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RESEARCH ARTICLE

Pushing the limits of glucose kinetics: how rainbow trout cope with a carbohydrate overload

Kevin Choi and Jean-Michel Weber*

ABSTRACT

Rainbow trout are generally considered to be poor glucoregulators. To evaluate this, exogenous glucose was administered to chronically hyperglycemic fish at twice the endogenous rate of hepatic production, and their ability to modulate glucose fluxes was tested. Our goals were to determine: (1) whether hyperglycemic fish maintain higher glucose fluxes than normal; (2) whether they can lower hepatic production (R_a glucose) or stimulate disposal (R_d glucose) to cope with a carbohydrate overload; and (3) an estimate of the relative importance of glucose as an oxidative fuel. Results show that hyperglycemic trout sustain elevated baseline R_a and R_d glucose of 10.6±0.1 µmol kg^{-1} min^{-1} (or 30% above normal). If 50% of R_d glucose was oxidized as in mammals, glucose could account for 36 to 100% of metabolic rate when exogenous glucose is supplied. In response to exogenous glucose, rainbow trout can completely suppress hepatic glucose production and increase disposal 2.6-fold, even with chronically elevated baseline fluxes. Such large changes in fluxes limit the increase in blood glucose to 2.5-fold and are probably mediated by the effects of insulin on glucose transporters 2 and 4 and on key enzymes of carbohydrate metabolism. Without this strong and rapid modulation of glucose kinetics, glycemia would rise four times faster to reach dangerous levels, exceeding 100 mmol l^{-1}. Such responses are typical of mammals, but rather unexpected for an ectotherm. The impressive plasticity of glucose kinetics demonstrated here suggests that trout have a much better glucoregulatory capacity than is usually portrayed in the literature.

KEY WORDS: Hepatic glucose production, Exogenous glucose, Glucoregulation, Carbohydrate metabolism, Continuous tracer infusion, Fish, *Oncorhynchus mykiss*

INTRODUCTION

Rainbow trout are generally considered to be poor glucoregulators because they normalize glycemia very slowly in glucose tolerance tests (Legate et al., 2001) and show limited sensitivity to insulin (Marín-Juez et al., 2014; Polakof et al., 2012). These observations are based on measurements of blood glucose concentrations that depend on changing rates of appearance in and disappearance from the circulation (R_a and R_d glucose). Earlier kinetics studies have reported baseline glucose fluxes in kelp bass, sea bass and tuna (Bever et al., 1977; Garin et al., 1987; Weber et al., 1986), as well as changes in flux during glucose tolerance tests in wolf fish and brown trout (Blasco et al., 1996; Machado et al., 1989). However, all these studies have relied on the bolus injection technique, which cannot quantify R_a and R_d separately. Adequate methods to measure fluxes by continuous tracer infusion were then developed for rainbow trout (Haman et al., 1997a; Haman and Weber, 1996) and they were used to start investigating capacity to regulate glucose production and disposal. These experiments show that trout can adjust R_a glucose in response to acute hypoxia (+45%) and lower temperature (−46%) (Haman et al., 1997b), to epinephrine (+100%) and propranolol (−32%) (Weber and Shanghavi, 2000), and to prolonged low-intensity swimming (−33%) (Shanghavi and Weber, 1999). Using stable isotopes, Felip and colleagues have also recently quantified carbohydrate incorporation/conversion in the tissues of rainbow trout after feeding them a high-carbohydrate diet (Felip et al., 2012). Under these nutritional conditions, they have shown that continuous low-intensity swimming improves the use of carbohydrates and lipids, thereby sparing proteins. The fact that trout can modulate glucose fluxes in response to changes in environmental conditions, catecholamines and exercise suggests that they may have a better ability for glucoregulation than previously thought. Unfortunately, insufficient information on the regulation of R_a and R_d is presently available to assess their true capacity for glucose homeostasis.

In comparison, mammalian glucoregulation has received extensive attention (Triplitt, 2012). The multiple mechanisms that allow fine regulation of glucose fluxes from hepatic production to muscle uptake and metabolism (insulin, glucagon, glucose transporters and muscle hexokinase) have been well characterized (Shrayyef and Gerich, 2010; Wasserman et al., 2011). Measurements of glucose kinetics in diabetic humans have showed that R_a and R_d can both be chronically stimulated (Meyer et al., 1998), and the infusion of exogenous glucose has been used to test the limits of mammalian plasticity for glucose fluxes. In miniature pigs, exogenous glucose causes a dramatic suppression of endogenous R_a and a large increase in R_d (Muller et al., 1988). The same response has been reported in dogs and humans receiving an oral load of glucose (Abumrad et al., 1982; Ferrannini et al., 1985; Jackson et al., 1986; Kelley et al., 1988), but the capacity of fish to modulate glucose fluxes has never been tested with an exogenous supply. Therefore, the goals of this study are to explore the limits of glucose kinetics in rainbow trout using hyperglycemic animals (potentially showing elevated glucose fluxes) and the infusion of exogenous glucose at twice the baseline rate of hepatic production. More specifically, we want to determine: (1) whether hyperglycemic trout maintain higher glucose fluxes than their normoglycemic counterparts; (2) whether they can lower endogenous glucose production or stimulate glucose disposal to cope with exogenous supply; and (3) the relative importance of circulating glucose as an oxidative fuel. We anticipate that hyperglycemic trout maintain higher glucose fluxes than normoglycemic controls, but have limited ability to modulate R_a and R_d when exogenous glucose is provided.

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RESULTS

Steady-state glucose kinetics of hyperglycemic fish

Metabolic rate ($\dot{M}_{O_2}$), blood glucose concentration and glucose specific activity of hyperglycemic animals receiving no exogenous glucose (but infused with trace amounts of [6-^{3}H]glucose) are shown in Fig. 1. Tracer infusion was started 1 h before time 0, to reach isotopic steady state. Over the next 4 h, $\dot{M}_{O_2}$ remained stable (P>0.05; Fig. 1A) and averaged 63.7±0.4 µmol O_2 kg^{-1} min^{-1}. Blood glucose concentration also remained constant (P=0.654; Fig. 1B) and averaged 12.5±0.04 mmol l^{-1}, or more than twice the levels of normoglycemic trout (Polakof et al., 2012; Weber and Shanghavi, 2000). Blood glucose specific activity only showed a minor increase after 3 h (P<0.05; Fig. 1C), but remained constant at all other times, averaging 435±5.1 Bq µmol^{-1}. Fig. 2 compares baseline glucose fluxes of hyperglycemic fish (this study) with those of normoglycemic fish from a previous study (Weber and Shanghavi, 2000). The glucose fluxes of hyperglycemic fish averaged 10.6±0.1 µmol kg^{-1} min^{-1} throughout the experiments. Even though ANOVA detected a minor, overall change in glucose flux over time (P<0.05), Dunnett's *post hoc* test was unable to identify specific means that were statistically different from baseline (time 0). All the glucose fluxes measured in hyperglycemic fish were higher than those from normoglycemic fish (Weber and Shanghavi, 2000), which averaged 7.9±0.07 µmol kg^{-1} min^{-1} (P<0.05).

Glucose kinetics of hyperglycemic fish supplied with exogenous glucose

$\dot{M}_{O_2}$, blood glucose concentration and glucose specific activity of hyperglycemic animals receiving exogenous glucose are shown in Fig. 3. Infusion of trace amounts of [6-^{3}H]glucose was started 1 h before time 0, to reach isotopic steady state, and infusion of exogenous (unlabeled) glucose was started at time 0. Over the next

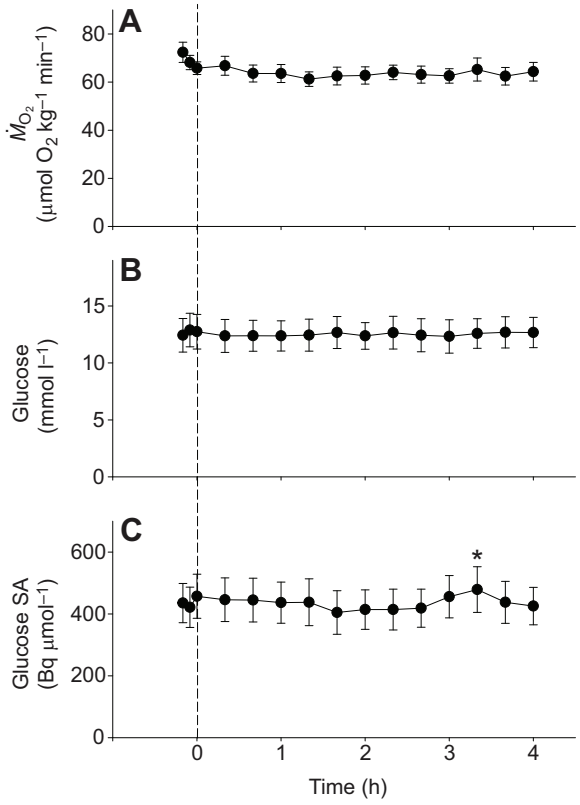

Fig. 1. Glucose kinetics of resting rainbow trout. Glucose kinetics were measured by continuous infusion of [6-^{3}H]glucose (started at time −1 h). (A) Metabolic rate. (B) Blood glucose concentration. (C) Blood glucose specific activity (SA). Values are means±s.e.m. (N=7). * indicates significant difference from baseline (time 0; P<0.05).

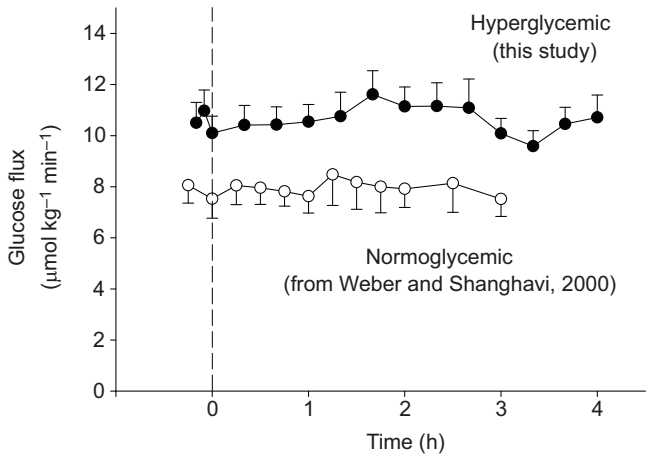

Fig. 2. Glucose fluxes of resting hyperglycemic and normoglycemic rainbow trout. Data for normoglycemic fish are from Weber and Shanghavi, 2000. All animals were in steady state with matching rates of glucose production and glucose disposal (at each time point, glucose flux=R_a glucose=R_d glucose). All means were different (P<0.05) between the normoglycemic and hyperglycemic groups (statistics not shown on figure). Values are means±s.e.m. (N=13 for normoglycemic and N=7 for hyperglycemic trout).

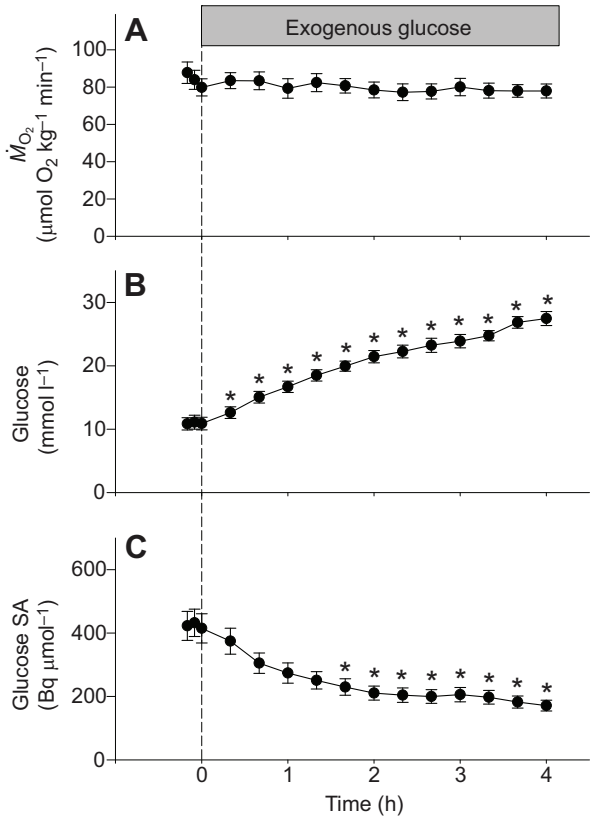

Fig. 3. Effects of exogenous glucose infusion on glucose kinetics in resting rainbow trout. (A) Metabolic rate. (B) Blood glucose concentration. (C) Blood glucose specific activity (SA). These parameters were monitored during measurement of glucose kinetics by continuous infusion of [6-^{3}H] glucose. Infusion of tracer was started at time −1 h and infusion of exogenous, unlabeled glucose was started at time 0. Values are means±s.e.m. (N=8). * indicates significant difference from baseline (time 0; P<0.05).

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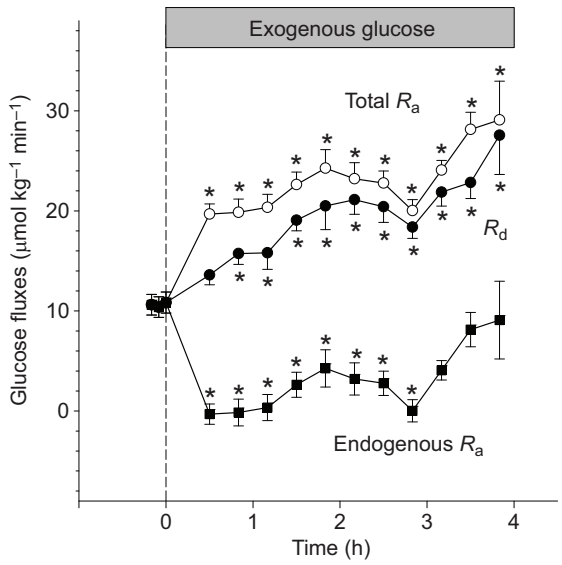

Fig. 4. Effects of exogenous glucose infusion on glucose fluxes of resting rainbow trout. The measured total rate of appearance of glucose (total R_a; open circles) is the sum of endogenous glucose production by the fish liver (endogenous R_a; filled squares) and the infusion rate of exogenous, unlabeled glucose. The rate of glucose disposal (R_d glucose) is shown by filled circles. Values are means±s.e.m. (N=8). * indicates significant difference from baseline value (P<0.05).

4 h, $\dot{M}_{O_2}$ remained stable (P>0.05; Fig. 3A) and averaged 79.7±0.6 μmol O_2 kg^{-1} min^{-1}. Blood glucose concentration increased steadily from a baseline value of 10.9 mmol l^{-1} to 27.5 mmol l^{-1} at the end of the experiment (P≤0.001; Fig. 3B). Blood glucose specific activity decreased from 423±46 Bq μmol^{-1} to 171±17 Bq μmol^{-1}. All specific activities measured after 1.5 h were lower than baseline (P≤0.001; Fig. 3C). Fig. 4 shows the glucose fluxes of hyperglycemic fish supplied with exogenous glucose. Measured total R_a (=rate of endogenous hepatic glucose production+rate of exogenous glucose administration) increased progressively from 10.6 μmol kg^{-1} min^{-1} to 29.1 μmol kg^{-1} min^{-1} (P≤0.001). R_d glucose increased from 10.6 μmol kg^{-1} min^{-1} to a maximum of 27.6 μmol kg^{-1} min^{-1} after 4 h (P≤0.001). Endogenous R_a glucose rapidly decreased from 10.6 μmol kg^{-1} min^{-1} to 0.4 μmol kg^{-1} min^{-1} (P≤0.001) before returning to baseline over the last hour of the experiment. Table 1 summarizes the changes in blood glucose and lactate concentration, endogenous R_a glucose and R_d glucose in hyperglycemic fish receiving no exogenous glucose (controls) as well as those infused with exogenous glucose.

Relative changes in R_a and R_d glucose

Changes in the endogenous R_a glucose of fish receiving exogenous glucose relative to the R_a glucose of those receiving no exogenous glucose (all hyperglycemic) are presented in Fig. 5. Exogenous glucose supply caused the rapid and complete suppression of endogenous R_a glucose in hyperglycemic fish for the first 2.5 h (P≤0.001). However, endogenous R_a glucose returned to levels that were not different from baseline during the last hour of the experiment (P>0.05).

Changes in the R_d glucose of hyperglycemic fish receiving no glucose and of those receiving exogenous glucose relative to the R_d glucose of normoglycemic fish are presented in Fig. 6. Hyperglycemic fish receiving no exogenous glucose maintained constant R_d glucose over time (P>0.05) at an average level of 134±2% of normoglycemic controls. Hyperglycemic fish receiving exogenous glucose progressively increased relative R_d glucose from 132 to 291% of normoglycemic controls (P≤0.001). All the values for hyperglycemic fish (with or without exogenous glucose) were higher than for normoglycemic controls (P<0.05; statistics not indicated on Fig. 6).

Relative importance of glucose as an oxidative fuel

The potential relative contribution of glucose oxidation to total aerobic metabolism in hyperglycemic trout is shown in Fig. 7. Calculations were made after assuming that 50% of R_d glucose is oxidized as in mammals (see Discussion for details). Fish receiving no glucose could support 50.3±0.9% of $\dot{M}_{O_2}$ with glucose oxidation (Fig. 7A). For animals receiving exogenous glucose, the relative contribution of glucose oxidation to $\dot{M}_{O_2}$ increased from 36 to 100% throughout the experiment (Fig. 7B).

Impact of flux regulation on blood glucose concentration

To evaluate the effects of the changes in glucose kinetics reported here, Fig. 8 provides a comparison of observed concentrations with theoretical concentrations if glucose fluxes had not responded to the administration of exogenous glucose. Three different scenarios were used to calculate these hypothetical changes in glycemia: (1) if R_a glucose had not been suppressed; (2) if R_d glucose had not been stimulated; and (3) if both R_a and R_d had remained constant throughout the infusion of exogenous glucose. Observed blood glucose concentrations reached 27.5 mmol l^{-1} after 4 h of exogenous glucose infusion. However, hypothetical fish would have reached 59 (if R_a was not suppressed), 66 (if R_d was not stimulated) and 107 mmol l^{-1} (if glucose fluxes had not responded at all to exogenous supply).

DISCUSSION

This study shows that rainbow trout have the ability to stop hepatic glucose production completely and to stimulate glucose disposal several fold when coping with an exogenous glucose challenge. Such large changes in glucose kinetics are particularly striking because they were elicited in hyperglycemic animals that already maintain elevated baseline glucose fluxes. If 50% of R_d was oxidized in these fish, as in mammals, glucose oxidation could

Table 1. Initial, intermediate and final values for blood metabolite concentrations and glucose flux of rainbow trout supplied with exogenous glucose

	Control			Exogenous glucose		
	Initial	Intermediate (2 h)	Final (4 h)	Initial	Intermediate (2 h)	Final (4 h)
Glucose (mmol l^{-1})	12.4±1.5 (7)	12.4±1.2 (7)	12.7±1.3 (7)	10.9±1.0 (8)	21.5±1.0 (8)*	27.5±1.1 (8)*
Lactate (mmol l^{-1})	0.8±0.2 (7)	0.8±0.2 (7)	1.0±0.2 (7)	1.2±0.3 (8)	1.2±0.3 (8)	1.1±0.3 (8)
Endogenous R_a glucose (μmol kg^{-1} min^{-1})	10.5±0.8 (7)	11.1±0.8 (7)	10.7±0.9 (7)	10.6±1.0 (8)	3.2±1.6 (8)*	9.1±3.9 (8)
R_d glucose (μmol kg^{-1} min^{-1})	10.5±0.8 (7)	11.1±0.8 (7)	10.7±0.9 (7)	10.6±1.0 (8)	21.1±1.5 (8)*	27.6±3.9 (8)*

Rates of endogenous glucose appearance or hepatic production (R_a glucose) and rates of glucose disposal (R_d glucose) are given. Values are means±s.e.m. (N). * indicates significant difference from initial values within each group (P<0.05).

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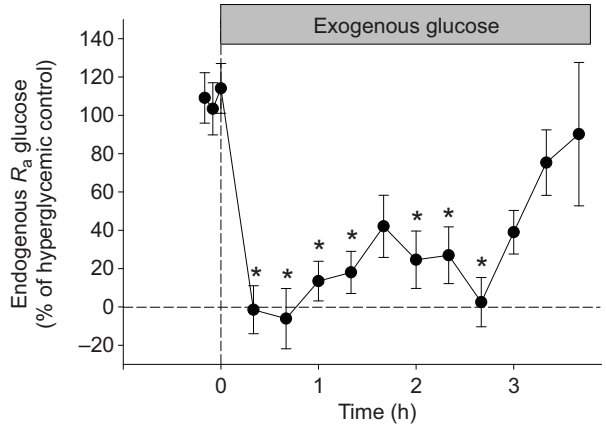

Fig. 5. Percentage of the endogenous R_a glucose of control hyperglycemic fish shown by fish supplied with exogenous glucose. R_a glucose in treatment fish supplied with exogenous glucose is shown as a percentage of control hyperglycemic fish, which received no exogenous glucose (filled symbols in Fig. 2). Dotted line (0%) indicates complete inhibition of endogenous R_a (=hepatic glucose production) in the fish supplied with exogenous glucose. Values are means±s.e.m. (N=7). * indicates significant difference from baseline (P<0.05).

account for 36 to 100% of metabolic rate when exogenous glucose is supplied. When receiving glucose at twice the normal rate of hepatic production for 4 h, trout only show a 2.5-fold increase in glycemia even though current literature describes them as poor glucoregulators. If they were unable to modulate glucose fluxes, their circulating glucose levels would actually increase 10-fold. Therefore, rainbow trout have a much better capacity for glucoregulation than previously suggested by simple monitoring of glycemia in glucose tolerance tests.

Suppression of hepatic glucose production

Hyperglycemic trout are able to suppress hepatic glucose production completely and for several hours during exogenous glucose infusion (Figs 4 and 5), even though baseline fluxes are already elevated (Fig. 2). Such a total inhibition of endogenous R_a is characteristic of the mammalian response to exogenous glucose

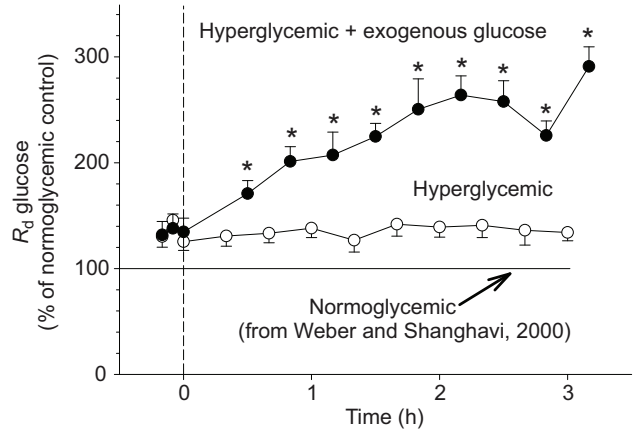

Fig. 6. Percentage of the R_d glucose of normoglycemic fish shown by hyperglycemic fish receiving no exogenous glucose and hyperglycemic fish supplied with exogenous glucose. Values are means±s.e.m. (N=7 for hyperglycemic trout and N=8 for hyperglycemic trout supplied with exogenous glucose). * indicates significant differences from baseline (time 0; P<0.05). All values for hyperglycemic trout (with or without exogenous glucose supply) are significantly higher than 100% (statistics not indicated on figure; P<0.05).

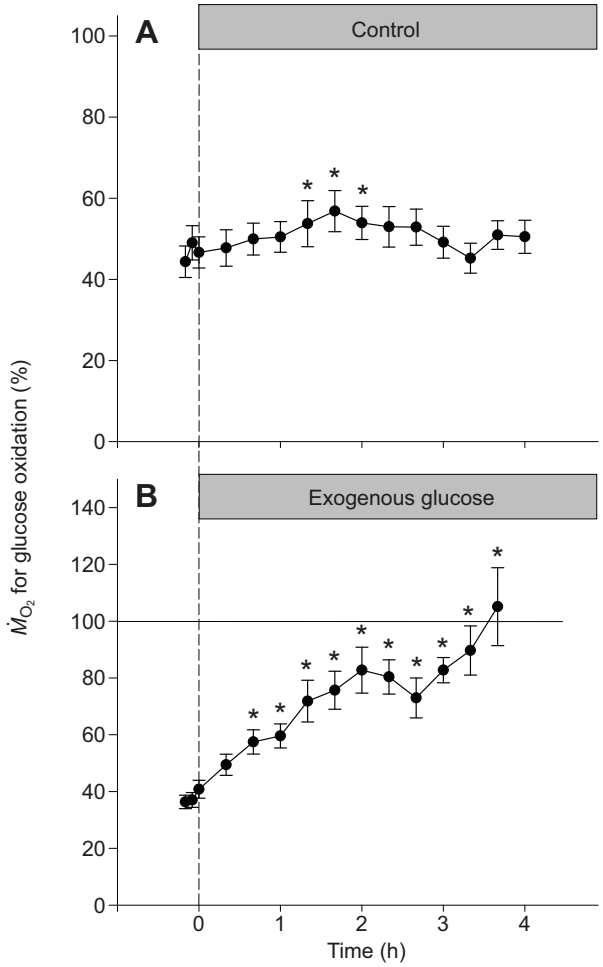

Fig. 7. Percentage of total metabolic rate accounted for by glucose oxidation. (A) Control fish. (B) Fish receiving exogenous glucose. Values were calculated assuming that 50% of R_d glucose was oxidized as reported for resting mammals (see Discussion). Values are means±s.e.m. (N=7 for control and N=8 for exogenous glucose). * indicates significant difference from baseline (P<0.05).

(Abumrad et al., 1982; Ferrannini et al., 1985; Jackson et al., 1986; Kelley et al., 1988; Muller et al., 1988), but was unexpected in trout. The liver can only produce glucose in two ways: glycogenolysis and gluconeogenesis. In humans, glycogenolysis is initially responsible for most of the glucose produced in the post-absorptive state, but it is progressively replaced by gluconeogenesis as liver glycogen reaches depletion (Shrayyef and Gerich, 2010; Wasserman, 2009). In fish, the relative importance of the two pathways has not been established, and both may contribute to R_a glucose under our conditions. For carnivorous species like rainbow trout, however, gluconeogenesis from amino acid precursors is known to play an important role (Kirchner et al., 2003; Polakof et al., 2012). What are the mechanisms responsible for suppressing glycogenolysis and/or gluconeogenesis in trout responding to exogenous glucose? Current information shows that insulin plays a major role in blocking R_a glucose in animals undergoing acute elevation in glycemia. In mammals as well as trout, hepatic production is decreased when high insulin levels inhibit glucose release in the circulation by terminating the transcription of glucose 6-phosphatase (G6Pase) (Polakof et al., 2010a; Rojas and Schwartz, 2014). Insulin-mediated inhibition of glycogen phosphorylase (GPase) also suppresses glycogenolysis in trout (Enes et al., 2009; Polakof et al., 2010b) and

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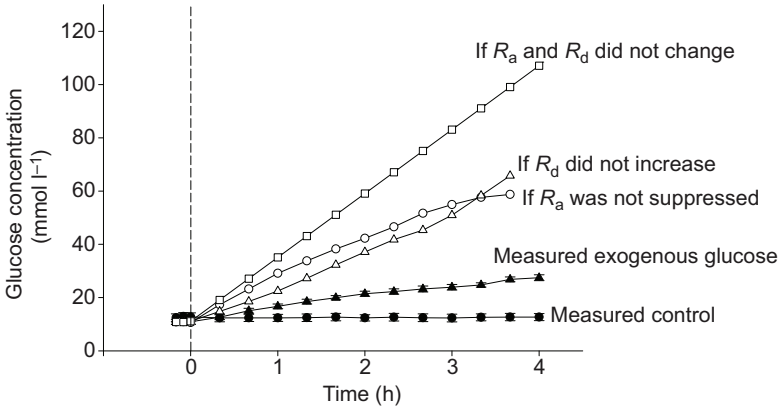

Fig. 8. Comparison of measured blood glucose concentrations with theoretical values calculated for hypothetical fish that fail to regulate their glucose fluxes when exogenous glucose is provided. Measured blood glucose concentrations are presented for control fish and for those receiving exogenous glucose. Theoretical concentrations are given for three different scenarios: (1) if R_a glucose had not been suppressed; (2) if R_d glucose had not been stimulated; and (3) if both, R_a and R_d, had remained at baseline throughout the infusion of exogenous glucose.

mammals (Shrayyef and Gerich, 2010). Similarly, gluconeogenesis is inhibited by high levels of insulin, which inhibits transcription of mammalian phosphoenolpyruvate carboxykinase (PEPCK) (Rojas and Schwartz, 2014). In trout, however, insulin-mediated inhibition of PEPCK only occurs when the fish are fed a high-protein diet (40–60%) (Cowey et al., 1977a,b; Enes et al., 2009). This mechanism was probably operating here, because our fish were fed a diet containing 46% protein. More research is needed to determine the relative importance of glycogenolysis and gluconeogenesis as well as the exact mechanisms responsible for the suppression of hepatic glucose production in fish.

Stimulation of glucose disposal

Rainbow trout are able to increase R_d 2.6-fold in response to exogenous glucose (Figs 4 and 6) and this large change in kinetics was unexpected for an animal generally considered to be glucose intolerant (Polakof et al., 2012). Glucose disposal can be stimulated by increasing the blood-to-tissue concentration gradient or membrane permeability to glucose. Many interdependent factors including insulin, glucose transporters (GLUTs), enzymes that use glucose as a substrate, hyperglycemia and catecholamines could all play a role in modulating glucose gradient or membrane permeability. In fish, insulin is known to affect GLUTs and the activity of several key enzymes of glucose metabolism. It causes an increase in glucose permeability in both fish (Marín-Juez et al., 2014) and mammals (Wasserman et al., 2011) by moving GLUT4 from intracellular stores to the membranes of myocytes and adipocytes. Insulin also stimulates GLUT2 activity in trout liver (Polakof et al., 2010b), whereas hyperglycemia plays this role in the brain (Polakof et al., 2012). When glucose leaves the circulation and enters tissues, it is first phosphorylated. In trout, this process is accelerated by insulin-mediated activation of hexokinase in the liver (Polakof et al., 2010b) and hyperglycemia-mediated activation of glucokinase in brain and liver (Enes et al., 2009; Polakof et al., 2012). Insulin also stimulates the intracellular disposal of glucose phosphate by activating glycogen synthase to replenish glycogen stores (Polakof et al., 2010a,b). Epinephrine has also been shown to stimulate R_d glucose in resting trout (Weber and Shanghavi, 2000), but it is unclear whether the administration of exogenous glucose is accompanied by an increase in catecholamines. Finally, some glucose is probably lost in urine because the kidney is not able to deal with such levels of hyperglycemia. The rate of appearance of glucose in urine was previously quantified in adult rainbow trout that increased glycemia to 36 mmol l^{-1} after infusion of exogenous glucose, and it reached a maximal value of 2.1 µmol kg^{-1} min^{-1} (Bucking and Wood, 2005). Even though glycemia only increased to 28 mmol l^{-1} in our experiments, the same rate of urinary loss would have accounted for 7.6% of R_d (=2.1/27.6 µmol kg^{-1} min^{-1}).

Capacity for glucoregulation

An important goal of this study was to test the glucoregulatory ability of rainbow trout by subjecting them to a strong glycemic stress. After 4 h of infusion, the fish were able to limit glucose accumulation to a 2.5-fold increase in circulating concentration (Fig. 3B). This was achieved through major changes in glucose kinetics: the complete suppression of endogenous R_a for the first 3 h and a 160% increase in R_d (Fig. 4). To illustrate the consequences of these changes in flux, Fig. 8 shows calculated values for glycemia if glucose kinetics had not been modulated. If only partial changes in kinetics had occurred (i.e. if only R_a or only R_d had responded), glycemia would have increased 6-fold, whereas no modulation of fluxes at all would have resulted in a 10-fold increase to 107 mmol l^{-1} (Fig. 8). For comparison, a 2 h infusion of exogenous glucose at only once the normal rate of hepatic production in miniature pigs caused a 20% increase in glycemia (Muller et al., 1988). Even though the glucose challenge imposed on the pigs was a lot weaker (half the infusion rate for half the time) and took place in a 37°C endotherm with high basal metabolic rate instead of a 13°C ectotherm, these mammals with good glucoregulatory capacity were unable to avoid hyperglycemia. Our study shows that rainbow trout are actually able to minimize glycemic stress through rapid regulation of glucose fluxes. Experiments where exogenous glucose and insulin were infused also support the idea that trout are good glucoregulators (Polakof et al., 2010b), but this capacity is abolished when the fish are fed a high carbohydrate diet (Polakof et al., 2011).

Chronic hyperglycemia

We reasoned that chronically hyperglycemic trout with elevated baseline glucose fluxes would help to explore potential limits in the capacity to modulate glucose kinetics. Resting blood glucose concentrations were increased 2.3-fold (Fig. 1) and baseline glucose fluxes 1.3-fold (Fig. 2) compared with normoglycemic fish (Weber and Shanghavi, 2000). Interestingly, these animals mimic the metabolism of some diabetic patients who show a 2-fold increase in blood glucose concentration and a 1.3-fold increase in baseline hepatic production over healthy humans (Meyer et al., 1998). In trout, this state of chronic hyperglycemia can be achieved by feeding on a high-fat diet that reduces glucose phosphorylation (lower glucokinase and hexokinase activities in liver, muscle and white adipose tissue), decreases hepatic lipogenesis (via inhibition of fatty acid synthase and glucose-6-phosphate dehydrogenase) and

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increases hepatic glucose release (upregulation of G6Pase) (Figueiredo-Silva et al., 2012). It is clear that growing conditions were different for the fish of this study compared with the normoglycemic animals used previously (Weber and Shanghavi, 2000). The chronically hyperglycemic fish measured here show metabolic characteristics similar to type II diabetics (Figueiredo-Silva et al., 2012) and, therefore, would be expected to have impaired capacity for glucoregulation. Regardless, they were able to modulate glucose fluxes extremely well and normoglycemic fish subjected to the same glucose challenge should probably be able to cope even better.

Glucose as an oxidative fuel

R_d glucose is the sum of glucose oxidation and non-oxidative disposal. Therefore, it is a measure of the highest possible rate of glucose oxidation when non-oxidative disposal is nil. In the resting state, mammals only oxidize about 50% of R_d glucose as reported for rats (43%) (Brooks and Donovan, 1983), dogs (30–50%) (Paul and Bella Issekutz, 1967; Wasserman et al., 1992) and humans (40–60%) (Glamour et al., 1995; Katz et al., 1992). The fraction of R_d glucose actually oxidized in resting trout has never been measured, but is most likely less than 100%, as in mammals. To start characterizing the potential importance of glucose as an oxidative fuel in trout, we have calculated the contribution of this fuel to metabolic rate, assuming that 50% of R_d glucose was oxidized as in resting mammals (Fig. 7). In control fish, glucose would only cover about 50% of $\dot{M}_{O_2}$ (Fig. 7A), but in the fish receiving exogenous glucose, this fuel alone could support total metabolic rate after 4 h (Fig. 7B). At the end of the exogenous supply experiments, a maximum of 50% R_d glucose can be oxidized and, therefore, at least 50% of R_d must go to non-oxidative disposal. If other fuels than glucose are also oxidized, the relative importance of glucose oxidation will decrease below 50% of R_d glucose and non-oxidative disposal will increase accordingly.

Conclusions

Resting hyperglycemic trout maintain high enough rates of glucose production and disposal to account for 36% of metabolic rate, but reach 100% when exogenous glucose is supplied for a few hours. With their baseline glucose fluxes chronically elevated, they can completely suppress hepatic production and boost disposal by 160% to minimize the effects of a massive glucose challenge. Such responses are typical of mammals, but unexpected for an ectotherm. They are probably mediated by the effects of insulin on GLUT2 and GLUT4, as well as on key enzymes of carbohydrate metabolism. However, almost nothing is known about the endocrine regulation of glucose fluxes in fish. Sorting out the roles of insulin, hyperglycemia and GLUTs strikes us as an important challenge for future work. Without these large and rapid changes in glucose fluxes, trout glycemia would have increased four times faster than observed here to reach dangerous levels exceeding 100 mmol l^{-1} within hours. This study shows that trout capacity for glucoregulation is actually much better than generally described in the literature.

MATERIALS AND METHODS

Animals

Rainbow trout (471±34 g; N=15) (*Oncorhynchus mykiss* Walbaum 1792) were purchased from Linwood Acres Trout Farm (Campbellcroft, Ontario, Canada) where they were fed commercial food pellets (5.5 Optimum mix from Corey Nutrition Company, Fredericton, NB, Canada). This feed contains 26% lipids (with 15% carbohydrates) and it has been shown that such a high-fat diet causes persistent hyperglycemia in rainbow trout (Figueiredo-Silva et al., 2012). The fish were held in a 1200 liter flow-through tank in dechlorinated Ottawa tap water maintained at 13°C, and were exposed to a 12 h:12 h light:dark photoperiod. They were acclimated to these conditions for a minimum of 2 weeks before experiments. The animals were randomly divided into a control group and a group receiving exogenous glucose. All the procedures were approved by the Animal Care Committee of the University of Ottawa and adhered to the guidelines established by the Canadian Council on Animal Care.

Catheterization

Fish were fasted for 24 h prior to surgery. They were anesthetized with unbuffered ethyl 3-aminobenzoate methanesulfonate (MS-222; 60 mg l^{-1}) and doubly cannulated with BTPE-50 catheters (Instech Laboratories, Plymouth Meeting, PA, USA) in the dorsal aorta as described previously (Haman and Weber, 1996). The catheters were kept patent by flushing with Cortland saline containing 50 U ml^{-1} heparin (Sigma-Aldrich, St Louis, MO, USA). Only animals with a hematocrit >20% after recovery from surgery were used in experiments.

Swim tunnel respirometry

Even though all experiments were performed in resting animals, overnight recovery and subsequent measurements were made in a 90 liter swim tunnel respirometer (Loligo Systems, Tjele, Denmark) 36 h after the cessation of feeding, keeping water velocity at 0.5 body lengths s^{-1}. This very low speed minimizes stress; it requires no swimming and allows the fish to rest quietly at the bottom of the respirometer. The swim tunnel was filled with the same quality water as the holding tank and kept at 13°C. $\dot{M}_{O_2}$ was measured by intermittent flow respirometry using galvanic oxygen probes connected to a DAQ-PAC-G1 instrument controlled with AutoResp software (version 2; Loligo Systems). The probes were calibrated before measurements using air-saturated water (20.9% O_2).

Glucose kinetics

The catheters were made accessible through the swim tunnel lid by channeling them through a water-tight port. The rates of glucose appearance (R_a) and glucose disposal (R_d) were measured by continuous infusion of [6-^{3}H]glucose (PerkinElmer, Boston, MA, USA; 1.691 TBq $mmol^{-1}$). Infusates were freshly prepared immediately before each experiment by drying an aliquot of the solution obtained from the supplier under N_2 and resuspending in Cortland saline. A priming dose equivalent to 360 min of infusion was injected as a bolus at the start of each infusion (time −60 min) to reach isotopic steady state in <45 min (Shanghavi and Weber, 1999). Preliminary experiments indicated that this priming dose was sufficient to label the large glucose pool of hyperglycemic trout. For both groups (control and exogenous glucose), glucose kinetics were quantified by infusing labeled glucose at 1 ml h^{-1} using a calibrated syringe pump (Harvard Apparatus, South Natick, MA, USA). Infusion rates for labeled glucose averaged 4381± 322 Bq kg^{-1} min^{-1} (N=15) and these trace amounts only accounted for 0.00004% of the baseline rate of hepatic glucose production in normoglycemic fish (Shanghavi and Weber, 1999). In addition, the group receiving exogenous glucose was supplied with unlabeled glucose at a rate of 20 µmol kg^{-1} min^{-1} starting at time 0. The infusion pump rate was determined individually for each fish (~1 ml h^{-1}) to adjust for differences in body mass. This rate of exogenous glucose supply is equivalent to twice the baseline rate of endogenous glucose production by the liver measured in the control group. Blood samples (100 µl each) were taken after 50, 55 and 60 min (time 0) of tracer infusion to quantify baseline glucose kinetics, as well as every 20 min thereafter. The amount of blood sampled from each fish accounted for <10% of total blood volume. Samples were immediately deproteinized in 200 µl of perchloric acid (6% w/w) and centrifuged for 5 min at 12,000 rpm (Eppendorf 5415C, Brinkman, Rexdale, Canada). Supernatants were kept frozen at −20°C until analyses. Glucose fluxes measured here in adult hyperglycemic fish of both sexes (471±34 g body mass, fed a diet containing 26% fat) were compared with those of adult normoglycemic trout of both sexes (566±19 g body mass, fed a diet containing 11% fat) from a previous study (Weber and Shanghavi, 2000).

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Sample analyses

Blood glucose concentration was measured spectrophotometrically using a Spectra Max Plus384 Absorbance Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). To measure glucose activity, samples were dried under N_2 to eliminate labeled water and resuspended in distilled water. Radioactivity was measured by scintillation counting (Beckman Coulter LS 6500, Fullerton, CA, USA) in Bio-Safe II scintillation fluid (RPI Corp., Mount Prospect, IL, USA).

Calculations and statistics

Glucose fluxes were calculated using the classic equations of Steele (Steele, 1959). When glucose concentration remained at baseline levels, R_a and R_d were equal and the steady state equation was used to calculate them. When glucose concentration varied significantly from baseline, R_a and R_d were calculated separately using the non-steady state equations. The rate of endogenous glucose production (endogenous R_a glucose) was calculated by subtracting the rate of exogenous glucose infusion from the values measured for total R_a glucose. Statistical comparisons were performed using one-way repeated-measures analysis of variance (RM-ANOVA) with the Dunnett's *post hoc* test to determine which values were significantly different from baseline (SigmaPlot v12, Systat Software, San Jose, CA, USA). When the assumptions of normality or equality of variances were not met, Friedman's non-parametric RM-ANOVA on ranks was used or the data were normalized by $\log_{10}$ transformation before parametric analysis. Values are presented as means±s.e.m. and a level of significance of $P<0.05$ was used in all tests.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

K.C. and J.-M.W. designed the experiments. K.C. performed the measurements and analyzed the data. K.C. and J.-M.W. interpreted the results and wrote the paper.

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