Gene 532 (2013) 246–252

Contents lists available at ScienceDirect

Gene

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

Effects of myogenin on muscle fiber types and key metabolic enzymes in gene transfer mice and C2C12 myoblasts

Lin-Na Zhu, Yang Ren, Jing-Qing Chen, Yi-Zhen Wang *

Institute of Feed Science, Zhejiang University, The Key Laboratory of Molecular Animal Nutrition, Ministry of Education, Zhejiang Provincial Laboratory of Feed and Animal Nutrition, Hangzhou, Zhejiang, PR China

ARTICLE INFO

Article history:
Accepted 9 September 2013
Available online 17 September 2013

Keywords:
Myogenin
Muscle fiber types
Oxidative metabolism

ABSTRACT

Skeletal muscle fiber type composition is one of the important factors influencing muscle growth and meat quality. As a member of the myogenic transcription factors, myogenin (MyoG) is required for embryonic myoblast differentiation, but the expression of MyoG continues in mature muscle tissue of adult animals, especially in oxidative metabolic muscle, which suggests that MyoG may play a more extended role. Therefore, using MyoG gene transfer mice and C2C12 myoblasts as *in vivo* and *in vitro* models, respectively, we elected to study the role of MyoG in muscle fiber types and oxidative metabolism by using overexpression and siRNA suppression strategies. The overexpression of MyoG by DNA electroporation in mouse gastrocnemius muscle had no significant effect on fiber type composition but upregulated the mRNA expression ($P < 0.01$) and enzyme activity ($P < 0.05$) of oxidative succinic dehydrogenase (SDH). In addition, downregulation of the activity of the glycolytic enzymes lactate dehydrogenase (LDH, $P < 0.05$) and pyruvate kinase (PK, $P < 0.05$) was observed in MyoG gene transfer mice. *In vitro* experiments verified the results obtained in mice. Stable MyoG-transfected differentiating C2C12 cells showed higher mRNA expression levels of myosin heavy chain (MyHC) isoform IIX ($P < 0.01$) and SDH ($P < 0.05$), while the LDH mRNA was attenuated. The enzyme activities of SDH ($P < 0.01$) and LDH ($P < 0.05$) were similarly altered at the mRNA level. When MyoG was knocked down in C2C12 cells, MyHC IIX expression ($P < 0.05$) was decreased, but the mRNA level ($P < 0.05$) and the enzyme activity ($P < 0.05$) of SDH were increased. Downregulating MyoG also increased the activity of the glycolytic enzymes PK ($P < 0.05$) and hexokinase (HK, $P < 0.05$). Based on those results, we concluded that MyoG barely changes the MyHC isoforms, except MyHC IIX, in differentiating myoblasts but probably influences the shift from glycolytic metabolism towards oxidative metabolism both *in vivo* and *in vitro*. These results contribute to further understand the role of MyoG in skeletal muscle energy metabolism and also help to explore the key genes that regulate meat quality.

1. Introduction

Adult skeletal muscle fibers represent members of a heterogeneous population that inherently differ in their energy metabolism, contractile properties, and color. Muscle fiber types can be delineated according to differences in their structural and functional properties. To date, myosin heavy chain (MyHC) isoforms seem to represent the most appropriate markers for fiber type delineation. The following pure fiber types exist in adult mammalian skeletal muscles: slow-twitch oxidative type I (MyHCI), which metabolizes lipids as a source of energy, and three fast-twitch types, namely oxidative type IIA (MyHC IIA), oxido-glycolytic type IIX (MyHC IIX) (Pette and Staron, 1990; Schiaffino and Reggiani, 1994, 1996), and glycolytic type IIB (MyHC IIB), which contain higher amounts of glycogen and glucose, and predominantly use glycogen and glucose as fuel (Choi et al., 2007). In humans, the muscle fiber type profile, in part, dictate muscle performance, which is important in sports science (Costill et al., 1976; Gollnick et al., 1972), and is relevant to neuromuscular diseases as well as metabolic diseases, such as obesity and diabetes (Bikman et al., 2010; He et al., 2001). In farm animals, muscle fiber type composition is one of the main factors influencing many of the peri- and post-mortal biochemical processes and, thereby, meat quality (Chang et al., 2003; Klont et al., 1998; Lee et al., 2010; Ryu et al., 2005).

Extensive work has been conducted on the relationship between muscle fiber type composition and meat production and quality in pigs. The results have shown that both the contractile and metabolic nature of fibers likely influence meat quality (Werner et al., 2010). The intramuscular fat (IMF) content is an important factor that influences sensory quality. Oxidative fibers have been shown to contain more triglycerides, which represents a small proportion of IMF, than glycolytic fibers (Essengustavsson et al., 1994; Karlsson et al., 1999). In addition, the presence of oxidative fibers was positively related

Abbreviations: MyoG, myogenin; MyHC, myosin heavy chain; SDH, succinic dehydrogenase; LDH, lactate dehydrogenase; PK, pyruvate kinase; HK, hexokinase.

* Corresponding author at: College of Animal Science, Zhejiang University, Hangzhou 310058, PR China. Tel.: +86 571 88982815; fax: +86 571 88982650.
E-mail address: yzwang@zju.edu.cn (Y.-Z. Wang).

0378-1119/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2013.09.028

to color characteristics, better water-holding capacity, and better tenderness (Chang et al., 2003; Eggert et al., 2002). Type IIB fibers tend to be larger in diameter than other fiber types, and the proportion of MyHC IIB contributes to the increase in muscle mass (Ryu et al., 2008). However, in pigs, higher white (glycolytic) fiber percentages have been shown to correlate with the PSE (pale, soft, exudative) meat condition (Chang et al., 2003; Fiedler et al., 1999; Larzul et al., 1997). These fibers are more reliant on glycolytic pathways to produce energy for contraction and contain less myoglobin to store oxygen and may shift to anaerobic metabolism earlier, thereby, their metabolism contributes to a very fast pH decline by the degradation of glycogen to lactic acid, which results in muscle protein denaturation and PSE meat after slaughter (Hammelman et al., 2003; Ryu et al., 2005).

The swine industry breeding system has been based on the major objectives of high growth rate, low feed conversion, and high lean meat percentage. As a result, dramatic improvements in the efficiency of meat production have been made through genetic selection, breeding conditions, and improvement of nutrition (Merks, 2000; Sellier and Rothschild, 1991). In modern meat-type pigs, breeding selection has increased the abundance of MyHC IIB transcript with an unexpected deterioration in meat quality. In farm animals, a better control of meat quality is of major importance for producers and retailers to satisfy the consumers' requirement for a consistently good product. To be advantageous in achieving both high meat content and good meat quality, the regulation of muscle fiber type has become a research topic of great interest in animal science.

In our previous study, we used a microarray analysis to compare longissimus dorsi muscle gene expression profiles between Landra-

2.6. Western blotting for MyoG

The cellular protein was extracted using the T-PER Tissue Protein Extraction Reagent (Pierce, Thermo Fisher Scientific, USA). The total protein content was quantified using the Pierce BCA Protein Assay Kit (Thermo Scientific). The protein samples (50 μg) were electrophoresed through a 10% SDS-polyacrylamide gel followed by electrotransfer to nitrocellulose membranes (Millipore, Bedford, MA, USA). After blocking in defatted milk powder, the membranes were incubated with an anti-mouse MyoG antibody (Abcam) and an anti-mouse ß-actin antibody (Sigma) followed by an incubation in the presence of a peroxidase-labeled secondary antibody (Pierce, Thermo Fisher Scientific, USA). The signals were detected as chemical luminescence exposed to X-ray films using the ECL Western Blotting Detection System (Amersham Biosciences, Piscataway, NJ, USA).

2.7. Enzyme extraction and assay

We measured the maximal activities of the glycolytic enzymes lactate dehydrogenase (LDH, EC 1.1.1.27), pyruvate kinase (PK, EC 2.7.1.40), and hexokinase (HK, EC 2.7.1.1) as well as the maximal activities of the mitochondrial enzymes malate dehydrogenase (MDH, EC1.1.1.37) and succinate dehydrogenase (SDH, EC 1.3.99.1). The enzyme activities were measured using a UV/Vis spectrophotometer (Beckman DU 640) on cells and gastrocnemius muscle. The LDH and HK activities were assessed at 340 nm at 25 °C by measuring the use or production of NADH (Alzghoul et al., 2004; Kalousti et al., 1969). The PK activity was measured using an assay coupled to LDH following NADH as described previously (

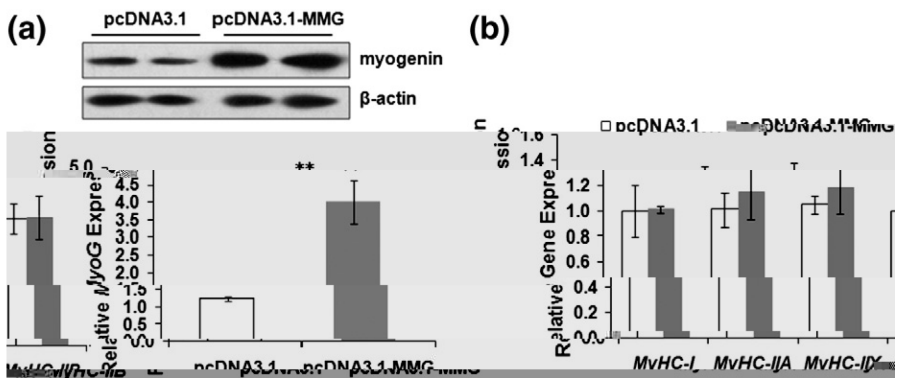


Fig. 2. (a) Quantitative RT-PCR and western blot analysis of MyoG in mouse gastrocnemius muscles at 10 days post-electroporation. (b) Relative mRNA levels of myosin heavy chain (MyHC) isoform genes in mouse gastrocnemius muscles at 10 days post-electroporation were determined using qRT-PCR. The data represent the fold change in mRNA expression relative to the control (pcDNA3.1 treatment).

of the other three MyHC isoforms (Fig. 4b). The variation trend of SDH, MDH, and LDH expression coincided with the results *in vivo*. The upregulation of SDH and the downregulation of LDH occurred in differentiating stably transfected clones at both the mRNA and enzyme activity levels (Fig. 5). In addition, glycolytic PK and HK activities revealed a downward trend.

Additionally, MyoG siRNA was transfected into C2C12 myoblasts, followed by the induction of cell differentiation, to inhibit the gene expression of MyoG. The gene and protein expression of MyoG were analyzed during the differentiation process. With the transfection of MyoG siRNA, the MyoG gene expression level significantly decreased compared with the Neg.-siRNA transfected C2C12 cells (Fig. 6a), and western blot analysis confirmed the decrease in MyoG protein amount. These results indicate that the expression of the MyoG gene and protein was successfully inhibited by the transfection of MyoG siRNA in the mouse differentiating myoblast cells.

Interestingly, when we analyzed the expression levels of the four MyHC isoform types, a decreasing trend of the four MyHC isoform types was found in the MyoG siRNA-transfected cells, but only the intermediate fiber type (MyHC IIX) reached a significant level (Fig. 6b).

The expression levels of MDH, SDH, and LDH in the MyoG siRNA-transfected cells were analyzed to investigate the effects of MyoG gene silencing on the mitochondria-related genes. MyoG silencing downregulated MDH and SDH and upregulated LDH gene expression. The SDH mRNA expression level was significantly lower in the MyoG siRNA-transfected cells compared with the negative siRNA transfected cells (Fig. 7a). MyoG siRNA transfection also inhibited the cellular SDH activity and increased PK and HK activities (Fig. 7b).

4. Discussion

MyoG is one of the myogenic regulatory factors (MRFs) that acts as key regulatory molecule during early muscle differentiation, and it has been suggested that MyoG may play a more extended role because its expression also persists in postmitotic mature muscles (Hughes et al., 1993). In this study, we applied overexpression and siRNA suppression strategies to investigate the influence of MyoG on muscle fiber type isoform expression and muscle oxidative metabolism. Using MyoG gene transfer mice as an *in vivo* biomodel, we observed that the overexpression of MyoG in glycolytic gastrocnemius muscles elevated the mRNA expression and the enzyme activity of the mitochondrial enzyme SDH as well as decreased the glycolytic enzyme activities of LDH and PK, which induced a shift from glycolytic metabolism to oxidative metabolism. Moreover, no significant change in fiber type specific MyHC isoform expression was observed in gastrocnemius muscles of MyoG gene transfer mice. SDH activity of the electron transport chain increased, which indicated the coordinated upregulation of mitochondrial proteins encoded by both nuclear and mitochondrial genes.

The present data suggest causality between MyoG and oxidative capacity in muscle, which is also supported by correlations found *in vivo* that MyoG is expressed more highly in fibers with high oxidative capacity and mitochondrial content than in glycolytic fibers (Hughes et al., 1993; Rescan et al., 1995). Changes in MyoG transcript and protein levels were similar in direction and magnitude to the changes in the metabolic enzymes following endurance training (Kadi et al., 2004; Siu et al., 2004), which coincides exactly with previous studies indicating that MyoG is involved in regulating the metabolic

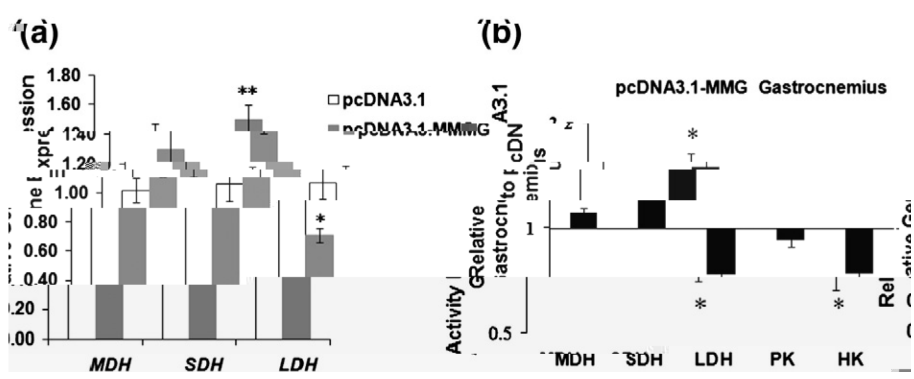


Fig. 3. (a) Quantitative RT-PCR analysis of mitochondria-related genes MDH, SDH, and LDH in mouse gastrocnemius muscles at 10 days post-electroporation. (b) Gastrocnemius muscles overexpressing MyoG show higher oxidative and lower glycolytic enzyme activity. Ten days after MyoG electroporation, mouse gastrocnemius muscles were processed for enzyme activity analysis. Values are expressed as the mean ± SEM. *$P < 0.05$; **$P < 0.01$.

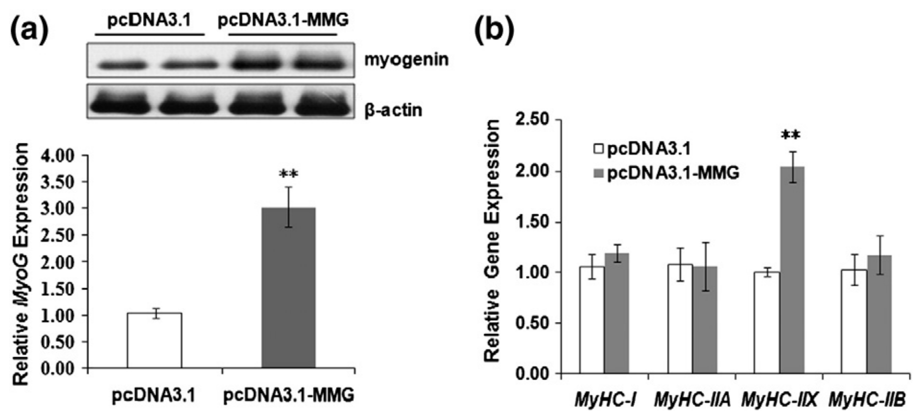


Fig. 4. (a) Quantitative RT-PCR and western blot analysis of MyoG in mouse differentiating myoblast cells stably transfected with pcDNA3.1-MyoG. Cells were cultured in differentiation medium for 3 days and subjected to qRT-PCR. The data represent the fold change in mRNA expression relative to the control. $^{**}P < 0.01$. (b) Relative mRNA levels of myosin heavy chain (MyHC) isoform genes in mouse differentiating myoblasts stably transfected with pcDNA3.1-MyoG were determined using qRT-PCR. The data represent the fold change in mRNA expression relative to the control. $^{*}P < 0.05$.

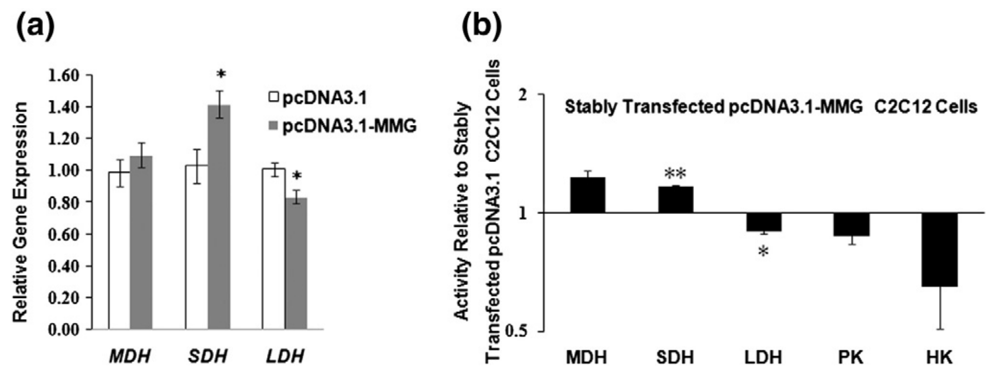


Fig. 5. (a) Quantitative RT-PCR analysis of mitochondria-related genes MDH, SDH, and LDH in mouse differentiating myoblasts stably transfected with pcDNA3.1-MyoG. Cells were cultured in differentiation medium for 3 days and subjected to qRT-PCR analysis of mitochondria-related genes MDH, SDH, and LDH. (b) C2C12 cells overexpressing MyoG showed higher oxidative and lower glycolytic enzyme activities. Cells stably transfected with pcDNA3.1-MyoG were cultured in differentiation medium for 3 days and processed for enzyme activity analysis. Values are expressed as the mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$.

processes intrinsic to muscle catabolism or anabolism (Loughna and Brownson, 1996; Marsh et al., 1997; Mozdziak et al., 1998). MyoG gene expression has been shown to be influenced by electrical activity (Alway et al., 2002; Merlie et al., 1994) and thought to be involved in a link in the pathway between electrical activity and acetylcholine receptor gene expression (Dutton et al., 1993). In addition, MyoG has been shown previously to regulate genes involved in oxidative metabolism and repress genes involved in glycolytic metabolism through histone deacetylase 4 (HDAC4) activity (Tang et al., 2009), which exhibits activity in the cytosol and mitochondria to regulate the acetylation of metabolic enzymes (Galmozzi et al., 2013). We propose that skeletal muscle MyoG regulated by neural electrical

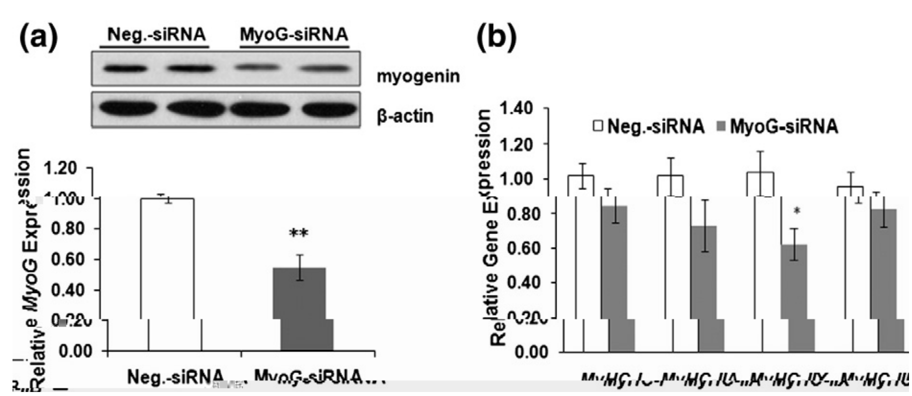
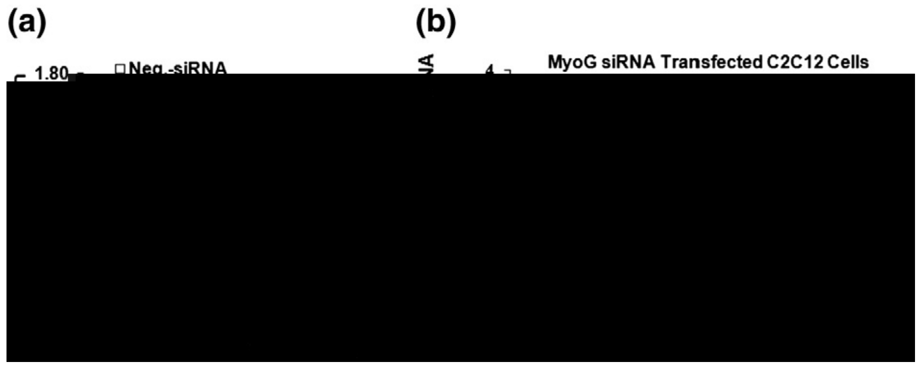


Fig. 6. (a) Quantitative RT-PCR and western blot analysis of MyoG in mouse differentiating myoblast cells transfected with MyoG-siRNA. After reaching confluency, the mouse C2C12 myoblast cells transfected with the MyoG-siRNA were cultured in differentiation medium for 3 days and subjected to qRT-PCR. The data represent the fold change in mRNA expression relative to the control (Neg.-siRNA). $^{**}P < 0.01$. (b) Relative mRNA levels of myosin heavy chain (MyHC) isoform genes in mouse differentiating myoblast cells transfected with MyoG-siRNA were determined using qRT-PCR. The data represent the fold change in mRNA expression relative to the control (Neg.-siRNA). $^{*}P < 0.05$.



[redacted]

Fig. 7. (a) Quantitative RT-PCR analysis of mitochondria-related genes MDH, SDH, and LDH in mouse differentiating myoblast cells transfected with MyoG-siRNA. The MyoG siRNA-transfected cells were cultured in differentiation medium for 3 days and subjected to qRT-PCR analysis of mitochondria-related genes MDH, SDH, and LDH. (b) C2C12 cells show lower oxidative and higher glycolytic enzyme activities after RNA interference of MyoG. The MyoG siRNA-transfected cells were cultured in differentiation medium for 3 days and processed for enzyme activity analysis. Values are expressed as the mean ± SEM. $^{*}P < 0.05$.

activity could represent a direct or indirect signal able to switch on genes responsible for altering processes that normally regulate mitochondrial biogenesis and the cell metabolic status of muscle fibers.

However, evidence indicates that MyoG acts in concert with the other MRFs in the regulation of fiber type. The manipulation of MyoG expression in transgenic mice, which is accompanied by a reciprocal downregulation of MyoD (Gundersen et al., 1995), causes an increase in oxidative metabolism in muscle fibers (Hughes et al., 1999). Moreover, transgenic mice lacking functional MyoD have shifts in fiber type in the oxidative direction (Seward et al., 2001). Our present work raised the possibility that the MyoG/MyoD ratio, rather than absolute levels, might be decisive in fiber type transformation (Hughes et al., 1993). MyoG overexpression in C2C12 myoblasts tended to upregulate the expression of four MyHC genes, while its knockdown downregulated their expression. However, the MyHC IIX isoform expression changed at a statistically significant level, which was distinct from the result of the *in vivo* experiment but in accordance with a report that low doses of MyoG promote the formation of fast-twitch fibers in cell culture (Alapat et al., 2009). A plausible explanation is that the *in vivo* DNA electroporation triggered a large number of postmitotic fibers, which differed from the *in vitro* C2C12 cell line. MyoG is best known for regulating skeletal muscle development during the embryonic and fetal stages of life (Molkentin and Olson, 1996) and mediates the terminal differentiation of embryonic myoblasts. Alterations in MyHC gene expression caused by MyoG manipulation *in vitro* were likely to be associated with the effect of MyoG on myoblast differentiation, which is consistent with a previous report that transient MyoG overexpression stimulated myoblast differentiation (Rochard et al., 2000). It is worth mentioning that high mitochondrial activity appears to be associated with the preliminary steps of myogenic differentiation, and the rise of mitochondrial activity just before the onset of terminal differentiation may characterize the irreversible engagement of myoblasts into terminal differentiation (Rochard et al., 2000).

The present data from the *in vivo* study support the conclusion drawn from studies on the effects of moderate endurance training: oxidative enzyme activity and MyHC type can be regulated independently in adult skeletal muscle (Kiens et al., 1993; Schluter and Fitts, 1994). Previous studies have suggested that some of the variations in fiber type characteristics and metabolic potentials within muscle can explain the variations in certain meat quality (Chang et al., 2003; Lee et al., 2010). Glycolytic processes in meat are the most important factors responsible for quality deterioration, including low ultimate pH and acidic meat with low water-holding capacity (Le Roy et al., 2000). The proportion of fast-twitch glycolytic fibers (MyHC IIB) was enhanced with the development of modern breeding technology including variety of breeding, which increased the growth rate and meat yield while decreasing the meat quality due to muscle glycolytic metabolism. Because oxidative enzyme activity and muscle fiber composition can be regulated independently, a balance could be acquired between the meat quality issues caused by the metabolic properties and the advantages of MyHC in meat yield.

In conclusion, our data suggest that overexpressing MyoG induces a metabolic enzyme activity shift from glycolytic to oxidative *in vitro* and *in vivo* and that MyoG suppression causes the opposite effect. In addition, MyoG does not affect muscle fiber type in mature muscle tissues but can change the MyHC IIX mRNA expression in differentiating myoblasts when the shift occurred in the slow direction. This study contributes to further understand the role of MyoG in skeletal muscle energy metabolism and also helps to explore the key genes regulating meat quality. Nonetheless, additional research is required to elucidate the cellular pathways and the molecular mechanisms that regulate metabolic changes induced by MyoG.

Conflict of interest

The authors declare that no conflicts of interest exits.

Acknowledgments

This work was supported by the Special Fund for Cultivation and Breeding of New Transgenic Organism (Grant No. 2009ZX08009-144B) and the Major State Basic Research Development Program of China (973 Program) (Grant No. 2012CB124705).

References

Alapat, D.V., Chaudhry, T., Ardakany-Taghavi, R., Kohtz, D.S., 2009. Fiber-types of sarcomeric proteins expressed in cultured myogenic cells are modulated by the dose of myogenin activity. Cell. Signal. 21, 128–135.

Alway, S.E., Degens, H., Lowe, D.A., Krishnamurthy, G., 2002. Increased myogenic repressor Id mRNA and protein levels in hindlimb muscles of aged rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 282, R411–R422.

Alzghoul, M.B., Gerrard, D., Watkins, B.A., Hannon, K., 2004. Ectopic expression of IGF-I and Shh by skeletal muscle inhibits disuse-mediated skeletal muscle atrophy and bone osteopenia in vivo. FASEB Journal 18, 221–223.

Arfman, N., Watling, E.M., Clement, W., Vanoosterwijk, R.J., Devries, G.E., Harder, W., Attwood, M.M., Dijkhuizen, L., 1989. Methanol metabolism in thermotolerant methylotrophic bacillus strains involving a novel catabolic nad-dependent methanol dehydrogenase as a key enzyme. Archives of Microbiology 152, 280–288.

Bikman, B.T., et al., 2010. Metformin improves insulin signaling in obese rats via reduced IKKβ action in a fiber-type specific manner. J. Obes. 2010.

Chang, K.C., et al., 2003. Relationships of myosin heavy chain fibre types to meat quality traits in traditional and modern pigs. Meat Sci. 64, 93–103.

Choi, Y.M., Ryu, Y.C., Kim, B.C., 2007. Influence of myosin heavy- and light chain isoforms on early postmortem glycolytic rate and pork quality. Meat Sci. 76, 281–288.

Costill, D.L., Daniels, J., Evans, W., Fink, W., Krahenbuhl, G., Saltin, B., 1976. Skeletal-muscle enzymes and fiber composition in male and female track athletes. J. Appl. Physiol. 40, 149–154.

Dutton, E.K., Simon, A.M., Burden, S.J., 1993. Electrical activity-dependent regulation of the acetylcholine-receptor delta-subunit gene, MyoD, and myogenin in primary myotubes. Proc. Natl. Acad. Sci. U. S. A. 90, 2040–2044.

Eggert, J.M., Depreux, F.F.S., Schinckel, A.P., Grant, A.L., Gerrard, D.E., 2002. Myosin heavy chain isoforms account for variation in pork quality. Meat Sci. 61, 117–126.

Essengustavsson, B., Karlsson, A., Lundstrom, K., Enfalt, A.C., 1994. Intramuscular fat and muscle-fiber lipid contents in halothane-gene-free pigs fed high or low-protein diets and its relations to meat quality. Meat Sci. 38, 269–277.

Fiedler, I., Ender, K., Wicke, M., Maak, S., von Lengerken, G., Meyer, W., 1999. Structural and functional characteristics of muscle fibres in pigs with different malignant hyperthermia susceptibility (MHS) and different meat quality. Meat Sci. 53, 9–15.

Galmozzi, A., et al., 2013. Inhibition of class I histone deacetylases unveils a mitochondrial signature and enhances oxidative metabolism in skeletal muscle and adipose tissue. Diabetes 62, 732–742.

Gollnick, P.D., Saltin, B., Saubert, C.W., Armstron, Rb, Piehl, K., 1972. Enzyme-activity and fiber composition in skeletal-muscle of untrained and trained men. J. Appl. Physiol. 33, 312–319.

Gundersen, K., Rabben, I., Klocke, B.J., Merlie, J.P., 1995. Overexpression of myogenin in muscles of transgenic mice — interaction with id-1, negative crossregulation of myogenic factors, and induction of extrasynaptic acetylcholine-receptor expression. Mol. Cell. Biol. 15, 7127–7134.

Guo, J., et al., 2011. Comparisons of different muscle metabolic enzymes and muscle fiber types in Jinhua and Landrace pigs. J. Anim. Sci. 89, 185–191.

Hammelman, J.E., Bowker, B.C., Grant, A.L., Forrest, J.C., Schinckel, A.P., Gerrard, D.E., 2003. Early postmortem electrical stimulation simulates PSE pork development. Meat Sci. 63, 69–77.

Hasty, P., et al., 1993. Muscle deficiency and neonatal death in mice with a targeted mutation in the myogenin gene. Nature 364, 501–506.

He, J., Watkins, S., Kelley, D.E., 2001. Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity. Diabetes 50, 817–823.

Heiden, M.G.V., et al., 2010. Evidence for an alternative glycolytic pathway in rapidly proliferating cells. Science 329, 1492–1499.

Ho, S.H., et al., 2004. Protection against collagen-induced arthritis by electrotransfer of an expression plasmid for the interleukin-4. Biochem. Biophys. Res. Commun. 321, 759–766.

Hughes, S.M., Taylor, J.M., Tapscott, S.J., Gurley, C.M., Carter, W.J., Peterson, C.A., 1993. Selective accumulation of MyoD and myogenin messenger-RNAs in fast and slow adult skeletal-muscle is controlled by innervation and hormones. Development 118, 1137–1147.

Hughes, S.M., Chi, M.M.Y., Lowry, O.H., Gundersen, K., 1999. Myogenin induces a shift of enzyme activity from glycolytic to oxidative metabolism in muscles of transgenic mice. J. Cell Biol. 145, 633–642.

Kadi, F., Johansson, F., Johansson, R., Sjostrom, M., Henriksson, J., 2004. Effects of one bout of endurance exercise on the expression of myogenin in human quadriceps muscle. Histochem. Cell Biol. 121, 329–334.

Kalousti, H.D., Stolzenb, F.E., Everse, J., Kaplan, N.O., 1969. Lactate dehydrogenase of lobster (*homarus americanus*) tail muscle. I. Physical and chemical properties. Journal of Biological Chemistry 244, 2891–2901.

Karlsson, A.H., Klont, R.E., Fernandez, X., 1999. Skeletal muscle fibres as factors for pork quality. Livest. Prod. Sci. 60, 255–269.

Kiens, B., Essengustavsson, B., Christensen, N.J., Saltin, B., 1993. Skeletal-muscle substrate utilization during submaximal exercise in man — effect of endurance training. J. Physiol. (Lond.) 469, 459–478.

Klont, R.E., Brocks, L., Eikelenboom, G., 1998. Muscle fibre type and meat quality. Meat Sci. 49, S219–S229.

Larzul, C., et al., 1997. Phenotypic and genetic parameters for longissimus muscle fiber characteristics in relation to growth, carcass, and meat quality traits in large white pigs. J. Anim. Sci. 75, 3126–3137.

Le Roy, P., et al., 2000. Comparison between the three porcine RN genotypes for growth, carcass composition and meat quality traits. Genet. Sel. Evol. 32, 165–186.

Lee, S.H., Joo, S.T., Ryu, Y.C., 2010. Skeletal muscle fiber type and myofibrillar proteins in relation to meat quality. Meat Sci. 86, 166–170.

Loughna, P.T., Brownson, C., 1996. Two myogenic regulatory factor transcripts exhibit muscle-specific responses to disuse and passive stretch in adult rats. FEBS Lett. 390, 304–306.

Marsh, D.R., Criswell, D.S., Carson, J.A., Booth, F.W., 1997. Myogenic regulatory factors during regeneration of skeletal muscle in young, adult, and old rats. J. Appl. Physiol. 83, 1270–1275.

Merks, J.W.M., 2000. One century of genetic changes in pigs and the future needs. In: Hill, W.G., Bishop, S.C., McGuirk, B., McKay, J.C., Simm, G., Webb, A.J. (Eds.), The challenge of genetic change in animal production. Br. Soc. Anim. Sci. Occ. Publ., pp. 8–19 (Occasional publication no. 27, Edinburgh).

Merlie, J.P., Mudd, J., Cheng, T.C., Olson, E.N., 1994. Myogenin and acetylcholine-receptor alpha-gene promoters mediate transcriptional regulation in response to motor innervation. J. Biol. Chem. 269, 2461–2467.

Miao, Z.G., Wang, L.J., Xu, Z.R., Huang, J.F., Wang, Y.R., 2009. Developmental changes of carcass composition, meat quality and organs in the Jinhua pig and Landrace. Animal 3, 468–473.

Molkentin, J.D., Olson, E.N., 1996. Defining the regulatory networks for muscle development. Curr. Opin. Genet. Dev. 6, 445–453.

Mozdziak, P.E., Greaser, M.L., Schultz, E., 1998. Myogenin, MyoD, and myosin expression after pharmacologically and surgically induced hypertrophy. J. Appl. Physiol. 84, 1359–1364.

Pette, D., Staron, R., 1990. Cellular and molecular diversities of mammalian skeletal muscle fibers. Reviews of Physiology, Biochemistry and Pharmacology, vol. 116. Springer, Berlin Heidelberg 1–76.

Rescan, P.Y., Gauvry, L., Paboeuf, G., 1995. A gene with homology to myogenin is expressed in developing myotomal musculature of the rainbow-trout and in-vitro during the conversion of myosatellite cells to myotubes. FEBS Lett. 362, 89–92.

Rochard, P., et al., 2000. Mitochondrial activity is involved in the regulation of myoblast differentiation through myogenin expression and activity of myogenic factors. J. Biol. Chem. 275, 2733–2744.

Ryu, Y.C., Choi, Y.M., Kim, B.C., 2005. Variations in metabolite contents and protein denaturation of the longissimus dorsi muscle in various porcine quality classifications and metabolic rates. Meat Sci. 71, 522–529.

Ryu, Y.C., et al., 2008. Comparing the histochemical characteristics and meat quality traits of different pig breeds. Meat Sci. 80, 363–369.

Schiaffino, S., Reggiani, C., 1994. Myosin isoforms in mammalian skeletal-muscle. J. Appl. Physiol. 77, 493–501.

Schiaffino, S., Reggiani, C., 1996. Molecular diversity of myofibrillar proteins: gene regulation and functional significance. Physiol. Rev. 76, 371–423.

Schluter, J.M., Fitts, R.H., 1994. Shortening velocity and atpase activity of rat skeletal-muscle fibers — effects of endurance exercise training. Am. J. Physiol. 266, C1699–C1713.

Sellier, P., Rothschild, M.F., 1991. Breed identification and development in pigs. In: Maijala, K. (Ed.), Genetic Resources of Pig, Sheep and Goat. World Animal Science. Elsevier Science Publishers, Amsterdam, The Netherlands, pp. 125–143.

Seward, D.J., Haney, J.C., Rudnicki, M.A., Swoap, S.J., 2001. bHLH transcription factor MyoD
uaffK. (Etell0(i)30.4651(g)0(Am)24.8(a7.5(S)7.2(30.181(ng)33.5(e)i.5(e)0(l)4719.1(P)0.4(s)19.1(i