Meat Science 82 (2009) 205–212

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Changes in muscle gene expression related to metabolism according to growth potential in young bulls

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ARTICLE INFO

Article history:
Received 5 August 2008
Received in revised form 8 January 2009
Accepted 9 January 2009

Keywords:
Muscle growth
Gene expression
Metabolism
Growth factors
Charolais bulls

ABSTRACT

To analyse the effects of genetic selection in favour of high muscle development on muscle gene expression, oligonucleotide microarrays were used to compare the transcriptome of *Longissimus thoracis* muscle from 15- and 19-month-old Charolais bull calves divergently selected for high (H) or low (L) muscle growth. Transcriptome data revealed that about two thirds of the genes involved in glycolysis were up-regulated at 15 and at 19 months of age in H animals. Lastly, some differentially expressed genes were associated with muscle mass in the carcass (*FGF6*, *PLD2*) independently of fat deposition and meat quality. Selection for muscle growth potential is associated with modified expression of some genes involved in growth, and also with increased expression of genes involved in glycolysis. Furthermore, this change in muscle metabolism is likely to be dissociated from fat deposition and beef quality, providing new criteria for genetic selection in favour of muscle growth.

1. Introduction

In France, genetic selection in farm animals, notably in cattle, has been directed towards high muscle development at the expense of fat in order to produce leaner carcasses and increase meat production. This selection may have influenced muscle characteristics and consequently meat quality. Indeed, studies performed on young Charolais bulls, divergently selected for muscle growth potential, showed that an increased lean to fat ratio was associated with more rapid muscle glycolysis and a lower intramuscular fat content (Renand, Berge, Picard, Robelin, Geay, & Krauss et al., 1994). High (H) growth potential animals had more fibres, a higher proportion of fast glycolytic fibres and a lower proportion of slow fibres than low (L) growth potential animals (Picard, Jurie, Duris, & Renand, 2006). In contrast, selection for growth capacity did not change the total collagen content but slightly increased the heat-labile collagen content (Renand et al., 1994). Slow-oxidative muscle characteristics were shown to be diminished using a transcriptomic approach on young Charolais bulls selected for muscle growth potential (Sudre et al., 2005). It can be argued that the muscle modifications driven by this genetic selection may favour tenderness thanks to the enhanced fast-glycolytic muscle properties (which favour meat ageing), but be detrimental to flavour because of the lower intramuscular fat content.

Nowadays, consumers are looking for highly consistent meat in terms of beef quality (tenderness, juiciness, flavour, colour and healthiness), so it is important to improve our understanding of the effects of genetic selection on muscle characteristics, with the ultimate objective of controlling the variability of meat quality. Functional genomics (including for instance microarray technology) provides new opportunities in meat science to study the influence of different production systems (growth path, nutrition level, grass-feeding) on biological characteristics of muscle related to beef quality (Eggen & Hocquette, 2004).

Therefore, to identify genes with expression profiles associated with muscle growth potential, the transcriptome of *Longissimus thoracis* muscle from 15- and 19-month-old Charolais bulls divergently selected for high or low muscle growth potential was analysed. To this end, long-oligonucleotide microarrays representing human and murine genes previously selected for their involvement in skeletal and cardiac muscle physiology were used. We have also characterized animals in terms of beef performance and muscle properties to relate the gene expression profiles to phenotypic characters.

2. Materials and methods

2.1. Animal and muscle samples

This study was conducted with 16 among 25 young Charolais bull calves (slaughtered at 15 or 19 months) from an INRA

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doi:10.1016/j.meatsci.2009.01.012

experimental herd of the INRA Experimental Unit of Bourges. The animals were raised and then slaughtered simultaneously and in the same conditions as described in Bernard et al. (2007). The calves were the progeny of 12 Charolais sires divergently selected for muscle growth capacity among 80 progeny-tested sires. Progeny testing had been conducted in this herd using 793 slaughtered bull calves. The sires were ranked on a synthetic index (SI) combining their breeding values (BV) for high muscle weight and low carcass fat percentage. The sires used to create the current generation of experimental animals were chosen from extremes of the distribution of the SI, so the progeny were expected to have the greatest possible divergence in genetic potential. The BV of the 25 experimental animals was estimated in an animal model using all available information. The calves were ranked on a similar SI as their sires. The 25 SI ranged from –3.5 to +2.8 with a standard deviation of 1.7. The 16 most extreme calves (with SI from –3.5 to –0.7 (–2.1 on average) on one hand and from +1.0 to +2.8 (+1.8 on average) on the other hand) were chosen: four per age characterized by high muscle growth capacity (H) and four per age characterized by low muscle growth capacity (L). A selection of *Longissimus thoracis* muscle (LT) was excised from each carcass within ten minutes post-mortem. The samples were immediately frozen in liquid nitrogen and stored at –80 °C pending analysis.

2.2. Biochemical studies

Muscle characteristics were assessed as described by Bernard et al. (2007). Glycolytic and oxidative energy metabolism was assessed by measuring, respectively, lactate dehydrogenase (LDH, EC 1.1.1.27) activity, and isocitrate dehydrogenase (ICDH, EC 1.1.1.42), citrate synthase (CS, EC 2.3.3.1) and cytochrome-*c* oxidase (COX, EC 1.9.3.1) activities. Enzyme activities were expressed in micromoles per minute per gram of muscle. Total collagen content was measured in lyophilized muscle powder and the data were expressed as micrograms of hydroxyproline per milligram of dry matter. The histological architecture of the muscle fibres was revealed by azorubin staining of serial cross-sections and the mean fibre area was calculated. Total lipids, triglycerides and phospholipids were analysed as described previously and expressed as milligrams per gram of muscle.

2.3. Microarray experiments and data analyses

Transcriptome analysis was performed using microarrays, prepared at the West Genopole transcriptome platform, on which 50-mer oligonucleotides probes (MWG Biotech) were spotted. The oligonucleotides were designed from 3861 human and 1557 murine genes implicated in normal and pathological skeletal and cardiac muscle. The 5418 genes represented on the microarray were spotted in triplicate with 2898 control spots (buffer and empty). These genes encode proteins involved in different biological processes according to Gene Ontology (http://cardioserve.nantes.inserm.fr/ptf-puce/myochips_fr.php).

Microarray experiments and data analyses were performed as described by Bernard et al. (2007). In brief, four RNA samples of 15 μg total RNA extracted from each muscle were reverse transcribed, labelled with Cy3 and hybridised to the microarray simultaneously with the Cy5-labelled reference pool. So, four chips were hybridised per LT sample. The concomitant use of additional within- and between-chip replicates (three and four respectively) allowed obtaining low and balanced values of both false positive and false negative rates (Le Meur et al., 2004). After washing, the microarrays were scanned with an Affymetrix 428 Array Scanner and the images were analysed using Genepix Pro 6.0 software (Axon Instruments, Inc). Data were filtered and normalised according to the *lowess fitness* method using MADSCAN (Le Meur et al., 2004). Genes that were differentially expressed between H and L animals were identified using Significance Analysis of Microarray (SAM) (Tusher, Tibshirani, & Chu, 2001). The results were expressed according to the fold change value (FC), which represents the H/L expression ratio. A gene was potentially declared to be up-regulated (FC > 1) or down-regulated (FC < 1) when its expression was higher or lower, respectively, in H animals. Only the genes with the highest fold-change and belonging to a biological pathway for which some differential expressions were confirmed by RT-PCR were declared up-regulated or down-regulated. The ontology of the differentially expressed genes and of all genes in microarray, notably biological process, was determined using the PANTHER classification system (Thomas et al., 2003).

The data discussed in this publication have been deposited in NCBIs Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GSE5561.

2.4. Real time RT-PCR

The differential expression of six genes revealed by the microarray data was checked by real time RT-PCR using a LightCycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics Gmbh, Mannhein, Germany). Primer sequences were designed with an annealing temperature of 60 °C using Primer3 software. The forward and reverse primers for the genes tested and for internal reference gene (*CDH11*) are reported in Table 1. PCR efficiency was tested for each primer pair by a 10-fold dilution series of purified cDNA. Each reaction was subjected to melting curve analysis to ensure the specificity and integrity of the PCR product.

2.5. Statistical analysis

For data related to carcass composition, muscle biochemistry data and genes declared differentially expressed following arrays and real time RT-PCR experiments, analysis of variance was performed using the GLM Procedure of the Statistical Analysis System Institute (1996). The tested effects included genotype, age and the genotype × age interaction. For RT-PCR data, the model also contained levels of a control gene (*CDH11*) as a covariable to control technical variability, this gene being not differentially expressed among animals (data not shown) (Hocquette & Brandstetter, 2002). All results were presented as adjusted means with appropriate standard errors of means (s.e.m). Differences between adjusted means were compared using the PDIFF option of SAS. For gene studies, this procedure allowed to confirm by usual statistical procedures that genes declared significantly differentially expressed were indeed characterized by different expression levels between genotypes.

In order to calculate the percentage of muscle growth variability explained by muscle characteristics and gene expression levels, a correlation study was performed using Statistica software (StatSoft, France).

Table 1
Primer sequences used in quantitative real-time PCR.

Gene Symbol	Forward primer (5′)	Reverse primer (3′)
ACO2	CTCCAACAACCTGCTCATTG	CTGGCAAAGCTCTTGGTGAT
CDH11	GGGTCCCTGAGCTCCTTAGA	AGATTCCTCAGAACGCCAGA
ENO3	AGCGTCTGGCCAAATACAAT	TGGTTGGCACCAGTGTTTTA
GAPDH	CGACCACTTTGTCAAGCTCA	TCAGGGCCTTAGAGATGGAA
IGF2	TTTCTCTCTCCGCTGCTCTC	TTAGGGAGGACGGGCTGT
LDHA	GCCGATTTGGCAGAAAGTAT	TCTGGATCCCCCAAAGTGTA
Pgm2	CTCGCATCATCTTTCGACTG	ACCACATCTGGCCTGTCTTC