Bovine myofiber characteristics are influenced by postweaning nutrition\textsuperscript{1,2}


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ABSTRACT: This study determined the extent to which bovine longissimus lumborum muscle (LLM) myofibers are influenced by nutrition for 120 d from weaning and the time-course of recovery after severe postweaning nutritional restriction. After weaning, 3 groups of Belmont Red cattle, a tropically adapted breed, were fed to achieve rapid growth (RG, ≥0.6 kg of BW gain/d; n = 16), slow growth (SG, 0.2 kg of BW gain/d; n = 17), or BW loss (WL, 10% loss of weaning weight; n = 17) over 120 d. They were then grazed as 1 group at pasture with forage supplementation for 600 d until slaughter at approximately 500 kg of BW. Samples of LLM were taken from 8 to 12 animals per treatment 6 d before (baseline) and 115, 204, 324, and 476 d after commencement of the study and from all cattle at slaughter (d 721). Myofiber characteristics were determined by immunocytochemical staining of myosin heavy chains. Cross-sectional areas (CSA) of the major myofiber types 1, 2A, and 2X in WL were reduced at d 115 compared with baseline and with the growth groups (all $P < 0.001$); however, there was little difference in the percentage of the different myofiber types (all $P > 0.10$). Differences in CSA of the major myofiber types between WL and the growth groups at 115 d were smallest for type 1 (slow oxidative) and greatest for type 2X (fast glycolytic). Consequently, the relative area (percentage of total myofiber area) of type 1 myofibers in WL was significantly greater at 115 d than in the growth groups ($P < 0.001$). During recovery from postweaning nutritional restriction, significant differences in major myofiber type percentages were not evident (all $P > 0.10$), and by 721 d CSA of myofiber types differed little between the treatment groups, although SG had greater CSA of type 1 ($P < 0.05$) and type 2A ($P < 0.01$) myofibers than WL and RG. At 721 d, the relative area of type 2A myofibers was less in WL compared with SG ($P < 0.01$) and RG ($P < 0.05$) and of type 2X myofibers greater ($P < 0.05$) in WL compared with SG. It is concluded that in the LLM of cattle undergoing severe nutritional restriction immediately postweaning, the size of the more glycolytic fiber types is more adversely affected than the more oxidative types, resulting in an increased relative area of type 1, slow oxidative myofibers. However, given adequate time and nutriment at pasture, LLM myofiber characteristics of cattle recovered to near normal after severe, chronic nutritional restriction immediately postweaning, consistent with earlier findings for beef quality.

Key words: cattle, growth, muscle, myofiber, nutrition, weaning

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doi:10.2527/jas.2009-1936

\textsuperscript{1}A subset of results from this study was presented in Lehnert et al. (2006).
\textsuperscript{2}The authors acknowledge funding from the Cooperative Research Centre for Cattle and Beef Quality, Australia (Project 1.2). The authors thank Bill van den Heuvel and Alan Day (CSIRO), who provided technical assistance; Keith Newby (NSW Department of Primary Industries), who performed tissue biopsies; and Stuart McClelland, Steve Sinclair, and Keryn Hutton (NSW Department of Primary Industries), who carried out procedures associated with muscle fiber typing. We also thank Brigitte Picard (INRA), who provided antibody S5 8H2.
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Received March 4, 2009.
Accepted June 11, 2009.
INTRODUCTION
Mating, calving, and weaning within pastoral systems are generally timed according to feed availability to optimize conception rates, lactational performance, and offspring growth. In northern Australia, cattle may undergo prolonged BW stasis or loss after weaning, then improved growth and at least partial compensation as conditions for growth of pasture improve (Tomkins et al., 2006).

Myofiber characteristics contribute to variation in muscle growth and in eating quality (Rehfeldt et al., 2000; Freking et al., 2004; Greenwood et al., 2006a,b). Myofiber characteristics may differ between muscles (Hunt and Hedrick, 1977), and with age and BW (Jurie et al., 1999), sex (Johnston et al., 1981), genotype (Wegner et al., 2000; Picard et al., 2006), and use of metabolic modifiers (Clancy et al., 1986; Vestergaard et al., 1994).

Nutrition and growth path may also affect myofiber characteristics (Seideman and Crouse, 1986; Picard et al., 1995) or may have little or no effect (Maltin et al., 1998; Hoch et al., 2005). Where effects are evident, they may not persist in the longer term (Allingham et al., 2001; Greenwood and Cafe, 2007). Despite these findings, the extent to which nutrition immediately postweaning influences myofiber characteristics in tropically adapted cattle in the short or long term has not been studied.

The objective of this research, therefore, was to test the hypotheses that myofibers in the longissimus lumborum muscle (LLM) of tropically adapted cattle are influenced by nutrition for 120 d from weaning and to examine the changes during recovery from severe postweaning nutritional restriction. The LLM was studied because of its commercial importance, its use in carcass assessment (AUS-MEAT, 1998; Perry et al., 2001) and beef quality (MSA, 1999) grading systems, and because eating quality characteristics had been previously examined for the LLM of the cattle within this study (Tomkins et al., 2006).

MATERIALS AND METHODS
This experiment, including all animal procedures, was carried out in accordance with the Commonwealth Scientific and Industrial Research Organisation (CSIRO) animal ethics guidelines.

Animals, Experimental Design, and Nutrition

The animals used in this study were a subset of those described by Tomkins et al. (2006). Briefly, 50 Belmont Red (Bos taurus composed of 50% Africander, 25% Hereford, and 25% Shorthorn) steers around 8 mo of age, with a BW of 205 ± 34 kg (mean ± SD), were randomly allocated to 3 groups. Diets were varied to achieve divergent growth paths. A BW loss (WL) group was fed low-quality grass hay (Angeleton grass, Dichanthium aristatum) ad libitum with average intake of 13 g of DM/kg of BW daily. A slow growth (SG) group was fed lucerne (Medicago sativa) hay at a restricted average intake of 19 g of DM/kg of BW daily. A rapid growth (RG) group was fed lucerne hay ad libitum, resulting in average intakes of 28 g of DM/kg of BW daily. These diets were fed for 120 d to achieve a 10% loss of weaning weight (0.22 kg of BW loss/d), growth of approximately 0.2 kg of BW gain/d, or rapid growth (≥0.6 kg of BW gain/d), for the WL, SG, and RG groups, respectively (Figure 1). This treatment period was followed by pasture feeding and forage supplementation for 601 d until the conclusion of the experiment 721 d after commencement, when the cattle averaged approximately 500 kg of BW (Figure 1). Details on effects of the nutritional treatments during and after the postweaning treatment periods on growth, carcass, and beef quality characteristics are also provided by Tomkins et al. (2006).

Sampling Times

Samples of LLM were collected at 6 time points: immediately before commencement of the experiment (d −6, baseline), toward the end of the nutritional treatments (d 115), and 84 (d 204), 204 (d 324), 356 (d 476), and 601 (d 721) d after cessation of the nutritional treatments (Figure 1). Muscle samples were obtained by biopsy at the first 5 sampling times and from carcasses after slaughter at the final sampling. Samples were obtained from 8 to 12 cattle within each treatment group at each time point up to d 476 and from 16 (SG) or 17 (WL and RG) cattle within each treatment group at d 721, as detailed in Figure 1.

Biopsy Procedure and Sample Preparation

Tissue was collected from the LLM of live cattle using an open biopsy procedure described by Allingham et al. (2001) and from carcasses postslaughter. For the biopsy, animals were restrained in a chute and given a mild sedating dose of xylazine hydrochloride (0.2 mg/kg of BW; Bomazine 100, Pharm Tech Pty. Ltd., Hornsby, New South Wales, Australia) intramuscularly. After sedation, the animals were anaesthetized with an intravenous injection of ketamine hydrochloride [2.0 mg/kg of BW; Parnell Ketamine Injection, Parnell Laboratories (Aust) Pty. Ltd., Alexandria, New South Wales, Australia], a short-acting general anesthetic. Approximately 5 g of tissue was excised as a rectangular block approximately 5 cm long × 1 cm wide × 2 cm deep from the superficial (dorsal) region of the LLM over the 12th to 13th ribs. After excision of the tissue, the muscle and subcutaneous fat were sutured and then the skin was sutured, and the animals given a 20-mL intramuscular injection of a broad spectrum antibiotic (Procaine penicillin 300 mg/mL; Propen, Ilium Veterinary Products, Smithfield, New South Wales, Australia). Sample collection alternated between the left and right sides at
successive biopsies on each animal, ensuring that sites previously biopsied were avoided by sampling at a site lateral to previous biopsy sites. All animals recovered unaided and without incident from anesthesia.

A tissue subsample of approximately 1 cm² × 2 cm deep was prepared, and any subcutaneous fat and fascia and approximately 5 mm of muscle were removed from the superficial (dorsal) surface of the LLM, leaving a block of muscle approximately 1 cm³ for immunohistochemistry and myofiber analyses. During sample preparation, the alignment of the sample was maintained to allow cryosections to be cut perpendicular to the direction of the muscle cells (Figure 2), commencing from the cranial surface of the tissue block. Each muscle sample was mounted using gum tragacanth (Sigma Chemical Company, St. Louis, MO; prepared 5% wt/vol in distilled, deionized H₂O) onto a cork block, with muscle fibers running perpendicular to the cork block. Samples were frozen by immersion in iso-pentane cooled to approximately −160°C in liquid nitrogen, before storage at −70°C.

Muscle Immunohistochemistry

Cross-sectional, 10-µm-thick, serial sections were cut from each frozen sample using a cryostat microtome (ThermoShandon AS 620 Cryostat SME, Thermotrace Ltd., Noble Park, Victoria, Australia). The sections were air-dried and stored at −20°C until commencement of the staining procedures. The sections were then thawed, fixed in 100% acetone, and recovered in 0.01 M PBS, pH 7.2, before application of blocking solution (10% nonimmune serum, Zymed Laboratories, South San Francisco, CA) for 10 min. The blocking solution and subsequent reagents were applied within a well created around each tissue section using a hydrophobic slide marking pen. A volume of 50 µL of diluted monoclonal antibody against slow or type 1 (clone WB-MHC, Novocastra, Newcastle upon Tyne, UK; diluted 1:100 in PBS), fast or type 2 (clone MY-32, Sigma; diluted 1:1,200 in PBS), and types 1, 2X, and 2B (clone S5 8H2; see Picard et al., 1998; Duris et al., 2000; Arguello et al., 2001; Reggiani and Mascarello, 2004; diluted 1:1,000 in PBS) myosin heavy chain (MHC) isoforms were applied to serial tissue sections and incubated for 1 h at 37°C in a humid chamber. Rabbit anti-laminin, affinity isolated antibody (Sigma; diluted 1:500 in PBS) was also included in the solution containing anti-type 1 MHC to allow cellular margins to be delineated (Figure 2). The antibodies were detected using a broad spectrum labeled-[strept]-avidin-biotin amplification system and the substrate chromagen, diaminobenzidine (Zymed Laboratories). The stained sections were then dehydrated and cleared using graded ethanolys and xylenes, and coverslips applied using a xylene-based mounting medium.

Myofiber Classification and Morphometry

Microscopic image analysis was used to classify and measure myofibers. An Axioskop2 Plus microscope fitted with Plan-Neofluar objectives and a Zeiss AxioCam digital camera (Carl Zeiss Pty. Ltd., Göttingen, Germany) was used to produce images. Images were generated using a 10× or 20× objective, depending on the size of the myofibers, which were captured using Zeiss Axiosion AC release 4.4 software and analyzed using Zeiss KSRun version 3.0 software (Carl Zeiss Pty Ltd.). Myofibers were classified visually from images generated using the software program as types 1, 2C (type 1 to type 2A intermediate), 2A, 2AX (type 2A to type 2X intermediate), and 2X (Figure 2) based on their stain-
The imaging program was calibrated for cross-sectional area (CSA) measurements using a stage micrometer (Leica Microsystems GmbH, Wetzlar, Germany) and the CSA of cells of each myofiber type determined by tracing the anti-laminin-stained margins of the cells using an electronic drawing tablet. Only cells that appeared to be cut perpendicular, not oblique, to the length of the myofibers were measured.

Myofiber types described previously as 2B and 2AB (Picard et al., 1998) were classified as types 2X and 2AX on the basis that antibody S5–8H2 binds to type 2B and type 2X MHC (Reggiani and Mascarello, 2004), but that limb and trunk muscles of cattle express type 2X MHC and little or no type 2B MHC (Tanabe et al., 1998; Maccatrozzo et al., 2004; Reggiani and Mascarello, 2004; Picard and Cassar-Malek, 2009). For each sample classified, the total area of each myofiber type relative to the total myofiber area and the average CSA for all myofibers were calculated from the percentage and average size of the myofiber types.

Myofibers were classified for each muscle sample from 2 fields across the 3 serial sections and CSA of cells measured in each field, as described above. On average, 274 myofibers were classified per sample, declining from an average of 321 cells per sample for baseline samples to 210 cells per sample at d 721. The average SD between duplicate fields within sample for percentage myofiber types was 2.4% for type 1, 0.5% for type 2C, 3.7% for type 2A, 1.2% for type 2AX, and 3.4% for type 2X. The average SD between duplicate fields within sample for myofiber CSA was 156 µm² for type 1, 292 µm² for type 2C, 297 µm² for type 2A, 534 µm² for type 2AX, and 323 µm² for type 2X.

Statistical Analyses

Myofiber characteristics and BW were analyzed using the statistical software package ASReml (Gilmour et al., 2006), which fits linear mixed models by REML. The repeated measurements of each myofiber characteristic over time from d 115 to 721 were analyzed using a cubic smoothing spline approach, which is appropriate when the response is nonlinear. The trend was partitioned into 2 components, fitting time as a fixed linear covariate and a random spline term to account for nonlinear smooth deviations about the linear trend (see Verbyla et al., 1999). Nutritional treatment (with the 3 levels, RG, SG, and WL) was fitted as a fixed effect, as was the interaction of nutrition with a linear time trend; with a random interaction of the spline terms with nutrition also fitted to estimate a smooth response to time for each of the treatments. Similarly, random terms identifying individual animals and the interaction with linear and spline terms were fitted to estimate a smooth response to time for each individual animal. Predictions were made at each of the sampling times, as well as a prediction of the slope of the linear

Figure 2. Immunocytochemical staining of myosin heavy chains (MHC) and laminin in bovine longissimus lumborum muscle. Representative myofibers are indicated: s, type 1 (= slow oxidative); a, type 2A (= fast oxidative-glycolytic); x, type 2X (= fast glycolytic); ax, type 2AX (= type 2A-type 2X intermediate). Classification of type 2AX myofibers was based on positive staining for the type 2 MHC antibody; intermediate staining for the type 1, 2B, and 2X MHC antibody; and negative staining for the type 1 MHC antibody. Type 2C myofibers, which had a prevalence of less than about 1% of myofibers, are not present in the field shown. Type 2C myofibers are type 1-type 2A intermediate, and classification was based on positive or intermediate staining for all 3 MHC antibodies.
response to time for each of the 3 nutritional treatment
groups during the 600-d forage feeding period from ces-
sation of the nutritional treatments to the end of the
experiment. The predictions were derived from data for
all animals from which samples and myofiber data were
obtained (Figure 1).

During the 600-d posttreatment forage feeding pe-
riod, the BW of the cattle increased substantially (Fig-
ure 1). Hence, we also included BW as a covariate in
the models used to determine the posttreatment effects
of nutrition to assess the extent to which BW influ-
enced the significance of nutritional effects on myofiber
characteristics. Statistical significance of effects was ac-
cepted at \( P < 0.05 \).

RESULTS

BW of Steers

Body weight of the nutritional groups differed \( (P <
0.001) \) at the end of the nutritional treatment period
(Figure 1). The BW of the SG cattle did not differ
significantly from the RG group at d 214 or thereafter.
The BW of the WL steers remained less than the RG
and SG steers until conclusion of the study at d 721 \( (P
< 0.05) \).

Percentage of Myofiber Types

During the posttreatment forage finishing period (d
121 to 721) the proportion of type 1 fibers increased
linearly \( (P < 0.01) \) from about 25 to 28\% of the total
(Figure 3a), whereas the proportion of type 2X declined
\( (P < 0.001) \) from about 55 to 45\% (Figure 3e). During
the early posttreatment period, WL cattle had less of
the intermediate or transitional type 2C myofibers than
RG cattle \( (P < 0.05) \), whereas at the conclusion of the
study the WL and SG cattle had more of this myofi-
ber type \( (P < 0.001) \) than RG cattle (Figure 3b). In
keeping with these differences, the average daily rate of
change of type 2C myofibers was greater in WL and SG
than RG cattle during the 600-d postnutritional treat-
ment period \( (P < 0.01) \). There was also a nonlinear
time trend \( (P < 0.001) \) for percentage of the interme-
diate or transitional type 2AX (Figure 3d) myofibers.
Body weight during the 600-d posttreatment period
had a significant positive effect on percentage of type
2A myofibers \( (P < 0.05) \) and tended \( (P < 0.10) \) to
negatively influence percentage of type 2X myofibers
(Figure 3e), indicative of an overall shift toward more
oxidative myofiber types with increasing BW. However,
there were no significant differences between nutritional
treatments in the percentage of myofiber types 1, 2A,
2AX, and 2X, or in the average daily rate of change of
these fibers \( (all \ P > 0.10) \). The significance of results
for percentage of myofiber types was not altered by
accounting for differences in BW between nutritional
treatments.
The slow oxidative, type 1 myofibers in WL were smaller than in SG and RG due to the nutritional treatments at 115 d ($P < 0.001$) and remained smaller than in RG until d 476 ($P < 0.05$) and SG to the conclusion of the study ($P < 0.05$, Figure 4a). Type 2C myofibers were smaller in WL ($P < 0.01$) and SG ($P < 0.05$) than in RG cattle due to the postweaning nutritional treatments, remained smaller in WL compared with RG at 214 d ($P < 0.05$), and did not differ significantly due to nutritional treatment thereafter (Figure 4b). Type 2A myofibers were smaller in WL than in SG and RG cattle at the conclusion of the nutritional treatments ($P < 0.001$, Figure 4c). The SG and RG groups diverged in size of type 2A myofibers during the posttreatment period, resulting in significant differences at d 476 ($P < 0.05$) and at the conclusion of the study ($P < 0.01$). By contrast, size of type 2A myofibers in the WL and RG groups converged during the posttreatment period and did not differ at the conclusion of the study ($P > 0.10$). The type 2AX myofibers did not differ significantly due to nutrition at the conclusion of the treatment period or during the early posttreatment period (all $P > 0.10$) but became increasingly smaller in WL compared with the other groups of cattle as the posttreatment period progressed, resulting in significant differences ($P < 0.05$) compared with the growth groups at d 721 (Figure 4d). Type 2X myofibers in WL cattle were smaller than in SG and RG cattle at the conclusion of the nutritional treatments ($P < 0.001$) and were smaller until d 476 compared with RG and to the conclusion of the study compared with SG (all $P < 0.01$, Figure 4e).

Average CSA for all myofibers was closely related to BW ($P < 0.001$), increasing over time for the SG and RG groups, but decreasing in WL during the nutritional treatment period as these steers lost BW, and then increasing more rapidly than for the other treatment groups until the end of the study (Figure 4f). Consequently, average CSA was smaller in WL than in the growth groups at the conclusion of the nutritional treatments ($P < 0.001$), then remained smaller in WL than in RG until d 324 (all $P < 0.001$) and smaller than in SG until 476 d (all $P < 0.05$), and did not differ between treatment groups at the conclusion of the study (all $P > 0.10$).

After accounting for differences in BW, CSA of type 1 myofibers were no longer significantly smaller in WL than in RG cattle at d 476 ($P > 0.10$). Similarly, after accounting for BW the effect of nutrition on type 2C myofibers in WL compared with RG cattle during the early posttreatment period (d 214) was no longer significant ($P > 0.10$). Also, the type 2X myofibers were of similar size in WL and SG cattle by d 476 ($P > 0.10$), after differences in BW were accounted for.
The average daily rate of change in myofiber CSA after the nutritional treatment period was greater in WL and SG than in RG cattle for myofiber types 1 ($P < 0.05$, Figure 4a), 2C ($P < 0.05$, Figure 4b), and 2A ($P < 0.01$, Figure 4c), and for all myofibers ($P < 0.01$, Figure 4f). The rate of change was greater in WL than in the growth groups for type 2X ($P < 0.01$, Figure 4e). However, after accounting for differences in BW, average daily rate of change no longer differed between SG and RG cattle for type 1 CSA or between any of the nutritional groups for type 2C (all $P > 0.10$).

Percentage of Total Myofiber Area (Relative Area)

The WL cattle had a greater relative area of type 1 myofibers than SG and RG cattle at the conclusion of the nutritional treatments ($P < 0.001$, Figure 5a), indicative of a shift toward more oxidative metabolism. However, the relative area of type 1 myofibers in the WL cattle declined markedly during the initial recovery period so that differences between treatment groups disappeared during the early postweaning period and the groups did not differ thereafter (all $P > 0.10$). For the SG and RG cattle the relative area of type 1 myofibers gradually increased with increasing BW over time ($P < 0.001$). Consequently, because WL had a significantly greater type 1 relative area at d 115, but not at d 721, the average rate of change over time for WL was significantly less than for SG and RG ($P < 0.05$), despite being similar from d 214 until conclusion of the study. Inclusion of BW in the statistical model had only a small effect, with the average rate of change for WL remaining significantly different from SG ($P < 0.01$), but not RG ($P > 0.05$), during the entire posttreatment period.

The relative area of the fast myofiber types (2C, 2A, 2AX, and 2X) did not differ significantly due to postweaning nutritional treatment at any time up to 324 d (all $P > 0.10$, Figure 5). From d 476 onward, there were some differences in the relative areas of type 2C, 2A, and 2X myofibers. Relative area of type 2C myofibers was greater in WL than in RG cattle at the conclusion of the study ($P < 0.05$), with a correspondingly greater rate of change for WL steers ($P < 0.05$, Figure 5b). The WL cattle had a less relative area of 2A myofibers than SG cattle at d 476 ($P < 0.05$) and than SG ($P < 0.01$) and RG ($P < 0.05$) cattle at the conclusion of the study, this difference being reflected in a decreased average rate of change in relative area of 2A myofibers in WL cattle ($P < 0.05$, Figure 5c). The SG cattle had a smaller relative area comprising type 2X myofibers from d 476 ($P < 0.05$) compared with WL cattle and also a more negative rate of change ($P < 0.05$, Figure 5e), indicative of a shift toward more glycolytic relative to oxidative metabolism.

After accounting for differences in BW, significant nutritional effects on the average daily rate of change in relative area of type 1 were no longer evident between

Figure 5. Predicted means ± SEM for percentage of total myofiber area (relative area) comprising a) type 1 (slow oxidative), b) type 2C (type 1-type 2A intermediate), c) type 2A (fast oxidative-glycolytic), d) type 2AX (type 2A-2X intermediate), and e) type 2X (fast glycolytic) myofibers in the longissimus lumborum muscle of cattle before, during, and after BW loss (▲), slow growth (■), or rapid growth (♦) as a result of postweaning nutritional treatments. Predicted values were formed from the smoothing spline models described in the Statistical Analyses subsection of the Materials and Methods. Numbers of steers for each treatment at each sampling time are detailed in Figure 1.
WL and RG ($P > 0.10$). The relative area of type 2A myofibers was no longer less in WL than in SG cattle at d 476 and no longer less in WL compared with RG cattle at the conclusion of the study ($P > 0.10$).

**DISCUSSION**

The results of this study demonstrate that severe nutritional restriction of tropically adapted cattle resulting in prolonged loss of BW after weaning increases the relative area comprising type 1 (slow oxidative) myofibers in the LLM compared with animals grown slowly or rapidly during the same period. Marked reductions in the CSA of myofibers were also evident in the WL compared with the better nourished groups of cattle, with the shift in the relative area of type 1 myofibers due principally to the greater magnitude of nutritional effects on the size of type 2X and type 2A myofibers compared with the slow oxidative, type 1 myofibers and not due to changes in the percentage of myofiber types in the LLM. We have also shown that during recovery from the postweaning nutritional restriction, differences in percentages of myofiber types were not evident. At the conclusion of the study, the average CSA of myofibers differed little between the nutritional treatment groups due to more rapid myofiber growth among the WL group during the posttreatment period. However, there were some more persistent effects of postweaning nutrition that related to differences in BW, whereas others, including a shift in the relative areas of type 2A and 2X myofibers, remained significant after accounting for differences in BW during the posttreatment period.

Severe nutritional restriction of cattle resulting in prolonged loss of BW after weaning resulted in an increase within the LLM in the relative area comprising type 1 (slow oxidative) myofibers compared with animals grown slowly or rapidly during the same period. Nutritional deprivation of cattle was associated with a more generalized loss of muscle mass. The weight of the LLM was reduced by 59% compared with an increase of 55% in the rapidly grown cattle during the 120 d treatment period, resulting in the underfed cattle having an LLM only 27% of the weight of that of their rapidly grown counterparts (Beef CRC, 2002). This was associated with a downregulation of expression of genes for muscle structural proteins, but with a lesser effect on expression of genes specific to slow oxidative myofibers compared with those for fast (type 2) myofibers and, more specifically, than with those for fast glycolytic (type 2X) myofibers (Lehnert et al., 2006). At the gross myofiber level the response of mammalian muscle to very severe nutritional restriction or starvation is generally consistent with the findings of the present study (see Lehnert et al., 2006), with a relative sparing of more oxidative types and preferential atrophy of more glycolytic types (Suzuki, 1973; Goldspink and Ward, 1979; White et al., 2000). This may be associated with increased ketogenesis (Klinhom et al., 2006) and may limit utilization of glucose and energy while enhancing ATP production per unit of glucose (see Lehnert et al., 2006). These findings indicate that the BW loss nutritional treatment imposed in the present study produced similar physiological responses in muscle to those observed during starvation.

The severity of the BW loss treatment, and the differential response to nutrition that can occur between muscles (Suzuki and Okada, 1976; Greenwood et al., 2006b) may have contributed to differences between the findings of the present study and those of other studies in cattle (Picard et al., 1995; Brandstetter et al., 1998; Maltin et al., 1998; Cassar-Malek et al., 2004; Hoch et al., 2005) in which less severe nutritional restriction was imposed. Despite this, Seideman and Crouse (1986) reported a similar shift in the relative area of myofiber types in the longissimus thoracis muscle due to less severe, chronic energy restriction from 8 mo (weaning) to 17 mo of age, consistent with a greater proportion of fast glycolytic myofibers with increased dietary energy levels (Suzuki and Okada, 1976; Johnston et al., 1981).

The present study also indicates some long-term shifts in myofiber characteristics due to nutrition during the treatment period, after the treatment period, or both. Some of the longer term effects of the postweaning nutritional treatments remained after accounting for differences in BW during the posttreatment period. At the conclusion of the present study (600 d posttreatment), the relative area of type 2A myofibers was less and of type 2X myofibers somewhat greater in the cattle that lost BW for 120 d after weaning compared with those grown slowly during the same period. The extent to which these findings are a direct result of the nutritional treatments, are indirect effects due to differences in subsequent growth and BW, or a combination of both, is unclear and remains to be determined. However, dietary restriction followed by more rapid growth affected beef tenderness in the study of Allingham et al. (1998), and in the study of Tomkins et al. (2006), the LLM of the cattle that lost BW tended to have better eating quality than the LLM of the slowly grown cattle [palatability score (see Watson et al., 2008) 54.3 vs. 47.8, respectively]. This latter finding was associated with a trend toward more intramuscular fat in the LLM of the WL cattle than of the SG cattle. Despite the above findings, the results of the present study appear to contrast with other studies in livestock in which reduced proportions of fast glycolytic (types 2X or 2B) and increased proportions fast oxidative-glycolytic (type 2A) myofibers due to genetics were associated with better meat quality (Rehfellt et al., 2000; Freking et al., 2004; Greenwood et al., 2006a,b). However, they do not preclude long-term differences between the nutritional treatment groups in other structural characteristics. Nor do they preclude differences in metabolic characteristics of muscle that contribute to meat quality that can, at least partially, be uncoupled from myofiber types characterized using the content of MHC isoforms.
(Gardner et al., 2007; Warner et al., 2007; Park et al., 2009). Furthermore, the present findings and those of Tomkins et al. (2006) do not preclude the possibility of the nutritional treatments affecting eating quality of beef earlier in the posttreatment period.

Overall, however, our findings suggest that the nutritional treatments imposed after weaning had relatively minor long-term effects on myofiber characteristics despite the severity of the WL treatment, which emphasizes the plasticity of bovine muscle and its capacity to recover mass and myofiber characteristics. This is consistent with other studies in Bos taurus cattle that have shown little or no effect on myofiber characteristics after recovery from nutritional restriction before birth (Greenwood and Cafe, 2007), from birth to weaning (Picard et al., 1995; Allingham et al., 2001; Greenwood and Cafe, 2007), or from weaning (Brandstetter et al., 1998; Cassar-Malek et al., 2004; Hoch et al., 2005). Our results are also generally consistent with findings in other mammalian species that show that nutritional restriction after weaning has little long-term effect on myofiber characteristics when animals are recovered for a prolonged period on adequate nutrition (Ashgar and Yeates, 1979; Bedi et al., 1982).

In conclusion, this study demonstrates that severe nutritional restriction of tropically adapted cattle for 120 d after weaning increases the relative area comprising type 1 (slow oxidative) myofibers in the LLM compared with cattle better nourished during the same period. By 600 d after the nutritional treatments, the overall average CSA for all myofibers in the LLM did not differ between the nutritional treatment groups. At this time, however, the relative area of type 2A myofibers was less and of type 2X myofibers was somewhat greater in the cattle that had lost BW compared with those grown slowly for 120 d from weaning. Some of the more persistent effects of postweaning nutrition related to differences in BW, whereas other longer term effects remained significant after accounting for differences in BW during the posttreatment period.

**LITERATURE CITED**


