Genetics and Molecular Research

Effects of muscle fiber type on glycolytic potential and meat quality traits in different Tibetan pig muscles and their association with glycolysis-related gene expression

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Genet. Mol. Res. 14 (4): 14366-14378 (2015)
Received May 18, 2015
Accepted August 19, 2015
Published November 13, 2015
DOI http://dx.doi.org/10.4238/2015.November.13.22

ABSTRACT. The myosin heavy chain (MyHC) composition, glycolytic potential, mitochondrial content, and gene expression related to energy metabolism were analyzed in eight muscles from Tibetan pigs, to study how meat quality develops in different muscle tissues. The muscles were classified into three clusters, based on MyHC composition: *masseter*, *trapezius*, and *latissimus dorsi* as 'slow-oxidative-type'; psoas major and semimembranosus as 'intermediate-type'; and longissimus dorsi, obliquus externus abdominis, and semitendinosus as 'fast-glycolytic-type'. The 'slow-oxidative-type' muscles had the highest MyHC I and MyHC IIA content ($P < 0.01$); 'intermediate-type' muscles, the highest MyHC IIx content ($P < 0.01$); and 'fast-glycolytic-type' muscles, the highest MyHC IIb content ($P < 0.01$). The pH values measured in 'slow-oxidative-type' muscles were higher than those in the other clusters were; however, the color of 'fast-glycolytic-type' muscles was palest ($P < 0.01$). Mitochondrial content increased in

the order: fast-glycolytic-type < intermediate-type < slow-oxidative-type. In the 'slow-oxidative-type' muscles, the expression levels of genes related to ATP synthesis were higher, but were lower for those related to glycogen synthesis and glycolysis. Mitochondrial content was significantly positively correlated with MyHC I content, but negatively correlated with MyHC IIb content. MyHC I and mitochondrial content were both negatively correlated with glycolytic potential. Overall, muscles used frequently in exercise had a higher proportion of type I fibers. 'Slow-oxidative-type' muscles, rich in type I fibers with higher mitochondrial and lower glycogen and glucose contents, had a higher ATP synthesis efficiency and lower glycolytic capacity, which contributed to their superior meat quality.

Key words: Glycolytic potential; Mitochondria; Muscle fiber type; Meat quality; Tibetan pigs

INTRODUCTION

Pig muscle is converted into pork through the metabolic process of glycolysis, which starts immediately after slaughter. The rate and extent of pH decline are associated with ultimate meat quality (Hamilton et al., 2003; Ryu and Kim, 2005; Scheffler and Gerrard, 2007). Glycolytic potential (GP) is a measure of all compounds present in the muscle that can be converted into lactate, and is a reliable and rapid predictor of pork quality (Miller et al., 2000; Fontanesi et al., 2008). In the live animal, GP is closely related to the composition of the myosin heavy chain (MyHC) (Klont et al., 1998). For example, Type IIb fibers have a higher glycogen and glucose content than Type I fibers (Choe et al., 2008), but are less rich in mitochondria than Type I and IIa fibers (Staron et al., 1984). Therefore, Type IIb fibers have a lower oxidative capacity and a higher rate of ATP consumption than Type I fibers (Men et al., 2012). A high GP means that muscles produce a high amount of lactate, which leads to a rapid decrease in pH. Moreover, the denaturation of actin and myosin in the muscle, caused by the decrease in pH, will alter the muscle protein matrix leading to changes in meat color, toughness, and water-holding capacity (Bendall and Swatland, 1988; Lonergan et al., 2007).

In studies on muscle fiber types according to breed, gender, age, and muscle function, Men et al. (2012) found that the proportions of MyHC fiber types I and IIa from selected breeds increased in the order DYL (Duroc x Yorkshire x Landrace crosses) < DZP (Duroc x Zhongbai crosses) < ZBP (Zhongbai pig) < JHP (Jinhua pig), whereas for MyHC IIb, the order was reversed. Regarding gender, Gutmann et al. (1970) found that the temporalis was a "fast-white" muscle in male guinea pigs, but a "fast-red" muscle in females. Novák et al. (2010) found that the soleus muscle of young rats contained significantly higher MyHC IIa and lower MyHC I levels than that of older rats. Differences in the biological function of muscles, such as workload, also influence the composition of fibers (Staron et al., 1984). Lefaucheur et al. (2004) found that the higher frequency movement of the rhomboideus led to significantly higher MyHC I and lower MyHC IIb contents than that of the longissimus dorsi, a muscle involving lower levels of exercise. However, there are few reports on meat quality as it varies in different muscles. With improving living standards in many developed countries, the price of pork depends on the cut and the muscles involved. In the present study, eight different skeletal muscles from Tibetan pigs were used to study the effect of muscle fiber type on GP and pork meat quality by analyzing the composition of the muscle fiber, the GP, the mitochondrial content, and their association with the expression of genes related to glycogen synthesis, ATP synthesis, and glycolysis.

MATERIAL AND METHODS

The experimental protocol was approved by the Animal Care and Ethics Committee of Sichuan Agricultural University, Sichuan, China, under permit No. DKY-S20093030.

Animals and treatments

Eight Tibetan pigs (60 ± 3.0 kg, 270 days old) were evaluated. All animals (and carcasses) were subjected to similar treatment and handling along with the whole experiment stage, both feeding and sampling. Just prior to slaughter the pigs were kept off feed, but given free access to water for 24 h, following which they were electrically stunned, exsanguinated, scalded, and rinsed. Samples were obtained from the core of eight muscle tissues: obliquus externus abdominis (OEA); longissimus dorsi (LOD); psoas major (PSM); semimembranosus (SMB); semitendinosus (SMD); masseter (MAS); latissimus dorsi (LAD); and trapezius (TRA). Samples were then rapidly frozen in liquid nitrogen for gene expression and biochemical analysis. At 45 min postmortem, samples were taken from the eight muscles for meat quality measurements. After 24 h of chilling, additional samples were taken to evaluate and compare meat quality traits.

Meat quality measurements

Values of muscle pH were measured with a pH meter (Orion Research Inc., Boston, MA, USA) at 45 min and 24 h postmortem. Color parameters were determined using a Minolta CR-300 colorimeter (Minolta Camera, Osaka, Japan) with an illuminant D65, a 10° standard observer, and a 2.5 cm port area. The meat color change values (ΔE*) were calculated as ΔE*=, where 'Δ' means the difference between colors measured at 45 min and 24 h postmortem. The pH change values (ΔP*) were calculated as ΔP*=, where pH_1 and pH_u represent the pH values measured at 45 min and 24 h postmortem, respectively.

ELISA analysis glycogen, glucose, glucose-6-phosphate, and lactate content

About 500 mg *longissimus dorsi* sample was weighed and homogenized in 500 mL 0.9% saline and then centrifuged at 4200 *g* for 10 min at 4°C. The supernatant was diluted 50 times to measure Gly (Glycogen), Glu (Glucose), G-6-P (Glucose-6-phosphate), and LA (Lactate) content by using standard commercial kits from BlueGene Biotech Co., Ltd. (Shanghai, China), following manufacturer recommendations. Optical density (OD) at 450 nm was immediately measured utilizing an ELISA microplate reader. A calibration curve was constructed using OD values corresponding to successive concentrations of the standard. Compound contents were expressed as mmol/g wet weight and GP values were calculated by the formula: GP = 2 x (Gly + Glu + G-6-P) + LA (Monin and Sellier, 1985).

Real-time polymerase chain reaction (PCR) analysis

The expression levels of selected genes were determined using quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis. Total RNA was extracted from the *longissimus dorsi* using the TRIZOL reagent (Invitrogen Corp, Carlsbad, CA) according to manufacturer instructions. Reverse transcription was performed using oligo (dT) random 6-mers

primers provided in the PrimeScript RT Master Mix kit (TaKaRa, Dalian, China), following manufacturer recommendations. The SYBR Premix Ex Taq kit (TaKaRa, Dalian, China) was used to perform q-PCR on a CFX96 Real-Time PCR detection system (Bio-Rad, Richmond, CA). Primer sequences for q-PCR are shown in Table 1.

Table 1. Primers used for PCR.

Gene symbol	Primer sequences (5' to 3')	Amplicon (bp)	Tm (°C)
*ACTB**	TCTGGCACCACACCTTCT TGATCTGGGTCATCTTCTCAC	114	60.0
*TBP**	GATGGACGTTCGGTTTAGG AGCAGCACAGTACGAGCAA	124	60.0
*TOP2B**	AACTGGATGATGCTAATGATGCT TGGAAAAACTCCGTATCTGTCTC	137	60.0
GYG	CTCTTGTGGCTTCTGTAGAAAGG AAAGGAATCTGCTCCCATGTAAT	157	53.0
GYS1	ATGACTCCTCGGACCCTATCTT TACCCATAACCGTGCATTCAGC	222	59.0
PRKAG3	CTTGGGCTGGTGGAAGAGAA CCCACGAAGCTCTGCTTCTT	269	60.6
PFK	GTTGAACGACCTCCAGAAAGC GGCGGACAACTCAGGAATAAAA	300	63.0
ACL	GAGGCAGCATCGAAACTTCA TCCCAACTTCTCCCATCACCC	165	63.0
PKM	TTAGCGGCAGCTTTGATAGTTC CACAATGACCACATCTCCCTTC	264	63.0
ATP5A1	TAGTAGATGCCCTTGGTAACGC CCAGTCTGTCGGTCGCCAATAA	202	63.0
ATP5B	GAATCCCTTCTGCGGTGGGTTAT GGCAGGAGCAGGGTCAGTCAAGT	149	60.0
COX1	ACTACTGACAGACCGCAACC TCCAATGGACATTATGGCTC	147	56.0
GCG	GAATCAACACCATCGGTCAAAT CTCCACCCATAGAATGCCCAGT	197	60.0
HK1	GGGTGAGGCTGGTCCGACTTAT CAGGCGGGTCAGGATTTCTTT	100	59.0
HK2	CAAGAGGAGGATGAAGTGGAAA CACTGGACGATGTGGTCAAAGAG	282	63.0
HK4	GGCTTCACCTTCTCCTTTCCC TCTCCGTTTGATGGCATCTCG	138	61.0
MyHC1	AGCCTCTTTCTTCTCCCAGGGAC ATCCAGGCTGCGTAACGCTCTT	384	55.6
MyHC2a	CACTTGCTAAGAGGGACCTCTGA ATCCAGGCTGCGTAACGCTCTTT	375	60.8
MyHC2b	CATCTGGTAACATAAGAGGTACA ATCCAGGCTGCGTAACGCTCTTT	429	59.6
MyHC2x	CTTTCCTCATAAAGCTTCAAGTTC ATCCAGGCTGCGTAACGCTCTT	398	57.5

*Housekeeping gene; Tm, Annealing temperature.

All measurements contained a negative control (without a cDNA template), and each RNA sample was analyzed in triplicate. Fifteen target genes: *GYG* (glycogenin); *GYS1* (glycogen synthase 1); *PRKAG3* (protein kinase, AMP-activated, gamma 3 non-catalytic subunit); *PFK* (Phosphofructokinase); *ATP5A1* (ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1); *ATP5B* (ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide); *ACL* (ATP-citrate synthase); *PKM* (pyruvate kinase); *HK1* (hexokinase 1); *HK2* (hexokinase 2); *HK4* (hexokinase 4); *MyHC I* (myosin heavy chain I); *MyHC IIa* (myosin heavy chain IIa); *MyHC IIx* (myosin heavy chain IIx); and *MyHC IIb* (myosin heavy chain IIb) were normalized to three

internal control genes (*ACTB*, *TBP*, and *TOP2B*) (Erkens et al., 2006). Relative expression levels of the target mRNAs were calculated using the ΔΔCt method (Livak and Schmittgen, 2001). The concentrations of MyHC mRNA were calculated by a standard-curve equation and the ratio of each MyHC mRNA was expressed as 100%X. The relative copy numbers of mitochondria were designed to be detected by cytochrome oxidase 1 (*COX1*) and glucagon gene (*GCG*) for mitochondrial DNA (mtDNA) and nucleic DNA, respectively. The ratio of COX1 to GCG within each sample was used to calculate mtDNA content.

Statistical analysis

Statistical analysis was performed using ANOVA in the SAS System (SAS 9.2, SAS Inst. Inc., Cary, NC). The model included tissues as the main effects. Duncan's multiple range test was applied to compare the mean values of the tissue samples. Mean values and standard errors are reported in successive Figures. Differences were considered significant if $P \leq 0.05$. Correlation among different indices was evaluated with Pearson bivariate analysis and a two-tailed test of significance.

RESULTS AND DISCUSSION

Composition of MyHC in eight muscle tissues from Tibetan pigs

The composition of MyHC in Tibetan pig muscle tissues is shown in Figure 1A-D.

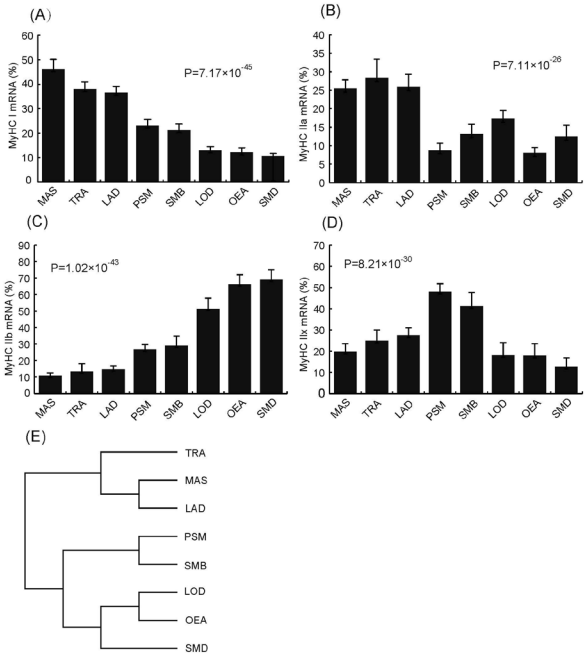

Figure 1. Composition of MyHC mRNA in eight Tibetan pig muscle tissues. **A.** MyHC I mRNA. **B.** MyHC IIa mRNA. **C.** MyHC IIb mRNA. **D.** MyHC IIx mRNA. **E.** Eight Tibetan pig muscle clustering based on MyHC composition. Data are reported as means ± SE. MAS: masseter; TRA: trapezius; LAD: latissimus dorsi; PSM: psoas major; SMB: semimembranosus; LOD: longissimus dorsi; OEA: obliquus externus abdominis; and SMD: semitendinosus.

The masseter, trapezius, and latissimus dorsi muscles had higher MyHC I and MyHC IIA mRNA concentrations than other muscles ($P < 0.01$) (Figure 1A and 1B). The longissimus dorsi, obliquus externus abdominis, and semitendinosus muscles had higher MyHC IIb concentrations than other muscles ($P < 0.01$) (Figure 1C). The absolute amounts of MyHC IIx were highest in psoas major and semimembranosus ($P < 0.01$) (Figure 1D). These results are consistent with previous reports. Chang et al. (2003) compared muscle fiber composition in four pig breeds, and found that the longissimus dorsi had higher MyHC IIb and lower MyHC IIa, IIx, and I contents than the psoas major. Sazili et al. (2005) also found that the MyHC I content in sheep meat increased in the order: longissimus dorsi < tensor fasciae latae < semitendinosus < supraspinatus < trapezius, but the order for MyHC II fibers was reversed.

Different muscle fiber types have different functions in energy metabolism and contraction: MyHC I is a slow contraction, oxidative type, whereas MyHC IIb is a fast contraction, glycolytic type (Schiaffino and Reggiani, 1996). Different muscle tissues are composed of different MyHC subtypes depending on the extent to which they are exercised and their energy requirements. The eight muscle tissues are classified based on MyHC composition in Figure 1E. The muscles clustered as the 'slow-oxidative-type' included the masseter, trapezius, and latissimus dorsi; the 'intermediate-type' included the psoas major and semimembranosus cluster; and the 'fast-glycolytic-type' included the longissimus dorsi, obliquus externus abdominis, and semitendinosus cluster. The results are consistent with the function of the muscle tissues; for example, the masseter is the major muscle responsible for chewing food and has the highest amount of movement. The 'slow-oxidative-type' muscle fiber is therefore necessary to provide sufficient energy and persistent contraction without fatigue. In contrast, the 'fast-glycolytic-type' is consistent with the major function of the longissimus dorsi in maintaining body shape, with comparatively less movement. Differences in energy metabolism and muscle contraction could contribute to GP and meat quality. Choe et al. (2008) found that MyHC I content was significantly negatively correlated with GP and positively correlated with ultimate pH values, but MyHC IIb showed reverse effects.

Glycolytic potential and meat quality in eight muscles from Tibetan pigs

As shown in Figure 2, the content of glycogen, G-6-P, glucose, and lactic acid were highest in 'slow-oxidative-type' muscle tissues (Figure 2A, B, C, and D).

There were significant differences in GP between the eight muscle tissues ($P < 0.01$). The average GP in the 'slow-oxidative-type' muscle tissues was lower than that in the 'intermediate-type' tissues ($P < 0.05$), and the average GP in 'intermediate-type' tissues was lower than that in the 'fast-glycolytic-type' tissues ($P < 0.05$) (Figure 2E). These results are consistent with those of Monin and Sellier (1985), who showed that the longissimus dorsi and semimembranosus muscles had higher GP than the rectus abdominis muscle in Penshire pigs. Choi et al. (2007) found that MyHC IIb fibers had higher glucose and glycogen contents, and therefore higher GP, than MyHC I fibers.

The measurements of meat quality from the eight muscle tissues are shown in Figure 2F. The pH_1 (45 min postmortem) and pH_u (24 h postmortem) values in muscles decreased in the order: 'slow-oxidative-type' > 'intermediate-type' > 'fast glycolytic-type.' However, the meat lightness (L^*) at 45 min and 24 h postmortem in 'fast-glycolytic-type' muscles was higher than that in the 'intermediate-type' and 'slow-oxidative-type' ($P < 0.05$). Similar results were reported by Melody et al. (2004), who found that the semimembranosus and psoas major muscles had higher values of pH_u and water-holding capacity than the longissimus dorsi. Owing to the dissociation of lactate in pork, pH values decrease with time. With muscle pH decreasing, sarcoplasmic proteins

could degenerate and change meat color from red to white. Moreover, the meat protein matrix would be altered, influencing muscle texture and reducing its water holding capacity. Therefore, pH values are a valuable index to measure complex quantitative traits in meat quality. Muscle fiber characteristics have direct effects on glycolytic potential and meat quality. Ryu and Kim (2005) found that increasing the proportion of MyHC IIb was related to increasing the postmortem metabolic rate, which caused subsequent deterioration of meat quality.

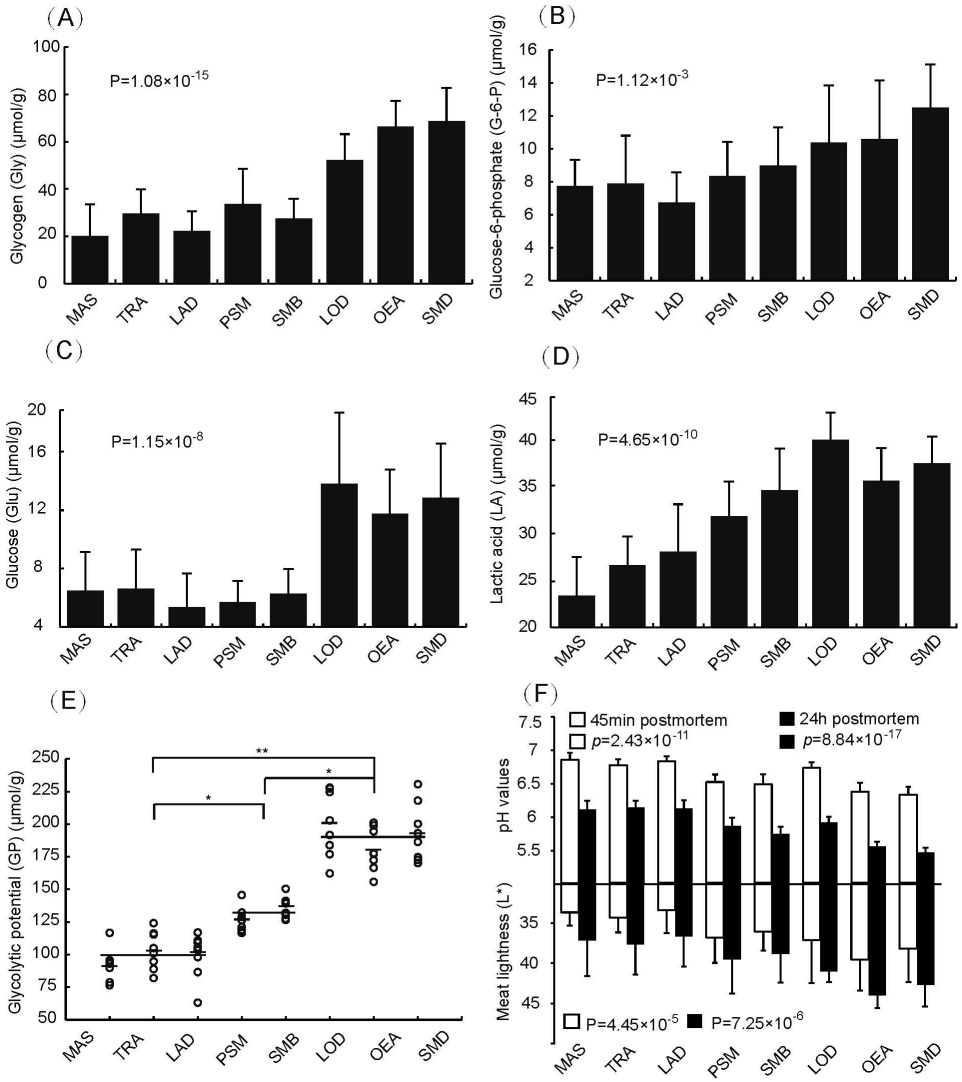

Figure 2. Glycolytic potential and meat quality in eight muscles of the Tibetan pig. A-D: The content of Glycogen (**A**); G-6-P (**B**); Glucose (**C**); and Lactate (**D**) in the eight muslces of Tibetan pig. (**E**): Glycolytic potential in eight muscles and average GP of each muscle cluster; short-line represents average of each muscle, long-line represents average of clustering muscles, *P < 0.05, **P < 0.01. (**F**): Meat quality of eight Tibetan pig muscles. White columns represent meat quality measured at 45 min postmortem, black columns represent meat quality measured at 24 h postmortem. Upper areas of the chart reflect pH values and lower areas reflect meat lightness (L*).

Mitochondrial content in eight muscles from Tibetan pigs

Mitochondria constitute the cellular energy factory that plays an important role in postmortem energy metabolism. The mitochondrial DNA copy numbers from each muscle type are shown in Figure 3.

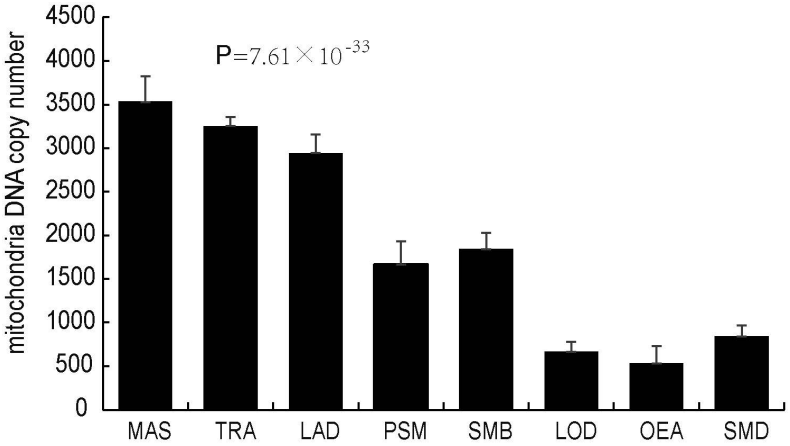

Figure 3. Mitochondrial DNA copy number in each muscle type. Values represent double of the ratio of the amount of mtCOX1 to GCG. Data are reported as means ± SD.

From our results, it seems that the mitochondrial DNA copy number in the different muscle types increased in the order: 'fast-glycolytic-type' < 'intermediate-type' < 'slow-oxidative-type'. These results are consistent with a previous study (Schwerzmann et al., 1989) that showed the mitochondrial volume density in muscle fibers was higher in the 'oxidative' soleus muscle than in the 'glycolytic' gracilis muscle.

The mitochondrial DNA copy number has different distributions according to muscle fiber type (Ingjer, 1979). Arany et al. (2007) found that MyHC I and IIa fibers were more oxidative and rich in mitochondria than IIb fibers. Mitochondrial DNA copy number has been found to be closely related to muscle energy metabolic status (Zong et al., 2002), which depends on the level of exercise of the muscle (Irrcher et al., 2003). Some interesting findings about the influence of exercise on mitochondrial content (Irrcher et al., 2003; Yan et al., 2011), showed that endurance exercise could induce MyHC IIb change to MyHC I, and thereby increase mitochondrial content. Therefore, in the present study the masseter muscle, having the highest mitochondrial content, was also associated with the highest exercise frequency in chewing food daily. Moreover, pyruvic acid can be broken down into lactate through glycolysis, or be transferred into mitochondria to produce CO_2 and water through oxidation. Therefore, to some extent, the density of mitochondria in muscle fibers determines energy metabolic type.

Correlations between muscle fiber type, mitochondrial content, GP, and meat quality traits

The correlations between muscle fiber type and mitochondrial content, and GP and meat quality traits are shown in Table 2.

Table 2. Coefficients of correlation among MyHC mRNA composition, mitochondrial content, GP, and meat quality traits.

Traits	MyHC1	MyHC2a	MyHC2b	MyHC2x	Glycogen	Glucose	LA	GP
MtDNA	0.31**	0.08	-0.27**	0.12*	-0.25*	-0.22*	-0.18*	-0.26**
Glycogen	-0.39**	-0.21*	0.43***	-0.15	1	0.33**	0.14*	0.38**
Glucose	-0.26**	-0.13*	0.34**	-0.08	0.36**	1	0.17*	0.27**
LA	-0.23*	-0.07	0.20**	-0.04	0.25**	0.26*	1	0.18**
GP	-0.47***	-0.16*	0.33**	-0.11*	0.40**	0.38**	0.12*	1
pH_1	0.12*	0.08	-0.23**	-0.10	-0.23**	-0.13*	-0.08	-0.24**
pH_u	0.29**	0.15*	-0.19**	0.08	-0.29**	-0.27**	-0.13*	-0.32**
ΔP	-0.06	0.07	0.16*	-0.04	0.18*	0.16*	0.06	0.17*
L^*_1	-0.19**	-0.05	0.23*	-0.13	0.25*	0.21**	0.12	0.26*
L^*_U	-0.21**	-0.16*	0.18**	-0.05	0.18**	0.18*	0.03	0.22*
ΔE	-0.05	0.03	0.14*	-0.16	0.11*	0.10	-0.08	0.12*

MtDNA, Mitochondrial DNA copy number; LA, Lactate content; GP, glycolytic potential; pH_1, measured at 45 min postmortem; pH_u, measured at 24 h postmortem; ΔP, pH value change at 45 min and 24 h postmortem; L^*_1, meat lightness measured at 45 min postmortem; L^*_u, meat lightness measured at 24 h postmortem; ΔE, meat lightness change at 45 min and 24 h postmortem. *P < 0.05, **P < 0.01, ***P < 0.001.

The MyHC I content had a strong negative correlation with GP (r = -0.47, P < 0.001) and glycogen (r = -0.39, P < 0.01). The MyHC IIb content had a strong positive correlation with glycogen (r = 0.43, P < 0.001), glucose (r = 0.34, P < 0.01), GP (r = 0.33, P < 0.01), and L^*_1 (r = 0.23, P < 0.05). However, the MyHC IIa and MyHC IIx contents were not strongly correlated with GP nor with meat quality. Moreover, mitochondrial content was positively correlated with MyHC I and MyHC IIx contents (r = 0.31, P < 0.01; r = 0.12, P < 0.05, respectively) and negatively correlated with MyHC IIb content (r = -0.27, P < 0.01). Interestingly, the mitochondrial content was also significantly correlated with glycogen, glucose, lactate, and GP (P < 0.01). Similar relationships reported by other studies (Chang et al., 2003; Hamilton et al., 2003; Hambrecht et al., 2005; Lefaucheur, 2006) have indicated that as the MyHC IIb content increased within muscle, the glycogen content and GP also increased, but meat quality decreased. These researchers also demonstrated that the variation in meat quality was caused by postmortem energy metabolism related to mitochondria that generate the ATP supply for every biological function. However, there is an apparent lack of reports about the relationship between mitochondrial content and GP, or meat quality. From our results, it can be inferred that the variation in meat quality from different muscle tissues, might be caused by differences in the proportions of muscle fiber types and in the mitochondrial content. For example, the comparatively high mitochondrial content in Type I fibers could suggest greater availability of pyruvic acid to generate ATP through the tricarboxylic acid cycle in mitochondria. Therefore, Type I fibers would have had less pyruvic acid to produce lactate through anaerobic glycolysis in cytoplasm.

Metabolism-related gene expression in eight muscle tissues from Tibetan pigs

As shown in Figure 4 A-K, the expression of metabolic-related genes showed differences between the eight muscle tissues.

The mRNA levels of the two genes encoding glycogenin (*GYG*) and glycogen synthase (*GYS1*) were significantly higher in ‘fast-glycolytic-type’ muscles than in ‘intermediate-type’ and ‘slow-oxidative-type’ muscles (P < 0.01) (Figure 4A and B). The mRNA levels of the *ACLY*, *ATP5B*, and *ATP5A1* genes for ATP synthesis were significantly higher in ‘slow-oxidative-type’ muscles than in ‘intermediate-type’ and ‘fast glycolytic-type’ muscles (P < 0.01) (Figure 4C, D, and E).

The mRNA levels of the four genes *HK1*, *HK2*, *HK4*, and *PFK* encoding rate-limiting enzymes in glycolysis were significantly lower in 'slow-oxidative-type' muscles than in 'intermediate-type' and 'fast-glycolytic-type' muscles (P < 0.01) (Figure 4F, G, H, and I). The mRNA levels of the *PRKAG3* gene, which responds to cellular metabolic stresses, was higher in 'fast-glycolytic-type' than in 'slow-oxidative-type' muscles (P < 0.01) (Figure 4J). However, the mRNA levels for gene-encoding pyruvate kinase showed no significant differences among the eight muscle tissues (P > 0.05) (Figure 4K). The expression patterns of the 11 genes (Figure 4L) showed that those for *ACLY*, *ATP5B*, and *ATP5A1* differed from the others.

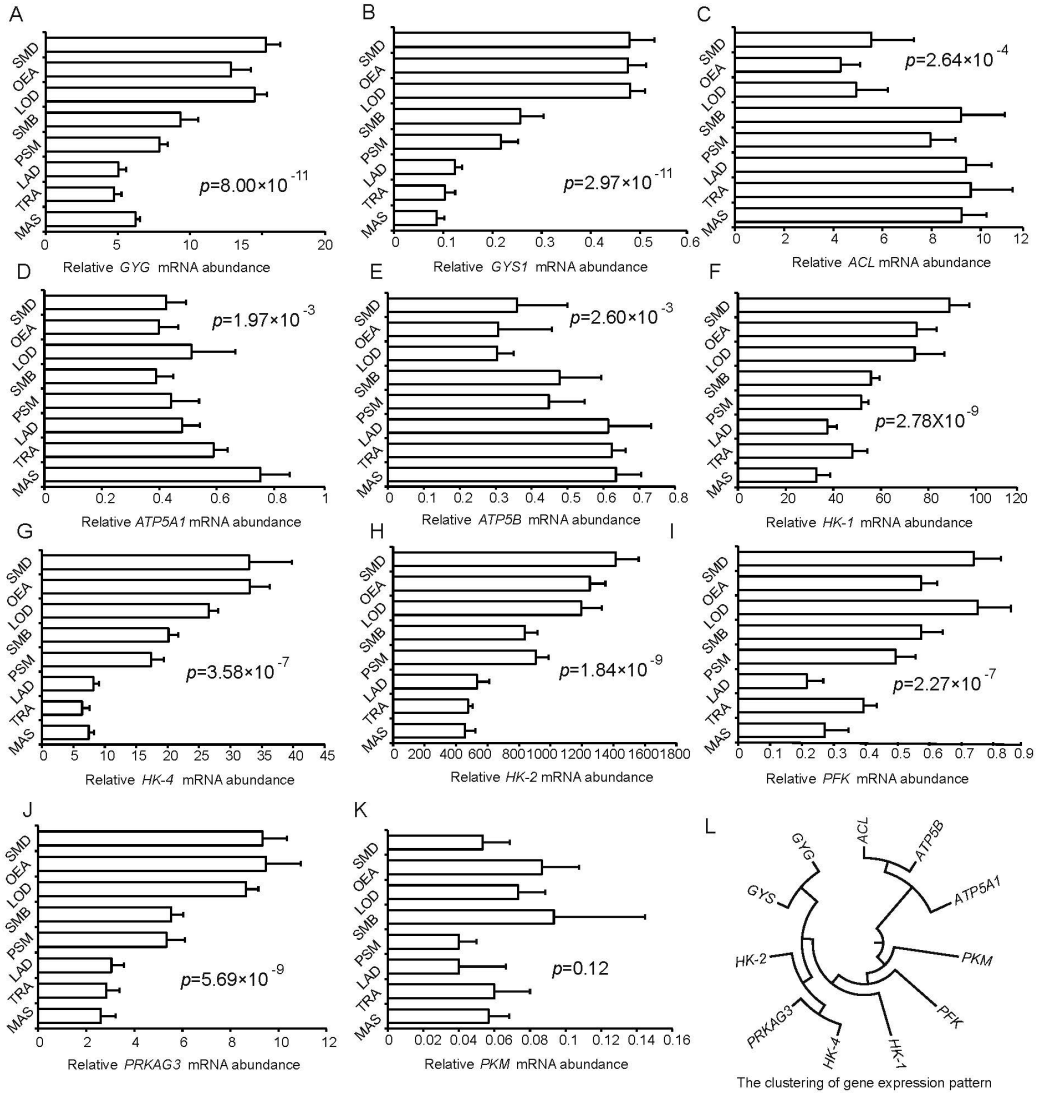

Figure 4. Metabolism-related gene expression in eight muscle tissues of Tibetan pigs. A-K: The genes GYG, GYS1, ACL, ATP5A1, ATP5B, HK-1, HK-2, HK-4, PFK, PRKAG3, and PKM expression in eight muscles. Relative quantification used ACTB, TBP, and TOP2B, three internal control genes to normalize. L: Clustering of gene expression patterns.

According to these expression patterns, the high levels of *GYG*, *GYS1*, *HK1*, *HK2*, *HK4*, *and PFK* mRNA in 'fast-glycolytic-type' muscles suggested that they had a higher glycogen content and rate of glycolysis. The glycogen synthase complex was composed of GYG (glycogenin) and GYS1 (glycogen synthase) proteins, which must both be present for the glycogen synthesis reaction. Sukigara et al. (2012) and McCue et al. (2008) found that mutation of the *GYS1* and *GYG* genes could cause serious diseases in humans, based on defects in muscle glycogen storage. Hexokinase (HK) is an important rate-limiting enzyme that catalyzes the reaction for the degradation of glycogen through glycolysis, and thus influences pork quality. Different types of HK isozymes exist in different tissues: HK-1 is the predominant isozyme in brain, HK-2 is mainly found in muscle tissues, and HK-4 is mainly found in hepatocytes and pancreatic islets. All three isozymes are adapted to facilitate glucose phosphorylation in different types of cell (Steinke et al., 2006). The results of the present study show that *HK-2* was the predominantly expressed mRNA in muscle tissues. This might be due to the high-altitude, hypoxic living conditions of the Tibetan pigs used. Gwak et al. (2005) found that hypoxia could stimulate the proliferation of human hepatoma cells through the induction of hexokinase II expression. PFK catalyzes the conversion of fructose 1-phosphate and ATP to fructose 1,6-bisphosphate and ADP, and according to our results, there was a correlation between *PFK* mRNA expression and GP. Pyruvate kinase (PKM) catalyzes the irreversible conversion of phosphoenolpyruvate and ADP to pyruvate and ATP. However, the related mRNA expression levels showed no significant differences among the eight muscle tissues studied, which supports the gene expression patterns in Figure 4L. Interestingly, Allison et al. (2003) found that pyruvate kinase capacity was not correlated with longissimus pH values and drip loss. Therefore, the expression level of PFK cannot readily explain the differences in glycolysis rates. Our findings revealed that the biceps femoris and longissimus dorsi muscles had higher glycogen and lactate contents, and GP but inferior meat quality compared to other muscle tissues. According to the expression of *ACLY*, *ATP5B*, and *ATP5A1* genes, we can conclude that the efficiency of producing ATP in 'slow-oxidative-type' muscles was higher than that in 'fast-glycolytic-type' type muscles. Although there are different metabolic types in muscle, concerning oxidative and glycolytic metabolism, the process of glycogen or glucose conversion to pyruvic acid was the same for both oxidative and anaerobic metabolism. However, pyruvic acid can be broken down into lactate through glycolysis, or can be transferred into mitochondria to produce CO_2 and water through oxidation. The anaerobic metabolism of glucose is inefficient and produces only two ATP molecules for each glucose molecule, whereas complete oxidative metabolism produces 38 ATP molecules (Gatenby and Gillies, 2004). Therefore, 'fast-glycolytic-type' muscles with a lower mitochondrial content need more glucose and glycogen molecules as raw materials for glycolysis, to satisfy the demand for ATP to allow tissues to maintain normal functions. Thus, the mitochondrial content has a major influence on the variation of meat quality in different muscle tissues.

CONCLUSION

Our results suggest that 'slow-oxidative-type' muscles, with generally higher levels of activity, have higher MyHC I and lower MyHC IIb contents than 'intermediate-type' and 'fast-glycolytic-type' muscles with lower levels of activity. The MyHC I content of muscles was positively correlated, and the MyHC IIb content was negatively correlated with mitochondrial content. Muscles with higher mitochondrial content (e.g. the masseter) were highly efficient in ATP synthesis and thus needed less glycogen and glucose for glycolysis, to provide energy. The relatively high levels of mRNA expression for genes related to glycogen synthesis (*GYG* and *GYS*), and genes related

to glycolysis (*HK1*, *HK2*, *HK4*, and *PFK*) contributed to the high glycogen content, glycolytic rate, and GP in 'fast-glycolytic-type' muscles. Our results provide more information exploring the effect of mitochondrial content in muscle fibers on pork quality.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The study was supported by the Initiation Foundation Project of the Chinese Education Ministry, the earmarked fund for China Agriculture Research System (#CARS-36-05B), and the Sichuan Sci & Tech Support Program (#2012NZ001 and 2011NZ0034).

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