Glucose homeostasis in rainbow trout fed a high-carbohydrate diet: metformin and insulin interact in a tissue-dependent manner

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Polakof S, Moon TW, Aguirre P, Skiba-Cassy S, Panserat S. Glucose homeostasis in rainbow trout fed a high-carbohydrate diet: metformin and insulin interact in a tissue-dependent manner. Am J Physiol Regul Integr Comp Physiol 300: R166–R174, 2011. First published November 10, 2010; doi:10.1152/ajpregu.00619.2010.—Carnivorous fish species such as the rainbow trout (Oncorhynchus mykiss) are considered to be “glucose intolerant” because of the prolonged hyperglycemia experienced after intake of a carbohydrate-enriched meal. In the present study, we use this species to study glucose homeostasis in fish chronically infused with the hypoglycemic agents, insulin, and metformin, and fed with a high proportion of carbohydrates (30%). We analyzed liver, skeletal muscle, and white adipose tissue (WAT), which are insulin- and metformin-specific targets at both the biochemical and molecular levels. Infusion with the combination of insulin and metformin can effectively utilize dietary glucose at the liver, resulting in lowered glycemia, increased insulin sensitivity, and glucose storage capacity, combined with reduced glucose output. However, in both WAT and skeletal muscle, we observed decreased insulin sensitivity with the combined insulin + metformin treatment, resulting in the absence of changes at the metabolic level in the skeletal muscle and an increased potential for glucose uptake and storage in the WAT. Thus, the poor utilization by rainbow trout of a diet with a high proportion of carbohydrates can at least be partially improved by a combined treatment with insulin and metformin, and the glucose intolerance observed in this species could be, in part, due to some of the downstream components of the insulin and metformin signaling pathways. However, the predominant effects of metformin treatment on the action of insulin in these three tissues thought to be involved in glucose homeostasis remain exclusive in this species.

fish; glucose homeostasis; muscle; liver; white adipose tissue; insulin; metformin

COMMERCIAL AQUACULTURE of most carnivorous fish species requires feeding these species a high-protein diet that currently is met with fish meal-based diets. The sustainability of this practice, which requires high quantities of wild fish is accepted today but not for the long term. Thus, it is recognized that the replacement of fish meal with plant raw materials represents a sustainable alternative for the stability and further expansion of aquaculture. However, such substitution remains problematic since feedstuffs of plant origin are naturally rich in carbohydrates, and species like the rainbow trout Oncorhynchus mykiss are considered to be “glucose intolerant” (21, 42) due mainly to the prolonged hyperglycemia experienced after a glucose load or intake of a carbohydrate-enriched meal (3, 24). This intolerance is not thought to be related to the absence of insulin, but, nonetheless, the mechanism(s) by which insulin regulates plasma glucose levels in fish remain speculative, and the relative contribution of the main peripheral tissues sensitive to this hormone remain to be clarified (22). Insulin receptors are present in the major insulin-responsive tissues, including white muscle, liver, and adipose tissues (4, 11, 27), and upregulation of insulin-binding and tyrosine kinase activities is observed after insulin treatment and carbohydrate-rich diets, respectively (2, 11, 27). Moreover, we have recently demonstrated that both fasted and high-carbohydrate-fed rainbow trout respond to increased circulating exogenous insulin levels and that insulin improved glucose distribution and uptake by peripheral tissues, enhancing the capacity of the animal to deal with a glucose load (31, 32).

Metformin is an antidiabetic drug used for the treatment of human type 2 diabetes to improve glucose homeostasis by enhancing the insulin sensitivity mainly of the liver and skeletal muscle (10). The major effect of metformin in mammals is to inhibit gluconeogenesis by downregulating hepatic gluconeogenic enzyme mRNA levels through activation of AMP-activated protein kinase (37). Metformin also improves glucose control by enhancing insulin-stimulated glucose disposal, increasing insulin receptor tyrosine kinase activity, enhancing glycogen synthesis activity, and increasing recruitment and activity of glucose facilitative transporter type 4 (GLUT4) in skeletal muscle (10). In adipose tissue, metformin promotes the reesterification of free fatty acids and inhibits lipolysis, thus indirectly improving insulin sensitivity through reduced lipotoxicity (10). Treatment with metformin was previously reported in two cyprinid species, the common carp (Cyprinus carpio) and the zebrafish (Danio rerio), both known to be glucose tolerant (8, 13). Recently, rainbow trout fed a high-carbohydrate diet plus metformin showed improved glucose homeostasis despite unexpected effects in liver and skeletal muscle metabolism, including induction of gluconeogenic and lipogenic gene expression (26). More recently, we demonstrated that in fasted trout, metformin exerted a paradoxical and negative interaction with insulin when glucose homeostasis was examined, given that when both substances were infused, metformin impaired the insulin signaling pathway and exerted a predominant effect on glucose-related genes (33). We hypothesized in this study that the nutritional status of the animal was responsible for these changes and the inability of metformin alone to generate the expected phenotype, i.e., improved glycemia control in glucose-injected trout.
The actions of metformin alone in fish as noted above remain to be fully elucidated, and the paradoxical interaction with insulin needs to be further explored. Thus, on the basis of the ability of metformin to ameliorate hyperglycemia in trout fed with a carbohydrate-enriched diet (26) and the long-term hypoglycemic effect of insulin on fasted trout (33), in the present study, we use this species as a model of “glucose-intolerant” fish to study dietary glucose homeostasis in fish chronically infused with insulin and metformin. Our main objective was to assess the ability of rainbow trout to cope with a high proportion of dietary carbohydrates in their diet when implanted with osmotic pumps infusing two compounds known to improve glucose homeostasis: insulin and metformin. Thus, in the present study, we combined two compounds known to ameliorate trout hyperglycemia (26, 33) to define a more appropriate fish model. Fish were fed a high-carbohydrate diet (30%) and infused with saline, insulin, metformin, or insulin plus metformin for 5 days. At the end of this period, specific insulin and metformin targets were analyzed at both biochemical and molecular levels, including plasma glycemia, mRNA levels and activities of glucose metabolism-related proteins, insulin signaling, and glycogen levels. We have studied these parameters in the two main tissues involved in glucose metabolism: the liver, and white skeletal muscle. Also, for the first time, fish effects were assessed in the white adipose tissue (WAT).

MATERIALS AND METHODS

Fish. Rainbow trout (Oncorhynchus mykiss Walbaum) were obtained from the Institut National de la Recherche Agronomique (INRA) experimental fish farm facilities of Donzacq (Landes, France). Fish were maintained in tanks kept in open circuits supplied with 17°C well- aerated water, under controlled photoperiod (12:12-h light-dark cycle) and fed a commercial diet during the acclimation period (T-3P classic, Trouv, France). Fish weight 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. Fish to be infused were food deprived for 48 h and then implanted with 1003D Alzet miniosmotic pumps (length 1.5 cm, diameter 0.6 cm, total mass ~0.5 g; Durect, Cupertino, CA) containing either saline (control, n = 6), metformin (n = 6; Sigma, St. Louis, MO), bovine insulin solution (Sigma, n = 6), or insulin plus metformin (n = 6). To avoid solubility problems between insulin and metformin in the same pump, fish receiving both substances were implanted with two pumps (one for insulin, one for metformin), while the rest of the groups were implanted also with an extra pump infusing only saline solution. Additionally, the use of mammalian insulin allows us to avoid the complications derived from the use of fish insulin, because most of the piscine species express at least two insulin isoforms (5, 20). Pump flow rates were determined to be 0.39 µl/h, which at 17°C should provide sustained release of 20 mg/kg·day−1 of metformin and 26 µg insulin-kg−1·day−1 (0.7 IU insulin-kg−1·day−1) for 11 days. Pumps were inserted into the peritoneal cavity through a 1.0-cm incision made in the ventral midline at ~2.0 cm rostral of the pelvic fin. The incision was closed with one stitch, and an antibiotic gel was applied topically to the incision area. Pumps were implanted in the morning and the next morning, fish were fed with a high-carbohydrate diet (30% dextrin, 57% fish meal, 10% fish oil, 1% attractant mix, 1% mineral mix, 1% vitamin mix). Fish were fed this meal once per day (at 9 AM, providing ~2% of the fish weight) for 5 days and sampled 6 h after the last meal to evaluate fish metabolism at the peak of the postprandial glycemia (25). No symptoms of stress, including alterations in feeding behavior, were observed as a consequence of pump implantation.

Tissue sampling. Trout were killed by a sharp blow to the head. Blood was removed from the caudal vein and centrifuged at 3,000 g for 5 min, and the recovered plasma was immediately frozen at −20°C until analyzed. Gut content of each fish was systematically checked to ensure that the fish sampled had consumed the diet. Liver, perivisceral white adipose tissue, and a sample of dorso-ventral white muscle were immediately dissected, weighed, and frozen in liquid nitrogen and kept at ~80°C pending analyses.

Biochemical and molecular analyses. Plasma glucose levels were determined using a commercial kit (Biomedica, France) adapted to a microplate format. Bovine insulin levels were measured using a commercial ELISA kit (Merckodia, Sweden) as in Polakof et al. (33). Tissue glycogen levels were determined following the method of Keppler et al. (15). Enzyme activities were assessed as previously presented (30).

Tissues used to assess enzyme activities were homogenized by ultrasonic disruption in 9 vol of ice-cold buffer containing 50 mmol/l Tris (pH 7.6), 5 mmol/l EDTA, 2 mmol/l 1,4-dithiothreitol, and a protease inhibitor cocktail (Sigma, St. Louis, MO; P-2714). The homogenate was centrifuged, and the supernatant was used immediately for enzyme assays. Enzyme activities were determined using a microplate spectrophotometer. Enzyme reaction rates were determined by the increase or decrease in absorbance of NAD(P)H at 340 nm. The reactions were started by the addition of supernatant (15 µl) at a preestablished protein concentration, omitting the substrate in control wells (final volume 265–295 µl), and allowing the reactions to proceed at 20°C for preestablished times (3–10 min). Enzyme activities are presented as milliunits (nmoles/min) per milligram protein. Homogenate protein was assayed in triplicate using the bicinchoninic acid method (47) with BSA (Sigma) as a standard. Enzyme activities were assessed at maximum rates following preliminary tests to determine optimal substrate and cofactor concentrations. G6PDH, HK, G6Pase, and PEPCK activities were estimated by changes in absorbance as in Ref. 30, while fatty acid synthase was assayed following the method described by Figueiredo-Silva et al. (9) adapted to trout tissues.

mRNA levels for proteins involved in glucose transport and metabolism were determined by real-time quantitative RT-PCR (q-PCR) (33), including GLUT4, hexokinase (HK), glucose 6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK), fatty acid synthase (FAS), glucose 6-phosphate dehydrogenase (G6PDH), and sterol regulatory element binding protein 1c-like (SREBP-1c-like). Primers were designed to overlap an intron where possible (Primer3 software) using known sequences in trout nucleotide databases (GenBank and INRA-Sigenae), as previously described (33). Quantification of the target gene transcripts was done using e1α gene expression as a reference (28), which was found to be stably expressed in this study. Relative quantification of the target gene transcript with the e1α reference gene transcript was made following the Pfaff method (28).

Protein extraction (20 µg of protein for liver and WAT and 40 µg for muscle) and Western blot analysis were assessed using anti-phospho-Akt Ser473 (anti-Akt antibodies (Cell Signaling Technology, Paris, France), which was previously demonstrated to cross-react with the rainbow trout Akt protein (33)).

Statistical analysis. Results are expressed as means ± SE (n = 6). Data were analyzed by one-way ANOVA. When necessary, data were log-transformed to fulfill the conditions of the ANOVA. 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 are presented in Fig. 1. As predicted, fish fed the high-carbohydrate diet and implanted with saline pumps (control group) were hyperglycemic (−9 mM) when compared with the glycemia of fish fed commercial diets (−5 mM; data not shown) (39). Surprisingly, fish with insulin pumps had glycemic values comparable with the control group. On the contrary, fish implanted with metformin-containing pumps alone or in combination with insulin displayed significantly lower glycemia than the control group, being 7 and 6.5 mM, respectively. Exogenous bovine insulin levels in the blood were consistent across fish with bovine insulin-containing pumps that demonstrated a higher phosphorylation status than the control (saline) group. For muscle (Fig. 2B) and WAT (Fig. 2C), the results for pAkt were similar with the insulin-implanted group displaying higher phosphorylation levels than the saline group (three- and twofold, respectively).

mRNA levels encoding hepatic enzymes involved in carbohydrate metabolism and their respective specific activities are presented in Fig. 3. G6Pase mRNA levels (Fig. 3A) were affected by the metformin treatment, alone or in combination with insulin, displaying lower values than the control group. However, G6Pase activities (Fig. 3B) were only reduced in fish with the metformin pumps. In contrast, mRNA levels of the other gluconeogenic enzyme, PEPCK (Fig. 3C), were not affected by metformin but were increased by insulin treatment. PEPCK activities (Fig. 3D) were more sensitive to the treatments, being stimulated by each, especially by the combination of insulin + metformin (twofold above the control). Enzymes involved in hepatic lipogenesis were differentially affected by the treatments. FAS mRNA levels (Fig. 3E) were significantly increased by the treatments containing metformin, with levels up to 10-fold above the control group. In contrast to the FAS mRNA levels, FAS activities (Fig. 3F) were more affected by the insulin treatment (threefold above the control) than those containing metformin, with moderate but significant increases. No changes in G6PDH mRNA levels (Fig. 3G) or activities (Fig. 3H) were noted for any group, except for the significant increase in mRNA levels of fish treated with insulin + metformin (five-fold above the control). We found similar results for the mRNA levels of SREBP-1c-like (Fig. 3I), a major upregulation (seven-fold) by the combination of insulin + metformin, and also by insulin alone but to lesser degree (twofold).

mRNA levels encoding muscle GLUT4 and HK, as well as HK activities are presented in Fig. 4. mRNA levels for both GLUT4 (Fig. 4A) and HK (Fig. 4B) were significantly upregulated in fish with metformin pumps. For HK activities (Fig. 4C), the results were similar to those for mRNA levels with the exception of an increased activity also in the insulin + metformin group.

mRNA levels encoding WAT enzymes involved in carbohydrate metabolism and their respective specific activities are presented in Fig. 5. GLUT4 mRNA levels (Fig. 5A) were
significantly increased in those treatments that included metformin, being up-regulated about twofold with respect to the control group. While HK mRNA levels (Fig. 5B) remained unchanged across treatments, HK activities (Fig. 5C) increased in the group infused with insulin + metformin. FAS mRNA levels (Fig. 5D) and activities (Fig. 5E) were differently affected by the treatments. While mRNA levels were upregulated in all treatments when compared with the control group, activities were increased only in fish receiving insulin infusion. G6PDH mRNA levels (Fig. 5F) and activities (Fig. 5G) were
regulated in different ways. G6PDH mRNA levels were downregulated in fish receiving metformin or insulin alone, while activities were enhanced in the groups infused with metformin alone or in combination with insulin. Finally, the mRNA levels of the transcription factor SREBP-1c-like (Fig. 5H) were particularly sensitive to the presence of metformin in the infusion medium being upregulated in both the metformin and insulin/metformin treatments, while insulin-alone resulted in decreased mRNA levels.

Glycogen levels in liver, skeletal muscle and WAT are presented in Fig. 6. Liver glycogen levels (Fig. 6A) were increased primarily when insulin was present, including insulin alone and in combination with metformin. On the contrary, glycogen levels in skeletal muscle (Fig. 6B) and WAT (Fig. 6C) decreased when compared with the control group in fish receiving metformin alone or in combination with insulin.

DISCUSSION

Metformin actions in rainbow trout, a “glucose intolerant” fish species, were previously unknown. While some of the actions previously documented in mammals, such as inhibition of hepatic glucose output are not found in trout, we previously showed an improved postprandial glycemic profile in metformin-fed trout probably linked to an increased lipogenic activity (26). Moreover, in fasted trout, metformin exerted a paradoxical and negative effect on insulin action (33). To unravel this paradox, we explored in the present study whether metformin alone or in combination with insulin lowered plasma glucose levels in trout fed a high-carbohydrate diet, showing that for the complete functional response to the treatment, the time of induction was important.

Lower glycemia levels in trout fed carbohydrates and infused with metformin. Rainbow trout infused with metformin for 5 days and fed a high-carbohydrate diet showed lower glycemia than fish fed the same diet and infused only with saline (vehicle). This lowering effect on glycemia exerted by metformin is in agreement with its use as an antidiabetic drug in mammals (17, 36). As far as we are aware, this experiment is the first where the gastrointestinal tract was not involved with metformin treatment in a chronic study. Thus, the results obtained in the present study are exclusively due to changes in glucose uptake and utilization by peripheral tissues, eliminating the known effects of this drug on intestinal glucose absorption and oxidation (1). This glycemic effect of metformin was previously observed in fish, although the administration method was different: transdermally for zebrafish embryos (8), in the food for trout (26), or intaperitoneally (acute) for carp (13). The fact that the lowering effects on glycemia were observed in trout fed with carbohydrates as opposed to a previous study using fasted trout injected acutely (intraperitoneally) with glucose (33), confirmed that the nutritional status of the animal is key for the metformin action and supports a possible indirect involvement of the gastrointestinal tract on glucose homeostasis (29).

Metformin administration in trout in this study and in the fasted trout study (25) did not affect the phosphorylation status of Akt in liver, skeletal muscle, or WAT (not previously tested). This is not too surprising, as the main signaling pathway utilized by metformin to exert its actions in mammals is thought to be LKB1 and the AMP-dependent protein kinase (37). Unfortunately, none of these proteins could be detected in our in vivo study utilizing mammalian antibodies.
The major effects of metformin in mammals include the inhibition of hepatic gluconeogenesis, increased glucose uptake into muscle, and an antilipolytic effect on adipose tissue (10). Although some of these effects are described in fish (8, 33), others like an increased FAS mRNA levels in liver seem to occur only in the piscine model (26). Metformin actions on fish WAT were previously not explored. Globally, we reported that rainbow trout infused with metformin showed an increased lipogenic potential.

Fig. 5. Effects of saline, metformin, insulin or insulin + metformin infusion (5 days) on the levels of mRNA transcripts encoding white adipose tissue (WAT) genes and enzyme activities in trout fed a high-carbohydrate diet (5 days). GLUT4 mRNA levels (A), HK mRNA levels (B), HK activities (C), FAS mRNA levels (D), FAS activities (E), G6PDH mRNA levels (F), G6PDH activities (G), and sterol regulatory element binding protein 1-like (SREBP-1c-like) mRNA levels (H). Results are presented as means ± SE (n = 6) and were analyzed by one-way ANOVA followed by a Student-Newman-Keuls comparison test. Different letters indicate significant differences between groups (P < 0.05). More details are provided in Fig. 3.
at both the biochemical and molecular levels in liver and WAT. The increased hepatic mRNA levels of FAS induced by metformin were previously reported in fed trout (26), and on the basis of the data presented here, the changes in this enzyme may be primarily responsible for the hypoglycemic effects of this drug in trout. Further support for these results was found with SREBP-1c-like, which followed the changes in lipogenic potential and is known to regulate lipogenesis at the molecular level (40). The fact that metformin has no effect on rat adipocyte lipogenesis (34) suggests that the mechanism by which metformin acts in fish could be different from that described in mammals. However, this is not the only metabolic event that could improve glycemic profiles under these conditions. On the basis of the lower activities and mRNA levels of hepatic G6Pase, a decreased capacity to export glucose to the blood from the liver is predicted. A lower ability to export glucose from the liver was previously reported in fasted trout infused with metformin (33) and is consistent with the effects described in mammals (18, 36). However, increased PEPCK activities, together with the previously reported high mRNA levels for this enzyme in trout-fed metformin (26) suggest that at least in salmonids, reduced hepatic glucose output is related to a decreased glycogen breakdown rather than with a reduced gluconeogenic potential, one of the known actions of metformin in mammals (16) and at least zebrafish (8). On the other hand, we found enhanced mRNA levels for GLUT4 in skeletal muscle and WAT, suggesting an increased potential for glucose uptake from plasma by peripheral tissues in metformin-infused trout.

**Infused insulin has no effect on plasma glucose levels in trout fed carbohydrates.** The major result obtained in trout infused with insulin and fed the high-carbohydrate diet was an absence in glycemic changes when plasma values were compared with the saline-infused control group. These results are surprising, since in other studies where insulin was administrated to fish fed similar diets, significant hypoglycemia was observed with respect to the saline-infused control group (23, 41). However, in both studies, the insulin dose was higher and acutely administrated in contrast to the present study in which physiological insulin doses (19) were infused over a longer time period. Chronic exposure to insulin is able to create insulin insensitivity and resistance in mammals, resulting in prolonged hyperglycemia and thus impaired glucose control (14). A similar phenomenon was described by us in the skeletal muscle of fasted rainbow trout, where Akt phosphorylation status was depressed in the insulin-infused fish (32). However, in the present study, the nutritional status of the animal seems to be key in protecting the insulin signaling pathway from constant insulin stimulation, since Akt phosphorylation was enhanced in the insulin-treated fish when compared with the control group in both skeletal muscle and WAT, the two insulin-sensitive tissues assessed. Actually, some of the parameters assessed, including increased hepatic glycogen levels and lipogenic potential in liver and WAT, suggest that insulin was exerting its predictable effects (22). On the other hand, the absence of changes in genes related to glucose uptake and phosphorylation and changes in glycogen content in insulin-sensitive peripheral tissues (skeletal muscle and WAT) indicates that some other reported effects of the hormone (4, 22) are not taking place under the conditions of our study. The duality of these results related to global glucose homeostasis in rainbow trout must be investigated in future studies. However, excess circulating glucose due to the high-carbohydrate proportion in the food cannot be discarded as responsible, at least in part, for the lack of changes in glycemia in the insulin-infused trout.

**Insulin plus metformin effects on carbohydrate-fed trout are tissue-dependent.** We hypothesized in this study that the combination of the antidiabetic drug metformin and the hypoglycemic hormone insulin would improve glucose homeostasis in rainbow trout fed a high-carbohydrate diet. The effects exerted by the combination of metformin and insulin were strongly tissue dependent, and often a combination between the effects of the product infused individually. Globally, we found that none of the effects exerted by metformin alone on glucose metabolism in skeletal muscle is observed when the drug is combined with insulin.
Actually, the absence of changes in glucose uptake, phosphorylation, and glycogen storage matches the results obtained with insulin alone, suggesting a predominant action of insulin on this tissue, traditionally known to be insulin sensitive (12, 22). The exception was that Akt phosphorylation status was not stimulated even when insulin was infused, in agreement with a previous study in fasted trout (33). Despite the recurrent negative interaction between metformin and insulin in this species, the mechanism by which this takes place remains to be elucidated. In this case, we suggest that whatever mechanism is responsible, it does not involve the phosphorylation status of Akt, because the phosphorylation status was similar to that observed in the insulin-infused group. As opposed to the muscle results, metformin exerts predominant effects on WAT glucose metabolism. Some of the parameters not affected by insulin, including glucose uptake and glycogen levels, were increased by the combination of metformin and insulin, as observed in the metformin-alone group. On the other hand, as occurred in skeletal muscle, the infusion of metformin together with insulin blocked Akt phosphorylation noted with insulin alone, confirming the multisissie negative effect of this drug on the insulin-signaling pathway. The fact that in trout, WAT is also an insulin-sensitive tissue (4, 22) gives more importance to this interaction, suggesting that the glucose homeostasis in this species could be compromised at least in the WAT when metformin is coadministered with the insulin. Finally, the changes observed in the liver did not correspond clearly to any of the treatments alone. Thus, while Akt phosphorylation was not altered by insulin or metformin alone, their combination resulted in a significantly higher Akt phosphorylation status than in the control group. This result agrees with that described in mammals where metformin is utilized to improve insulin action, reducing insulin resistance (7). On the other hand, the increased lipogenic potential observed with respect to the control group in both liver and WAT in the insulin plus metformin compared with the independent treatments, was not altered by the interaction. Other aspects of the hepatic glucose metabolism were differentially affected by the coinfusion of insulin and metformin: the glucose export capacity was reduced, as in the metformin-alone group, while the glycogen levels were increased, as in the insulin-alone group. As a whole, and in a very interesting way, we have found that the metformin plus insulin group presented all of the effects expected for the combined treatment, i.e., a better glycemc phenotype (lower glycemia levels) but also an increased ability to store the excess glucose as glycogen and lipids. Additionally, this is the first time in which we observed, in contrast to a previous study with fasted trout (33), an improved insulin sensitivity in fish infused with metformin and insulin, as occurs with the type II diabetic patients (35).

**Perspectives and Significance**

The present study had as its main objective to improve dietary glucose utilization and homeostasis in rainbow trout, a “glucose-intolerant” species. To accomplish this, trout were infused with metformin (a recognized antidiabetic drug) and insulin (hypoglycemic hormone) and fed a high proportion of carbohydrates; we expected that the combined effect of both compounds would have a positive impact on glucose homeostasis. Thus, trout infused with insulin and metformin can effectively utilize dietary glucose at the hepatic level, including lowered glycemia, increased insulin sensitivity, and glucose storage capacity, combined with reduced glucose output, as documented in mammals (6) and zebrafish (8). The fact that glucose utilization could be improved by the insulin plus metformin combination implies that some of the pathways involved in glucose utilization in the liver of the control group may not be working effectively or as in glucose-tolerant species, leading to the poor glucose utilization observed in trout fed with carbohydrates. However, the responses of the other tissues to the combined treatment did not necessarily follow that of the liver. In the two insulin-sensitive tissues (WAT and skeletal muscle), we observed decreased insulin sensitivity with the combined treatment, resulting in the absence of changes at the metabolic level in skeletal muscle and an increased potential for glucose uptake and storage in WAT. While some of our results demonstrated that part of the negative interactions observed previously in trout (33) were dependent on the nutritional status, there were important tissue-specific variations in the response resulting in an improvement in glycemia but not complete normoglycemia. We can, therefore, suggest that the poor utilization of a diet containing a high proportion of carbohydrate by the rainbow trout could at least be partially improved by a combination of insulin plus metformin and that the glucose intolerance observed in this species could be, in part, due to some of the downstream components of the insulin and metformin signaling pathways. The mammalian-like effect of metformin in trout also supports the hypothesis that at least some of the components involved in the metformin action in mammals (43) also exist in the trout. However, the predominant effects of metformin treatment on insulin actions, at least in some of the main tissues related with glucose homeostasis, remain to be elucidated in this species. The fact that this interaction takes place in one species in which dietary carbohydrate utilization is naturally low makes the use of these two products together a very helpful tool to better understand the basis of this paradoxical metabolic behavior. Moreover, our data demonstrated that while some of the expected metabolic pathways (gluconeogenesis) related with glucose homeostasis were affected by metformin and insulin, other unsuspected pathways were also altered, such as lipogenesis. This could be very important because while metformin-induced lipogenesis could improve the postprandial glycemic profiles in trout, this pathway is also inhibited by high-fat diets (currently used in aquaculture), which in combination with a high-carbohydrate diet could lead to persistent hyperglycemia in this species (unpublished observations S. Polakof and S. Panserat). Further studies are needed to better understand the contribution of lipogenesis in fish glucose homeostasis, including the selection of fish lines where lipogenesis is enhanced or where new effectors of this pathway are investigated (38).

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.
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