GLUCOSE KINETICS OF HYPERGLYCEMIC RAINBOW TROUT: EFFECTS OF EXOGENOUS GLUCOSE AND EXERCISE

KEVIN CHOI

Thesis submitted to the Faculty of Graduate and Postdoctoral Studies in partial fulfillment of the requirements for the Master of Science degree in Biology

Ottawa-Carleton Institute of Biology, Faculty of Sciences, University of Ottawa

© Kevin Choi, Ottawa, Canada, 2015

Title: Glucose Kinetics of Hyperglycemic Rainbow Trout:

Effects of Exogenous Glucose and Exercise

Author: Kevin Choi, B.Sc.H

Thesis advisor: Jean-Michel Weber, Ph.D., Department of Biology, University of Ottawa

Research funded in part by: Natural Science and Engineering Research Council

GLUCOSE KINETICS OF HYPERGLYCEMIC RAINBOW TROUT: EFFECTS OF EXOGENOUS GLUCOSE AND EXERCISE

SUMMARY

This thesis investigates the ability of rainbow trout to modulate hepatic glucose production (R_a) and disposal (R_d). My goals were to determine: (1) if resting trout can modulate fluxes to cope with exogenous glucose; (2) how fluxes change during graded swimming; (3) how exogenous glucose affects swimming kinetics; and (4) if exogenous glucose affects cost of transport or performance. Results show that resting trout suppress R_a completely and stimulate R_d from 10.6 to 27.6 μmol kg^{-1} min^{-1}. During swimming, fluxes increase from 15.6 to 21.9 μmol kg^{-1} min^{-1}, but only at speeds >2.4 BL s^{-1}. When given glucose, trout suppress R_a from 16.4 to 4.1 μmol kg^{-1} min^{-1} and stimulate R_d from 16.4 to 40.1 μmol kg^{-1} min^{-1}. Glucose lowers metabolic rate but does not affect critical swimming speed. Therefore, this research shows that rainbow trout have a much better capacity for glucoregulation than generally suggested by current literature.

RÉSUMÉ

Cette thèse examine la capacité de la truite arc-en-ciel à modifier son taux de production hépatique (R_a) et son taux d'utilisation du glucose (R_d). Mes buts étaient de déterminer : (1) si la truite au repos peut ajuster ses flux pour faire face au glucose exogène; (2) comment les flux changent au cours de la nage d'intensité croissante; (3) comment le glucose exogène affecte la cinétique pendant la nage; et (4) si le glucose exogène a un impact sur le coût du transport ou la performance. Les résultats montrent que la truite au repos arrête complètement R_a et stimule R_d de 10.6 à 27.6 μmol kg^{-1} min^{-1}, mais seulement à des vitesses >2.4 longueurs de corps par seconde. Quand elle reçoit du glucose, la truite diminue R_a de 16.4 à 4.1 μmol kg^{-1} min^{-1} et stimule R_d de 16.4 à 40.1 μmol kg^{-1} min^{-1}. Le glucose exogène abaisse le taux métabolique mais ne modifie pas la vitesse critique de nage. En conséquence, cette recherche montre que la truite arc-en-ciel possède une bien meilleure capacité de glucorégulation que généralement suggérée par la litérature actuelle.

ACKNOWLEDGEMENTS

I would like to dedicate this thesis to my family, Dr. Yong Choi, Mrs. Cindy Choi and Nicholas Choi, for the love and support they provided throughout my life and academic career. They always believed I would achieve my goals and never let me doubt my abilities.

I would like to thank Dr. Jean-Michel Weber for giving me the opportunity to complete a master's degree. Having no prior research experience, he provided tremendous support and guidance over the past two years, making this one my most memorable experiences.

I would like to thank Soo-Min Kim who has always been there for me, providing support throughout my degree and in my personal life. I would also like to thank my lab mates for their continued support: Teye Omlin, Alyssa Gonzalez, Eric Vaillancourt, Luke De Freitas, Eric Turenne, Jill Madigan, Chelsea Hill and Tessa Blanchard.

Finally, I would like to thank Bill Fletcher and Christine Archer for their help in animal care along with Dr. John Lewis and Dr. Tom Moon (thesis committee members) for their guidance.

This thesis was supported by an NSERC discovery grant to Dr. Jean-Michel Weber.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: General Introduction

INTRODUCTION

This thesis investigates the ability of rainbow trout (*Oncorhynchus mykiss* Walbaum) to modulate glucose fluxes. As a species generally considered to be glucose intolerant, little is known about the role of hepatic glucose production (R_a glucose) and disposal (R_d glucose) in glucoregulation. There are only a few studies that have started to explore their flux modulation ability (Haman et al., 1997b; Shanghavi and Weber, 1999; Weber and Shanghavi, 2000), but nothing has attempted to push their glucoregulation to the limits. Therefore, this thesis aims to address how well trout can regulate glucose fluxes in response to exogenous glucose and graded exercise.

Overview of fuel selection and energy production

Adenosine 5'-triphosphate (ATP) is the universal energy currency used by all animals to maintain basal metabolism for survival. Despite its importance, ATP is not stored in large quantities as it is synthesized on demand to match the energetic needs of the animal. Therefore, various fuels (lipids, carbohydrates, and proteins) must be readily available to replenish ATP at a rate matching its utilization. These fuels are selected based on the metabolic demand of the animal as each confers its own costs and benefits. Lipids are the most abundant (~90% of total energy reserve) because they can be stored without water making them light and energy dense (Weber, 2011). However, they are slow to mobilize and can only be metabolized aerobically (Weber, 1999). In contrast, carbohydrates are stored in small quantities due to their mass, but can be metabolized anaerobically and produce ATP at higher maximal rates (Weber, 1999). Proteins are not normally used for energy production because they play important structural and functional roles, as well as lead to the production of toxic ammonia as a metabolic waste product (Weber, 2011).

Importance of glucose as a metabolic fuel

At rest, lipids are usually the fuel of choice owing to their high energy density per molecule (Weber, 2011). However, certain tissues rely extensively on glucose even during this low metabolic state. In resting mammals, glucose is mainly used by the brain (45-60% of total glucose used), skeletal muscle (15-20%), kidney (10-15%), blood cells (5-10%), splanchnic organs (3-6%) and adipose tissue (2-4%) (Shrayyef and Gerich, 2010). Of these tissues, the brain is the largest user of glucose since other possible fuels (such as ketone bodies or free fatty acids) face transport limitations across the blood brain barrier (Shrayyef and Gerich, 2010). Without appropriate glucose levels, various complications can arise: hypoglycemia can cause severe nervous system damage, coma, and death, while hyperglycemia can lead to diabetes. During high intensity exercise, lipids cannot provide ATP fast enough and cells must rely on carbohydrates from circulation and intramuscular glycogen stores. Therefore, maintaining appropriate blood glucose concentration is important for basal physiological conditions as well as to support high intensity exercise.

Comparing glucoregulators: mammals versus trout

Given the importance of glucose, fine control over glucoregulation is paramount in mammals. Glucose tolerance tests have been used as a simple and rough estimate of glucoregulatory ability and early studies have used this method to compare different species. In mammals, a glucose load of 1 g glucose/kg body mass (as recommended by the World Health Organization) (Bergot, 1979; Horowitz et al., 1993) is administered, and blood glucose concentration is measured overtime. Results show that, on average, mammals take 1 to 3 h to restore baseline glucose concentrations (Abumrad et al., 1982; Andrikopoulos et al., 2008; Horowitz et al., 1993; Jackson et al., 1973; Kelley et al., 1988; Shrayyef and Gerich,

2010). In contrast to the rapid response seen in mammals, trout take 24 h to restore normoglycemia when using 0.25 g glucose/kg body mass (Legate et al., 2001). This observation combined with the limited effects of insulin on glucose transporters and enzymes associated with glucose metabolism (Marín-Juez et al., 2014; Polakof et al., 2012), suggests rainbow trout are poor glucoregulators. However, one of the major limitations of the glucose tolerance test is that it only measures blood glucose concentration. Glucose concentration and its changes depend on the rates of glucose appearance into and disposal from the circulation. R_a and R_d glucose can fluctuate, but glucose concentration only changes when there is a mismatch between them. Therefore, concentration measurements alone provide very little information on glucose kinetics.

Hyperglycemia

In the laboratory, rainbow trout can experience chronic or acute hyperglycemia, and both will be used in this thesis in an attempt to explore the limits of glucoregulation. I will use chronically hyperglycemic trout because I want to add another level of glycemic stress to further push their glucoregulatory limits. Diabetic humans can exhibit a 1.3-fold increase in baseline glucose fluxes over healthy humans (Meyer et al., 1998), and hopefully these hyperglycemic trout will also have elevated baseline fluxes, making any potential flux modulation that much more difficult. To achieve this, my trout will be fed a high fat diet (26%) which has been shown to cause persistent hyperglycemia by reducing glucose phosphorylation, hepatic lipogenesis and increasing hepatic glucose release (Figueiredo-Silva et al., 2012). In addition, I will induce acute hyperglycemic stress through the infusion of exogenous glucose. The infusion rate will be roughly twice their rate of hepatic glucose

production, which will ensure elevated glucose appearance in the circulation even if they are able to completely suppress endogenous production.

Glucose kinetics theory

To measure glucose fluxes, I will be using [6-^{3}H]glucose and Steele's steady and non-steady state equations (Steele, 1959). These equations simplify the animal to 2 compartments: the rapidly mixing pool (which comprises of plasma and extracellular fluid) and tissues. The rapidly mixing pool has a constant volume where the rate of glucose appearance (R_a) is equal to the rate of disposal (R_d). When labelled glucose is added to this rapidly mixing pool, the rate at which it is infused will eventually match the rate at which it leaves the pool. When this occurs, the animal is considered to be in isotopic steady state and the ratio of labelled glucose to unlabelled glucose (known as specific activity) is constant. Therefore, if we know the infusion rate of the labelled glucose (F = DPM/kg/min) as well as the specific activity (SA = DPM/μmol), we can calculate R_a (= R_d in μmol/kg/min) as

$$R_a = R_d = \frac{F}{SA}$$

When there is a change in glucose concentration over time, R_a and R_d are not equal. Therefore, the non-steady state equation must be used to calculate R_a and R_d separately as

$$R_a = \frac{F - pV\left(\frac{C_2 + C_1}{2}\right)\left(\frac{SA_2 - SA_1}{t_2 - t_1}\right)}{\left(\frac{SA_2 + SA_1}{2}\right)}$$

$$R_d = R_a - pV\left(\frac{C_2 - C_1}{t_2 - t_1}\right)$$

where pV is the pool volume of glucose (ml/kg), c_1 is the glucose concentration in the blood at time 1 and c_2 is the glucose concentration in the blood at time 2 for two consecutive time

samples (mmol l^{-1}), and t_1 and t_2 are the times at which blood samples are taken (min). Pool volume varies between different metabolites, but has been determined empirically to be 50 ml/kg for glucose (Haman et al., 1997b).

RESTING GLUCOSE KINETICS

Glucose flux modulation in trout

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, these studies have relied on the bolus injection technique which has severe limitations: (1) flux calculations are based on the surface area under specific-activity decay curves that are difficult to measure accurately; (2) each experiment produces only one flux value; and (3) it assumes steady state and cannot quantify R_a and R_d separately (Omlin and Weber, 2010). Recently, the more modern continuous tracer infusion technique was developed to quantify glucose fluxes in rainbow trout as shown in Fig. 1.1, because it does not suffer from these limitations (Omlin and Weber, 2010). Baseline glucose kinetics studies in rainbow trout using this technique revealed a large variation in glucose fluxes, ranging from 6 to 38 μmol kg^{-1} min^{-1} (Haman et al., 1997a; Haman and Weber, 1996). Since then, various stressors have been used on rainbow trout to test their ability to regulate fluxes and are summarized in Fig. 1.2 (Haman et al., 1997b; Shanghavi and Weber, 1999; Weber and Shanghavi, 2000). The fact that trout can modulate glucose fluxes in response to changes in environmental conditions, catecholamines and exercise suggest that

they may have a better ability for glucoregulation than previously thought. Unfortunately, insufficient information on the regulation of R_a and R_d glucose is presently available to assess their true capacity for glucose homeostasis.

Mammalian glucoregulation

In contrast to the limited information available in trout, mammalian flux regulation has received extensive attention (Triplitt, 2012). 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 (-70%) and a large increase in R_d (150%) (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 exogenous supply. In mammals, rapid changes in glucose fluxes are mostly regulated via insulin that can suppress gluconeogenic enzymes, increase glucose transporter density in membranes, and stimulate glycogen synthesis (Shrayyef and Gerich, 2010). However, trout appear to show limited sensitivity to insulin (Moon, 2001), and, therefore, it is unclear whether or to what extent trout are able to modulate their fluxes.

GLUCOSE KINETICS DURING EXERCISE

Effects of exercise on glucose kinetics

In mammals, glucose contributes significantly to energy metabolism in working muscles (Shrayyef and Gerich, 2010). During aerobic exercise, glucose oxidation accounts for 10 to 40% of metabolic rate (Wahren et al., 1971; Weber et al., 1996) and glucose fluxes

can be stimulated 5-fold over resting values (Romijn et al., 2000; Weber et al., 1996). By contrast, the role of glucose in fish metabolism has not been clearly characterized. Its true importance in trout remains unknown because glycemia shows high sensitivity to various hormones, water osmolarity, and diet, suggesting that glucose is an important substrate (Polakof et al., 2012). However, the opposite could also be argued because trout are renowned for their poor glucoregulation (Legate et al., 2001; Moon, 2001). In addition, they decrease glucose fluxes during prolonged, low intensity swimming, whereas mammals doing equivalent exercise show a 2- to 4-fold increase (Shanghavi and Weber, 1999). This suggests that glucose plays a minor role as a metabolic fuel during swimming, but the glucose kinetics of fish has never been measured at high exercise intensities. The only study to have attempted glucose measurements in trout at high swimming speeds reported a 1.9-fold increase in red muscle glucose concentration (Richards et al., 2002). Unfortunately, no information on kinetics can be drawn from this datum.

Pushing the limits of glucose disposal

Trout are considered to have a limited capacity to stimulate disposal rates because only epinephrine has been shown to increase R_d glucose by a maximum of 2-fold (Weber and Shanghavi, 2000). Because mammals show the greatest stimulation of disposal during intense exercise or exogenous glucose infusion, it would be interesting to assess how trout would respond to the same stresses. With the limited information available, it is unclear how much trout are able to increase their disposal rates. In mammals, providing exogenous glucose during exercise allows them to stimulate R_d glucose to higher values compared to exercise alone (Angus et al., 2002; Howlett et al., 1998; Marmy-Conus et al., 1996; McConell et al., 1994) or at rest with only exogenous supply (Ferrannini et al., 1985; Jackson

et al., 1986; Muller et al., 1988). Therefore, submitting trout to a combination of intense swimming and exogenous glucose supply is predicted to reveal their true capacity for glucose disposal.

Glucose as an ergogenic aid

Most studies testing the effects of exogenous glucose on human athletic performance show a significant improvement, although some report no change or even a decrease (Cermak and van Loon, 2013). These types of performance improvement range from increases in overall endurance (32-44%) to improvement in time trial experiments (30-50%) (Febbraio et al., 2000; Tabata and Kawakami, 1991; Wright et al., 1991). However, it is not known whether raising glucose availability could increase critical swimming speed (U_{crit}) in trout, because a previous study with exogenous lactate showed no improvement (Omlin et al., 2014). Exogenous glucose could also affect fuel selection by causing a switch from lipids to carbohydrates. Therefore, it could potentially decrease metabolic rate (MO_2) because 15% to 30% less oxygen is needed to produce the same amount of ATP when oxidizing carbohydrates rather than lipids (Schippers et al., 2012; Welch et al., 2007). This in turn could result in a lower cost of transport (COT) measured as the amount of oxygen used to move one unit body mass by one unit distance (Teulier et al., 2013). The combination of MO_2 and U_{crit} measurements will allow me to determine changes in aerobic and anaerobic metabolism, respectively, when trout are given exogenous glucose.

GOALS OF THESIS

This thesis includes a series of *in vivo* experiments where continuous tracer infusion is used to quantify changes in the rates of hepatic glucose production and glucose disposal during various challenges. It is divided in two parts designed to test the ability of rainbow trout to regulate glucose fluxes. In the first part (Chapter 2), I 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) what is the relative importance of circulating glucose as an oxidative fuel. In the second part (Chapter 3), I want to determine: (1) the effects of graded swimming on the glucose kinetics of hyperglycemic rainbow trout; (2) how the supply of exogenous glucose modulates the changes in glucose flux caused by exercise alone; and (3) whether exogenous glucose increases performance (U_{crit}) or decreases COT. Finally, in Chapter 4, a general discussion of all the results is provided to summarize what was learned about glucose flux modulation in rainbow trout.

Figure 1.1. Diagram of a doubly cannulated rainbow trout. The solid purple line is used for the continuous infusion of the radiotracer and the dotted purple line is used for the infusion of unlabelled, exogenous glucose. The solid orange line is used for blood sampling. Dots (•) represent catheter entry sites in the artery. Catheters are not drawn to scale. Figure was modified from (Haman and Weber, 1996).

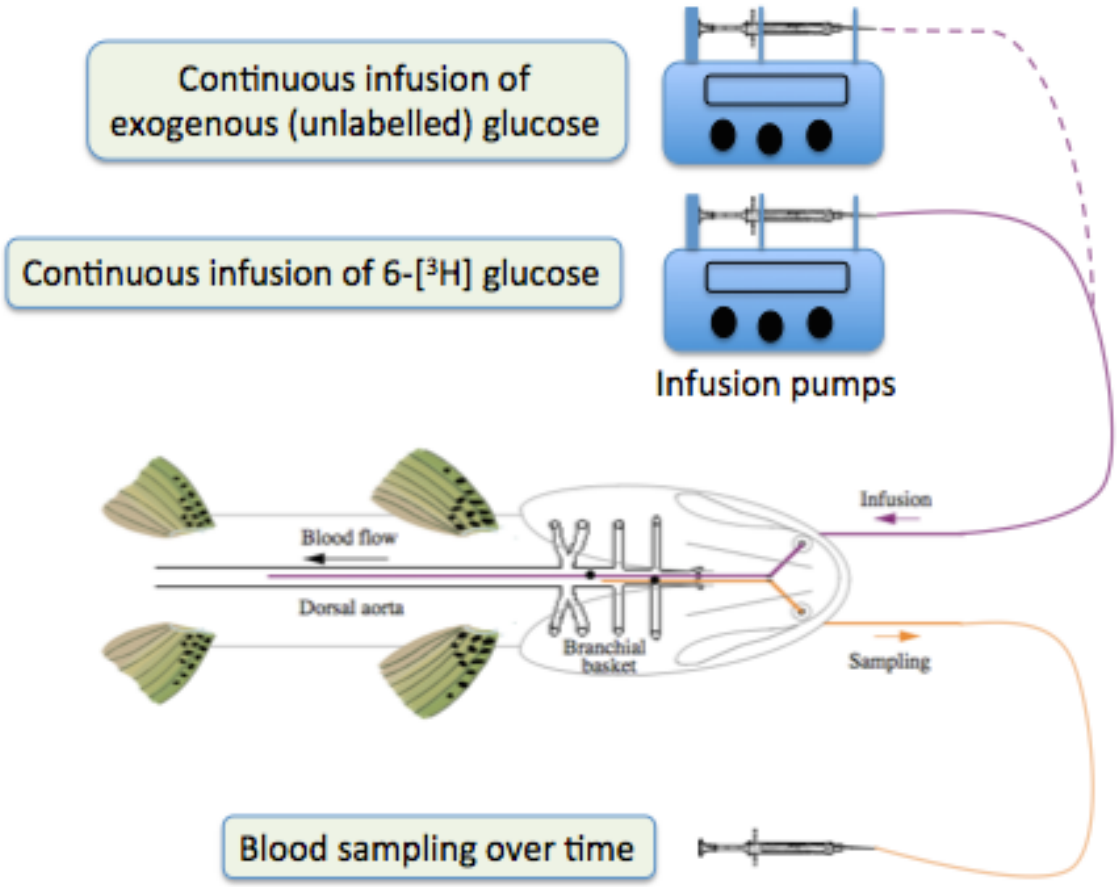
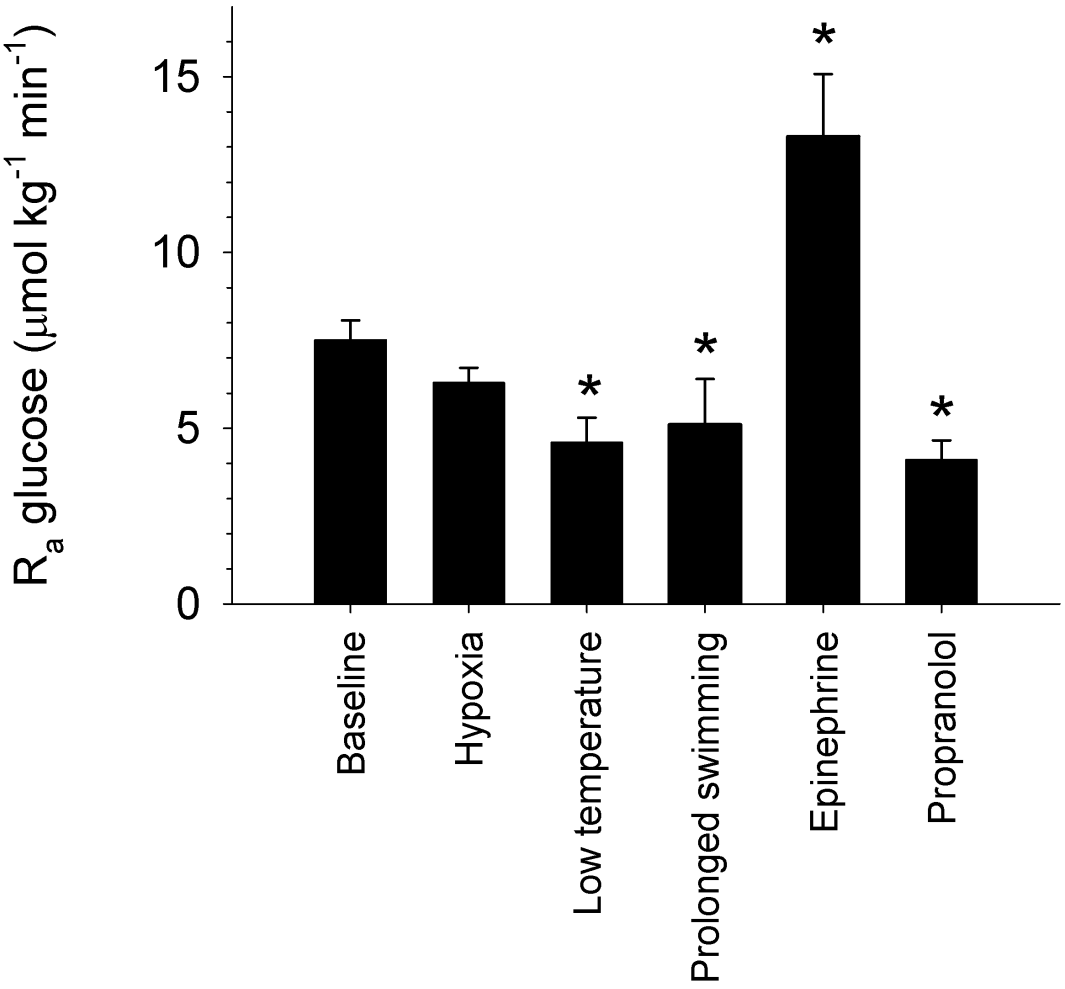

Continuous infusion of exogenous (unlabelled) glucose
Continuous infusion of 6-[3H] glucose
Infusion pumps
Infusion
Blood flow
Dorsal aorta
Branchial basket
Sampling
Blood sampling over time

Figure 1.2. Effects of various treatments to date on the rate of hepatic glucose production in rainbow trout. Low temperature, prolonged swimming and propranolol decrease R_a while epinephrine stimulates it. Data for hypoxia and low temperature are from (Haman et al., 1997b), exercise is from (Shanghavi and Weber, 1999), and epinephrine and propranolol is from (Weber and Shanghavi, 2000). The baseline value presented is the average of all the baseline values measured in the studies listed above. * indicate significant differences from the baseline values measured in each individual study ($P<0.05$).


R_a glucose (μmol kg^{-1} min^{-1})
15
10
5
0
*
*
*
*
Baseline
Hypoxia
Low temperature
Prolonged swimming
Epinephrine
Propranolol

CHAPTER 2: Pushing the limits of glucose kinetics:

How rainbow trout cope with a carbohydrate overload

Based on:

Kevin Choi and Jean-Michel Weber

The Journal of Experimental Biology (doi: 10.1242/jeb.125716)

Biology Department, University of Ottawa, Ottawa, Canada

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 into, 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 of these studies relied on the bolus injection technique that 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 showed 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). The fact that trout can modulate glucose fluxes in response to changes in environmental conditions, catecholamines and exercise suggest 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 fish capacity to modulate glucose fluxes has never been tested with 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, I 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) what is the relative importance of circulating glucose as an oxidative fuel. I 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.

METHODS

Animals

Rainbow trout (471±34 g; *N*=15) (*Oncorhynchus mykiss* Walbaum) 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,

New Brunswick, Canada). This feed contains 26% lipids 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 1,200 L flow-through tank in dechlorinated Ottawa tap water maintained at 13^{o}C, and were exposed to a 12:12 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 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 L swim tunnel respirometer (Loligo Systems, Tjele, Denmark) keeping water velocity at 0.5 body lengths per second. 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^{o}C. Metabolic rate (MO_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 6 h 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 labelled glucose at 1 ml h^{-1} using a calibrated syringe pump (Harvard Apparatus, South Natick, MA, USA). Infusion rates for labelled glucose averaged 4381±322 Bq kg^{-1} min^{-1} (*N*=15) and these trace amounts accounted for only 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 unlabelled 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.

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 labelled 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 v.12, 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.

RESULTS

Steady-state glucose kinetics of hyperglycemic fish

Metabolic rate (MO_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. 2.1. Tracer infusion was started 1 h before time 0 to reach isotopic steady-state. Over the next 4 h, MO_2 remained stable ($P>0.05$; Fig. 2.1A) and averaged 63.7±0.4 μmol O_2 kg^{-1} min^{-1}. Blood glucose concentration also remained constant ($P=0.654$; Fig. 2.1B) and averaged 12.5±0.04 mmol l^{-1}, or more than twice the levels of normoglycemic trout (Weber and Shanghavi, 2000). Blood glucose specific activity only showed a minor increase after 3 h ($P<0.05$; Fig. 2.1C), but remained constant at all other times, averaging 435±5.1 Bq μmol^{-1}. Fig. 2.2 compares baseline glucose fluxes of hyperglycemic fish (this study) with those of normoglycemic fish from a previous study (Weber and Shanghavi, 2000). The glucose flux 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) that averaged 7.9±0.07 μmol kg^{-1} min^{-1} ($P<0.05$).

Glucose kinetics of hyperglycemic fish supplied with exogenous glucose

Metabolic rate (MO_2), blood glucose concentration and glucose specific activity of hyperglycemic animals receiving exogenous glucose are shown in Fig. 2.3. 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 (unlabelled) glucose was started at time 0. Over the next 4 h, MO_2 remained stable (P>0.05; Fig. 2.3A) 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±1.0 to 27.5±1.1 mmol l^{-1} at the end of the experiment (P<0.001; Fig. 2.3B). Blood glucose specific activity decreased from 423±46 to 171±17 Bq μmol^{-1}. All specific activities measured after time 1.5 h were lower than baseline (P<0.001; Fig. 2.3C). Fig. 2.4 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±1.0 to 29.1±3.9 μmol kg^{-1} min^{-1} (P<0.001). R_d glucose increased from 10.6±1.0 to a maximum of 27.6±3.9 μmol kg^{-1} min^{-1} after 4 h (P<0.001). Endogenous R_a glucose rapidly decreased from 10.6±1.0 to 0.4±1.3 μmol kg^{-1} min^{-1} (P<0.001) before returning to baseline over the last hour of the experiment. Table 2.1 summarizes the changes in blood glucose and lactate concentration, 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 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. 2.5. 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. 2.6. Hyperglycemic fish receiving no exogenous glucose maintained their R_d glucose constant over time (P>0.05) at an average level of 134±2% of normoglycemic controls. Hyperglycemic fish receiving exogenous glucose progressively increased their 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. 2.6).

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. 2.7. Calculations were made following 2 different assumptions: either that 100% or that only 50% of R_d glucose is oxidized. If 100% is oxidized, glucose by itself could account for total MO_2 for fish receiving exogenous glucose as well as those that do not (all values > 100%). If 50% of R_d is oxidized, hyperglycemic fish receiving no glucose could support 50.3±0.9% of MO_2 with glucose oxidation (Fig. 2.7A). For fish receiving exogenous glucose and oxidizing 50% of R_d, the relative contribution of glucose oxidation to MO_2 would go from 36 to 100% throughout the experiment (Fig. 2.7B).

Impact of flux regulation on blood glucose concentration

To evaluate the effects of the changes in glucose kinetics reported here, Fig. 2.8 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. In these fish, glucose oxidation alone could account for total metabolic rate, even without exogenous supply. 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 2.4, 2.5), even though baseline fluxes are already elevated (Fig. 2.2). Such a total inhibition of R_a is characteristic of the mammalian response to exogenous glucose (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 produces 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 my conditions. 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 mammals (Shrayyef and Gerich, 2010). Similarly, gluconeogenesis is inhibited by high insulin that stops the 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; Cowey et al., 1977b; Enes et al., 2009). This mechanism was probably operating here because my 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 by 2.6-fold in response to exogenous glucose (Figs 2.4, 2.6) and this large change in kinetics was unexpected for an animal generally considered as 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 (GLUT), 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; Polakof et al., 2010b). 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.

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: the infusion of exogenous glucose at twice the normal rate of hepatic production. After 4 h of infusion, the fish were able to limit glucose accumulation to a 2.5-fold increase in circulating concentration (Fig. 2.3B). This was achieved through major changes in glucose kinetics: the complete suppression of R_a for the first 3 h and a 160% increase in R_d (Fig. 2.4). To illustrate the consequences of these changes in flux, Fig. 2.8 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 by 6-fold, whereas no modulation of fluxes at all would have resulted in a 10-fold increase to 107 mmol l^{-1} (Fig. 2.8). 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 these pigs was a lot weaker (half the infusion rate for half the time), these mammals with good glucoregulatory capacity were unable to avoid hyperglycemia. My study shows that rainbow trout are actually able to minimize glycemic stress through rapid regulation of glucose fluxes.

Chronic hyperglycemia

I reasoned that chronically hyperglycemic trout with elevated baseline glucose fluxes would help explore potential limits in the capacity to modulate glucose kinetics. Resting

blood glucose concentrations were 2.3-fold greater (Fig. 2.1B) and baseline glucose fluxes 1.3-fold higher (Fig. 2.2) than in normoglycemic fish (Weber and Shanghavi, 2000). This conclusion was drawn from direct comparisons with (Weber and Shanghavi, 2000) that had an average blood glucose concentration of approximately 5.4 mmol l^{-1}. However, looking at multiple studies on normoglycemic rainbow trout, the average blood glucose concentration is approximately 5.3 mmol l^{-1} (N=72) (Haman and Weber, 1996; Haman et al., 1997b; Shanghavi and Weber, 1999; 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 (glucokinase and hexokinase in liver, muscle and white adipose tissue), hepatic lipogenesis (fatty acid synthase and glucose-6-phosphate dehydrogenase) and increases hepatic glucose release (glucose-6-phosphatase) (Figueiredo-Silva et al., 2012). It is clear that growing conditions were different for the fish of this study compared to normoglycemic animals used previously (Weber and Shanghavi, 2000). The chronically hyperglycemic fish measured here show metabolic characteristics similar to type II diabetic humans (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 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, I calculated the contribution of this fuel to metabolic rate, assuming that either 100% or only 50% of R_d was oxidized (Fig. 2.7). If 100% of R_d is oxidized, endogenous glucose alone could account for the metabolic rate of a resting fish. If 50% of R_d is oxidized (as in resting mammals), glucose would only cover about 50% of MO_2 (Fig. 2.7A). In the fish receiving exogenous glucose, this fuel alone could support total metabolic rate, even if only 50% of R_d was oxidized (Fig. 2.7B). At the end of the exogenous supply experiments, a maximum of 50% R_d 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% R_d and non-oxidative disposal will increase accordingly.

CONCLUSIONS

Resting hyperglycemic trout maintain high enough rates of glucose production and disposal to account for total metabolic rate, even without exogenous supply. 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 were probably mediated by the effects of insulin on GLUT2 and 4, 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 me as an important challenge for future work. Without these large and rapid changes in glucose fluxes, trout glycemia would have increased 4 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.

Table 2.1. Initial, intermediate (2 h) and final values (4 h) for blood metabolite concentrations and glucose fluxes of rainbow trout supplied with exogenous glucose or not (controls). Rates of 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*). * indicate significant differences from initial values within each group ($P<0.05$).

	Control			Exogenous Glucose		
	Initial	2 Hours	Final	Initial	2 Hours	Final
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)
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) *

Figure 2.1. Metabolic rate (MO_2) (A), blood glucose concentration (B), and blood glucose specific activity (C) of resting rainbow trout (control group) during measurement of glucose kinetics by continuous infusion of [6-^{3}H]glucose (started at time -1 h). Values are means ± s.e.m. (*N*=7). * indicate significant differences from baseline (time 0; $P<0.05$).

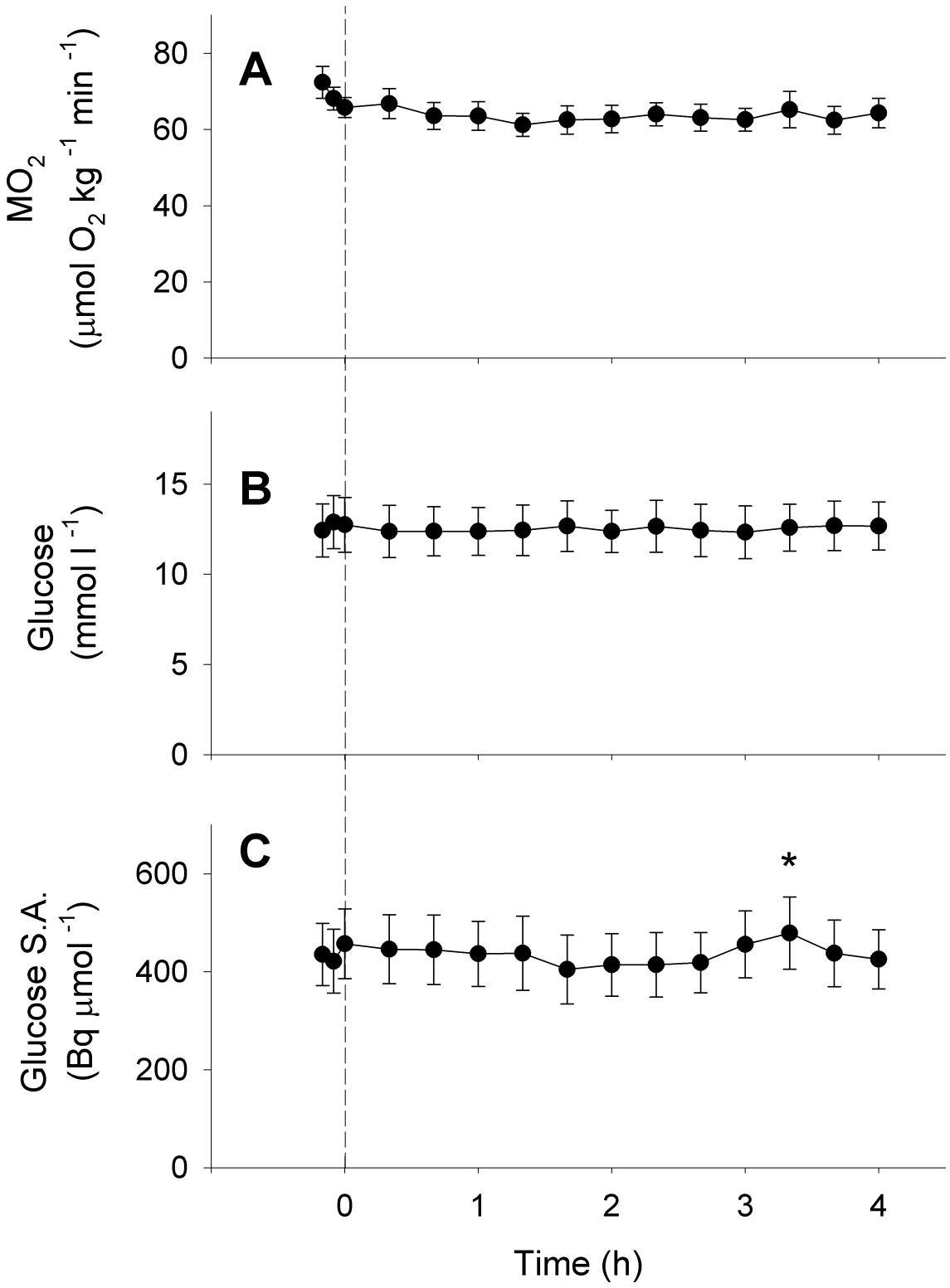

A
B
C
MO_2 (µmol O_2 kg^{-1} min^{-1})
Glucose (mmol l^{-1})
Glucose S.A. (Bq µmol $^{-1}$)
Time (h)
*

Figure 2.2. Glucose fluxes of resting, hyperglycemic trout (control group; closed circles) compared with the fluxes of resting, normoglycemic trout (open circles) (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).

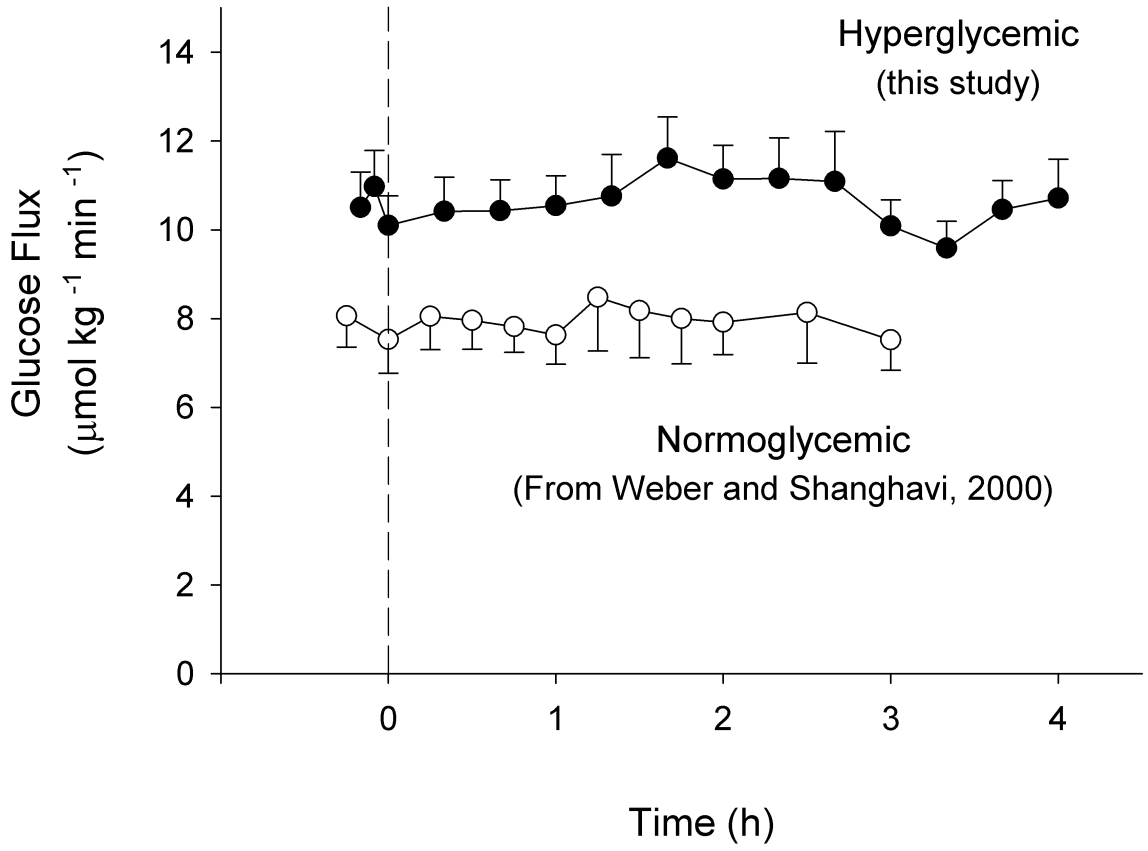

Hyperglycemic
(this study)
Normoglycemic
(From Weber and Shanghavi, 2000)
Glucose Flux
(μmol kg -1 min -1)
Time (h)
0
2
4
6
8
10
12
14
0
1
2
3
4

Figure 2.3. Effects of exogenous glucose infusion on metabolic rate (MO_2) (A), blood glucose concentration (B), and blood glucose specific activity (C) in resting rainbow trout. 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, unlabelled glucose was started at time 0. Values are means ± s.e.m. (*N*=8). * indicate significant differences from baseline (time 0; $P<0.05$).

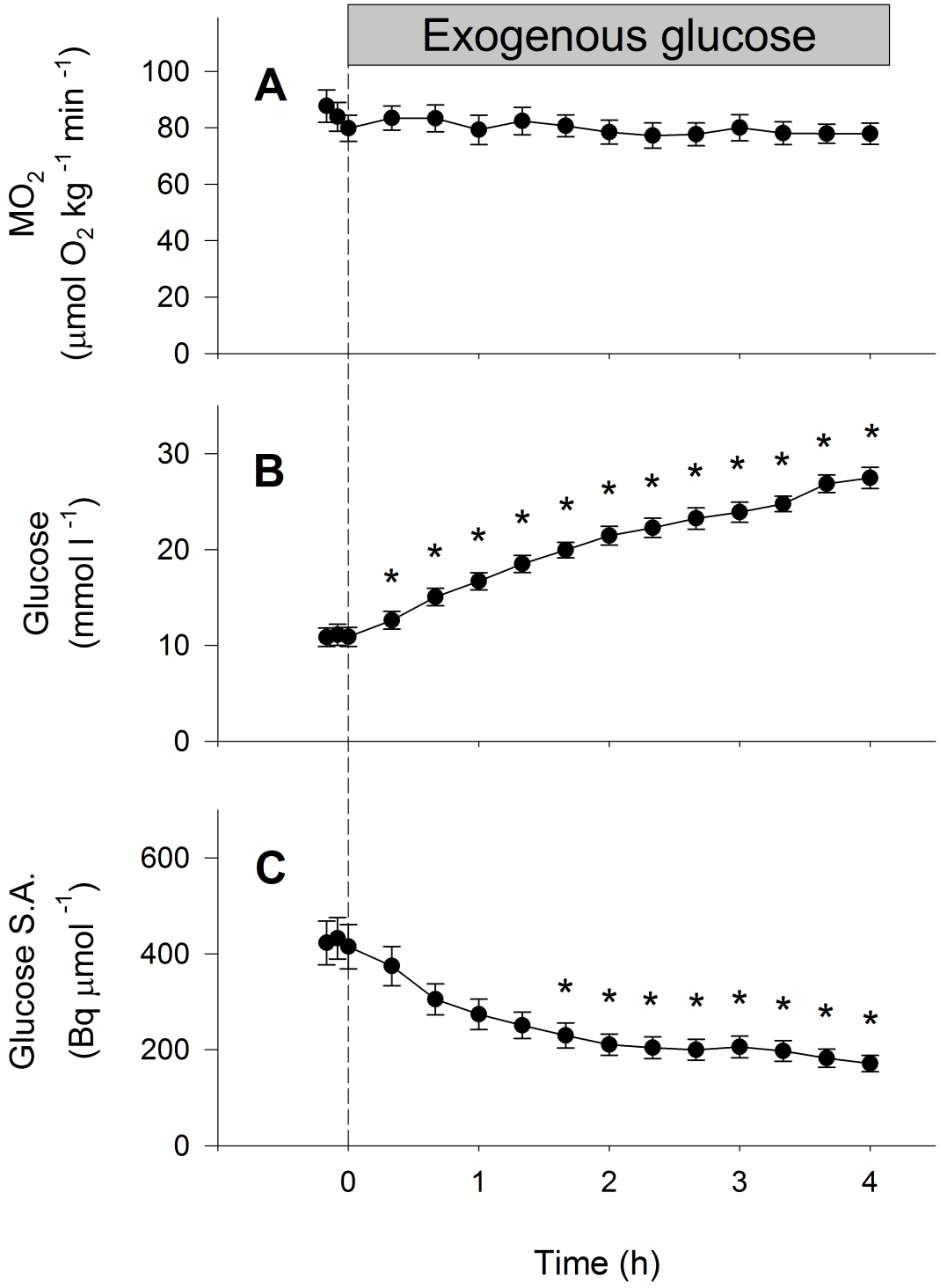

Exogenous glucose
A
B
C
MO_2 (µmol O_2 kg^{-1} min^{-1})
Glucose (mmol l^{-1})
Glucose S.A. (Bq µmol $^{-1}$)
Time (h)

Figure 2.4. Effects of exogenous glucose infusion on the 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, unlabelled glucose. The rate of glucose disposal (R_d glucose) is shown with filled circles. Values are means ± s.e.m. (*N*=8). * indicate significant differences from baseline values ($P<0.05$).

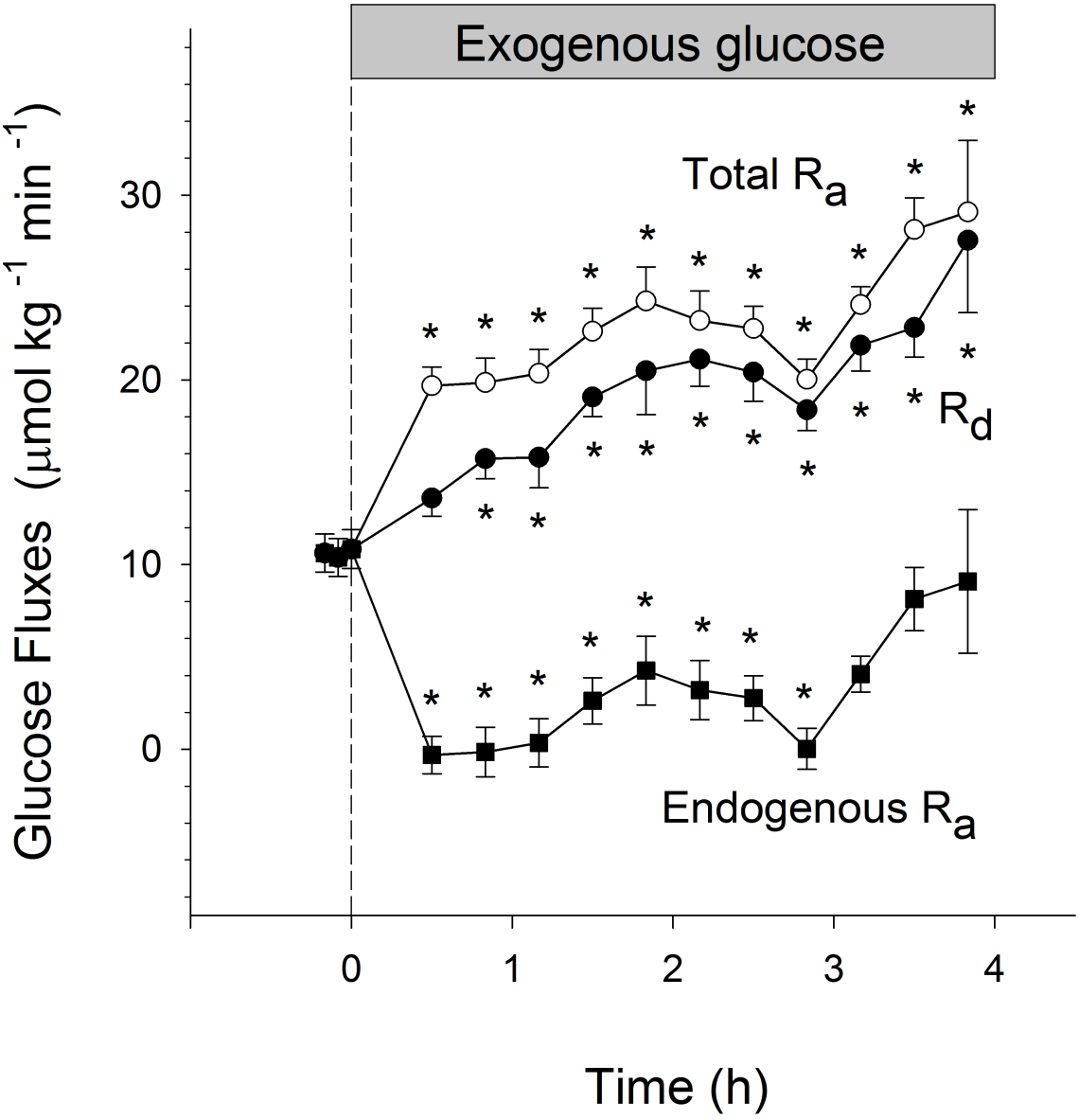

Exogenous glucose
Total R_a
R_d
Endogenous R_a
Glucose Fluxes (μmol kg^{-1} min^{-1})
Time (h)
0
10
20
30
0
1
2
3
4

Figure 2.5. Percentage of the R_a glucose of control hyperglycemic fish (receiving no exogenous glucose) shown by treatment fish (supplied with exogenous glucose). 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). * indicate significant differences from baseline ($P<0.05$).

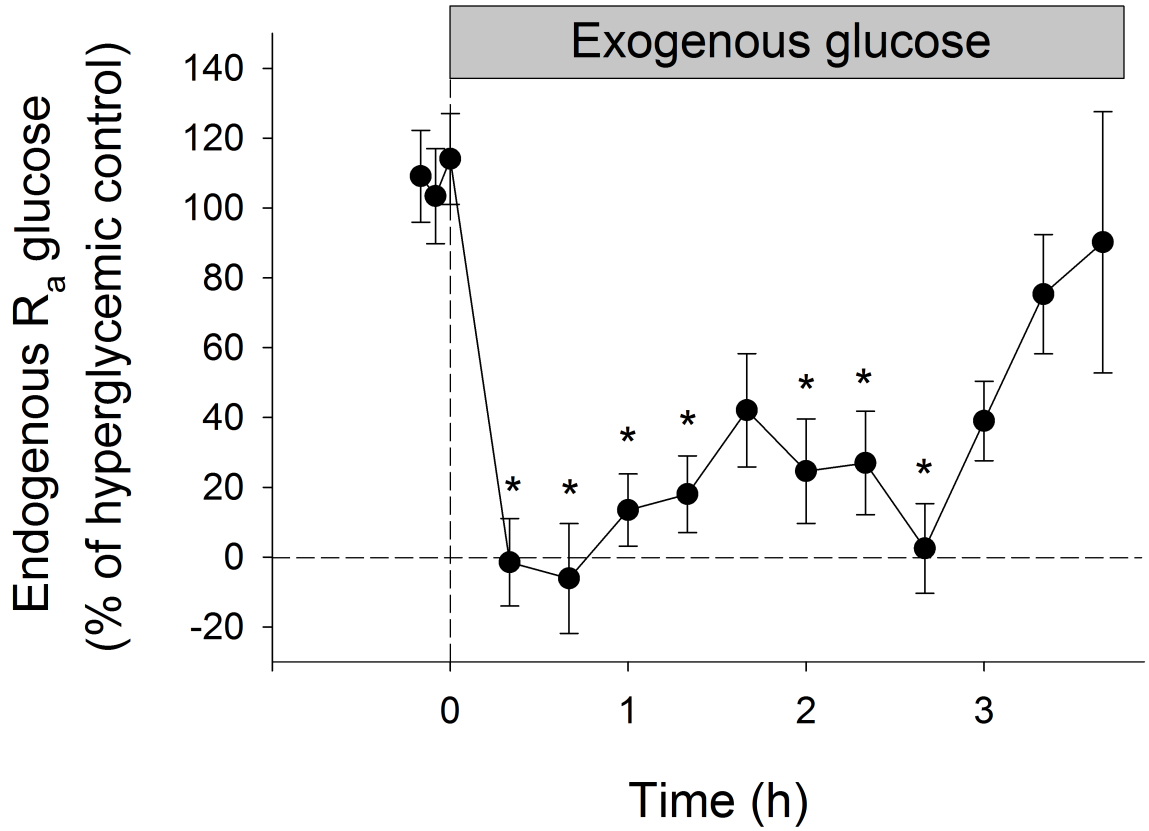

Exogenous glucose
Endogenous R_a glucose
(% of hyperglycemic control)
140
120
100
80
60
40
20
0
-20
0
1
2
3
Time (h)
*
*
*
*
*
*
*

Figure 2.6. Percentage of the R_d glucose of normoglycemic fish (horizontal line) shown by hyperglycemic fish receiving no exogenous glucose (open circles) and hyperglycemic fish supplied with exogenous glucose (closed circles). Values are means ± s.e.m. (N=7 for hyperglycemic trout and N=8 for hyperglycemic trout supplied with exogenous glucose). * indicate 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).

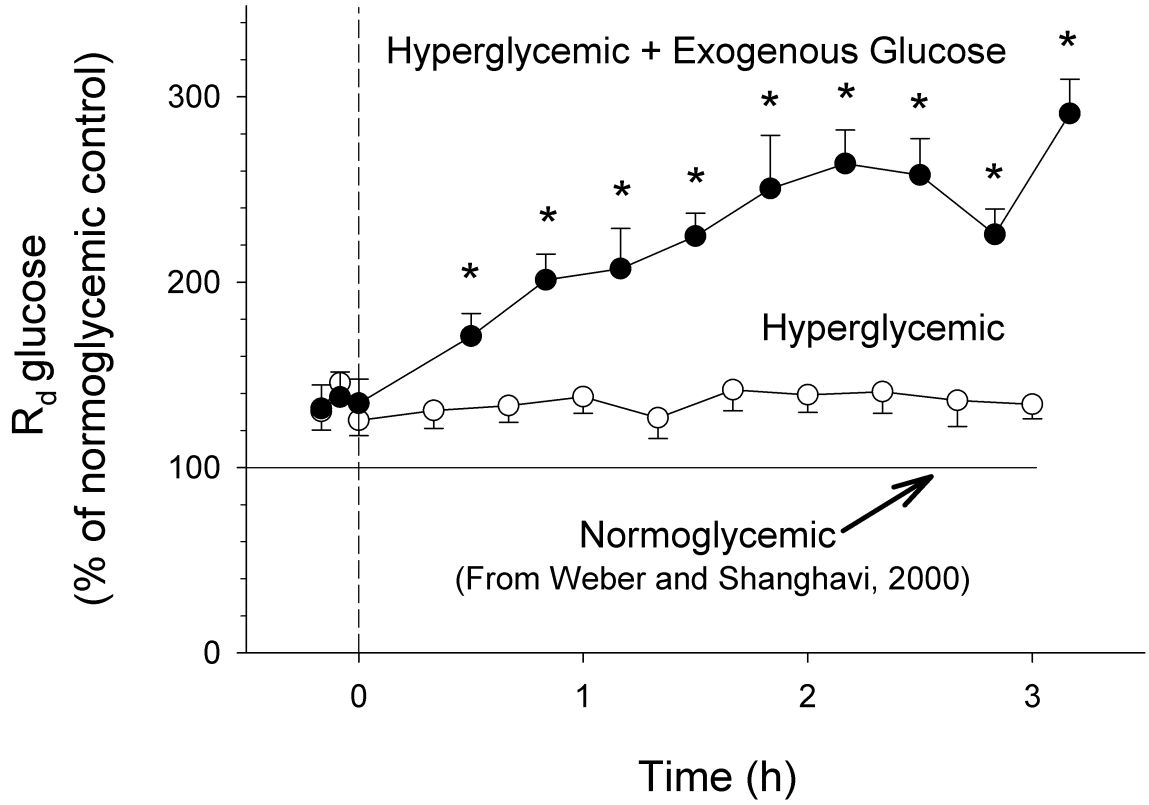

Hyperglycemic + Exogenous Glucose
Hyperglycemic
Normoglycemic
(From Weber and Shanghavi, 2000)
R_d glucose
(% of normoglycemic control)
Time (h)
300
200
100
0
0
1
2
3
*

Figure 2.7. Calculated percentages of total metabolic rate (MO_2) accounted for by glucose oxidation in control fish (A) and in fish receiving exogenous glucose (B). Values were calculated assuming that either 100% (closed circles) or only 50% (open circles) of R_d glucose was oxidized. Values are means ± s.e.m. (*N*=7 for control and *N*=8 for exogenous glucose). * indicate significant differences from baseline ($P<0.05$).

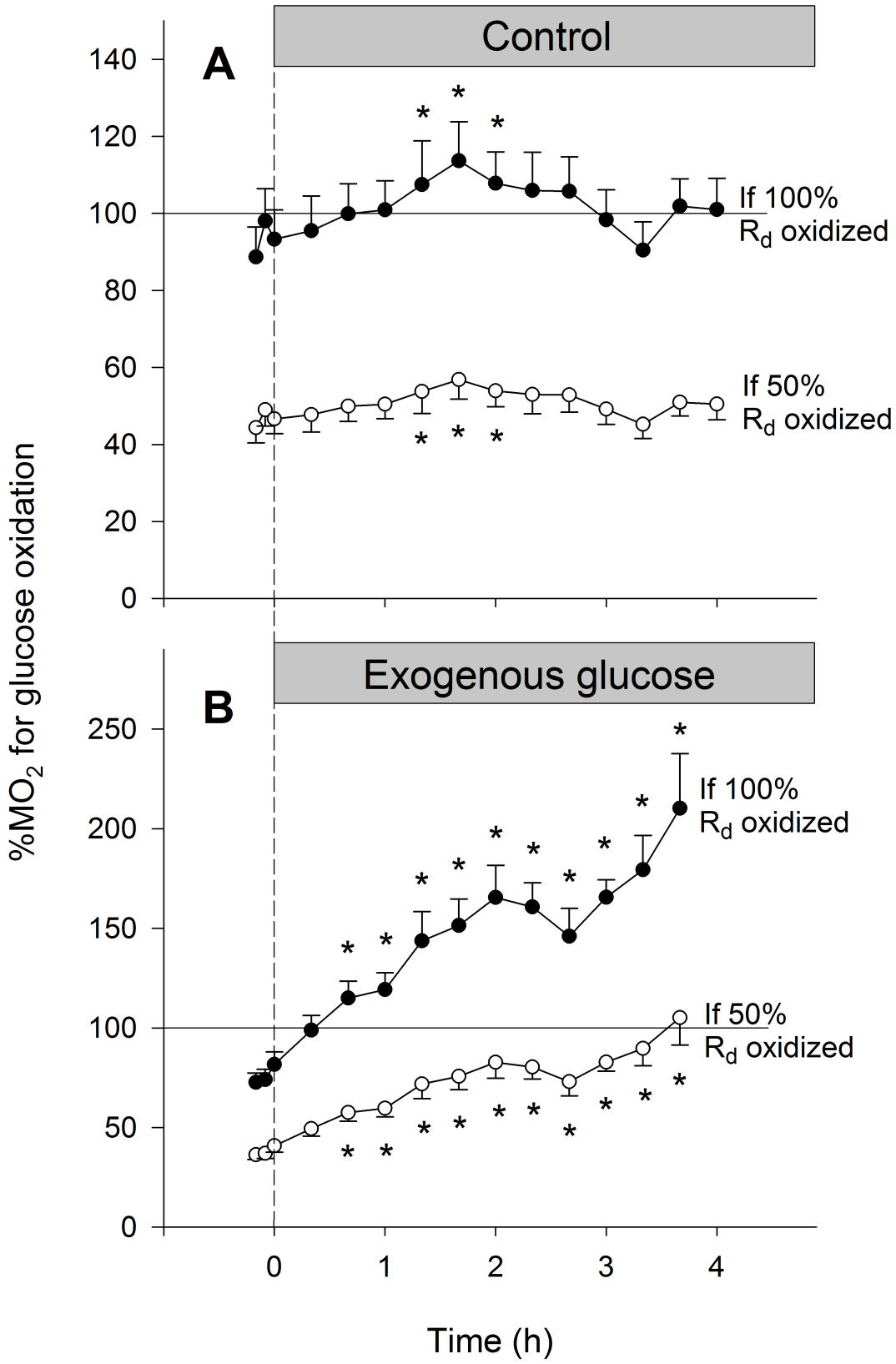
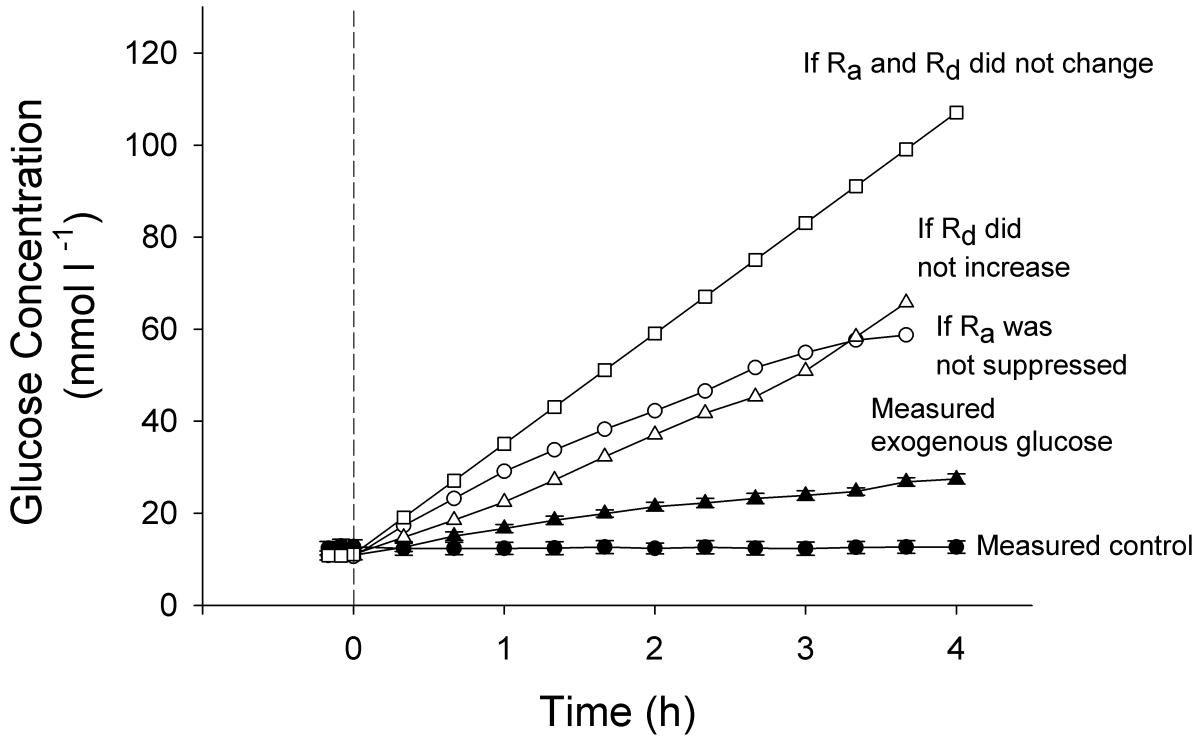

Control
A
140
120
100
80
60
40
20
0
If 100%
R_d oxidized
If 50%
R_d oxidized
Exogenous glucose
B
250
200
150
100
50
0
If 100%
R_d oxidized
If 50%
R_d oxidized
0
1
2
3
4
Time (h)
$\%MO_2$ for glucose oxidation

Figure 2.8. Comparison of measured blood glucose concentrations with theoretical values calculated for hypothetical fish that would fail to regulate their glucose fluxes when exogenous glucose is provided. Measured blood glucose concentrations are presented for control fish (closed circles) and for those receiving exogenous glucose (closed triangles). Theoretical concentrations are given for 3 different scenarios: (1) if R_a glucose had not been suppressed (open circles); (2) if R_d glucose had not been stimulated (open triangles); and (3) if both, R_a and R_d, had remained at baseline throughout the infusion of exogenous glucose (open squares).


Glucose Concentration
(mmol l -1)
120
100
80
60
40
20
0
0
1
2
3
4
Time (h)
If Ra and Rd did not change
If Rd did
not increase
If Ra was
not suppressed
Measured
exogenous glucose
Measured control

CHAPTER 3: Coping with exogenous glucose overload: Glucose kinetics of rainbow trout during graded swimming

Based on:

Kevin Choi and Jean-Michel Weber

American Journal of Physiology, in review.

Biology Department, University of Ottawa, Ottawa, Canada

INTRODUCTION

In mammals, glucose plays an essential role as a fuel for the brain and contributes significantly to energy metabolism in working muscles (Shrayyef and Gerich, 2010). During aerobic exercise, glucose oxidation accounts for 10 to 40% of metabolic rate (Wahren et al., 1971; Weber et al., 1996) and glucose fluxes can be stimulated 5-fold over resting values (Romijn et al., 2000; Weber et al., 1996). By contrast, the role of glucose has not been clearly characterized in fish metabolism and the evidence available for a model species like rainbow trout is still ambiguous. The high sensitivity of trout glycemia to various hormones, water osmolarity, and diet suggests that glucose is an important substrate (Polakof et al., 2012). However, the opposite could be argued because trout are renowned for their poor glucoregulation (Moon, 2001) and only normalize glycemia very slowly in glucose tolerance tests (Legate et al., 2001). In addition, they decrease glucose fluxes during prolonged, low intensity swimming, whereas mammals doing equivalent exercise show a 2- to 4-fold increase (Shanghavi and Weber, 1999). This suggests that glucose plays a minor role as a fuel for locomotion, but the glucose kinetics of fish have never been measured at high exercise intensities when carbohydrates become preferred.

Trout were previously considered to have a limited capacity to modulate glucose fluxes because the only changes demonstrated were a 2-fold increase with epinephrine (Weber and Shanghavi, 2000), and more minor effects of temperature (Haman et al., 1997b) and low intensity swimming (Shanghavi and Weber, 1999). However, in Chapter 2, I put the plasticity of trout glucose kinetics to a more stringent test by infusing exogenous glucose at twice the baseline rate of hepatic production in hyperglycemic animals (Choi and Weber, 2015). These fish show impressive responses by completely suppressing endogenous production (R_a glucose) and stimulating disposal by 2.6-fold (R_d glucose). Such mammal-

like regulation is particularly surprising because it occurs in hyperglycemic fish with chronically elevated baseline glucose fluxes. Under such conditions, it is unclear whether R_d glucose is pushed to its upper limit because mammals receiving exogenous glucose can boost disposal to higher levels during exercise (Angus et al., 2002; Howlett et al., 1998; Marmy-Conus et al., 1996; McConell et al., 1994) than at rest (Ferrannini et al., 1985; Jackson et al., 1986; Muller et al., 1988). Most human studies testing the effects of exogenous glucose on athletic performance show an improvement, although some report no change or even a decrease (Cermak and van Loon, 2013). It is not known whether enhancing glucose availability could increase critical swimming speed (U_{crit}) in trout, but a previous study testing exogenous lactate showed no effect (Omlin et al., 2014). The supply of exogenous glucose could also affect fuel selection by causing a switch from lipids to carbohydrates. Therefore, it could potentially decrease metabolic rate (MO_2) because 15% to 30% less oxygen is needed to produce the same amount of ATP when oxidizing carbohydrates rather than lipids (Schippers et al., 2012; Welch et al., 2007). This could result in a lower metabolic cost of transport (COT) measured as the amount of oxygen needed to move one unit body mass by one unit distance (Teulier et al., 2013). Therefore, the goals of this study are: (1) to quantify the effects of graded swimming on the glucose kinetics of hyperglycemic rainbow trout; (2) to determine how the supply of exogenous glucose modulates the changes in glucose flux caused by exercise alone; and (3) to see whether exogenous glucose increases swimming performance (U_{crit}) or decreases COT. I anticipate that glucose fluxes will show a greater decrease at high swimming speeds than previously observed during sustained, low-intensity exercise (Shanghavi and Weber, 1999), that exogenous glucose will suppress R_a and stimulate R_d to higher values than reported for rest in Chapter 2 (Choi and Weber, 2015), and that it will fail to improve U_{crit} but decrease COT.

METHODS

Animals

Rainbow trout (316±12 g; *N*=22) (*Oncorhynchus mykiss* Walbaum) were purchased from Linwood Acres Trout Farm (Campbellcroft, Ontario, Canada) where they were fed commercial food pellets (26% lipids) (5.5 Optimum mix from Corey Nutrition Company, Fredericton, New Brunswick, Canada). The fish were held in a 1,200 L flow-through tank in dechlorinated Ottawa tap water maintained at 13^{o}C, and were exposed to a 12:12 h light:dark photoperiod. The acclimation period under these conditions was a minimum of 2 weeks. The animals were randomly divided into two groups: "control" and "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

After 24 h of fasting, fish were anesthetized with 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 flushed with Cortland saline containing 50 U ml^{-1} heparin (Sigma-Aldrich, St Louis, MO, USA) to prevent any blood clots from forming. Only animals with a hematocrit >20% after recovery from surgery were used in experiments.

Swim tunnel respirometry and U_{crit} protocol

After surgery, each animal was allowed to recover overnight in a 90 L swim tunnel respirometer (Loligo Systems, Tjele, Denmark) at 13^{o}C with a water velocity of 0.5 body length

per second (BL s^{-1}). This low water flow rate minimizes stress and requires no swimming to remain stationary. Metabolic rate (MO_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). Both groups (with or without exogenous glucose) performed a stepwise critical swimming speed (U_{crit}) protocol as detailed in (Teulier et al., 2013).

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 as described in Chapter 2. A priming dose equivalent to 6 h 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). For both experimental groups, glucose kinetics were quantified by infusing labelled glucose at 1 ml h^{-1} using a calibrated syringe pump (Harvard Apparatus, South Natick, MA, USA). Infusion rates for labelled glucose averaged 5952±209 Bq kg^{-1} min^{-1} (*N*=22) and these trace amounts only accounted for 0.00005% 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 unlabelled glucose at a rate of 20 µmol kg^{-1} min^{-1}. The exact infusion rate (~1 ml h^{-1}) was determined individually for each fish to adjust for differences in body mass. Blood samples (100 µl each) were taken after 50, 55 and 60 min of tracer infusion to quantify baseline glucose kinetics (and to confirm isotopic steady state), as well as every 20 min thereafter corresponding to the

stepwise increase in swimming speed. 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.

Sample analyses

Blood glucose concentration was measured spectrophotometrically using a Spectra Max Plus384 Absorbance Microplate Reader (Molecular Devices, Sunnyvale, CA, USA), and 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

Critical swimming speed (U_{crit}), total cost of transport (TCOT), and net cost of transport (NCOT) were calculated as previously described in (Teulier et al., 2013). TCOT is the total amount of oxygen required to move one unit body mass by one unit distance, which includes the cost of sustaining life in resting tissues. NCOT is the oxygen cost to power locomotion alone and excludes all resting costs. Glucose fluxes were calculated using the equations of Steele (Steele, 1959). The steady state equation was used to calculate baseline flux under resting conditions while the non-steady state equation was used during exercise to calculate R_a and R_d glucose separately. The rate of endogenous glucose production (endogenous R_a) was calculated by subtracting the rate of exogenous glucose infusion from measured values for total R_a glucose. Statistical comparisons were performed using one- or two-way repeated-measures analysis of variance (RM-ANOVA) with the Dunnett's *post hoc* test to determine which values were significantly different from control, or a two-tailed t-test

to compare U_{crit} between treatment groups (SigmaPlot v.12, 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.

RESULTS

Metabolic rate, critical swimming speed, and cost of transport

Metabolic rate (MO_2), total cost of transport (TCOT), and net cost of transport (NCOT) for control fish (no exogenous glucose) and fish receiving exogenous glucose are shown in Fig. 3.1. MO_2 was lower in fish receiving exogenous glucose than in controls ($P=0.040$, Fig. 3.1A), but increased with swimming speed in both groups ($P<0.001$). MO_2 increased from a resting value of 67.4±5.0 to a maximum of 149±31 μmol kg^{-1} min^{-1} in the controls, and from 54.3±2.6 to 169±5.9 μmol kg^{-1} min^{-1} in fish receiving glucose. Exogenous glucose had no effect on U_{crit} that was 2.3±0.1 BL s^{-1} in controls and 2.3±0.2 BL s^{-1} in the treatment group ($P=0.873$). TCOT was lower in fish receiving glucose than in controls ($P=0.036$; Fig. 3.1B). It decreased with speed from 5.0±0.5 to 2.0 μmol O_2 kg^{-1} m^{-1} in the control fish ($P<0.001$), and from 3.8±0.2 to a minimal value of 2.2±0.1 μmol O_2 kg^{-1} m^{-1} in fish receiving glucose ($P<0.001$). NCOT was not different between the treatment groups ($P=0.595$; Fig. 3.1C). It increased from 0.4±0.2 to 1.3±0.3 μmol O_2 kg^{-1} m^{-1} in the control fish ($P<0.001$), and from 0.2±0.1 to a maximal value of 1.7±0.1 μmol O_2 kg^{-1} m^{-1} in fish receiving glucose ($P<0.001$).

Carbohydrate metabolism in control fish

In control fish receiving no exogenous fuel, glucose concentration was independent of swimming speed and remained constant at 15.6±0.4 mmol l^{-1} throughout the experiment (P=0.305; Fig. 3.2A). Lactate concentration increased from a resting value of 1.0±0.2 to a maximum of 3.1 mmol l^{-1} at the highest speed (P=0.002; Fig. 3.2A). Glucose specific activity decreased with speed from 415±35 to 235 Bq μmol^{-1} (P=0.039; Fig. 3.2B). Fig. 3.3 shows the effects of graded swimming on glucose fluxes. Because glucose concentration remained constant, R_a and R_d glucose were never different from each other (P>0.05) and they showed the same changes with speed. R_a glucose remained constant until 2.4 BL s^{-1} where there was an increase from 15.6±1.4 to 22.1 μmol kg^{-1} min^{-1} (P=0.012). R_d glucose followed the same pattern with an increase from 15.6±1.4 to 21.8 μmol kg^{-1} min^{-1} at speeds greater than 2.4 BL s^{-1} (P=0.047).

Carbohydrate metabolism in fish receiving exogenous glucose

In fish receiving exogenous fuel, glucose concentration increased from a resting value of 17.7±1.6 to a maximum of 43.8±11.3 mmol l^{-1} at the highest speed (P<0.001; Fig. 3.4A). Lactate concentration increased from a resting value of 1.1±0.4 to a maximum of 3.1±1.3 mmol l^{-1} at the highest swimming speed (P<0.001; Fig. 3.4A). Glucose specific activity decreased with speed from 390±44 to 145±56 Bq μmol^{-1} (P<0.001; Fig. 3.4B). Fig. 3.5 shows the effects of graded swimming and exogenous glucose on glucose fluxes. Measured total R_a (= rate of endogenous hepatic glucose production + rate of exogenous glucose administration) increased progressively from 16.4±1.6 to 42.7±13.4 μmol kg^{-1} min^{-1} as exogenous glucose was supplied and speed increased (P<0.001). R_d glucose increased from 16.4±1.6 to 40.1±13 μmol kg^{-1} min^{-1} as speed

increased (P<0.001). Endogenous R_a glucose decreased from 16.4±1.6 to 4.1±1.7 μmol kg^{-1} min^{-1} before increasing to 22.7±13.4 μmol kg^{-1} min^{-1} at higher speeds than 2.6 BL s^{-1} (P<0.001). Table 3.1 summarizes the changes in MO_2, blood glucose and lactate concentration, R_a glucose and R_d glucose in fish receiving no exogenous glucose (controls) as well as those infused with exogenous glucose.

Relative changes in R_d glucose with exercise

Control fish receiving no exogenous fuel decreased R_d glucose to 81.1±6.9% of baseline values before increasing to 195% at higher speeds than 2.4 BL s^{-1} (P=0.017) (Fig. 3.6). Fish receiving exogenous fuel increased R_d glucose progressively from 100 to 275±143% of resting values before exogenous glucose was supplied (P<0.001).

Potential contribution of glucose oxidation to MO_2

The relative importance of glucose as an oxidative fuel was calculated after assuming either that 100% or only 50% of R_d glucose is oxidized (100% R_d provides the highest possible contribution of glucose to metabolic rate; 50% R_d is the average value observed in resting mammals). For the control group, the relative contribution of glucose to MO_2 varied with swimming speed (P=0.002; Fig. 3.7A). If 100% of R_d is oxidized, the maximal possible contribution of glucose decreases progressively from 136±16% to 51±11%, before increasing to 111% at speeds higher than 2.4 BL s^{-1}. If 50% of R_d is oxidized, the maximal contribution of glucose decreases from 67.9±7.8% to a minimum of 25.5±5.4%, before rising back to 55.5% at speeds higher than 2.4 BL s^{-1}. In the fish receiving exogenous glucose, the relative contribution of this fuel to MO_2 did not vary with swimming speed (P=0.12; Fig. 3.7B). If 100% of R_d is

oxidized, glucose alone could account for total MO_2 because all calculated values are above 100%. If 50% of R_d is oxidized, the relative contribution of glucose to MO_2 would average 88.3±4.4%.

Impact of flux regulation on blood glucose concentration

To evaluate the effects of the changes in glucose kinetics reported here, Fig. 3.8 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 43.8 mmol l^{-1} after ~4 h of exogenous glucose infusion. However, hypothetical fish would have reached 74 (if R_a was not suppressed), 77 (if R_d was not stimulated), and 114 mmol l^{-1} (if glucose fluxes had not responded at all to exogenous supply).

DISCUSSION

This study shows that the glucose fluxes of rainbow trout are only stimulated by strenuous swimming (1.4-fold), but remain unaffected at exercise intensities below 2.5 BL s^{-1}. By comparison, mammals already start increasing fluxes at low work intensities to reach up to 5-fold baseline during intense exercise. When trout receive exogenous glucose during swimming, they are able to stimulate disposal more strongly than with exogenous supply alone or exercise alone, reaching maximal values of 40 μmol kg^{-1} min^{-1}. In addition, they suppress hepatic glucose

production, but not completely as they do at rest in Chapter 2. Supplying extra carbohydrates in the form of glucose does not improve swimming performance because U_{crit} remains constant as observed previously for exogenous lactate (Omlin et al., 2014). However, the metabolic rate of fish receiving exogenous glucose is consistently lower than in controls and their total cost of transport is reduced accordingly. A potential switch from lipids to more carbohydrates may allow them to use oxygen more efficiently for ATP production because carbohydrate oxidation is accomplished with a higher P/O ratio than lipid oxidation (Schippers et al., 2012; Welch et al., 2007).

Glucose kinetics during graded swimming

This study is the first to characterize the effects of swimming intensity up to U_{crit} on the glucose fluxes of rainbow trout. The only information previously available was that, unlike mammals, trout progressively decrease R_a and R_d glucose during sustained low intensity exercise (-33% over 4 h) (Shanghavi and Weber, 1999). Therefore, I had anticipated that intense swimming would cause an even stronger inhibition of glucose fluxes. This hypothesis is rejected because they show a 40% increase at the highest swimming speeds (Fig. 3.3). In mammals, both sustained and incremental exercise gradually increase R_a glucose (Romijn et al., 2000; Weber et al., 1996). This stimulation of hepatic production is mainly mediated by circulating glucagon and catecholamines that stimulate gluconeogenesis and glycogenolysis (Galbo et al., 1975). Instead of a progressive increase in R_a with work intensity, trout first show a non-significant trend towards a decrease before an abrupt stimulation, but only when they reach maximal intensities above 2.5 BL s^{-1} (Fig. 3.3). Plasma epinephrine may play an important role in regulating this response because it decreases below baseline during low intensity swimming, potentially explaining the inhibition of R_a

observed previously (Shanghavi and Weber, 1999) as well as the non-significant trend seen here (Fig. 3.3). Low epinephrine levels have been shown to impair gluconeogenesis and glycogenolysis in isolated trout hepatocytes (Wright et al., 1989). Overall, these observations support the idea that glucose is not a critical muscle fuel during submaximal swimming. At exercise intensities close to U_{crit}, a sudden increase in circulating catecholamines and/or glucagon may be responsible for the stimulation of R_a glucose. These hormones activate hepatic glucose production (Polakof et al., 2012), but more experiments will be needed to characterize their exact role during high speed swimming.

Pushing the limits of glucose disposal

This study demonstrates that trout are able to increase R_d glucose from 16 to 22 μmol kg^{-1} min^{-1} at high swimming speeds (Fig. 3.3). This can be caused by insulin-independent migration of GLUT4 from intracellular stores to the membranes of myocytes and adipocytes in response to exercise (Marín-Juez et al., 2014). When intense exercise and exogenous glucose are combined, trout can push R_d even higher to record values of 40 μmol kg^{-1} min^{-1} (Figs 3.5, 3.6) and this result is consistent with the mammalian response (Howlett et al., 1998; Marmy-Conus et al., 1996; McConell et al., 1994). Exogenous glucose can cause GLUT4 migration that is mediated by elevated insulin levels (Marín-Juez et al., 2014), which also activate liver GLUT2 (Polakof et al., 2010b), glycolysis (Enes et al., 2009; Polakof et al., 2012; Polakof et al., 2010b) and glycogen synthesis (Polakof et al., 2010a; Polakof et al., 2010b). In addition, exogenous glucose causes further hyperglycemia that activates brain GLUT2 (Polakof et al., 2012) and maintains higher blood-to-tissue glucose gradients that

drive disposal. The simultaneous effects of exercise, insulin, and hyperglycemia provide the necessary signals to reach these highest rates of glucose disposal.

Exogenous glucose inhibits hepatic glucose production

Trout receiving exogenous glucose are able to lower hepatic glucose production by 75% for several hours during graded exercise (Fig. 3.5). This response is not as dramatic as in the resting state when R_a glucose is completely suppressed (Chapter 2), but is still surprising for an animal widely considered as a poor glucoregulator. Current information shows that elevated insulin levels caused by strong hyperglycemia play a major role in inhibiting R_a glucose (Banos et al., 1998). In mammals as well as trout, insulin reduces hepatic glucose production by inhibiting glucose 6-phosphatase (Polakof et al., 2010a; Rojas and Schwartz, 2014) and glycogen phosphorylase (Enes et al., 2009; Polakof et al., 2010b; Shrayyef and Gerich, 2010). In mammals, insulin also inhibits phosphoenolpyruvate carboxykinase (Rojas and Schwartz, 2014), but this only occurs in trout when they are fed a high protein diet (40-60%) (Cowey et al., 1977a; Cowey et al., 1977b; Enes et al., 2009). This mechanism could have acted in my study because the trout diet contained 46% protein. During exercise, exogenous glucose does not cause the complete inhibition of R_a as it does at rest. This is probably because swimming triggers additional hormonal signals that are absent at rest like increases in circulating glucagon and catecholamines (Galbo et al., 1975). These hormones can stimulate R_a by activating glycogenolysis and gluconeogenesis (Polakof et al., 2012). This occurs in mammals and explains why they can only decrease R_a to baseline values during exercise (Angus et al., 2002; Howlett et al., 1998; Marmy-Conus et al., 1996; McConell et al., 1994), but completely suppress it at rest (Ferrannini et al., 1985; Jackson et al., 1986; Muller et al., 1988).

Swimming performance and energetics

The metabolic rate of trout receiving exogenous glucose is significantly lower compared to trout receiving no glucose (-16%) (Fig. 3.1A). In Chapter 2, I found the opposite effect where fish receiving exogenous glucose showed elevated MO_2. In this experiment, trout were at rest and this could have made them more sensitive to their surroundings, causing greater stress compared to if they were swimming. In Chapter 3, trout were swimming and presumably are less stressed, which may explain why their MO_2 did not increase when infused with exogenous glucose. If MO_2 did decrease in response to the glucose infusion, this would suggest that the animal is using a greater proportion of glucose because it takes between 15% to 30% less oxygen to produce the same amount of ATP when oxidizing carbohydrates compared to lipids (Schippers et al., 2012; Welch et al., 2007). These fish also have a lower total cost of transport (TCOT = basal metabolic cost + cost of locomotion) (Fig. 3.1.B) while net cost of transport (NCOT = cost of only locomotion) is unaffected (Fig. 3.1C). This may indicate that the increase in carbohydrate use is only occurring at rest, which is unexpected since trout have various fuel sources they can utilize in this low metabolic state. As exercise intensity increases, trout will have to use carbohydrates to sustain the high ATP demand (Weber, 2011). Since there is no difference in NCOT when given glucose, this suggests that trout are not utilizing the excess circulatory glucose and are most likely relying on intramuscular carbohydrates at these high speeds. This is supported by the fact that there is no improvement in U_{crit} when given exogenous glucose. The cost of transport calculations were based on rates of oxygen consumption, and I ignored the contribution of anaerobic metabolism at very high swimming speeds. However, an earlier paper measuring lactate kinetics, using similar sized trout that achieved similar MO_2 and

lactate concentrations, estimated this error . They found that cost of transport could have been underestimated by 1 to 6.7% at the highest swimming speeds (Teulier et al., 2013).

Capacity for glucoregulation

In Chapter 2, rainbow trout showed a good ability to regulate glucose fluxes and to minimize the effect of a glucose load in the resting state (Choi and Weber, 2015). Here, graded exercise could provide more of a regulatory challenge given the constantly changing demand for glucose. Even among mammals who are normally considered as good glucoregulators, some species are unable to maintain glycemia and triple blood glucose concentration during intense exercise (Weber et al., 1996). Against all expectation, trout showed perfect glucoregulation by maintaining constant blood glucose levels at all swimming speeds including U_{crit} (Fig. 3.2). In the second part of this study, I have evaluated how they cope with the simultaneous stresses of graded exercise and exogenous glucose. After 4 h of glucose infusion, they were able to limit glucose accumulation to a 2.5-fold increase in circulating concentration (Fig. 3.4A). This was achieved through major changes in glucose kinetics: the strong inhibition of endogenous R_a (-75%) for the first ~3 h, and a 145% increase in R_d (Fig. 3.5). To illustrate the consequences of these changes in flux, Fig. 3.8 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 by 4.3-fold, whereas no modulation of fluxes at all would have resulted in a 6.4-fold increase to 114 mmol l^{-1} (Fig. 3.8). This study shows that rainbow trout subjected to graded swimming are able to modulate glucose fluxes rapidly to minimize glycemic stress.

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 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). However, during exercise, this can increase up to 93% as seen in humans (Brooks, 1998). The fraction of R_d glucose actually oxidized in swimming trout has never been measured, but is likely to fall in the range of 50% to 100% depending on the work intensity. To start characterizing the potential importance of glucose as an oxidative fuel in trout, I have calculated the contribution this fuel could make to total metabolic rate, assuming that either 100% or only 50% of R_d is oxidized (Fig. 3.7). In fish receiving no exogenous glucose, this fuel alone could not account for total metabolic rate at speeds above 1.8 BL s^{-1} (Fig. 3.7A). At such high exercise intensities, most of the ATP is produced from carbohydrates, and, therefore, a significant contribution from intramuscular glycogen is expected to make up the shortfall. In fish receiving exogenous glucose, a maximum of 50% R_d can be oxidized between 0.8 to 1.8 BL s^{-1} (Fig. 3.7B) because it is enough to explain 100% of MO_2. At these speeds, therefore, at least 50% of R_d must go to non-oxidative disposal. If fuels other than glucose are also oxidized, the relative importance of glucose oxidation will decrease below 50% of R_d and non-oxidative disposal will increase accordingly. In future experiments, the direct measurement of glucose oxidation will be needed to quantify the exact contribution of glucose oxidation to the metabolic rate of swimming fish.

CONCLUSIONS

High intensity swimming does not inhibit glucose fluxes like steady low-intensity exercise, but causes a parallel 40% stimulation of R_a and R_d glucose. Because the rates of glucose production and disposal are stimulated in synchrony, rainbow trout are able to show perfect glucoregulation even as they reach U_{crit}. The increase in R_a is probably mediated by glucagon and catecholamines, while insulin-independent movement of GLUT4 stimulates R_d. When fish are given exogenous glucose during swimming, they can boost disposal to record values as they deal with the high total R_a caused by exogenous supply and residual hepatic production. Under these conditions, R_d glucose is probably stimulated by a combination of signals including exercise itself, insulin, and hyperglycemia that activate GLUT4, GLUT2, and key enzymes of carbohydrate metabolism. Fish receiving exogenous glucose show a lower MO_2 because they might be using relatively more carbohydrates that allow them to produce ATP at a higher P/O ratio. However, this high glucose availability does not improve U_{crit}, suggesting that they are unable to use the extra fuel during maximal exercise. This study shows that rainbow trout have a remarkable capacity to adjust glucose fluxes that allows them to cope with the cumulative stresses of a glucose overload and graded exercise.

Table 3.1. Initial (rest) and final values (U_{crit}) for metabolic rate (MO_2), blood metabolite concentrations, and glucose fluxes in swimming trout supplied with exogenous glucose or not (controls). R_a glucose: rate of glucose appearance or (endogenous) hepatic production; R_d glucose: rate of glucose disposal. Values are means ± s.e.m (*N*). * indicate significant differences from initial values within each group ($P<0.05$).

	No exogenous glucose (controls)		Exogenous Glucose	
	Initial	Final	Initial	Final
MO_2 (μmol O_2 kg^{-1} m^{-1})	67.4±5.0 (10)	124 (1) *	54.3±2.6 (12)	169±5.9 (2) *
Glucose (mmol l^{-1})	15.1±1.0 (10)	12.7 (1)	17.7±1.6 (12)	43.8±11 (2) *
Lactate (mmol l^{-1})	1.0±0.2 (10)	3.1 (1)	1.1±0.4 (12)	3.1±1.3 (2)
Endogenous R_a Glucose (μmol kg^{-1} min^{-1})	15.6±1.2 (10)	22.1 (1)	16.4±1.6 (12)	22.7±13 (2)
R_d Glucose (μmol kg^{-1} min^{-1})	15.6±1.2 (10)	21.8 (1)	16.4±1.6 (12)	40.1±13 (2) *

Figure 3.1. Metabolic rate (MO_2) (A), total cost of transport (TCOT) (B), and net cost of transport (NCOT) (C) in fish receiving no exogenous glucose (control group; filled circles) and fish receiving exogenous glucose (open circles) during graded swimming. Values are means ± s.e.m. (N=10 for controls and 12 for exogenous glucose). Within each treatment group, MO_2 and NCOT increased with speed while TCOT decreased (P<0.001). MO_2 and TCOT were lower for exogenous glucose than for controls (P<0.05), but no significant difference in NCOT was detected between treatments.

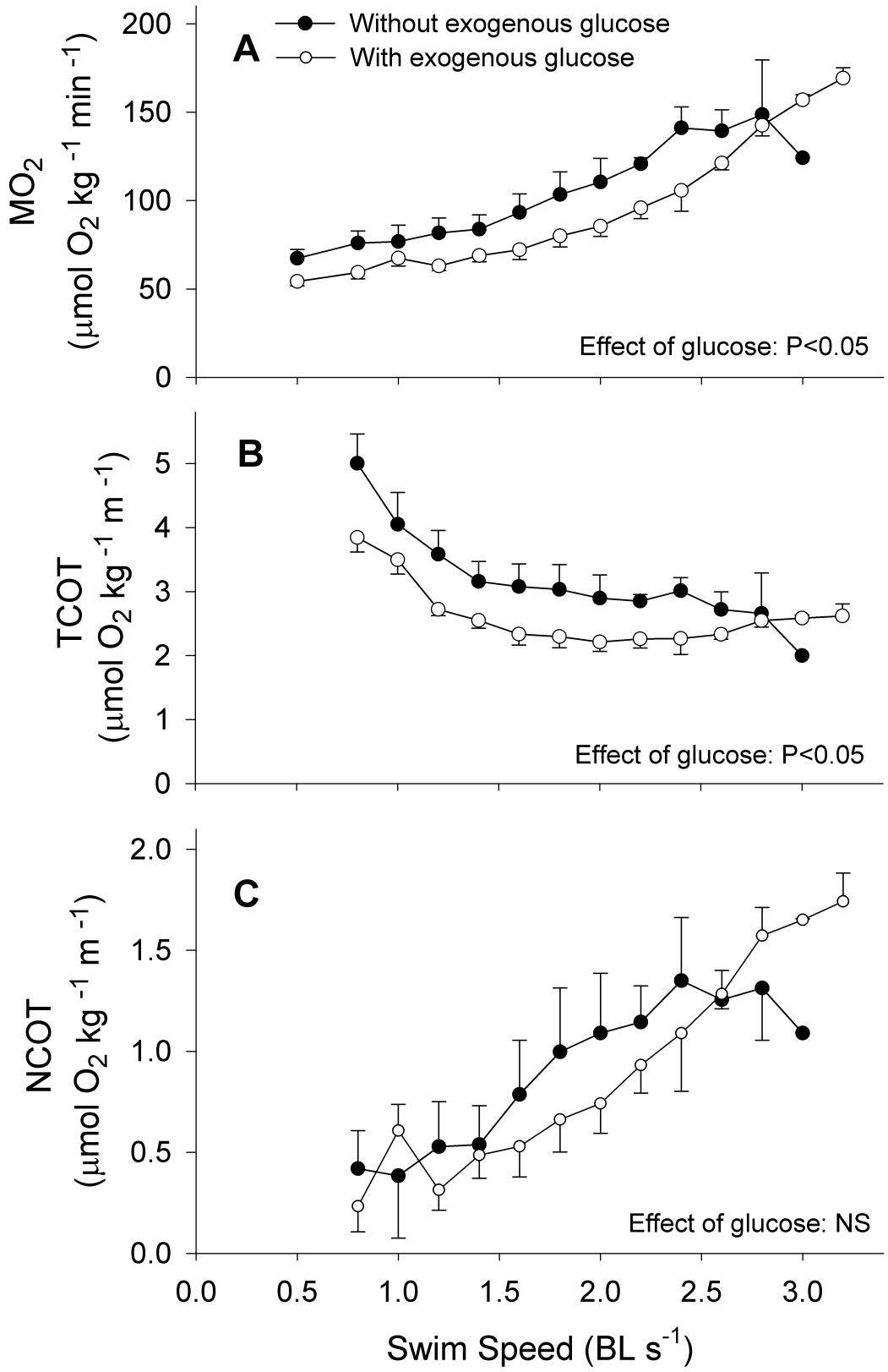

A
Without exogenous glucose
With exogenous glucose
MO2 (µmol O2 kg-1 min-1)
200
150
100
50
0
Effect of glucose: P<0.05
B
TCOT (µmol O2 kg-1 m-1)
5
4
3
2
1
0
Effect of glucose: P<0.05
C
NCOT (µmol O2 kg-1 m-1)
2.0
1.5
1.0
0.5
0.0
Effect of glucose: NS
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Swim Speed (BL s-1)

Figure 3.2. Blood glucose and lactate concentrations (A), and blood glucose specific activity (B) in control fish receiving no exogenous glucose during graded swimming. Values are means ± s.e.m. Numbers indicated under mean glucose values are sample sizes *N* for each speed (*N* decreases with speed because individual fish reached different maximal exercise intensities). Lactate concentration increased with speed while glucose specific activity decreased with speed ($P<0.05$). Dunnett's *post hoc* test could not identify specific means that were statistically different from baseline.

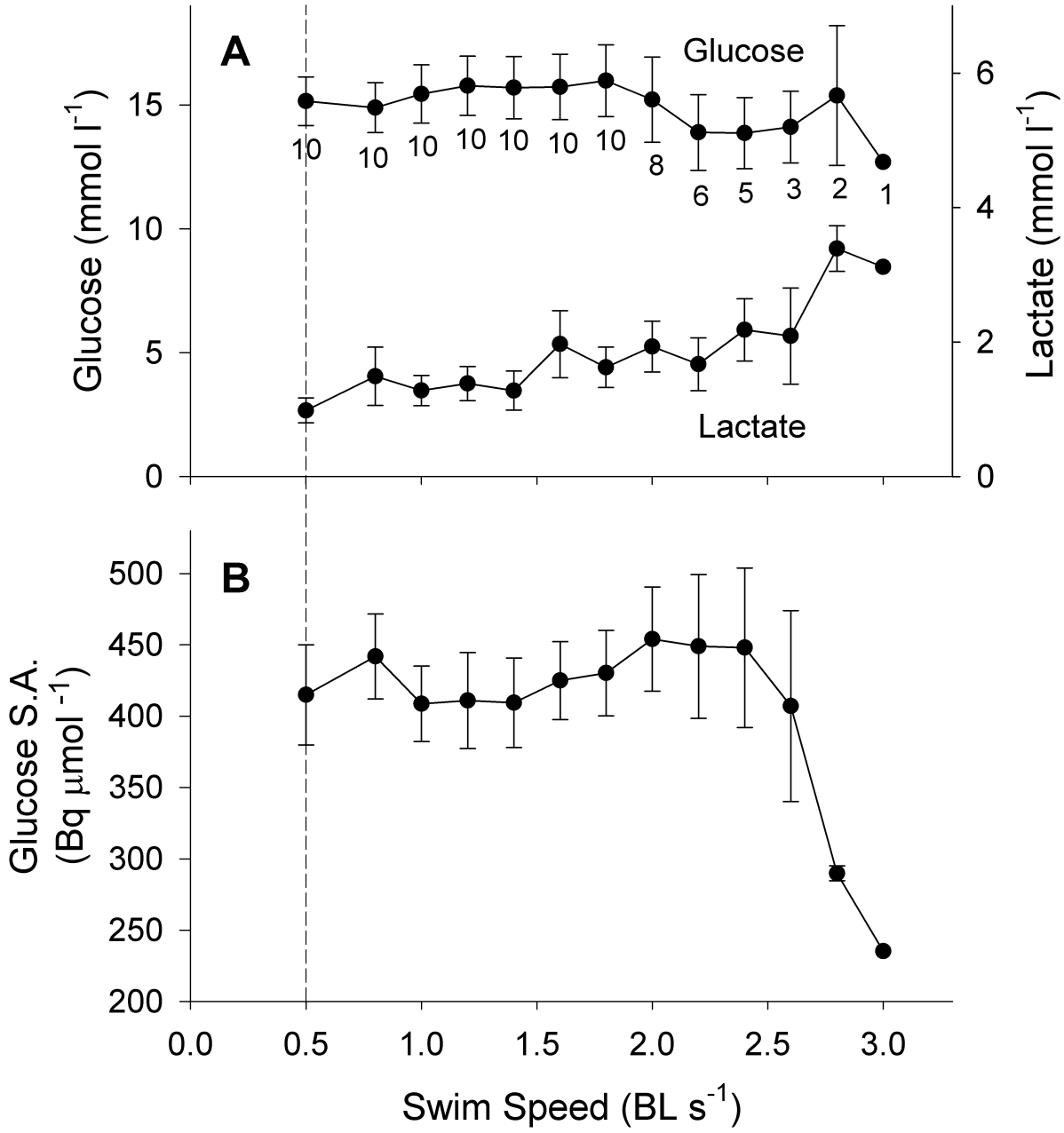

A
Glucose
Lactate
Glucose (mmol l-1)
Lactate (mmol l-1)
10
10
10
10
10
10
10
8
6
5
3
2
1
B
Glucose S.A.
(Bq µmol -1)
Swim Speed (BL s-1)

Figure 3.3. Effects of graded swimming on the glucose fluxes of control fish receiving no exogenous glucose. The rate of endogenous (hepatic) glucose production (Endogenous R_a) is shown with open circles, and the rate of glucose disposal (R_d) with filled circles. Values are means ± s.e.m. (N=10 below 1.8 BL s^{-1}, but <10 at higher speeds because individual fish reached different maximal exercise intensities). Both endogenous R_a and R_d increased with speed (P<0.05). Dunnett's *post hoc* test could not identify specific means that were statistically different from baseline.

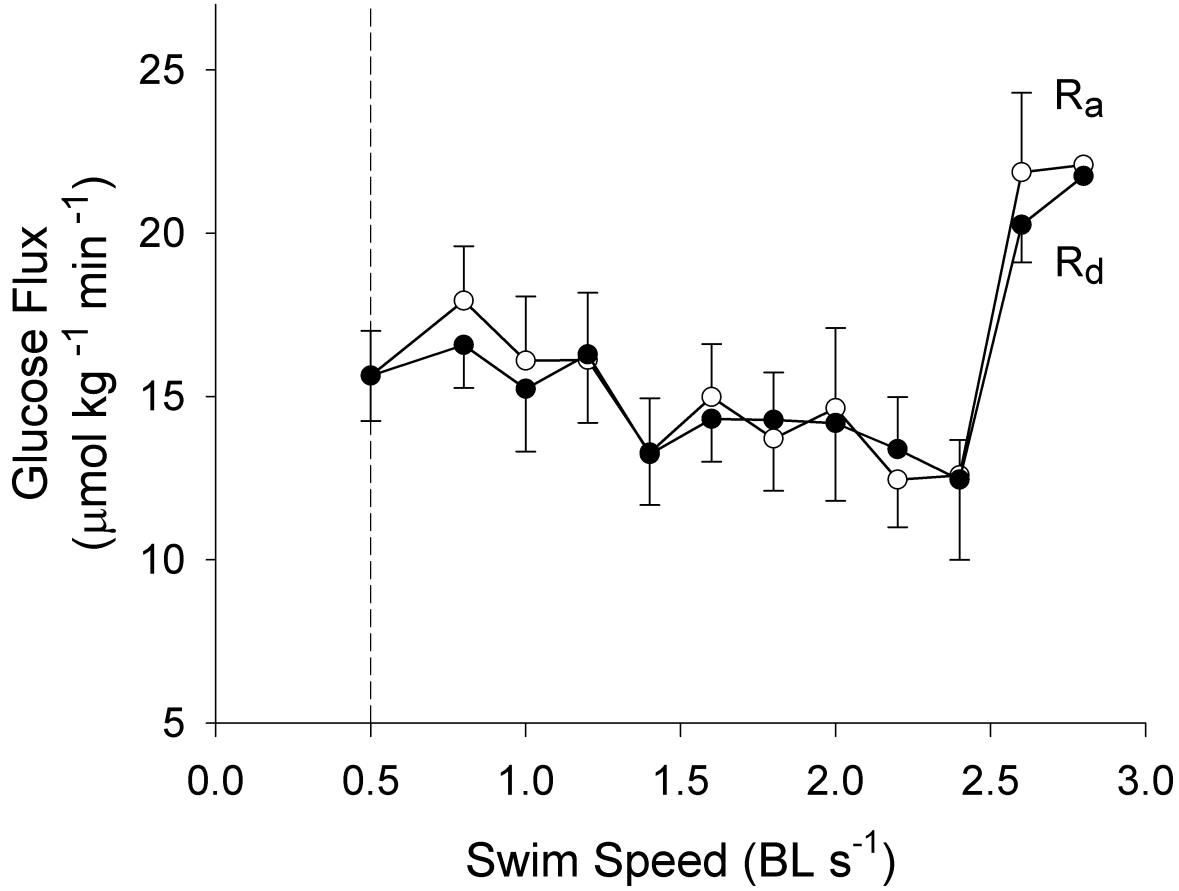

Glucose Flux
(μmol kg-1 min-1)
25
20
15
10
5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Swim Speed (BL s-1)
Ra
Rd

Figure 3.4. Blood glucose and lactate concentrations (A), and blood glucose specific activity (B) in fish receiving exogenous glucose during graded swimming. Values are means ± s.e.m. Numbers indicated under mean glucose values are sample sizes for each speed. These 3 parameters changed with speed (P<0.001) and * indicates significant differences from baseline. For lactate concentration, Dunnett's *post hoc* test could not identify specific means that were different from baseline.

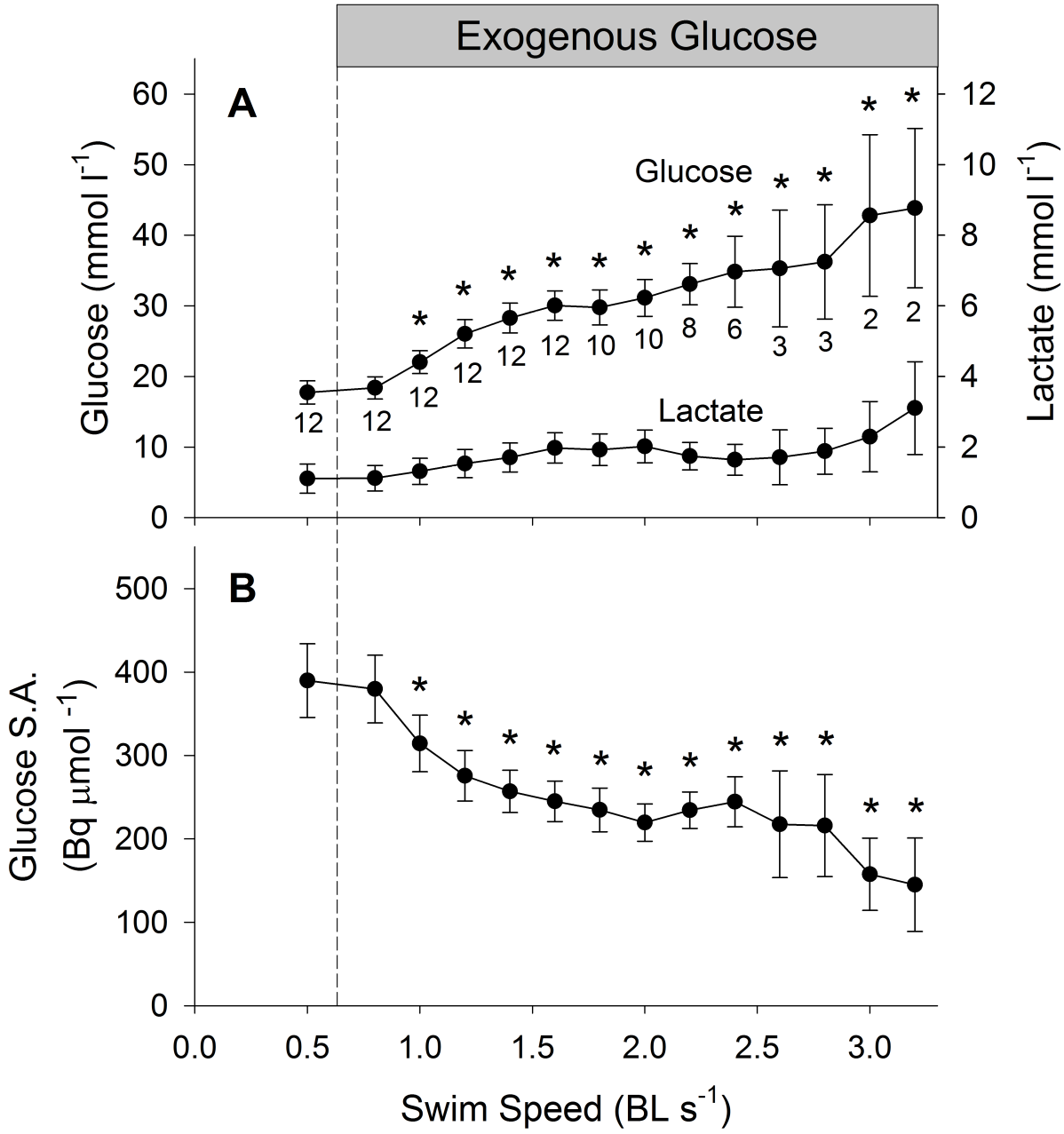

Exogenous Glucose
A
B
Glucose
Lactate
Glucose (mmol l-1)
Lactate (mmol l-1)
Glucose S.A. (Bq µmol -1)
Swim Speed (BL s-1)
0
10
20
30
40
50
60
2
4
6
8
10
12
100
200
300
400
500
0.0
0.5
1.0
1.5
2.0
2.5
3.0
12 12 12 12 12 10 10 8 6 3 3 2 2

Figure 3.5. Effects of graded swimming on the glucose fluxes of fish receiving exogenous glucose. The measured total rate of appearance of glucose (Total R_a; filled triangles) is the sum of endogenous glucose production (Endogenous R_a; open circles) and exogenous glucose supply. The rate of glucose disposal (R_d) is indicated with filled circles. Values are means ± s.e.m. (N=12 below 1.6 BL s^{-1}, but <10 at higher speeds because individual fish reached different maximal exercise intensities). Total R_a, endogenous R_a and R_d glucose increased with speed (P<0.001), and * indicates differences from baseline for each parameter.

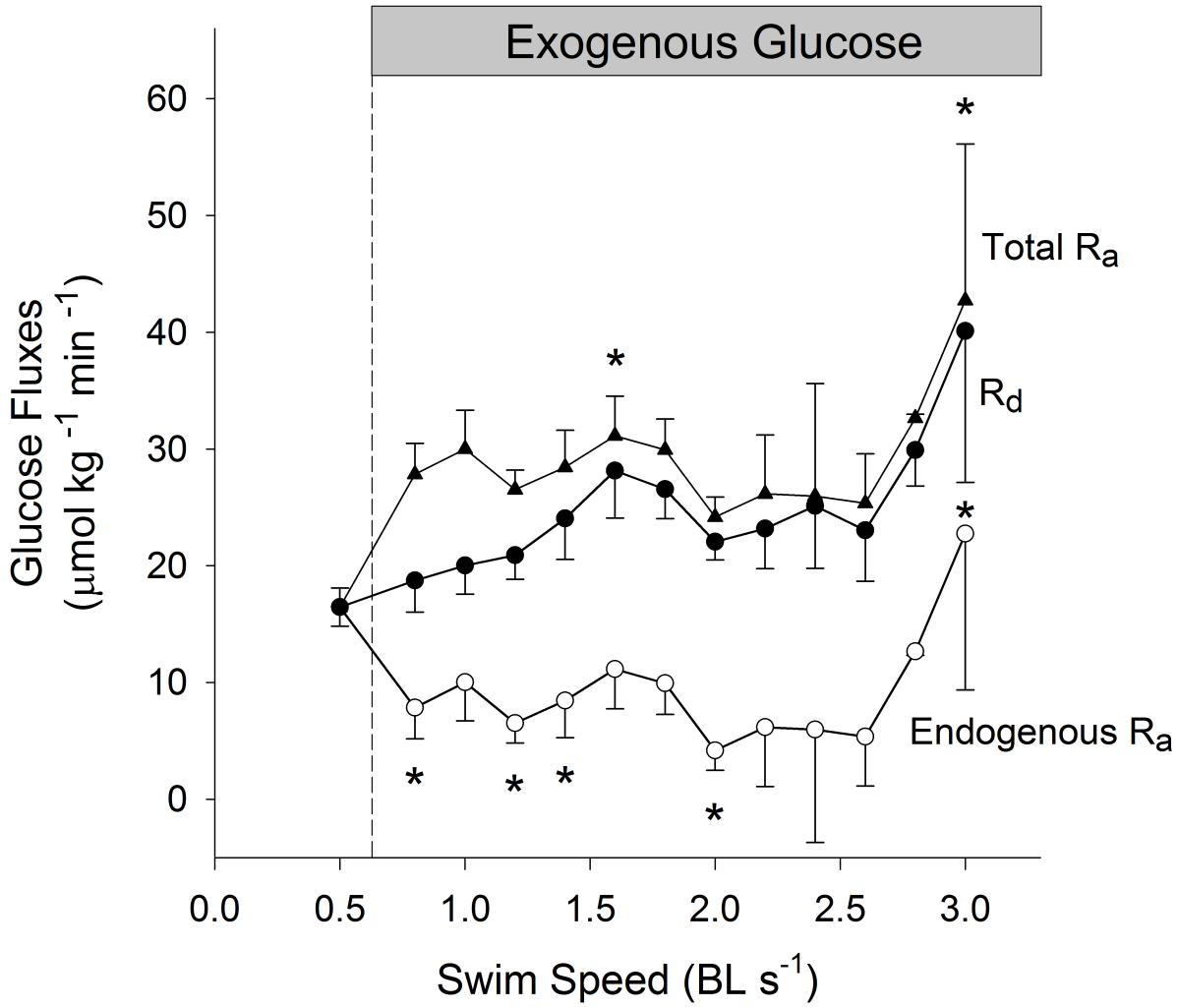

Exogenous Glucose
Glucose Fluxes
(μmol kg -1 min -1)
60
50
40
30
20
10
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Swim Speed (BL s-1)
Total Ra
Rd
Endogenous Ra
*
*
*
*
*
*
*

Figure 3.6. Relative changes in R_d glucose for control fish receiving no exogenous glucose (filled circles; N=10) and fish supplied with exogenous glucose (open circles; N=12). Values are means ± s.e.m. Speed had an effect on relative R_d glucose in both treatment groups (P<0.05). Dunnett's *post hoc* test could not identify specific means that were different from baseline.

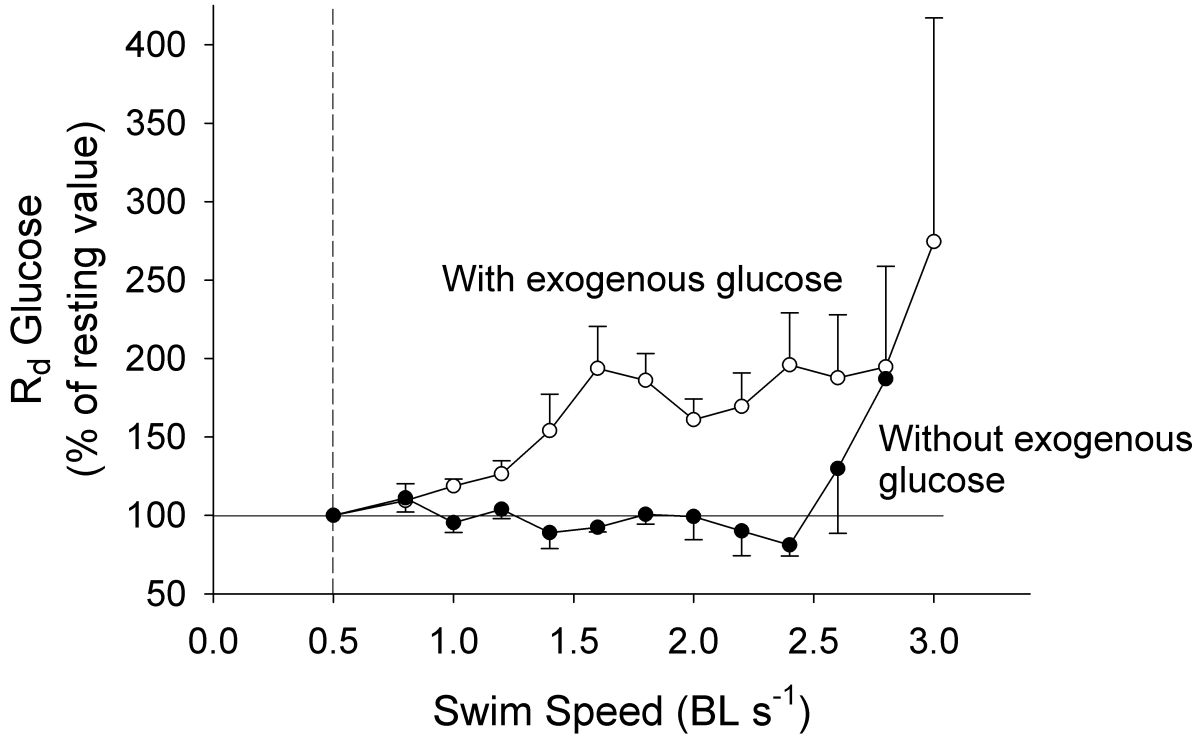

400
350
300
250
200
150
100
50
R_d Glucose
(% of resting value)
With exogenous glucose
Without exogenous
glucose
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Swim Speed (BL s^{-1})

Figure 3.7. Calculated percentages of metabolic rate (MO_2) that could be accounted for by glucose oxidation in control fish receiving no exogenous glucose (A) and fish receiving exogenous glucose (B) during graded swimming. Values were calculated assuming that either 100% (filled circles) or only 50% (open circles) of R_d glucose was oxidized. Values are means ± s.e.m. (N=10 for controls and 12 for exogenous glucose). Percentage of MO_2 changed in control fish with speed (P<0.05) and Dunnett's *post hoc* test could not identify specific means that were different from baseline.

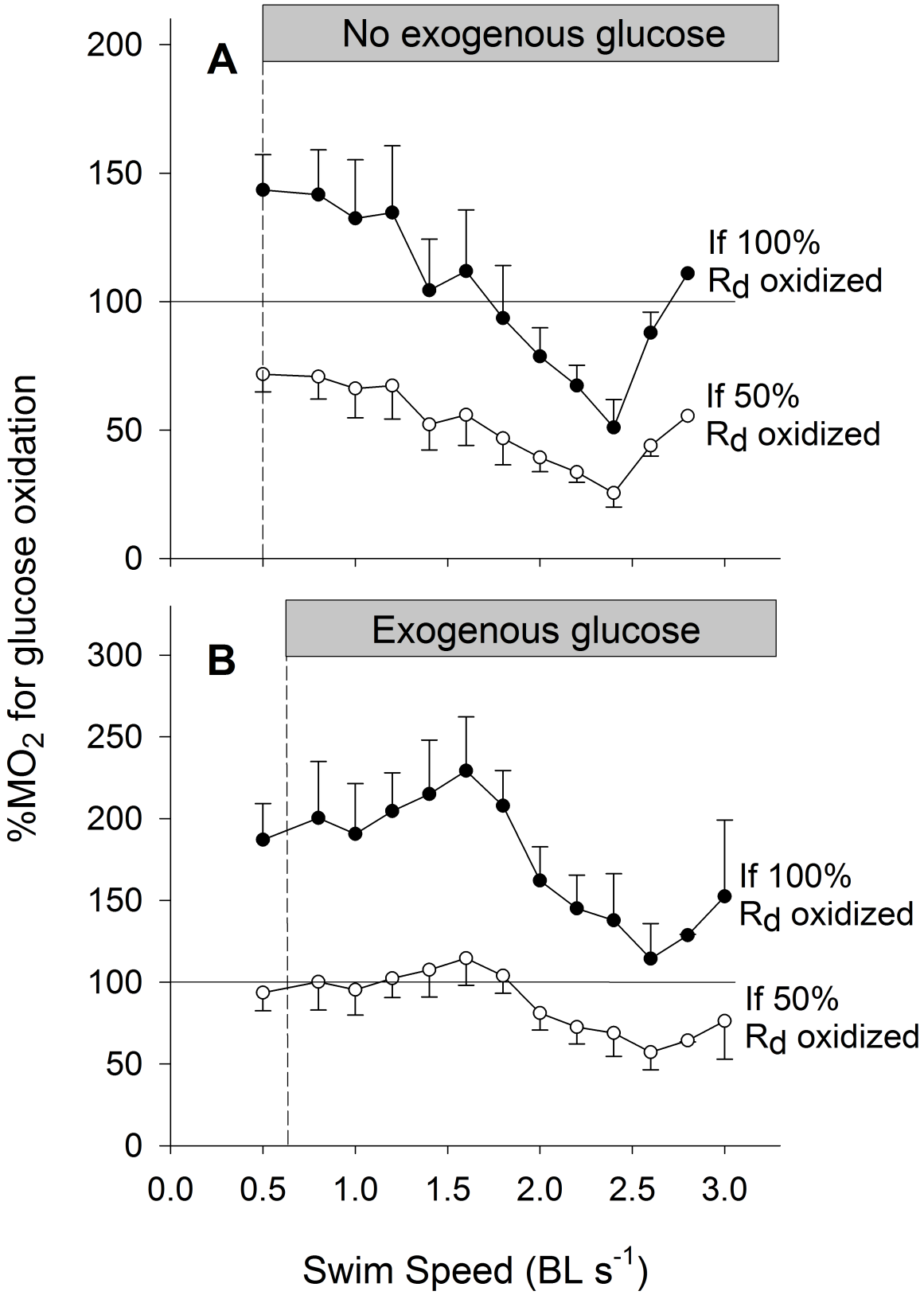
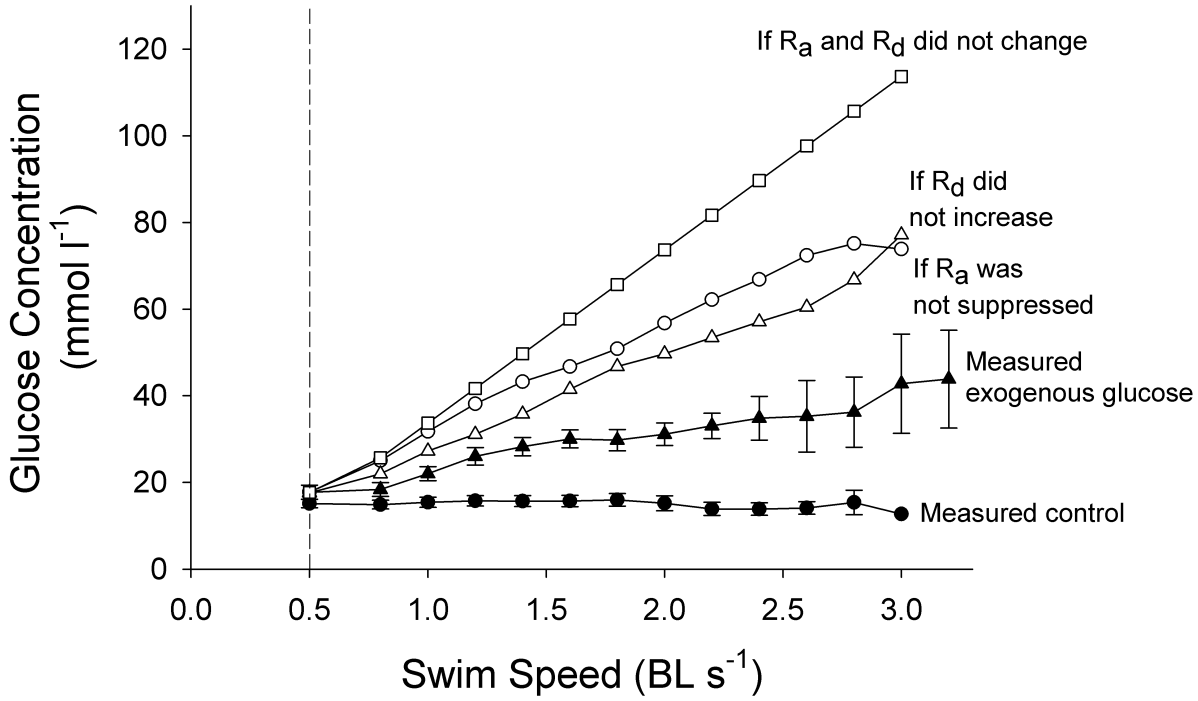

A
No exogenous glucose
200
150
100
50
0
If 100%
Rd oxidized
If 50%
Rd oxidized
B
Exogenous glucose
300
250
200
150
100
50
0
If 100%
Rd oxidized
If 50%
Rd oxidized
%MO2 for glucose oxidation
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Swim Speed (BL s-1)

Figure 3.8. Comparison of measured blood glucose concentrations with theoretical values calculated for hypothetical fish that would fail to regulate their glucose fluxes when exogenous glucose is provided during graded swimming. Measured blood glucose concentrations are presented for control fish (closed circles) and for those receiving exogenous glucose (closed triangles). Theoretical concentrations are given for 3 different scenarios: (1) if R_a glucose had not been suppressed (open circles); (2) if R_d glucose had not been stimulated (open triangles); and (3) if both, R_a and R_d, had remained at baseline throughout the infusion of exogenous glucose (open squares).


If R_a and R_d did not change
If R_d did not increase
If R_a was not suppressed
Measured exogenous glucose
Measured control
Glucose Concentration (mmol l^{-1})
Swim Speed (BL s^{-1})
0
20
40
60
80
100
120
0.0
0.5
1.0
1.5
2.0
2.5
3.0

CHAPTER 4: General Conclusions

THESIS OVERVIEW

The main goal of this thesis was to assess the glucoregulatory capacity of rainbow trout by measuring glucose kinetics during a glucose challenge. Rainbow trout have generally been considered as poor glucoregulators based on glucose tolerance tests (Legate et al., 2001) and show limited sensitivity to insulin (Marín-Juez et al., 2014; Polakof et al., 2012). However, measurements of kinetics can provide more information on their ability to glucoregulate than the simple monitoring of glycemia. In the first series of experiments (Chapter 2), I evaluated how resting rainbow trout can modulate their rates of hepatic glucose production (R_a glucose) and disposal (R_d glucose) when they are challenged with a large glucose load. This was done in hyperglycemic trout that maintain elevated baseline fluxes because, under these conditions, they face a more difficult task than normoglycemic fish with lower baseline fluxes. I effectively tried to push the fish to the limits of their capacity for glucoregulation. Given their reputation for poor glucoregulation, I anticipated that they would have limited ability to modulate fluxes in response to exogenous glucose.

Contrary to expectations, I established that trout have a surprisingly good ability to modulate their fluxes under resting conditions. Therefore, in a second series of experiments (Chapter 3), I determined whether graded exercise with or without exogenous glucose supply would cause even more extreme changes in glucose kinetics. Only one study had looked at glucose fluxes in swimming trout, and this was done at low intensity for several hours of submaximal exercise. This earlier study showed that trout decrease their glucose fluxes whereas mammals exercising at equivalent intensities increase them by 2- to 4-fold (Shanghavi and Weber, 1999). It is also well known that humans exercising at high intensities increase glucose fluxes very strongly (Romijn et al., 2000), but this had not been measured in trout. Therefore, I wanted to determine whether trout would also decrease R_a

and R_d during intense exercise (as they do during low intensity swimming) or, alternately, whether they respond like mammals and stimulate fluxes. I anticipated that glucose fluxes would continue to decrease as swimming speed increased.

Another important goal of Chapter 3 was to see if R_d glucose could be stimulated to higher values than measured during intense swimming, or with exogenous glucose at rest. In mammals, the combination of exercise and exogenous glucose increases R_d to higher values compared to exercise alone (Angus et al., 2002; Howlett et al., 1998; Marmy-Conus et al., 1996; McConell et al., 1994) or exogenous supply at rest (Ferrannini et al., 1985; Jackson et al., 1986; Muller et al., 1988). Finally, I also wanted to determine if the supply of extra glucose would affect swimming energetics and performance by measuring potential changes in cost of transport (COT) and critical swimming speed (U_{crit}), respectively. In humans, most studies investigating the effects of exogenous glucose supply on athletic performance show a significant improvement (Cermak and van Loon, 2013). In trout, the combination of exercise and exogenous fuel supply has only been tested using lactate (Omlin et al., 2014), but no changes in COT or U_{crit} could be demonstrated .

SUMMARY OF PRINCIPAL FINDINGS

CHAPTER 2: *Effects of exogenous glucose supply on the glucose kinetics of hyperglycemic rainbow trout under resting conditions.*

1. Hyperglycemic rainbow trout maintain elevated baseline glucose fluxes of 10.6±0.1 μmol kg^{-1} min^{-1} compared to normoglycemic trout (7.95±0.08 μmol kg^{-1} min^{-1}) (Weber and Shanghavi, 2000).

2. Resting rainbow trout can suppress hepatic glucose production from 10.6±1.0 to 0.4±1.3 μmol kg^{-1} min^{-1} during exogenous glucose infusion. They are also able to stimulate disposal from 10.6±1.0 to 27.6±3.9 μmol kg^{-1} min^{-1}.
3. In 4 h of exogenous glucose infusion, glycemia only increases by 2.5-fold. If trout were to respond with partial modulation of fluxes, blood glucose concentration would increase 6-fold from 10 mmol l^{-1} to 59 mmol l^{-1} (if R_a was not suppressed) and to 66 mmol l^{-1} (if R_d was not stimulated). With no flux modulation at all, blood glucose concentration would increase by more than 10-fold to reach dangerous levels of 107 mmol l^{-1}.

CHAPTER 3: *Glucose kinetics of hyperglycemic trout during graded swimming with or without supply of exogenous glucose.*

1. During graded exercise, glucose concentration remains constant and fluxes only increase at high speeds exceeding 2.4 BL s^{-1} from 15.6±1.4 to 22.1 μmol kg^{-1} min^{-1}.
2. Exogenous glucose decreases the metabolic rate (MO_2) of swimming fish (-16%) as well as the total cost of transport (TCOT). However, the net cost of transport (NCOT) and U_{crit} remain unaffected.
3. Trout increase R_d glucose from 16.4±1.6 to the highest rate measured to date of 40.1±13 μmol kg^{-1} min^{-1} when given exogenous glucose during intense exercise. Endogenous R_a glucose is partly suppressed at submaximal speeds from 16.4±1.6 to 4.1±1.7 μmol kg^{-1} min^{-1} before increasing to 22.7±13 μmol kg^{-1} min^{-1} at speeds higher than 2.6 BL s^{-1}.

GENERAL DISCUSSION

Resting glucose fluxes: mammals versus fish

Are the baseline glucose fluxes of fish different from those of mammals? Comparing absolute flux rates directly could be misleading because glucose movements within different organisms are greatly dependent on basal metabolic rate (BMR). Because mass-specific BMR varies with body size, I have plotted the relationship between resting glucose fluxes and body mass on a log-log scale for the different species of fish and mammals measured to date (Fig. 4.1). In this figure, therefore, it is possible to separate differences due to mass from those caused by other factors. In particular, evaluating differences between endotherms and ectotherms can be done more easily when size effects are accounted for. In mammals, there is a negative linear correlation that allows for reasonable prediction of resting glucose fluxes from body mass (r^2=0.853; P<0.001) (Weber et al., 1997). The lower glucose fluxes of large mammals reflect their lower mass-specific metabolic rate (Makarieva et al., 2008). These large animals require less fuel and energy per unit body mass to sustain life. In fish, no correlation between body mass and glucose fluxes can be demonstrated, partly because sample size is very low, because fish fluxes were measured at different temperatures, and also possibly because various tracer methods (some rather unreliable, like bolus injection (Omlin and Weber, 2010)) were used. In general, however, fish have lower fluxes compared to mammals of the same mass (all fish values are below the mammalian regression line), as expected for ectotherms with lower metabolic rates (White and Kearney, 2013). Determining whether fish glucose fluxes vary with body size in a consistent way will require flux measurements using a reliable tracer method (continuous infusion) on multiple species covering a larger range of body sizes, and to account/correct for all confounding factors independent of size (e.g. temperature).

Glucose flux modulation

I was able to show that hyperglycemic trout maintain elevated baseline fluxes compared to normoglycemic fish (Fig. 4.2), which was caused by a high fat diet that stimulates hepatic glucose production (Figueiredo-Silva et al., 2012). This made them an interesting experimental model to investigate glucose kinetics: hyperglycemic trout show some of the metabolic characteristics of type II diabetes patients, and would therefore be expected to have a particularly limited capacity for regulating glucose fluxes, compared to their normoglycemic counterparts. I anticipated that these animals would only be able to respond weakly to an exogenous glucose challenge and to graded swimming. Surprisingly, trout showed a strong stimulation in R_a glucose during high intensity exercise, but maintained glycemia perfectly constant. This large change in flux may have been caused by catecholamines and glucagon being released through exercise, resulting in increased glycogenolysis and gluconeogenesis (Galbo et al., 1975; Polakof et al., 2012) (Fig. 4.2A). When trout were provided with exogenous glucose at rest and during exercise, they were able to suppress hepatic glucose production, most likely using insulin as the main signal to inhibit gluconeogenic and glycogenolytic enzymes (Enes et al., 2009; Polakof et al., 2010a; Polakof et al., 2010b) (Fig. 4.2A).

Disposal rates also show a high degree of plasticity which was a surprise given the amount of time it takes trout to restore glycemia in glucose tolerance tests (Legate et al., 2001). During graded exercise (without exogenous supply), they stimulate R_d glucose through insulin-independent movement of GLUT4 from intracellular stores to the membrane (Marín-Juez et al., 2014) (Fig. 4.2A). When trout are given exogenous glucose, they can further stimulate their disposal rate. At rest (with exogenous glucose), trout can completely suppress their endogenous R_a, so that R_d only has to match the rate of exogenous glucose

supply to achieve perfect glucoregulation. Stimulation of disposal is presumably regulated through insulin-induced movement of GLUT4 and activation of hepatic GLUT2, as well as stimulation of enzymes involved in glucose metabolism (Enes et al., 2009; Marín-Juez et al., 2014; Polakof et al., 2010a; Polakof et al., 2010b). During exercise (with exogenous supply), R_d is stimulated to record values because it has to match both exogenous supply and residual endogenous production. Unlike at rest, hepatic R_a is not completely suppressed during swimming possibly due to the effects of released glucagon or catecholamines (Galbo et al., 1975; Polakof et al., 2012). Therefore, trout have to use both exercise- and insulin-induced mechanisms to cope with the high total R_a (Enes et al., 2009; Marín-Juez et al., 2014; Polakof et al., 2010a; Polakof et al., 2010b). 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). Glycemia increased to 28 mmol l^{-1} in my resting experiment (Chapter 2) and 43.8 mmol l^{-1} in my swimming experiment (Chapter 3), therefore, the same rate of urinary loss would have accounted for 7.6% (=2.1/27.6 μmol kg^{-1} min^{-1}) and 5.2% of R_d (=2.1/40.1 μmol kg^{-1} min^{-1}), respectively.

Correlation between glucose concentration and fluxes

Before accurate flux measurements became available, changes in metabolite concentration were routinely used to draw conclusions about the metabolic responses of fish to various stimuli. Unfortunately, changes in concentration often provide limited or misleading information about changes in flux (Haman et al., 1997b). I wanted to evaluate

whether changes in blood glucose concentration could be used to predict changes in flux when exogenous glucose is given in resting or swimming trout. Therefore, I have plotted the relationship between blood glucose concentration and glucose fluxes for both situations (Fig. 4.3). When exogenous supply is given, R_a glucose exhibits a similar response at rest (Fig. 4.3A) or during exercise (Fig. 4.3B), although the magnitude of the effect varies. In both cases, there is a sudden decrease in R_a glucose that eventually reversed as concentration increases. The same R_a is prevalent at blood glucose concentrations of 11 and 27 mmol l^{-1} in resting trout, and, therefore, it is not possible to use concentration as an index of flux in this case (Fig. 4.3A). In contrast, combined R_d glucose values for rest and exercise show a much more linear association with glucose concentration (r^2=0.839; P<0.001; Fig. 4.3C). This would allow for a reasonable prediction of R_d based on glycemia when exogenous glucose is supplied to resting or swimming animals. The overlap between the resting and exercise relationships also suggests that even though swimming increases systemic blood flow (Randall and Daxboeck, 1982), glucose is not being taken up by muscle cells more rapidly than at rest. This observation is supported by the fact that U_{crit} is not improved in fish receiving exogenous glucose, indicating that trout are not relying on circulatory glucose at high swimming speeds.

GENERAL CONCLUSIONS

This thesis addresses important questions regarding the capacity of rainbow trout to modulate glucose fluxes, a species generally considered as a poor glucoregulator. Through *in vivo* experiments, I have demonstrated that rainbow trout have the ability to make large and rapid adjustments to glucose fluxes in response to exogenous glucose, much like mammals.

At rest, their ability to suppress hepatic glucose production completely and to strongly stimulate glucose disposal was unexpected, especially in hyperglycemic animals that already maintain elevated baseline fluxes. This rapid modulation allows them to limit the increase in glycemia to only 2.5-fold (Fig. 2.8). During graded exercise (without exogenous glucose), trout show perfect glucoregulation by maintaining constant glycemia regardless of swimming speed (Fig. 3.2A). This is impressive because some mammals are unable to glucoregulate during intense exercise (Weber et al., 1996). Finally, during graded exercise with exogenous glucose, trout can suppress hepatic glucose production and stimulate disposal to record values to limit the increase in glycemia to only 2.5-fold (Fig. 3.8). Overall, the results from my thesis show that rainbow trout have a much better capacity to glucoregulate than generally suggested by current literature.

FUTURE DIRECTIONS

The next generation of experiments should focus on the hormonal control of glucose fluxes. Data from Chapter 2 show that trout can completely suppress hepatic glucose production in response to exogenous glucose. In mammals, this response is mainly controlled by insulin and it would be interesting to assess whether insulin levels are also elevated and play the same role in trout. In Chapter 3, I observed that glucose fluxes show a non-significant trend towards a decrease at submaximal swimming speeds, and a previous study reported that epinephrine levels decrease during sustained low-intensity exercise (Shanghavi and Weber, 1999). Characterizing the role of epinephrine in maintaining baseline glucose fluxes and possibly inhibiting them at low swimming speeds would be useful. Understanding how glucose fluxes are strongly stimulated when the fish approach U_{crit} will require the

measurement of glucagon and catecholamine levels to determine the relative roles of these hormones. Beta-blockers could be used as a tool to separate the effects of glucagon and catecholamines. Finally, determining the exact pathways and specific tissues responsible for the record increase in glucose disposal observed in swimming fish supplied with exogenous glucose will be an important challenge for future work.

Figure 4.1. Relationship between body mass and resting glucose fluxes in mammals (rats, guinea pigs, rabbits, cats, monkeys, pigs, dogs, goats, seals, sheep, humans, reindeer, ponies, cows, and horses) (Weber et al., 1997) and fish (Bever et al., 1977; Choi and Weber, 2015; Garin et al., 1987; Haman and Weber, 1996; Machado et al., 1989; Shanghavi and Weber, 1999; Weber et al., 1986; Weber and Shanghavi, 2000). A linear regression is plotted for the mammalian data along with a 95% confidence interval: $y=-0.228x+1.525$; $r^2=0.853$; $P<0.001$.

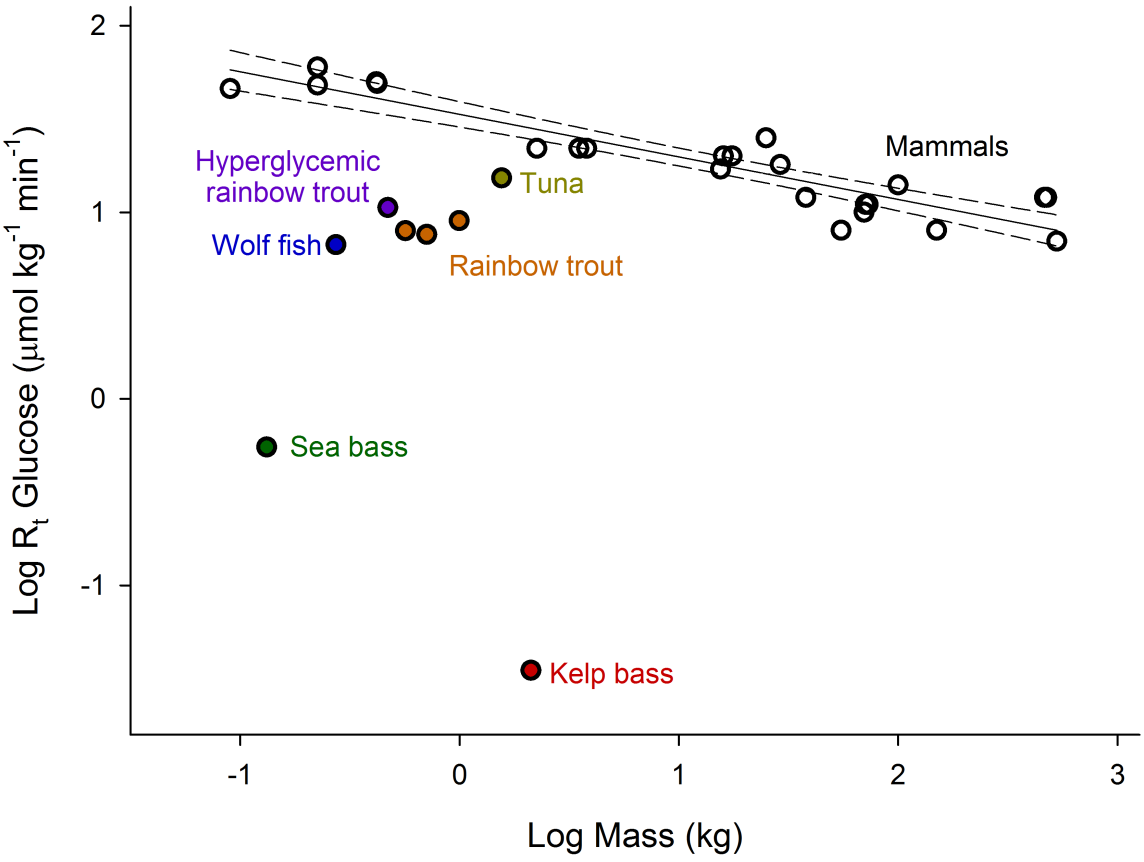

Log R_t Glucose (μmol kg^{-1} min^{-1})
Log Mass (kg)
2
1
0
-1
-1
0
1
2
3
Mammals
Hyperglycemic
rainbow trout
Tuna
Wolf fish
Rainbow trout
Sea bass
Kelp bass

Figure 4.2. Effects of the various parameters tested in this thesis on the rates of hepatic glucose production and glucose disposal presented as absolute values (A) and as a percent of normoglycemic values (B). Data for normoglycemic fish are from (Weber and Shanghavi, 2000). Values are means ± s.e.m. (N=13 for normoglycemia; N=7 for hyperglycemia; N=8 for exogenous glucose; N=10 for swimming; N=12 for exogenous glucose + swimming). * indicate significant differences from normoglycemic values (P<0.05).

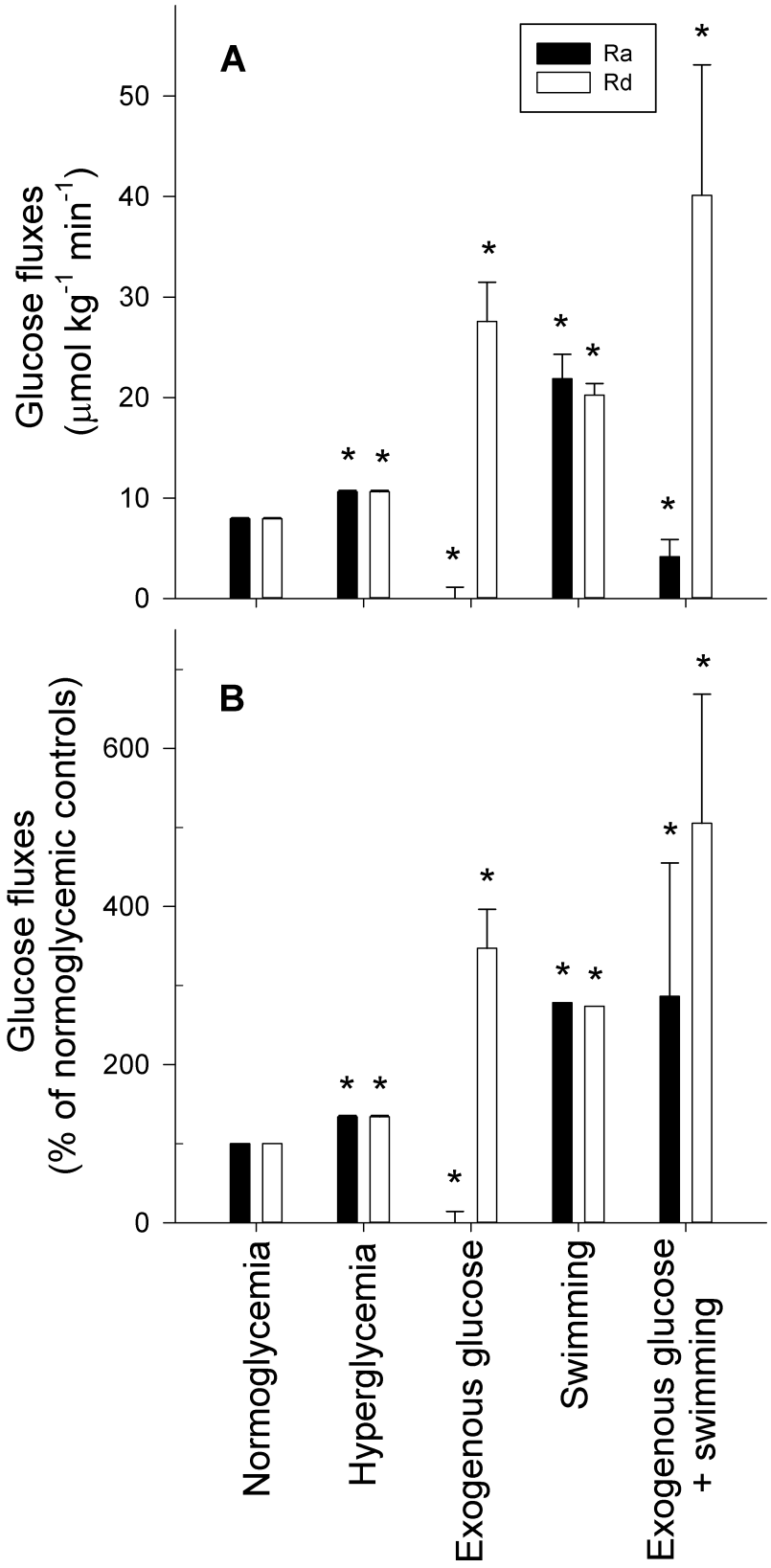
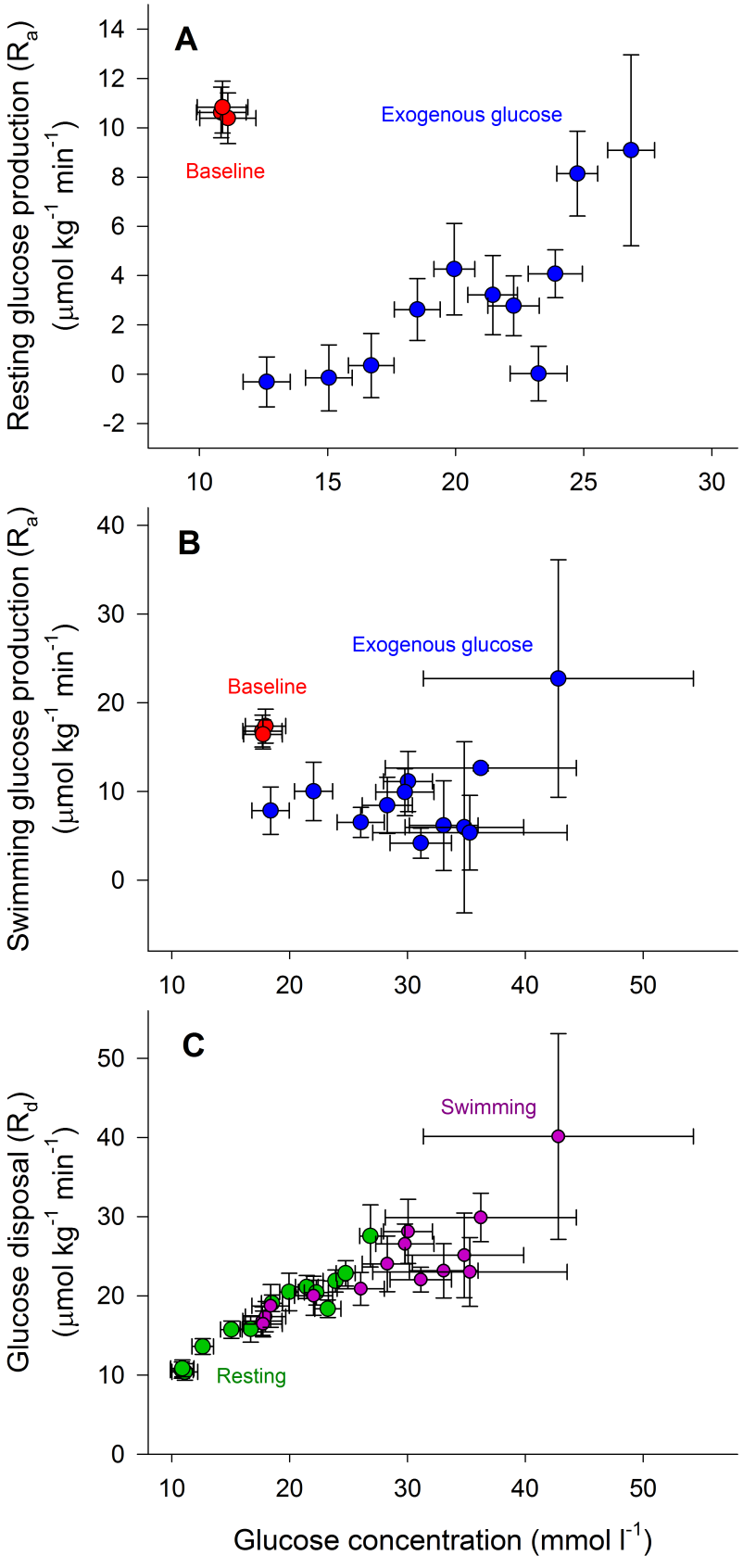

A
Ra
Rd
Glucose fluxes
(μmol kg-1 min-1)
0
10
20
30
40
50
B
Glucose fluxes
(% of normoglycemic controls)
0
200
400
600
Normoglycemia
Hyperglycemia
Exogenous glucose
Swimming
Exogenous glucose
+ swimming

Figure 4.3. Relationship between blood glucose concentration and the rate of: (A) hepatic glucose production at rest; (B) hepatic glucose production during graded swimming; (C) disposal at rest and during swimming. Values are means ± s.e.m. (*N*=8 for resting; *N*=12 for swimming). A linear regression equation was determined for the disposal rate graph (C) but was not plotted on the figure: $y=0.690x+4.555$; $r^2=0.839$; $P<0.001$.


A
Exogenous glucose
Baseline
Resting glucose production (Ra)
(µmol kg-1 min-1)
B
Exogenous glucose
Baseline
Swimming glucose production (Ra)
(µmol kg-1 min-1)
C
Swimming
Resting
Glucose disposal (Rd)
(µmol kg-1 min-1)
Glucose concentration (mmol l-1)

REFERENCES

Abumrad, N. N., Cherrington, A. D., Williams, P. E., Lacy, W. W. and Rabin, D. (1982). Absorption and disposition of a glucose load in the conscius dog. *American Journal of Physiology* **262**, E398-406.

Andrikopoulos, S., Blair, A. R., Deluca, N., Fam, B. C. and Proietto, J. (2008). Evaluating the glucose tolerance test in mice. *Am J Physiol Endocrinol Metab* **295**, E1323-32.

Angus, D. J., Febbraio, M. A. and Hargreaves, M. (2002). Plasma glucose kinetics during prolonged exercise in trained humans when fed carbohydrate. *Am J Physiol Endocrinol Metab* **283**, E573-E577.

Banos, N., Baro, J., Castejon, C., Navarro, I. and Gutierrez, J. (1998). Influence of high-carbohydrate enriched diets on plasma insulin levels and insulin and IGF-I receptors in trout. *Regulatory Peptides* **77**, 55-62.

Bergot, F. (1979). Effects of dietary carbohydrates and of their mode of distribution on glycemia in rainbow trout (Salmo gairdneri Richardson). *Comparative Biochemistry and Physiology* **64A**, 543-547.

Bever, K., Chenoweth, M. and Dunn, A. (1977). Glucose turnover in kelp bass (Paralabax sp.): in vivo studies with [6-3H, 6-14C]glucose. *American Journal of Physiology* **232**, R66-R72.

Blasco, J., Fernandez-Borras, J., Marimon, I. and Requena, A. (1996). Plasma glucose kinetics and tissue uptake in brown trout in vivo: effect of an intravascular glucose load. *Journal of Comparative Physiology* **165**, 534-541.

Brooks, G. A. (1998). Mammalian fuel utilization during sustained exercise. *Comparative Biochemistry and Physiology Part B* **120**, 80-107.

Brooks, G. A. and Donovan, C. M. (1983). Effect of endurance training on glucose kinetics during exercise. *Am. J. Physiol.* **244**, E505-E512.

Bucking, C. and Wood, C. M. (2005). Renal regulation of plasma glucose in the freshwater rainbow trout. *J Exp Biol* **208**, 2731-9.

Cermak, N. M. and van Loon, L. J. (2013). The use of carbohydrates during exercise as an ergogenic aid. *Sports Med* **43**, 1139-55.

Choi, K. and Weber, J.-M. (2015). Pushing the limits of glucose kinetics: How rainbow trout cope with a carbohydrate overload. *Journal of Experimental Biology*.

Cowey, C. B., Higuera, M. d. l. and Adron, J. W. (1977a). The effect of dietary composition and of insulin on gluconeogenesis in rainbow trout (Salmo gairdneri). *Br J Nutr*, 385-395.

Cowey, C. B., Knox, D., Walton, M. J. and Adron, J. W. (1977b). The regulation of gluconeogenesis by diet and insulin in rainbow trout (Salmo gairdneri). *British Journal of Nutrition* **38**, 463-470.

Enes, P., Panserat, S., Kaushik, S. and Oliva-Teles, A. (2009). Nutritional regulation of hepatic glucose metabolism in fish. *Fish Physiol Biochem* **35**, 519-39.

Febbraio, M. A., Chiu, A., Angus, D. J., Arkinstall, M. J. and Hawley, J. A. (2000). Effects of carbohydrate ingestion before and during exercise on glucose kinetics and performance. *Journal of Applied Physiology* **89**, 2220-2226.

Ferrannini, E., Bjorkman, O., Jr., G. A. R., Pilo, A., Olsson, M., Wahren, J. and DeFronzo, R. A. (1985). The disposal of an oral glucose load in healthy subjects. *Diabetes* **34**, 580-588.

Figueiredo-Silva, A. C., Panserat, S., Kaushik, S., Geurden, I. and Polakof, S. (2012). High levels of dietary fat impair glucose homeostasis in rainbow trout. *J Exp Biol* **215**, 169-78.

Galbo, H., Holst, J. J. and Christensen, N. J. (1975). Glucagon and plasma catecholamine responses to graded and prolonged exercise in man. *Journal of Applied Physiology* **38**, 70-76.

Garin, D., Rombaut, A. and Freminet, A. (1987). Determination of glucose turnover in sea bass dicentrarchus labrax. comparative aspects of glucose utilization. *Comparative Biochemistry and Physiology* **87**, 981-988.

Glamour, T. S., McCullough, A. J., Sauer, P. J. J. and Kalhan, S. C. (1995). Quantificaation of carbohydrate oxidation by respiratory gas exchange and isotopic tracers. *Am. J. Physiol.* **268**, E789-E796.

Haman, F., Powell, M. and Weber, J.-M. (1997a). Reliability of Continuous Tracer Infusion for Measuring Glucose Turnover Rate in Rainbow Trout. *J Exp Biol* **200**, 2557-2563.

Haman, F. and Weber, J.-M. (1996). Continuous Tracer Infusion to Measure In Vivo Metabolite Turnover Rates in Trout. *J Exp Biol* **199**, 1157-1162.

Haman, F., Zwingelstein, G. and Weber, J.-M. (1997b). Effects of hypoxia and low temperature on substrate fluxes in fish: plasma metabolite concentrations are misleading. *American Journal of Physiology* **273**, R2046-R2054.

Horowitz, M., Edelbroek, M. A. L., Wishart, J. M. and Straathof, J. W. (1993). Relationship between oral glucose tolerance and gastric emptying in normal healthy subjects. *Diabetologia* **36**, 857-862.

Howlett, K., Angus, D., Proietto, J. and Hargreaves, M. (1998). Effect of increased blood glucose availability on glucose kinetics during exercise. *Journal of Applied Physiology* **84**, 1413-1417.

Jackson, R. A., Peters, N., Advani, U., Perry, G., Rogers, J., Brough, W. H. and Pilkington, T. R. E. (1973). Forearm glucose uptake during the oral glucose tolerance test in normal subjects. *Diabetes* **22**, 442-458.

Jackson, R. A., Roshania, R. D., Hawa, M. I., Sim, B. M. and DiSilvio, L. (1986). Impact of glucose ingestion on hepatic and peripheral glucose metabolism in man: An analysis based on simultaneous use of the forearm and double isotope techniques. *Journal of Clinical Endocrinology and Metabolism* **63**, 541-549.

Katz, H., Homan, M., Butler, P. and Rizza, R. (1992). Use of [3-3H]glucose and [6-14C]glucose to measure glucose turnover and glucose metabolism in humans. *Am. J. Physiol.* **263**, E17-E22.

Kelley, D., Mitrakou, A., Marsh, H., Schwenk, F., Benn, J., Sonnenberg, G., Arcangeli, M., Aoki, T., Sorensen, J., Berger, M. et al. (1988). Skeletal muscle glycolysis, oxidation, and storage of an oral glucose load. *The Journal of Clinical Investigation* **81**, 1563-1571.

Legate, N. J., Bonen, A. and Moon, T. W. (2001). Glucose tolerance and peripheral glucose utilization in rainbow trout (Oncorhynchus mykiss), American eel (Anguilla rostrata), and black bullhead catfish (Ameiurus melas). *Gen Comp Endocrinol* **122**, 48-59.

Machado, C. R., Garofalo, M. A. R., Roselino, J. E. S., Kettelhut, I. C. and Migliorini, R. H. (1989). Effect of fasting on glucose turnover in a carnivorous fish (Hoplias sp). *American Journal of Physiology* **256**, R612-R615.

Makarieva, A. M., Gorshkov, V. G., Li, B. L., Chown, S. L., Reich, P. B. and Gavrilov, V. M. (2008). Mean mass-specific metabolic rates are strikingly similar across life's major domains: Evidence for life's metabolic optimum. *Proc Natl Acad Sci U S A* **105**, 16994-9.

Marín-Juez, R., Capilla, E., Carvalho-Simoes, F., Camps, M. and Planas, J. V. (2014). Structural and Functional Evolution of Glucose Transporter 4 (GLUT4): A Look at GLUT4 in Fish. In *Glucose Homeostasis*, (ed. L. Szablewski), pp. 37-67: InTech.

Marmy-Conus, N., Fabris, S., Proietto, J. and Hargreaves, M. (1996). Pre-exercise Glucose Ingestion and Glucose Kinetics During Exercise. *Journal of Applied Physiology* **81**, 853-857.

McConell, G., Fabris, S., Proietto, J. and Hargreaves, M. (1994). Effect of carbohydrate ingestion on glucose kinetics during exercise. *Journal of Applied Physiology* **77**, 1537-1541.

Meyer, C., Stumvoll, M., Nadkarni, V., Dostou, J., Mitrakou, A. and Gerich, J. (1998). Abnormal renal and hepatic glucose metabolism in type 2 diabetes mellitus. *J. Clin. Invest.* **102**, 619-624.

Moon, T. W. (2001). Glucose intolerance in teleost fish: fact or fiction? *Comparative Biochemistry and Physiology* **129**, 243-249.

Muller, M. J., Moring, J. and Seitz, H. J. (1988). Regulation of hepatic glucose output by glucose in vivo. *Metabolism* **37**, 55-60.

Omlin, T., Langevin, K. and Weber, J.-M. (2014). Exogenous lactate supply affects lactate kinetics of rainbow trout, not swimming performance. *Am J Physiol Regul Integr Comp Physiol* **307**, R1018-R1024.

Omlin, T. and Weber, J. M. (2010). Hypoxia stimulates lactate disposal in rainbow trout. *J Exp Biol* **213**, 3802-9.

Paul, P. and Bella Issekutz, J. (1967). Role of extramuscular energy sources in the metabolism of the exercising dog. *J. Appl. Physiol.* **22**, 615-622.

Polakof, S., Moon, T. W., Aguirre, P., Skiba-Cassy, S. and Panserat, S. (2010a). Effects of insulin infusion on glucose homeostasis and glucose metabolism in rainbow trout fed a high-carbohydrate diet. *J Exp Biol* **213**, 4151-7.

Polakof, S., Panserat, S., Soengas, J. L. and Moon, T. W. (2012). Glucose metabolism in fish: a review. *J Comp Physiol B* **182**, 1015-45.

Polakof, S., Skiba-Cassy, S., Choubert, G. and Panserat, S. (2010b). Insulin-induced hypoglycaemia is co-ordinately regulated by liver and muscle during acute and chronic insulin stimulation in rainbow trout (Oncorhynchus mykiss). *J Exp Biol* **213**, 1443-52.

Randall, D. J. and Daxboeck, C. (1982). Cardiovascular changes in the rainbow trout (Salmo gairdneri Richardson) during exercise. *Can. J. Zool.* **60**, 1135-1140.

Richards, J. G., Mercado, A. J., Clayton, C. A., Heigenhauser, G. J. F. and Wood, C. M. (2002). Substrate utilization during graded aerobic exercise in rainbow trout. *J Exp Biol* **205**, 2067-2077.

Rojas, J. M. and Schwartz, M. W. (2014). Control of hepatic glucose metabolism by islet and brain. *Diabetes, Obesity and Metabolism* **16**, 33-40.

Romijn, J. A., Coyle, E. F., Sidossis, L. S., Rosenblatt, J. and Wolfe, R. R. (2000). Subtrate metabolism during different exercise intensities in endurance-trained women. *Journal of Applied Physiology* **88**, 1707-1714.

Schippers, M.-P., Ramirez, O., Arana, M., Pinedo-Bernal, P. and McClelland, Grant B. (2012). Increase in Carbohydrate Utilization in High-Altitude Andean Mice. *Current Biology* **22**, 2350-2354.

Shanghavi, D. S. and Weber, J.-M. (1999). Effects of Sustained Swimming on Hepatic Glucose Production of Rainbow Trout. *J Exp Biol* **202**, 2161-2166.

Shrayyef, M. Z. and Gerich, J. E. (2010). Normal Glucose Homeostasis. In *Principles of Diabetes Mellitus*, (ed. L. Poretsky), pp. 19-35: Springer US.

Steele, R. (1959). Influences of glucose loading and of injected insulin on hepatic glucose output. *Annals of the New York Academy of Sciences* **82**, 420-430.

Tabata, I. and Kawakami, A. (1991). Effects of Blood Glucose Concentration on Ratings of Perceived Exertion During Prolonged Low-Intensity Physical Exercise. *Japanese Journal of Physiology* **41**, 203-215.

Teulier, L., Omlin, T. and Weber, J. M. (2013). Lactate kinetics of rainbow trout during graded exercise: do catheters affect the cost of transport? *J Exp Biol* **216**, 4549-56.

Triplitt, C. L. (2012). Examining the mechanisms of glucose regulation. *Am J Manag Care* **18**, S4-S10.

Wahren, J., Felig, P., Ahlborg, G. and Jorfeldt, L. (1971). Glucose metabolism during leg exercise in man. *Journal of Clinical Investigation* **50**, 2715-2725.

Wasserman, D. H. (2009). Four grams of glucose. *Am J Physiol Endocrinol Metab* **296**, E11-21.

Wasserman, D. H., Kang, L., Ayala, J. E., Fueger, P. T. and Lee-Young, R. S. (2011). The physiological regulation of glucose flux into muscle in vivo. *J Exp Biol* **214**, 254-62.

Wasserman, D. H., Lacy, D. B., Bracy, D. and Williams, P. E. (1992). Metabolic regulation in peripheral tissues and transition to increased gluconeogenic mode during prolonged exercise. *Am. J. Physiol.* **263**, E345-E354.

Weber, J.-M. (1999). Energy cycle in vertebrates: From food to ATP. In *Nature Encyclopedia of Life Sciences*: Chichester: John Wiley & Sons, Ltd.

Weber, J.-M., Brill, R. W. and Hochachka, P. W. (1986). Mammalian metabolite flux rates in a teleost: lactate and glucose turnover in tuna. *American Journal of Physiology* **250**, R452-R458.

Weber, J.-M., Fournier, R. and Grant, C. (1997). Glucose kinestics of the virginia opossum: possible implications for predicting glucose turnover in mammals. *Comparative Biochemistry and Physiology* **118**, 713-719.

Weber, J.-M., Roberts, T. J., Vock, R., Weibel, E. R. and Taylor, C. R. (1996). Design of the oxygen and substrate pathways III. Partitioning energy provision from carbohydrates. *Journal of Experimental Biology* **199**, 1659-1666.

Weber, J.-M. and Shanghavi, D. S. (2000). Regulation of Glucose Production: Role of Epinephrine In Vivo and in Isolated Hepatocytes. *Am J Physiol Regul Integr Comp Physiol* **278**, R956-R963.

Weber, J. M. (2011). Metabolic fuels: regulating fluxes to select mix. *J Exp Biol* **214**, 286-94.

Welch, K. C., Jr., Altshuler, D. L. and Suarez, R. K. (2007). Oxygen consumption rates in hovering hummingbirds reflect substrate-dependent differences in P/O ratios: carbohydrate as a 'premium fuel'. *J Exp Biol* **210**, 2146-53.

White, C. R. and Kearney, M. R. (2013). Determinants of inter-specific variation in basal metabolic rate. *J Comp Physiol B* **183**, 1-26.

Wright, D. A., Sherman, W. M. and Dernbach, A. R. (1991). Carbohydrate feedings before, during, or in combination improve cycling endurance performance. *Journal of Applied Physiology* **71**, 1082-1088.

Wright, P. A., Perry, S. F. and Moon, T. W. (1989). Regulation of hepatic gluconeogenesis and glycogenesis by catecholamines in rainbow trout during environmental hypoxia. *Journal of Experimental Biology* **147**, 169-188.