Brown adipose tissue development and metabolism in ruminants[1]

S. B. Smith*[2], G. E. Carstens*, R. D. Randel*, H. J. Mersmann‡, and D. K. Lunt†

*Department of Animal Science, Texas A&M University, College Station 77843; †Texas A&M University Agricultural Research Center, McGregor 76657; and ‡USDA, ARS, Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030

ABSTRACT: We conducted several experiments to better understand the relationship between brown adipose tissue (BAT) metabolism and thermogenesis. In Exp. 1, we examined perirenal (brown) and sternum s.c. adipose tissue in 14 Wagyu × Angus neonates infused with norepinephrine (NE). Perirenal adipocytes contained numerous large mitochondria with well-differentiated cristae; sternum s.c. adipocytes contained a few, small mitochondria, with poorly developed cristae. Lipogenesis from acetate was high in BAT but barely detectable in sternum s.c. adipose tissue. In Exp. 2, we compared perirenal and tailhead adipose tissues between NE-infused Angus (n = 6) and Brahman (n = 7) newborn calves. Brahman BAT contained two-to-three times as many total β-receptors as Angus BAT. The mitochondrial UCP1:28S rRNA ratio was greater in Brahman BAT than in BAT from Angus calves. Lipogenesis from acetate and glucose again was high, but lipogenesis from palmitate was barely detectable. Tailhead s.c. adipose tissue from both breed types contained adipocytes with distinct brown adipocyte morphology. In Exp. 3, three fetuses of each breed type were taken at 96, 48, 24, 14, and 6 d before expected parturition, and at parturition. Lipogenesis from acetate and glucose in vitro decreased 97% during the last 96 d of gestation in both breed types, whereas the UCP1 gene expression tripled during gestation in both breed types. At birth, palmitate esterification was twice as high in Angus than in Brahman BAT and was at least 100-fold higher than in BAT from NE-infused calves from Exp. 2. Uncoupling protein-1 mRNA was readily detectable in tailhead s.c. adipose tissue in all fetal samples. In Exp. 4, male Brahman and Angus calves (n = 5 to 7 per group) were assigned to 1) newborn treatment (15 h of age), 2) 48 h of warm exposure (22°C) starting at 15 h of age, or 3) 48 h of cold exposure (4°C) starting at 15 h of age. Brahman BAT adipocytes shrank with cold exposure, whereas Angus BAT adipocytes did not. Similarly, BAT from neonatal lambs (Exp. 5; n = 6 per group) was depleted of lipid in response to cold exposure, although UCP1 gene expression persisted. In Exp. 4, NE stimulated lipogenesis from palmitate in BAT incubated in vitro. Lipogenesis from palmitate was higher in Angus than in Brahman BAT, and increased with both warm and cold exposure. These studies suggest that BAT from Brahman calves may be exhausted of lipid shortly after birth during times of cold exposure.

Key Words: β-Adrenergic Receptors, Bovidae, Brown Adipose Tissue, Lipogenesis, Morphology, Uncoupling Protein

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Introduction

Bos indicus (e.g., Grey Brahman) is a tropical breed whose calves exhibit greater cold-induced mortality than *Bos taurus* (e.g., Angus) calves (Josey et al., 1987). Brown adipose tissue (**BAT**) thermogenesis is an important mechanism of heat production in newborn mammals to support homeothermy and maintain survival of newborn calves (Himms-Hagen, 1990). For this reason, the mortality rate of newborn calves is in part dependent on the activation of BAT nonshivering thermogenesis (Carstens et al., 1997).

Bos indicus purebred calves exhibit a greater decline in rectal temperature in response to short-term (120 min) cold exposure than *Bos indicus* crossbred cattle (Godfrey et al., 1991). Similarly, Carstens et al. (1998) demonstrated that newborn Brahman calves exhibit lesser norepinephrine-stimulated (peak) thermogenesis than newborn Angus calves. The mechanism responsible for the lesser ability of *Bos indicus* calves to gener-

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[2]Correspondence: 2471 TAMU (phone: 979-845-3939; fax: 979-458-2702; e-mail: sbsmith@tamu.edu).

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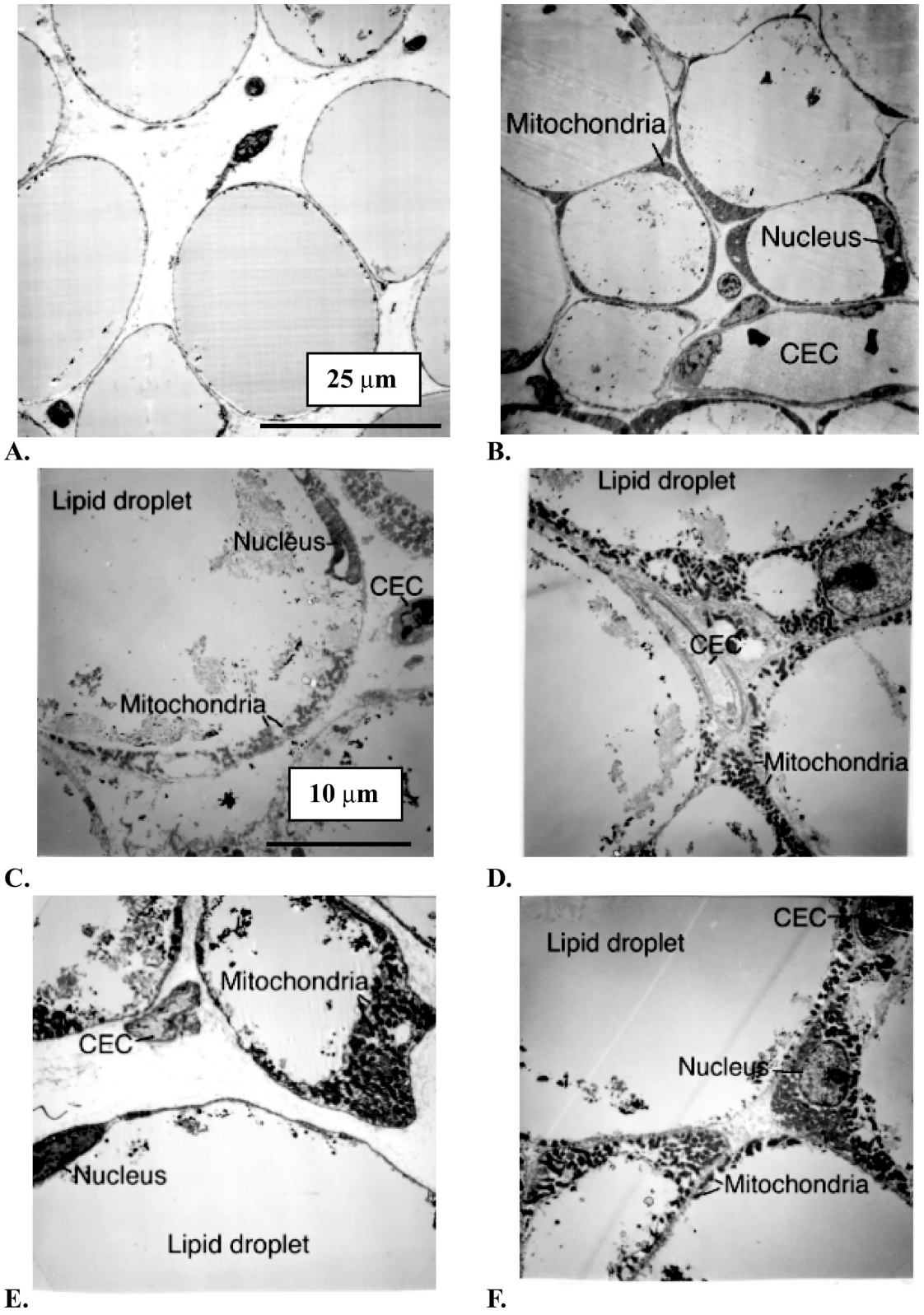

Figure 1. Transmission electron micrographs of s.c. (A, C, E) and perirenal (B, D, F) adipose tissues from newborn Wagyu × Angus (A, B) (Exp. 1), or from Angus (C, D), and Brahman (E, F) calves (Exp. 2). Large lipid droplets, adipocyte nuclei, and tightly packed mitochondria are evident in all perirenal adipose tissue samples. No brown adipocytes are evident in sternum s.c. adipose tissue from Wagyu × Angus calves, whereas abundant brown adipocytes are apparent in tailhead s.c. adipose tissue from Angus and Brahman calves. In E, a brown adipocyte is seen adjacent to a white adipocyte. CEC = capillary endothelial cell. Scale bar in A also applies to B; scale bar in C also applies to D through F.

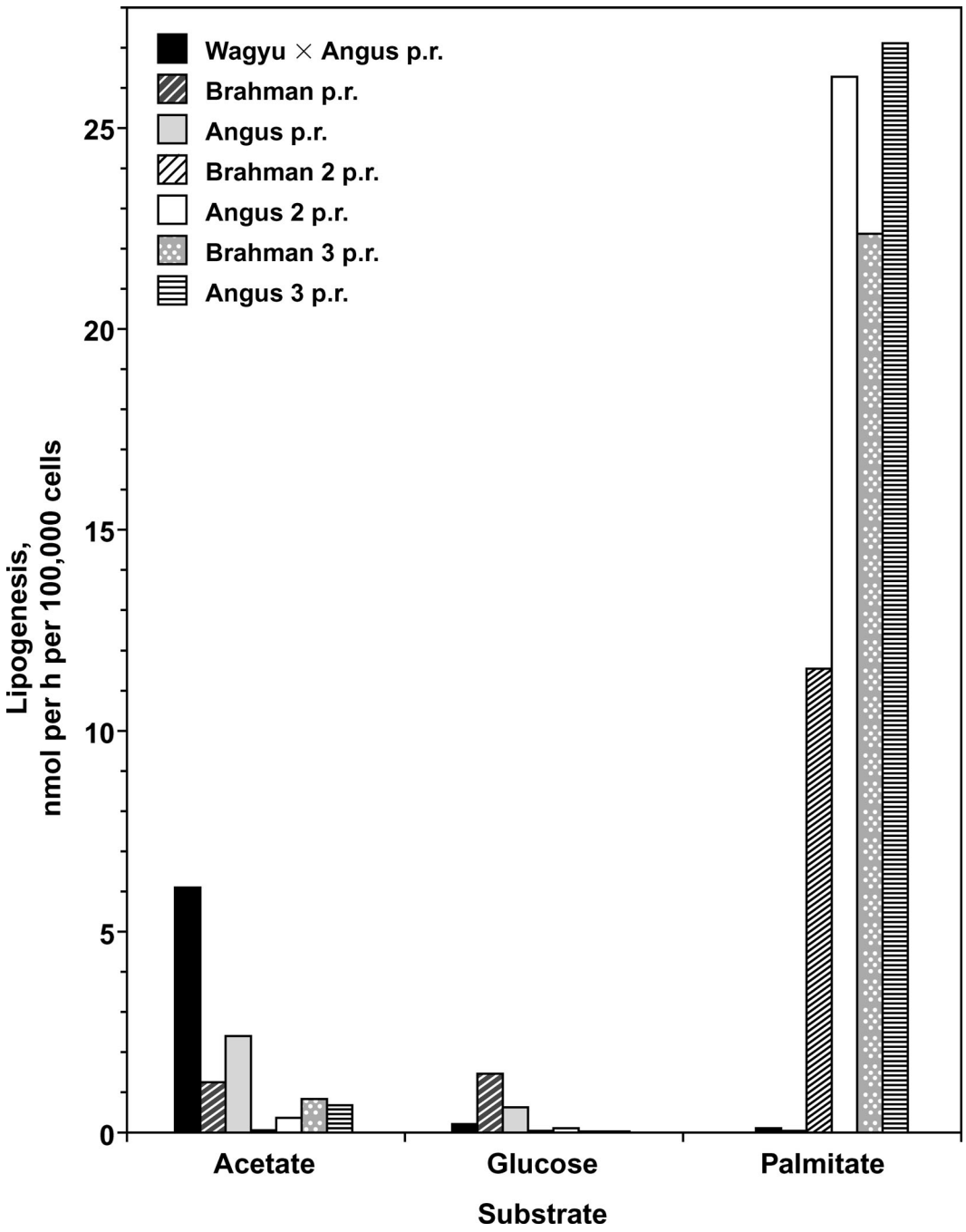

Figure 2. Lipogenesis from acetate, glucose, and palmitate in vitro in bovine perirenal (p.r.) brown adipose tissue (BAT). Data are summarized for Exp. 1 through 4. Palmitate incorporation into lipids was not measured in BAT from the Wagyu × Angus calves (Exp. 1). Lipogenesis from acetate in BAT from the NE-infused Wagyu × Angus, Angus, and Brahman calves (Exp. 1 and 2) was higher than that in BAT from 5-h-old and 15-h-old, non-infused Angus and Brahman calves (Exp. 3 and 4). Lipogenesis from palmitate was markedly higher in BAT from non-infused calves, and under those conditions, accounted for virtually all of the lipid accumulation in vitro. Palmitate incorporation into lipids was greater in Angus than in Brahman BAT in samples from both groups of noninfused calves (Exp. 3 and 4; 2 p.r. and 3 p.r.).

ate heat by nonshivering thermogenesis may lie in a reduced ability of BAT from *Bos indicus* calves to utilize stored lipids (Godfrey et al., 1991). The objectives of these experiments were 1) to provide a description of the morphological and metabolic characteristics that are unique to BAT from newborn calves, 2) to characterize differences and similarities in BAT from Angus and Brahman newborn calves, 3) to describe the ontogenic development of BAT in fetal calves (including expression of uncoupling protein-1 [**UCP1**]), and 4) to empha-

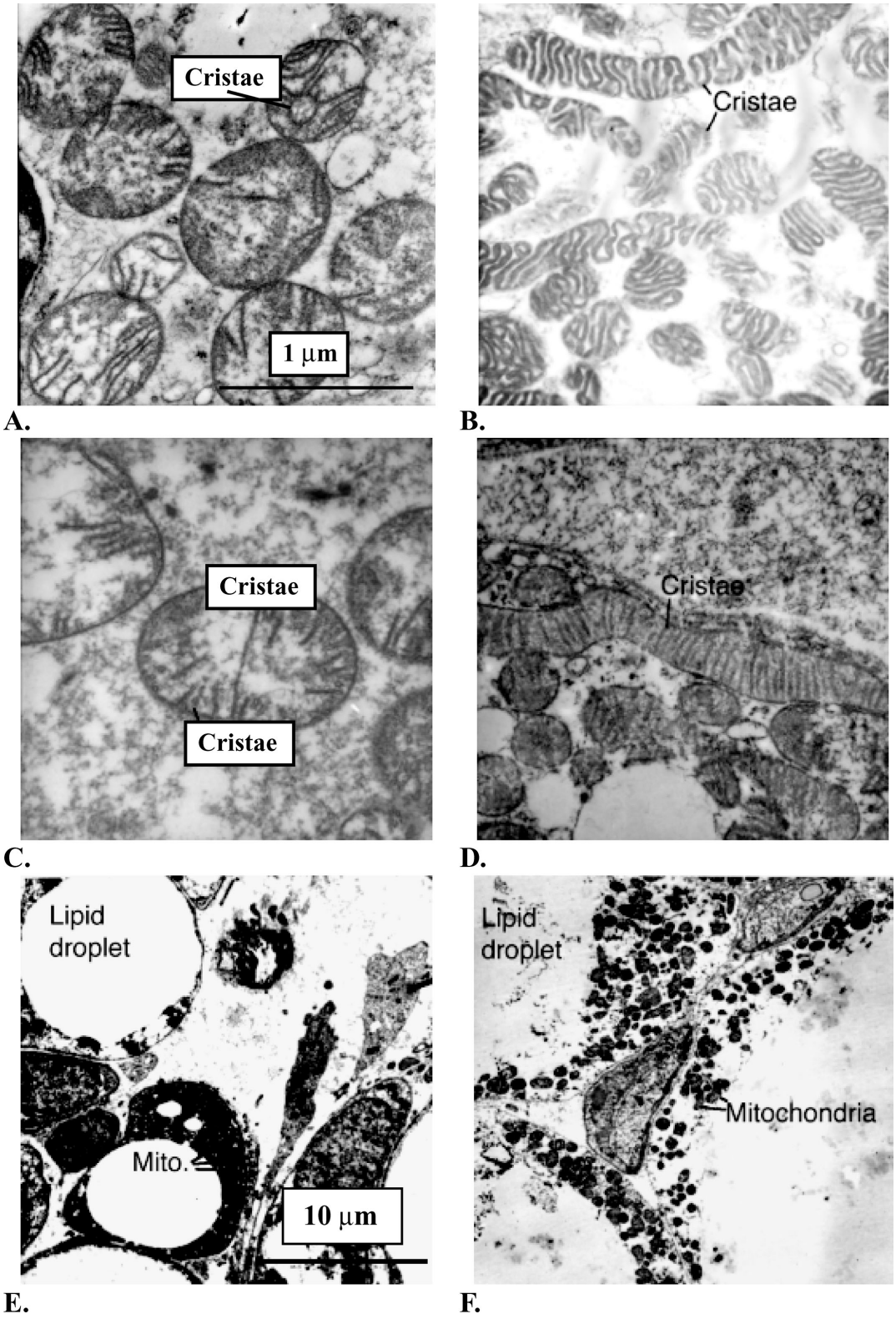

Figure 3. Transmission electron micrographs of perirenal (brown) adipocyte mitochondria from Angus (A, B) and Brahman (C through F) calves (Exp. 3). Samples were taken either at 190 d of gestation (A, C, E) or at birth (approximately 290 d of gestation; A, D, F). At the beginning of the third trimester, mitochondria are large and spherical, with poorly defined cristae (A, C). Adipocytes have a large central lipid vacuole, with smaller lipid droplets completely encased in mitochondria. By parturition, some mitochondria have become profoundly elongated, with highly convoluted, differentiated cristae. However, their cross-sectional circumference is less than that observed in mitochondria from fetal samples. Usually only a single lipid vacuole (droplet) is apparent at birth. Scale bar in A also applies to C through D. The scale bar in F also applies to E.

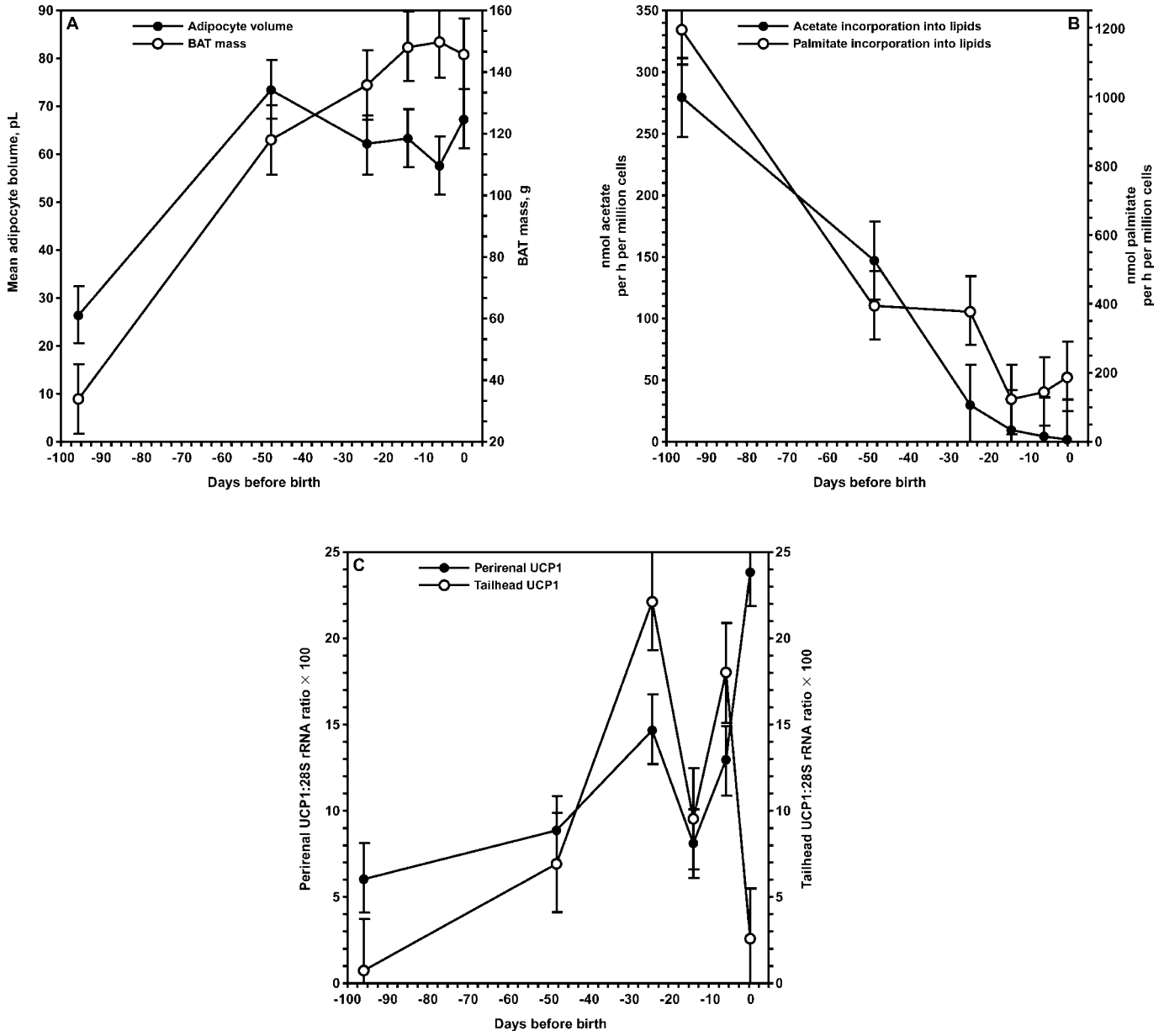

Figure 4. Characteristics of bovine perirenal adipose tissue during the last trimester (Exp. 3). A) Perirenal brown adipose tissue (BAT) mass increased to 48 d before birth, after which time there was no increase in mass. Brown adipocyte volume increased in a similar fashion. B) Lipogenesis in vitro from acetate and palmitate was extremely high at 96 d before birth, and rates plummeted during the next 48 d in utero. C) The concentration of uncoupling protein-1 (UCP1) increased during the last trimester in perirenal adipose tissue but was nearly undetectable at birth in tailhead s.c. adipose tissue. Overall standard error for each trait is attached to its respective symbol (n = 6 per time point; data pooled across breeds).

size the importance of BAT lipid metabolism during the prenatal and perinatal periods.

Materials and Methods

Experiment 1: Morphological and Metabolic Characteristics of Bovine Brown Adipose Tissue. This experiment initially was designed to test the effects of prepartum protein restriction on BAT function in calves (Martin et al., 1997). However, there was no effect of protein restriction of the dams, so this aspect of the study will not be described herein.

Cows used for Exp. 1 were fed individually diets containing 7 or 9% CP for 98 d. At approximately 6 h of age, Wagyu × Angus calves (n = 14) were infused with 20 μg/kg BW norepinephrine (**NE**) in the jugular vein for subsequent measurement of NE-stimulated thermogenesis (not reported here) (Martin et al., 1997). Brown adipose tissue was obtained from the calves at 12 h of age for the measurement of UCP1 gene expression,

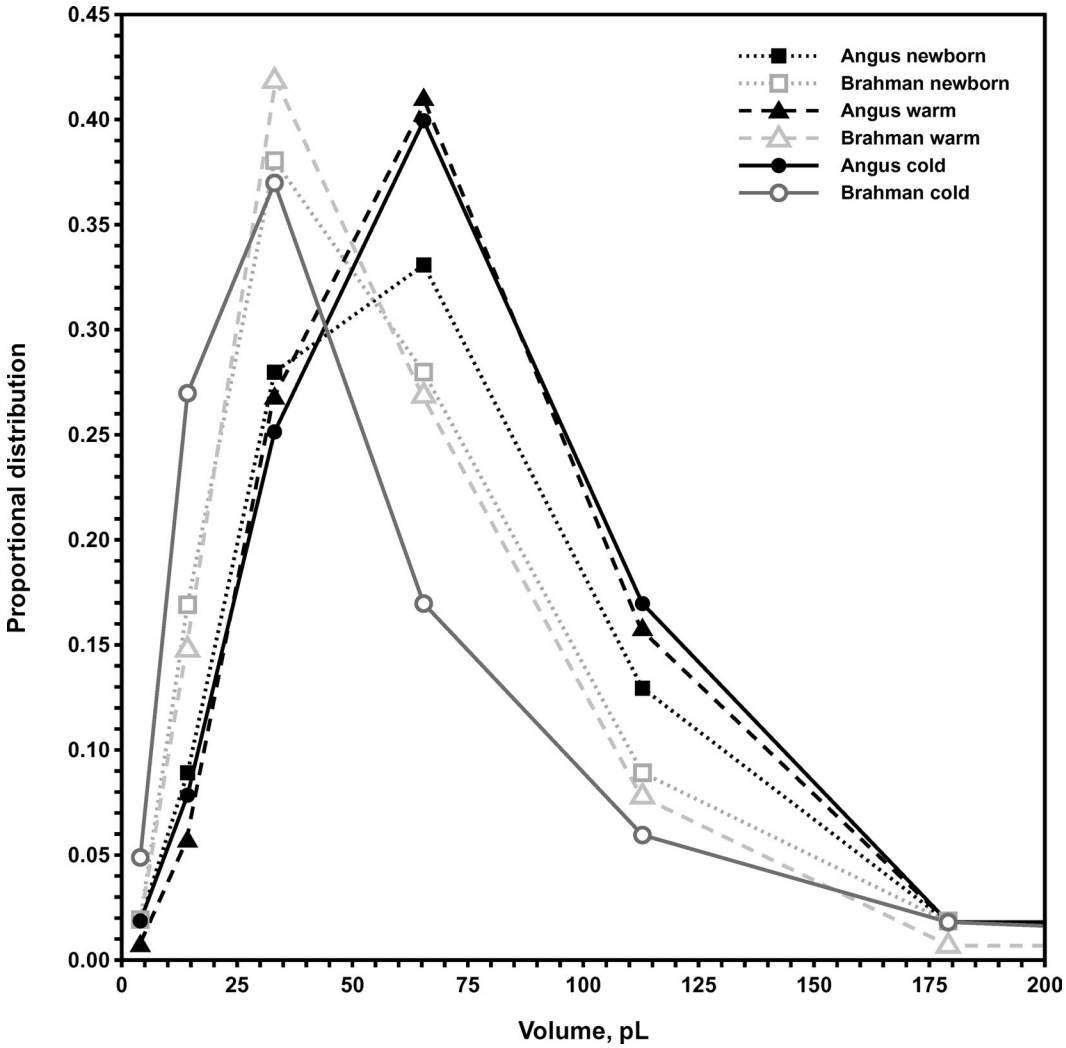

Figure 5. Adipocyte volume distributions for Angus and Brahman newborn calves (15 h old), newborn calves held at 22°C for 48 h (warm), and newborn calves held at 4°C for 48 h (cold) (Exp. 4). Treatment, $P = 0.09$; breed, $P = 0.004$; treatment × breed, $P = 0.26$ (n = 5 to 7 calves per breed and treatment).

morphology, and lipid metabolism. In this and subsequent experiments, UCP1 mRNA was measured by Northern analysis or slot blot analysis, or both, as described below. Morphology was documented both by transmission electron microscopy (described below) and by osmium fixation of s.c. and/or brown adipose tissues (May et al., 1994). To quantify and characterize lipid metabolism in vitro, perirenal adipose tissue slices were incubated for 2 h at 37°C with either 10 m*M* acetate plus 1 μCi of [1-^{14}C]acetate or 10 m*M* glucose plus 1 μCi [U-^{14}C]glucose. Additional details are provided below.

Experiment 2: Brown Adipose Tissue from Angus and Brahman Newborn Calves. Perirenal and tailhead s.c. adipose tissues were collected from Angus (n = 6) and Grey Brahman calves (n = 7) and used for the same measurements as in Exp. 1 (Miller et al., 1999a). As in Exp. 1, calves had been infused with NE before collection of BAT at approximately 12 h of age. For the measurement of lipogenesis, flasks contained either 10 m*M* acetate and 10 m*M* glucose plus 1 μCi of [1-^{14}C]acetate per flask, or a combination of 10 m*M* glucose plus 1 μCi of [U-^{14}C]glucose, 0.75 m*M* sodium palmitate plus 0.5 μCi of [9,10-^{3}H]palmitate, and 30 mg/mL fatty acid-poor bovine serum albumin. The affinity and number of β-adrenergic receptors (**β-AR**) were measured in perirenal brown adipose tissue, using [^{125}I]iodocyanopindolol as a ligand (Mersmann and McNeel, 1992). No attempt was made to separate subtypes.

Experiment 3: Ontogenic Development of Bovine Brown Adipose Tissue. Fetuses (n = 3 per breed type at each time point) from Angus or Grey Brahman mature cows were taken at 96, 48, 24, 14, and 6 d before expected parturition, and at parturition (Landis et al., 2002). Samples were obtained when the calves were 5 h of age; calves were not infused with NE before sample collection. Uncoupling protein-1 gene expression and adipocyte morphology in samples of perirenal and s.c. adipose tissues were measured at each interval, as were lipid metabolism in the perirenal adipose tissues at each fetal period. For the measurement of lipogenesis,

flasks contained either 10 m*M* acetate and 10 m*M* glucose plus 1 μCi of [1-^{14}C]acetate per flask, or a combination of 10 m*M* glucose plus 1 μCi of [U-^{14}C]glucose, 0.75 m*M* sodium palmitate plus 0.5 μCi of [9,10-^{3}H]palmitate, and 30 mg/mL fatty acid-poor bovine serum albumin. Additional details are provided below.

Experiment 4: Postnatal Response of Bovine Brown Adipose Tissue to Warm and Cold Exposure. Male Angus and Brahman calves (n = 5 to 7 per breed type per treatment) were assigned randomly to one of three experimental groups: 1) newborn (15 h of age), 2) 48 h of 22°C exposure starting at 15 h of age, and 3) 48 h of 4°C exposure starting at 15 h of age. Calves were not infused with NE before sample collection. The warm- and cold-treatment calves were housed in rooms maintained at 22 and 4°C, respectively. Tailhead s.c. adipose tissue was collected for morphological measurements and UCP1 gene expression, whereas perirenal BAT samples were obtained for the morphological measurements, UCP1 gene expression, and in vitro incubations. For this experiment, flasks contained 1) 5 m*M* sodium acetate, 5 m*M* glucose, and 1 μCi of [1-^{14}C]acetate; 2) 5 m*M* sodium acetate, 5 m*M* glucose, 0.75 m*M* sodium palmitate, 30 mg/mL BSA and 0.5 μCi of [3-^{3}H]palmitate plus 1 μCi of [U-^{14}C]glucose; or 3) 5 m*M* sodium acetate, 5 m*M* glucose, 0.75 m*M* sodium palmitate, 30 mg/mL BSA and 0.5 μCi of [1-^{14}C]palmitate for measurement of palmitate oxidation. These flasks also contained 10^{-3}, 10^{-6}, or 10^{-9} *M* norepinephrine plus 0.5 m*M* theophylline. The measurement of conversion of palmitate to CO_2 and total lipids is described below.

Experiment 5: Postnatal Response of Ovine Brown Adipose Tissue to Warm and Cold Exposure. This experiment examined the effects of postpartum environmental temperature in neonatal lambs. Lambs were obtained within 2 h from twin-bearing ewes (n = 8). Within twin pair, lambs were assigned at birth to a cold (6°C) or warm (28°C) environmental chamber for 48 h. Brown adipose tissue was collected and was analyzed for UCP1 mRNA concentration and histology.

General Procedures

Institutional Approval. All experiments were approved individually by the Texas A&M University Laboratory Animal Care Committee.

Animals and Diets. With the exception of Exp. 1, mature Angus and Grey Brahman cows were grazed on bermudagrass hay and 0.91 kg/d of a cottonseed meal-based range cube (20% CP). Ewes in Exp. 5 consumed a similar diet, except that some received Cu supplementation. Gravid cows and ewes were monitored every 4 h, and calves and lambs were collected before suckling and given pooled colostrum. Calves and lambs that were kept in environmental chambers (Exp. 4 and 5) were fed pooled colostrums (40 mL/kg BW). Calves and lambs were killed with an overdose of sodium pentobarbital.

Sample Preparation for Histology. Samples were prepared immediately for histological analysis. Tissue samples (approximately 100 mg) were sliced into 1-mm size pieces and placed into a primary fixative of 3% glutaraldehyde:0.08 *M* sodium cacodylate buffer (pH 7.4). One perirenal sample from each age group within each breed type (n = 12 total) was postfixed in 2% osmium tetroxide, stained with Enbloc stain (2% uranyl-acetate in methanol), and embedded in Epon/Araldite. The embedded samples were sectioned to an approximately 70-nm thickness and were photographed at 60 kV with a transmission electron microscope (10C; Zeiss, Jena, Germany).

Preparation and Analysis of RNA. Total RNA was isolated from perirenal adipose tissue samples by the guanidine thiocyanate-phenol-chloroform extraction procedure. Purity and yield were determined by the ratio of absorbance at 260 and 280 nm. Uncoupling protein-1 mRNA was determined by Northern blot and slot blot analysis.

For Northern blot analysis, 40 μg of total RNA was denatured at 68°C, separated by electrophoresis on a 1.0% agarose gel containing formaldehyde and capillary transferred to nylon Hybond N^{+} membrane (Amersham Life Science, Inc., Arlington Heights, IL). Transfer efficiency was checked by ethidium bromide UV visualization. The membrane was baked at 80°C for 2 h and UV cross-linked (UV Stratalinker 1800; Stratagene Cloning System, La Jolla, CA). The blot was prehybridized with 0.1 g/L salmon sperm DNA for 2 h at 55°C. A UCP1 mRNA was generated by PCR. The template DNA was the bovine calf UCP1 1.4-kb cDNA (generously provided by L. Casteilla, Centre de Rechere, CNRS, Toulouse, France) linearized with *Eco*R1. The primers were 5′-CTC AGC GGG CCT AAC GAC-3′ and 5′-GTT TGT TTT TCA CCA GGG-3′, which produced a PCR product approximately 350 bp in size. The PCR-generated UCP1 probe was radiolabeled with [α-^{32}P]dCTP by random primer method (Gibco BRL Life Technologies, Grand Island, NY) and hybridized to the RNA. The blot was rinsed once with 0.1% SDS in 2× SSC (300 m*M* NaCl, 30 m*M* trisodium acetate), at 42°C for 15 min and applied to Kodak X-AR5 X-ray film (Eastman Kodak, Rochester, NY) for 10 d. To test the efficacy of the PCR-generated UCP probe, perirenal adipose tissue was obtained from two newborn and two 7-d cold-adapted calves from a separate study. As a negative control, RNA was extracted from adult bovine longissimus dorsi muscle that had been snap-frozen in liquid nitrogen. The RNA was extracted and Northern blot analysis was performed as described above.

A commercial slot blot apparatus (Schleicher & Schuell, Keene, NH) was used to quantify the relative amounts of UCP mRNA in the perirenal adipose tissue samples. Ten micrograms of total RNA were incubated at 65°C for 5 min in 3 vol (vol/vol) of the following solutions: 500 μL of formamide, 162 μL of formaldehyde (37% solution), and 100 μL of 10× MOPS (0.02 *M* 3-[N-morpholino] propane sulfonic acid, 0.005 *M* sodium acetate, 0.005 *M* ETDA, pH = 7.0). Samples were chilled on ice and 1 vol of cold 20× SSC (3 *M* NaCl and 0.3 *M*

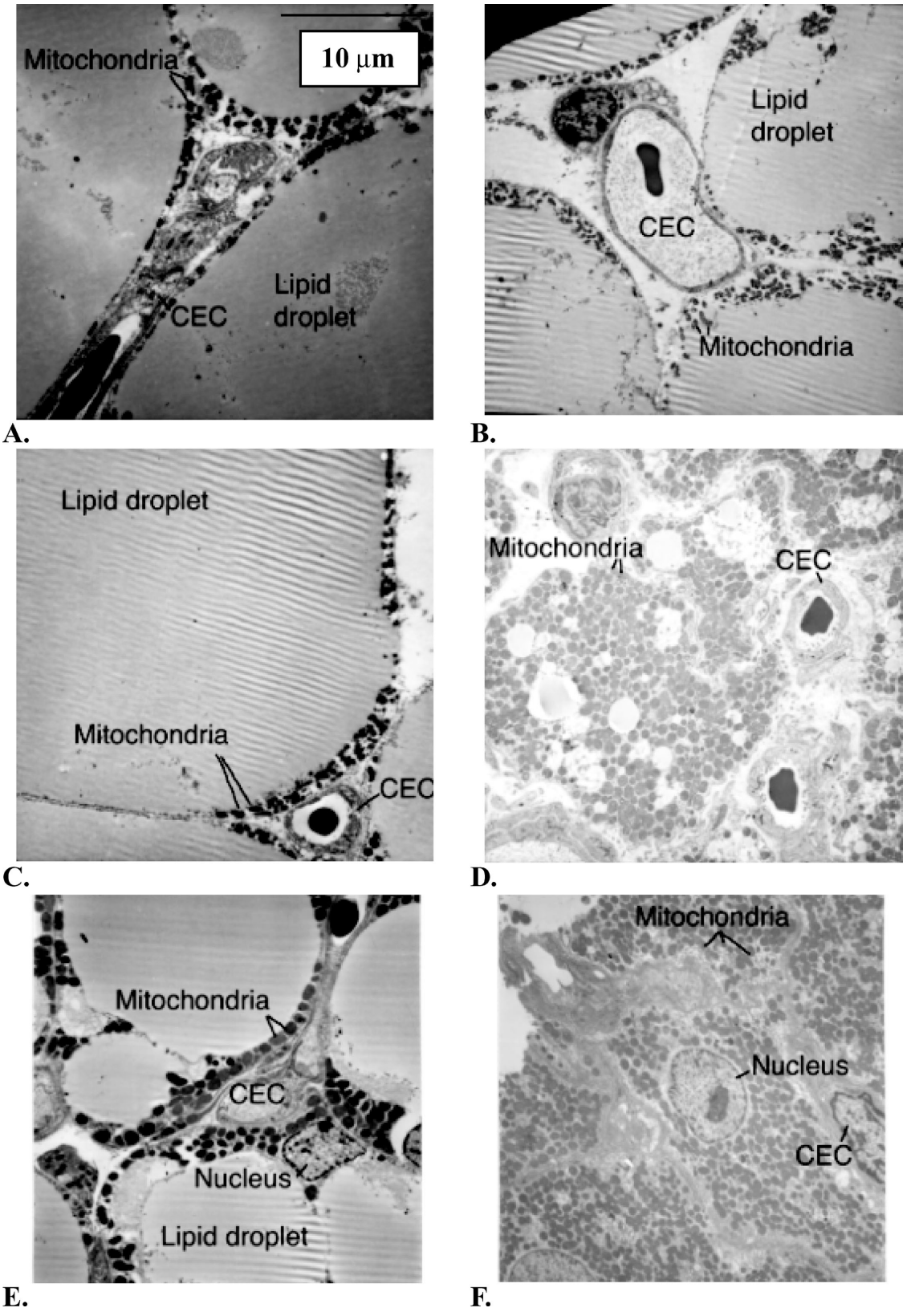

Figure 6. Perirenal (brown) adipocytes from Angus (A, B) and Brahman (C, D) newborn calves (Exp. 4) or newborn lambs (E, F; Exp. 5). Calves were held either at 22°C (A, C) or 4°C (B, D) for 48 h. Lambs were held at either 6°C (E) or 28°C (F) for 48 h. Both calves and lambs were fed pooled colostrums while in the environmental chambers. There was no apparent loss of lipid in Angus brown adipocytes, whereas lipid droplets had been almost completely consumed after 48 h of cold treatment in Brahman calf and lamb brown adipocytes. CEC = capillary endothelial cell. The scale bar in A also applies to B through F.

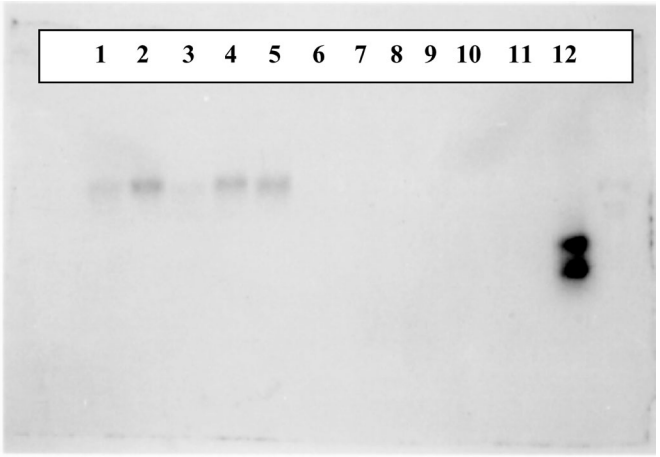

Figure 7. Northern blot analysis of UCP1 mRNA in 2-d cold- and warm-exposed lambs. The RNA was obtained as described from perirenal adipose tissue of lambs exposed for 48 h postnatally to 6°C (Lanes 1 to 5) or 28°C (Lanes 6 to 10; no bands apparent; Exp. 5). Lane 11 contains RNA from bovine longissimus muscle. Lane 12 contains DNA from a PCR reaction that used the calf UCP cDNA as template. The upper band in Lane 12 is the 300-bp PCR-UCP1 probe, whereas the lower band corresponds to unincorporated primers. Lane 13 (barely visible) contains the *EcoR1*-excised calf UCP1 partial cDNA (1.4 kb) (Casteilla et al., 1989). Northern blots were performed with 40 μg of total RNA (Lanes 1 to 11) or 5 ng of DNR (Lanes 12 and 13).

trisodium acetate, pH 7.0) was added. The RNA was applied to a nylon Hybond N^+ membrane in the slots of the manifold. The blot was processed as described above except that a duplicate blot also was hybridized with a radiolabeled cDNA for the 28S rat ribosomal RNA. After hybridization, rinsing, and autoradiography, the slot blot and Northern blot were scanned using the LKB 2202 Ultroscan laser densitometer (Bromma, Sweden) and the intensities of the bands were determined. For slot blots, density of UCP mRNA bands was corrected by ribosomal RNA.

Lipogenesis. Substrate selections and concentrations are described in detail above. Adipose tissue samples (50 to 100 mg) were incubated in duplicate for each substrate combination for each animal. In addition to the substrates indicated above, flasks contained oxygenated Krebs-Henseleit calcium-free buffer with 10 m*M* HEPES buffer (pH 7.35 to 7.40). Samples were incubated for 2 h at 37°C in a shaking water bath, and reactions were terminated with 3 mL of 10% trichloroacetic acid. Lipids were extracted essentially as described by Folch et al. (1957) in chloroform:methanol (2:1, vol/vol), and incorporation of substrates into lipids was measured by scintillation spectrometry. Some flasks contained hanging center wells with fluted filter paper for the measurement of CO_2. After 2 h of incubation, 0.5 mL of 2 *N* H_2SO_4 was injected into the medium and 0.2 mL of 2 *N* NaOH was injected into the hanging center well. Flasks were shaken at 37°C for an additional 2 h, the hanging center wells were transferred to 20-mL scintillation vials, and 2 mL of distilled-deionized water was added. After 30 min, 10 mL of a commercial scintillation fluid was added and disintegrations per minute were counted by scintillation spectrometry.

Statistical Analyses. Data were analyzed by two-factor analysis of variance by the SuperAnova program (Abacus Concepts, Inc., Berkeley, CA) when samples were taken at different ages or from calves held at different temperatures postnatally. Otherwise, data were analyzed by one-factor analysis of variance. All data are reported as means ± SEM.

Results and Discussion

Josey et al. (1987) reported that calves with greater Brahman inheritance exhibited higher morbidity and mortality rates during the neonatal period, especially as ambient temperature decreased. Angus calves exhibit approximately 25% higher peak metabolic rates (i.e., after NE infusion) than Brahman calves (Carstens et al., 1997; Martin et al., 1999a), which may contribute to their greater cold tolerance. It was our hypothesis that reduced BAT thermogenesis in Brahman calves may be related to a defect in their ability to store and/or utilize fatty acids in their BAT. Experiment 1, which used Wagyu × Angus newborn calves, provided our initial information about the histology of bovine white and brown adipose tissue at birth. Experiment 5 allowed a comparison of the effects of postpartum environmental temperature on BAT dedifferentiation in lambs and calves.

Experiment 1: Morphology and Lipid Metabolism. Although both perirenal and sternum s.c. adipocytes were mostly unilocular, perirenal adipocytes contained numerous large mitochondria with well-differentiated cristae; sternum adipocytes contained a few, very small mitochondria with poorly developed cristae, indicating that sternum s.c. adipose tissue in these Wagyu × Angus newborn calves was a white adipose tissue depot (Figure 1A, B) (Gemmell et al., 1972).

As demonstrated for fetal lambs (Vernon et al., 1981), acetate is the primary precursor for de novo fatty acid biosynthesis in newborn calves. Fatty acid biosynthesis from acetate was high in perirenal BAT (6.5 nmol acetate incorporated·10^5 $cells^{-1}$·h^{-1}) (Figure 2) but barely detectable in sternum s.c. adipose tissue (0.3 nmol acetate incorporated·100 mg^{-1}·h^{-1}; not shown). Glucose conversion to lipids, although low, was higher ($P < 0.05$) in s.c. adipose tissue (0.44 nmol glucose incorporated·10^5 $cells^{-1}$·h^{-1}) than in perirenal BAT (0.20 nmol glucose incorporated·10^5 $cells^{-1}$·h^{-1}). Sternum adipocytes also were smaller than perirenal adipocytes (11 vs. 27 pL). These data have been reported in detail by Martin et al. (1997). The rate of acetate incorporation into total lipids in sternum s.c. adipose tissue was substantially lower than we measured in a subsequent

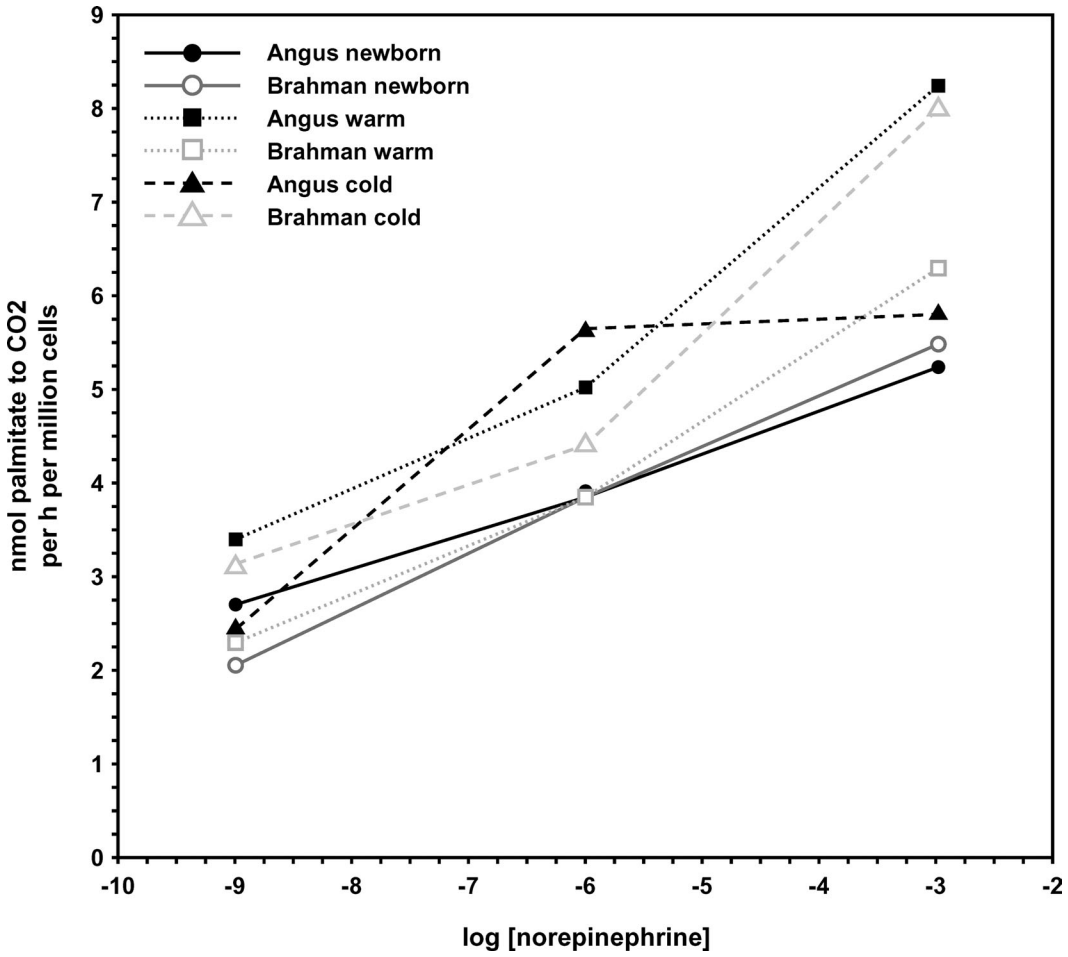

Figure 8. Norepinephrine-stimulated palmitate oxidation in perirenal adipose tissue from Angus and Brahman newborn calves (15 h old), newborn calves held at 22°C for 48 h (warm), and newborn calves held at 4°C for 48 h (cold; Exp. 4). Norepinephrine stimulated palmitate oxidation in a dose-dependent manner, but there was no effect of breed type ($P > 0.15$) or cold exposure ($P > 0.28$) on CO_2 production from palmitate (n = 5 to 7 calves per breed type and treatment). Lipogenesis from acetate was greater ($P < 0.05$) in BAT from calves held at warm or cold temperatures than in BAT from newborn calves. Data are plotted as a function of the log of the media norepinephrine concentrations (molar).

study in tailhead s.c. adipose tissue of 20-d-old Angus steers (approximately 14 nmol acetate incorporated·10^5 cells^{-1}·h^{-1}; Martin et al., 1999b). This may have been due to differences in sampling sites, age of the calves when sampled, or breed.

Experiment 2: Angus vs. Brahman Brown Adipose Tissue. In Exp. 2, Angus and Brahman BAT did not differ in perirenal brown adipocyte volume (46 vs. 53 pL, respectively). Large lipid droplets, adipocyte nuclei, and tightly packed mitochondria were evident in all perirenal adipose tissue samples, and perirenal adipose tissue is the major brown adipose tissue site in newborn calves (4 to 5% BW). Although no brown adipocytes were evident in sternum s.c. adipose tissue from Wagyu × Angus calves (Figure 1A), abundant brown adipocytes were apparent in tailhead s.c. adipose tissue from Angus and Brahman calves (Figure 1C, E). In Figure 1E, a brown adipocyte with an extraordinary abundance of mitochondria is seen adjacent to an obviously white adipocyte. These micrographs indicate that Angus and Brahman tailhead s.c. adipose tissue originally developed as BAT. We cannot discern from this information whether some portion of the brown adipocytes had dedifferentiated, and then redifferentiated into white adipocytes, or whether the white adipocytes arose from a separate line of preadipocytes. However, early research (Gemmell et al., 1972; Alexander, 1978; Vernon et al., 1981) suggested the former possibility. Gemmell et al. (1972) reported an exhaustive investigation of the interconversion of brown adipocytes to white adipocytes in neonatal lambs. They observed no degenerating brown adipocytes; rather, Gemmell et al. (1972) demonstrated a continuum of adipocytes with characteristics of both brown and white adipocytes.

Brahman BAT contained two-to-three times as many β-AR as Angus BAT (689 vs. 324; $P < 0.05$), although the dissociation constant (K_d) was not different between breed types (approximately 750 pM). The concentration

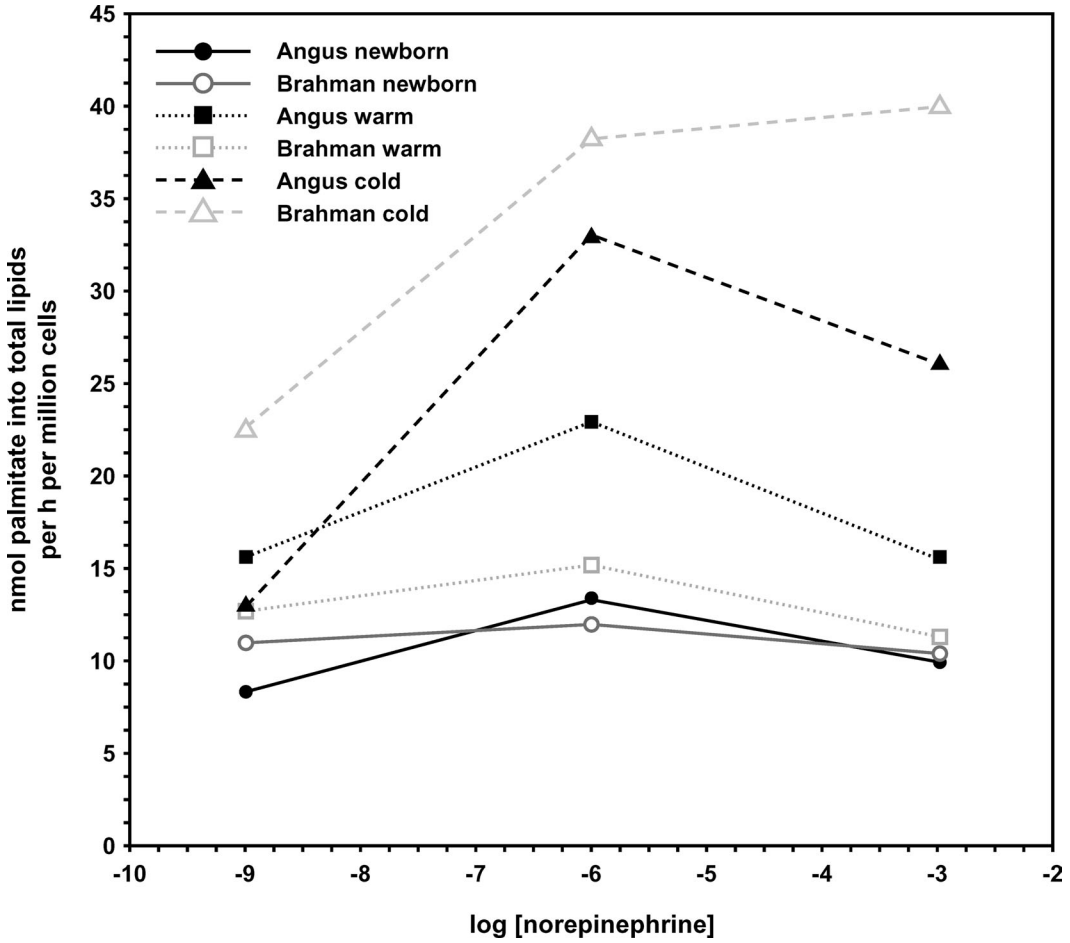

Figure 9. Norepinephrine-stimulated lipogenesis from palmitate in perirenal adipose tissue from Angus and Brahman newborn calves (15 h old), newborn calves held at 22°C for 48 h (warm), and newborn calves held at 4°C for 48 h (cold) (Exp. 4). Norepinephrine stimulated lipogenesis from palmitate nearly twofold but only in BAT from cold-exposed calves ($P < 0.05$; n = 5 to 7 calves per breed type and treatment). There was no effect of breed ($P > 0.30$). Data are plotted as a function of the log of the media norepinephrine concentrations (molar). Cold-exposed calves had greater rates of lipogenesis ($P < 0.01$) than newborn or warm-exposed calves.

of UCP1 mRNA was greater in Brahman BAT than in BAT from Angus calves. However, lipogenesis from acetate was greater in Angus BAT than in Brahman BAT (3 vs. 1 nmol acetate incorporated·10^5 cells^{-1}·h^{-1}; $P < 0.05$; Figure 2). The incorporation of glucose into total lipids was similar to rates for acetate incorporation (approximately 1 nmol glucose incorporated·10^5 cells^{-1}·h^{-1}), whereas palmitate incorporation was quite low (0.09 nmol palmitate incorporated·10^5 cells^{-1}·h^{-1}). The Angus and Brahman calves had been infused with NE before sample collection, and lipogenic rates were similar to those observed in the NE-infused Wagyu × Angus calves of Exp. 1. These data have been reported in Martin et al. (1999a).

Experiment 3: Ontogenic Development of Brown Adipose Tissue. At the beginning of the third trimester (approximately 190 d of gestation), perirenal adipose tissue mitochondria were large and spherical, with poorly defined cristae (Figure 3A, C). At this stage, Angus (Figure 3A) and Brahman (Figure 3C) mitochondria and adipocytes were indistinguishable. Adipocytes contained a large central lipid vacuole, with smaller lipid droplets completely encased in mitochondria (Figure 3E). By parturition, some mitochondria had become profoundly elongated, with highly convoluted, differentiated cristae (Figure 3B, D), and the adipocytes were unilocular (Figure 3F). However, their cross-sectional circumference was less than that observed in mitochondria from fetal samples. At birth, mitochondrial cristae of Angus calves were apparently continuous and highly convoluted (Figure 3B), whereas Brahman mitochondrial cristae usually were neither as convoluted nor as dense (Figure 3D).

Perirenal BAT mass increased from 96 to 48 d before birth, after which time there was no increase in mass (Figure 4A). Brown adipocyte volume increased in a similar fashion, indicating that most of the increase in BAT mass was in response to lipid filling of brown adipocytes. Alexander (1978) reported that the mass of perirenal adipose tissue in fetal Merino sheep increased

by approximately 34% over the last 3 wk of gestation. Vernon et al. (1981) documented a 40% increase in adipocyte volume of fetal lambs in the last 4 wk of pregnancy, indicating that most if not all of the increase in perirenal BAT mass in fetal lambs was due to adipocyte hypertrophy. Angus BAT adipocytes were smaller than Brahman BAT adipocytes at birth (56 vs. 78 pL; data are pooled across breed type in Figure 4).

Lipogenesis from acetate and palmitate in vitro at the beginning of the third trimester was extremely high (250 nmol acetate incorporated·10^6 cells^{-1}·h^{-1}; 1,200 nmol palmitate incorporated·10^6 cells^{-1}·h^{-1}; Figure 4B). Lipogenesis from both acetate and palmitate decreased over 80% during the last 96 d of gestation in both breed types. Vernon et al. (1981) demonstrated reductions in fatty acid synthesis from acetate and glucose in fetal ovine perirenal BAT that were similar in magnitude to those we observed. Palmitate esterification accounted for 98% of total glycerolipid synthesis in vitro in the newborn calves and was a primary contributor to lipid filling throughout gestation.

There was no difference in perirenal UCP1 mRNA concentration between breed types in Exp. 3 (Figure 4C). Nor was there a significant difference in the pattern of UCP1 gene expression between Brahman and Angus fetuses during gestation (data not separated for breed types). Thus, we found no evidence for differences in BAT gene expression between these breed types during the last trimester. Perirenal BAT UCP1 mRNA increased nearly fivefold during the last trimester, consistent with the extensive differentiation and elongation of mitochondria observed over this period. The timing of UCP1 gene expression differed in Exp. 3 and that reported by Casteilla et al. (1989). They previously reported that UCP1 gene expression was nearly undetectable at 180 d of fetal life, which provided the basis for our initial sampling time; UCP1 mRNA concentration did not increase appreciably until 259 d of gestation (Casteilla et al., 1989).

Tailhead s.c. UCP1 mRNA was barely detectable by birth (Figure 4C), but tended to be greater overall (P = 0.09) in Angus than in Brahman BAT. If UCP1 activity in s.c. adipose tissue persists after birth, then s.c. adipose tissue may contribute more to thermogenesis in Angus newborn calves than in Brahman calves. These data suggest that bovine s.c. adipose tissue originates as BAT and involutes to white adipose tissue postnatally, which is consistent with the findings of Exp. 2. This also is consistent with Trayhurn et al. (1993), who detected uncoupling protein (by Western analysis) in hind limb s.c. adipose tissue of goats up to 14 d postnatally. Trayhurn et al. (1993) were unable to detect UCP1 mRNA in newborn goats, indicating that the protein persisted several days after the mRNA had degraded. The persistence of brown adipocyte morphology (Martin et al., 1999a), in spite of nearly undetectable UCP1 mRNA (this study), indicates that bovine s.c. BAT develops in a pattern similar to that of goats. It should be noted that Casteilla et al. (1989) detected neither UCP1 protein nor mRNA in s.c. adipose tissue of calves. The data for Exp. 3 have been reported in detail by Landis et al. (2002).

Experiments 4 and 5: Brown Adipose Tissue of Warm- and Cold-Exposed Calves and Lambs. Exposure of 15-h-old Angus calves to either 22 or 4°C for 48 h tended to increase perirenal adipocyte volume (Figure 5). Brahman perirenal adipocytes were smaller than Angus adipocytes at birth and shrank ($P < 0.05$) in response to cold treatment. The reduction in adipocyte volume in Brahman BAT adipocytes was caused by delipidation during cold exposure (Figure 6C, D). Only small lipid droplets remained after cold exposure in Brahman BAT, whereas there was no apparent loss of lipid in Angus brown adipocytes after cold exposure (Figure 6A, B).

After 48 h at 28°C, brown adipocytes from newborn lambs were smaller, and contained more lipid droplets, than did brown adipocytes from newborn calves held for 48 h at 22°C (Figure 6E vs. 6C). There were virtually no lipid droplets apparent after 48 h of 6°C exposure in brown adipocytes from lambs (Figure 6F). These results were similar to those observed for Brahman BAT (Figure 6C, D). Gemmell et al. (1972) reported extensive lipid depletion in brown adipocytes from fasted lambs held for 2 d at 26°C. Apparently, the lambs in Exp. 5 received sufficient nutrients to maintain the lipid composition of their BAT at warm ambient temperature.

Whereas cold exposure depleted lamb brown adipocytes of lipid, it sustained UCP1 gene expression (Figure 7). There was detectable UCP1 mRNA in all samples from cold-exposed lambs, whereas UCP1 mRNA was undetectable in BAT from warm-exposed lambs. Both effects probably were caused by continuous, cold-induced secretion of NE (Trayhurn et al., 1987).

Norepinephrine stimulated palmitate oxidation in a dose-dependent manner, but there was no effect of breed type ($P > 0.15$) or cold exposure ($P > 0.28$) on CO_2 production from palmitate (Figure 8). Lipogenesis from acetate was greater ($P < 0.05$) in BAT from calves held at warm or cold temperatures than in BAT from newborn calves (not shown). Norepinephrine stimulated lipogenesis from palmitate nearly twofold, but only in BAT from cold-exposed calves ($P < 0.05$; Figure 9).

Brown adipose tissue obtains fatty acids from four sources (Himms-Hagan, 1990): 1) its own endogenous triacylglycerol (**TAG**) stores, 2) hydrolysis of plasma lipoproteins via lipoprotein lipase, 3) NEFA from the blood, and 4) the synthesis of NEFA from glucose. Alexander et al. (1968) reported that availability of circulating metabolites did not limit the metabolic response of lambs to cold exposure. The situation may differ in bovine neonates. Godfrey et al. (1991) demonstrated that cold-stressed neonatal Brahman (*Bos indicus*) calves did not utilize energy-containing constituents in the blood (such as glucose, lactate, TAG, protein, and NEFA) to control body temperature as efficiently as crossbred *Bos indicus*/*Bos taurus* calves.

Carstens et al. (1997) demonstrated that Brahman calves have higher circulating NEFA than Angus calves at birth, and there is a greater increase in NEFA in response to NE infusion in Brahman than in Angus calves. Taken together with the greater density of β-AR on Brahman BAT, the data suggest that Brahman calves are too effective in mobilizing lipids in response to cold challenge, and may exhaust their BAT lipid stores early postnatally, especially when exposed to the cold.

Implications

We hypothesized that we would detect differences between Angus and Brahman fetuses in molecular and morphological aspects of perirenal brown adipose tissue development that would explain documented differences in nonshivering thermogenesis between breeds. The primary finding of our investigations is that Brahman brown adipose tissue more rapidly mobilizes stored lipids. This may in part be responsible for the high mortality of Brahman calves born during severe winter conditions.

Literature Cited

Alexander, G. 1978. Quantitative development of adipose tissue in foetal sheep. Aust. J. Biol. Sci. 31:489–503.

Alexander, G., S. C. Mills, and T. W. Scott. 1968. Changes in plasma glucose, lactate and free fatty acids in lambs during summit metabolism and treatment with catecholamines. J. Physiol. 198:277–289.

Carstens, G. E., E. H. McPhail, S. Kahl, and S. B. Smith. 1998. Brown adipose tissue thermogenesis and ontogeny in Bos indicus and Bos taurus cattle. Pages 47–50 in Energy Metabolism of Farm Animals. K. J. McCraken, E. F. Unsworth, and A. R. G. Wylie, ed. CAB International, New York.

Carstens, G. E., P. C. Mostyn, M. A. Lammoglia, R. C. Vann, R. C. Apter, and R. D. Randel. 1997. Genotypic effects on norepinephrine-induced changes in thermogenesis, metabolic hormones, and metabolites in newborn calves. J. Anim. Sci. 75:1746–1755.

Casteilla, L., O. Champigny, F. Bouillaud, J. Robelin, and D. Ricquier. 1989. Sequential changes in the expression of mitochondrial protein mRNA during the development of brown adipose tissue in bovine and ovine species: Sudden occurrence of uncoupling protein mRNA during embryogenesis and its disappearance after birth. Biochem. J. 257:665–671.

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

Gemmell, R. T., A. W. Bell, and G. Alexander. 1972. Morphology of adipose cells in lambs at birth and during subsequent transition of brown to white adipose tissue in cold and in warm conditions. Am. J. Anat. 133:143–164.

Godfrey, R. W., S. D. Smith, M. J. Guthrie, R. L. Stanko, D. A. Neuendorff, and R. D. Randel. 1991. Physiological responses of newborn *Bos indicus* and *Bos indicus* × *Bos taurus* calves after exposure to cold. J. Anim. Sci. 69:258–263.

Himms-Hagen, J. 1990. Brown adipose tissue thermogenesis: Interdisciplinary studies. FASEB J. 4:2890–2898.

Josey, M. J., L. V. Cundiff, R. M. Koch, K. E. Gregory, and G. L. Hahn. 1987. Mortality and cold tolerance of calves with different proportions of Bos indicus to Bos taurus inheritance. Pages 334–337 in Proc. 8th Symp. Biometerology and Aerology, Clay Center, NE.

Landis, M. D., G. E. Carstens, E. G. McPhail, R. D. Randel, K. K. Green, L. Slay, and S. B. Smith. 2002. Ontogenic development of brown adipose tissue in Angus and Brahman fetal calves. J. Anim. Sci. 80:591–601.

Martin, G. S., G. E. Carstens, T. L. Taylor, C. R. Sweatt, A. G. Eli, D. K. Lunt, and S. B. Smith. 1997. Prepartum protein restriction does not alter norepinephrine-induced thermogenesis or brown adipose tissue function in newborn calves. J. Nutr. 127:1929–1937.

Martin, G. S., M. D. King, G. E. Carstens, A. G. Eli, H. J. Mersmann, and S. B. Smith. 1999a. Metabolism and morphology of brown adipose tissue from Brahman and Angus newborn calves. J. Anim. Sci. 77:388–399.

Martin, G. S., D. K. Lunt, K. G. Britain, and S. B. Smith. 1999b. 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 tissue from Angus and Waygu crossbred steers. J. Anim. Sci. 72:3110–3117.

Mersmann, H. J., and R. L. McNeel. 1992. Ligand binding to the porcine adipose tissue β-adrenergic receptor. J. Anim. Sci. 70:787–797.

Trayhurn, P., M. Ashwell, G. Jennings, D. Richard, and D. S. Stirling. 1987. Effect of warm or cold exposure on GDP binding and uncoupling protein in rat brown fat. Am. J. Physiol. 252:E237–E245.

Trayhurn, P., M. E. A. Thomas, and J. S. Keith. 1993. Postnatal development of uncoupling protein, uncoupling protein mRNA, and GLUT4 in adipose tissue of goats. Am. J. Physiol. 265:R676–R682.

Vernon, R. G., J. P. Robertson, R. A. Clegg, and D. J. Flint. 1981. Aspects of adipose-tissue metabolism in foetal lambs. Biochem. J. 196:819–824.