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Biochemical and transcriptomic analyses of two bovine skeletal muscles in Charolais bulls divergently selected for muscle growth

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Abstract

This work aimed to investigate the consequences of muscle growth selection on muscle characteristics. An oxidative muscle (*Rectus abdominis*, RA) and a glycolytic one (*Semitendinosus*, ST) were studied in two groups of six extreme young Charolais bulls of high or low muscle growth. Mitochondrial activity was lower in muscles of bulls with high muscle growth. Transcriptomic studies allowed the identification of putatively differentially expressed genes. The differential expression between genetic types of two genes in RA (a heat shock protein and a thyroid receptor interacting protein) and of seven genes in ST (including LEU5, tropomyosin 2, and sarcosin) was confirmed by different statistical approaches or Northern blot analysis, as well as the differential expression of five genes (including PSMD4 and DPM synthase) between RA and ST. Both biochemical and transcriptomic results indicate that selection on muscle growth potential is associated with reduced slow-oxidative muscle characteristics. Further studies are required to understand the physiological importance of genes whose expression is changed by selection.

Keywords: Beef; Muscle growth; Transcriptomic analysis; Selection

1. Introduction

Skeletal muscle is a tissue of major economic importance for meat production. Therefore, genetic selection has been directed in favour of muscle development in order to produce lean carcasses with the ultimate objective of increasing the production of muscle quantitatively at the expense of fat. Selective breeding in cattle has effectively been in increasing muscle growth rates in beef cattle and this may have modified muscle characteristics (Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002).

Consumers are looking for safe bovine meat of high and consistent quality. These demands are forcing farmers and processors to reconsider their production system based on a higher quantitative production of beef (Tarrant, 1998) and to better understand consumer acceptance of beef (Dransfield, 2001). Meat eating quality traits, including tenderness and flavour, are affected by postmortem technological treatments applied to the carcass or to muscles (Culioli, 1999; Thompson, 2002),

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as well as by anatomical, physical and chemical characteristics of muscles. The latter depend upon the genetic potential of animals and production systems (Burrow, Moore, Johnson, Barendse, & Bindon, 2001; Geay, Bauchart, Hocquette, & Culioli, 2001).

Muscles are made up of a heterogeneous mixture of fibres, the characteristics of which differ with respect to their contractile and metabolic properties. The diversity of muscle fibre types dictates not only the energy requirements of the muscle as a whole, but also influences the final quality traits of meat in all species (Klont, Brocks, & Eikelenboom, 1998). Indeed, variability in muscle fibre types is associated with differences in fat content and fatty acid profile (which influence flavour and dietetic properties of meat), glycogen content (which determines to a large extent the ultimate pH of the meat) and proteolytic activity (which contributes to meat ageing and hence tenderness). Other muscle features are also known to influence meat eating quality. They include the characteristics of intramuscular connective tissue (content and solubility of collagen, etc.) which determine the basal toughness of meat (McCormick, 1999). Muscle fibre area and type, as well as collagen and lipid characteristics, explain a significant part of the variability in tenderness and flavour. One fourth to one third of the variability of 2-day mechanical strength and 15-day tenderness or flavour scores were found to be related to the variability in these muscle characteristics (Renand, Picard, Touraille, Berge, & Lepetit, 2001). Moreover, calpastatin activity at 24 h postmortem or the ratio of calpastatin to calpain activities are indicators of muscle fibre ageing, which may also help to predict the final tenderness of beef (Koohmaraie et al., 1995). Similarly, intramuscular fat plays a major role in beef flavour (Denoyelle, 1995). Up to now, only the most important muscle characteristics have been studied; but the major part of the total variability in meat quality, such as tenderness and flavour, remains independent of these muscle characteristics (Renand et al., 2001).

The determinants of meat eating quality are thus not yet fully understood, but it is well accepted that the intrinsic characteristics of muscle play an important role (Geay et al., 2001). Moreover, a number of studies indicate that some of the meat quality traits are moderately heritable and also variable between genotypes (Burrow et al., 2001). Consequently, a better understanding of this genetic variability could allow discriminatory tests for eating quality to be developed. It is therefore important to know more about the molecular, genetic and biochemical differences between animals that produce meat of different eating quality. To understand the way by which muscle characteristics are involved in eating quality and to identify new genes involved in this process, DNA array technology may be the method of choice. Indeed, new indicators can be demonstrated by large-scale measurements of gene characteristics and expression at the mRNA or protein levels (Eggen & Hocquette, 2004). This approach can be considered as a huge step forward compared to previous molecular biology analyses of meat focused on a very small number of genes (Ortigues-Marty, Hocquette, & Vermorel, 2001). Therefore, it is important to develop this innovative functional genomic approach in meat science.

One key question is to depict the consequences of long-term selection for muscle growth (Koohmaraie et al., 2002) on the relationship of muscle characteristics to meat quality traits. Therefore, we undertook a first study using two complementary strategies: (i) the biochemical assays of known muscle proteins (enzyme activity, etc.), (ii) the identification of differentially expressed genes in muscles using array technology. Unfortunately, cDNA libraries and related arrays were not available in the bovine species at the beginning of this study. We previously demonstrated the feasibility of using an heterologous approach in cattle (Sudre et al., 2003) with cDNA arrays comprising 1339 printed PCR products representing muscle-specific human cDNA clones (Piétu et al., 1996). Here, we report results obtained both by expression profiling and biochemical assays performed on different muscle samples.

2. Materials and methods

2.1. Animals and muscle samples

The experiment used 64 young Charolais bulls. They were all born to pure bred Charolais cows from an INRA experimental herd, weaned at 32 weeks and then kept in an open shed. They were fed a complete pelleted diet distributed ad libitum with a limited amount of straw until slaughter. Half of the animals were slaughtered at 15 months of age and half at 19 months of age. At slaughter, the warm carcass and the internal fat depot weights were recorded. The next day the 6th thoracic rib joint was dissected and the carcass composition (muscle and fat contents) was estimated using the prediction equation proposed by Robelin and Geay (1975).

Bull calves were progeny of 25 Charolais sires divergently selected on their muscle growth capacity among 60 progeny tested sires (High [H]: $n=12$; Low [L]: $n=13$). Progeny testing had been previously conducted in this herd with 793 slaughtered bull calves. The sires were ranked on a synthetic index combining their breeding value for a high muscle weight and a low carcass fat percentage. The sires used for creating the current generation of experimental animals were chosen from extremes of the distribution of this selection index: muscle weight and fat percentage expected breeding values of +18 kg and −1.1%, respectively, for H sires and −17 kg and +0.9%, respectively, for L sires (with a genetic SD of 17 kg and 1.5%, respectively, for muscle weight and fat percentage).