MYOSTATIN AND ADIPOGENIC TRANSCRIPTION FACTOR EXPRESSIONS DURING SKELETAL MUSCULAR GROWTH OF JAPANESE BLACK CATTLE

M. Shibata*[1], K. Matsumoto[1], K. Aikawa[1], T. Muramoto[1], S. Muroya[2], M. Ohe[2], I. Nakajima[2], K. Chikuni[2], S. Fujimura[3] and M. Kadowaki[3]

[1] National Agricultural Research Center for Western Region (WeNARC), Ohda, Shimane, 694-0013, Japan, [2] National Institute of Livestock and Grassland Science, Tsukuba, Ibaraki, 305-0901, Japan, [3] Faculty of Agriculture, Niigata University, Niigata, 950-2181, Japan, Email: shibatam@affrc.go.jp

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Introduction

Myostatin is classified as a member of the transforming growth factor-β superfamily and identified as a specific negative regulator of skeletal muscle growth in myostatin gene null mice, which have reduced rates of body fat accumulation (McPherron *et al.*, 1997). Similarly, natural myostatin mutations in cattle have been shown to be associated with a double-muscling phenotype (Grobet *et al.*, 1997). Although we (Shibata *et al.*, 2003) and others have reported myostatin gene expression in fetal bovine and calf skeletal muscle, detailed long-term developmental changes in myostatin gene expression in cattle have not been characterized. The present study investigates the association of myostatin in the formation of skeletal muscle in Japanese Black Cattle (JBC). We report developmental changes in the myostatin gene and adipogenic transcription factor expression from the fetal to the fattening period.

Materials and Methods

Skeletal muscles were obtained by slaughter or by biopsy from JBC which had been bred at the WeNARC. Donor cattle at 8 months gestation were slaughtered and fetuses obtained. *Musculus (M.) Semitendinosus* tissue was obtained by biopsy, under local anesthesia, from JBC aged 4, 10, 16, 22, and 28 months. All collected samples were rapidly frozen in liquid nitrogen and stored at –80°C until RNA extraction. Total RNA was extracted from muscle tissues using the TRIZOL reagent according to the manufacturer's protocol. First strand cDNA was synthesized from 3 μg of total RNA using SuperScript II RNase H⁻ reverse transcriptase with oligo dT primer. After reverse transcription, expressions of target genes were determined by quantitative real time PCR with a TaqMan probe using an ABI 7700 detection system, or a semi-quantitative reverse transcription (RT)-PCR. Relative target genes mRNA levels were normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Results and Discussion

We report long-term developmental changes in myostatin gene expression in *M. Semitendinosus* of JBC up to the fattening period. The results showed two peaks: the highest in the fetal stage and the second at 16 months of age (Figure 1). *M. Semitendinosus*, *M. Semimembranosus* and *M. Biceps femoris*, all present in the hindlimbs, increase in weight in double-muscled cattle (Boccard and Dumont, 1974); further, the muscular hypertrophy that is characteristic of these cattle was more marked in hindlimbs than forelimbs (Dumont, 1982). Higher expression of the gene was always found in *M. Semitendinosus* compared to *M. Longissimus lumborum* of JBC (Shibata *et al.*, 2003). These results suggest that hindlimb development at middle of fattening period in bred cattle may need the potential control of muscle growth by myostatin.

To investigate the relationship between myostatin and fat accumulation in skeletal muscle, expressions of the adipogenic transcription factors, CCAAT/enhancer binding protein (C/EBPα) and peroxisome proliferator -activated receptor (PPARγ2), were measured at different stages of skeletal muscle development by semiquantitative RT-PCR. PPARγ2 expression at 16 months was higher than at any other stage, but C/EBPα level did not change between the different stages (Figure 2). The fat content of double-muscled cattle is lower than that of normal cattle (Shahin *et al.*, 1985) and, interestingly, study with myostatin null mice recently revealed that a decrease in body fat accumulation results from a reduction of adipogenesis and, accordingly, decreased leptin secretion (Lin *et al.*, 2002). This suggests that myostatin is involved in the regulation of adiposity. It is interesting that higher PPARγ2 gene expression was observed at 16 months, compared with other stages in JBC. A previous report indicated that fat deposition in dressed JBC carcasses was slow until 15 months, and then dramatically increased at 15 to 16 months. The increase in fat accumulation in skeletal muscle has been shown to occur at 15–20 months in JBC (Mitsuhashi *et al.*, 1987). These findings suggest that myostatin may be related to marbling formation at the middle of the fattening period in JBC.

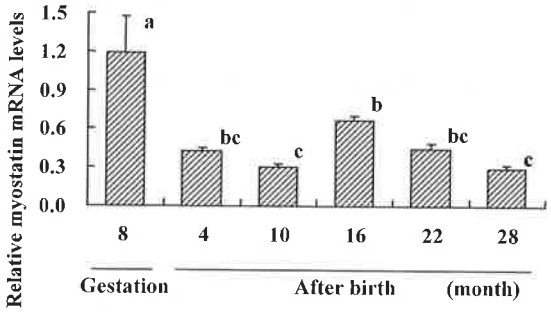

Figure 1: Developmental changes in myostatin gene expression in *M. Semitendinosus* of Japanese Black Cattle by real time PCR. Relative myostatin mRNA levels were normalized with GAPDH mRNA. Bars represent the means ± SEM of 3 to 8 animals. Values with different letters (a - c) are significantly different ($P<0.05$).

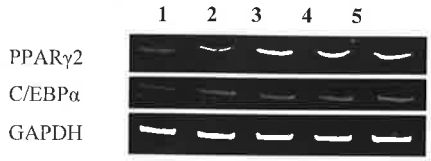

Figure 2: Polyacrylamide gel electrophoresis of RT-PCR products amplified from *M. Semitendinosus* with gene specific primer pairs. Lane 1: 4 months, Lane 2: 10 months, Lane 3: 16 months, Lane 4: 22 months, Lane 5: 28 months after birth.

Conclusion

The present study revealed high expressions of both myostatin and PPARγ2 genes in *M. Semitendinosus* at 16 months. This increase in genes may be related to marbling formation in the fattening period.

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