Different regulation of insulin on glucose and lipid metabolism in 2 strains of gibel carp

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Abstract

To test the hypothesis that response to insulin by regulating glucose and lipid metabolism in gibel carp A strain may be different from that in DT strain, bovine insulin was injected into both strains of gibel carp after previous fasting for 48 h. The results showed that insulin induced hypoglycemia at 3 h in 2 strains, and that this was coupled with increased expression of glucose transporters (GLUT2 in the liver and GLUT1, GLUT4 in the muscle) and glycolytic enzyme (HK2 in the muscle) in both strains. Insulin induced increased glycolysis (GK) and fatty acid oxidation (ACO3 in the liver and CPT1a, ACO3 in the muscle) in the DT strain. Conversely, very strong lipogenic capacity, as indicated by higher mRNA levels of transcription factor of fatty acid anabolism (SREBP1) and lipogenic enzymes (ACC, ACLY, and FAS) and decrease lipolytic capacity as indicated by lower mRNA levels of fatty acid oxidation enzymes in the liver (ACO3) and muscle (CPT1a and ACO3) detected in the A strain after insulin injection. Higher plasma insulin levels and decreased plasma free fatty acid levels were detected at 8 h post insulin injection in A strain induced hypoglycemia. However, plasma glucose levels returned to baseline and no effect on fatty acid levels in the DT strain was observed in response to insulin treatment at the same point in time. These insulin-strain interactions demonstrated that insulin induced different changes in glucose and lipid metabolism in these 2 strains as expected.

1. Introduction

It is well known that glucose turnover rate in fish is 20–100 times lower than in mammals of equivalent body mass (Weber and Zwingelstein, 1995). Consequently, teleost fish are generally considered glucose intolerant due to the hyperglycemia that usually persists for a long time after high glucose flux (Moon, 2001). It was previously believed that this lack of effective glucose clearance was caused by a low level of blood insulin. However, this has been shown to be incorrect, as insulin levels in fish (0.2–5 nM) tend to be higher than in mammals (Mommsen and Plisetskaya, 1991). Insulin, is an anabolic hormone that plays pivotal roles in the regulation of glucose homeostasis, stimulation of stimulating cell growth and differentiation, promotion of lipogenesis, glycogen and protein synthesis, and inhibition of lipolysis, glycogenolysis and protein breakdown in mammals (Saltiel and Kahn, 2001; Hernandez-Sanchez et al., 2006).

Numerous studies have been investigated to reveal insulin effects on fish metabolism by subjecting fish to exogenous insulin, including fish insulin, porcine insulin and bovine insulin (Perez et al., 1989; Plagnes-Juan et al., 2008; Polakof et al., 2010c). These studies have all shown that insulin administration led to hypoglycemia effectively, concurrent with decreased plasma free fatty acids level (Mommsen and Plisetskaya, 1991; Polakof et al., 2012). It has been suggested that regulation of insulin in fish was tissue-specific and that peripheral tissue utilization was important for glucose homeostasis (Polakof et al., 2010a). The liver is considered a principal insulin-sensitive organ for regulation of the intermediary metabolic response to nutritional state (Klover and Mooney, 2004). Generally, hepatic glycogen deposition is depleted and liver glycolysis is up-regulated by insulin, but gluconeogenic genes are not always depressed by insulin in certain species of fish (Mommsen and Plisetskaya, 1991; Polakof et al., 2010c). As in mammals, insulin stimulates synthesis of fatty acids in the fish liver through up-regulation of the expression of lipogenic genes, but it is accompanied by unchanged or increased fatty acids oxidation in rainbow trout, which is contrary to mammals (Polakof et al., 2011; Jin et al., 2014). Muscle represents almost 50% of the body weight in fish and therefore plays a crucial role in the regulation of glucose homeostasis (Moon, 2001). Muscle glycogen synthesis and storage is increased in fish after insulin injection (Sánchez-Gurmaches et al., 2010). However, no changes or inhibited muscle glycolysis have been observed in rainbow trout subjected to bovine insulin (Polakof et al., 2010b; Jin et al., 2014). Insulin administration induces lipogenic action in lamprey and rainbow trout, concurrent with down-regulation of fatty acid oxidation (Kao et al., 1999; Polakof et al., 2010a). However, information regarding the effects of insulin in fish on glucose and lipid metabolism is contradictory. This may be because of different species, varying insulin doses, routes of administration and nutritional status (Polakof et al., 2012). Most injection studies have been conducted using pharmacological doses of the hormone, while only a few have investigated physiological concentrations (Polakof et al., 2010a). Moreover, most studies have used intraperitoneal or intravenous administration and have focused on carnivorous fish, such as rainbow trout, European eel, sea bass and catfish, although a few experiments investigated common carp, but used intramuscular injection or aortic cannulation (Van Raaij et al., 1995; Polakof et al., 2012). Overall, information regarding the effects of intraperitoneally injected insulin on omnivorous fish and genotype are scarce.

Gibel carp (*Carassius gibelio*), which is an omnivorous species widely distributed from northern Europe to Asia, is one of the most important economical freshwater aquaculture species

in China with an annual production of up to 2.6 million tons (Gui and Zhou, 2010). Different strains were selected for rapid growth based on the natural gynogenetic population of gibel carp, such as the CAS III strain (A strain) (Wang et al., 2011). In recent years, the A strain has become the most popular gibel carp species in the aquaculture because of its fast growth. The Dongting strain (DT strain), however, is a natural wild strain with slow growth, which does not favor aquaculture. Because the differences in growth traits between the A strain and DT strain may be linked to differences in the ability to use carbohydrates, and because the metabolism of carbohydrate utilization can be regulated by insulin, it is here hypothesized that the effect of insulin on glucose and lipid metabolism could differ between the A and DT strains. Therefore, to investigate the regulation of glucose homeostasis by insulin in these 2 strains, fish from A strain and DT strain were subjected to intraperitoneal administration with physiological doses of bovine insulin. We then examined basal plasma metabolite levels (glucose, triglycerides and free fatty acids) before injection and levels at 3 time intervals after injection. Glycogen level, mRNA levels of genes involved in glycolysis, gluconeogenesis, lipogenesis and fatty acid β -oxidation were analyzed in the liver and muscle. Transcript levels of transcription factor SREBP1c were also evaluated, as were those of glucose facilitative transporter GLUT2 in the liver and the glucose facilitative transporters GLUT1 and GLUT4 in the white skeletal muscle. To our knowledge, this is the first study of the effects of insulin on different genotypes in omnivorous fish. The results presented herein will improve our understanding of the relationship between insulin and gene background with respect to regulation of glucose and lipid metabolism in fish.

2. Materials and methods

2.1 Experimental design and sampling procedure

The study was performed using 2 strains of gibel carp, A strain and DT strain. Fish (A strain body weight 48.4 ± 1.6 g; DT strain body weight 58.4 ± 1.3 g) were maintained in tanks with 25°C well-aerated water and fed a commercial diet (No. 718, Haid Group, China). The experimental protocol was approved by the ethics committee of the Institute of Hydrobiology, Chinese Academy of Sciences.

Fish were deprived of food for 48 h before the injection in order to ensure that their digestive tract was empty and that they had a basal level of plasma metabolites. Fish were lightly anaesthetized with MS-222 (100 mg/L, Sigma-Aldrich, USA) and weighed, after which they were administered an intraperitoneal injection of bovine insulin (2.0 IU/kg, Sigma-Aldrich, USA) or saline solution alone at 100 μ L/100 g body mass, then distributed into triplicate tanks per treatment. Six fish per treatment (from 3 tanks) were anaesthetized and sampled at 0, 3, 8 and 12 h after injection. Blood was quickly removed from the caudal vein using syringes rinsed with heparin sodium (0.2%), then centrifuged at 3000 g for 10 min. The recovered plasma was immediately frozen and kept at -20°C until analysis. The entire liver and a sample of dorso-anterior white skeletal muscle were dissected and cut into small pieces, which were immediately frozen in liquid nitrogen, then stored at -80°C until analysis.

2.2 Biochemical analysis

Liver and muscle glycogen levels were analyzed in samples (n=6) at 8 h after insulin or saline injection according to manufacturer's instructions (Glycogen Colorimetric Assay Kit, Biovision, U.S.). Briefly, six individual samples per experimental condition were used as biological replicates. Frozen tissues (10 mg) were homogenized on ice with 200 μ l water). Homogenates were boiled for 10 min, after which they were centrifuged at 18,000 g for 10 min. The resulting supernatants were then used immediately for glycogen assays. For the assays, 2 μ l samples were added to a 96-well plate, after which 48 μ l of hydrolysis buffer was added. Next, glycogen standard was diluted from 0 μ g to 2.0 μ g per well. Hydrolysis enzyme mix was then added to glycogen standards and samples. The reaction was subsequently incubated for 30 min at room temperature after which 50 μ l of reaction mix (46 μ l development buffer, 2 μ l development enzyme mix and 2 μ l oxiRed probe) was added to each well. Finally, the reaction was incubated for another 30 min at temperature without the light, after which the absorbance at OD 570 nm was measured.

Plasma glucose (Glucose kit, Wako, Japan), triglycerides (Triglycerides kit, Wako, Japan) and free fatty acids (NEFA kit, Wako, Japan) levels were determined in all samples (n=6) using commercial kits adapted to a microplate format according to the manufacturer's recommendations. Bovine insulin level was measured using a commercial ELISA kit (Mercodia, Sweden).

2.3 Gene expression analysis

Analyses of mRNA levels were performed on samples from the liver and skeletal muscle collected at 8 h after injection with insulin or saline solution (n=6 for each tissue and strain). Total RNA was extracted using Trizol reagent (Invitrogen, USA) according to the manufacturer's recommendations. One microgram of the resulting total RNA was reverse transcribed into cDNA using the SuperScript III RNaseH-reverse transcriptase kit (Invitrogen, USA) and random primers (Promega, USA) according to the instructions of each manufacturer. Target gene expression levels were determined by quantitative real-time RT-PCR, using specific primers (Table.1).

Quantitative RT-PCR was conducted using a LightCycle 480 II system (Roche, Switzerland) with SYBR Green I Master Mix. The transcript levels assessed were glucokinase (GK), hexokinase 2 (HK2), 6-phosphofructokinase (6PFK) and pyruvate kinase (PK) for glycolysis; glucose-6-phosphatase (G6Pase), fructose 1,6-bisphosphatase (FBPase) and phosphoenolpyruvate carboxykinase (PEPCK) for gluconeogenesis; glucose facilitative transporter type 1 (GLUT1), type 2 (GLUT2) and type 4 (GLUT4) for glucose transport; sterol regulatory element binding protein 1 (SREBP1) for transcription factor mediating insulin effect; ATP citrate lyase (ACLY), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) for lipogenesis; carnitine palmitoyl transferase 1A (CPT1a) and acyl-CoA oxidase 3 (ACO3) for fatty acid β -oxidation. α -tubulin and elongate factor 1 α (EF-1 α) were used as non-regulated reference genes in liver and muscle respectively where their expression was stable. Relative quantification of target gene expression was performed using the mathematical model described by Pfaffl (2001).

2.4 Statistical analysis

Results from plasma metabolite levels (n=6), glycogen content (n=6) and gene expression (n=6) are expressed as the means $\pm$ SEM and were analyzed using 2-way ANOVA to assess differences induced by insulin vs. saline injections, strains (A strain vs. DT strain) and interactions. When an

interaction was significant, the means were compared using the Student-Newman-Keuls multiple comparison test. For all statistical analyses, the level of significance was considered at $P < 0.05$.

3. Results

3.1 Plasma metabolite levels

Plasma metabolites at 0 h, 3 h, 8 h and 12 h after intraperitoneal injection with insulin or saline solution are presented in Table 2. Bovine insulin levels were high at 3 h post insulin injection, then drastically decreased afterwards in both strains. The levels of bovine insulin in A strain were significantly higher than in DT strain (around 25 times and 5 times, respectively) at 8 h and 12 h. Interactions between injection and genotype were denoted based on the glucose level at each time point. Insulin administration led to a significant decrease in plasma glucose at 3 h post injection in both strains. Glycemia value returned to baseline by 12 h in A strain, but increased to basal levels 8 h after insulin injection in DT strain. Plasma triglycerides levels were higher in DT strain than A strain, regardless of injection. Insulin injection decreased plasma free fatty acids levels in both strains at 3 h. Decreased free fatty acids levels were still found in A strain at 8 h post insulin administration, but not in DT strain.

3.2 Tissue glycogen content

As shown in Fig. 1A, a significant interaction between injection and strain was observed in liver glycogen level, with insulin injection strongly inhibiting glycogen storage in DT strain, but inducing no change in A strain. Similarly, glycogen content in muscle remained unaltered in both strains (Fig. 1B).

3.3 Glucose metabolism

Relative transcript levels of glucose transporter and key glycolytic enzymes in the liver sampled at 8 h after injection are presented in Fig. 2A. Transcript levels of GLUT2 were significantly up-regulated by insulin in both strains. In muscle, the expression of glucose transporters GLUT1 and GLUT4 was induced by insulin in both strains (Fig. 2B). The transcript levels of GLUT4 was lower in A strain than in DT strain, regardless of injection. As shown in Fig. 3A, there was a significant interaction between injection and strain on GK mRNA levels ($P < 10^{-3}$), with a strong induction of GK expression by insulin in DT strain. Additionally, 6PFK transcript levels were higher in the liver of DT strain than A strain, regardless of injection. The abundance of PK transcripts was unaltered by genotype and insulin injection. Cellular mRNA levels encoding HK2 were significantly enhanced in the muscle by insulin at 8 h after administration (Fig. 3B). Genotype and insulin had no effect on 6PFK mRNA level. PK expression was higher in DT strain than in A strain, regardless of injection.

Changes in the relative mRNA transcripts of gluconeogenic enzymes in the liver sampled at 8 h after injection are shown in Fig. 4. No significant difference in G6Pase, FBPase and PEPCK mRNA level was found regardless of injection and strain.

3.4 Lipid metabolism

As shown in Fig. 5A, a significant interaction between injection and strain was observed for SREBP1, ACC, ACLY and FAS mRNA levels in the liver. Insulin induced a marked increase in the expression of all these lipogenic genes in A strain, while unaltered transcript levels were observed in DT strain after insulin administration. In terms of lipogenesis in the muscle, enhanced SREBP1 mRNA level were induced by insulin in both strains, while DT strain showed a higher SREBP1 mRNA level than A strain (Fig. 5B). However, A strain had a greater ACC mRNA transcript level than DT strain. No changes in ACLY expression were observed, regardless of injection and strain. A significant injection $\times$ strain interaction on FAS mRNA level with enhanced FAS expression induced by insulin was observed for A strain.

Changes in mRNA levels of lipolytic enzymes in the liver are shown in Fig. 6A. No significant differences in CPT1a mRNA level were found, regardless of injection and strain. With respect to transcript levels of ACO3, an interaction was observed between injection and strain, with a slight decrease induced by insulin in A strain, and an insulin reverse effect observed in DT strain. Changes in relative mRNA transcript levels of key enzymes involved in fatty acid oxidation in muscle are presented in Fig. 6B. A significant inhibition of CPT1a expression by insulin was found in A strain, while its expression was slightly enhanced in DT strain. A similar pattern was also observed for ACO3 mRNA level in muscle.

4. Discussion

In the present study, plasma glucose drastically decreased 3 h after the administration of physiological doses of insulin in both strains, indicating that gibel carp could effectively respond to exogenous insulin. These findings are in agreement with those of a previous investigation of rainbow trout (Plagnes-Juan et al., 2008; Polakof et al., 2010c; Jin et al., 2014). In the A strain, glucose levels required 12 h after insulin injection to return to baseline, and in the DT strain, they required 8 h, indicating that the A strain had a different ability to regulate glucose homeostasis in response to changes in insulin than the DT strain did. On the contrary, the same recovery period was observed in different rainbow trout lines (Jin et al., 2014). In this way, the duration and degree of hypoglycemia was found to vary both among and within species (such as varying by strain). Similarly, free fatty acids concentrations decreased at 3 h after insulin injection, supporting insulin function as an anabolic hormone (Polakof et al., 2010a). Many factors, including strain, significantly alter hematological parameters in fish (Mccarthy, et al., 1973). Herein a significant difference in basal levels of plasma glucose, triglycerides, and free fatty acids was found between the A strain and DT strain, which may be attributed to their different genotypes.

As in mammals, the existence of different members of glucose transporters (GLUTs) that mediated glucose across plasma membranes by insulin has been reported in fish (Navarro et al., 2006). In the present study, the up-regulated GLUT2, GLUT1 and GLUT4 transcript levels indicated that insulin stimulated glucose uptake in both the liver and muscle of A strain and DT strain, which may be responsible for hypoglycemia in both strains after the injection. These results are in line with the increase GLUT1 and GLUT4 expression stimulated by insulin in rainbow trout (Díaz et al., 2009). Liver glycogen content decreased after insulin administration in DT strain, which is comparable to recovery of the glycemia level in DT strain at 8 h post insulin injection,

and may be due to increased glucose production in response to insulin-induced hypoglycemia (Lecavalier et al., 1989). This glycogen depletion in DT strain is in agreement with the results observed for many other fish species (Navarro et al., 2006). However, no change in muscle glycogen was observed after insulin injection, suggesting the lack of an efficient response of the liver to insulin in this tissue, which is consistent with the results observed for rainbow trout (Jin et al., 2014). Glucose phosphorylation led by hexokinase is a prerequisite for entry of glucose the glycolytic pathway and to its conversion to glycogen (Legate, et al., 2001). Concurrent with unaltered muscle glycogen levels in both strains, HK2 expression increased by insulin probably facilitates the glycolytic process, which was consistent with increases in GLUT transcription levels. This increase indicates a rapid response to insulin stimulation via high relative mass of the tissue in gibel carp. GK, a key glycolytic enzyme involved in the first step in glycolysis in the liver, was regulated by insulin. In the present study, GK expression was markedly induced by insulin in DT strain after injection, indicating up-regulated glycolytic potential after insulin injection in this strain. However, no response of GK was observed in A strain, reaffirming that insulin may not be sufficient to induce GK in fish (Panserat et al., 2014). The results of the present study showed that glucose uptake potential indicated by GLUT4 mRNA levels and glycolytic potential reflected by hepatic 6PFK and muscular PK transcript levels were lower in A strain, indicating weaker ability of glucose homeostasis regulation by this physiological process in comparison to DT strain. As significant injection $\times$ strain interactions were observed in liver glycogen content and GK mRNA level, these findings could be due to the different regulation of glucose metabolism by insulin in these two gibel carp strains.

In mammals, insulin increases transcription of glycolytic enzymes, while it inhibits the gene expression of the enzymes in gluconeogenesis (Saltiel and Kahn, 2001). Contrary to the well-known inhibition of gluconeogenesis by insulin in mammals, unaltered gluconeogenic enzymes were observed in both strains, suggesting that hypoglycemia induced by insulin was not due to inhibition of the gluconeogenic process, which is in accordance with the lack of an effect on expression of gluconeogenic genes in rainbow trout after insulin injection (Jin et al., 2014). The insulin induced hypoglycemia observed herein may be caused by increased glucose uptake by the liver and muscle, unaltered gluconeogenesis and up-regulated glycolysis in muscle in both gibel carp strains. However, the response of glycogen and the glycolytic process in the liver differed between A strain and DT strain.

In general, insulin promotes free fatty acids uptake in fish (Caruso and Sheridan, 2011). This effect was confirmed in the present study by the significant decrease in free fatty acid levels in the plasma at 3 h post insulin treatment in both strains. Hypoglycemia and decreased plasma free fatty acids level at 8 h post insulin injection were only observed in A strain, which can likely be attributed to the very low plasma concentrations of bovine insulin in this strain at this sampling time. Indeed, it is well known that the metabolic relationship between glucose and fatty acids is reciprocal and not dependent (Randle, 1998). Specifically, extra glucose can be converted to fatty acids and triglycerides by lipogenesis, and the production of lipogenic enzymes is positively correlated with the transcription of the corresponding genes (Koo et al., 2001). SREBP-1 is a membrane-bound transcriptional factor that regulates key enzymes involved in fatty acid biosynthesis (Minghetti et al., 2011). In mouse liver and culture cells, increases in fatty acids occur via the induction of lipogenic gene expression (such as FAS and acetyl CoA carboxylase), which are mediated by induction of transcription factor SREBP1 (Shimano et al., 1999; Shimano,

2001). The liver is the major lipogenic tissue in fish (Polakof et al., 2012), and *de novo* synthesis of lipids from glucose in fish liver has been confirmed as a mechanism to control glucose homeostasis (Bou et al., 2016). Enhanced hepatic lipogenesis was observed in rainbow trout with high fat muscle content, demonstrating a better ability to control hyperglycemia induced by dietary carbohydrates (Skiba-Cassy et al., 2009; Kamalam et al., 2012). In the present study, the decreased plasma free fatty acids concentration in A strain was supported by a higher rate of lipogenesis and lower lipolysis at 8 h post insulin injection. A dramatic induction of SREBP1 was observed in A strain that occurred concomitantly with the up-regulation of transcript levels of the key enzymes for fatty acid synthesis (ACC, ACLY and FAS) in the liver of this strain following insulin injection, supporting the lipogenic role of this hormone. Insulin increased SREBP-1 gene expression in the muscle and not the liver of the DT strain carp, but it did not induce any significant changes in the expression of lipogenic enzymes in either type of tissue, which was in agreement with rainbow trout receiving a pharmacological dose or a physiological dose of insulin (Plagnes-Juan et al., 2008; Jin et al., 2014). The finding that insulin did not alter plasma free fatty acid levels after 8 h of insulin treatment may linked to the low free fatty acids uptake, which was caused by low bovine insulin concentration in the plasma. In this way, it can be concluded that the action of insulin on lipid synthesis in the DT strain is different from that in the A strain.

Fatty acid oxidation is an important source of energy in liver and white muscle in fish (Tocher, 2003). ACO and CPT1a are the key regulatory enzymes in peroxisomal and mitochondrial β -oxidation respectively (Kerner and Hoppel, 2000). Studies in rainbow trout have shown that oxidation of free fatty acids was down-regulated by insulin (Jin et al., 2014). In the present study, transcript abundance of genes related to ACO3 and CPT1a decreased in the liver of the A strain, suggesting the anti-lipolytic role of insulin in this strain (Polakof et al., 2012). However, in line with results observed in rainbow trout, the mRNA level of CPT1a was enhanced in the DT strain that received an intraperitoneal injection of insulin (Polakof et al., 2010a). These different change profiles in the A strain and DT strain may be due to the free fatty acid levels in the plasma at 8 h after insulin injection. Consistent with the decreased plasma free fatty acid concentration, fatty acid oxidation was decreased in the A strain. Conversely, insulin tended to up-regulate fatty acid β -oxidation in the DT strain in line with increased free fatty acid levels in the plasma at the same time. Taken together, these results suggest that fatty acid synthesis and oxidation induced by insulin are regulated differently in the two different strains of gibel carp.

Concurrent with the increased lipogenic and decreased fatty acid oxidation in A strain at 8 h post insulin injection, the plasma triglycerides level was also increased at this time point, confirming *de novo* hepatic lipogenesis is an important source of triglycerides (Ginsberg et al., 2005). However, induction of triglycerides in the plasma of DT strain at the same time point with unaltered lipogenic genes expression and increased fatty acid oxidation should be further investigated. DT strain exhibited higher basal levels of triglycerides than A strain, which may be attributed to their different genetic backgrounds (Mccarthy, et al., 1973).

In conclusion, the results of the present study revealed how glucose and lipid metabolisms are regulated by insulin in different genotypes of gibel carp from a physiological point of view. The potential increase of glucose uptake by liver and muscle are attributed to the hypoglycemia observed after insulin injection in two gibel carp strains. Enhanced fatty acid synthesis ability and inhibited fatty acid oxidation potential was observed in A strain at 8 h after insulin administration, supporting the observed decrease in free fatty acids. However, enhanced glycolysis and fatty acid

oxidation was found in DT strain, but not in A strain. In addition, decreased liver glycogen content appeared to responsible for the recovery from hypoglycemia at 8 h post insulin treatment in this strain. Therefore, insulin regulates glucose and lipid metabolism via a different mechanism in these 2 strains of gibel carp. To the best of our knowledge, this is the first study to investigate insulin effects on lipid metabolism and glucose metabolism related to the genotype of gibel carp, which will improve our understanding of the interaction between insulin regulation and the genetic background in fish.

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TABLE LEGENDS

Table 1 Primers used for PCR.

Table 2 Plasma metabolite levels in A strain and DT strain after intraperitoneal (IP) administration of insulin (2.0 IU/kg) or saline solution.

Data are the means $\pm$ SEM (n=6). The effect of injection type, strain and interaction were analyzed by 2-way ANOVA ($P<0.05$) followed by a Student-Newman-Keuls multiple comparison test. Mean values not sharing a common lowercase letter are significantly different from each other. Within each injection treatment (column wise), significant differences at different time points (3 h, 8 h and 12 h) are represented by different uppercase letter (one-way ANOVA, $P<0.05$).

FIGURE LEGENDS

Fig. 1 Glycogen content analysis in liver (A) and muscle (B) of gibel carp from A strain (grey bars) and DT strain (black bars) 8 h after IP administration of insulin or saline solution.

Data are the means $\pm$ the SEM (n=6) and were determined using 2-way ANOVA, followed by a Student-Newman-Keuls multiple comparison test. Differences were considered significantly different at $P<0.05$.

Fig. 2 Gene expression of glucose transporters in the liver (A) and muscle (B) of gibel carp from A strain (grey bars) and DT strain (black bars) 8 h after IP administration of insulin or saline solution.

Glucose transporter type 2 (GLUT2), glucose transporter type 1 (GLUT1) and glucose transporter 4 (GLUT4) mRNA levels were measured using real-time quantitative RT-PCR. Results are expressed as the means $\pm$ SEM (n=6) and were determined by 2-way ANOVA ($P<0.05$), followed by a Student-Newman-Keuls multiple comparison test.

Fig. 3 Gene expression of selected glycolytic enzymes in the liver (A) and muscle (B) of gibel carp from A strain (grey bars) and DT strain (black bars) 8 h after IP administration of insulin or saline solution.

Glucokinase (GK), Hexokinase 2 (HK2), 6-phosphofructokinase (6PFK) and pyruvate kinase (PK) mRNA levels were measured using real-time quantitative RT-PCR. Results are expressed as the means $\pm$ SEM (n=6) and were determined by 2-way ANOVA ($P<0.05$), followed by a Student-Newman-Keuls multiple comparison test. Mean values not sharing a common lowercase letter are significantly different from each other.

Fig. 4 Gene expression of selected gluconeogenic enzymes in the liver of gibel carp from A strain (grey bars) and DT strain (black bars) 8 h after IP administration of insulin or saline solution.

Glucose-6-phosphatase (G6Pase), fructose 1,6-bisphosphatase (FBPase) and phosphoenolpyruvate carboxykinase (PEPCK) mRNA levels were measured using real-time quantitative RT-PCR.

Results are expressed as the means $\pm$ SEM (n=6) and were determined by 2-way ANOVA ($P<0.05$), followed by a Student-Newman-Keuls multiple comparison test.

Fig. 5 Gene expression of selected enzymes and transcription factors involved in NADPH generation and lipogenesis in the liver (A) and muscle (B) of gibel carp from A strain (grey bars) and DT strain (black bars) 8 h after IP administration of insulin or saline solution.

SREBP1 (sterol regulatory element binding protein 1), ATP citrate lyase (ACLY), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) mRNA levels were measured using real-time quantitative RT-PCR. Results are expressed as the means $\pm$ SEM (n=6) and were determined by 2-way ANOVA ($P<0.05$), followed by a Student-Newman-Keuls multiple comparison test. Mean values not sharing a common lowercase letter are significantly different from each other.

Fig. 6 Gene expression of selected fatty acid oxidation enzymes in the liver (A) and muscle (B) of gibel from A strain (grey bars) and DT strain (black bars) 8 h after IP administration of insulin or saline solution.

Carnitine palmitoyl transferase 1A (CPT1a) and acyl-CoA oxidase 3 (ACO3) mRNA levels were measured using real-time quantitative RT-PCR. Expression values were normalized by β -tubulin-transcripts. Results are expressed as the means $\pm$ SEM (n=6) and were determined by 2-way ANOVA ($P<0.05$), followed by a Student-Newman-Keuls multiple comparison test. Mean values not sharing a common lowercase letter are significantly different from each other.

Table 1

Function classifications	Gene name	Sense and antisense primer (5'-3')	Genbank accession number	Product size
Internal reference	α -tubulin	TCCTTCAACACCTTCTTCAGTGAGAC AGCTGCTCAGGGTGGAAACAGC	JX4135181	134 bp
	Elongation factor 1 alpha (EF-1 α)	GTTGGAGTCAACAAGATGGACTCCAC CTTCCATCCCTTGAACCAGCCCAT	AB056104	198 bp
Glycolysis	Glucokinase (GK)	GAGGAGATGCGTAAGGTGGAGCT TTCTCATACAGCTGATGTCCAGGGTT	KX898498	167 bp
	Hexokinase 2 (HK2)	TATGAGAAGATGATCAGCGGGATGTACC ATCACACGTGCTGCTGATCAAGCCCA	KX898499	225 bp
	6-phosphofructokinase (6PFK) (Liver)	ACACCGGATGCCGCAGAAGCA TCGATCTCTCCGGTCACATACTCG	KX898500	105 bp
	6-phosphofructo-1-kinase (6PFK) (Muscle)	TAGCTATCATGAACGTTGGAGCTCC CTTTGCCTGTCCAGCCTCCAACA	KX898501	180 bp
	Pyruvate kinase (PK) (Liver)	GCATCTGTGTCTGCTGGACATCGA TGAGAGCCGTGAGAGAAGTTCAGTC	KX898502	144 bp
	Pyruvate kinase (PK) (Muscle)	ATCATGGAGGCCAGCGATGGCAT GGCTTCTTGATCATGCTCTCCAACAT	KX898503	167 bp
	Glucose transporter type 1 (GLUT1)	CACTGTTGTGTCTCTGTTTGTGG AGATTGCTATGATGCTAATGTAGGACATC	KY328741	158 bp
Glucose transport	Glucose transporter type 2 (GLUT2)	CTCGTGGATGAGCTACCTCAGCAT CCCTGACTGAAGATCTCCGCCA	KX898504	111 bp
	Glucose transporter type 4 (GLUT4)	CACTCTCTGTGGCCATCTTCTCCAT	KY328742	241 bp

		TCACCAACATACATGGGCACCAATCCT		
Gluconeogenesis	Glucose-6-phosphatase (G6Pase)	CCTTACTGGTGGGTCCATGAGACT	KX898505	90 bp
		TGGGCCGGTCTCACAGGTCAT		
	Fructose 1,6-bisphosphatase (FBPase)	CACAAATGTTACAGGTGACCAGGTGAA	GU593002	193 bp
		AATGTTTGAAGAGCCATCCAGAGGGT		
	Phosphoenolpyruvate carboxykinase (PEPCK)	AGACAAACCCTCATGCCATGGCAAC	KX898506	226 bp
		GGGTCTATGATGGGGCACTGG		
Lipid synthesis	Sterol regulatory element binding protein 1 (SREBP1)	GGCCCTCTACTGCGTGGCACA	KX898507	194 bp
		ACCACCATTTGGAGTGAGGGTCAC		
	ATP citrate lyase (ACLY)	AGTTTGGCCACGCTGGAGCTTGT	KX898508	112 bp
		CCCAGCTCATCGAAGCTCTTGG		
	Acetyl-CoA carboxylase (ACC)	GAGCTGTCTATCAGAGGAGACTTCA	KF499584	139 bp
		GACGCTCGGCCTGCATCTTCT		
	Fatty acid synthase (FAS)	CCACACCATGGACCCACAGCT	KF511494	158 bp
		CTGGGTCTTTACTGAAGGCCTCT		
Fatty acids β -oxidation	Carnitine palmitoyl transferase 1 isoform a (CPT1a)	GAAGCTCATCAGGCTGTGGCCTT	KX898509	113bp
		TTCCAGGAGTGAAGTCCGGAGAG		
	Acyl-CoA oxidase 3 (ACO3)	TGTGGAGGACACGGTTACCTTGC	KX898510	115bp
		AGTTGCTGGTCTGCTGCAGAAGG		

Table 2

Plasma metabolite	Post-injection time	A strain		DT strain		<i>P</i>		
		Saline	Insulin	Saline	Insulin	Injection	Strain	I×S
Insulin (μg/L)	3 h	-	196.44±11.95 ^A	-	166.89±8.19 ^A	-	0.07	-
	8 h	-	17.39±4.05 ^B	-	0.69±0.09 ^B	-	<0.01	-
	12 h	-	3.34±0.77 ^C	-	0.61±0.09 ^B	-	<0.01	-
Glucose (mmol/L)	0 h	1.70±0.08	1.70±0.08 ^A	2.33±0.18	2.33±0.18 ^{AB}	-	0.03	-
	3h	2.19±0.14 ^b	1.12±0.15 ^{cB}	3.13±0.37 ^a	0.96±0.15 ^{cC}	<0.01	0.23	0.03
	8h	2.09±0.09 ^b	0.67±0.15 ^{cB}	2.68±0.11 ^a	2.56±0.18 ^{aA}	<0.01	<0.01	<0.01
	12h	2.06±0.15 ^b	2.13±0.18 ^{bA}	2.71±0.09 ^a	1.92±0.14 ^{bB}	0.02	0.16	<0.01
Triglycerides (mmol/L)	0 h	0.62±0.12	0.62±0.12	2.18±0.04	2.18±0.04	-	<10 ⁻⁴	-
	3h	0.60±0.20	0.75±0.17	2.10±0.12	2.24±0.08	0.35	<0.01	0.96
	8h	0.43±0.12	0.51±0.11	1.99±0.06	2.35±0.07	0.03	<0.01	0.14
	12h	0.61±0.16	0.82±0.12	2.38±0.05	2.49±0.08	0.16	<0.01	0.67
Free fatty acids (mEq/L)	0 h	0.25±0.04	0.25±0.04 ^A	0.64±0.06	0.64±0.06 ^B	-	<10 ⁻³	-
	3h	0.35±0.07	0.09±0.01 ^B	0.59±0.08	0.14±0.03 ^C	<0.01	<0.01	0.69
	8h	0.34±0.05 ^c	0.07±0.01 ^{dB}	0.62±0.04 ^b	0.90±0.08 ^{aA}	0.83	<0.01	<0.01
	12h	0.26±0.03	0.27±0.05 ^A	0.85±0.15	0.93±0.04 ^A	0.59	<0.01	0.68

Fig. 1

A

B

Fig. 2

A

B

Fig. 3

Fig. 4

Fig. 5

A

B

Fig. 6

A

B