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AGE-DEPENDENT CHANGES IN BOVINE SKELETAL MUSCLE TRANSCRIPTOMIC PROFILE

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The postnatal growth of muscle tissue occurs by hypertrophy comprising satellite cells proliferation, differentiation and protein turnover. The highest rate of skeletal muscle gains and protein synthesis in bulls occurs in the period between 180 and 360 days of postnatal life. However, genes which are responsible for quantitative and qualitative changes in skeletal muscle during this period are not identified up to date. The aim of our study was to compare the changes in transcriptomic profile of skeletal muscle (*m. semitendinosus*) in 12 Polish Black and White bulls between 6 and 12 month of life. For experimental purposes we used bovine cDNA microarray (the NBFGC EST collection) which contains 18,263 unique genes, derived from many different tissue types and various physiologically important states within these tissues. Our results revealed 53 genes which expression changed in the same manner depending on age of all examined pairs of animals. Thirty two of these genes showed at least 2-fold difference in expression between two analyzed age points. Age-dependent up-regulation was the most pronounced in the case of following genes: similar to MAD2L1 binding protein, similar to thymocyte potein thy28 isoform 1, similar to type I inositol-1,4,5-triphosphate 5-phosphatase, similar to nucleoside diphosphate kinase 6, proline rich 14, similar to transcription factor E2-alpha and phospholipase C gamma 1. The highest age-dependent decrease of the transcript was observed in the case of: similar to ubiquitin carboxy-terminal hydrolase L1, similar to latent TGF-beta binding protein 3 precursor, phospho-mannomutase 2, CD74 antigen, simlar to BCL6 co-represor-like 1, plateled/endothelial cell adhesion molecule (PECAM1), necdin, zygin, tight junction protein 3, ankyrin and apolipoprotein-L3. Although the role of the most of above genes and interactions between products of their expression is not clear at the moment, the significance of their response between 6 and 12 month of age may indicate their involvement in growth, development and metabolic changes in bovine skeletal muscle during the first year of postnatal life.

Key words: *cDNA microarray, transcriptome, skeletal muscle, muscle development, cattle*

INTRODUCTION

It is commonly accepted that the mass of skeletal muscle is dependent on the number and size of the myofibers. The final muscle mass is achieved by hyperplasia during prenatal and hypertrophy during postnatal period of the individuals life. Fiber number and final point of fiber formation seems to be stable among species and is relative to cell number after proliferation phase. In cattle the number of muscle fibers is fixed at 240 day of fetal life (1). Transcriptome analysis of bovine muscles during ontogenesis showed the importance of the last three months of gestation in myogenesis (2). Fiber size is highly correlated with satellite cells quantity, nutrients, hormones and growth factors availability. It is an established knowledge that the influence of specific hormones and growth factors is needed for muscle growth and maturation. It is also well known that postnatal myofiber hypertrophy is directly linked with increased level of DNA due to division and fusion of satellite cells with myofibers. For this reason there is an increased protein production and visualized muscle growth by hypertrophy. It has previously been shown that muscle development and maturation processes are kept under the influence of somatotropic axis which acts through GH and IGFs (IGF receptor concentration, IGFBPs activity) proteins, which in turn are able to stimulate protein synthesis in many different tissues and within muscle tissue (for review see: Oksbjerg *et al.* (3)).

Robelin and Tulloh (1) assessed that between 0 and 542th day from birth an average total body mass of Friesian bulls increased from 41 kg to 534 kg E.B.W. (Empty Body Weight). Simultaneously muscle mass increment was 36.6% of E.B.W. from day 0, through 45% of E.B.W. at 120th day, to 41% of E.B.W. at 534th day of bulls life. It means that skeletal muscle mass in this period increased from 15 kg at birth day, through 48.6 kg at 120th day, to 222.22 kg at 534th day of life. This findings showed that skeletal muscle to E.B.W. ratio increased in the first period of bulls life and decreased from about 6th month of life. The cause of this phenomenon is parallel increment of fat and connective tissues deposition. However, absolute skeletal muscle mass augmented in this period nearly 15 times. Robelin and Tulloh (1) studies revealed that the daily gain of skeletal muscle tissue is the highest (about 400g/day) from about 180th to about 300th day of life after which they observed decrease in daily gain of this tissue. At the same time period they observed slow increment of fatty tissue (about 100 g/day) with a pick about 600th day of life. The daily lipid deposition became higher then protein retention as early as 220 days and the daily fatty tissue growth became higher then muscle growth at 450 days (1). Above data indicate that the highest dynamics in skeletal muscle growth and qualitative changes in muscle composition occur between 6th and 12th month of life. However, the metabolic processes and genes responsible for regulation of muscle growth in this period are still obscure.

The availability of microarray technology for most production animal species provides new opportunities for researchers to generate global gene expression

profiles. Microarrays have been used to elucidate gene function in knockout mice, evaluate breed differences and the effects of hormones and diet on transcriptome of skeletal muscle (4 - 6).

The aim of our study was to compare the changes in transcriptomic profile of skeletal muscle (*m. semitendinosus*) in Polish Black and White bulls between 6th and 12th month of life. For experimental purposes we used bovine cDNA microarray (the NBFGC EST collection) which contains 18,263 unique genes, derived from many different tissue types and various physiologically important states within these tissues (7). In the course of this study we were able to select 53 genes with more then 1.75-fold difference: 19 up-regulated and 34 down-regulated, all of which seem to be involved in muscle development and maturation.

MATERIAL AND METHODS

Animals and Muscle Samples

Six 6-month-old bulls and six 12-month-old bulls of the same breed (Polish Black and White) were breed at housing unit of Institute of Genetics and Animal Breeding of Polish Academy of Science in Jastrzębiec. They were maintained in standard conditions and fed with standard diet. The average body weight before slaughter of 6-month and 12-month-old bulls was 197.6 kg and 381.4 kg, respectively. The samples of *m. semitendinosus* were obtained during slaughter, immediately frozen in liquid nitrogen and stored at -80°C until analyzed.

RNA Isolation and Validation

Total RNA from muscle sample was isolated using Total RNA kit (A&A Biotechnology, Poland) according to the manufacturer's protocol. Isolated RNA samples were dissolved in RNase-free water and RNA quantity was measured using NanoDrop (NanoDrop Technologies, USA). The samples with adequate amount of RNA were treated with DNase I to eliminate DNA contamination. Subsequently the samples were purified using RNeasy MiniElute Cleanup Kit (Qiagen, Germany). The samples were again analyzed using BioAnalyzer (Agilent, USA) to measure final RNA quality and integrity.

Probes Labelling

Total RNA (10 µg) was reverse-transcribed using SuperScript Plus Indirect cDNA Labelling kit (Invitrogen, USA) according to the manufacturer's protocol. Single strand cDNA was labeled with Alexa 555 or Alexa 647 dyes (Invitrogen, USA). Efficiency of dyes incorporation was measured using NanoDrop (NanoDrop Technologies, USA). Afterwards the samples were randomly paired (one sample from 6-month-old bulls and one sample from 12-month-old bull) in one tube and hybridized.

Hybridization

Before hybridization microarray slides NGFBC (Centre of Animal Functional Genomics, Michigan State University, USA) (7) were prepared following the manufacturer's protocol. Hybridization was performed using automatic hybridization station HybArray12 (PerkinElmer,

USA). Slides were fixed in hybridization chambers and after o-ring conditioning probes were added. Hybridization of slides was performed using 18 hours step-down hybridization protocol provided by producer. After hybridization slides were automatically washed.

Hybridization Signal Detection, Quantification and Analysis

Acquisition and analysis of hybridization intensities were performed using microarray scanner ScanArray HT and ScanExpress software (PerkinElmer, USA). Different types of values were obtained for quantification of the dot intensity. Due to experimental variations in specific activity of cDNA target preparations or exposure time that might alter the signal intensity, data from different hybridizations were automatically normalized (LOWESS method) by ScanExpress software. For further analysis the ratio of mean spot after subtraction of background signals for each of two experimental samples was chosen. The average ratio of six slides were calculated and the changes of average ratio higher then 1.75-fold were considered significant. Genes names were identified using MSU NBFGC internet data base and BLAST software.

RESULTS

The comparison of the transcriptomic profiles of *m. semitendinosus* between 6th and 12th month bulls revealed 53 genes with the change of expression depending on age of examined animals. In Table 1 and 2 are presented only these genes, which expression changed in the same manner (up-regulated or down-regulated) in six examined pairs of bulls (6 vs. 12 month old). Thirty two of these genes showed at least 2-fold difference in expression between two analyzed age points.

Characteristics of up-regulated genes

Nineteen genes were up-regulated with age, which was manifested with 1.75-4.01-fold increase in their expression (*Table 1*). Among them the highest response was observed in the case: *Bos taurus* similar to MAD2L1 binding protein, *Bos taurus* similar to thymocyte protein thy28 isoform 1, *Bos taurus* hypothetical protein LOC614230, *Bos taurus* similar to Type I inositol-1,4,5-trisphosphate 5-phosphatase, *Bos taurus* similar to Nucleoside diphosphate kinase 6 (NDK 6), *Bos taurus* secreted and transmembrane protein 1B mRNA, *Bos taurus* proline rich 14 (PRR14), PREDICTED: *Bos taurus* similar to Transcription factor E2-alpha, *Homo sapiens* F-box and leucine-rich repeat protein 17 (FBXL17), *Bos taurus* similar to solute carrier family 29 (nucleoside transporters), *Bos taurus* sulfide dehydrogenase like (SQRDL) and *Bos taurus* phospholipase C, gamma 1 (PLCG1).

MAD2L1 binding protein - mitotic arrest deficient 2 - plays a crucial role in the spindle checkpoint induced apoptosis. In case of gastric cancer it has been reported that down-regulation of this protein may results in inhibition of apoptosis. Spindle inhibitors and DNA-damage agents can activate mitochondrial pathway and induce apoptosis of gastric cancer cells with normal MAD2 expression. We revealed over 4-fold increase expression of MAD2L1 binding protein. MAD2 is an essential component of the mitotic spindle checkpoint

Table 1. Age-dependent up-regulation of gene expression in bovine skeletal muscle (n=6)

No.	Gene ID NBFGC	GenBank Accession Number	Gene Name	Fold
1.	NBFGC_BE721247	BC118446	*Bos taurus* similar to MAD2L1 binding protein	4.01 ↑
2.	NBFGC_BE665937	XM_868467	*Bos taurus* similar to thymocyte protein thy28 isoform 1	3.36 ↑
3.	NBFGC_AW425668	XM_876600	*Bos taurus* hypothetical protein LOC614230	2.55 ↑
4.	NBFGC_BE485247	XM_866984	*Bos taurus* similar to Type I inositol-1,4,5-trisphosphate 5-phosphatase	2.45 ↑
5.	NBFGC_BE664298	XM_586294	*Bos taurus* similar to Nucleoside diphosphate kinase 6 (NDK 6)	2.44 ↑
6.	NBFGC_BF651339	BK005437	*Bos taurus* secreted and transmembrane protein 1B	2.40 ↑
7.	NBFGC_AW426526	NM_001035396	*Bos taurus* proline rich 14 (PRR14)	2.34 ↑
8.	NBFGC_BE682623	XM_609091	PREDICTED: *Bos taurus* similar to Transcription factor E2-alpha	2.28 ↑
9.	NBFGC_BE237392	NM_022824	*Homo sapiens* F-box and leucine-rich repeat protein 17 (FBXL17)	2.27 ↑
10.	NBFGC_BE754356	XM_610062	*Bos taurus* similar to solute carrier family 29 (nucleoside transporters)	2.26 ↑
11.	NBFGC_AW632206	NM_001040511	*Bos taurus* sulfide dehydrogenase like (SQRDL)	2.04 ↑
12.	NBFGC_BE663518	NM_174425	*Bos taurus* phospholipase C, gamma 1 (PLCG1)	2.01 ↑
13.	NBFGC_BE749228	XM_611199	*Bos taurus* similar to interphase cyctoplasmic foci protein 45	1.97 ↑
14.	NBFGC_AW432009	XM_591546	*Bos taurus* similar to transducer of regulated CREB protein 3	1.96 ↑
15.	NBFGC_AW435571	XM_583477	PREDICTED: *Bos taurus* hypothetical LOC506950	1.94 ↑
16.	NBFGC_BE721627	XM_589917	*Bos taurus* similar to leucine rich repeat containing 36	1.91 ↑
17.	NBFGC_AW486562	XM_877026	*Bos taurus* similar to kinesin 2 60/70kDa isoform 2	1.86 ↑
18.	NBFGC_BE722297	XM_591279	*Bos taurus* similar to phosphatidylinositol-binding clathrin assembly protein	1.85 ↑
19.	NBFGC_AW447791	XM_865538	*Bos taurus* similar to ADP-ribosylation factor 6	1.79 ↑

pathway. It was previously shown to be associated with drug resistance of tumor cells. Du *et al.* (8) indicate that down regulation of MAD2 could promote the drug resistance of gastric cancer cells and inhibit anticancer drugs induced-apoptosis by up-regulating Bcl-2 and interfering the mitochondrial apoptosis pathway.

Thymocyte protein Thy28 - has recently been identified to be involved in apoptosis of avian lymphocyte. *Thy28* gene product is highly conserved among vertebrates and plants and appears to be implicated in apoptosis induction in human lymphoma cells. Muscle, lung, heart contains low levels of Thy28 proteins (9). Down-regulation of mouse Thy28 (mThy28) protein expression appears to be accompanied by apoptotic processes. Thymocytes from mice contain moderate amount of mThy28 protein and undergo proliferation, differentiation, or apoptosis during murine thymic maturation (10).

Type I inositol-1,4,5-trisphosphate 5-phosphatase - The water-soluble InsP3 releases Ca^{2+} from ER but is eventually degraded to Ins(1,4)P2 by a phosphomonoesterase, InsP3 5-phosphatase. Maximal Ins(1,4,5)P3 5'-phosphatase activity was significantly increased in the immature vs. adult tracheal smooth muscle (11). Skeletal muscle triads are possessing the whole set of enzymes of the phosphatidylinositol (PI)-linked signal generating pathway, PI-kinase, PI(4)P-kinase, and PI(4,5)P_2-phospholipase C (PLC). The activities of these enzymes are comparable to those found in other cell types for which a functional role of the PI-pathway in intracellular signal transduction has been established. For skeletal muscle an unequivocal function and an initiating signal for Ins(1,4,5)P_3-liberation is still unknown. However, the observed Ca-dependency of PLC activity suggests that here Ins(1,4,5)P_3 production is a consequence rather than a cause of increasing cytosolic Ca^{2+} (12).

Nucleoside diphosphate kinase (Ndk) also known as myokinesin - is an important ubiquitous enzyme that generates nucleoside triphosphates (NTPs) or their deoxy derivatives by terminal phosphotransfer from NTPs such as ATP or GTP to any nucleoside diphosphate or its deoxy derivative. NTPs, particularly GTP, are important for cellular macromolecular synthesis and signalling mechanisms (13). The structures of NDP kinases are highly conserved from *Escherichia coli* to human (43% identity), and they are believed to be a housekeeping enzyme essential for DNA and RNA synthesis. In addition, NDP kinases have been shown to have additional regulatory functions for growth and developmental control, signal transduction, and tumor metastasis suppression (14).

Proline rich 14 (PRR14) - there are at least two calmodulin (CaM) binding sites and a proline-rich domain (PRD) that may be important in the tyrosine kinase-mediated regulation of the calcium channel. The calcium binding protein, CaM, appears to play an important role in both Ca^{2+}-mediated inactivation of the channel as well as in the facilitation of Ca^{2+} entry (15).

Transcription factor E2-alpha (Immunoglobulin enhancer-binding factor E12/E47, Transcription factor 3, TCF-3, Immunoglobulin transcription factor 1, Transcription factor ITF-1) - Heterodimers between TCF3 and tissue-specific basic helix-loop-helix (bHLH) proteins play major roles in determining tissue-specific cell fate during embryogenesis, like muscle or early B-cell differentiation. Dimers bind DNA on E-box motifs: 5'-CANNTG-3'. It is also known to bind to the kappa-E2 site in the kappa immunoglobulin gene enhancer (UniProtKB/Swiss-Prot data base).

F-box and leucine-rich repeat protein 17 (FBXL17) - Members of the F-box protein family, such as FBXL17, are characterized by an approximately 40-amino acid F-box motif. SCF complexes formed by SKP1, cullin (CUL1), and F-box proteins, act as protein-ubiquitin ligases. F-box proteins interact with SKP1 through the F-box, and they interact with ubiquitination targets through other protein interaction domains (16).

Equilibrative nucleoside transporters (solute carrier family 29), SLC29A1, ENT1 - specific transport proteins required for the transfer of hydrophilic nucleosides across cell membranes. The equilibrative transporters are expressed ubiquitously. They have transmembrane domains with contains sites for phosphorylation by protein kinase CK2 (casein kinase II). Nucleoside transporter expression by mammalian cells has also been directly correlated with growth rate and CK2-mediated phosphorylation may be a factor in this regulation. By regulating the concentration of adenosine available to cell surface receptors, they influence many physiological processes ranging from cardiovascular activity to neurotransmission (17, 18).

Phospholipase C gamma 1 (PLCG1) - Phospholipase C-g1, a tyrosine kinase substrate, hydrolyses phosphatidylinositol 4,5-bisphosphate to produce inositol 1,4,5-trisphosphate and diacylglycerol, which act as second messenger molecules to mobilize intracellular calcium and activate protein kinase C, respectively. Chattopadhyay and Carpenter (19) have investigated the role of phospholipase C-gamma1 in anoikis, which is a cell death, induced by the loss of extracellular matrix adhesion.

Characteristics of down-regulated genes

Thirty four genes were down-regulated with age, which was shown as a 1.76-4.04-fold decrease in their expression (*Table 2*). Among them the highest effect was observed in the case of: *Bos taurus* similar to ubiquitin carboxy-terminal hydrolase L1, *Bos taurus* similar to Latent TGF beta binding protein 3 precursor, *Bos taurus* similar to CG32369-PB isoform B, *Bos taurus* similar to IQ motif containing E, *Bos taurus* phosphomannomutase 2, *Bos taurus* CD74 antigen (CD74), PREDICTED: *Macaca mulatta* rho-related BTB domain containing 3, *Bos taurus* similar to BCL6 co-repressor-like 1, *Bos taurus* similar to methyltransferase-like protein 1 isoform a, *Homo sapiens* acyl-Coenzyme A binding domain containing 5 (ACBD5), *Bos taurus* platelet/endothelial cell adhesion molecule (PECAM1), *Bos taurus* similar to Serine/threonine protein phosphatase 4 catalytic subunit, *Bos taurus* similar to RNA-binding protein S1, serine-rich domain, *Bos taurus* similar to myotubularin-related protein 3 isoform c, PREDICTED: *Bos taurus* similar to WHSC1L1 protein isoform long, *Bos taurus* necdin (Ndn), *Bos taurus* zygin 1 isoform 1 (FEZ1), *Bos taurus* tight junction protein 3 (zo3), *Bos taurus* similar to ankyrin repeat and SOCS box-containing 8 and *Bos taurus* similar to Apolipoprotein-L3 (TNF-inducible protein CG12-1).

Ubiquitin carboxy-terminal hydrolase (UCH-L1), official name: Ubiquitin thiolesterase - Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is a deubiqutinating enzyme (DUB) that regulates ubiquitin-dependent metabolic pathways. Deubiquitinating enzymes are a large group of proteins that cleave ubiquitin-protein bonds. Potentially, DUBs may act as negative and positive regulators of the ubiquitin system. In addition to ubiquitin recycling, they are

Table 2. Age-dependent down-regulation of gene expression in bovine skeletal muscle (n=6)

No.	Gene ID NBFGC	GenBank Accession Number	Gene Name	Fold
1.	NBFGC_AW669632	XM_592241	*Bos taurus* similar to ubiquitin carboxy-terminal hydrolase L1	4.04 ↓
2.	NBFGC_AW668650	XM_593232	*Bos taurus* similar to Latent TGF beta binding protein 3 precursor	3.96 ↓
3.	NBFGC_BE721548	XM_580625	*Bos taurus* similar to CG32369-PB, isoform B	3.55 ↓
4.	NBFGC_BE667821	XM_871154	*Bos taurus* similar to IQ motif containing E	3.34 ↓
5.	NBFGC_BG688783	BC102817	*Bos taurus* phosphomannomutase 2	3.04 ↓
6.	NBFGC_AW326084	BT021489	*Bos taurus* CD74 antigen (CD74)	2.74 ↓
7.	NBFGC_BE721547	XM_001090858	PREDICTED: *Macaca mulatta* rho-related BTB domain containing 3	2.53 ↓
8.	NBFGC_AW345575	XM_590977	*Bos taurus* similar to BCL6 co-repressor-like 1	2.50 ↓
9.	NBFGC_BG693350	XM_879945	*Bos taurus* similar to methyltransferase-like protein 1 isoform a	2.43 ↓
10.	NBFGC_BF605499	NM_145698	*Homo sapiens* acyl-Coenzyme A binding domain containing 5 (ACBD5)	2.43
11.	NBFGC_BE588599	NM_174571	*Bos taurus* platelet/endothelial cell adhesion molecule (PECAM1)	2.34 ↓
12.	NBFGC_AW437483	XM_881209	*Bos taurus* similar to Serine/threonine protein phosphatase 4 catalytic subunit	2.31 ↓
13.	NBFGC_BE758270	XM_881395	*Bos taurus* similar to RNA-binding protein S1, serine-rich domain	2.29 ↓
14.	NBFGC_BF606237	XM_866127	*Bos taurus* similar to myotubularin-related protein 3 isoform c	2.23 ↓
15.	NBFGC_BF073584	XM_865899	PREDICTED: *Bos taurus* similar to WHSC1L1 protein isoform long	2.20 ↓
16.	NBFGC_BG691155	NM_001014982	*Bos taurus* necdin (Ndn)	2.14 ↓
17.	NBFGC_AW668876	NM_001024522	*Bos taurus* zygin 1 isoform 1 (FEZ1)	2.07 ↓
18.	NBFGC_BE479044	XM_584210	*Bos taurus* tight junction protein 3 (zo3)	2.07 ↓
19.	NBFGC_BF599904	XM_869496	*Bos taurus* similar to ankyrin repeat and SOCS box-containing 8	2.04 ↓
20.	NBFGC_BF774349	XM_596688	*Bos taurus* similar to Apolipoprotein-L3 (TNF-inducible protein CG12-1)	2.02 ↓
21.	NBFGC_BE664610	BC114825	*Bos taurus* similar to brain zinc finger protein	1.98 ↓
22.	NBFGC_BE846129	NM_001024511	*Bos taurus* transcriptional adaptor 3-like isoform b (TADA3L)	1.97 ↓
23.	NBFGC_AW336601	BC102483	*Bos taurus* similar to abhydrolase domain containing 1 (predicted)	1.95 ↓
24.	NBFGC_AW425316	AY644517	*Bos taurus* T cell receptor gamma cluster 1 (TCRG1) gene	1.94 ↓
25.	NBFGC_AW655457	BC020568	*Homo sapiens* chromosome 10 open reading frame 54	1.92 ↓
26.	NBFGC_AW668953	XM_587874	PREDICTED: *Bos taurus* similar to Rho-related GTP-binding protein RhoN	1.90 ↓
27.	NBFGC_BF076751	XM_617423	*Bos taurus* similar to CG7958-PA, isoform A	1.89 ↓
28.	NBFGC_BF606725	BC111206	*Bos taurus* glutamate-cysteine ligase, modifier subunit	1.86 ↓
29.	NBFGC_AW478322	XM_864015	*Bos taurus* similar to T-complex associated-testis-expressed 1-like	1.84 ↓
30.	NBFGC_BE722985	NM_174547	*Bos taurus* guanylate cyclase 2C (GUCY2C)	1.84 ↓

31.	NBFGC_AW660294	AK024224	*Homo sapiens* cDNA FLJ14162 fis, clone NT2RM4002504	1.83 ↓
32.	NBFGC_BE751919	BC109513	*Bos taurus* similar to small glutamine-rich tetratricopeptide	1.82 ↓
33.	NBFGC_BE751885	XM_864110	*Bos taurus* similar to Death-associated protein kinase 1	1.77 ↓
34.	NBFGC_BF600247	XM_586712	PREDICTED: *Bos taurus* similar to Ubiquitin carboxyl-terminal hydrolase 22	1.76 ↓

involved in processing of ubiquitin precursors, in proofreading of protein ubiquitination and in disassembly of inhibitory ubiquitin chains. A point mutation (I93M) in the gene encoding this protein is implicated as the cause of Parkinson's disease in one kindred. Furthermore, a polymorphism (S18Y) in this gene has been found to be associated with a reduced risk for Parkinson's disease. The gene is also associated with the Alzheimer's disease, and required for normal synaptic and cognitive function (20).

Latent TGF-beta binding protein 3 precursor - Transforming growth factor-beta (TGF-beta) is produced by most cells in large latent complexes of TGF-beta and its propeptide (LAP) associated with a binding protein. The latent TGF-beta binding proteins (LTBPs-1, -2 and -3) mediate the secretion and, subsequently, the association of latent TGF-beta complexes with the extracellular matrix (ECM). Transforming growth factor-beta (TGF-beta) is secreted as latent high molecular mass complexes from producer cells. The N-terminal precursor remnant, also called latency-associated peptide (LAP), forms a non-covalently linked complex with TGF-beta and confers the latency to TGF-beta. In many cell types, latent TGF-beta binding protein-1 (LTBP-1) is disulphide-linked to LAP, and forms complexes of more than 230 kDa. In addition, LTBP-2 and -3, which are structurally similar to LTBP-1, can be part of latent TGF-beta complexes (21).

IQ motif - First identified as a CaM binding motif in neuromodulin by Storm and colleagues (22) the IQ motif was first characterized in myosins by Cheney and Mooseker (23). Proteins that contain IQ motifs typically bind calmodulin in the absence of calcium, although there are some exceptions. IQ motifs frequently occur in tandem, such as in the myosins, although the binding stoichiometry for calmodulin to multiple IQ motifs is unclear. It has been suggested that 'complete' IQ motifs (containing the G and second basic residue) do not require calcium to bind calmodulin; binding of incomplete IQ motifs (lacking the second basic residue) is calcium dependent (24, 25).

Phosphomannomutases (PMMs) - They are cytosolic enzymes crucial for the glycosylation of glycoproteins. In humans, two highly conserved PMMs exist: PMM1 and PMM2. *In vitro* both enzymes are able to convert mannose-6-phosphate (mannose-6-P) into mannose-1-P, the key starting compound for glycan biosynthesis (26). Thiel *et al.* (27) in their studies indicate that Pmm2 is essential for early development of mice.

CD74 antigen - CD74 is an integral membrane protein that was thought to function mainly as an MHC class II chaperone. However, CD74 was recently shown to have a role as an accessory-signaling molecule. Starlets *et al.* (28) studies demonstrated that CD74 regulates B-cell differentiation by inducing a pathway leading to the activation of transcription mediated by the NF-kappaB p65/RelA homodimer and its coactivator, TAF(II)105. They show that CD74 stimulation with anti-CD74 antibody leads to an induction of a signaling cascade resulting in NF-kappaB activation, entry of the stimulated cells into the S phase, elevation of DNA synthesis, cell division, and augmented expression of BCL-X(L). These studies therefore demonstrate that surface CD74 functions as a survival receptor.

Rho GTPases are molecular switches that control a wide variety of signal transduction pathways in all eukaryotic cells. They are known principally for their pivotal role in regulating the actin cytoskeleton, but their ability to influence cell polarity, microtubule dynamics, membrane transport pathways and transcription factor activity is probably just as significant (29).

BCL6 co-repressor-like 1 - BCL6 also call LAZ3, ZBTB27 is expressed in mature B-cells and required for germinal center formation. However, its ubiquitous expression, with predominant levels in skeletal muscle, suggests that it may act outside the lymphoid system. Findings of Albagli-Curiel *et al.* (30) suggest that *LAZ*3 could play a role in muscle differentiation. Together with some results reported in other cell types, they propose that *LAZ*3 may contribute to events common to various differentiation processes, possibly the induction and stabilization of the withdrawal from the cell cycle. Induction of apoptosis by BCL-6 was preceded by down-regulation of apoptosis repressors BCL-2 and BCL-X(L). These results suggest that BCL-6 induces apoptosis by regulating the expression of these apoptosis-regulating genes (31).

Methyltransferase-like protein 1 isoform a - A substrate for protein kinase B (PKB)a in HeLa cell extracts was identified as methyltransferase-like protein-1 (METTL1), the orthologue of trm8, which catalyses the 7-methylguanosine modification of tRNA in *Saccharomyces cerevisiae*. PKB and ribosomal S6 kinase (RSK) both phosphorylated METTL1 at Ser27 in vitro. Ser27 became phosphorylated when HEK293 cells were stimulated with insulin-like growth factor-1 (IGF-1) and this was prevented by inhibition of phosphatidyinositol 3-kinase (32).

Acyl-Coenzyme A binding domain - binds medium- and long-chain acyl-CoA esters with very high affinity and may function as an intracellular carrier of acyl-coa esters. It is also able to displace diazepam from the benzodiazepine (bzd) recognition site located on the gaba type a receptor. Therefore it is possible that this protein also acts as a neuropeptide to modulate the action of the gaba receptor (UniProtKB/Swiss-Prot data base).

Platelet/endothelial cell adhesion molecule (PECAM1) - the signal delivered via platelet endothelial cell adhesion molecule-1 (PECAM-1) seems to contribute to the resistance of this cell population to starvation, and it is related to the

maintainance of mitochondrial metabolism. Indeed, this molecule, originally described as an adhesion receptor belonging to the immunoglobulin superfamily, capable of homophilic and heterophilic interactions, turned out to be a signalling molecule, containing an immunoreceptor tyrosine-based inhibitory motifs (ITIM) within its cytoplasmic domain. In particular, it has been shown that PECAM-1 binds to different kinases and phosphatases, including the phosphatidylinositide-3-kinase that phosphorylates Akt, which, in turn can upregulate transcription and function of antiapoptotic proteins, such as Bcl-2 and Bcl-xl or Al, responsible for the rescue from mitochondrial apoptosis. The possible role of PECAM-1 engagement in the prevention of starvation-induced apoptosis of HPC precursors and in the maintainance of their survival is discussed. This protein is a cell adhesion molecule expressed on platelets and at endothelial cell intercellular junctions (33 - 35).

Serine/threonine protein phosphatase 4 (PPX, PP4) - the PPX/PP4 Ser/Thr protein phosphatases belong to the type 2A phosphatase subfamily and are present in most eukaryotic organisms. It is a novel regulatory subunit of PP4, which is expressed ubiquitously but abundantly in mesangial cells. Its pathophysiologic role in mesangial cells and glomerulus remains unknown. As PP4 is an essential protein for nucleation, growth, and stabilization of microtubules at centrosomes/spindle pole bodies during cell division, PP4[Rmeg] may play a role in regulation of mitosis in mesangial cells (36).

RNA-binding protein S1 - the ribosomal protein S1 - plays critical roles in translation initiation and elongation in *Escherichia coli* and is believed to stabilize mRNA on the ribosome - it is a potent activator of transcriptional cycling in vitro. Sukhodolets *et al.* (37) propose that, *in vivo*, cooperative interaction of multiple RNA-binding modules in S1 may enhance the transcript release from RNA polymerase, alleviating its inhibitory effect and enabling the core enzyme for continuous reinitiation of transcription.

Myotubularin-related protein 3 isoform c - dephosphorylates proteins phosphorylated on Ser, Thr, and Tyr residues and low molecular weight phosphatase substrate paranitrophenylphosphate (UniProtKB/Swiss-Prot data base).

Necdin (Ndn) - is a 325-amino-acid residue protein encoded by a cDNA clone isolated from neurally differentiated embryonal carcinoma cells. Ectopic expression of necdin induces growth arrest of proliferative cells. Necdin binds to major transcription factors E2F1 and p53, suggesting that necdin exerts its functions through the interactions with these cell-cycle-regulating factors (38). Brunelli and Cossu (39) revealed that in undifferentiated mesoangioblast cells first response to TGF-beta1 stimulation is the simultaneous up-regulation of *necdin* and *msx2*. These results indicate that, at least *in vitro*, these two genes are early targets of TGF-beta1.

Zygin 1 isoform 1 (FEZ1) - the protein designated FEZ1 (fasciculation and elongation protein zeta-1) consisting of 393 amino acid residues shows a high Asp/Glu content and contains several regions predicted to form amphipathic helices.

Northern blot analysis has revealed that FEZ1 mRNA is abundantly expressed in adult rat brain and throughout the developmental stages of mouse embryo. Combined with the recent finding that a human FEZ1 protein is able to complement the function of UNC-76 necessary for normal axonal bundling and elongation within axon bundles in the nematode, these results suggest that FEZ1 plays a crucial role in the axon guidance machinery in mammals by interacting with PKCζ (40).

Tight junction protein 3 (zo3) - The tight junction (TJ) is the outermost component of the junctional complex. It is responsible for controlling the passage of ions and molecules through the paracellular pathway, and maintaining a polarized distribution of lipids and proteins between the apical and basolateral plasma membranes (41).

Ankyrin repeat and SOCS box-containing 8 (ASB8) - Liu *et al.* (42) have cloned a new member of human ankyrin repeat and SOCS box containing protein family (ASB). Some members of suppressor of cytokine signaling (SOCS) family were negative regulators of the signaling initiated by cytokines, hormones, and growth factor through blocking JAK-STAT pathway or cytokine receptor. Previous studies suggested that some SOCS proteins may target the bound protein to proteasomal degradation, and that the process was dependent on the ability of the SOCS box recruiting their interacting partners to a core ubiquitination complex via interacting with Elongin B and Elongin C.

Apolipoprotein L3 (Apolipoprotein-L3, Apolipoprotein L-III, ApoL-III, TNF-inducible protein CG12-1, CG12_1) - Apolipoprotein L (APOL) proteins belong to the high density lipoprotein family, which plays a central role in cholesterol transport. The cholesterol content of membranes is important in cellular processes such as modulating gene transcription and signal transduction both in the adult brain and during neurodevelopment. Horrevoets *et al.* (43) identified APOL3, which they designated CG12-1, using differential display analysis to identify genes induced in human umbilical vein endothelial cells (HUVECs) incubated with monocyte-conditioned medium and/or stimulated by tumor necrosis factor-alpha (TNFA).

DISCUSSION

The results of the present study revealed unknown age-dependent transcriptomic response in skeletal muscle of Polish Black and White bulls. The first report describing transcriptome analysis in bovine muscles during ontogenesis was published by Sudre *et al.* (2). However this study was performed on macroarray with 1339 human skeletal muscle cDNA clone inserts and pooled RNA samples. Our study was focused on comparison of transcriptomic profile in *m. semitendinosus*, which is rather white glycolytic muscle, between bulls at 6 and 12 month of age. We used specific bovine cDNA microarray with 18,263 genes to compare the transcriptome in 6 pairs of animals at different age. As a reliable response we assumed only this expression pattern which occurred in all

Table 3. Functional classification of age-dependent transcriptomic response in skeletal muscle of bulls. Only genes with at least 2-fold expression change were classified.

Physiolological function	Gene
Cell proliferation and differntiation	*Bos taurus* Transcription factor E2-alpha ↑ *Bos taurus* Latent TGF beta binding protein 3 precursor ↓ *Bos taurus* Necdin ↓ *Bos taurus* BCL6 co-repressor-like 1 ↓ *Bos taurus* Phosphomannomutase 2 ↓
Mitosis/apoptosis	*Bos taurus* MAD2L1 binding protein ↑ *Bos taurus* Thymocyte protein thy28 isoform 1 ↑ *Bos taurus* Hypothetical protein LOC614230 ↑ *Bos taurus* Nucleoside diphosphate kinase 6 ↑ *Bos taurus* CD74 antigen ↓ *Bos taurus* BCL6 co-repressor-like 1 ↓
Signal transduction	*Bos taurus* Phospholipase C gamma 1 ↑ *Bos taurus* Nucleoside diphosphate kinase 6 ↑ *Bos taurus* Type I inositol-1,4,5-trisphosphate 5-phosphatase ↑ *Bos taurus* Ankyrin repeat and SOCS box-containing 8 ↓ *Bos taurus* IQ motif containing E ↓ *Bos taurus* Methyltransferase-like protein 1 isoform a ↓ *Macaca mulatta* Rho-related BTB domain containing 3 ↓ *Bos taurus* CD74 antigen ↓ *Bos taurus* Apolipoprotein-L3 ↓ *Bos taurus* Necdin ↓ *Bos taurus* Platelet/endothelial cell adhesion molecule ↓
Protein turnover	*Homo sapiens* F-box and leucine-rich repeat protein 17 ↑ *Bos taurus* Ubiquitin carboxy-terminal hydrolase L1 ↓ *Bos taurus* CG32369-PB isoform B ↓
Function of plasma membrane	*Bos taurus* Solute carrier family 29 (ENT1) ↑ *Bos taurus* Type I inositol-1,4,5-trisphosphate 5-phosphatase ↑ *Bos taurus* Tight junction protein 3 ↓ *Macaca mulatta* Rho-related BTB domain containing 3 ↓ *Bos taurus* Platelet/endothelial cell adhesion molecule ↓ *Bos taurus* CD74 antigen ↓
Function of cytoskeleton	*Bos taurus* Serine/threonine protein phosphatase 4 catalytic subunit ↓ *Bos taurus* Myotubularin-related protein 3 isoform c ↓ *Macaca mulatta* Rho-related BTB domain containing 3 ↓ *Bos taurus* Necdin ↓ *Bos taurus* Zygin 1 isoform 1 ↓
Transcription and translation	*Bos taurus* Nucleoside diphosphate kinase 6 ↑ *Bos taurus* Transcription factor E2-alpha ↑ *Bos taurus* RNA-binding protein S1 serine-rich domain ↓
Ca2+ binding and transport	*Bos taurus* Proline rich 14 ↑ *Bos taurus* Type I inositol-1,4,5-trisphosphate 5-phosphatase ↑ *Bos taurus* IQ motif containing E ↓ *Bos taurus* Necdin ↓
Lipid metabolism	*Bos taurus* Phospholipase C gamma 1 ↑ *Bos taurus* Acyl-Coenzyme A binding domain containing 5 ↓ *Bos taurus* Apolipoprotein-L3 ↓

examined pairs of animals with a change of expression at least 1.75-fold. Using this system of transcriptome analysis we found 53 reactive genes: 19 of them were up-regulated (*Table 1*), whereas 34 down-regulated (*Table 2*) with age.

An interpretation of these results is difficult because the physiological role of the most identified genes in skeletal muscle growth and development is unknown. Using available literature data we classified genes with the highest response (above 2-fold) into functional gene groups involved in: cell proliferation and differentiation, mitosis/apoptosis, signal transduction, protein turnover, function of plasma membrane, function of cytoskeleton, transcription and translation, Ca^{2+} binding and transport and lipid metabolism (*Table 3*). Performed analysis revealed that the highest number of genes was engaged in signal transduction (12 genes), function of plasma membrane (7 genes), mitosis/apoptosis (6 genes) and proliferation and differentiation (5 genes). The shift towards down-regulation of gene expression in skeletal muscle of elder bulls may reflect the tendency to compromise of muscle gains and protein synthesis rate occurring between 6^{th} and 12^{th} month of age (1).

Although the role of the most of above genes and interactions between products of their expression is not clear at the moment and was not shown in myofibers or satellite cells, the significance of their response between 6 and 12 month of age may indicate their involvement in growth, development and metabolic changes in bovine skeletal muscle during the first year of postnatal life. Further investigations are needed to confirm and explain their real role in muscle ontogenesis.

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