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In the online version of this paper, the regression equations reported in the captions of Figs 3 and 4 (p. 2736) are incorrect. The correct captions, together with the original figures, are shown below.

The print version of the article is unaffected by this error.

The authors apologise to readers for any inconvenience this may have caused.

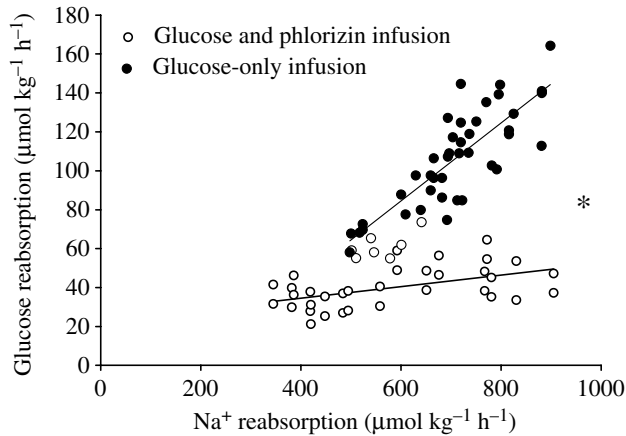


Fig. 3. Glucose reabsorption ($\mu\text{mol kg}^{-1} \text{h}^{-1}$) vs Na^+ reabsorption ($\mu\text{mol kg}^{-1} \text{h}^{-1}$) in fish infused with glucose-only or phlorizin and glucose together. * indicates significantly different ($P < 0.05$) regression line slopes. Points are values for individual fish at different times, not mean values. Regression equations: for glucose only group, $y = 0.200x - 35.471$ ($r^2 = 0.8489$) and for glucose + phlorizin treated group, $y = 0.031x + 58.047$ ($r^2 = 0.018$).

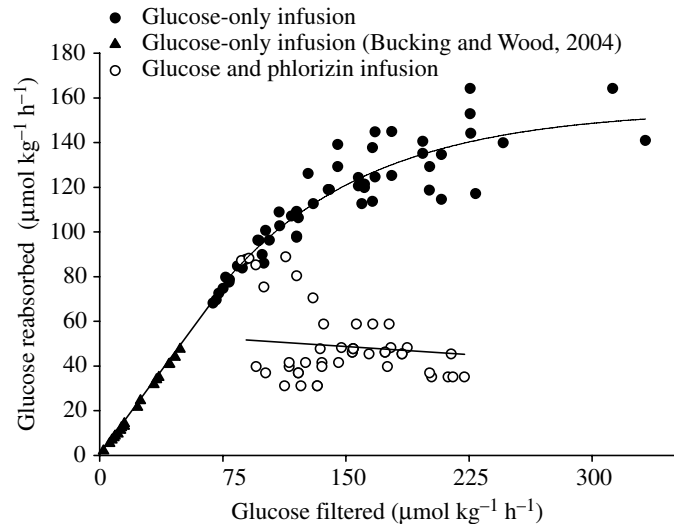


Fig. 4. Glucose filtration ($\mu\text{mol kg}^{-1} \text{h}^{-1}$) vs glucose reabsorption ($\mu\text{mol kg}^{-1} \text{h}^{-1}$) in fish infused with glucose-only or phlorizin and glucose together. Points are values for individual fish at different times, not mean values. See text for details. Regression equations: for glucose only group, $y = 162.01 - [171 / (1 + 0.00004x^{227.7})]$ ($r^2 = 0.9657$) and for glucose + phlorizin treated group, $y = 0.29x + 22.586$ ($r^2 = 0.2584$).

Renal regulation of plasma glucose in the freshwater rainbow trout

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Summary

This study examined the effects of prolonged hyperglycemia on renal handling of glucose and explored the *in vivo* pharmacological effects of phlorizin on glucose transport in the rainbow trout. The transport of glucose was examined by experimentally elevating the rate of renal glucose reabsorption *via* infusion of the fish with exogenous glucose at a rate of $70 \mu\text{mol kg}^{-1} \text{h}^{-1}$ and by inactivating the glucose transporters *via* the simultaneous administration of phlorizin ($1 \mu\text{mol kg}^{-1} \text{h}^{-1}$). Glucose was reabsorbed against a concentration gradient, until plasma glucose levels reached $\sim 22 \mu\text{mol l}^{-1}$ and the transport maximum of glucose in the kidney ($\sim 145 \mu\text{mol kg}^{-1} \text{h}^{-1}$) was exceeded. At this point, glucose was lost to the urine, resulting in glucosuria. Glucosuria affected water reabsorption, approximately doubling the water clearance ratio, and resulted in osmotic diuresis. This in turn reduced Na^+ reabsorption, increasing the amount lost to

the urine from 0.5% to 2% of the filtered load. Glucose reabsorption was found to be correlated with Na^+ reabsorption, though the latter was almost 10-fold higher than glucose transport rates. Phlorizin treatment reduced glucose reabsorption, although it did not block it entirely until 48–72 h of infusion. The glucosuria resulting from the blockade of the glucose transporters resulted in a similar osmotic diuresis and a greater Na^+ loss to the urine (9% of filtered load). The results are discussed with respect to the net renal ‘wasting’ of glucose and the detrimental osmoregulatory and ionoregulatory effects associated with glucosuria caused by carbohydrate-rich diets.

Key words: carbohydrate diet, glucose transport maximum, Na^+ reabsorption, *Oncorhynchus mykiss*, phlorizin, renal function.

Introduction

Despite the fact that most fish are considered to behave as diabetic-like animals, experiencing prolonged hyperglycemia after ingesting a carbohydrate-rich meal (Palmer and Ryan, 1972; Furuichi and Yone, 1981; Wilson, 1994; Wright et al., 1998), *in vivo* renal handling of glucose by fish has rarely been examined directly. Carbohydrates found in fish feed are generally various forms of starches obtained from grains, such as corn or wheat (Horn, 1998). These starches are broken down into their monosaccharide components, one of which is glucose, and absorbed by the gastrointestinal tract of the fish (Horn, 1998).

Rainbow trout (*Oncorhynchus mykiss* Walbaum), like many other species of fish, have difficulty peripherally utilizing glucose obtained from their diets, which results in slow plasma clearance (Legate et al., 2001). As a consequence, carbohydrates are considered to be of limited value in the nutrition of fish such as the rainbow trout (Phillips and Brockway, 1959; Legate et al., 2001). However, due to recent findings that commercial fish feeds, manufactured with fish meal and oils, may be contributing to the contamination of farmed fish stocks with PCBs (polychlorinated biphenyls) and other toxins (Hites et al., 2004), there is renewed interest to

explore alternative sources of food energy such as carbohydrates. Replacing traditionally used fish meal with cheaper carbohydrates would also reduce feed production costs and demands on depleting wild fish stocks, which are the current source of the feed (Naylor et al., 2000).

However, carbohydrates, such as glucose, may be lost in the urine of fish due to their inability to clear the nutrient from the plasma (Wright et al., 1998). In mammals, prolonged hyperglycemia induces a cascade of three events in the nephron, as summarized by Massry and Glassocks (2001). First, the filtered glucose load exceeds the transport capabilities of the proximal tubule, resulting in glucosuria. Thereafter, glucose gains access to more distal portions of the nephron, creating a net suppression of water reabsorption, a condition referred to clinically as osmotic diuresis. If osmotic diuresis persists, the final event is the reduction in Na^+ reabsorption by the nephron, resulting in natriuresis. Whether these three events occur in the kidney of a freshwater fish in response to hyperglycemia is unknown.

Work by Friere et al. (1995) involving brush-border membrane vesicles (BBMV) isolated and prepared from the proximal tubule has aided in characterizing the glucose

transporter in the rainbow trout kidney. The BBMVs displayed a single Na⁺-dependent D-glucose co-transport system that bound Na⁺ and glucose in a 1:1 stoichiometry and appeared to use the energy from Na⁺ and voltage gradients to transport glucose in a manner similar to that in mammals.

Phlorizin (phloretin-2'-β-glucoside) is a flavonoid present in apples and derived from phloretin (phloretin-2'-O-glucose). In mammals, phlorizin is a competitive inhibitor of glucose uptake *via* Na⁺/D-glucose co-transporters (Alvarado et al., 1964; Leung et al., 2000; Crespy et al., 2002), and the physiological effect *in vivo* appears to be primarily the inhibition of renal tubular reabsorption of filtered glucose. This is accomplished by phlorizin binding to the transporter and inhibiting the conformational change necessary for glucose transport (Horsburgh et al., 1978). In fish, phlorizin has been reported to inhibit Na⁺-dependent glucose transport in BBMVs from the proximal tubule of the kidney (Friere et al., 1995).

The goals of the present study were to examine the effects of prolonged hyperglycemia on the renal handling of glucose and to explore the *in vivo* pharmacological effects of phlorizin on glucose transport in the rainbow trout. The study was designed to increase the rate of renal tubular glucose reabsorption by exogenous glucose loading and thereby determine the plasma threshold and the transport maximum (TmG) of the freshwater trout kidney. We hypothesized that once these thresholds are surpassed, osmotic diuresis, and possibly natriuresis, would ensue. Also, in view of the likely coupling of glucose reabsorption to Na⁺ reabsorption, the effects of altering the glucose transporter's level of functioning on Na⁺ reabsorption was explored, as well as the effect of phlorizin on both processes.

Materials and methods

Experimental animals

Rainbow trout (233±4 g) from Humber Springs Trout Farm (Orangeville, Ontario, Canada) were acclimated to seasonal temperatures (10–13°C) and dechlorinated Hamilton tapwater [Ca²⁺=1.8 mmol l⁻¹; Cl⁻=0.8 mmol l⁻¹; Na⁺=0.6 mmol l⁻¹; Mg²⁺=0.5 mmol l⁻¹; K⁺=0.04 mmol l⁻¹; titration alkalinity (to pH 4.0) = 1.9 mequiv. l⁻¹; total hardness=140 mg l⁻¹ as CaCO₃; pH 8.0]. The fish were fed daily with 5-point-sized commercial trout pellets (Martin Mills, Ontario, Canada) at a 2% body mass ration until one week before surgery, when feeding was suspended. The crude carbohydrate content of the diet was reported by the manufacturers as 30%.

Experimental protocol

While fish were anaesthetized with MS-222 (0.07 g l⁻¹; Sigma, St Louis, MO, USA) and artificially ventilated on an operating table, dorsal aortic (Soivio et al., 1972) and internal urinary (Curtis and Wood, 1991; Wood and Patrick, 1994) catheters were implanted. The dorsal aortic catheters (Clay-Adams PE-50, Sparks, MA, USA) were filled with 0.3 ml of Cortland saline (Na⁺=140 mmol l⁻¹; Cl⁻=130 mmol l⁻¹; K⁺=5 mmol l⁻¹; Ca²⁺=1 mmol l⁻¹, Mg²⁺=2 mmol l⁻¹, glucose=

5.5 mmol l⁻¹; pH 7.8; Wolf, 1963) containing 50 i.u. ml⁻¹ of lithium heparin (Sigma) and sealed. The internal urinary catheters (heat-molded Clay-Adams PE-60) were drained by gravity into glass vials placed 3 cm below the water line. By placing the catheters inside the bladder, any reabsorptive/secretory role of the bladder is prevented and therefore the function of the kidney is solely examined.

The fish were then placed in individual darkened flux boxes and allowed to recover for 24 h, during which time the functionality of the catheters was assessed. Fish in which both catheters were operational were deemed to be experimentally viable. Such fish were then injected, *via* the dorsal aorta catheter, with 17 μCi (0.629 MBq) of [1,2-³H]polyethylene glycol (PEG-4000; New England Nuclear, Boston, MA, USA) in 0.66 ml of Cortland saline, followed by an additional wash of 0.3 ml of Cortland saline (Curits and Wood, 1991; McDonald and Wood, 1998). The [³H]PEG-4000 was allowed to equilibrate for 12 h before starting the experiment. [³H]PEG-4000 is the glomerular filtration rate (*GFR*) marker of choice in teleost fish because it undergoes the least radioautolysis, metabolic breakdown or post-filtration reabsorption relative to other *GFR* markers (Beyenbach and Kirschner, 1976; Erickson and Gingrich, 1986; Curtis and Wood, 1991).

The fish were separated into two treatment groups (*N*=7 each). The first group received a constant infusion of 140 mmol l⁻¹ glucose supplemented with an NaCl solution to achieve a typical plasma osmolality of 280 mOsmol kg⁻¹. The second treatment group received the same infusion solution with an additional 2 mmol l⁻¹ phlorizin added. The infusions, *via* a Gilson Minipulse peristaltic pump and at a rate of 500 μl kg⁻¹ h⁻¹ were begun after allowing the [³H]PEG-4000 to equilibrate for 12 h and were continued for 72 h. Blood samples (100 μl) were taken every 12 h and centrifuged at 13 000 g for 30 s to separate plasma and red blood cells, and urine samples were collected at the same time – i.e. over 12 h periods. Plasma and urine samples were immediately frozen in liquid nitrogen and stored at –80°C for later analysis.

Analytical techniques

Glucose concentrations in the plasma and urine were measured enzymatically (hexokinase, glucose-6-phosphate dehydrogenase) using a commercial kit (Sigma, 301A). The [³H]PEG-4000 radioactivities of the plasma (25 μl) and urine (1 μl) were measured by diluting with double-distilled water to a total volume of 5 ml, then adding 10 ml of ACS scintillation fluor (Amersham, UK). The samples were then counted in an LKB Rackbeta 1217 Counter (Turku, Finland). Tests demonstrated that quenching was uniform and therefore no correction was necessary. Na⁺ concentrations in plasma and urine were measured using a Varian 1275 Atomic Absorption Spectrophotometer (Walnut Creek, CA, USA).

Calculations

Urinary excretion rates (*U*) of any substance (*X*) were calculated as:

$$\dot{U}_x = [X]_u \times UFR \quad (1)$$

using measured values of urine flow rates (UFR) and urine concentrations of the substance ($[X]_u$). Clearance rates (\dot{C}_x) were calculated as the excretion rate of X divided by its concentration in the blood plasma ($[X]_p$):

$$\dot{C}_x = ([X]_u \times UFR) / [X]_p \quad (2)$$

Therefore, glomerular filtration rates (clearance rates of [3H]PEG-4000) were calculated as the excretion of [3H]PEG-4000 radioactivity in the urine (c.p.m._u) divided by its concentration in the blood plasma (c.p.m._p):

$$GFR = (c.p.m._u \times UFR) / c.p.m._p \quad (3)$$

[3H]PEG-4000 radioactivity exhibited a steady decline over time. Therefore, the concentration in blood plasma was taken as the mean of measurements at the start and end of each 12 h period.

The filtration rate (FR ; also known as filtered load) of a substance $[X]$ at the glomeruli was calculated as:

$$FR_x = [X]_p \times GFR \quad (4)$$

and the net tubular reabsorption rate (TR) of X as:

$$TR_x = \dot{U}_x - FR_x \quad (5)$$

The clearance ratio (relative clearance) of a substance (CR_x ; Wood, 1995) relates the clearance of X to the GFR or, in other words, its excretion rate (\dot{U}_x) relative to its filtration rate (FR_x):

$$CR_x = ([X]_u \times UFR) / ([X]_p \times GFR) \quad (6)$$

A CR_x of more than 1 indicates that secretion of X has occurred on a net basis; less than 1 indicates that reabsorption of X has occurred on a net basis.

All rates were related to fish body mass, i.e. ml kg⁻¹ h⁻¹.

Statistics

Data have been generally reported as means \pm S.E.M. (N =number of fish), unless otherwise stated. Regression analysis was used to determine if there was a significant relationship between two variables. Regression lines were fitted by the method of least squares, and the significance ($P < 0.05$) of the slope was assessed. Significant ($P < 0.05$) differences between two regression line slopes were calculated, according to Zar (1974), by an analysis of covariance (ANCOVA). Significant differences between treatment means were evaluated using Student's paired and unpaired t -tests as appropriate ($P < 0.05$), with Bonferroni correction for multiple comparisons (Nemenyi et al., 1977). Significant differences between time points were evaluated with an analysis of variance (ANOVA) followed by a *post-hoc* test (LSD). All statistical tests were run using SPSS, version 10.

Results

Prior to infusion, plasma glucose concentrations were around 10 mmol l⁻¹ for both treatment groups (Fig. 1A). The

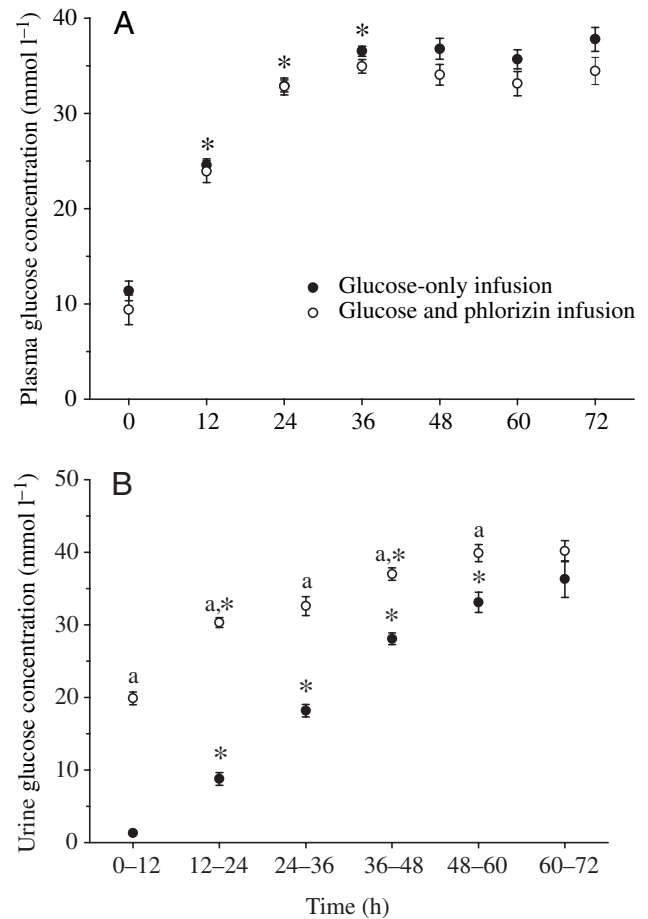


Fig. 1. (A) Plasma glucose concentrations (mmol l⁻¹). (B) Urine glucose concentrations (mmol l⁻¹). Values are means \pm 1 S.E.M. ($N=7$). * indicates a significant difference ($P < 0.05$) between the indicated value and the previous 12 h value; ^a indicates a significant difference ($P < 0.05$) between the indicated value and the glucose-only infusion value at the same time point.

glucose infusions raised plasma glucose levels markedly in both treatment groups regardless of the presence or absence of phlorizin, such that there was no significant difference between them at any time. After 36 h, the plasma concentration reached a plateau of 35.3 ± 2.5 mmol l⁻¹ (both treatments averaged over 36–72 h, $N=14$), which remained unchanged for the duration. The concentration of glucose in the urine also increased over time for both groups, as seen in Fig. 1B. For urine collected over the first 12 h of infusion, the glucose-only infusion group exhibited a urine concentration of 1.31 ± 0.66 mmol l⁻¹, which is close to detection limits of the assay. However, the urine concentration of glucose rose significantly over the next 48 h before reaching a maximum value of 36.3 ± 2.1 mmol l⁻¹ ($N=7$; 72 h). Even within the first 12 h of infusion, the effect of phlorizin was evident, resulting in a significant elevation of urine glucose to 17.1 ± 1.6 mmol l⁻¹ ($N=7$), which was considerably higher than that of the glucose-only infusion group. The phlorizin-treated group maintained a significantly ($P < 0.01$) higher urine glucose concentration for the next 48 h,

Table 1. Comparison of UFR, GFR, glucose excretion, glucose reabsorption and Na⁺ reabsorption rates between the two infusion groups

	0–12	12–24	24–36	36–48	48–60	60–72
Glucose-only infusion						
UFR (ml kg ⁻¹ h ⁻¹)	2.39±0.15	2.66±0.12	2.84±0.18*	3.29±0.32*	2.84±0.34	3.47±0.12*
GFR (ml kg ⁻¹ h ⁻¹)	5.76±0.40 ^b	5.95±0.34 ^b	5.05±0.53 ^b	5.26±0.21 ^b	4.37±0.84*	4.58±0.91*
Glucose excretion (μmol kg ⁻¹ h ⁻¹)	3.39±1.76	26.40±5.27*	56.02±6.27*	92.54±11.43*	93.48±14.11*	127.17±11.43*
Glucose filtration (μmol kg ⁻¹ h ⁻¹)	109±14	175±8*	176±19*	191±8*	143±4*	168±26*
Glucose reabsorption (μmol kg ⁻¹ h ⁻¹)	106±13	149±8*	121±16*	98±8	50±8*	51±10*
Na ⁺ reabsorption (μmol kg ⁻¹ h ⁻¹)	746±97	609±79	665±103	672±84	582±74	522±90*
Glucose and phlorizin infusion						
UFR (ml kg ⁻¹ h ⁻¹)	2.69±0.17	2.97±0.28	2.53±0.44	3.04±0.46	2.89±0.37	3.52±0.30*
GFR (ml kg ⁻¹ h ⁻¹)	6.21±0.63 ^b	5.28±0.29 ^{*,b}	3.91±0.59 ^{*,b}	5.26±0.67 ^b	4.28±0.43 ^{*,b}	4.46±0.54*
Glucose excretion (μmol kg ⁻¹ h ⁻¹)	53.42±5.99 ^a	91.89±11.42 ^{*,a}	85.53±16.87 ^a	114.27±17.84	117.01±15.61	143.39±15.81
Glucose filtration (μmol kg ⁻¹ h ⁻¹)	107±13	157±18*	133±20	180±21*	128±17	154±29
Glucose reabsorption (μmol kg ⁻¹ h ⁻¹)	54.19±7.82 ^a	65.51±10.83 ^a	47.65±8.47 ^a	65.91±12.32 ^a	11.97±4.49a*	11.42±8.05 ^{a,*}
Na ⁺ reabsorption (μmol kg ⁻¹ h ⁻¹)	831±68	617±53*	535±78*	667±60*	424±45*	509±95*

Values are means ± S.E.M. (*N*=7). * indicates a significant difference (*P*<0.05) between the indicated time period and the 0–12 h time period; ^a indicates a significant difference (*P*<0.05) between the mean phlorizin infusion values and the glucose-only infusion values at the same time point; ^b indicates a significant difference (*P*<0.05) in the glomerular filtration rate (*GFR*) values from the urine flow rate (*UFR*) values in the same infusion group.

increasing to reach a final plateau of 40.4±1.9 mmol l⁻¹ (*N*=7), not significantly different from the glucose-only group at this time. Notably, the final urine concentrations for both groups were not different from the final plasma glucose concentrations measured in this study. Also notable is the fact that the final glucose excretion rates in both groups (127–143 μmol kg⁻¹ h⁻¹) were higher than the glucose infusion rates (70 μmol kg⁻¹ h⁻¹) (Table 1). The plasma glucose rates appear to be fluctuating slightly as a result of the high glucose excretion rates (Fig. 1A). However, the true nature of the phenomenon is not apparent in the time course of the experiment and, if further time points were examined, a decrease might become evident.

UFR increased significantly over time to a similar degree within each treatment (Table 1). As with glucose excretion rates, the final *UFR*s (water excretion rates) showed a greater elevation above background (~1 ml kg⁻¹ h⁻¹) than the infusion rates (0.5 ml kg⁻¹ h⁻¹). *GFR* was also not significantly different between the two treatment groups (Table 1), although *GFR* decreased over the course of the experiment as *UFR* increased. However, for most time points of the experiment, *GFR* was significantly higher than *UFR*, indicating that reabsorption of water had occurred (Table 1), with the exception of the final experimental period (60–72 h) in both treatments.

The filtration rate of glucose during the glucose-only infusion increased over time, peaking at 36–48 h (Table 1). The same pattern was seen during the phlorizin infusion, with filtration rates peaking at 36–48 h, and for the course of the experiment there was no significant difference between the two groups. The final filtration rates were 60% higher than the initial filtration rates during 0–12 h.

As with the urine concentrations discussed earlier, the effect

of phlorizin was immediately apparent when examining the glucose excretion rates. During the first 12 h, excretion rates were 16 times higher in the phlorizin treatment than in the glucose-only treatment (Table 1). Both groups showed an increase in excretion over time, eventually becoming similar in value.

Glucose reabsorption for the glucose-only infusion group experienced a significant decrease at 48 h following an initial increase (Table 1), ending at 50% of the rate during the first 12 h. The glucose reabsorption rates for the phlorizin-exposed group were significantly lower than the glucose-only infusion group at all time points, ranging from 50 to 75% lower, and they underwent a similar decrease from 48 h onwards.

Glucose clearance ratios showed a similar pattern to glucose excretion rates (Fig. 2A). During the glucose-only infusions, the clearance ratio started out at 0.028±0.015, which shows an almost complete reabsorption of glucose (approximately 97%). As plasma glucose concentrations rose over time to reach a plateau, the clearance ratios rose steadily as well, eventually reaching ~0.75 (only 25% reabsorption of glucose) by the final time period. During the phlorizin treatment, the clearance ratio at 12 h had already reached 0.5 (50% reabsorption of glucose), and by 48 h had reached a value (0.9) not statistically different from 1.0, which would indicate that glucose was being filtered with the same efficiency as the [³H]PEG 4000 and was not being reabsorbed.

Na⁺ clearance ratios were low (0.005–0.1; indicating >90% reabsorption) and increased significantly over time during both treatments (Fig. 2B). In the glucose-only treatment, net reabsorption decreased from ~99.5% to 97.5%; it decreased to ~91% in the phlorizin treatment. The Na⁺ clearance ratios for the phlorizin-infused group were significantly elevated from

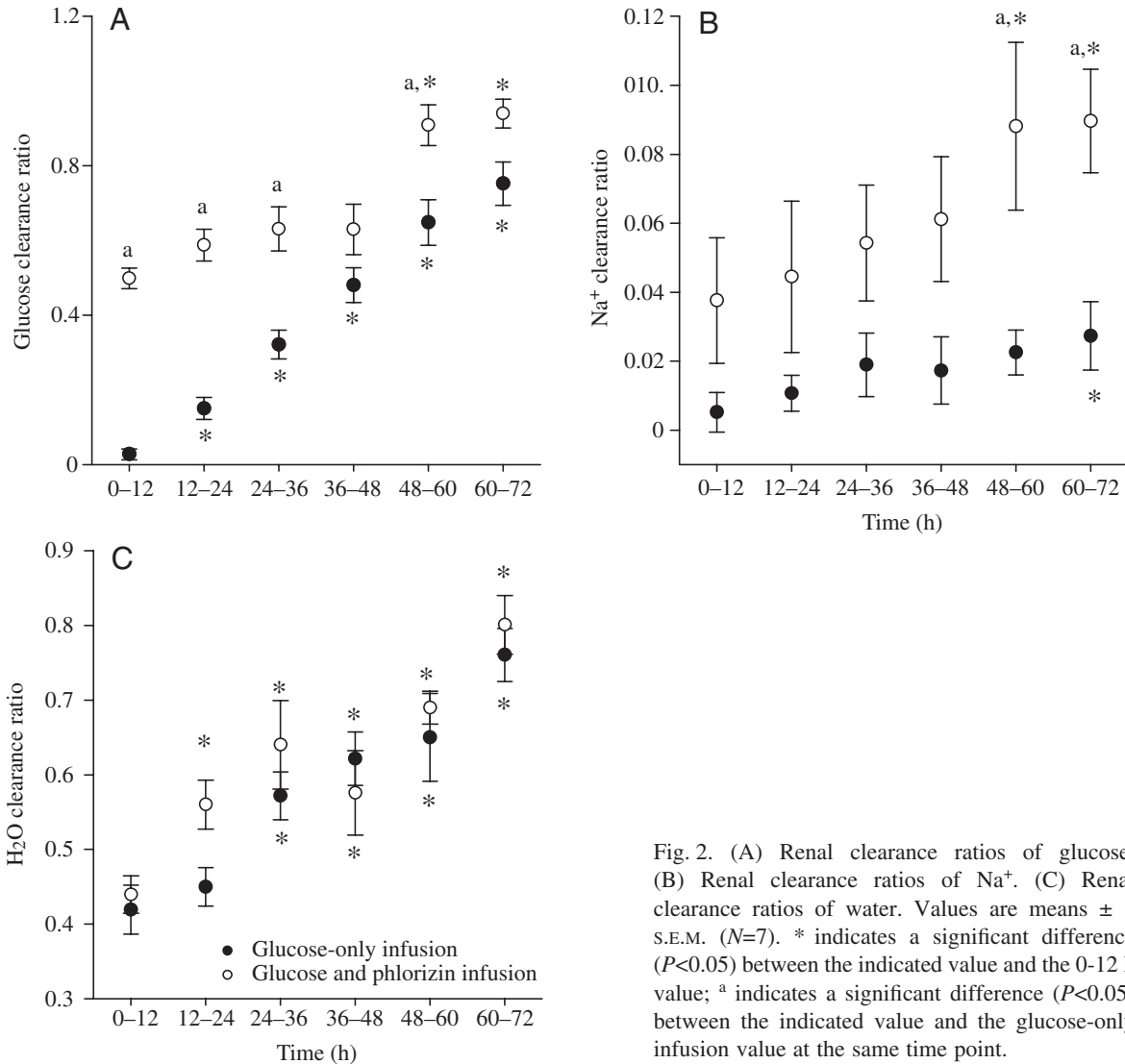


Fig. 2. (A) Renal clearance ratios of glucose. (B) Renal clearance ratios of Na⁺. (C) Renal clearance ratios of water. Values are means ± 1 S.E.M. (N=7). * indicates a significant difference ($P < 0.05$) between the indicated value and the 0-12 h value; ^a indicates a significant difference ($P < 0.05$) between the indicated value and the glucose-only infusion value at the same time point.

48 h onwards when compared with the glucose-only treatment group. Water clearance ratios were not different at any time point between the two treatments but they did show an increase over time, effectively doubling from the starting values (Fig. 2C). Thus, water reabsorption decreased from approximately 55% to 20%.

Glucose and Na⁺ reabsorption were well correlated during the glucose-only infusion (Fig. 3). Fig. 3 also shows that Na⁺ reabsorption was almost 10-fold higher than glucose reabsorption and that the phlorizin treatment effectively eliminated the relationship between Na⁺ and glucose reabsorption.

Fig. 4 shows a combination of results from this experiment and from a similar experiment using much lower glucose concentrations for infusion that was reported earlier (Bucking and Wood, 2004). During the glucose-only infusions, glucose was initially reabsorbed entirely as filtered loads increased, but then reabsorption rates appeared to reach a plateau as filtration rates continued to rise. This leveling off at high values suggests

that a transport maximum has been reached. For the phlorizin treatment, glucose reabsorption was independent of the filtered glucose load over the range for which data were available and averaged approximately 35% of the plateau value in the glucose-only treatments.

A graphical representation of the glucose filtration, excretion and reabsorption rates *versus* plasma glucose concentrations is shown in Fig. 5. The threshold plasma concentration at which glucose started to be lost to the urine because the reabsorption rate reached saturation was approximately 22 mmol l⁻¹. The leveling off of the glucose reabsorbed line indicates the TmG of the transporter and was approximately 145 $\mu\text{mol kg}^{-1} \text{h}^{-1}$.

Discussion

The handling of glucose by the rainbow trout kidney appears to be well correlated with the handling of Na⁺ (Fig. 3). While these data do not prove dependency, they do support the conclusions made by Friere et al. (1995) that glucose

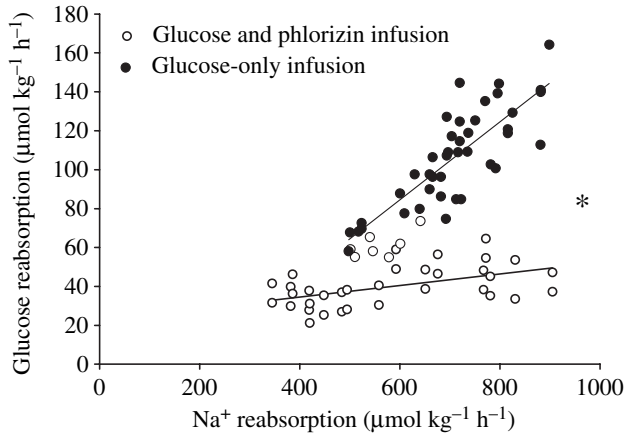


Fig. 3. Glucose reabsorption ($\mu\text{mol kg}^{-1} \text{h}^{-1}$) vs Na^+ reabsorption ($\mu\text{mol kg}^{-1} \text{h}^{-1}$) in fish infused with glucose-only or phlorizin and glucose together. * indicates significantly different ($P < 0.05$) regression line slopes. Points are values for individual fish at different times, not mean values. Regression equations: for glucose only group, $y = 162.01 - [171 / (1 + 0.00004x)^{227.7}]$ ($r^2 = 0.9657$) and for glucose + phlorizin treated group, $y = 0.29x + 22.586$ ($r^2 = 0.2584$).

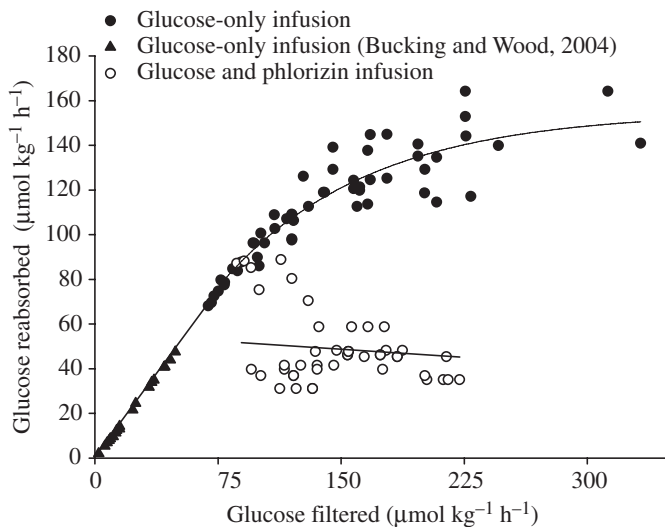


Fig. 4. Glucose filtration ($\mu\text{mol kg}^{-1} \text{h}^{-1}$) vs glucose reabsorption ($\mu\text{mol kg}^{-1} \text{h}^{-1}$) in fish infused with glucose-only or phlorizin and glucose together. Points are values for individual fish at different times, not mean values. See text for details. Regression equations: for glucose only group, $y = 0.200x - 35.471$ ($r^2 = 0.8489$) and for glucose + phlorizin treated group, $y = 0.031x + 58.047$ ($r^2 = 0.018$).

transporters in the proximal tubule of the rainbow trout kidney are Na^+ -dependent D-glucose co-transporters. However, the rate of Na^+ reabsorption was considerably higher than the rate of glucose reabsorption, suggesting that most Na^+ transporters were not involved in the reabsorption of glucose. Certainly, phlorizin blockade of glucose reabsorption did not significantly alter net Na^+ reabsorption relative to the glucose-only infusion group (Fig. 3; Table 1). Indeed, Nishimura et al. (1983) concluded that the distal portion of the nephron in the rainbow

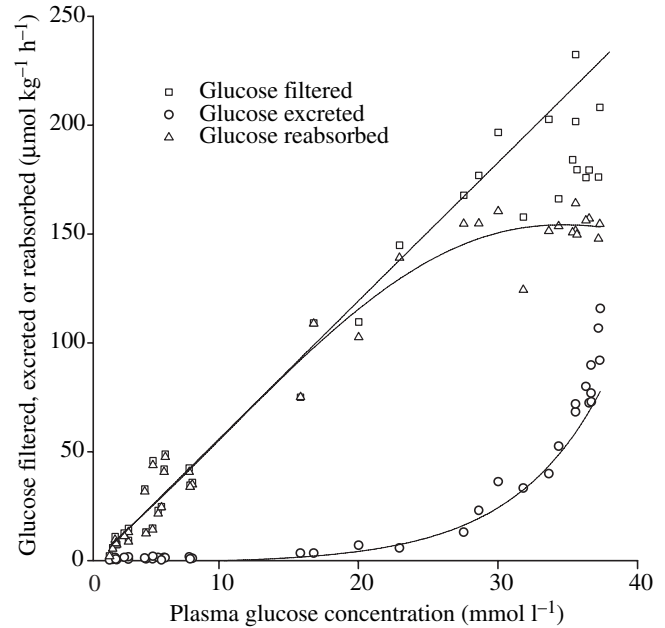


Fig. 5. Relationships of glucose filtration, excretion and reabsorption rates to plasma glucose concentration in the rainbow trout, allowing estimation of the *in vivo* glucose transport maximum from the plateau in glucose reabsorption. Points are for fish infused with glucose only and represent individual fish at different times, not mean values. See text for details. The equation of the regression line for glucose filtered is $y = 5.547x - 2.224$ ($r^2 = 0.9615$, $P < 0.05$), where y is the glucose filtration rate ($\mu\text{mol kg}^{-1} \text{h}^{-1}$) and x is the plasma glucose concentration (mmol l^{-1}). The equation of the regression line for glucose excreted is $y = 0.942 + \log(1.207) \times 1.207^x$ ($r^2 = 0.9321$, $P < 0.01$), where y is the glucose excretion rate ($\mu\text{mol kg}^{-1} \text{h}^{-1}$) and x is the plasma glucose concentration (mmol l^{-1}). The equation of the regression line for glucose reabsorbed is $y = -96.182 + 246.349e^{-0.5 \{[(x-33.715)/23.302]^2\}}$ ($r^2 = 0.9149$, $P < 0.05$), where y is the glucose reabsorption rate ($\mu\text{mol kg}^{-1} \text{h}^{-1}$) and x is the plasma glucose concentration (mmol l^{-1}).

trout kidney was responsible for diluting the urine produced in the proximal tubule. This was accomplished through Na^+ (and other electrolyte) reabsorption and the fact that the epithelium appeared to have reduced permeability to water. The apparent permeability to water could be increased, however, by increasing the osmotic pressure in the lumen of the tubule (Nishimura et al., 1983).

The infusion of glucose raised plasma glucose levels well beyond those normally seen in rainbow trout (Wright et al., 1989). At normal to moderately elevated plasma glucose concentrations ($5\text{--}20 \text{ mmol l}^{-1}$), glucose reabsorbed was a linear function of glucose filtered (Figs 4, 5). However, as the hyperglycemia worsened, the relationship between reabsorption and filtration approached an asymptote as a result of the TmG of glucose being reached (Fig. 4). Above a certain blood glucose level (threshold value), excretion becomes a linear function of the amount filtered (or plasma concentration), and reabsorption reaches a plateau. Consequently, a plot of urinary excretion rate against plasma

Table 2. Transport maximum of glucose (TmG) in rainbow trout, and species comparison of peak plasma glucose concentrations

Species	TmG ($\mu\text{mol kg}^{-1} \text{h}^{-1}$)	Peak plasma glucose concentration* (mmol l ⁻¹)	Experimental procedure, administration of glucose
Rainbow trout	145 [†]	22 [†]	<i>In vivo</i> , infusion
	58 ^a	–	<i>In situ</i> , perfused kidney
	75 ^b	–	<i>In situ</i> , perfused kidney
	170 ^c	–	<i>In vivo</i> , infusion
	–	22 ^d	<i>In vivo</i> , bolus injection
	–	15 ^e	<i>In vivo</i> , chronic dietary
	–	25 ^f	<i>In vivo</i> , bolus dietary
	–	20 ^g	<i>In vivo</i> , chronic dietary
American eel	–	35 ^d	<i>In vivo</i> , bolus injection
Black bullhead catfish	–	12 ^d	<i>In vivo</i> , bolus injection
Tilapia	–	18 ^h	<i>In vivo</i> , bolus injection

*Peak in plasma glucose concentration after ingestion/injection of various concentrations of glucose.
[†]Present study; ^aAmer and Brown (1995); ^bBrown et al. (2000); ^cGray and Brown (1985); ^dLegate et al. (2001); ^eBergot (1979); ^fPalmer and Ryan (1972); ^gPhillips et al. (1948); ^hWright et al. (1998).

glucose concentration yields a threshold value at which glucose is lost to the urine, and a plot of urinary reabsorption rates against plasma glucose concentration yields a sigmoidal curve, as shown in Fig. 5, from which a TmG can be calculated (Massry and Glassocks, 2001).

The TmG found in this study is similar to findings in another *in vivo* study (Gray and Brown, 1985; Table 2). However, it is approximately double compared with findings on *in situ* perfused trout kidney studies (Amer and Brown, 1995; Brown et al., 2000; Table 2). Part of these differences could be accounted for by variations in experimental temperatures, as TmG varies with temperature, with higher temperatures yielding higher TmGs (MacKay and Beatty, 1967). However, it seems likely that part of the discrepancy could reflect more efficient renal function *in vivo* than in perfused-organ experiments.

The plasma threshold value at which glucose started to be lost to the urine was found to be 22 mmol l⁻¹. This is similar to other peak plasma values found in the rainbow trout, after ingestion of glucose-rich diets or injection of glucose-rich solutions, although different when compared with other species (Table 2). The minor differences seen within rainbow trout might be attributed to experimental procedure, as the highest plasma values were seen with bolus injections and infusions, while the lowest value was seen in a chronic dietary exposure. This could be due to differential activation of physiological processes, depending on the route of exposure. Indeed, Hemre et al. (1995) observed that plasma glucose levels in Atlantic salmon rose only marginally after oral ingestion, while intraperitoneal administration resulted in prolonged hyperglycemia.

A possible explanation for the difference observed between species (Table 2) could involve variations in 'glucose space', where glucose space is the space into which glucose is dispersed and includes plasma and extracellular fluid (Legate et al., 2001). Glucose space has been shown to vary between species, ranging from 100 to 400 ml kg⁻¹ (Garin et al., 1987),

although only a limited number of species have been examined to date. Another possible explanation for the differences in glucose plasma peaks is a possible difference in the renal glucose transport maximum between species. There are reported differences in many renal transport processes between species, including those involved with glucose, especially when comparing marine and freshwater fish (Dantzler, 1989). In addition, there exists evidence of differential handling of dietary starches between species. Perch have been shown to increase intestinal glucose absorption when fed high starch diets, while rainbow trout remained unchanged (Buddington, 1987). Krogdahl et al. (2004) have also shown that Atlantic salmon and rainbow trout exhibit differential handling of dietary starches, wherein rainbow trout were observed to have a higher capacity to digest and absorb starches compared with Atlantic salmon.

As the hyperglycemia created from the infusions increased, glucose was lost to the urine as a consequence of the transport maximum being reached, resulting in glucosuria as expected. As mentioned in the Introduction, this is the first event of three that occurs in the kidney during hyperglycemia in mammals. The second event, the decrease in reabsorption of water over time, was also present in the rainbow trout kidney, as evidenced by the increasing clearance ratio for water (Fig. 2C). This is probably due to the increasing osmotic pressure in the distal portion of the nephron due to the increasing concentration of glucose in the urine (Massry and Glassocks, 2001). This increase in osmotic pressure raises the apparent permeability of the distal nephron to water, as mentioned earlier (Nishimura et al., 1983).

The third event that occurs in mammalian kidneys, the loss of Na⁺ to the urine, became clearly apparent in the last 12–24 h of the study (Table 1; Fig. 2B). Natriuresis caused by osmotic diuresis is the final occurrence in the kidney in response to hyperglycemia and tends to show a delayed response (Massry and Glassocks, 2001). This is supported by the phlorizin treatment group, which exhibited a decrease in Na⁺

reabsorption 12 h before the glucose-only treatment. The former group was experiencing elevated glucosuria compared with the glucose-only infusion group over a longer period of time (Fig. 2A). Overall, the freshwater rainbow trout kidney displays similar trends in response to hyperglycemia when compared with the mammalian kidney.

Despite the desire to include carbohydrates in fish food, there exists evidence that carbohydrate levels must be closely controlled when incorporated into the feed. Reduced growth rates have been observed in some fish species fed carbohydrate-free diets (Anderson et al., 1984; Degani et al., 1986). Conversely, feeding excessive dietary carbohydrates to fish has been shown to adversely affect an array of physiological parameters, such as growth and liver size and function (Hilton and Atkinson, 1982; Dixon and Hilton, 1985). These deleterious effects have been attributed to the poor peripheral utilization, or poor peripheral uptake, of glucose and other carbohydrates by the tissues or to insulin production and binding difficulties (Hilton and Atkinson, 1982; Legate et al., 2001).

The elevated levels of glucose found in the plasma of the rainbow trout after a carbohydrate-rich meal or injection are close to the plasma concentration at which the transport maximum is reached in this study (Fig. 5; Table 2; Philips et al., 1948; Palmer and Ryman, 1972; Bergot, 1979; Legate et al., 2001). This indicates that fish fed high starch diets may lose some of the glucose to the environment *via* urinary excretion. For aquaculture, this would affect the amount of starch or glucose incorporated into diets because, above a certain concentration of glucose, it would be of no use to, and possibly detrimental to the health of, the fish. The present study indicates that disturbances in ionoregulation and osmotic regulation associated with glucosuria may contribute to these deleterious effects.

Although the phlorizin infusion at $1 \mu\text{mol kg}^{-1} \text{h}^{-1}$ was effective in reducing the reabsorption of glucose, it did not immediately block it entirely (Table 1). Glucose excretion rates only became equal to filtration rates after 48 h of phlorizin infusion, as shown by the clearance ratios in Fig. 2A and Table 1.

Administration of $1\text{--}10 \mu\text{mol kg}^{-1} \text{h}^{-1}$ of phlorizin caused complete inhibition of glucose transport in several *in vitro* studies conducted in fish (Pritchard and Kleinzeller, 1976), while previous studies on mammals have encountered complete blockade of glucose reabsorption at rates of $<1 \mu\text{mol phlorizin kg}^{-1} \text{h}^{-1}$ (Horsburgh et al., 1978; Silverman et al., 1970). The slow onset of blockade in the present study was probably due to competition. Indeed, Kimmich (1990) found that at glucose concentrations much greater than phlorizin concentrations, glucose was able to out-compete phlorizin for access to the transporter despite having a much lower binding affinity for the transporter. This was probable in the present experiment as the glucose concentration was 70 times greater than the phlorizin concentration in the infusion solution.

In summary, constant infusion of glucose into the plasma of the rainbow trout results in persistent hyperglycemia. As a

result, the kidney filters glucose at rates that exceed the transport maximum of the nephrons, resulting in glucosuria. This causes osmotic diuresis and natriuresis. As freshwater fish are hyperosmotic to their environment, this poses a detrimental risk to their osmotic and ionic regulation. With regard to the desire to incorporate carbohydrates into the diets of farmed fish, this should be approached with caution as, above a certain concentration, glucose obtained from the diet will result in glucosuria.

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