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RESEARCH ARTICLE

High levels of dietary fat impair glucose homeostasis in rainbow trout

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SUMMARY

This study was designed to assess the effects of dietary fat levels on glucose homeostasis in rainbow trout under prolonged hyperglycaemia induced by high carbohydrate intake. Trout were fed identical amounts of one of two iso-energetic diets containing either a low (LFD, 3%) or a high fat level (HFD, 20%) and similar amounts of digestible carbohydrates (26–30%) for 14 days. While a single high fat meal reduced glycaemia compared with a low fat meal, the consumption of a high fat diet for 14 days resulted in prolonged hypergylcaemia and reduced plasma glucose clearance in response to an exogenous glucose or insulin challenge. The hyperglycaemic phenotype in trout was characterised by a reduction of the activities of lipogenic and glucose phosphorylating enzymes with a concomitant stimulation of enzymes involved in glucose production in the liver and reduced glycogen levels in the white muscle. Impaired glucose tolerance (IGT) was further associated with a significant reduction of insulin receptor substrate 1 (IRS1) protein content in muscle, and with a poor response of HFD fed fish to an exogenous insulin load, suggestive of impaired insulin signalling in trout fed with a HFD. To our knowledge, this is the first study showing that a teleost can also develop a high fat-induced IGT, characterised by persistent hyperglycaemia and reduced insulin sensitivity, established symptoms of IGT and the prediabetic insulin-resistant state in mammals. Our results also provide evidence that persistent hyperglycaemia after a high carbohydrate meal stems from a metabolic interaction between dietary macronutrients rather than from high carbohydrate intake alone.

Key words: high fat diet, glucose homeostasis, rainbow trout, insulin sensitivity.

INTRODUCTION

Impaired glucose tolerance (IGT) and impaired fasting glucose form an intermediate stage in the development of diabetes mellitus in mammalian species including man (McGarry, 1994). Besides carbohydrate intake, the relative proportions of dietary macronutrients also appear to play a major role in the development of IGT. Elevated levels of circulating free fatty acids (FFA), following the consumption of a high fat diet (HFD) are known to impair insulin function (Kraegen et al., 1986; Pedersen et al., 1991; Wang et al., 2002). This is further supported by the demonstration that increased levels of circulating FFA, through intravenous lipid infusion, induce insulin resistance (IR) in rodent and human skeletal muscle (Dresner et al., 1999; Pan et al., 1997; Shulman, 2000). As a result, feeding a HFD to mammalian models has been confirmed as a useful approach for the study of metabolic disorders such as IGT, IR, obesity and type 2 diabetes (Ahren et al., 1999; Kraegen et al., 1986; Storlien et al., 1997).

Rainbow trout (*Oncorhynchus mykiss*) is considered to be glucose intolerant, and is characterised by a prolonged hyperglycaemia following a glucose load or carbohydrate-rich meal (Moon, 2001). The limited ability of trout to regulate glycaemia seems to relate to an expected induction of glycolytic capacity and an unexpected non-inhibition of the gluconeogenic potential in the liver by insulin or dietary carbohydrates (Panserat et al., 2001; Polakof et al., 2011b). The reduced number of insulin receptors in muscle and the low affinity of glucose transporter proteins for glucose are also proposed as possible causes of the low carbohydrate use in fish (Gutiérrez et al., 2006; Moon, 2001; Navarro et al., 1999). However, fasted rainbow trout were recently demonstrated to show efficient use of exogenous glucose and insulin sensitivity (Polakof et al., 2010). This finding suggests that the poor utilisation of dietary carbohydrates and the persistent hyperglycaemia in this species stems from an interaction between the dietary components rather than from an intrinsically unregulated glucose homeostasis. In fact, the interaction between dietary carbohydrates and lipids is well known to adversely affect glucose metabolism in mammals, eventually causing IGT (Randle, 1998). In fish, attempts to understand the nutritional origin of glucose intolerance has mainly focused on the carbohydrate/protein ratio (Kirchner et al., 2003) without considering the lipid content of the diet, despite observations of increased glycaemia after the consumption of a HFD, as seen in salmonids (Hemre and Sandnes, 1999; Mazur et al., 1992), grouper (Epinephelus coioides) (Cheng et al., 2006) and sunshine bass (*Morone chrysops*×*M. saxatilis*) (Hutchins et al., 1998), sometimes associated with hyperinsulinaemia (Shearer et al., 1997). To our knowledge, only one study has suggested a link between the abnormally high hepatic gluconeogenic potential in rainbow trout and the dietary lipid content (Panserat et al., 2002). Additionally, long-term palmitate feeding has been reported to induce hyperinsulinaemia, hyperglycaemia and loss of insulin sensitivity in the omnivorous Indian perch (*Anabas testudineus*), classic symptoms of the development of insulin resistance in mammals (Barma et al., 2006), suggesting that the mechanisms mediating the control and possible disturbance of glucose homeostasis are being conserved through evolution (Polakof et al., 2011b). However, for the past two decades, feed formulations for intensively farmed fish have tended to increase both fat (Company et al., 1999; Grisdale-Helland and Helland, 1997; Hillestad and Johnsen, 1994) and

digestible carbohydrate (Fernández et al., 2007; Kaushik and Oliva-Teles, 1985; Venou et al., 2003) content as non-protein energy sources in order to spare protein for growth and reduce nitrogen loss to the aquatic environment. The positive protein-sparing effects of increased dietary lipid and digestible carbohydrate are, however, often associated with adverse effects in terms of fat deposition (Company et al., 1999; Jobling et al., 1998) and with the development of IGT in fish (Bergot, 1979; Cheng et al., 2006; Hutchins et al., 1998; Polakof et al., 2010).

Therefore, in the present study, we hypothesised that a high dietary fat level combined with a carbohydrate-rich diet may have detrimental effects on the metabolic control of glucose homeostasis in rainbow trout. In order to evaluate this possible metabolic interaction between dietary fat and carbohydrates, we fed rainbow trout for 14 days with a HFD (20% fat) and the time course changes in glycaemia were followed at 1, 7 and 14 days in comparison with a group fed a high protein, low fat diet (LFD; 3% fat). The two diets contained similar amounts of digestible carbohydrates (26–30%). In order to evaluate changes (related to diet and time of feeding) in the control of glucose homeostasis, fish were also subjected to glucose (GTT) and insulin (ITT) tolerance tests in the fasted state during the course of the feeding trial. To understand the metabolic origin of the diet-induced hyperglycaemic phenotype, key enzymes of the glucose and lipid metabolic pathways, glycogen levels and proteins involved in the insulin-signalling pathway were evaluated.

Table 1. Ingredients and analysed composition of the diets fed to rainbow trout

Ingredients (%)	LFD	HFD
Gelatinised starch[a]	30.0	24.3
Fat blend[b]	1.0	19.1
Protein blend[c]	66.0	35.9
Cellulose[d]	0.0	15.0
Other[e]	3.0	5.7
Analysed composition		
Dry matter (DM) (% diet)	92.4	94.9
Digestible protein (% DM)	49.1	26.4
Digestible lipid (% DM)	3.1	19.9
Digestible carbohydrate (% DM)[f]	30.9	26.0
Digestible energy (kJ g^{-1} DM)	18.3	18.7

[a]Gelatinised corn starch (Roquette, Lestrem, France).
[b]Lipid blend (see Materials and methods, % diet): 1% (low fat diet, LFD) and 14.1% (high fat diet, HFD) rapeseed oil (Daudruy, Dunkerque, France) and 5% (HFD) fish oil (Sopropêche, Lorient, France).
[c]Protein blend (% protein blend): 50% fishmeal (Sopropêche), 16.5% soybean protein concentrate (Sopropêche); 16.5% pea protein concentrate (Roquette), 16.5% wheat gluten (Roquette) and 0.5% DL methionine (Ajinomoto Eurolysine, Paris, France).
[d]Cellulose (Rettenmeier et Sohne, Rosenberg, Germany).
[e]Other (% diet): 2% Diamol (indigestible marker, Diamol GM, Franz Bertram, Hamburg, Germany), 1% premix (INRA UPAE, Jouy en Josas, France) and (for HFD) 0.4% $CaCO_3$, 1.8% $Ca(HPO_4)_2$, 0.5% Na_2CO_3.
[f]Calculated as (DE_{total}–$DE_{protein}$–DE_{lipid})/17.7 (where DE is digestible energy), using caloric values (kJ g^{-1}) of 23.7, 39.6 and 17.7 for protein, fat and carbohydrate, respectively.

MATERIALS AND METHODS

Animal care

Juvenile rainbow trout (*O. mykiss*, Walbaum) were obtained from the INRA experimental fish farm of Donzacq (Landes, France) and fed a commercial trout diet during the pre-experimental period (Skretting France, 40% crude protein and 24% crude fat as fed). The feeding trial was conducted in a recirculating rearing system (INRA, St-Pée-sur-Nivelle, France) at a water temperature of 17°C and controlled photoperiod (12 h L:12 h D). The fish were weighed and sorted prior to stocking in order to obtain homogeneous groups of fish of similar (100±10 g) individual body mass (24 fish per 70 l tank). The experiments were conducted following the Guidelines of the National Legislation on Animal Care of the French Ministry of Research (decree no. 2001-464 of 29 May 2001) and was approved by the Ethics Committee of INRA (according to INRA 2002-36 of 14 April 2002).

Feeding trials

Prior to the feeding trials, fish were food deprived for 72 h – the time required to ensure the complete emptying of the digestive tract. After this period, trout were fed for 1 (single test meal), 7 or 14 days with two experimental diets with identical digestible energy (DE, 18 kJ g^{-1} of dry matter) containing 3% digestible fat and 49% digestible protein (LFD) or 20% digestible fat and 26% digestible protein (HFD). Digestible nutrient and energy contents of the diets were determined previously in our laboratory and are detailed in Table 1. The digestible carbohydrate content of the diets was 30.9 g (LFD) and 26 g (HFD) per 100 g of dry matter. The digestible carbohydrate content of the diets was calculated as (DE_{total}–$DE_{protein}$–DE_{lipid})/17.7, using caloric values of 23.7, 39.6 and 17.7 kJ g^{-1} for protein, fat and carbohydrates, respectively). In order to assess the ability of fish fed a HFD to reverse their glycaemic profiles, 1 week after the beginning of the experiment, the HFD group was divided into two sub-groups, which were fed thereafter (1 week) with either a HFD or a LFD (HFD→LFD). Triplicate groups of fish were fed identical (pair feeding) amounts of feed (~1.5 g per 100 g body mass per day) once a day (09:00 h) in order to avoid possible differences in voluntary food intake induced by the fat content (Saravanan et al., 2011). Blood was sampled from six individual fish per treatment before the meal (0 h) and at postprandially at 3, 6, 12 and 24 h on days 1, 7 and 14. Blood samples were taken by caudal vein puncture and collected into tubes using heparin as an anti-coagulant.

For tissue collection, six trout per group were anaesthetised with 2-phenoxyethanol (0.05%) and killed by a sharp blow on the head 6 h after feeding (on days 1, 7 and 14). Liver and a sample of white muscle (hypaxial muscle taken anterior of the dorsal fin) and perivisceral white adipose tissue (WAT) were immediately dissected, frozen in liquid nitrogen and kept at –80°C for subsequent analysis. To ensure that the fish sampled had effectively consumed the diet, the gut content was systematically checked.

GTT and ITT

In order to analyse the effect of dietary treatment on the ability of trout to deal with exogenous glucose, fish were subjected to intraperitoneal GTT at different points during the feeding trial. For this, six fish per treatment were injected intraperitoneally (5 ml kg^{-1}) with saline solution containing 500 mg glucose kg^{-1}, 24 h after the last meal (on days 2, 8 and 15). Blood was collected at 0 (prior to the injection), 3, 6 and 12 h after glucose administration. The area under the curve for glucose ($AUC_{glucose}$) was calculated based on the trapezoidal rule (NCSS 2007, LLC, Kaysville, UT, USA). The capacity to clear glucose (K_G) was determined with the formula: $K_G=(0.693\times100)/T_{1/2}$, where $T_{1/2}$ is the half-life of plasma glucose decay obtained with the formula: $T_{1/2}=\ln2/\omega$. The constant ω of plasma glucose disintegration was obtained with the formula: $\omega=(\ln C_1-\ln C_2)/T_2-T_1$, with glucose concentration C_1 at time T_1 (3 h) and C_2 at T_2 (12 h). At the end of the trial, 24 h fasted trout (day 15)

were injected intraperitoneally with 1.5 IU of bovine insulin kg^{-1} (Sigma) to evaluate the response of peripheral tissues to an insulin challenge. Blood was then collected from six fish per treatment at 0 (prior to the administration), 3, 6 and 9 h after insulin administration. During the ITT, insulin sensitivity (K_{ITT}) was estimated from the rate constant for glucose, calculated between 9 and 3 h as above. The $AUC_{glucose}$ was calculated using the trapezoidal method as above.

Analytical methods

Blood glucose levels were determined with one drop of blood using the Accu-Check™ glucometer (Roche, Boulogne-Billancourt, France). Plasma FFA (non-esterified fatty acids, NEFA C; Wako Chemicals GmbH, Neuss, Germany) and triacylglycerides (TAG, Biomerieux, Marcy-L'Etoile, France) levels were determined using a colorimetric commercial kit adapted to microplates. Fatty acid synthase (FAS; EC 2.3.1.85), glucose 6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), hexokinase (HK; EC 2.7.1.1), glucokinase (GK; EC 2.7.1.2) and glucose 6-phosphatase (G6Pase; EC 3.1.3.9) activities were determined as described previously (Figueiredo-Silva et al., 2009). Enzyme activity (IU), defined as μmoles of substrate converted to product per min, at an assay temperature of 37°C, was expressed per mg of soluble protein (specific activity). Soluble protein content was determined according to the Bradford method, (Bradford, 1976) using a Bio-Rad protein assay kit (Munich, Germany) with bovine serum albumin as a standard. Glycogen levels were determined as described previously (Keppler et al., 1974).

Muscle protein extraction (40 μg) and western blots were developed using anti-phospho-Akt Ser473 (Cell Signaling Technology Inc., Danvers, MA, USA), anti-IRS-1 (Abcam, Paris, France) and anti-β-tubulin (Cell Signaling Technology Inc.). Insulin receptor substrate 1 (IRS1) and Akt protein level was assessed in the skeletal muscle, the main insulin-sensitive tissue. Anti-phospho-Akt Ser473 (Polakof et al., 2010), anti-β-tubulin (Polakof et al., 2010) and anti-IRS1 (Seiliez et al., 2011) have been shown to successfully cross-react with rainbow trout Akt, β-tubulin and IRS1 protein, respectively.

Statistical analysis

The results are expressed as means ± s.e.m. (N=6), and were analysed by one-way ANOVA at each sampling point with diet as the independent factor or by two-way ANOVA with time and diet as independent variables (SigmaStat; SPSS, Chicago, IL, USA). *Post hoc* comparisons were made using a Student–Newman–Keuls test, and differences were considered statistically significant at $P<0.05$. When necessary, data were log transformed to fulfill the conditions of the ANOVA.

RESULTS

Time course of changes in blood glucose levels and plasma TAG and FFA levels after HFD *vs* LFD consumption

Fig. 1 shows the diet- and time-induced changes in blood levels of glucose (Fig. 1A), TAG (Fig. 1B) and FFA (Fig. 1C). On day 1, fish fed a single HFD meal had higher circulating levels of FFA and TAG (Fig. 1B and C) and lower circulating levels of glucose (Fig. 1A and Fig. 2A) relative to fish fed a single LFD meal. Lower glucose levels were also apparent 6 h after the glucose challenge (Fig. 2B) in fish fed a single HFD meal compared with those fed a LFD meal (day 1). After 7 days of feeding, circulating glucose and TAG increased in HFD-fed trout, whereas circulating glucose decreased in LFD-fed trout without significant changes in TAG or FFA levels over time. The effect of the diets on glycaemic profiles was reversed after 7 days of feeding (Fig. 1A, Fig. 2C). However, no difference in the glycaemic profile between the HFD and LFD groups was observed in response to the GTT at this time (day 7, Fig. 2D). Feeding HFD for one additional week (day 14) confirmed the results found for glycaemia at day 7, being even more pronounced, as seen by AUC values that are 2-fold higher after 1 week and 3-fold higher after 2 weeks. This is also evidenced by the IGT following glucose injection (Fig. 2F) together with persistent high circulating levels of glucose and FFA (Fig. 1A,C). Interestingly, feeding a LFD (for 1 week) after feeding a HFD for 1 week improved glucose clearance (Fig. 2E) and the response to exogenous insulin challenge was similar to that of the LFD group (Fig. 3B). However, postprandial $AUC_{glucose}$

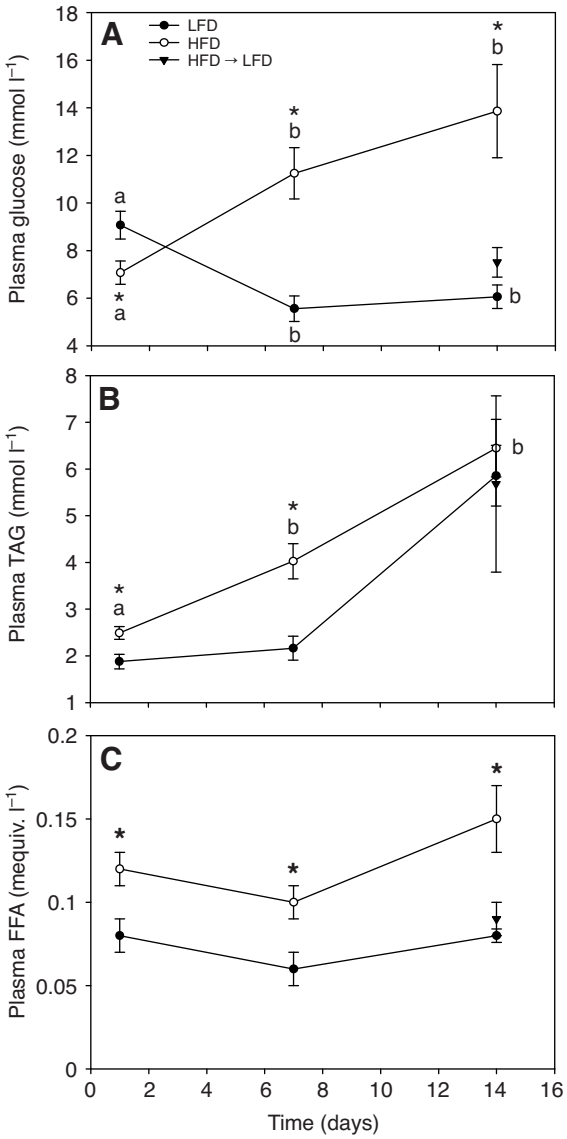

Fig. 1. Time course of changes in plasma glucose (A), triacylglyceride (TAG, B) and free fatty acid (FFA, C) levels in rainbow trout fed a low fat diet (LFD) or a high fat diet (HFD) for 14 days, or in trout fed for 7 days with HFD and for the other 7 days with LFD. Samples were taken 6 h after the meal. Results are expressed as means ± s.e.m. (N=6) and were analysed by two-way ANOVA followed by Student–Newman–Keuls comparison test. *Significant difference between HFD group and other diet groups ($P<0.05$). LFD *vs* HFD: A, $P<0.001$; B, $P<0.02$; C, $P<0.02$. Different letters indicate differences between times within the same diet (LFD or HFD).

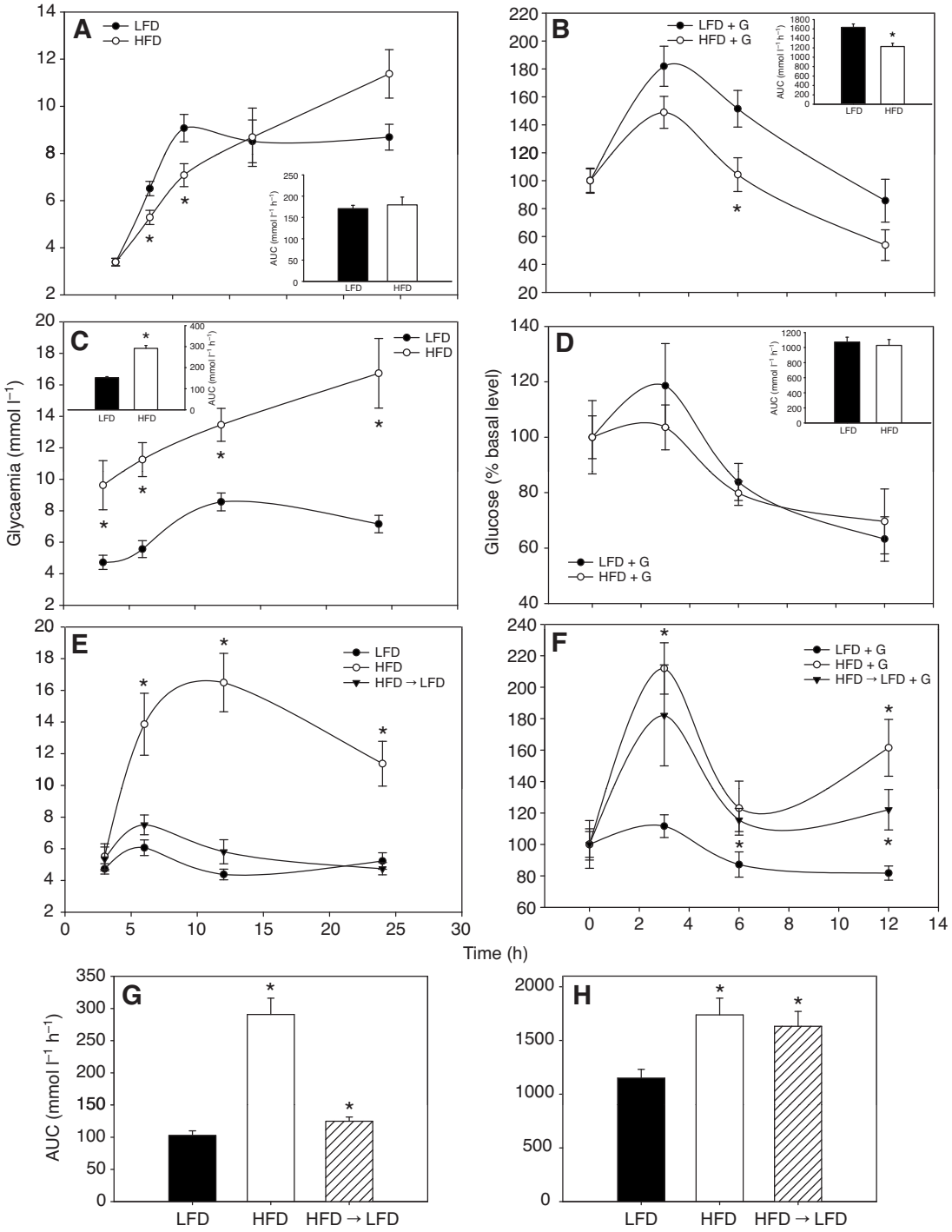

Fig. 2. Postprandial blood glucose levels in trout fed a LFD or a HFD for 1 (A), 7 (C) or 14 days (E) and subjected to a glucose tolerance test (GTT) 24 h after the last meal (B, D and F, respectively). Insets represent the area under the curve for glucose ($AUC_{glucose}$). Fish were either fed the same diet (LFD or HFD) for 2 weeks or were fed the HDF for 1 week then switched to the LFD for 1 week (HFD→LFD). The corresponding postprandial glycaemia is shown in E and the GTT in F. The respective AUC values are represented in G and H. Results are expressed as means ± s.e.m. (N=6) and were analysed by two- or one-way ANOVA followed by Student–Newman–Keuls comparison test. When necessary, data were log transformed to fulfill the conditions of the ANOVA. *Significant difference (P<0.05) with respect to the LFD.

(Fig. 2G) and response to GTT were not statistically different from the HFD group (Fig. 2H).

It is noteworthy that the negative effect of HFD on glucose disappearance rate (K_G) and the half-life of plasma glucose decay ($T_{1/2}$) was significant after 7 days of feeding. The consumption of HFD reduced K_G and increased $T_{1/2}$ ~3 times (Table 2), and contrasts with the similar K_G and $T_{1/2}$ found over time in fish fed the LFD. Moreover, the lower K_{ITT} in the HFD group relative to the LFD or HFD→LFD groups (Fig. 3B) is evidence of the reduced sensitivity of the HFD group to insulin after 14 days of feeding.

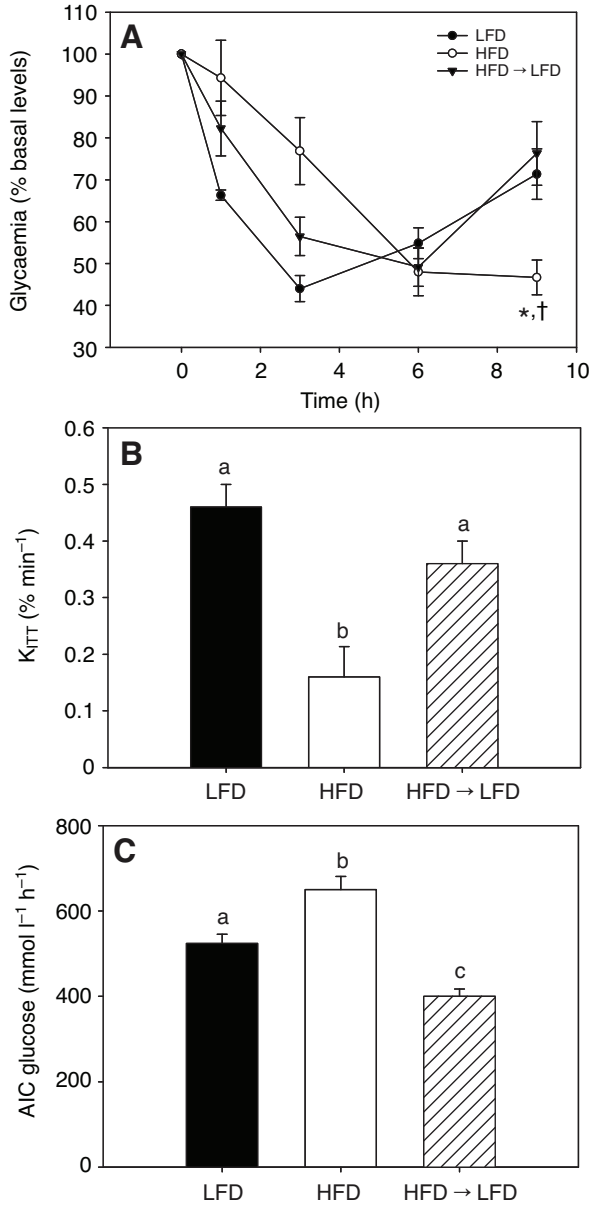

Fig. 3. Glycaemic parameters following the insulin tolerance test (ITT, 1.5 IU bovine insulin kg^{-1}) in trout fed for 14 days with a LFD or HFD or in trout fed for 7 days with a HFD and for the other 7 days with a LFD. B and C represent the insulin sensitivity (K_{ITT}) and $AUC_{glucose}$. Samples were taken before (0) and 3, 6 and 9 h after the injection. Results are expressed as means ± s.e.m. (N=6). Data for A were analysed by two-way ANOVA followed by Student–Newman–Keuls comparison test. Significant differences from LFD (*P<0.05) or HFD→LFD (†P<0.05) at each time are indicated. Data for K_{ITT} and $AUC_{glucose}$ were analysed by one-way ANOVA followed by Student–Newman–Keuls comparison test. Different letters indicate differences between diets (P<0.05).

Table 2. The capacity to clear glucose (K_G) and half-life of plasma decay ($T_{1/2}$) in trout fed for 1, 7 or 14 days with low (LFD) or high fat diet (HFD)

K_G (% h^{-1})	1 day	7 days	14 days
LFD	9.1±1.3[a]	7.1±0.6[a]	7.0±0.7[a]
HFD	12.6±1.9[a]	2.6±0.4*[,b]	2.7±0.7*[,b]
HFD→LFD	n.d.	n.d.	5.05±0.98
$T_{1/2}$ (h)			
LFD	8.5±1.3[a]	10.1±0.9[a]	10.3±0.9[a]
HFD	6.1±0.8[a]	29.5±4.1*[,b]	20.0±4.0*[,b]
HFD→LFD	n.d.	n.d.	17.1±3.9

Trout were subjected to a glucose tolerance test (GTT, 500 mg kg^{-1}) 24 h after the last meal, and blood was sampled before and 3, 6 and 12 h after the injection. The HFD→LFD group was fed for 7 days with the HFD and 7 days with the LFD. See Materials and methods for more details.

Results are means ± s.e.m. (N=6) and were analysed by 2-way ANOVA followed by the Student–Newman–Keuls comparison test.

*P<0.05, significantly different from LFD group. Different letters indicate significant difference among sampling times for the same diet. n.d., no data.

Effect of HFD *vs* LFD on glycogen levels, key enzymes involved in glucose and lipid metabolism and insulin-signalling pathway

The hepatic activity of HK, GK (Fig. 4A and D) and G6PDH (Fig. 5C) was higher following a single LFD meal than following a single HFD meal (day 1). The response of metabolic key enzymes to the different nutritional conditions was also affected after 7 days of feeding. At the hepatic level, fish fed the HFD down-regulated HK (Fig. 4A) and the lipogenic enzymes FAS and G6PDH (Fig. 5A,C), while G6Pase activity was up-regulated (Fig. 4E), compared with the LFD group. Diet-induced changes in metabolism persisted over time for FAS and G6PDH activity, but not for HK or G6Pase activity, which was similar between the diets after 14 days of feeding. Hepatic GK activity, which was not different after 7 days of feeding (Fig. 4D) was significantly higher with the LFD compared with the HFD after 14 days of feeding. Although feeding a LFD for 1 week after feeding a HFD for 1 week improved glucose clearance (Fig. 2E) and the response to exogenous insulin challenge, diet-induced changes in metabolism of the HFD→LFD group were not statistically different from those found with the HFD group.

While glucose and lipid metabolic pathways responded differently to the HFD and LFD in the liver, this response was less clear in muscle and WAT. Yet, FAS activity in WAT induced by a single meal was higher with the LFD than with the HFD (day 1, Fig. 5B). Over the entire feeding period, sustained lower HK activity was also found in muscle and WAT of fish fed the HFD compared with those fed the LFD (Fig. 4B,C). In addition, glycogen levels were significantly higher in liver (Fig. 6A) and lower in muscle (Fig. 6B) and WAT (Fig. 6C) of fish fed the HFD compared with those fed the LFD for14 days. At day 14, the adverse effect of the HFD on glycaemic control was associated with a reduced IRS1 protein content but not with changes in Akt phosphorylation status (at Ser473) in white muscle of rainbow trout (Fig. 7).

DISCUSSION

The so-called glucose intolerance observed in carnivorous fish has been the subject of numerous studies over the last few years (Moon, 2001; Wilson, 1994), but remains little understood. The present study shows for the first time that reducing the fat content in the diet may improve the glycaemic control in carnivorous fish fed high carbohydrate diets. This stems from our finding that the persistent postprandial hyperglycaemia, classically observed in trout fed a high carbohydrate diet, was observed only in fish fed the HFD and not the LFD, suggesting an interaction between the effect of high carbohydrate and high fat on glucose utilisation and sensitivity to insulin in trout.

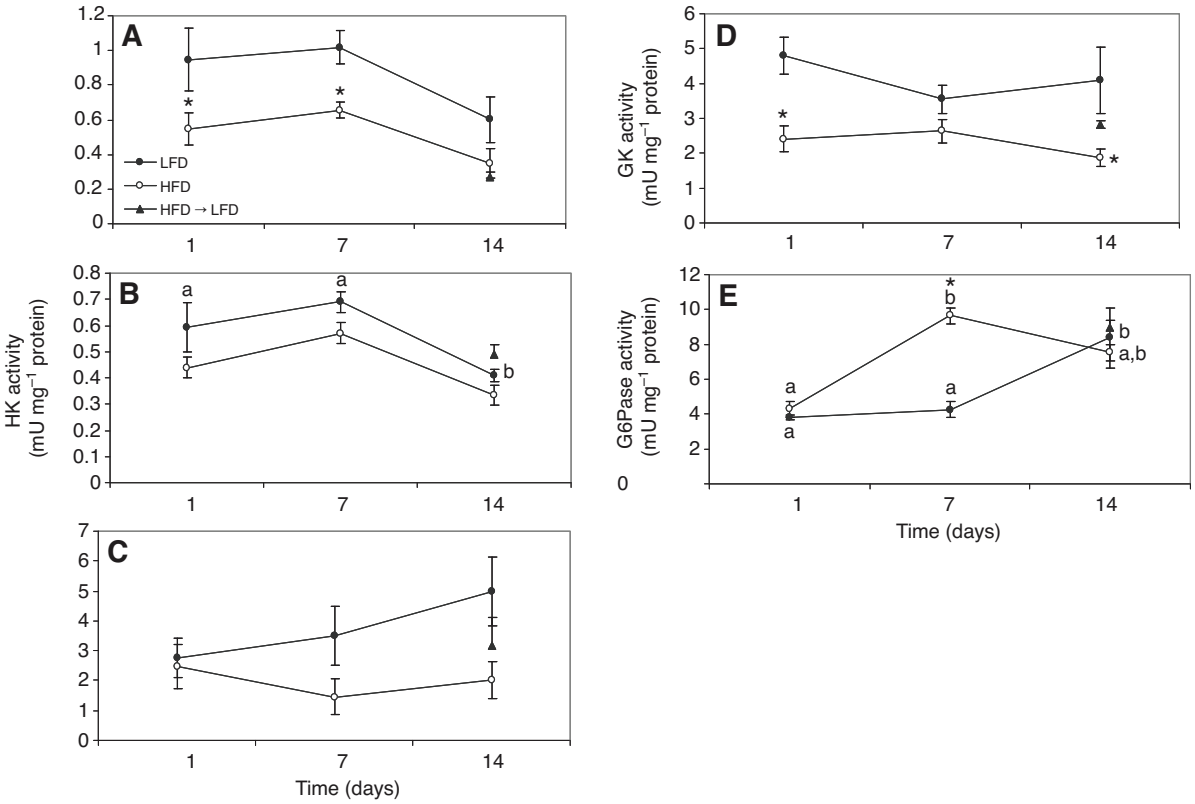

Fig. 4. Time course of changes in hexokinase (HK) activity in liver (A), muscle (B) and white adipose tissue (WAT) (C), and of glucokinase (GK) activity (D) and glucose 6-phosphatase (G6Pase) activity (E) in liver of rainbow trout fed a LFD or HFD for 14 days, or in trout fed for 7 days with HFD and for the other 7 days with LFD. Samples were taken 6 h after the meal. Results are expressed as means ± s.e.m. (N=6) and were analysed by two-way ANOVA followed by Student–Newman–Keuls comparison test. *Significant difference (P<0.05) from LFD at each time. Different letters indicate differences between times (1, 7 or 14 days) for each diet (LFD or HFD). LFD *vs* HFD: A, P<0.001; B, P=0.016; C, P=0.021; D, P<0.001; E, P=0.01 (P-values correspond to the 2-way ANOVA analysis).

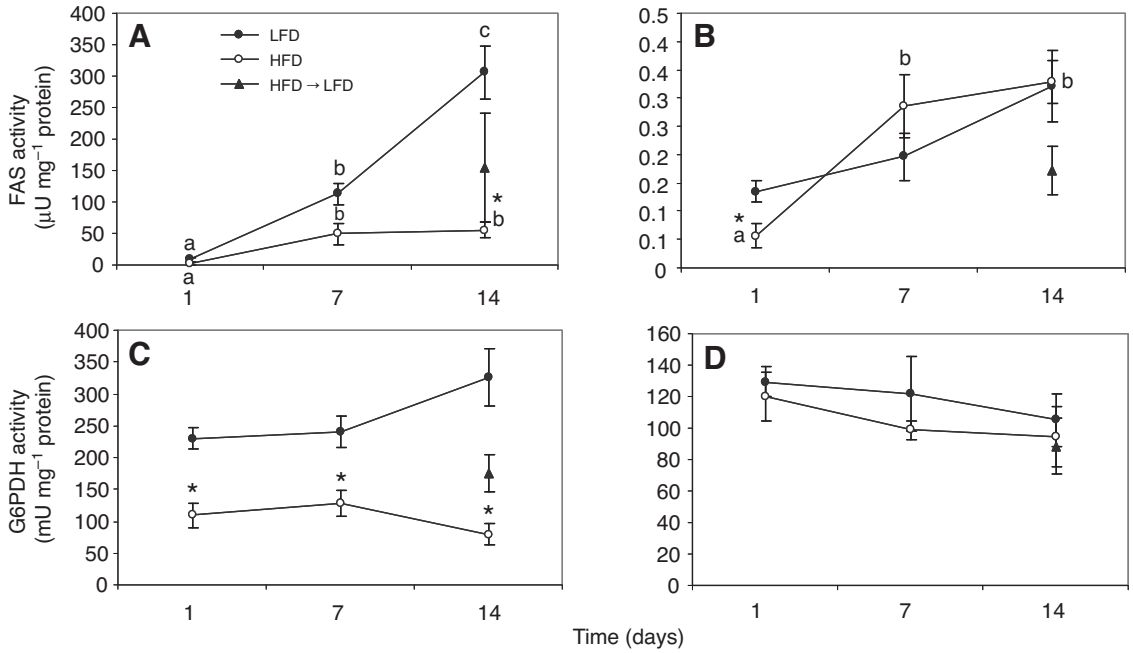

Fig. 5. Time course of changes in fatty acid synthase (FAS) and glucose 6-phosphate dehydrogenase (G6PDH) activity in liver (A and C) and WAT (B and D) of rainbow trout fed a LFD or HFD for 14 days, or in trout fed for 7 days with HFD and for the other 7 days with LFD. Samples were taken 6 h after the meal. Results are expressed as means ± s.e.m. (N=6) and were analysed by two-way ANOVA followed by Student–Newman–Keuls comparison test. *Significant difference (P<0.05) from LFD at each time. Different letters indicate differences between times (1, 7 or 14 days) for each diet (LFD or HFD). LFD *vs* HFD: A, P<0.05; B, P=0.341; C, P<0.01; D, P=0.229 (P-values correspond to the 2-way ANOVA analysis).

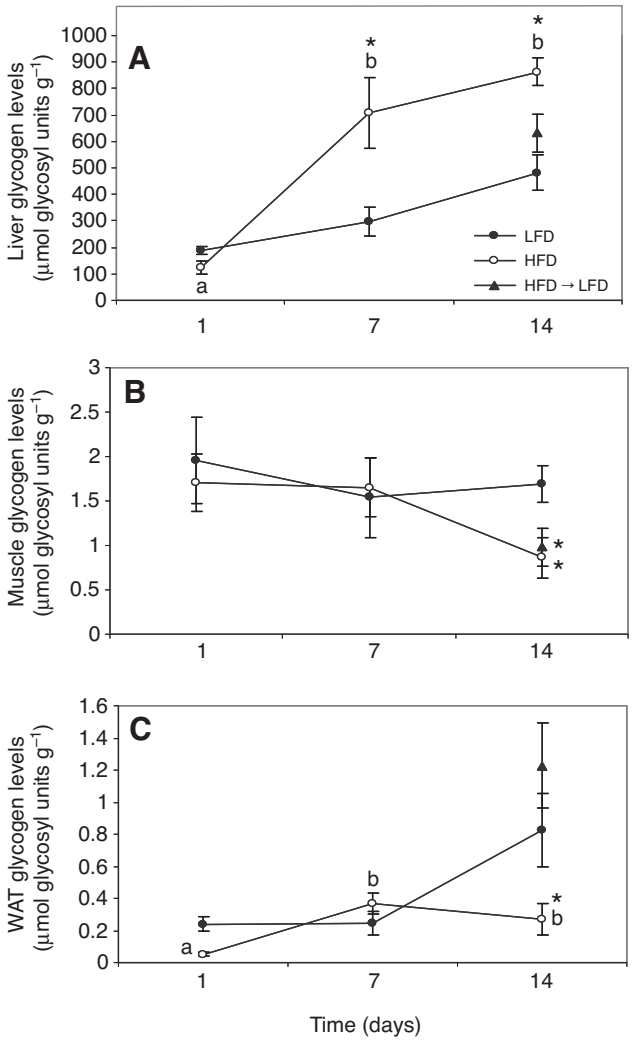

Fig. 6. Glycogen levels in liver (A), muscle (B) and WAT (C) of rainbow trout fed a LFD or HFD for 1, 7 or 14 days, or in trout fed for 7 days with HFD and for the other 7 days with LFD. Samples were taken 6 h after the meal. Results are expressed as means ± s.e.m. (N=6) and were analysed by two-way ANOVA followed by Student–Newman–Keuls comparison test. *Significant different (P<0.05) from LFD at each time. Different letters indicate differences between times (1, 7 or 14 days) for each diet (LFD or HFD). LFD *vs* HFD: A, P<0.001; B, P=0.296; C, P=0.032 (P-values correspond to the 2-way ANOVA analysis).

High fat, high carbohydrate diet induces reversible postprandial hyperglycaemia and impaired glucose tolerance in comparison with low fat, high carbohydrate diet

Impaired glucose tolerance in trout fed a HFD was accompanied by a concomitant increase in circulating FFA and TAG, in line with reports in mammals that IGT might stem from a metabolic interaction between carbohydrates and lipids (Randle, 1998). This elevation of plasma FFA levels corroborates previous studies in which the elevation of circulating FFA through intravenous lipid infusion (Dresner et al., 1999; Pan et al., 1997; Shulman, 2000) or following the consumption of a HFD (Kraegen et al., 1986; Pedersen et al., 1991; Wang et al., 2002) was associated with the development of IGT and IR in mammals. Several other studies in fish with high dietary fat have similarly shown the occurrence of hyperglycaemia (Cheng et al., 2006; Hemre and Sandnes, 1999; Hutchins et al., 1998; Mazur et al., 1992). However, to our knowledge, none of these studies considered the possibility of an interaction between the effects of high dietary fat and carbohydrate on the control of glycaemia. In our study, trout fed a carbohydrate-rich diet clearly had an improved glycaemic control with the LFD compared with the HFD. Indeed, in trout fed the LFD, postprandial $AUC_{glucose}$ was reduced by up to 35% from day 1 (Fig. 2A) to day 14 (Fig. 2G). A similar reduction (~30%) in the $AUC_{glucose}$ following the GTT confirms the positive and progressive (day 1 to day 14) effect of the LFD on glucose tolerance and thus dietary glucose utilisation. In contrast, feeding the HFD for 2 weeks dramatically increased (~40%) the postprandial $AUC_{glucose}$ over time and also decreased the glucose clearance following a GTT, confirming the time-dependent development of the IGT. Our results are in accordance with the development of IGT observed in 100 day palmitate-fed Indian perch (Barma et al., 2006) and in mammalian models (rodents) (Thiess et al., 2004; Wang et al., 2002; Winzell and Ahren, 2004) in which IGT developed after 1–2 weeks on the HFD. Importantly, the HFD-induced hyperglycaemia seen in the present study was found to be reversible, at least at the postprandial and ITT level, as the glycaemia control and insulin sensitivity were recovered by feeding the LFD to fish initially fed the HFD. The development of this nutritionally induced IGT in fish is of major importance given the current tendency to concomitantly increase dietary fat and carbohydrate levels in feed of farmed carnivorous fish like salmonids (Company et al., 1999; Hillestad and Johnsen, 1994).

Metabolic events linked to the HFD-induced hyperglycaemia

Over the 2 weeks, postprandial hyperglycaemia in fish fed the HFD was associated with an increased capacity for hepatic glucose production (G6Pase activity), reduced peripheral glucose phosphorylation (GK/HK activity in liver, muscle and WAT) and lowered hepatic lipogenic potential (FAS and G6PDH activity). Nevertheless, in fish fed the LFD, G6Pase was also strongly stimulated from day 7 to day 14, possibly because of the dietary protein level in the LFD (49%) relative to the HFD (26%) (Kirchner et al., 2003; Moon and Foster, 1995). Despite its higher G6Pase activity, our data show that the high protein level in the LFD did not negatively affect glycaemic control, and that G6Pase was even down-regulated in this group of fish. Other studies have demonstrated the inhibitory effect of a high fat intake on glycolytic enzymes in mammals (Jump and Clark, 1999; Jump et al., 1994) and its stimulatory effect on hepatic gluconeogenic enzymes in both mammals (Fanelli et al., 1993) and fish (Panserat et al., 2002). Yet, this is the first study showing reduced glycolytic activity related to high fat intake in fish. The difference in the lipid content of the diets used here (HFD/LFD ~6-fold) was higher than in our previous study (ratio of ~2.4) (Panserat et al., 2002), which may explain why in the present study the HFD was found to reduce the activity of glucose phosphorylating enzymes, as seen in liver, muscle and WAT. Despite this reduced activity of glucose phosphorylating enzymes in the liver of trout fed the HFD, hepatic glycogen levels increased, favouring the idea that the storage of excess glucose as hepatic glycogen does not actually contribute to the control of glycaemia but simply reflects the glycaemic profile. This concept is not new in trout and has been observed recently in insulin-induced hypoglycaemic fish (Polakof et al., 2010). In both muscle and WAT, where gluconeogenesis plays a minor role, the inhibition of HK by the HFD was accompanied by depleted glycogen levels. We recently demonstrated that *in vivo* insulin treatment strongly increased the glycogen content in muscle of rainbow trout through the up-regulation of glycogen synthase activity (Polakof et al., 2010).

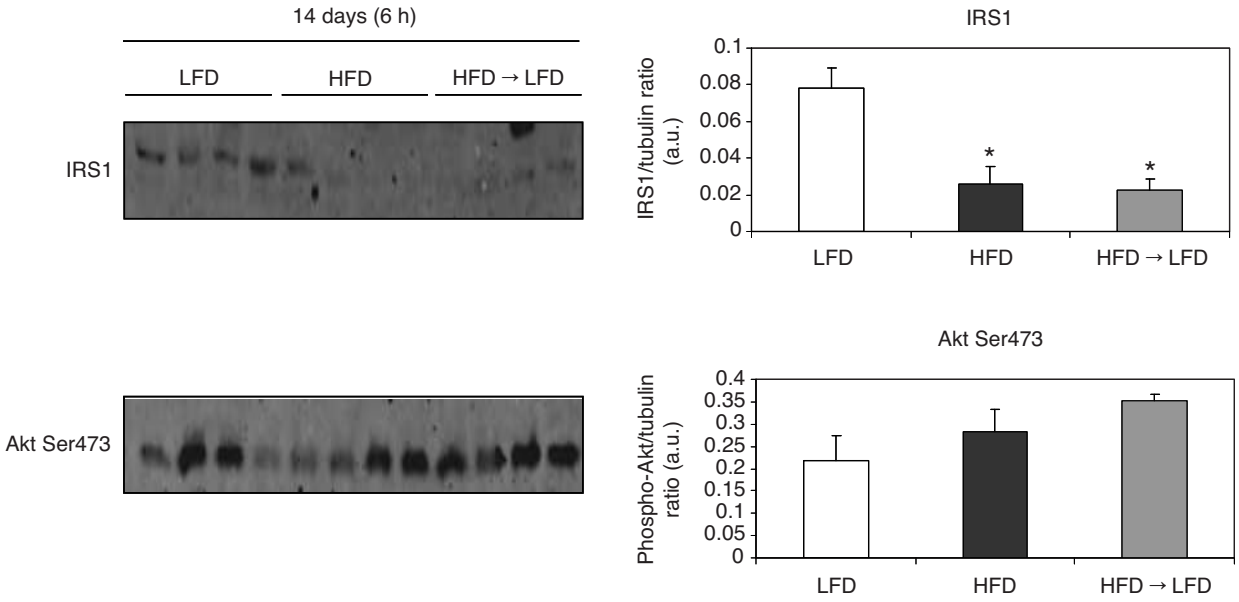

Fig. 7. Insulin receptor substrate 1 (IRS1) protein levels and Akt phosphorylation status (at Ser473) in muscle of rainbow trout fed a LFD or HFD for 14 days. Graphs represent the ratio between IRS1 and the total amount of the reference protein (β-tubulin). *Significant difference (P<0.05) from LFD. Samples were taken 6 h after the meal. Gels were loaded with 40 μg total protein per lane.

When glucose is taken up from the plasma by the liver, it is either stored as glycogen or oxidised through glycolysis and the pentose phosphate pathway, and ultimately used for FA synthesis through lipogenesis. In the present study, a 40-fold time course increase in hepatic FAS activity was observed in fish fed the LFD, whereas time course changes in lipogenic activity remained small in trout fed the HFD. It seems, therefore, that the regulation of plasma glucose is more efficient when carbohydrates are channelled into the lipogenic pathway, decelerating gluconeogenesis, as suggested before in rainbow trout (Panserat et al., 2009; Polakof et al., 2009; Polakof et al., 2011a) and in omnivorous fish (Shimeno et al., 1993). Similar to findings in rodents (Schwarz et al., 2003) and humans (Hudgins et al., 1998) fed a LFD, the enhanced lipogenesis in trout fed the LFD was accompanied by an increase in circulating TAG towards the end of the trial (day 14). Unlike in the liver, lipogenesis was not regulated by the diet in WAT, which maintained a more constitutive lipogenic potential. Hence, the reduced glucose phosphorylation potential and glycogen storage in muscle, together with the reduced lipogenesis in liver, probably caused the progression from hyperglycaemia to IGT and also reduced insulin sensitivity in trout after 14 days of HFD feeding. Although these metabolic changes are most likely to be related to the high fat and starch content of the HFD, we are also aware that the diets are not isoproteic, and therefore a possible role of dietary protein in glucose homeostasis cannot be ruled out in the present study. In this sense, although in omnivorous (Ausman et al., 1972; Belo et al., 1976) and herbivorous (Gerrits et al., 2008) mammalian species very low protein levels can have a detrimental effect on glycaemic control, recent studies on a feline model have demonstrated that this is not the case for carnivorous animals (Verbrugghe et al., 2010). Also in fish, a 10% reduction in dietary protein level (45% to 35%) showed no detrimental effect on glycaemia or on key enzymes of glucose metabolism (Figueiredo-Silva et al., 2009). Indeed, high protein rather than low protein intake has been reported to enhance gluconeogenesis and promote insulin resistance in mammalian species (Tremblay et al., 2007). The fact that the development of IGT in trout was associated with low protein intake (HFD) rather than high protein intake (LFD) favours the idea that the hyperglycaemic phenotype in trout is more closely related to differences in dietary fat level (3% *vs* 20%) than to differences in dietary protein level (50% *vs* 26%).

IRS1 degradation in muscle of trout fed the HFD as an indicator of early insulin-signalling defects

Although the precise involvement of insulin-signalling defects in the development of insulin resistance remains unclear even in mammals, IRS proteins are considered to be key molecules in the insulin-signalling cascade (White, 1997). In this respect, a reduction of the IRS1 protein level in insulin-sensitive tissues, like skeletal muscle, has been proposed as one of the mechanisms inducing IR in mammals (Zhande et al., 2002) and fish (Seiliez et al., 2011). Because of its relatively high mass in relation to total body mass, white skeletal muscle represents the major insulin-sensitive target tissue in trout, as in mammals, being hence of prime importance for glucose homeostasis (Gutiérrez et al., 2006). The assessment of skeletal muscle IRS1 status, as an attempt to elucidate an earlier insulin-signalling defect, showed that IGT in trout fed a HFD was indeed associated with a strong decrease in IRS1 at day 14. This is consistent with the inhibition of IRS1 and of phosphoinositide 3-kinase (PI3 kinase) phosphorylation in myocytes seen in palmitate-fed Indian perch (Barma et al., 2006) and the reduction of the Tyr phosphorylation of IRS1 in chronically insulin-infused trout (Seiliez et al., 2011). Insulin-resistant status in fasted rainbow trout has also been associated with reduced Akt phosphorylation (Polakof et al., 2010). In the present study, Akt was, however, unaffected by the consumption of the HFD in muscle, suggesting that different proteins involved in the same signalling pathway, i.e. IRS1, Akt, can be affected differently during the development of IR. Yet, our results provide evidence for a link between high fat consumption and early insulin-signalling defects in trout muscle.

Conclusions and perspectives

To our knowledge, this is the first study in a non-mammalian vertebrate showing that the combination of a high proportion of fat

in a high carbohydrate diet may result in the development of IGT and a reduced sensitivity to exogenous insulin in trout. The present study demonstrates that rainbow trout fed a HFD for 14 days exhibited (reversible) hyperglycaemia, IGT and reduced insulin sensitivity, which closely resembles the glucose-intolerant and pre-diabetic insulin-resistant state described in mammals (McGarry, 1994). The metabolic origin of this condition, although not completely understood, seems to be related to reduced peripheral glucose phosphorylation, an increased potential to export hepatic glucose and the inability to store excess circulating glucose as fat through lipogenesis. Moreover, degradation of IRS1 was found in the main insulin-sensitive tissue (the skeletal muscle) in fish fed a HFD. The results of this study offer new perspectives on farmed rainbow trout from a nutritional and physiological standpoint: (i) a reduction in dietary fat content could improve glycaemic control in carnivorous fish fed a high carbohydrate diet, allowing the carbohydrate component of the diet to be increased, which represents an advantage in terms of the effort to reduce the reliance on marine fat sources in aquaculture, and (ii) our results show for the first time that a teleost also can develop a high fat-induced IGT, characterised by persistent hyperglycaemia and reduced insulin sensitivity. Rainbow trout might then constitute a potential model to elucidate the impact of dietary macronutrient composition on carbohydrate and lipid metabolism and on the development of physiopathologies such as metabolic syndrome, obesity and type 2 diabetes mellitus.

LIST OF ABBREVIATIONS

AUC	area under the curve
FAS	fatty acid synthase
FFA	free fatty acid
G6Pase	glucose 6-phosphatase
G6PDH	glucose 6-phosphate dehydrogenase
GK	glucokinase
GTT	glucose tolerance test
HFD	high fat diet
HK	hexokinase
IGT	impaired glucose tolerance
IR	insulin resistance
IRS1	insulin receptor substrate-1
ITT	insulin tolerance test
LFD	low fat diet
TAG	triacylglycerides
WAT	white adipose tissue

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