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Preproinsulin expression, insulin release, and hepatic glucose metabolism after a glucose load in the omnivorous GIFT tilapia *Oreochromis niloticus*

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ABSTRACT

This study was performed to investigate the time course of changes in the expression of preproinsulin (*ins*) and insulin-degrading enzyme (*ide*), plasma insulin level and hepatic glucose metabolism after a glucose tolerance test (GTT) in genetically improved farmed Nile tilapia (GIFT), *Oreochromis niloticus*. Male adult fish were intraperitoneally injected with 1 g glucose/kg of body weight after 24 h of food deprivation, and then subjected to sampling at 0, 1, 2, 4, 6 and 12 h after the GTT. Compared with the control (4.3 mM/L), plasma glucose level peaked at 1 h (14.8 mM/L), decreased during 2–4 h (5.7–8.3 mM/L), and was restored after 6 h of the GTT. Although the expression of *ins1*, *ins2* and *ide* were not affected in the Brockman body, plasma insulin level concomitantly increased with the increase of plasma glucose level during 1–4 h after the glucose injection. The expression of glucose transporter 1 sharply increased at 1 h after the GTT, indicating that hepatic glucose uptake was stimulated. The mRNA level of glucokinase was up-regulated during 1–4 h, and the activity of phosphofructokinase increased during 2–4 h, suggesting that hepatic glycolysis was active during 1–4 h after the glucose administration. The mRNA level of glycogen synthase 1 increased at 1 h, and liver glycogen level accumulated at 4 h after the GTT. Although the expression of glucose-6-phosphatase catalytic subunit a2 was suppressed during 1–2 h, neither the mRNA level of phosphoenolpyruvate carboxykinase 2 nor its activity was impacted by the glucose injection, which might have prolonged the glucose clearance of tilapia. Hepatic lipogenesis was effectively stimulated to dispose excess glucose absorbed by the hepatocytes, as up-regulation of mRNA levels of ATP citrate lyase a (during 1–4 h), acetyl-CoA carboxylase α (during 4–12 h) and fatty acid synthase (during 2–12 h) were accompanied by the GTT. Taken together, it was concluded that glucose was an effective insulin secretagogue in tilapia, and liver played an important role to clear the glucose load through stimulation of glucose uptake, glycolysis, glycogenesis and lipogenesis.

1. Introduction

Glucose is a central molecule for the metabolism of all vertebrates including fish, and it plays a pivotal role as fuel and metabolic substrate (Hemre et al., 2002; Wood and Trayhurn, 2003). However, fish is generally deemed glucose intolerant, given the much longer time it takes to clear a glucose load in contrast to mammals (Moon, 2001; Polakof et al., 2012). Moreover, glucose tolerance in fish is species-dependent and closely related to feeding habits, with lower glucose disposal for the carnivorous fish as compared with the omnivorous or herbivorous fish (Moon, 2001; Hemre et al., 2002). The precise reason for the sluggish glucose clearance of fish is still not fully understood, although a number of possible hypotheses such as lack of glucokinase (Walton and Cowey, 1982; Wilson, 1994), shortage of insulin production (Palmer and Ryman, 1972; Wilson and Poe, 1987), fewer insulin receptors (Parrizas et al., 1994) and devoid of insulin sensitive glucose transported 4 (Glut4) in peripheral tissues (Wright et al., 1998) were proposed. However, the advent of biochemical, immunoassay and molecular techniques has proved that the aforementioned hypotheses may be not the issues with respect to the glucose tolerance of fish (Mommsen and Plisetskaya, 1991; Panserat et al., 2000, 2014; Capilla et al., 2004, 2010; Andoh, 2016). There is increasing evidence that peripheral resistance to insulin action might be the ultimate reason in terms of glucose intolerance in fish (Alexander et al., 2006; Polakof et al., 2012).

In mammals, insulin-sensitive peripheral tissues such as liver,

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skeletal muscle and adipose tissue contribute greatly to the quick clearance of a glucose load. In fish, the hypoglycemic effect of insulin has already been well documented (Navarro et al., 2002; Caruso and Sheridan, 2011). However, the duration and degree of hypoglycemia and the metabolic responses of peripheral tissues after insulin administration are highly variable among fish species, and are strongly dependent on insulin dose, season, insulin origin and nutritional status (Polakof et al., 2012). In fish, in contrast to mammals, the mechanism by which insulin regulates plasma glucose level remains poorly understood, and the relative contribution of the main insulin-sensitive peripheral tissues remains to be clarified (Navarro et al., 2002; Polakof et al., 2012). Liver plays a central role in the glucose homeostasis, since it modifies the net glucose flux deriving it synchronously from the glucose production and utilization pathways (Enes et al., 2009; Jordan et al., 2010). Furuichi and Yone (1982) reported that the activities of both hepatic glycolytic phosphofructokinase and gluconeogenic glucose-6-phosphatase were stimulated by intraperitoneal (IP) injection of glucose in carp *Cyprinus carpio*, red sea bream *Pagrus major*, and yellowtail *Seriola quinqueradiata*. Enes et al. (2012) postulated that glucose uptake in the liver was stimulated by IP glucose load as liver glycogen accumulated in white sea bream *Diplodus sargus*. However, hepatic *glut1* mRNA level was decreased in response to IP glucose administration in grouper *Epinephelus coioides* (Liu et al., 2017). To the best of our knowledge, a systematic molecular investigation on the hepatic glucose transport and utilization after an acute glucose load is scarce in fish to date. Moreover, most teleosts possess two non-allelic preproinsulin (*ins*) genes (Caruso and Sheridan, 2011), and the insulin-degrading enzyme (IDE) has been recently identified as a new target to treat type 2 diabetes mellitus in mammals (Maianti et al., 2014; Tang, 2016). However, scarce information is available regarding the potential roles of *ins* and *ide* genes in the glucose homeostasis of fish.

As typical omnivorous fish species, tilapias are the second most farmed fish group worldwide due to their rapid growth, strong disease resistance, high marketability and relatively stable market prices (Ng and Romano, 2013). Among these related fish species, Nile tilapia *O. niloticus* is the most popular one cultured worldwide, which account for about 69.3% of the total yield of world tilapias (FAO (Food and Agriculture Organization of the United Nations), 2016). The genetically improved farmed Nile tilapia (GIFT strain) was developed by the International Center for Living Aquatic Resources Management (ICLARM) from selected breeding stocks from Africa and Asia (Tendencia et al., 2006), and it has been widely cultured in China in recent years. Lin et al. (1995) had reported that plasma insulin level was sensitive to an oral administration of glucose in hybrid tilapia *O. niloticus* × *O. aureus*. Mansour et al. (1998) had identified the *ins1* gene of Nile tilapia, and islet xenotransplantation in the Nile tilapia-to-mouse model strongly indicated that glucose intolerance might be resulted from peripheral resistance to insulin action (Wright et al., 2000; Alexander et al., 2006). The genome sequence of Nile tilapia is available (Brawand et al., 2014), which makes it a good candidate to study the nutrition regulatory mechanisms for omnivorous fish species. In our previous study, we have established sensitive molecular indicators for hepatic glucose metabolism of GIFT tilapia during postprandial nutrition status transition (Chen et al., 2017). In this study, thus we further investigated the effects of an acute glucose load on the expression of *ins1*, *ins2*, and *ide* in the Brockman body, plasma insulin level, and hepatic glucose transport and utilization. The results of this study would help us to preliminarily understand the transcription, degradation and secretion of insulin, and ascertain the role of liver in the glucose homeostasis after glucose administration in the omnivorous tilapia.

2. Materials and methods

2.1. Experimental fish

One hundred and twenty male juvenile GIFT tilapia (about 47.7 ± 4.3 g/fish) were purchased from Xiema Hatchery (Chongqing, China), and maintained in a closed indoor recirculating system consisted of two large rectangular glass tanks (700 L) with continuous filtered freshwater and aeration in Southwest University. Fish were cultured 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 analyzed proximate composition of the diet on the basis of dry matter was as follows: moisture: 6.86%; crude protein: 33.2%; crude lipid: 7.37%; ash: 9.49%. Fish, who exhibited very slow growth rate as compared with their companions, were abandoned during the acclimatization period.

2.2. Glucose tolerance test

In mature tilapia, much of the endocrine pancreas is consolidated into large, grossly visible islets known as Brockmann bodies (BBs), which are enveloped by a thin layer of exocrine pancreas and embedded in adipose tissue (Yang et al., 1999). To isolate the BBs easily, relatively big tilapia (average body weight: 562 ± 63 g/fish) were used in this study. Fish were fasted for 24 h after the last meal, and subjected to an acute glucose tolerance test (GTT). Fish (six replicates per group) were randomly selected, anaesthetized with about 60 mg/L MS-222 (Sigma, St Louis, USA), individually weighed, injected intraperitoneally with 1 g glucose/kg body weight, and immediately transferred to separate rectangular tanks. Another batch of tilapia (four replicates per group) was injected with equivalent volumes of saline solution only as a control. Fifteen g glucose was diluted in 0.85% sterile saline solution until the total volume was 50 mL. In general, it took < 10 min to finish the glucose injection within each group. After the injection, fish in groups were collected and anaesthetized for blood collection from caudal vein with heparinized sterile syringes at 1, 2, 4, 6 and 12 h, respectively. A third group of fish (six replicates) was sacrificed at time 0, at the end of the 24 h fasting period. BBs of the fish were isolated with an enzymatic method following the procedures of Yang and Wrigh (1995). Liver samples of these fish were then dissected, immediately frozen in liquid nitrogen, and transferred to − 80 °C until used for enzymatic and gene expression analysis. Blood samples were immediately centrifuged (4500 rpm, 10 min) at 4 °C (Eppendorf centrifuge 5430 R, Hamburg, Germany), and plasma was separated and stored at − 20 °C until used for analysis of biochemical indices.

The sampling time point of this study was chosen based on a pretest, in which plasma glucose level of tilapia returned to basal level at about 6 h after the glucose injection. During the period of GTT, water temperature, dissolved O_2, pH and ammonia were maintained at about 29.6 ± 2.6 °C, 7.62 ± 0.33 mg/L, 7.21 ± 0.38 and 0.08 ± 0.02 mg/L, respectively. 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. Proximate composition of the diet

Proximate composition analysis consisted of determining moisture, protein, lipid and ash contents of the diet using standard methods (AOAC, 1995).Crude protein ($N \times 6.25$) was determined by the Kjeldahl method after an acid digestion using an auto-Kjeldahl System (Hanon, Jinan, China). Crude lipid was determined by the ether-extraction method. Moisture was determined by oven drying at 105 °C for 24 h. Ash was determined using a muffle furnace at 550 °C for 24 h.

2.4. Plasma biochemical analysis

The levels of plasma glucose and triglyceride were detected by spectrophotometric assay using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The protein concentration of the plasma was determined by the biuret method. The