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A review of recent findings on myostatin, a gene which controls muscle growth

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ABSTRACT

The enlarged muscles of certain breeds of cattle, such as the Belgian Blue, result from a marked increase in the number of normal sized muscle fibres. Originally insulin-like growth factors (IGFs) were implicated in this myofibre hyperplasia. Recently it has been reported that mice lacking a myostatin gene, a member of the TGFβ super family, have enhanced skeletal muscle mass resulting from increased muscle fibre number and size. Mutations in this gene have been found in double muscled cattle, indicating that myostatin is an inhibitor of muscle growth. Myostatin is expressed early in gestation and then maintained to adulthood in certain muscles. Myostatin expression in bovine muscle is highest during gestation when muscle fibres are forming and some of the myogenic regulatory factors have elevated expression over the same period as myostatin. Myostatin and MyoD, myogenic regulatory factors expressed in muscle have been shown to differ between normal and hypertrophied muscle cattle breeds. This evidence strongly suggests that lack of functional myostatin is associated with an increase in fibre number which then results in a marked increase in potential muscle mass in double muscled cattle.

Keywords: myostatin; muscle growth.

INTRODUCTION

Highly muscled cattle, sheep and pigs have resulted from intense selection over a number of generations. Cattle exhibiting muscle hypertrophy have been identified in a number of different breeds such as Aberdeen Angus, Belgian Blue and white, Black and White Polish, Piedmontese and Charolais and have been called "double muscled", "doppelender", "a groppa doppia" and "culard" (Boccard, 1981). This paper focuses on the recent identification of the myostatin gene and its association with highly muscled breeds of cattle.

The muscle hypertrophy was originally thought to originate in Dutch cattle and then to have gradually diffused into British and European breeds and then to the USA and other countries. It is mainly associated with an increase in muscle fibre number rather than the enlargement of individual muscle fibres. The increase in muscle fibre number occurs during prenatal development and results in a doubling of muscle fibres in "double muscled cattle" (Gerrard *et al.,* 1991). Double muscled cattle also have a higher proportion of white muscle fibres (West, 1974), a higher number of bunched terminal axons (Swatland, 1973) and a lower collagen content than normal muscled cattle (Boccard, 1981). Although all muscles in double muscled animals have increased numbers of muscle fibres, only some of the muscles show an increase in weight when the increased muscle fibres are of normal size. Other muscles are smaller in double muscled cattle when compared with normal cattle which results from smaller fibres. An example of this is the M. *vastus lateralis* which is 14% larger and the M. *vastus medialis* which is 38% smaller than normal muscled cattle (Boccard, 1981). These surprising differences in relative muscle size occur after birth and appear to be associated with work induced hypertrophy (Martyn *et al*., 1997). Muscle from double muscled cattle also has lower collagen and connective tissue content and this has been associated with a 20-30% reduction of hydroxyproline (Hanset, 1982) and a lower proportion of stable non-reducible cross linked collagen (Bailey, 1982) when compared with normal muscled cattle. Arthur (1995) has recently reviewed the literature on the effects of double muscling on meat quality and concluded that most of the recent reports indicate that meat from double muscled cattle is more tender than that from normal breeds when the meat has been correctly chilled and aged.

This review will focus mainly on the Belgian Blue breed which consistently shows extreme double muscling.

The inheritance of double muscling in Belgian Blue cattle has been identified as a monogenic autosomal segregation pattern (Hanset and Michaux, 1985; Charlier *et al*., 1995). The muscular hypertrophy (mh) locus has been termed "partially recessive" because a single copy of the allele can have some effect, although the full double muscled phenotype requires the cattle to be homozygous. Gene mapping (Charlier *et al*., 1995) of the Belgian Blue cattle localised the mh gene to the centromeric end of the bovine chromosome 2 (BTA2) linkage group. The mh locus in the Piedmontese breed was localised gene to a 3- to 5-cM interval near the centromere of BTA2 (Casas *et al*., 1997) close to the position of the a-collagen type III (COL3A1) locus.

Recently a growth differentiation factor-8 (GDF-8), a member of the TGFβ superfamily, was disrupted in mice and the GDF-8 null mice were significantly larger than wild-type animals, with the increase in bodyweight coming from a 2-3 fold increase in muscle mass. As GDF-8 seemed to function as an inhibitor of muscle growth it was renamed myostatin (McPherron *et al*., 1997). The myostatin gene has been mapped to the same interval as the mh locus by genetic linkage (Smith *et al*., 1997). These findings indicate that the myostatin gene and the muscular hypertrophy gene are one and the same.

In double muscled Belgian Blue cattle an 11 bp mutation in the myostatin gene has been identified and a point mutation was identified in the Piedmontese breed (Kambadur *et al*., 1997). More recently, Grobet *et al*., (1998) have identified seven DNA sequence polymorphisms of which five were predicted to disrupt the function of myostatin. These studies clearly demonstrate that the double muscling phenotype in cattle is genetically heterogeneous and involves several mutations in the myostatin gene. The 11 bp deletion in the Belgian Blue cattle results in the loss of three amino acids which causes a frame shift after amino acid 274. The frame shift leads to a stop codon after amino acid 287 that is predicted to produce a truncated and biologically inactive protein. In the Piedmontese breed a mutation at position 941 bp results in the loss of a cysteine at aa 314. This cysteine has been shown to be essential in other TGFb family members in order to form a cysteine knot which stabilises the TGFb dimer. Overall these studies indicated that myostatin is probably the mh locus and that myostatin acts as an inhibitor of muscle development by limiting muscle fibre number and to some extent muscle fibre size.

A further level of control of muscle development by myostatin has been identified in the compact hypermuscular mouse (Szabo *et al*., 1998). The mutation that causes the increase in muscle mass is a deletion in the pro-peptide region, which precedes the proteolytic processing site of mouse myostatin. The pro-peptide region, by analogy with TGF-bs, may be involved in the folding, secretion and regulation of the targeting of myostatin (Miyazono *et al*., 1991) which could decrease the biological activity of myostatin but not completely remove it.

Studies on the expression of myostatin have shown that myostatin is expressed in cattle muscles from day 16 of gestation and throughout development until adulthood. The expression of myostatin in adult muscles is less than during fetal development and expression varies among adult muscles (Kambadur *et al*., 1997). Increased expression of myostatin during the embryonic period relates directly to the gestational stage when primary myoblasts are starting to fuse and differentiate into myofibres and the secondary myoblasts are initially proliferating and then fusing (Oldham *et al*., 1998). The expression of myostatin in the fetus and post-natally may also be associated with muscle fibre type as double muscled cattle have a greater number of fast glycolytic fibres than normal cattle. The variable expression of myostatin in adult muscles is difficult to relate to phenotypic differences which predominately seem to be associated with early development, ie. fibre number and type.

Expression of mutant myostatin mRNA is higher in double muscled Belgian Blue cattle than the normal cattle (Kambadur *et al*., 1997). This indicates that there may be negative feedback mechanism in muscle involving myostatin. This feedback mechanism may involve the myogenic regulatory transcription factors such as MyoD, because mRNA expression in muscle is higher in double muscled cattle during development than in normal muscled cattle (Oldham *et al*., 1998).

Recombinant myostatin has been shown to inhibit the proliferation of myoblasts in culture (Bass *et al*., 1998). Myostatin is localised in the nucleus of proliferating myoblasts in culture and predominantly in the cytoplasm, when the myoblasts fuse to form myotubes (Somers *et al*., 1998). In intact muscle fibres localisation of myostatin shows a striated pattern which appears to be associated with the myosin in the sarcomere (Somers *et al*., 1998). The differential localisation of myostatin at different developmental stages may be related to changing functions of myostatin.

The discovery that the myostatin gene is associated with and probably directly involved in the control of fibre development is an exciting observation. But how can this be of benefit to the NZ meat industry and the NZ economy? The key to the utilisation of these discoveries is the combining of techniques in molecular biology with sophisticated reproductive technologies which are available in NZ. Genetic markers of myostatin could be used to identify superior muscled breeding stock at a young age and specific engineering of the myostatin gene could enable stock with required levels of muscling to be generated. Further, an option which NZ is unlikely to take up but which our North American competitors will, is the therapeutic manipulation of "anti" myostatin during fetal and post-natal development. There are also possible medical benefits from the studies of the myostatin gene in muscle degenerative diseases, with muscle wasting which result from cancer and AIDS and also muscle repair in athletes, horses and greyhounds.

The identification of the role that myostatin plays in muscle development, growth and maintenance is one small part of the complex interactive system which controls muscle growth and function. The unravelling of these interactive systems will not only provide an understanding of muscle physiology, but will also provide opportunities which will benefit the meat industry and the treatment of muscular degenerative diseases and injuries.

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