferation occurred even at the relatively advanced age of the animals at slaughter. The greater rate of DNA replication (and, putatively, cell proliferation) of adipocytes from Wagyu cattle may explain the greater capacity of these cattle to accumulate adipose tissue, especially as marbling adipose tissue.

Table 3. Lipogenic enzyme activities and lipogenesis in vitro in subcutaneous and intramuscular adipose tissue from Angus and Wagyu crossbred steers

	Subcutaneous		Intramuscular			
Item	Angus	Wagyu	Angus	Wagyu	SEM	Significance[a]
Enzyme activity	nmol/(min·10^5cells)$^{-1}$					
Fatty acid synthetase	5.6	15.5	3.7	.7	3.3	B × D
NADP-malate dehydrogenase	65.1	89.9	34.3	57.6	11.3	D
G-6-P dehydrogenase[b]	1,154	1,544	1,055	788	139	B × D
6-PG dehydrogenase[c]	520	638	275	273	52	D
Lipogenesis in vitro	nmol/(2 h·10^5 cells)$^{-1}$					
Acute (0 h)	180.3	160.0	86.5	47.1	22.2[d]	D × T
36 h of culture	74.2	65.3	34.8	25.6	22.2[d]	—

[a]D = depot effect ($P < .05$); B × D = breed × depot interaction ($P < .05$). D × T = depot × culture time interaction.
[b]Glucose-6-phosphate dehydrogenase.
[c]6-Phosphogluconate dehydrogenase.
[d]Pooled standard error.

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