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Molecular and metabolic adaption of glucose metabolism in the red and white muscle of the omnivorous GIFT tilapia *Oreochromis niloticus* to a glucose load

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ABSTRACT

In this experiment, Genetically improved farmed Nile tilapia *Oreochromis niloticus* were intraperitoneally injected with 1 g glucose/kg of body weight or saline. Red and white muscle tissues were collected at 0, 1, 2, 4, 6 and 12 h after the glucose tolerance test (GTT) or saline injection, and the time course of changes in molecular and metabolic adaption of glucose metabolism of these two tissues were evaluated. The results showed that the expression of insulin-responsive glucose transporter 4 (*glut4*) was up-regulated at 4 h after the GTT in the red muscle, implying an increase of glucose uptake. However, the expression of *glut4* in the white muscle did not change with glucose load. The glycolysis of red muscle in tilapia was stimulated during 2–4 h after the GTT, as the expression of hexokinase 1b (*hk1b*), *hk2*, phosphofructokinase muscle type a (*pfkma*) and *pfkmb* and the activity of HK and PFK increased. By contrast, only the expression of *hk1b* was up-regulated at 6 h after the GTT in the white muscle. The mRNA level of glycogen synthase 1 (*gys1*) and glycogen content increased at 2 and 6 h, respectively after the GTT in the red muscle, suggesting that glucose storage was provoked. However, glycogen content in the white muscle was not impacted by GTT. Lipogenesis was stimulated in the red muscle as reflected by up-regulated expression of acetyl-CoA carboxylase α (*accα*) (during 2–4 h) and *accβ* (during 4–12 h) with GTT. In the white muscle, however, the expression of *accα* was not changed, and mRNA level of *accβ* was not up-regulated until 6 h after the GTT. Taken together, it was concluded that the glycolytic and glycogen synthesis mechanisms in the red muscle were highly regulated by an acute glucose load while those in the white muscle were less responsive to this stimulus.

1. Introduction

Glucose is used as an energy source in most organisms, from bacteria to humans including fish (Wood and Trayhurn, 2003). Fish are thought to have a lower ability to utilize dietary carbohydrate, especially when compared with mammals (Hemre et al., 2002). Given the longer duration of persistent hyperglycemia after a glucose load, fish are generally deemed as glucose intolerant (Moon, 2001; Polakof et al., 2012). In addition, glucose tolerance of fish is closely related to feeding habits, with poor glycoregulatory capacity for the carnivorous fish as compared with omnivorous or herbivorous fish (Hemre et al., 2002; Kamalam et al., 2017). Glucose tolerance test (GTT) is a flexible way to determine the glycoregulatory capacity in fish, which is indicative of their dietary carbohydrate utilization efficiency (Conde-Sieira et al., 2015). Similar to the results observed in mammals, glucose load is generally effective to trigger insulin secretion in fish (Lin et al., 1995; Blasco et al., 1996; Enes et al., 2012; Chen et al., 2018; Liu et al., 2018). Thus, it is postulated that peripheral resistance to insulin action might be the reason for the poor glucose tolerance in fish (Alexander et al., 2006; Polakof et al., 2012). In mammals, the main insulin-sensitive peripheral tissues (liver, skeletal muscle and adipose tissue) contribute greatly to clear the glucose load. In fish, however, the relative contribution of these tissues remains unclear (Polakof et al., 2012).

The molecular and metabolic adaption of liver to a glucose load is extensively studied in fish. Despite the differences in fish species, and the dose and route of glucose administration, GTT generally provokes glucose storage as glycogen and glycolysis while inhibits gluconeogenesis in the liver (Choi and Weber, 2015; Deck et al., 2017; Chen

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et al., 2018; Jin et al., 2018). As for white muscle, enhanced glucose storage and utilization are associated with GTT in several fish species (Blasco et al., 1996; Garcia-riera and Hemre, 1996; Deck et al., 2017; Li et al., 2018; Liu et al., 2018), but opposite results were also observed (Peres et al., 1999; Liu et al., 2017; Jin et al., 2018). Despite a low glucose uptake rate, muscle tissue is considered as the main site of glucose uptake, as it comprises almost half of the body weight of most fish (Blasco et al., 1996; Moon, 2001). Fish have two distinct types of muscle fibers, the red (also called slow) and white (also called fast) muscle (Greek-Walker and Pull, 1975; Johnston and Moon, 1980). As outlined above, contradictory results are reported regarding the glucose utilization of white muscle to GTT in fish, and it is still unclear that if red and white muscle play different roles in the glucose homeostasis of fish. Thus, it is necessary to systematically investigate how red and white muscle adapt molecularly and metabolically to a glucose load in fish.

Tilapia are the second most farmed fish group worldwide due to fast growth rate, tolerance of stocking density and high marketability (Ng and Romano, 2013). In 2016, Nile tilapia *Oreochromis niloticus* accounts for about 71.2% of the total yield of world tilapia production (FAO, 2017). In China, the Genetically Improved Farmed Nile Tilapia (GIFT) strain is the most popular in tilapia aquaculture. The genome sequence of Nile tilapia is available (Brawand et al., 2014), which makes it a good candidate to investigate regulatory mechanisms of nutrients for omnivorous fish. Our previous results suggest that liver plays an important role in clearing the glucose load in GIFT tilapia (Chen et al., 2018). However, the roles of red and white muscle in the glucose homeostasis are remaining unclear in tilapia. Thus, we further investigated the molecular and metabolic adaption of glucose metabolism in the red and white muscle of tilapia to GTT in the present study. The results of this study would help to ascertain if red and white muscle play different roles in the glucose homeostasis of fish.

2. Materials and methods

2.1. Experimental fish and acclimatization

Male juvenile GIFT tilapia (about 47.7 g/fish) were purchased from Xiema Hatchery (Chongqing, China), and were kept in a closed indoor recirculating system in the Key Laboratory of Freshwater Fish Reproduction and Development in Southwest University. The culture system was comprised of two rectangular glass tanks (700 L) with continuous aeration. Before formal experiment, fish were acclimatized in our facility for about one year, during which they were fed a commercial extruded diet to apparent satiation manually once daily at 9:00. The nutrient composition of the diet on the dry matter basis was: moisture, 6.86%; crude protein, 33.2%; crude lipid, 7.37%; ash, 9.49%; carbohydrate, 49.94%.

2.2. Glucose tolerance test

Tilapia (average body weight: 562 ± 63 g/fish) were food deprived for 24 h after the last feeding, and were subjected to an acute glucose tolerance test (GTT). The procedures of GTT were performed according to our previous study (Chen et al., 2018). Briefly, 30 fish (six replicates per treatment) were randomly selected, anaesthetized, individually weighed, injected intraperitoneally with 1 g glucose/kg body weight (BW) diluted in 0.85% sterile saline, and transferred to separate rectangular tanks. Another 30 fish (six replicates per treatment) were injected with equivalent volumes of saline solution only as negative control. Six fish were sacrificed at the end of the 24 h fasting period. Red muscle along the lateral line and white muscle of fish were collected before (time 0) and at 1, 2, 4, 6 and 12 h after glucose or saline injection, respectively. After taken, muscle tissues were immediately frozen in liquid nitrogen and transferred to −80 °C until used for enzymatic and molecular analysis. Water quality parameters during the experimental period were detailed in our previous study (Chen et al., 2018). All the experiments were conducted under the standard code of protocol for the Care and Use of Laboratory Animals in China. This research was approved by the Animal Ethics Committee of Southwest University.

2.3. Glycogen and enzymatic activity assay

The glycogen level and activities of hexokinase (HK) and phosphofructokinase (PFK) in the muscle tissues were measured spectrophotometrically using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China). Muscle tissues was hydrolyzed for glycogen extraction in 3 volumes of ice cold buffer supplied by the kit, and glycogen level was expressed as mg/g wet tissue. Muscle tissue was homogenized with a tissue homogenizer in 9 volumes of ice-cold PBS for HK activity assay or specific buffer supplied by the PFK assay kit. The homogenates were centrifuged at 2500 and 8000 *g* (4 °C for 10 min) for HK and PFK assay respectively, and the supernatants were used immediately for enzymatic analysis. The activity of HK is coupled with the reaction of glucose-6-phosphate dehydrogenase. One unit (U) of HK activity is defined as the generation of 1 mM NADPH per min at 37 °C, while one U of PFK activity is represented as the generation of 1 nM fructose-1, 6-diphosphate and 1 nM ADP per min at 25 °C. The protein concentration of the supernatant solution was determined by the biuret method, using bovine serum albumin as the standard.

2.4. Identification of target genes

The isolation and identification of target genes involved in glycolipid metabolism of tilapia were performed according to our previous study (Feng et al., 2019). Available transcriptome data of tilapia skeletal muscle (Brawand et al., 2014) were referenced to select relatively high expressed key gene paralogs involved with glucose transport and utilization. Thus, glucose transporters (*glut1a* and *glut4*, also called as *slc2a1a* and *slc2a4*, respectively), glycolytic hexokinase (*hk1a*, *hk1b* and *hk2*) and phosphofructokinase (*pfkma* and *pfkmb*: muscle type a and b of *pfk*, respectively), glycogenic glycogen synthase (*gys1*) and lipogenic acetyl-CoA carboxylase *α* (*acca and accβ*) were chosen as the target genes in this study.

2.5. RNA extraction, cDNA synthesis and real-time PCR analysis

The procedures of RNA extraction, RNA quality assessment and cDNA synthesis followed our previous study (Chen et al., 2018). The primer sets used for real-time PCR analysis were designed using Primer Premier 6 (Premier Biosoft Int., USA) with at least one primer in each set flanking the intron-exon boundary (Table 1). The real-time PCR and linear standard curves building were accomplished with the ABI-7500 fast real-time PCR system (Applied Biosystems, USA) as detailed by our previous study (Chen et al., 2018). The relative abundance of mRNA transcripts was evaluated using the formula: $R = 2^{-\Delta\Delta Ct}$ as described by Livak and Schmittgen (2001). The geometric mean of the copy numbers of β-actin was used to normalize the gene expression data. The amplification efficiency of target genes in the present study varied between 97% and 103%.

2.6. Statistical analysis

Data at each sampling point are shown as mean ± standard error (SE) of six individua fish (n = 6). All the data were first tested for the normality of distribution with one-sample Kolmogorov-Smirnov test and homogeneity of variances with the Levene test. When necessary, data were root-transformed to meet the standard of normal distribution. Differences with time in the glucose or saline injected fish were analyzed by one-way ANOVA. Differences between glucose- and saline-injected fish at each sampling point were also analyzed by one-way

Table 1
Primers used in the real-time PCR analysis.

Genes	Forward primer (5′–3′)	Reverse primer (3′–5′)	Product size (bp)	Accession no.
glut1a	GTTGGAACTGCGGTGATTGGCT	ATAGCAACAGCGATGGACCACAC	167	ENSONIT00000018608
glut4	GCTGTCCGTTGCCATCTTCTCC	TGTCAAGCCAGATGCCAATCCA	223	ENSONIT00000023890
hk1a	TGCCACTGCTACACTGAAGATGC	TCCTCGGGCGTGTCGTAGATTT	183	ENSONIT00000021491
hk1b	TTCTCTTTCCCGTGTGCCCAAAC	GTGACGCAGTTCCTCCATGTAGC	273	ENSONIT00000000240
hk2	CAGCACGGAACTCCATGATGACC	GCACAAATGTGGGCAGCATCTTG	176	ENSONIT00000019964
pfkma	CCGTGAGAGCCACAGTCAGAGT	TGCCGTCTCCACCGATTACACA	264	ENSONIT00000014964
pfkmb	GGTCGCATCTTCGCCAACTCTC	AGCCTTAGCCACCACTGTGTCTT	143	ENSONIT00000024076
gys1	GAGGCATCTACACCGTCATCCA	GCTCGCTCTTCCAGGAGTCTAA	276	ENSONIT00000024419
acca	GGAGTTCGGACAGCACCTATGAA	AGCAGGAGAAGCAACAGTGAAGT	155	ENSONIT00000002411
accβ	TGCCGACATTCCCACACTACCA	CCTCGTCTACATCGTGAACACATCC	137	ENSONIT00000017001
β-actin	CAGTGCCCATCTACGAG	CCATCTCCTGCTCGAAGTC	198	ENSONIT00000010701

glut, glucose transporter; *hk*, hexokinase; *pfkm*, phosphofructokinase (muscle type); *gys*, glycogen synthase; *acc*, acetyl-CoA carboxylase.

ANOVA. Significant differences among groups were determined by the Tukey's multiple range test, and the minimal significant level was set at 0.05. All the statistical analyzes were done with SPSS 17 for Windows (SPSS Inc, Chicago, USA).

3. Results

3.1. The expression of glucose transporters

The expression of glucose transporters in the red and white muscle of tilapia after glucose or saline injection are shown in Fig. 1. Compared with 0 h, the mRNA levels of *glut1a* in the red muscle were significantly down-regulated at 2 and 6 h after glucose and saline injection, respectively ($P < 0.05$, Fig. 1A). The expression of *glut4* in the red muscle markedly increased and peaked at 4 h after the glucose load ($P < 0.05$), but was not impacted by saline injection ($P > 0.05$, Fig. 1B). The transcript level of *glut4* in the red muscle of the glucose injected fish were significantly higher than the saline injected group since the injection time was over 2 h ($P < 0.05$). In the white muscle, the mRNA levels of *glut1a* were significantly down-regulated at 12 h after glucose or saline injection ($P < 0.05$, Fig. 1C). Neither glucose load nor saline injection changed the expression of *glut4* in the white muscle of tilapia ($P > 0.05$, Fig. 1D).

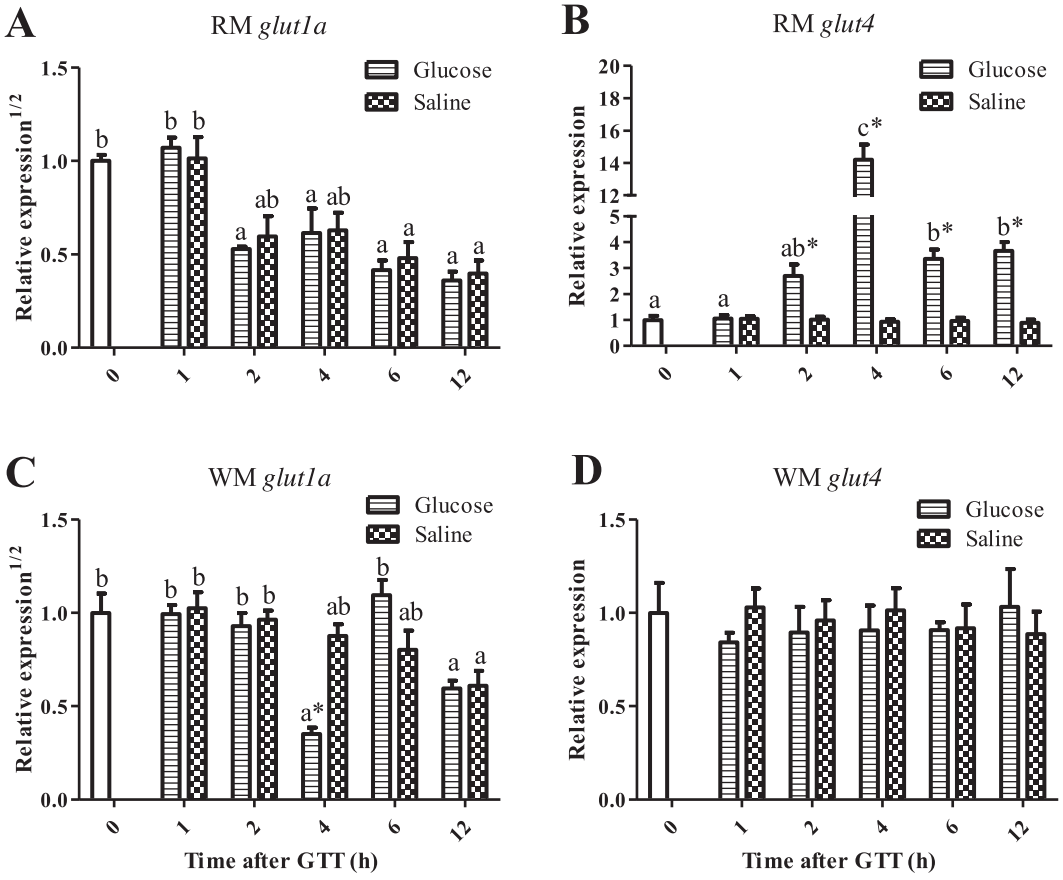

Fig. 1. Relative expression of glucose transporters in the red and white muscle of tilapia after glucose or saline injection Data are expressed as means ± SE (n = 6). Different letters indicate significant differences among the glucose or saline injected group with sampling time. * means significant difference between the glucose and saline injected fish at each sampling point. Fraction number as superscript on the vertical coordinate represents root transformation of the data. RM: red muscle; WM: white muscle; *glut*, glucose transporter.

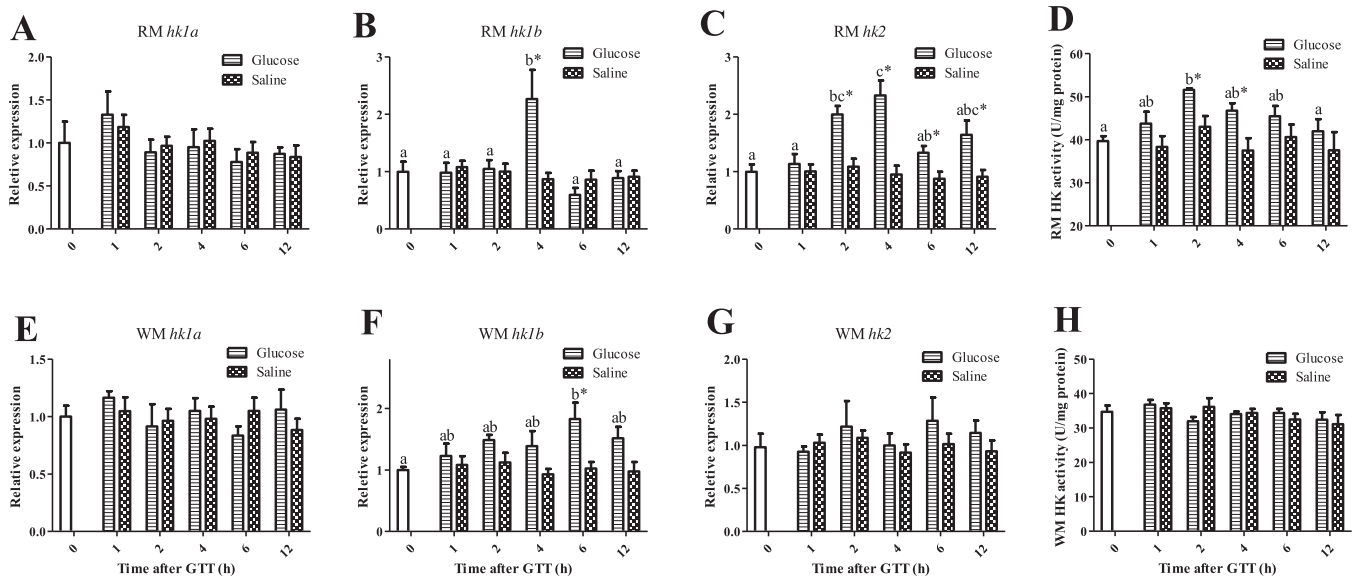

Fig. 2. Relative expression of hexokinase and its activity in the red and white muscle of tilapia after glucose or saline injection Data are expressed as means ± SE (n = 6). Different letters indicate significant differences among the glucose or saline injected group with sampling time. * means significant difference between the glucose and saline injected fish at each sampling point. RM: red muscle; WM: white muscle; *hk*, hexokinase.

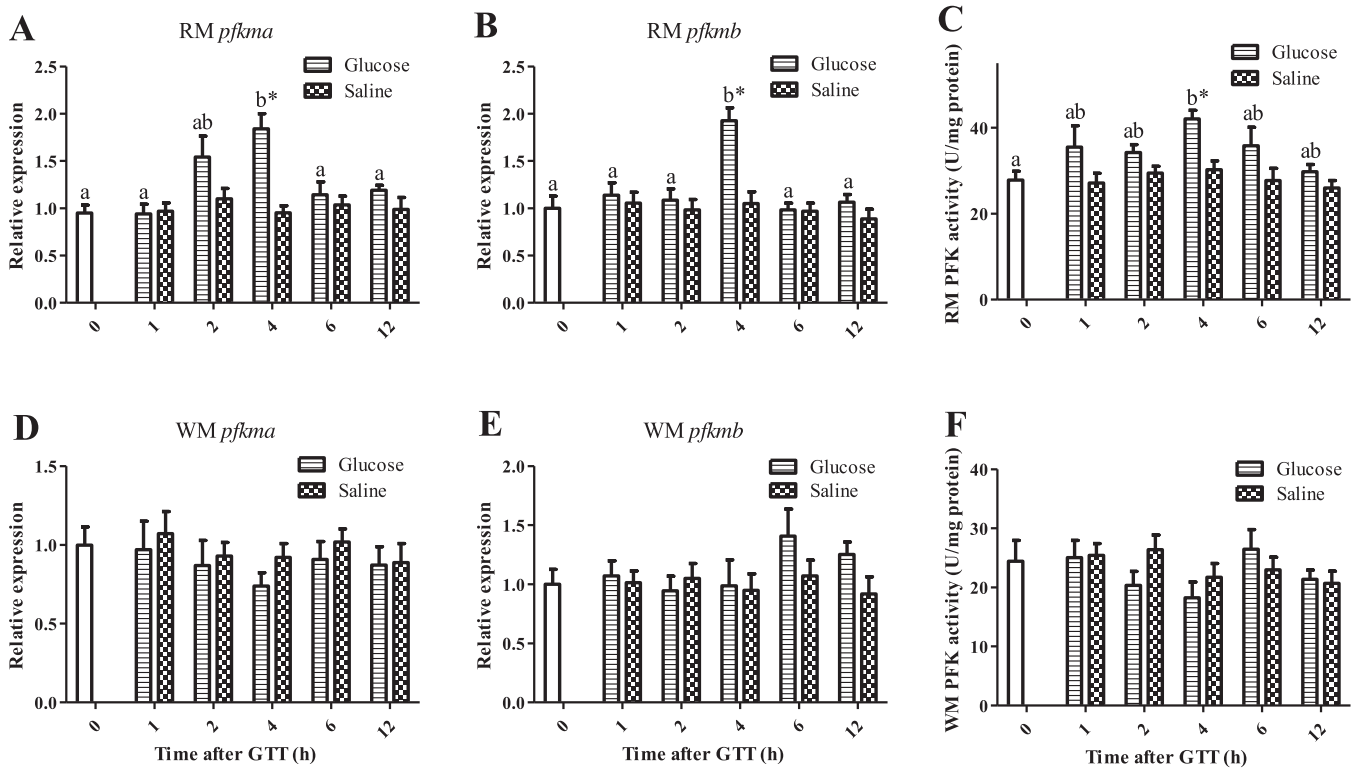

Fig. 3. Relative expression of phosphofructokinase and its activity in the red and white muscle of tilapia after glucose or saline injection Data are expressed as means ± SE (n = 6). Different letters indicate significant differences among the glucose or saline injected group with sampling time. * means significant difference between the glucose and saline injected fish at each sampling point. RM: red muscle; WM: white muscle; *pfkm*, phosphofructokinase muscle type.

3.2. The expression of *hk* paralogs and HK activity

The expression of *hk* paralogs and HK activity in the red and white muscle of tilapia are presented in Fig. 2. The mRNA level of *hk1a* in the red muscle was not affected by glucose load or saline injection ($P > 0.05$, Fig. 2A). Compared with 0 h, the expression of *hk1b* in the red muscle was up-regulated by 2.27-fold at 4 h ($P < 0.05$, Fig. 2B), while the transcript level of *hk2* increased by 1.99- and 2.33-fold at 2 and 4 h after the GTT respectively ($P < 0.05$, Fig. 2C). HK activity of the red muscle was significantly improved by 30.0% at 2 h, and then was recovered after 4 h of the glucose load ($P > 0.05$, Fig. 2D). Neither the expression of *hk1b* and *hk2*, nor HK activity in the red muscle were impacted by saline injection ($P > 0.05$). The transcript level of *hk2* in the red muscle of the glucose injected fish were significantly higher than the saline injected group since the injection time was over 2 h, while HK activity of the glucose load group was markedly higher than

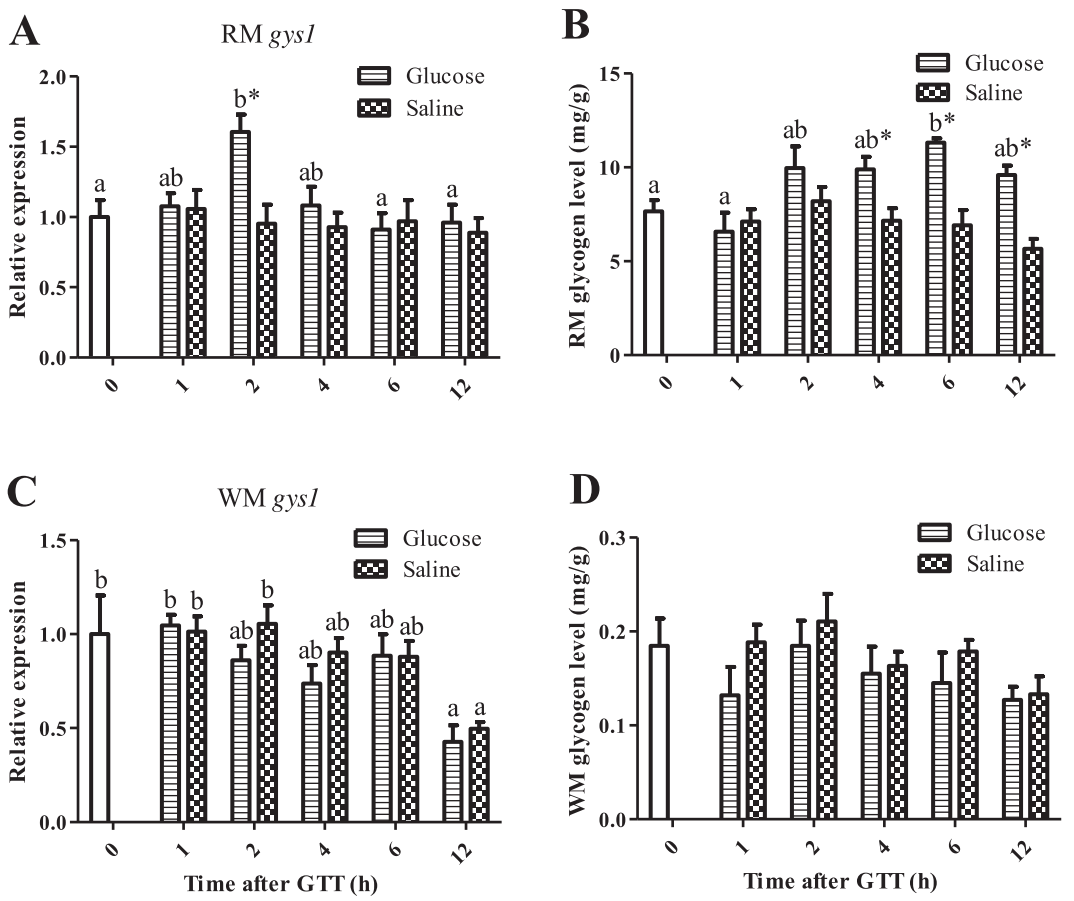

Fig. 4. Relative expression of glycogen synthase 1 and glycogen level in the red and white muscle of tilapia after glucose or saline injection Data are expressed as means ± SE (n = 6). Different letters indicate significant differences among the glucose or saline injected group with sampling time. * means significant difference between the glucose and saline injected fish at each sampling point. RM: red muscle; WM: white muscle; *gys*, glycogen synthase.

the saline injected fish during 2–4 h ($P < 0.05$). In the white muscle, only the mRNA level of *hk1b* was affected, and it was markedly up-regulated at 6 h after the GTT ($P < 0.05$, Fig. 2F). Neither the expression of *hk1a* (Fig. 2E) and *hk2* (Fig. 2G), nor HK activity (Fig. 2H) in the white muscle were impacted by glucose injection ($P > 0.05$). Saline injection did not changed the expression of *hk* paralogs or HK activity in the white muscle of tilapia ($P > 0.05$).

3.3. The expression of pfk paralogs and PFK activity

The expression of *pfk* paralogs and PFK activity in the red and white muscle of tilapia after glucose or saline injection are shown in Fig. 3. Compared with 0 h, the expression of *pfkma* (Fig. 3A) and *pfkmb* (Fig. 3B) were significantly up-regulated by 1.68- and 1.93-fold, respectively in the red muscle of tilapia at 4 h after the GTT ($P < 0.05$). Concomitantly, the activity of PFK in the red muscle markedly increased by 51.1% ($P < 0.05$, Fig. 3C). The expression of *pfk* paralogs and PFK activity were not impacted by saline injection in the red muscle ($P > 0.05$). Neither glucose load nor saline injection changed the expression of *pfkma* (Fig. 3D) and *pfkmb* (Fig. 3E), and PFK activity (Fig. 3F) in the white muscle of tilapia ($P > 0.05$).

3.4. The expression of gys1 and glycogen level

The mRNA level of *gys1* and glycogen content in the red and white muscle of tilapia are presented in Fig. 4. In the red muscle, the expression of *gys1* was up-regulated by 1.61-fold at 2 h ($P < 0.05$, Fig. 4A), while glycogen accumulation was significantly increased by 47.7% at 6 h after the GTT ($P < 0.05$, Fig. 4B). However, both *gys1* expression and glycogen content of the red muscle were not impacted by saline injection ($P > 0.05$). The expression of *gys1* in the glucose injected group was markedly higher than that of the saline injected group at 2 h, while glycogen content in the red muscle of tilapia with glucose load was significantly higher than the saline injected fish during 6–12 h ($P < 0.05$). The average glycogen level of red muscle (9.16 mg/g) was about 58.2 times higher than that of white muscle (0.15 mg/g). In the white muscle, the expression of *gys1* was markedly down-regulated at 12 h after glucose or saline injection ($P < 0.05$, Fig. 4C) as compared with 0 h, but the glycogen level was not affected by glucose load or saline injection ($P > 0.05$, Fig. 4D).

3.5. The expression of acc paralogs

The mRNA levels of *acc* paralogs in the red and white muscle of tilapia with glucose or saline injection are shown in Fig. 5. Compared with 0 h, the expression of *accα* was markedly up-regulated by 2.85- and 3.25-fold at 2 and 4 h respectively in the red muscle ($P < 0.05$, Fig. 5A), while the transcript level of *accβ* significantly increased by 2.41–2.73 folds during 4–12 h after the GTT ($P < 0.05$, Fig. 5B). However, saline injection did not change the expression of *accα* and *accβ* in the red muscle of tilapia. In the white muscle, the expression of *accα* was not affected by the glucose injection ($P > 0.05$, Fig. 5C), but the mRNA level of *accβ* was markedly up-regulated during 6–12 h after the GTT ($P < 0.05$, Fig. 5D). The expression of *acc* paralogs were not impacted by saline injection in the white muscle of tilapia.

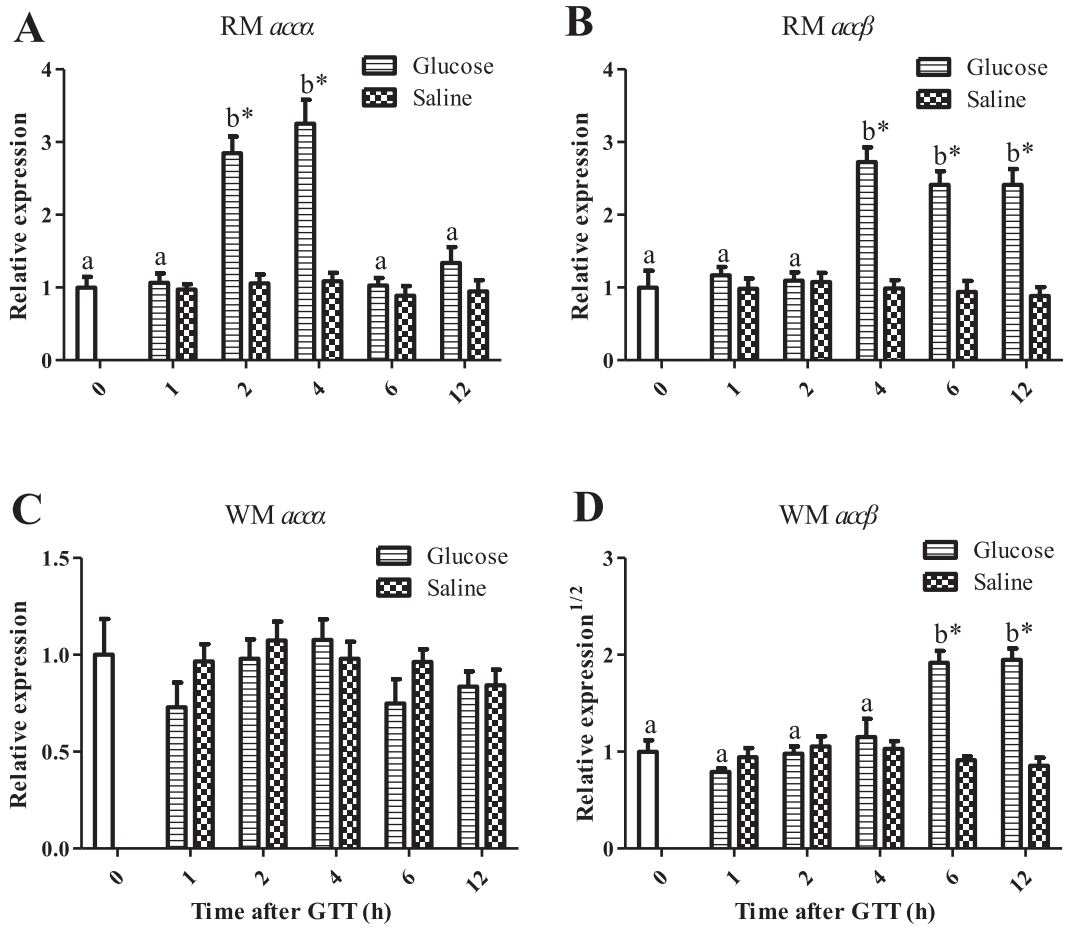

Fig. 5. Relative expression of acetyl-CoA carboxylase in the red and white muscle of tilapia after glucose or saline injection Data are expressed as means ± SE (n = 6). Different letters indicate significant differences among the glucose or saline injected group with sampling time. * means significant difference between the glucose and saline injected fish at each sampling point. Fraction number as superscript on the vertical coordinate represents root transformation of the data. RM: red muscle; WM: white muscle; *acc*, acetyl-CoA carboxylase.

4. Discussion

Prior to GTT, a pretest was performed to evaluate the glucose clearance of tilapia during 0–36 h after an intra-peritoneal (IP) injection of 1 g glucose/kg BW, and it was found that plasma glucose level returned to basal level earlier than 6 h after glucose treatment. Thus, an experimental period of 12 h was chosen to check the molecular and metabolic adaption of tilapia in response to a glucose load in this study, and similar experimental design in terms of GTT was also employed in hybrid tilapia (Lin et al., 1995), omnivorous gibel carp *Carassius gibelio* (Jin et al., 2018) and herbivorous blunt snout bream *Megalobrama amblycephala* (Xu et al., 2017, 2018). Our previous results showed that plasma glucose level increased during 1–4 h, and plasma insulin level increased concomitantly in tilapia after the glucose load (Chen et al., 2018). However, plasma glucose level of the saline injected fish did not change with sampling time, suggesting that minor stress was associated with glucose injection or sampling (Chen et al., 2018). In the present study, the time course of changes in glucose metabolism of tilapia muscle tissues in the saline injected fish were also analyzed to discriminate the differences due to food deprivation from glucose load.

In mammals, GLUT1 is responsible for the low level of basal glucose uptake in all cells (Zhao and Keating, 2007). The major cellular mechanism for disposal of an exogenous glucose load is insulin-stimulated glucose transport into skeletal muscle principally through GLUT4 (Huang and Czech, 2007). In tilapia, GLUT1 also functions as an ubiquitous basal level glucose transporter (Hrytsenko et al., 2010). In the present study, it seemed that an inhibition of basal glucose uptake in the red muscle was attributed to feed deprivation rather than glucose load, as reflected by similar *glut1a* expression patterns between the glucose and saline injected fish. Nonetheless, the expression of *glut4* was indeed up-regulated by GTT, suggesting an enhanced glucose uptake in response to increasing plasma insulin level in the red muscle of tilapia. However, both the expression of *glut1a* and *glut4* were insulin independent in the white muscle of tilapia. In line with our study, the expression level of *glut4* positively correlated with plasma insulin level in the red muscle rather than in the white muscle of carnivorous brown trout *Salmo trutta* (Capilla et al., 2002). However, Blasco et al. (1996) reported that glucose uptake rates in the red and white muscle of brown trout were stimulated by three and four folds, respectively after intravascular infusion of 0.5 g glucose/kg BW. In omnivorous juvenile gibel carp *Carassius gibelio*, the expression of *glut1* was not affected, while mRNA level of *glut4* was down-regulated by IP injection of 0.5 g glucose/kg BW in the white muscle (Jin et al., 2018). The discrepancies in different studies might be due to the differences in fish species, fish size, and the route and dose of glucose load.

HK and PFK are two important rate-limiting enzymes of the glycolysis pathway (Wegener and Krause, 2002; Kim and Dang, 2005). In the present study, the glycolysis of red muscle in tilapia was stimulated during 2–4 h after the GTT, as the expression of *hk1b*, *hk2*, *pfkma* and *pfkmb* and the activity of HK and PFK increased. By contrast, only the expression of *hk1b* was up-regulated at 6 h after the GTT, while the mRNA levels of *hk1a*, *hk2*, *pfkma* and *pfkmb* and HK activity as well as PFK activity in the white muscle did not change, suggesting that glycolysis was poorly impacted by glucose treatment in the white muscle

of tilapia. In gibel carp, the expression of *hk2* was also not affected, but the expression of *pfk* was down-regulated and up-regulated at 3 and 8 h, respectively in the white muscle after glucose injection (Jin et al., 2018).

Excess glucose can be stored as glycogen reserves in fish (Enes et al., 2009). In the present study, glucose storage was provoked in the red muscle of tilapia, as the mRNA level of *gys1* and glycogen content increased at 2 and 6 h, respectively after the GTT. In the white muscle of tilapia, the down regulation of *gys1* expression was due to extending time of food deprivation rather than glucose load, and glycogen content did not change with glucose treatment. Employing similar dose and route of glucose administration, glycogen level were also poorly affected in the white muscle of gilthead seabream *Sparus aurata* and European seabass *Dicentrarchus labrax* (Peres et al., 1999), while enhanced glycogen storage was evidenced in the white muscle of turbot *Scophthalmus maximus* (Garcia-riera and Hemre, 1996). The inconsistences in these studies might be attributed to the differences in the species, age and body size of the fish.

Although lipogenesis is not directly linked to glucose metabolism, this pathway converts excess glucose into fatty acids and contributes to glucose homeostasis (Polakof et al., 2012). In the present study, lipogenesis was stimulated in the red muscle of tilapia as reflected by up-regulated expression of *acca* (during 2–4 h) and *accβ* (during 4–12 h) after the GTT. However, only the mRNA level of *accβ* was up-regulated after 6 h of the GTT in the white muscle. After an IP injection of 0.5 g glucose/kg BW, the mRNA level of *acc* was not impacted in the white muscle of CAS III strain of gibel carp. In the Dongting strain of gibel carp, however, up-regulated expression of *acc* was observed in the white muscle (Jin et al., 2018).

Our previous results showed that the average glycogen content in the liver was about 66.6 mg/g (Chen et al., 2018). In this study, the average glycogen level in the red and white muscle of tilapia was 9.16 and 0.15 mg/g, respectively. In the adipose tissue of tilapia, the glycogen level was about 0.86 mg/g (Chen et al., unpublished data). Thus, liver is the main site of glycogen deposition in tilapia, followed by red muscle, adipose tissue, and white muscle in decreasing order. In the liver of tilapia, the glucose transport and utilization were sharply stimulated during 1–4 h after the GTT (Chen et al., 2018). The glucose utilization was improved during 2–6 h, especially at 4 h in the red muscle. It seemed that the adaption of glucose metabolism to GTT in the red muscle was a little slower than in the liver of tilapia. In the present study, the protein content of red and white muscle in tilapia did not differ by much (data not shown), and similar basal HK and PFK activity were evidenced in these two tissues. Considering that white muscle accounts for ≥ 95% of total skeletal muscle in tilapia (Alexander et al., 2006), white muscle was responsible for much more glycolytic activity compared to the red muscle on an absolute basis. Therefore, the glucose disposal of red muscle after glucose treatment was limited by its small proportion to white muscle, although its glycogen content was > 60 times higher than in the white muscle. Moreover, the glucose transport and utilization of white muscle was poorly impacted by GTT in tilapia. By contrast, skeletal muscle is the major site for the disposal of an exogenous glucose load in mammals (Huang and Czech, 2007). Thus, the limited metabolic and molecular adaption of glucose metabolism in the white muscle contributed greatly to the longer duration of hyperglycemia in tilapia after the GTT.

Taken together, it was concluded that the glycolytic and glycogen synthesis mechanisms in the red muscle were highly regulated by an acute glucose load whereas those in the white muscle were less responsive to this stimulus.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.03.010.

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