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Effects of insulin infusion on glucose homeostasis and glucose metabolism in rainbow trout fed a high-carbohydrate diet

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SUMMARY

The origin for the poor glucose utilization in carnivorous fish species fed high carbohydrate diets remains under debate. In the present study, we have fed rainbow trout a diet containing 30% carbohydrate for 1 or 5 days. In both cases, fish were implanted with mini-osmotic pumps releasing 0.7 i.u. kg^{-1} day^{-1} bovine insulin, and mRNA transcripts and the protein phosphorylation status of proteins controlling glycemia and glucose-related metabolism were studied in fish killed 6 h after the last meal. We demonstrate that when the exposure occurs over a short term (30 h), insulin exerts beneficial actions on trout glucose homeostasis, including a lowered glycemia and increased hepatic lipogenic and glycogenic potentials. However, when trout were fed for 5 days, these beneficial actions of insulin infusion were no longer observed. Thus, the increased lipogenic potential observed after one single meal was not present, and this together with the increased glycogenesis and the decreased glucose exported to the blood from the liver explains the lack of hypoglycemic action of insulin. The fact that insulin improved glucose homeostasis when administrated over a short time period implies that endogenous insulin secretion is inadequate in trout to deal with this amount of dietary carbohydrates. Moreover, the fact that a longer exposure to insulin resulted in a reduced response indicates that the rainbow trout is sensitive to insulin, re-enforcing the hypothesis that the hyperglycemia observed following a high carbohydrate meal is an insulin secretion issue rather an insulin action issue.

Key words: insulin, fish, dietary carbohydrate, glucose utilization, glucose metabolism.

INTRODUCTION

Carnivorous fish species, including rainbow trout (*Oncorhynchus mykiss*), are traditionally considered as glucose intolerant (Moon, 2001; Wilson, 1994), primarily owing to the prolonged hyperglycemia experienced after a glucose load or the intake of a carbohydrate-enriched meal (Bergot, 1979; Palmer and Ryman, 1972). At the metabolic level, some of the enzymes involved in glucose metabolism, such as glucokinase (liver), phosphofructokinase (liver and muscle) and pyruvate kinase (liver and muscle), are inducible by dietary carbohydrates, as described in mammalian systems (Fideu et al., 1983; Panserat et al., 2000a; Panserat et al., 2001c), yet an atypical regulation of other actors involved in glucose metabolism is reported. For example, a reduced capacity for glucose phosphorylation by hexokinase in fish muscle compared with in mammals is reported (Kirchner et al., 2005). Moreover, a carbohydrate-rich diet in rainbow trout does not affect the activity or gene expression of key enzymes of gluconeogenesis, including glucose 6-phosphatase (G6Pase), fructose 1,6-bisphosphatase (FBPase) and phosphoenolpyruvate carboxykinase (PEPCK) (Kirchner et al., 2008; Panserat et al., 2001a; Panserat et al., 2000b; Panserat et al., 2001b).

Although a deficiency in insulin secretion was initially thought to be the basis for poor glucose utilization following a glucose load (Furuichi and Yone, 1981; Palmer and Ryman, 1972), plasma insulin levels in piscine species were later found to be even higher than those in mammals (Mommsen and Plisetskaya, 1991). Insulin functions primarily as an anabolic hormone by stimulating postprandial glucose uptake by liver and skeletal muscle, depressing rates of hepatic gluconeogenesis, and activating glycogenesis and lipogenesis. However, the fact that some carnivorous species remained 'glucose intolerant', even when insulin was effectively secreted (Moon, 2001), and that insulin levels correlate with fish mass (Sundby et al., 1991b) suggests also that this hormone can have an important role on fish growth and development (Mommsen and Plisetskaya, 1991). In fish, although the most prominent response to exogenous insulin injection is hypoglycemia (Ince, 1983), both the magnitude and duration of this effect are dependent on insulin type and dose, the route of injection, the season, nutritional state and previous nutritional history (Ince, 1983; Mommsen and Plisetskaya, 1991). The mechanism by which insulin regulates plasma glucose levels in fish remains unclear, as does the relative contribution of each of the main peripheral tissues that are sensitive (liver, muscle and adipose tissue) to this hormone (Navarro et al., 2006).

The data relating to insulin effects in fasted fish are abundant (reviewed by Mommsen and Plisetskaya, 1991). We previously demonstrated that the carnivorous rainbow trout responded in a predictable mammalian fashion to insulin in the fasted state, and no evidence of glucose intolerance was observed under these conditions (Polakof et al., 2010b). However, information regarding insulin effects on fed fish are scarce, with hepatic glycogenolysis being the most common effect observed in fish fed diets containing 20 to 57% carbohydrates (Machado et al., 1988; Ottolenghi et al., 1982; Sundby et al., 1991c).

Thus, based on the available information, we hypothesize that the poor utilization of dietary carbohydrates in rainbow trout is

related to a problem of either insulin secretion or insulin action. Therefore, the objectives of the present study on the carnivorous rainbow trout were: (1) to study the metabolic effects of continuous exogenous insulin infusion in fish fed with a high carbohydrate diet for one (test meal) or 5 days; and (2) to evaluate the ability of insulin to improve trout glycemic control when fed with a high carbohydrate diet. In addition, for the first time the three main insulin targets, skeletal muscle, white adipose tissue (WAT) and liver, were studied at both the biochemical and the molecular levels to analyze the potential molecular origins of the plasma glucose profiles observed in fish fed with a high carbohydrate diet. Thus, glycemia, glycogen levels and the mRNA levels of the main proteins involved in glucose metabolism were studied in liver, skeletal muscle and WAT.

MATERIALS AND METHODS

Fish

Immature rainbow trout (*Oncorhynchus mykiss* Walbaum) were obtained from the INRA experimental fish farm facilities of Donzacq (Landes, France). Fish were maintained in tanks kept in open circuits with 17°C well-aerated water and a controlled photoperiod (light:dark 12 h:12 h), and were fed with a standard trout commercial diet during the acclimation period (T-3P classic, Trouw, France). Fish mass was 200±10 g. The experiments were conducted following the Guidelines of the National Legislation on Animal Care of the French Ministry of Research (Decret no. 2001-464 of May 29, 2001) and were approved by the Ethics Committee of INRA (according to INRA 2002-36 of April 14, 2002).

Experimental protocols

For sustained hormone infusions, fish were food deprived for 48 h and then implanted with 1003D Alzet mini-osmotic pumps (Alza, Durect Corp., Cupertino, CA, USA) containing either saline (control, N=12) or a bovine insulin solution (N=12) (Sigma Chemical Co., St Louis, MO, USA). Owing to the lack of commercially available piscine insulin, bovine insulin was administered. Bovine insulin was demonstrated in early fish studies to modify glycemia *in vivo* (Ince and Thorpe, 1978; Inui and Gorbman, 1977; Warman and Bottino, 1978), and more recently to modify transcript levels of genes known to be insulin sensitive (Polakof et al., 2010a; Polakof et al., 2010b). Additionally, the use of mammalian insulin avoids the complications of multiple piscine isoforms of insulin (Caruso et al., 2008; Mommsen et al., 2002). Pump flow rate was established to be 0.39 µl h^{-1}, which at 17°C should provide sustained release of 0.7 i.u. kg^{-1} day^{-1} insulin for 11 days. Fish were first anesthetized, body mass estimated, and pumps inserted into the peritoneal cavity through a 1.0-cm incision made in the ventral midline at *ca.* 2.0 cm rostral of the pelvic fins. The incision was closed with one stitch and an antibiotic gel applied topically to the incision area. Pumps were implanted in the morning then fish were allowed to recover. The next morning (24 h later), fish were fed with a diet containing a high level of carbohydrate (30% dextrin, 57% fish meal and 10% fish oil), and 6 h later (30 h from implantation), six animals from each treatment group were sampled (30 h group). The remaining fish (six per group) were fed the same high-carbohydrate diet for 4 additional days, and then were sampled 6 h after their last meal (5 day group).

Tissue and blood sampling

Trout were sacrificed by a sharp blow on the head. Blood was removed from the caudal vessels and centrifuged (3000 ***g***, 5 min); the recovered plasma was immediately frozen and kept at –20°C pending analyses. The gut contents of each fish were systematically checked to assert that the fish sampled had effectively consumed the diet. Liver, perivisceral WAT and a sample of dorsoventral white muscle were immediately dissected, weighed and frozen in liquid nitrogen and kept at –80°C pending analyses.

Molecular and biochemical analyses

Plasma glucose levels were determined using a commercial kit (Biomérieux, France) adapted to a microplate format. Bovine insulin levels were measured using a bovine-specific commercial ELISA kit (Mercodia, Uppsala, Sweden). Tissue glycogen levels were determined following the method of Keppler et al. (Keppler et al., 1974).

Tissue mRNA levels of proteins involved in glucose transport and metabolism were determined by real-time quantitative RT-PCR (q-PCR) (Polakof et al., 2009). The transcripts assessed were GLUT4 (glucose facilitative transporter type 4), GK (glucokinase), HK (hexokinase), PK (pyruvate kinase), 6PF1K (6-phophofructo-1-kinase), G6Pase1 (glucose 6-phosphatase 1), FBPase (fructose 1,6-bisphosphatase), PEPCK (phosphoenolpyruvate carboxykinase), FAS (fatty acid synthase), G6PDH (glucose 6-phosphate dehydrogenase) and SREBP-1c-like (sterol regulatory element binding protein 1-like). Primers were designed to overlap an intron where possible (Primer3 software) using known sequences found in trout nucleotide databases (GenBank and INRA-Sigenae), as previously described (Polakof et al., 2009). Quantification of the target gene transcript level was performed using *ef1*α gene expression as reference (Pfaffl, 2001), which was found to be stably expressed in this study. Relative quantification of the target gene transcript with the *ef1*α reference gene transcript was made following the Pfaffl method (Pfaffl, 2001).

Protein extraction (20 µg of protein for liver and WAT, and 40 µg for muscle) and western blotting were undertaken using anti-phospho-Akt Ser^{473} and anti-Akt antibodies (Cell Signaling Technology, Ozyme, St Quentin-en-Yvelines, France), which we previously demonstrated cross-react with rainbow trout Akt protein (Polakof et al., 2009).

Statistical analysis

Results are expressed as means ± s.e.m. (N=6). Data were analyzed by one-way ANOVA. When necessary, data were log transformed to fulfill the conditions of the analysis of variance. *Post hoc* comparisons were made using a Student–Newman–Keuls test, and differences were considered statistically significant at P<0.05.

RESULTS

Plasma glucose levels in fish fed one high carbohydrate meal and implanted with saline pumps were hyperglycemic (10.5±1.2 mmol l^{-1}) 30 h post-meal compared with fish fed the commercial diet (~5 mmol l^{-1}) (see Capilla et al., 2003; Panserat et al., 2000a) and with fasted trout implanted with saline pumps (~4.5 mmol l^{-1}) (see Polakof et al., 2010b). Moreover, fish implanted with pumps delivering bovine insulin and fed once with the carbohydrate meal showed significantly lower glycemic levels (7.5±0.4 mmol l^{-1}) than did the control group holding saline pumps and fed with carbohydrates. Bovine insulin levels were constant across all trout with insulin pumps, and averaged 6 ng ml^{-1} (data not shown). When trout were fed for an additional 4 days (5 days total) with a high carbohydrate diet, plasma glycemia was independent of insulin infusion: 9.1±0.4 for the control and 9.0±0.4 mmol l^{-1} for insulin-infused fish.

Hepatic mRNA transcript levels encoding enzymes involved in carbohydrate metabolism are shown in Fig. 1. When fish were exposed to insulin for 30 h and fed once with 30% carbohydrate,

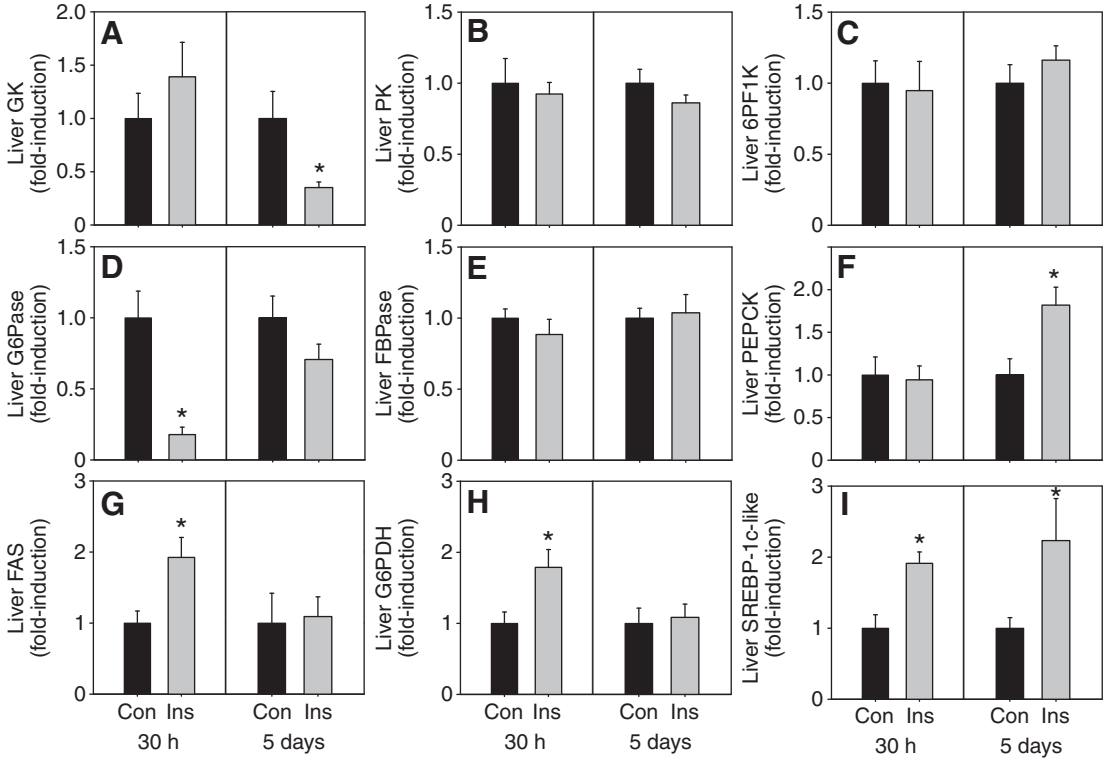

Fig. 1. Effects of insulin infusion (30 h or 5 days) on the levels of mRNA transcripts encoding hepatic genes in trout fed a high-carbohydrate diet. (A) Glucokinase (GK), (B) pyruvate kinase (PK), (C) 6-phophofructo-1-kinase (6PF1K), (D) glucose 6-phosphatase (G6Pase), (E) fructose 1,6-bisphosphatase (FBPase), (F) phosphoenolpyruvate carboxykinase (PEPCK), (G) fatty acid synthase (FAS), (H) glucose 6-phosphate dehydrogenase (G6PDH) and (I) sterol regulatory element binding protein 1-like (SREBP-1c-like). mRNA levels were estimated using real-time RT-PCR. Expression levels were normalized to elongation factor 1α (EF1α)-expressed transcripts, which did not change under the experimental conditions and which are presented as fold change against the saline solution-treated group set to 1. Results are presented as means + s.e.m. (N=6) and were analyzed by one-way ANOVA followed by a Student–Newman–Keuls comparison test. Asterisks indicate a significant difference (P<0.05). Con, control; Ins, insulin.

mRNA levels of glycolytic enzymes including GK (Fig. 1A), PK (Fig. 1B) and 6PF1K (Fig. 1C) were unaffected compared with those of the saline-control group. Gluconeogenic enzyme mRNA levels were differentially affected by insulin infusion with G6Pase (Fig. 1D) being strongly downregulated (approximately eightfold), and FBPase (Fig. 1E) and PEPCK (Fig. 1F) remaining unchanged. mRNA levels of enzymes involved in lipogenesis, including FAS (Fig. 1G) and G6PDH (Fig. 1H), as well as the transcription factor SREBP-1c-like (Fig. 1I), were enhanced by the insulin treatment. After 5 days of carbohydrate feeding, trout receiving insulin infusions showed lower mRNA levels for GK than did the saline controls, whereas PEPCK and SREBP-1c-like transcript levels were enhanced. The activation by insulin of hepatic FAS and G6PDH mRNA was abolished at day 5.

Few changes were observed in the relative transcript levels of genes encoding muscle proteins involved in carbohydrate metabolism (Fig. 2). Except for an increased GLUT4 (Fig. 2A) mRNA level in trout with insulin pumps and fed once, no other changes were observed in transcript levels for muscle HK (Fig. 2B), PK (Fig. 2C) or 6PF1K (Fig. 2D) between the insulin and saline groups. The change noted at 30 h for GLUT4 disappeared when trout were fed the high carbohydrate diet for 5 days; no other changes were observed in muscle transcripts at 5 days.

WAT mRNA levels encoding proteins involved in carbohydrate metabolism are presented in Fig. 3. After 30 h of infusion, insulin was without effects on the mRNA levels of proteins involved in glucose transport (GLUT4, Fig. 3A) and glycolysis (HK, Fig. 3B; 6PF1K, Fig. 3D), with the exception of PK (Fig. 3C), which was downregulated (approximately twofold) when compared with the saline-infused group. However, unlike the short-term treatments, when fish were treated for 5 days, GLUT4 and HK mRNA levels remained unchanged between the saline- and insulin-treated groups. Concerning the lipogenic pathway, whereas mRNA levels for FAS (Fig. 3E) remained unaffected, transcripts for G6PDH (Fig. 3F) were regulated by insulin infusion up to 3-fold above the levels of the saline control. When fish were fed the high-carbohydrate meal for 5 days and were insulin infused, PK mRNA levels were upregulated compared with those of the saline-control group, whereas the 6PF1K mRNA levels were slightly reduced. Similarly, opposing results were observed in the lipogenic pathway after longer-term feeding. Thus, whereas FAS mRNA transcripts were upregulated (approximately threefold) by insulin infusion, G6PDH mRNA levels were downregulated at 5 days.

Glycogen levels were variable between tissues (Fig. 4). In fish receiving insulin infusions for 30 h, glycogen levels were increased in liver (Fig. 4A), reduced in WAT (Fig. 4C) and unchanged in muscle (Fig. 4B). After 5 days of high-carbohydrate feeding, only hepatic glycogen was affected by insulin infusion, with levels higher than those of the saline control and higher than those at the 30 h time point.

The phosphorylation status of Akt (serine-473) in liver, skeletal muscle and WAT are reported in Fig. 5. In trout fed a high-carbohydrate diet and insulin infused for 30 h or 5 days, the Akt phosphorylation status was unaltered in liver (Fig. 5A) when compared with that of the saline control. However, increased Akt phosphorylation was observed in both muscle (Fig. 5B) and WAT (Fig. 5C) after both 30 h and 5 days of treatment.

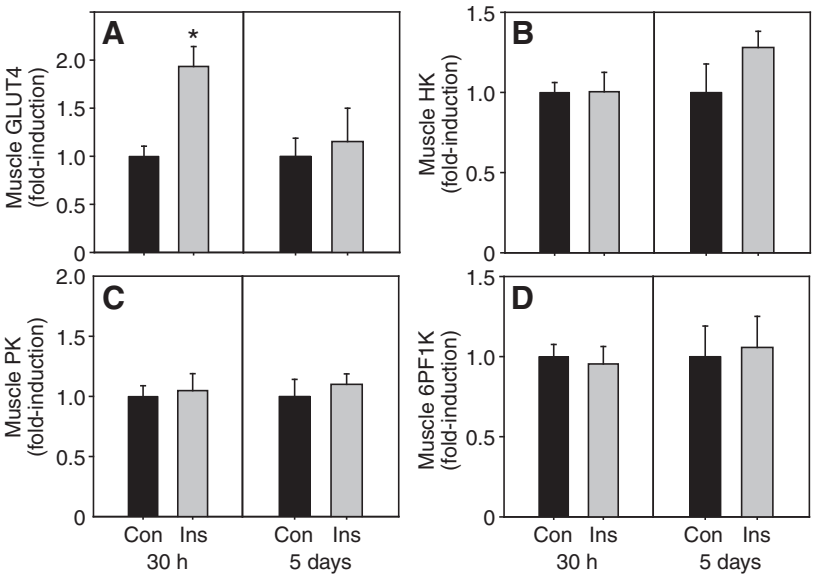

Fig. 2. Effects of insulin infusion (30 h or 5 days) on the levels of mRNA transcripts encoding muscle genes in trout fed a high-carbohydrate diet. (A) Glucose facilitative transporter type 4 (GLUT4), (B) hexokinase (HK), (C) pyruvate kinase (PK) and (D) 6-phophofructo-1-kinase (6PF1K). See Fig. 1 legend for details. Results are presented as means + s.e.m. (N=6) and were analyzed by one-way ANOVA followed by a Student–Newman–Keuls comparison test. Asterisks indicate a significant difference (P<0.05). Con, control; Ins, insulin.

DISCUSSION

The metabolic origin for the low dietary glucose utilization in carnivorous fish species such as rainbow trout remains a matter of debate (Panserat, 2009; Wilson, 1994). Although trout apparently possess an adequate ability to deal with glucose in the fasted state (Moon, 2001; Polakof et al., 2010b), when fed a high-carbohydrate diet they are less able to trigger appropriate metabolic responses to maintain normoglycemia (Enes et al., 2008; Hemre et al., 2002; Krogdahl et al., 2005; Panserat, 2009). Trout are able to secrete insulin in the postprandial state (Navarro et al., 2002), but when fed a carbohydrate-enriched meal they do not increase insulin levels significantly in the plasma when compared with carbohydrate-free

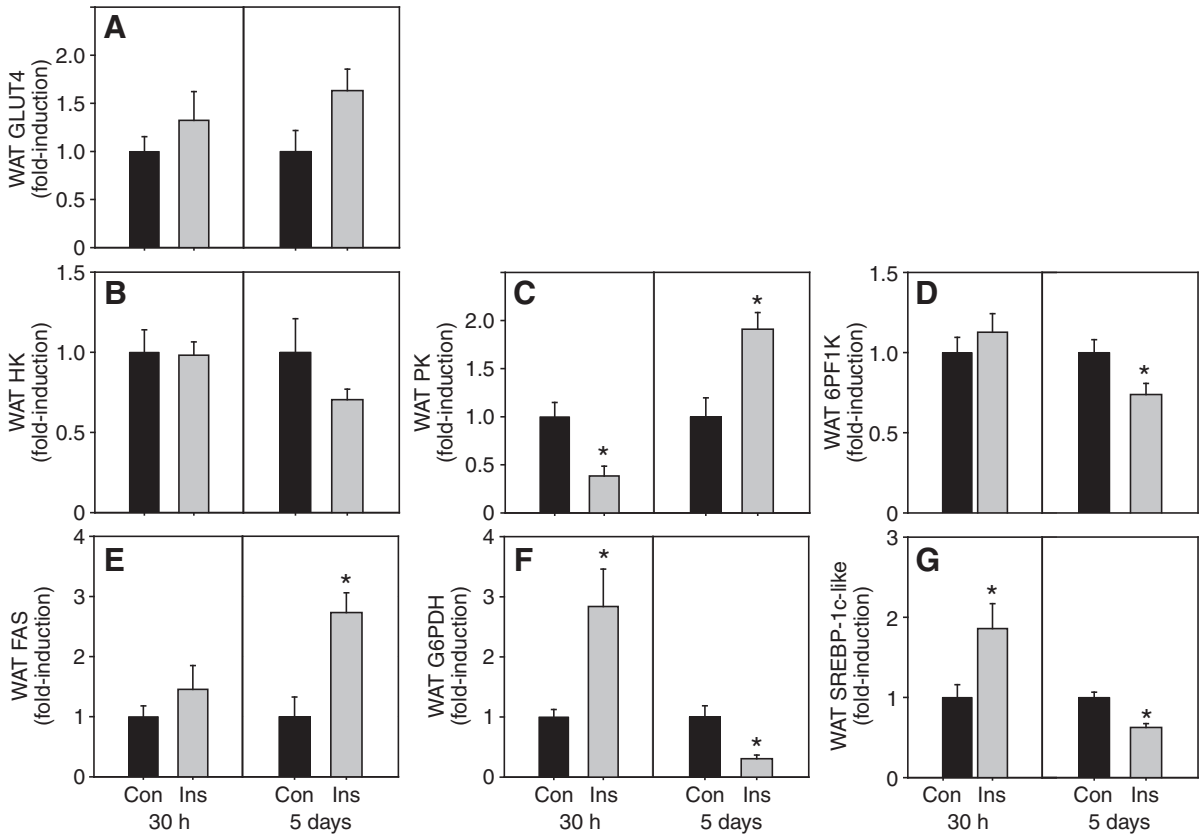

Fig. 3. Effects of insulin infusion (30 h or 5 days) on the levels of mRNA transcripts encoding white adipose tissue (WAT) genes in trout fed a high-carbohydrate diet. (A) Glucose facilitative transporter type 4 (GLUT4), (B) hexokinase (HK), (C) pyruvate kinase (PK), (D) 6-phophofructo-1-kinase (6PF1K), (E) fatty acid synthase (FAS), (F) glucose 6-phosphate dehydrogenase (G6PDH) and (G) sterol regulatory element binding protein 1-like (SREBP-1c-like). See Fig. 1 legend for details. Results are expressed as means + s.e.m. (N=6) and were analyzed by one-way ANOVA followed by a Student–Newman–Keuls comparison test. Asterisks indicate a significant difference (P<0.05). Con, control; Ins, insulin.

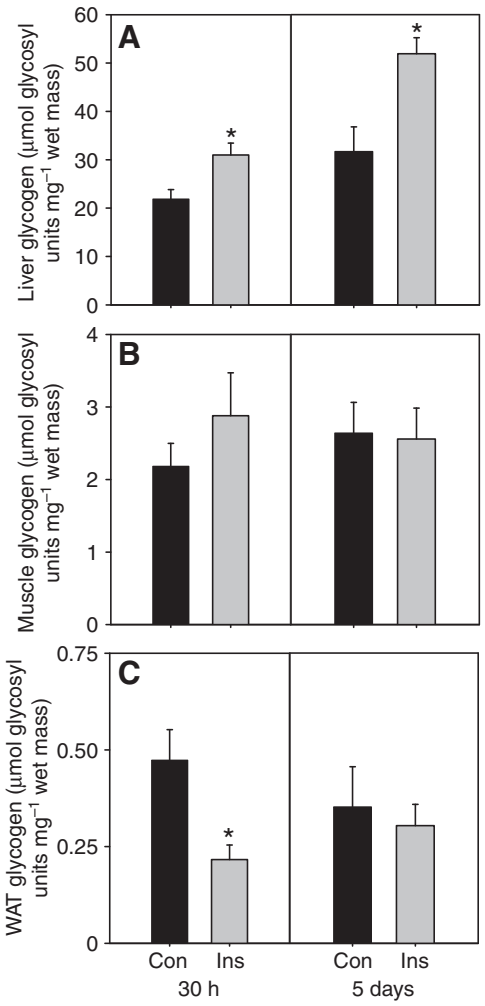

Fig. 4. Effects of insulin infusion (30 h or 5 days) on (A) liver, (B) white muscle and (C) white adipose tissue (WAT) glycogen levels in trout fed a high-carbohydrate diet. Results are expressed as means + s.e.m. (N=6) and were analyzed by one-way ANOVA followed by a Student–Newman–Keuls comparison test. Asterisks indicate a significant difference (P<0.05). Con, control; Ins, insulin.

fed trout (Capilla et al., 2003). Thus, in the present study insulin was delivered at a constant rate using implanted pumps while the trout were fed for 1 or 5 days with a carbohydrate-enriched diet to determine whether exogenous insulin could improve glycemia in this 'glucose-intolerant' species. Bovine insulin levels were estimated at 6 ng ml^{-1} across all trout implanted with pumps containing insulin; these circulating insulin values are relatively low when compared with trout plasma insulin values estimated by the partly homologous RIA analysis (bonito) (Capilla et al., 2003; Novoa et al., 2004), but within the physiological range when compared with fully homologous RIA analysis (salmon) (Sundby et al., 1991a; Sundby et al., 1991b).

Insulin improves glycemia after feeding one high-carbohydrate meal

Rainbow trout fed one carbohydrate-enriched meal 24 h after being implanted with an insulin pump showed a lower glycemia 6 h after feeding than did trout infused only with saline. These results are similar to those found by Sundby et al. in Atlantic salmon fed with a diet containing 19% carbohydrates and receiving a single insulin administration (Sundby et al., 1991c), even though plasma insulin (exogenous) levels were higher than in the present study. Other studies reported similar results, but comparisons are often difficult as the diets used contained a lower carbohydrate content (~5%), or the insulin dose was pharmacological and the animal model different (omnivorous species) (Ottolenghi et al., 1982).

Trout with insulin pumps for 30 h displayed liver changes that help to explain the lower glycemia exhibited in these fish compared with the saline-control group. In particular the liver demonstrated increased lipogenic potential and glycogen deposition. The enhanced transcript levels for key enzymes (FAS and G6PDH) and transcriptional factors involved in lipogenesis (SREBP-1c-like) support an increased lipogenic potential as previously reported in trout, with insulin and glucose as major regulators (Cowley and Sheridan, 1993; Polakof et al., 2009). The increased glycogen levels previously reported in catfish (Ottolenghi et al., 1982), and the decreased G6Pase mRNA levels shown here, further support the idea that insulin is stimulating glycogen deposition and depressing glucose export to the blood, thus preventing the hyperglycemia observed in the saline-control group. These results are further supported by the report that the repression of the G6Pase1 gene by glucose is overcome by insulin (Polakof et al., 2009); thus, it is not surprising that the poor inhibition of the G6Pase gene in trout fed carbohydrates (Panserat et al., 2001a) could be increased by the addition of insulin, improving glycemic control in insulin-infused trout. Other aspects of hepatic glucose metabolism were unaffected by insulin treatment, including mRNA levels of proteins involved in glycolysis and gluconeogenesis. These results are not surprising as enzymes involved in glycolysis are generally poorly affected at the molecular level by either dietary carbohydrates (Panserat et al., 2001c), insulin (Polakof et al., 2010b), or the combination of glucose and insulin.

Changes in the white muscle parameters of trout were minimal whether trout were continuously infused with insulin or with saline. Only GLUT4 mRNA levels were increased, as reported in other studies in which fish were fed with high-carbohydrate diets (Díaz et al., 2009; Polakof et al., 2010b), providing further evidence that the fed status and/or nutrient interactions might be responsible for these GLUT4 differences. Moreover, the phosphorylation and thus activation of Akt in white muscle indicates that at least part of the insulin signaling pathway is affected, supporting a possible stimulation of glucose uptake through GLUT4 (the mRNA levels of which were enhanced) (Yeaman et al., 2001) and further improvements to glycemia.

Metabolic information regarding carbohydrate metabolism in fish WAT is scarce (Mommsen and Plisetskaya, 1991; Navarro et al., 2006). Although the action of insulin on trout adipocyte glucose uptake was reported (Bouraoui et al., 2010), data regarding the effect of insulin *in vivo* on WAT glucose metabolism remain scarce. However, no major changes were found in mRNA transcript levels for proteins involved in glucose transport or glycolysis, whereas glycogen levels paradoxically decreased in insulin-infused trout. Although the insulin dependency of these processes remains to be elucidated, the fact that P-Akt was increased in insulin-infused fish compared with the saline-control group supports a possible role for insulin in the WAT, as reported in the *in vitro* study of Bouraoui et al. (Bouraoui et al., 2010).

Longer-term insulin infusion has no impact on glycemia in high-carbohydrate-fed trout

Trout subjected to a 5-day insulin infusion and fed a carbohydrate-enriched diet showed no glycemic differences when compared with

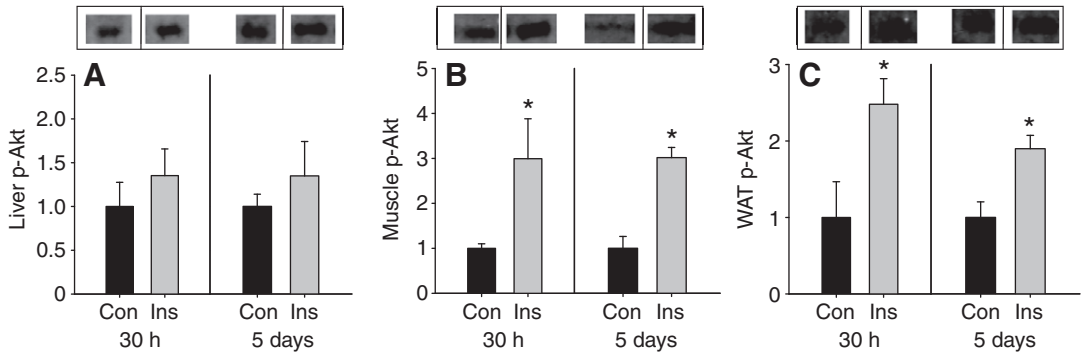

Fig. 5. Effects of insulin infusion (30 h or 5 days) on (A) liver, (B) white muscle and (C) white adipose tissue (WAT) Akt phosphorylation status (western blot analysis) in trout fed a high-carbohydrate diet. Gels were loaded with 20 μg total protein per lane. Protein phosphorylation levels were normalized to tissue β-tubulin levels and are indicated as fold-change compared with the saline-treated group. Results are expressed as means + s.e.m. (N=6) and were analyzed by one-way ANOVA followed by a Student–Newman–Keuls comparison test. Asterisks indicate a significant difference (P<0.05). Con, control; Ins, insulin.

trout receiving only saline. The lack of glycemic change might be related to the longer hormone exposure period and thus a decreased tissue sensitivity associated with changes in insulin receptors on the membrane (Baños et al., 1998) and/or adaptation to the diets in the insulin levels and tissue metabolic outputs (Novoa et al., 2004). At the metabolic level, transcripts of enzymes involved in hepatic glycolysis and gluconeogenesis were unaffected by the insulin treatment even though hepatic glycogen levels remained elevated. The absence of glycogen changes might reflect the decreased hepatic GK mRNA levels and the lack of change in G6Pase mRNA levels, potentially preventing glucose export to the blood. In comparison with the single meal result, the other main difference found after 5 days of feeding is the lack of effect of the insulin treatment on lipogenic enzyme mRNA levels. A potentially reduced lipogenesis could explain the maintenance of glycemia experienced by the insulin-infused group during the long-term experiment when compared with the control (Panserat et al., 2009; Skiba-Cassy et al., 2009).

Few changes were noted in the metabolic actors studied in skeletal muscle at the molecular level, except for the higher P-Akt levels. We previously reported that, in fasted trout insulin infused for 4 days, Akt phosphorylation status was reduced, making the tissue less sensitive at the metabolic level (Polakof et al., 2010b). The fact that no negative effect was found in the present study supports the involvement of the nutritional status of the animal; specifically, we hypothesize that the fed state prevents insulin desensitization of trout muscle exposed to a longer-term insulin infusion. Because no major changes were noted in other metabolic factors, additional studies are needed to test this hypothesis.

Despite the maintenance of an enhanced P-Akt in WAT with longer-term insulin infusion, no changes in WAT glycogen levels were found. By contrast, increased levels of FAS mRNA were observed, even when transcript levels of G6PDH and SREBP-1c-like were downregulated. Even though G6PDH activities were not assessed in this study, we do not find that G6PDH activities are influenced by insulin in trout WAT (S. Polakof, unpublished observations), which suggests that the apparent uncoupling between lipogenesis and NAPDH donors might be restricted to the molecular level. Only minor changes were observed for mRNA levels of proteins involved in glucose transport and metabolism in WAT. Globally, despite a marked sensitivity to insulin, no major changes were found in WAT glucose metabolism at the molecular level that could explain how insulin regulates glycemia in trout, which suggests that insulin might be regulating other aspects of the WAT metabolism, including the lipid pathways (Albalat et al., 2005; Navarro et al., 2006).

Does insulin infusion improve carbohydrate use in trout?

The utilization of dietary carbohydrates in carnivorous fish remains a matter of debate (Hemre et al., 2002; Wilson, 1994). In this study, we tested the ability of exogenous insulin administration to improve glucose homeostasis in rainbow trout fed a high-carbohydrate diet. We demonstrate that when the exposure occurs over a short term (30 h), insulin exerts beneficial actions on trout glucose homeostasis, including a lowered glycemia, and increased hepatic lipogenic and glycogenic potentials compared with the saline-control group. Surprisingly, no positive response was found in white muscle metabolism, even with evidence for the activation of the insulin-signaling pathway. When trout were fed for 5 days rather than for 1 day, these beneficial actions of insulin infusion were no longer observed. Although the continuous exposure of fasted trout to insulin was previously reported to result in insulin insensitivity (Polakof et al., 2010b), this do not seems to be the case in the present study, at least at the level of Akt phosphorylation status. However, we cannot overlook that the chronic exposure to insulin might lead to impaired insulin action without affecting insulin sensitivity as reported in mammals (McGuinness et al., 1990). At the metabolic level, the increased lipogenic potential observed after one single meal was not present after 5 days of feeding and, together with the increased glycogenesis and decreased glucose exported to the blood from the liver, seems to be the major explanation for the lack of hypoglycemic action of insulin. As a whole, we have found that when trout are fed a high-carbohydrate diet, the main actions of insulin are at the hepatic level, whereas changes in white muscle and WAT are minor, or are possibly related to other metabolic pathways not measured here (Albalat et al., 2005; Sánchez-Gurmaches et al., 2010). The fact that insulin improved glucose homeostasis when administrated over a short time period (at least up to 30 h) implies that endogenous insulin secretion is inadequate in trout to deal with this amount of dietary carbohydrates. This hypothesis is supported by the minor increase in circulating insulin levels in fish fed with high- compared with low-carbohydrate diets (Capilla et al., 2003), as well as by the fact that amino acids are stronger secretagogues of insulin than of glucose in fish (Andoh, 2007; Ronner and Scarpa, 1987), and could help to explain the poor dietary carbohydrate utilization in this carnivorous species (Hemre et al., 2002; Wilson, 1994). Thus, we hypothesize that the poor response shown by trout to insulin is related to the low sensitivity of the Brockmann Bodies insulin secretion system to glucose rather than to the insulin secretion system *per se.* Moreover, the fact that a longer exposure to insulin resulted in a reduced response indicates

that the rainbow trout is sensitive to insulin, re-enforcing the hypothesis that the hyperglycemia observed following a high-carbohydrate meal is an insulin secretion issue rather an insulin action issue. To dissociate these explanations, further studies are needed that focus on the insulin secretion pathway.

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