Myosin Heavy Chain Gene Expression in Bovine Fetuses and Neonates Representing Genotypes with Contrasting Patterns of Growth (43882)

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Abstract. **Fetal and neonatal myosin heavy chain (MHC) gene expression was examined in bovine genotypes that differed in their postnatal growth pattern and mature size. Pregnancies were established that would be expected to produce early-, intermediate-, or late-maturing postnatal growth. Fetal skeletal and cardiac muscles were collected at 100 and 200 days of gestation and at 30 days of age. Muscle tissue was analyzed for relative levels of MHC RNA and protein. Longissimus muscle MHC RNA/μg RNA was greater at the 100-day time point for the intermediate maturity type ($P < 0.05$), which differed from the 200-day time point where the early maturity type had the greater RNA level ($P < 0.05$). Triceps muscle MHC RNA/μg RNA weights differed due to genotype at 200 days gestation but did not differ at 100 days gestation or at 30 days postnatal. Ventricular muscle MHC RNA did not differ due to genotype at any of the three developmental stages. Differences due to maturity type in MHC protein/mg DNA were observed at 30 days of age, but no differences due to maturity type were observed at the prenatal time points. These results indicate that bovine fetal skeletal muscle MHC RNA production can be influenced by genotype and that genotype may be an important factor for future studies examining the role of external influences on fetal muscle growth.**

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Myosin is a major myofibrillar protein of skeletal muscle, representing approximately 25% of total muscle protein (1). The myosin molecule is a large hexameric protein that consists of two heavy chain subunits (MHC), two alkali light chain subunits and two regulatory light chain subunits (2). Myosin heavy chain gene expression has been demonstrated to be under developmental and stage-specific control (3, 4, 5), and MHC gene families have been identified in a number of species, including the bovine (6).

It has been well established for the bovine that different breeds can have diverse postnatal growth patterns, with early-maturing breeds reaching puberty at a younger chronological age than late-maturing breeds (7). In addition, birth weight in the bovine has been demonstrated to be positively correlated with postnatal growth rate (8), which suggests that breeds with different postnatal growth patterns may also differ in their prenatal growth. Because events that may influence prenatal muscle growth may be related to postnatal growth, we were interested in examining the influences that genotype may have on fetal muscle development. The objective of this study was to determine if MHC gene expression differed during gestation due the postnatal maturity type of the animal. The

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approach used was to establish pregnancies that would allow the comparison of the fetal MHC gene expression of cattle that are typically early maturing and small- to medium-framed adults to those that are late maturing and of a large adult frame size.

Materials and Methods

Establishment of Pregnancies and Tissue Collection. Pregnancies were established using embryo transfer such that fetuses and neonates of Angus × Angus were utilized for the early maturity type, Angus × Chianina for the intermediate maturity type and Chianina × Chianina for the late maturity type (9). A total of 13 fetuses were examined at the 100-day time point with four for the early-maturing growth pattern, four for the intermediate-maturing growth pattern, and five for the late-maturing growth pattern; 13 fetuses at the 200-day time point: four for early, five for intermediate, and four for late; and 11 neonates at the 30-day postpartum time point: four for early, three for intermediate, and four for late. Sires and donor cattle were chosen to represent the early or late maturity postnatal growth patterns based on published expected progeny difference estimates and adult phenotypic characteristics. Recipient females were Hereford × Angus heifers of similar age and size. The use of immature moderate-sized females as recipients raises the possibility that maternal size could limit the growth of fetuses in late gestation. Birth weights of the present study did not differ due to maturity type and were not correlated with maternal height or weight prior to 232 days of gestation (9). A correlation coefficient of 0.84 was observed, however, for birth weight and maternal weight for the late maturity type, which suggests some constraint on fetal growth may have occurred. This contrasts with a negative correlation between birth weight and recipient height; therefore, the relationship between birth weight and maternal size is somewhat unclear. Although this does not rule out the possibility that maternal size may influence late gestation fetal growth, data from previous studies have shown that maternal genotype has little effect on fetal growth before 200 days of gestation (9, 10).

Fetal tissue was collected at 100 and 200 days of gestation and neonatal tissue at 30 days of age. The 100-day gestation time point was chosen to represent a period where fetal weight gain is at a slow rate. This contrasts with the 200-day gestation time point, when the rate of fetal weight gain is near maximal. By 30 days after birth, the postnatal growth patterns typically begin to emerge. Tissues collected for determination of nucleic acid content and MHC protein content were stored at −90°C until analysis. Nucleic acid concentrations were determined by the Schmidt-Thannhauser method as described by Munro and Fleck (11), and protein concentrations were determined by the method of Bradford (12).

Butterfield and Berg (13) classified the postnatal longissimus as having a high-average growth impetus and the triceps brachii muscle as having a low growth impetus. Because these muscles differ in their postnatal growth, they were selected for the present study in order to examine possible differences in prenatal and neonatal muscle growth. For comparative purposes, differences between ventricular and skeletal muscle were also assessed.

Hybridization Analysis. The fast quail MHC cDNA utilized for the probe contains an internal PstI site which divides a 230-bp translated region from a 360-bp region that contains mostly nontranslated sequences (14). Since greater homology between species and individual isoforms exists in translated regions (5, 15–17), the translated portion of the cDNA was used as a probe for assessment of MHC RNA levels. To verify that the cDNA probe would hybridize to the correct molecular weight RNA, RNA was fractionated under denaturing conditions by electrophoresis in 2.2 *M* formaldehyde/1% agarose gels (18), transferred to nitrocellulose membranes, and allowed to hybridize to a [^{32}P]-labeled 230-bp probe (Fig. 1). Hybridization of the 230-bp region of the fast quail MHC probe has been used previously to screen a bovine genomic CDNA library resulting in the identification of six unique MHC gene sequences (6).

Muscle RNA was obtained by homogenizing freshly excised tissue in 4 *M* guanidine thiocyanate which was then centrifuged through 5.7 *M* cesium chloride at 150,000*g*, 20°C, for 22 hr (19). RNA concentrations were determined spectrophotometrically at 260 nm. Prior to using RNA for Dot blots, aliquots representing equal amounts were electrophoresed thorough 2.2 *M* formaldehyde/1.0% acrose gels and stained with ethidium bromide. Comparison of the intensities of the 28 and 18 S bands were used to assess the integrity of the RNA and confirm that similar amounts were loaded for each sample. For the analysis of MHC RNA, aliquots of RNA were spotted onto

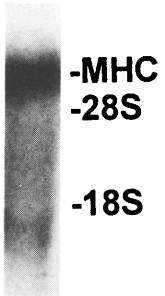

Figure 1. Hybridization of total skeletal muscle RNA to myosin heavy chain cDNA. The cDNA probe corresponds to a 3′ coding region of the embryonic quail fast MHC gene. Hybridization to the myosin heavy chain message is indicated at the arrow. Locations of 28 S and 18 S ribosomal RNAs are indicated.

nitrocellulose using a Bio-Rad Dot blot filtration manifold (Bio-Rad Laboratories, Rockville Center, NY) according to procedures described by White and Bancroft (20). Hybridizations were carried out at 42°C for 48 hr, after which filters were washed for 5 min in 0.2 *M* sodium chloride, 0.015 *M* sodium citrate (2 × SSC), 0.5% sodium dodecyl sulfate (SDS) at room temperature; 15 min in 2 × SSC, 0.1% SDS at room temperature; 30 min in 0.1 × SSC, 0.5% SDS at 37°C; and 30 min 0.1 × SSC, 0.5% SDS at 68°C. After a final rinse in 0.1 × SSC the filters were dried and then exposed for autoradiography at −70°C for 24–48 hr. Degree of hybridization was determined by laser densitometry of the resultant autoradiograms. Hybridization intensities were expressed as the area of peaks in absorbance units/μg RNA. Relative hybridization levels were normalized for DNA concentration of the tissue.

Analysis of Myosin Heavy Chain Protein Content. Muscle samples were prepared for polyacrylamide electrophoresis by homogenizing approximately 50 mg (actual weights recorded) of tissue in 8 *M* urea, 2 *M* thiourea, 0.05 *M* Tris (pH 6.8), 0.7 *M* 2-mercaptoethanol, and 3% lauryl sulfate (21). Aliquots of the muscle homogenates were applied to 5% polyacrylamide gels with 4% stacking gels (22). Following electrophoresis, gels were fixed and then stained with Coomassie blue and relative levels of MHC protein determined by laser densitometry of the MHC band. Relative levels of MHC protein are expressed as staining intensity in absorbance units/mg DNA.

Statistical Analysis. The experimental design was a 3 × 3 factorial arrangement with genotype, age, and genotype by age as the main effects. Least squares means were calculated using the General Linear Models procedure of SAS (23). The effect of calf sex was tested as a main effect and found not to be significant; therefore, it was not included in the final model. Differences in least squares means were determined using Tukey's Test when a treatment effect was significant. Unless otherwise specified, significance was accepted at $P < 0.05$.

Results

The longissimus muscle MHC RNA/μg RNA for the intermediate maturity type was greater compared to the late maturity type at 100 days of gestation ($P < 0.05$) (Fig. 2). The pattern was different however at 200 days of gestation, with the early maturity type having greater longissimus MHC RNA/μg RNA values than the intermediate or late maturity types. Although triceps MHC RNA/μg RNA was not significantly different due to genotype at 100 days of gestation, genotypic relationships were similar to that of the longissimus. This contrasts with triceps MHC RNA/μg RNA values at 200 days of gestation where the genotypic relationships were different for the triceps compared with the

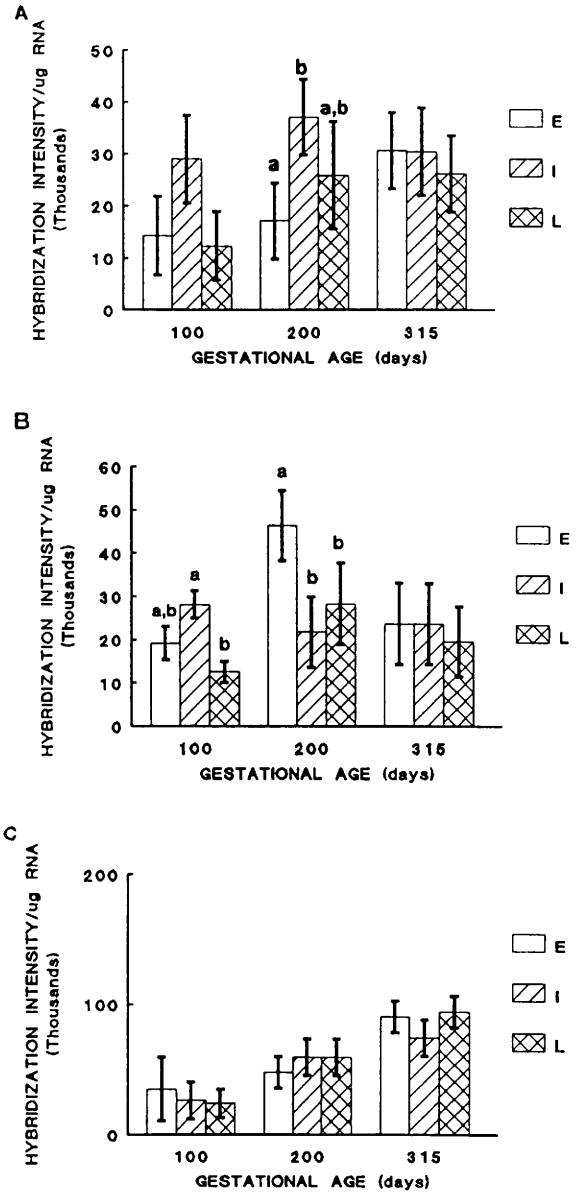

Figure 2. Relative hybridization of (A) triceps, (B) longissimus, and (C) ventricular muscle RNA to MHC cDNA probe/μg RNA. Data are expressed as least squares means ± SEM. Least squares means with differ letters differ ($P < 0.05$). Age 315 refers to days between breeding and 30 days of age. The number of animals represented by each bar is as follows: early maturity type—four at 100 days, five at 200 days, and four at 30 days postnatal; intermediate maturity type—four at 100 days, five at 200 days, and three at 30 days postnatal; late maturity type—five at 100 days, four at 200 days, and four at 30 days postnatal. Maturity types: E = Angus × Angus, I = Chianina × Angus, L = Chianina × Chianina.

longissimus. At 30 days of age, the genotypic relationships for MHC RNA/μg RNA, although not statistically different, were similar for the two skeletal muscles. No differences due to maturity type were observed for ventricular muscle.

No differences due to genotype for MHC protein/mg DNA were observed at the two gestational time points (Fig. 3). By 30 days of age, differences due to

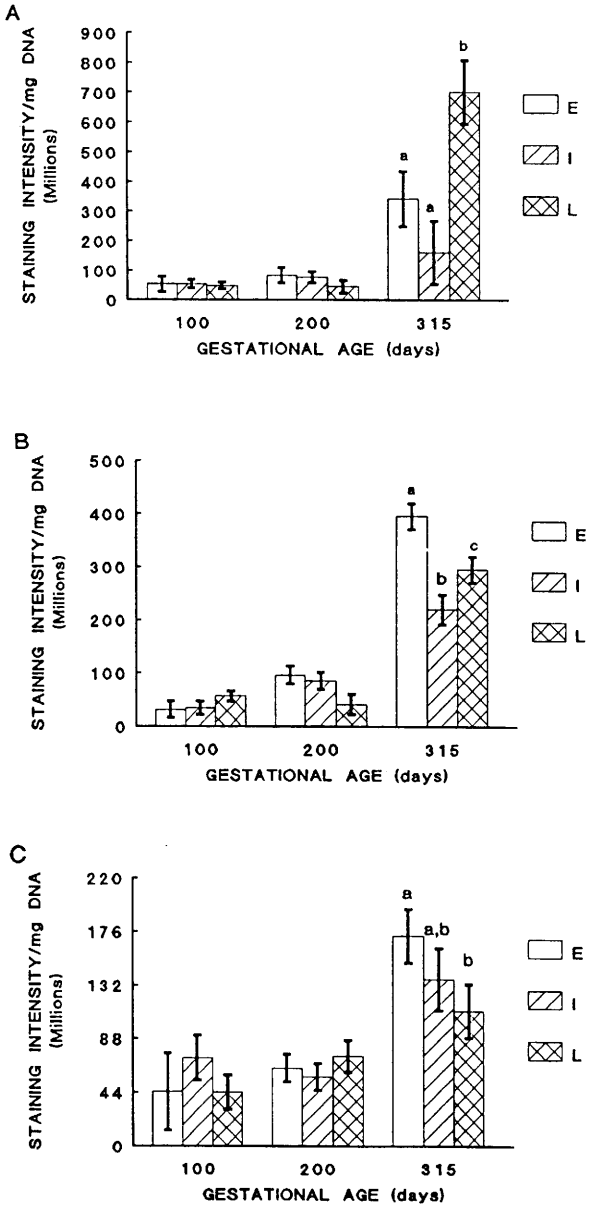

Figure 3. Relative levels of MHC protein/mg DNA in (A) triceps, (B) longissimus, and (C) ventricular muscle. Data are expressed as least squares means ± SEM. Least squares means within an age having different letters differ ($P < 0.05$). Age 315 refers to days between breeding 30 days of age. The number of animals represented by each bar is as follows: early maturity type—four at 100 days, five at 200 days, and four at 30 days postnatal; intermediate maturity type—four at 100 days, five at 200 days, and three at 30 days postnatal; late maturity type—five at 100 days, four at 200 days, and four at 30 days postnatal. Maturity types: E = Angus × Angus, I = Chianina × Angus, L = Chianina × Chianina.

genotype were evident, although the genotypic relationships for the two skeletal muscles were different. In addition, there was a 350% to 750% increase in the MHC protein/mg DNA levels at 30 days of age compared with the fetal time points.

Discussion

In the present study, differences in MHC RNA concentration due to genotype were observed at the two fetal time points. Previous studies have shown that no differences were observed due to genotype for muscle total muscle RNA concentration (9). Therefore, differences due to maturity type observed for MHC RNA/μg RNA are not due to an increase in total muscle RNA production, but are more likely due to differences in the activity of MHC genes. It is interesting to note that the genotypic relationships for MHC RNA/μg RNA at 200 days of gestation are different when the triceps brachii and longissimus muscles are compared. This suggests that the genotypic influence is different for the two muscles at this time. In contrast, genotypic relationships for skeletal MHC RNA concentration at 100 days of gestation and 30 days postnatal were nearly identical, suggesting that genotype influences MHC RNA concentration similarly for the two muscles at those time points. One could speculate that the different genotypic influences seen at 200 days of gestation are also a reflection differences in the growth patterns of the two muscles. These two muscles have been demonstrated to have different postnatal growth patterns. Butterfield and Berg (13) classified the longissimus muscle as having a high-average growth impetus (i.e., a muscle that would have an allometric growth coefficient of greater than 1.0 early in life and then not different from 1.0 later in life). The triceps muscle was classified a low or average impetus (for caput laterale and caput longum, respectively), with the allometric growth coefficient for that muscle being either below or not significantly different from 1.0 for the entire growth period. Swatland (24) observed that the rates of weight gain for the fetal pig triceps and longissimus differed at 60 days of gestation. In the present study, comparison of slopes of regression lines for the change in muscle weight to body weight ratios over time within genotype revealed that the triceps and longissimus muscles differed, suggesting differential growth during gestation for the two muscles (Table I). Although beyond the scope of this study, observations at more time points in future stud-

Table I. Probabilities That Slopes Differ for the Changes in Muscle to Body Weight Ratios with Age[a]

Maturity type[b]	Slope		*P* Value
	Triceps	Longissimus	
Early	0.0002	−0.0020	0.0305
Intermediate	−0.0027	−0.0037	0.0352
Late	−0.0036	−0.0065	0.0112

[a] Comparisons were made by testing for homogeneity of slopes for the regression of change in muscle to body weight ratio across time. Table values represent the probability that slopes differed.
[b] Maturity types: early = Angus × Angus; intermediate = Chianina × Angus; late = Chianina × Chianina.

ies may serve to demonstrate divergent development for different muscles. Therefore, genotype can influence MHC RNA concentrations during gestation, but the timing of this influence may differ for different muscles. Ventricular muscle MHC RNA concentration did not appear to be influenced by genotype in a way that suggested coordination of MHC gene expression with the two skeletal muscles.

In contrast to the differences observed for MHC RNA production, no differences due to genotype were observed for MHC protein/mg DNA for the two skeletal muscles until 30 days of age. Since MHC protein concentration did not differ due to genotype at the two fetal time points, the relationship between MHC RNA concentration and MHC protein concentration is not a simple one. Perhaps higher turnover rates of the MHC protein during fetal development results in protein levels that do not closely follow mRNA levels for the protein. Growth associated with higher rates of protein turnover has been reported for the fetal lamb (25) and in the postnatal rat (26). Also, in fetal rat diaphragm muscle, higher rates of total protein synthesis were observed to be more closely related to ribosomal concentration rather than translational activity, suggesting that protein synthesis in fetal rat striated muscle may not closely follow mRNA levels (27). The large increases in MHC protein/mg DNA observed between the fetal and gestational time points is consistent with the well-documented shift of growth due mainly to hyperplasia during early and midgestation to muscle growth due largely to protein synthesis and hypertrophy of cells in late gestation and early neonatal life (28). It is interesting to note, however, that there were no differences due to genotype in total protein concentrations of these muscles (9), which suggests that the genotypic differences seen in the present study are the result of somewhat specific influences on MHC protein production and are not reflections of genotypic differences in overall protein synthesis.

In conclusion, although the number of time points in the present study are limited, these data indicate that prenatal MHC RNA concentration can be influenced by genotype. The results of the present study suggest that genotype should be taken into account when evaluating external influences on fetal muscle growth in the bovine, since the timing of events that influence fetal muscle gene expression may differ in animals that have divergent postnatal growth patterns.

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