SERUM-MYOBLAST PROLIFERATION AND GENE EXPRESSION IN DOUBLE MUSCLED AND NORMAL CATTLE

D. E. GERRARD*, A. L. GRANT AND M. D. JUDGE

Department of Animal Sciences, Purdue University, West Lafayette, IN 47907
*Present address: Department of Food Science and Nutrition, University of Missouri–Columbia, Columbia, MO 65211

SUMMARY: Utilizing double muscled cattle as a model for studying myoblast proliferation and differentiation has revealed differences between normal (NM) and double muscled (DM) fetuses in serum mitogenic activity and tissue insulin–like growth factor–II (IGF–II) gene expression. These differences may play a role in the development of cattle with muscle hypertrophy.

INTRODUCTION: Double muscled cattle possess nearly 40% more muscle fibers than normal cattle (MacKellar, 1968). Fetal blood–borne growth factors modulate myoblast proliferation and differentiation in vitro (reviewed by Florini et al., 1991). The type and amount of growth factor changes with time in the serum of developing mammalian fetuses (Moses et al., 1980; Daughaday et al., 1982). Alterations in serum growth factor profiles have been assessed by several researchers using myoblast cultures as a bioassay (Kotts et al., 1967a,b; White et al., 1988, 1989; Gerrard et al., 1992a).

Insulin like growth factor II (IGF–II) is subject to profound regulation during fetal development of rodents (Lund et al., 1986). Serum and tissue concentrations of IGF–II fluctuate dramatically throughout gestation. Studies by Florini et al. (1991) suggest that autocrine production of IGF–II is required during myoblast differentiation. Changes in the expression of a growth factor closely tied to myogenesis may be responsible for muscle fiber hyperplasia in DM cattle. Therefore, the objectives of this study were to compare the serum mitogenic activity and the expression of IGF–II in liver and skeletal muscle of developing NM and DM fetuses.

MATERIAL and METHODS: Fetal blood samples from sixty beef or dairy (NM) fetuses of various ages were collected from pregnant cows slaughtered in a commercial slaughter plant. Double muscled fetal blood was taken from twelve fetuses of pregnant cows slaughtered in the Purdue University abattoir. Fetal age was recorded by crown–rump length (CRL; measured from the ischium to the poll of the cranium) and fetuses were assigned to a CRL representing time of gestation (<25, 26–50, 51–75, >75 cm).

A subclone (ELC5) of the rat L6 myoblast was obtained from Dr. C. Smith (Lilly Research Laboratories, Greenfield, IN) and used as a bioassay for determining serum–induced myoblast proliferation. Incorporation of ^{3}H–thymidine into cultured proliferating myoblasts was used to indication of proliferative activity.

Muscle samples were taken from the semitendinosus muscle of fetuses ranging in CRL of 12.5 to 90 cm for estimation of apparent muscle fiber number. Quantification of the apparent muscle fiber number was performed using an Image Analyzer (Cambridge Quantiquet 570, Chicago, IL).

Muscle and liver samples were also taken. Total RNA extraction by the acid guanidine thiocyanate phenol chloroform procedure (Chomczynski and Sacchi, 1987) was performed. Northern blot analysis was conducted using muscle and liver RNA as described by Grant et al. (1991). Dot blots were generated to determine relative changes in abundance of selected mRNAs. The cDNAs used in this study were rat IGF–II (Dr. M. Rechler, National Institue of Health) and rat beta actin (Dr. L. Kedes, University of Southern California).

Relative mRNA abundance, tritiated thymidine uptake, mean fiber area and apparent fiber number estimates were subjected to an ANOVA procedure of SAS (1988) to determine main effects of muscling and age. Differences between means were determined by the least square means of the SAS procedure.

RESULTS and DISCUSSION: Although not statistically significant at all time points, serum–stimulated myoblast replication tended to increase with CRL for both NM and DM fetuses (Figure 1). Normal muscled fetal serum–induced thymidine incorporation in L6 myoblasts was greatest at CRL > 50, least at CRL $\leq$ 25 and intermediate for 26–50 cm CRL (P<.05). Thymidine uptake by L6 myoblasts was greater with serum from DM fetuses than NM fetuses at CRL $\leq$ 25, 26–50 and 51–75 cm (P<.05) and tended to be greater at >75cm.

Greater myoblast proliferation observed in the presence of serum from DM fetuses over NM fetuses may result from the presence of a DM–specific serum factor which is responsible for the expression of bovine muscle fiber hyperplasia. More likely, an aberrant level of growth factor is present during a critical stage of myogenesis. Preliminary experiments showed that the L6 myoblasts used in this study did not respond to two well known mitogens, FGF and TGF–beta. This does not eliminate the possibility of TGF–beta or FGF mediating the serum reponse, but suggest other growth factors are responsible for increased myoblast replication in this system. In contrast to the age–associated increases in myoblast proliferation wherein IGF–I is the suspected mitogen, IGF–II probably mediates the difference in early muscle hyperplasia.

Mean fiber area decreased dramatically from 100 to 20 um2 between 15 and 38 cm CRL (Figure 2). Simultaneously, a 10-f increase in apparent muscle fiber number was observed at ~38 cm CRL (Figure 3). Swatland and Kieffer (1974) showed sim increases in muscle fiber number between 10 and 42 cm CRL of fetal bovine development.

Figure 1. Means and standard errors of incorporation of ^{3}H-thymidine into replicating myoblasts cultured in the presence of medium containing 10% sera from developing double muscled (DM) and normal muscled (NM) fetuses. Means bearing similar letters do not differ (P>.05).

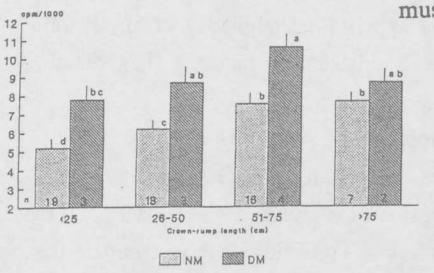


Figure 2. Mean fiber area of muscle fibers within the developing semitendinosus muscle of 10 double muscled (DM) and 19 normal muscled (NM) fetuses.

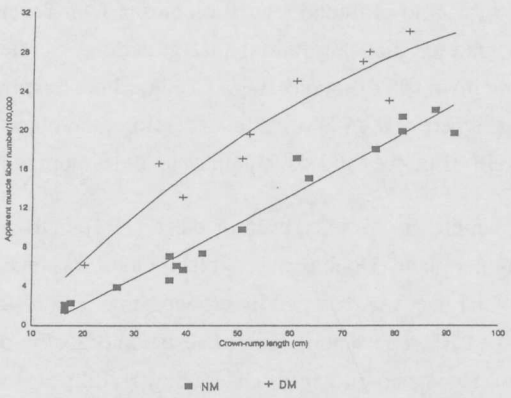
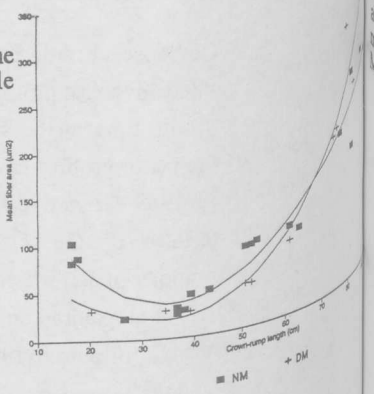


Figure 3. Estimated apparent muscle fiber numb the developing semitendinosus muscle of 10 dou muscled (DM) and 19 normal muscled (NM) fetuses



IGF-II mRNA was detected in muscle and liver tissues of both DM and NM fetuses (Figure 4). Rat IGF-II cDNA used in t study hybridized to transcripts of 4.5, 3.6, 2.75, 2.5, 1.6 and 1.15 kb. Although multiple transcripts were observed for rIGF-II cD the 4.5 kb transcript was most abundant. An additional 6 kb transcript was also observed on autoradiographs with longer expo times. The rat beta-actin cDNA hybridized to the 2.1 kb bovine beta actin mRNA in both liver and muscle. Some cross-hybridiz of the beta-actin cDNA to muscle alpha-actin mRNA occurred.

Figure 4. Autoradiogram from northern blot analysis of bovine liver and muscle tissues using a rat IGF-II cDNA (lanes 1,2,4 and 7 fetal liver; 3, 5, 6 and 8 fetal muscle).

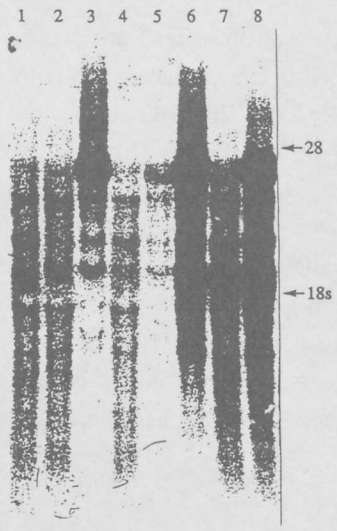


Figure 5. Means and standard errors of the relative abundance of IGF-II mRNA in the liver of developing normal muscled (NM) fetuses. Means bearing similar letters do not differ (P>.05).

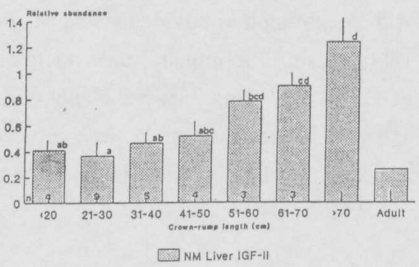
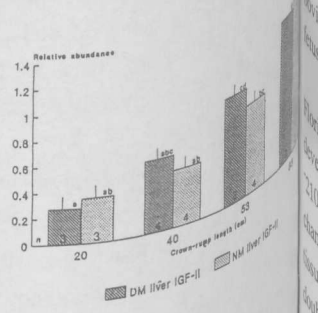


Figure 6. Means and standard errors of the re abundance of IGF-II mRNA in the liver of devel normal muscled (NM) fetuses. Means bearing s letters do not differ (P>.05).



Relative hybridization intensities for the IGF–II cDNA were greater for muscle than liver tissues, possibly from dilution of liver IGF–II mRNA by other liver mRNAs. Figures 5 and 6 indicate liver IGF–II expression increased with CRL in both NM and DM fetuses (P<.05) but no differences were observed between DM and NM fetuses. Expression of IGF–II in muscle tissue of developing bovine fetuses decreased (P<.05) with age post conception (Figure 7). Normal muscle IGF–II expression tended to fall after a crown–rump length of 70 cm was reached. This crown–rump length represents approximately 200 d pc. Interestingly, this time point coincides with the time bovine myofiber hyperplasia concludes (Swatland and Kieffer, 1974).

Figure 7. Means and standard errors of the relative abundance of IGF–II mRNA in the semitendinosus muscle of developing normal muscled (NM) fetuses. Means bearing similar letters do not differ (P>.05).

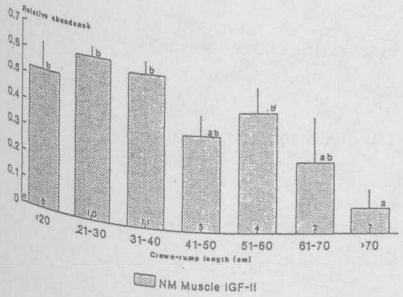
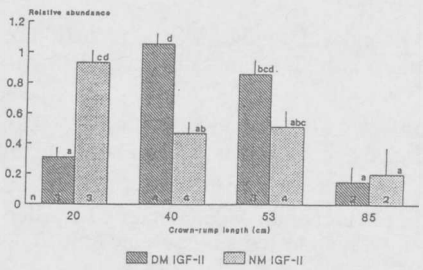


Figure 8. Means and standard errors of the relative abundance of IGF–II mRNA in the semitendinosus muscle of developing double muscled (DM) and normal muscled (NM) fetuses. Means bearing similar letters do not differ (P>.05).



To reduce the possibility of confounding muscle IGF–II expression with age, NM fetuses were compared to DM fetuses with similar CRL. At 20 cm CRL (approximately 100 d post–conception), muscle IGF–II expression was greater (P<.05) in NM fetuses than DM fetuses (Figure 8). Conversely, at 40 cm CRL, muscle IGF–II was greater (P<.05) for DM than NM fetuses. The reason for this disrupt change in expression of IGF–II in muscle is unknown. Since IGF–II is a mitogen of embryonic myoblasts (McFarland et al., (991) and DM cattle develop the potential for extreme muscularity through hyperplastic muscle cell growth (Swatland and Kieffer, (974; Ashmore et al., 1974), IGF–II may cause hyperplasia of myoblasts. Alternatively, with the compelling evidence recently shown by Florini et al. (1991) that paracrine production of IGF–II is imperative for myogenesis, increased muscle IGF–II expression may affect the onset of terminal differentiation. This suggests that the onset of terminal differentiation in the muscle of developing DM fetuses is delayed during the onset of secondary fiber formation compared to NM fetuses. Delayed expression of IGF–II may allow for continued mitotic activity of myoblasts from DM fetuses which could result in greater muscle fiber production.

CONCLUSIONS: Double muscled cattle provide a useful model for studying muscle development. Mechanisms controlling the development of an animal with 30% more muscle represents a biological phenomenon worth exploiting. Muscle cell hyperplasia in bovine fetuses occurs between 85 and 210 d post–conception. Serum–induced mitogenic activity is greater in DM fetuses than in NM fetuses during a time of muscle fiber hyperplasia. Serum growth factors may play a role in development of the DM phenotype.

Increased muscle IGF–II expression during development in bovine fetuses is consistent with myoblast culture results reported by Florini et al. (1991). Although time points prior to 13 cm CRL were not available, changes in muscle IGF–II expression during the development of bovine skeletal muscle may represent different stages myogenesis. Furthermore, muscle fiber number was established by 210 d pc which coincided with a significant decrease in the abundance of IGF–II mRNA. These data suggest that IGF–II expression changes with the maturation of prenatal muscle tissue in vivo and support the hypothesis that the time of IGF–II expression in muscle tissue may dictate ultimately the number of muscle fibers that bovine fetuses possess at birth. These experiments support the use of double muscled cattle as an excellent model for studying myogenesis. Further work to identify the source and type of growth factors present during the development of DM fetuses which result in increased myoblast proliferation may lead to better understanding the complex mechanisms controlling myogenesis.

REFERENCES:

Ashmore, C.R. W. Parker, H. Stokes and L. Doerr. 1974. Comparative aspects of muscle fiber types in fetuses of the normal [illegible] "double muscled" cattle. Growth 38:501.

Chomczynski, P. and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloro[illegible] extraction. Anal. Biochem. 162:156.

Daughaday, W.H., K.A. Parker, S. Borowsky, B. Tirvedi and M. Kapadia. 1982. Mesurement of somatomedin-related peptides in [illegible] neonatal,and maternal rat serum by insulin-like growth factor (IGF) I radioimmunoassay, IGF-II radioreceptor assay (RRA) [illegible] multiplication-stimulating activity RRA after acid-ethanol extraction. Endocrinology 110:575.

Florini, J.R., D.Z. Ewton and K.A. Magri. 1991. Hormones, growth factors and myogenic differentiation. Ann. Rev. Physiol. 53:201.

Florini, J.R., K.A. Magri, D.Z. Ewton, P.L. James, K. Grindstaff and P.S. Rotwein. 1991. "Spontaneous" differentiation of skel[illegible] myoblasts is dependent upon autocrine secretion of insulin-like growth factor-II. J. Biol. Chem. 266:15917.

Grant, A.L., W.G. Helferich, S.A. Kramer, R.A. Merkel and W.G. Bergen. 1991. Administration of growth hormone to pigs alters [illegible] amount of insulin-like growth factor-I mRNA in liver and skeletal muscle. J. Endo. 130:331.

Gerrard, D.E. and M.D. Judge. 1992a. Induction of myoblast proliferation in L6 myoblast cultures by fetal serum of double muscled [illegible] normal cattle. J. Anim. Sci. (submitted)

Gerrard, D.E., A.L. Grant, and M.D. Judge. 1992b. Growth factor gene expression in skeletal muscle during the development of [illegible] muscled and normal cattle. J. Endo. (submitted)

Kotts, C.E., F. Buonomo, M.E. White, C.E. Allen and W.R. Dayton. 1987a. Stimulation of in vitro muscle cell proliferation by [illegible] from swine injected with porcine growth hormone. J. Anim. Sci. 64:623.

Kotts, C.E., M.E. White, C.E. Allen, F. Martin and W.R. Dayton. 1987b. A statistically standardized muscle cell culture bioass[illegible] measuring the effect of swine serum on muscle cell proliferation. J. Anim. Sci. 64:615.

Lund, P.K., B.M. Moats-Statts, M.A. Hynes, J.G. Simmons, M. Jansen, A.J. D'Ercole and J.J. Van Wyk. 1986. Somatomedin C/insulin-like growth factor-I and insulin-like growth factor-II mRNA's in rat fetal tissues. J. Biol. Chem. 261:14539.

MacKeller, J.C. 1968. Muscular hypertrophy in South Devon cattle. Fellowship thesis, Royal College of Veterinary Surgeons, Lond[illegible] In: Structure and Development of Meat Animals. Prentice-Hall, Inc. New Jersey.

McFarland, D.C., J.E. Pesall and K.K. Gilkerson. 1991. Postnatal and embryonic myoblasts derived from the turkey. J. Anim. [illegible] 69(Suppl. 1):290 (Abstr.).

Moses, A.C., S.P. Nissely, P.A. Short, M.M. Rechler, R.M. White, A.B. Knight and O.Z. Higa. 1980. Increased levels [illegible] multiplication-stimulating activity, an insulin-like growth factor, in fetal rat serum Proc. Natl. Acad. Sci. 77: 3649.

Robelin, J., A Lacourt, D. Bechet, M. Ferrara, Y. Briand and Y. Geay. 1991. Muscle differentiation in the bovine fetus: a histolog[illegible] and histochemical approach. Growth, Development and Ageing 55:151.

SAS. 1988. SAS User's Guide: Statistics. SAS Inst. Inc., Cary, NC.

Swatland, H.J., and N.M. Kieffer. 1974. Fetal development of the double muscled condition in cattle. J. Anim. Sci. 38:752.

White, M.E., C.E. Allen and W.R. Dayton. 1988. Effect of sera from fed and fasted pigs on proliferation and protein turnover [illegible] cultured myogenic cells. J. Anim. Sci. 66:34.

White, M.E., D.H. Kretchmar, C.E. Allen and W.R. Dayton. 1989. Partial purification of a serum fraction from fasted pigs that inhib[illegible] proliferation of cultured myogenic cells. J. Anim. Sci. 67:3144.