

## RELIABILITY OF CONTINUOUS TRACER INFUSION FOR MEASURING GLUCOSE TURNOVER RATE IN RAINBOW TROUT

FRANÇOIS HAMAN, MARK POWELL AND JEAN-MICHEL WEBER\*

*Biology Department, University of Ottawa, 30 Marie Curie, Ottawa, Ontario, Canada K1N 6N5*

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### Summary

Glucose plays a fundamental role in mammalian energetics but its contribution as a metabolic fuel is not well established for fish; the accurate *in vivo* measurement of glucose flux is essential to determine the importance of this substrate in the energy budget of teleosts. Therefore, the goal of the present study was to verify the reliability of the continuous tracer infusion method for estimating glucose turnover rate in rainbow trout *Oncorhynchus mykiss*. Our secondary goals were to determine whether glucose flux can be estimated more accurately from plasma or from whole-blood samples, and to obtain an estimate of renal glucose production. Continuous infusions of [6-<sup>3</sup>H]glucose were performed in hepatectomized and intact animals. In some hepatectomized individuals, liver glucose production was replaced by a pump infusing unlabelled glucose at a known rate. Renal glucose production was measured in

hepatectomized fish where liver glucose production was not replaced, and it averaged  $1.1 \pm 0.1 \mu\text{mol kg}^{-1} \text{min}^{-1}$  (mean  $\pm$  S.E.M.,  $N=5$ ). Results show that glucose turnover rate is quantified accurately by continuous tracer infusion and that glucose flux can be estimated equally well from plasma (error of  $-0.7 \pm 4.9\%$ ) and from whole-blood (error of  $-5.7 \pm 2.9\%$ ) samples (means  $\pm$  S.E.M.,  $N=7$ ). This study provides the first experimental validation of continuous tracer infusion in fish, and shows that this method could become a powerful tool to investigate hormonal regulation of glucose metabolism in live teleosts.

Key words: glucose metabolism in fish, *in vivo* substrate fluxes, hepatic glucose production, non-steady-state glucose kinetics, tracer methodology, rainbow trout, *Oncorhynchus mykiss*.

### Introduction

Glucose metabolism plays a fundamental role in mammalian energetics, but its contribution as a cellular fuel is not well established for fish (Garin *et al.* 1987; Weber and Zwingelstein, 1995). No teleost tissue is known to use glucose preferentially (Moon and Foster, 1995), and the role of this metabolic fuel is generally considered to be relatively minor in fish (Van den Thillart, 1986). The accurate *in vivo* measurement of glucose flux is essential to determine the importance of this metabolite in the total energy budget of teleosts. In this context, we have recently described a new method of continuous tracer infusion to quantify glucose turnover rate in rainbow trout *Oncorhynchus mykiss* (Haman and Weber, 1996). This technique uses a double cannulation of the dorsal aorta that allows simultaneous blood sampling and isotope infusion. Surprisingly, the glucose turnover rates measured in trout using this new method were 4–9 times higher than previously published values obtained with the less versatile, but commonly used, bolus injection technique. In mammals, Allsop *et al.* (1978, 1979) showed that continuous infusion was more reliable than bolus injection in estimating glucose turnover rate. The validation technique used by these

authors to verify the reliability of flux measurements consisted of surgically removing all natural endogenous glucose sources (i.e. the liver and kidneys) and replacing them by an external pump infusing unlabelled glucose at a known rate. In the present study, we used a similar experimental approach to determine the accuracy of the continuous infusion technique in rainbow trout. An infusion of unlabelled and labelled glucose was carried out on hepatectomized trout to verify whether the known rate of unlabelled glucose entry from the pump can be measured accurately by our tracer dilution technique. Because nephrectomy cannot be performed in trout, a parallel series of experiments was carried out in hepatectomized animals without infusing unlabelled glucose to provide an estimate of renal glucose production in anaesthetized fish.

Glucose flux is calculated from the rate of tracer infusion and glucose specific activity in the circulation (Steele, 1959). This specific activity can be measured either in plasma or in whole blood, and the values obtained with both approaches are usually identical for most mammalian species, probably because equilibration across the membranes of the red blood cells is achieved rapidly. In contrast, erythrocytes of some fish

\*Author for correspondence (e-mail: jmweber@oreo.uottawa.ca).

species, including rainbow trout, show a very slow carrier-mediated glucose transport (Bolis *et al.* 1971; Nikinmaa and Tiihonen, 1994; Tse and Young, 1990), and it is unclear whether glucose specific activity is the same in plasma and inside the red blood cells during a continuous infusion of labelled glucose. Therefore, the three goals of this study were to verify the reliability of the continuous infusion method for quantifying glucose turnover rate in rainbow trout, to provide a first estimate of glucose production by fish kidney, and to determine whether glucose flux is assessed more accurately by measuring specific activity in plasma or in whole blood.

## Materials and methods

### Animals

Rainbow trout *Oncorhynchus mykiss* (Walbaum) of both sexes (mass 470–1060 g) were obtained from Linwood Acres Trout Farm (Campbellcroft, Ontario, Canada) and held in 550 l flow-through tanks at 15 °C. They were kept in dechloraminated, well-oxygenated Ottawa tap water under a 12 h:12 h L:D photoperiod. The animals were acclimated to these conditions for at least 1 month before experiments, and they were fed Purina trout chow three times a week until satiation.

### Catheterizations

A double cannulation of the dorsal aorta was performed under anaesthesia (0.1 g l<sup>-1</sup> ethyl-*N*-aminobenzoate sulphonic acid, MS-222, buffered with 0.2 g l<sup>-1</sup> bicarbonate) as described previously (Haman and Weber, 1996). Cannulated animals were either kept anaesthetized, and hepatectomized to validate the tracer infusion method (group R where glucose was replaced and group NR where it was not; see below), or they were taken out of anaesthesia to measure glucose turnover rate in intact animals (i.e. without removing the liver; group INT).

### Experiments with hepatectomized animals

Following cannulation, a ventral incision was made to expose the abdominal cavity. This incision was extended frontally to create a small opening in the pericardial membrane for monitoring heart rate, which was used as an indicator of the general health of the fish. Heart rate averaged 60±1.3 beats min<sup>-1</sup> and did not vary significantly during experiments (analysis of variance,  $P=0.31$ ). The hepatic portal vein, the hepatic vein and the coeliac artery were ligated, and the liver was removed and weighed. Liver mass was later subtracted from body mass for the calculation of turnover rate. Hepatectomized animals were then divided into two groups. In the first group, unlabelled glucose was infused using a pump to replace normal hepatic glucose production (group R: unlabelled glucose replacement) while in the second group, liver glucose production was not replaced (group NR: no replacement). Measurements in NR animals were designed to quantify residual glucose production by the kidney because this organ could not be surgically removed in our experiments (see Discussion).

Once cannulated and hepatectomized, R and NR animals remained on the surgical table and were maintained under anaesthesia throughout the experiments. Following the injection of a priming dose equivalent to 120 min of infusion, a continuous infusion of [6-<sup>3</sup>H]glucose (New England Nuclear 1.6 TBq mmol<sup>-1</sup> or Amersham 1.11 Tq mmol<sup>-1</sup>) was started at 1 ml h<sup>-1</sup> using a calibrated syringe pump (Harvard Apparatus, South Natick, MA, USA) (Haman and Weber, 1996). The infusate was prepared daily by drying a sample of [6-<sup>3</sup>H]glucose under N<sub>2</sub> and dissolving it in Cortland saline (Wolf, 1963). Infusion rates ranged from 33 841 to 96 928 disintegrations min<sup>-1</sup> kg<sup>-1</sup> min<sup>-1</sup>. Blood samples (600 µl each) were drawn 30, 40, 50 and 60 min after the beginning of infusion. For the R group, 240 mmol l<sup>-1</sup> unlabelled glucose was added to the infusate to replace natural hepatic glucose production. At a pump infusion rate of 1 ml h<sup>-1</sup>, the artificial entry rate of unlabelled glucose was therefore 4 µmol min<sup>-1</sup>. Since the same absolute infusion rate of unlabelled glucose was used for all the animals in the R group, mass-specific infusion rates ranged from 4.0 to 6.8 µmol glucose kg<sup>-1</sup> min<sup>-1</sup> and averaged 5.1±0.3 µmol glucose kg<sup>-1</sup> min<sup>-1</sup> (mean ± S.E.M.,  $N=7$ ). These rates of unlabelled glucose infusion were chosen deliberately to be lower than the resting turnover rates previously measured in intact, unanaesthetized animals (Haman and Weber, 1996) because MS-222 depresses glucose metabolism (Mazeaud *et al.* 1977). Preliminary experiments showed that infusing unlabelled glucose at between 10 and 15 µmol kg<sup>-1</sup> min<sup>-1</sup> (the normal values measured in intact animals) in anaesthetized, hepatectomized trout causes hyperglycaemia.

### Experiments with intact animals

After cannulation, the fish were allowed to recover for 24 h in an opaque acrylic box in which tracer infusions were subsequently performed. Rates of glucose turnover and oxygen consumption were then measured as described previously (Haman and Weber, 1996). Infusion rates of [6-<sup>3</sup>H]glucose ranged from 95 561 to 222 300 disintegrations min<sup>-1</sup> kg<sup>-1</sup> min<sup>-1</sup>, and blood samples (600 µl each) were drawn 40, 50, 60 and 70 min after beginning the infusion.

### Blood sample analysis

Measurements were made on plasma and whole-blood samples for R and INT animals, but only on plasma for NR animals. For R and INT animals, blood samples were immediately divided into two fractions: 300 µl was centrifuged to separate the plasma and another 300 µl was mixed with 500 µl of perchloric acid (PCA, 8 %) and centrifuged to obtain PCA extracts. Haematocrit was measured by centrifuging heparinized micro-haematocrit capillary tubes for 5 min at 11 000 revs min<sup>-1</sup>. Samples were then stored at -20 °C and analyzed within 2 weeks. Plasma and whole-blood glucose concentrations were determined spectrophotometrically on a Milton Roy, Spectronic 1001 plus at 340 nm (Bergmeyer, 1985). Plasma glucose activity was measured by drying 30 µl of plasma under N<sub>2</sub> at 55 °C in 20 ml scintillation vials.

Perchloric acid extracts were first neutralized with 3 mol l<sup>-1</sup> K<sub>2</sub>CO<sub>3</sub> in 0.5 mol l<sup>-1</sup> triethanolamine hydrochloride. Samples (60 µl each) of the neutralized extract were then placed in scintillation vials and dried under N<sub>2</sub>. Preliminary experiments with [6-<sup>3</sup>H]glucose standards showed that neutralization of the PCA extract is essential to avoid significant losses of tritium activity (up to 50%), probably caused by <sup>3</sup>H exchange from glucose to water at the extremely low pH reached during the drying process. Once dry, plasma and PCA extract samples were resuspended in 1 ml of water and counted in 10 ml of ACS II scintillation fluid (Amersham, Oakville, Ontario, Canada) on a Tri-Carb 2500 counter (Packard, Canada) with external quench correction. Glucose turnover rate ( $R_t$ ) was then calculated using the steady-state equation of Steele (1959).

#### Statistical analyses

Overall differences between plasma and whole-blood measurements, and between hepatectomized animals with glucose replacement and those without glucose replacement (R *versus* NR animals), were assessed using two-way analyses of variance (ANOVAs) with replication. For each sampling time, the Bonferroni *t*-test was used to detect potential differences between measured glucose turnover rates in plasma or whole blood and the known rate of unlabelled glucose entry from the pump ( $R_{inf}$ ). All values given are means  $\pm$  S.E.M.

### Results

In all experiments, the selected priming dose and the delay between the start of infusion and the first blood sample were adequate to reach isotopic steady state. Glucose concentration and specific activity did not change significantly throughout the measurement period (Figs 1, 2;  $P > 0.05$ ). Results for hepatectomized animals with glucose replacement (R) and without glucose replacement (NR) are presented in Fig. 1 and Table 1. In group R, the turnover rates measured in plasma or in whole blood were not significantly different from the artificial, mass-specific glucose replacement rates from the pump ( $R_{inf}$ ) (Fig. 1C;  $P > 0.05$ ). Per cent errors between measured rates and  $R_{inf}$  were calculated even though the mean values were not significantly different (Table 1). Mean per cent differences were  $+21.6 \pm 5.4\%$  (plasma) and  $+16.6 \pm 3.5\%$  (whole blood) when no correction was made for glucose production by the kidney (Table 1). These differences were reduced to  $-0.7 \pm 4.9\%$  (plasma) and  $-5.7 \pm 2.9\%$  (whole blood) after subtracting glucose production by the kidney (Table 1).  $R_{inf}$  and per cent difference between measured and true rates were not correlated (plasma,  $r^2 = 0.01$ ,  $P = 0.95$ ; whole blood,  $r^2 = 0.13$ ,  $P = 0.50$ ). For NR animals, glucose concentration, specific activity and  $R_t$  averaged  $2.7 \pm 0.2$  mmol l<sup>-1</sup>,  $120\,900 \pm 40\,884$  disints min<sup>-1</sup> µmol<sup>-1</sup> and  $1.1 \pm 0.1$  µmol kg<sup>-1</sup> min<sup>-1</sup>, respectively ( $N = 5$ ; Fig. 1). These three parameters were significantly different between R and NR animals ( $P < 0.05$ ).

Results for intact animals (INT) are presented in Fig. 2 and Table 2. Their metabolic rate ( $\dot{M}_{O_2}$ ) remained steady throughout the experiments ( $P > 0.05$ ) and averaged

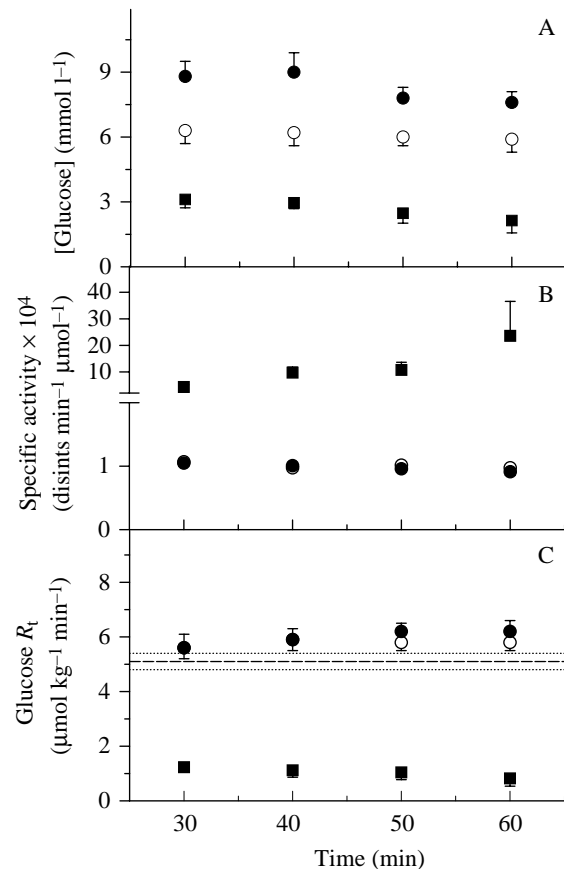


Fig. 1. Glucose concentration (A), specific activity (B) and turnover rate  $R_t$  (C) measured in plasma (filled symbols) or in whole blood (open symbols) during continuous infusions of [6-<sup>3</sup>H]glucose in hepatectomized, anaesthetized rainbow trout with (group R, circles,  $N = 7$ ) and without (group NR, squares,  $N = 5$ ) glucose replacement. In C, the dashed and dotted lines represent mean  $\pm$  S.E.M. mass-specific unlabelled glucose replacement rate from the pump ( $R_{inf}$ ). Values are means  $\pm$  S.E.M. (only indicated when wider than symbols). Open circles are not visible when completely overlapping with filled circles (B and C).

$46.9 \pm 2.3$  µmol O<sub>2</sub> kg<sup>-1</sup> min<sup>-1</sup> ( $N = 8$ ). The rates of glucose turnover determined from specific activities measured in plasma or in whole blood were the same for hepatectomized (R) (Fig. 1; Table 1) and intact animals (INT) (Fig. 2; Table 2) (plasma *versus* whole blood,  $P > 0.05$ ). Glucose concentrations measured in both plasma and whole blood were the same in INT animals (Fig. 2A,  $P > 0.05$ ), but different in R animals (Fig. 1A,  $P < 0.05$ ).

### Discussion

The present study shows that fish glucose dynamics can be quantified *in vivo* with confidence because continuous tracer infusion gives an accurate estimate of glucose turnover rate, perhaps the most fundamental parameter of carbohydrate metabolism. In hepatectomized trout in which normal liver glucose production was artificially replaced, estimates of

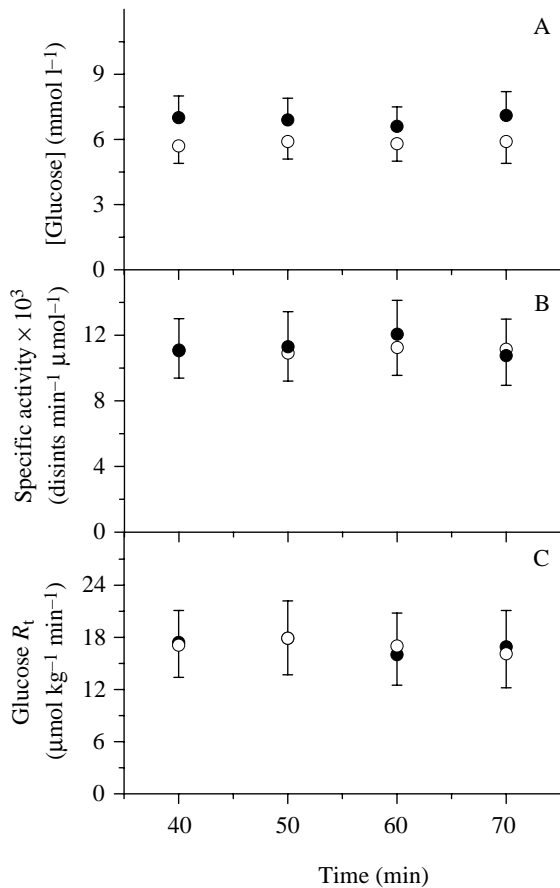


Fig. 2. Glucose concentration (A), specific activity (B) and turnover rate  $R_t$  (C) measured in plasma (filled symbols) or whole blood (open symbols) during continuous infusions of  $[6\text{-}^3\text{H}]\text{glucose}$  in intact, unanaesthetized rainbow trout (i.e. non-hepatectomized, group INT). Values are means  $\pm$  S.E.M.,  $N=8$ , except 70 min point in A for which  $N=7$ .

turnover rate obtained by continuous tracer infusion were not significantly different from the actual unlabelled glucose replacement rates imposed by the glucose pump (Fig. 1; Table 1). Furthermore, turnover rates calculated from plasma and from whole blood were identical, demonstrating that both analytical approaches are adequate to measure glucose specific activity in turnover experiments (Figs 1, 2; Tables 1, 2).

Two important observations led us to perform this study. First, numerous articles have reported fish glucose turnover rates under the assumption that the tracer techniques used to quantify these rates were accurate (Garin *et al.* 1987; Haman and Weber, 1996; Weber and Zwingelstein, 1995). However, neither bolus injection nor continuous infusion has been validated for fish models. Second, the infusion technique we have recently introduced yields much higher turnover rates than have been measured using bolus injection over the last 20 years, raising doubts on the validity of both methods. Here, we show that glucose fluxes measured by continuous infusion are accurate, and this observation indicates that previous values obtained by bolus injection underestimate true rates. A number

of reasons why rates may have been underestimated in bolus injection studies have been discussed previously (Weber and Haman, 1996). Briefly, adequate mixing of the bolus was not achieved in studies where the isotope was administered intraperitoneally, or the first part of the decay curve was missed because blood sampling was started too late after injection. In some cases, the animals were under anaesthesia during the measurements, a state in which carbohydrate metabolism is known to be depressed. In other cases, a constant blood glucose concentration was not achieved even though steady state is an important assumption of the bolus-injection method. In studies using carbon tracers, glucose recycling may have kept specific activity abnormally high in the tail of the decay curve, thereby overestimating the surface area under that curve and underestimating turnover rate. Future studies should focus on using the more versatile continuous infusion method that allows non-steady-state conditions as well as multiple flux measurements in the same experiment. For these reasons, we did not carry out a parallel validation of the bolus injection technique because this experimental approach is obsolete and will eventually be completely replaced by the continuous infusion method. For the same reasons, continuous infusion has been used in mammalian studies almost exclusively over the last three decades (Wolfe, 1992).

Why trout have such a high glucose turnover rate is not obvious. Our results show that only a small fraction of this glucose flux must be channelled towards oxidation because 40% of this flux would be sufficient to account for the total oxygen consumption if the animal were using glucose as its sole oxidative fuel. Clearly, much less than 40% of the total glucose flux must actually be oxidized because lipids and proteins are probably used preferentially for energy metabolism in the resting state. We can only speculate on the metabolic fate of most of the glucose produced. In fish, high rates of non-oxidative glucose disposal could be related to high rates of glucose cycling in the liver. This substrate cycle may be necessary to maintain high rates of hepatic glycogen turnover and continuously balance carbohydrate reserves between the different regions of this organ. From the resting state, a high glucose turnover rate coupled with a low oxidation rate would allow the animal to initiate exercise simply by reducing glucose cycling and increasing oxidation, without changing total glucose flux. A high resting turnover rate would also allow rapid replenishment of glycogen reserves during recovery from strenuous exercise. Direct measurements of glucose cycling and oxidation will be needed to test these hypotheses.

Published rates of glucose production measured *in vitro* are lower than quantified here *in vivo*, possibly because isolated liver cells do not release glucose as rapidly when they are placed out of their normal hormonal environment. Assuming that liver mass represents 1.2% of body mass and that hepatocytes account for 90% of total liver mass, we have extrapolated rates of hepatic glucose production per kilogram body mass from *in vitro* measurements. Data on isolated hepatocytes (Mommsen, 1986; Mommsen *et al.* 1988) yield

Table 1. Glucose concentration, specific activity (SA) and turnover rate ( $R_t$ ) in plasma (pl) and whole blood (wb) during continuous infusion of [6- $^3$ H]glucose in hepatectomized rainbow trout where normal liver glucose production was replaced by a pump infusing unlabelled glucose (group R)

Fish	$M_b$ (g)	[Glucose] <sub>pl</sub> (mmol l <sup>-1</sup> )	[Glucose] <sub>wb</sub> (mmol l <sup>-1</sup> )	SA <sub>pl</sub> (disints min <sup>-1</sup> μmol <sup>-1</sup> )	SA <sub>wb</sub> (disints min <sup>-1</sup> μmol <sup>-1</sup> )	$R_{inf}$ (μmol kg <sup>-1</sup> min <sup>-1</sup> )	$R_{t,pl}$ (μmol kg <sup>-1</sup> min <sup>-1</sup> )	$R_{t,wb}$ (μmol kg <sup>-1</sup> min <sup>-1</sup> )	Uncorrected % error (pl)	Uncorrected % error (wb)	Corrected % error (pl)	Corrected % error (wb)
1	854	6.6±0.2	4.2±0.1	7 742±325	9 457±149	4.7	6.8±0.2	5.6±0.1	+44.7	+19.1	+21.3	-4.3
2	777	8.3±2.7	6.2±1.5	11 074±432	10 781±174	5.1	5.5±0.2	5.7±0.1	+7.8	+11.8	-13.7	-9.8
3	723	7.9±0.2	6.6±0.1	9 401±341	9 994±473	5.5	7.0±0.2	6.6±0.3	+27.3	+20.0	+7.3	0.0
4	592	8.6±0.4	6.4±0.2	10 916±327	10 906±87	6.8	7.3±0.2	7.3±0.0	+7.4	+7.4	-8.8	-8.8
5	854	8.0±0.2	7.1±0.4	9 336±205	9 473±454	4.7	5.9±0.1	5.8±0.1	+25.5	+23.4	+2.1	0.0
6	863	9.4±0.5	6.9±0.3	10 908±168	11 465±377	4.6	5.0±0.1	4.8±0.1	+8.7	+4.3	-15.2	-19.6
7	1012	8.5±0.3	5.7±0.3	8 938±288	9 039±326	4.0	5.2±0.2	5.2±0.2	+30.0	+30.0	+2.5	+2.5
Mean	811	8.2	6.2	9 759	10 159	5.1	6.1	5.9	+21.6	+16.6	-0.7	-5.7
S.E.M.	50	0.3	0.4	474	341	0.3	0.4	0.3	5.4	3.5	4.9	2.9

Body mass ( $M_b$ ), rate of unlabelled glucose replacement from the pump ( $R_{inf}$ ) and % error between measured ( $R_t$ ) and known ( $R_{inf}$ ) rates are also indicated.

Per cent error =  $[(R_t - R_{inf})/R_{inf}] \times 100$ . Corrected % error was calculated by subtracting extra-hepatic glucose production from  $R_t$  (mean  $\pm$  S.E.M. extra-hepatic glucose production was  $1.1 \pm 0.1$  μmol kg<sup>-1</sup> min<sup>-1</sup>, measured in the NR group in which liver glucose production was not replaced, see Fig. 1C).

Values are means  $\pm$  S.E.M. ( $N=4$  blood samples).

Table 2. Glucose concentration, specific activity (SA) and turnover rate ( $R_t$ ) in plasma (pl) or whole blood (wb) during continuous infusions of [6- $^3$ H]glucose in intact rainbow trout (i.e. not hepatectomized, INT group) at 15 °C

Fish	$M_b$ (g)	Hct (%)	$\dot{M}O_2$ (μmol kg <sup>-1</sup> min <sup>-1</sup> )	[Glucose] <sub>pl</sub> (mmol l <sup>-1</sup> )	[Glucose] <sub>wb</sub> (mmol l <sup>-1</sup> )	SA <sub>pl</sub> (disints min <sup>-1</sup> μmol <sup>-1</sup> )	SA <sub>wb</sub> (disints min <sup>-1</sup> μmol <sup>-1</sup> )	$R_{t,pl}$ (μmol kg <sup>-1</sup> min <sup>-1</sup> )	$R_{t,wb}$ (μmol kg <sup>-1</sup> min <sup>-1</sup> )
1	858	30	43.9±1.2	6.4±0.1	4.8±0.1	13 398±368	13 279±361	8.1±0.2	8.2±0.2
2	800	31	44.0±1.8	3.6±0.1	2.7±0.0	16 876±361	16 694±248	7.1±0.1	7.2±0.1
3	779	31	47.9±1.2	8.9±0.2	7.6±0.2	2 930±10	2 911±97	37.7±1.5	37.7±1.2
4	781	21	58.7±1.0	5.1±0.2	4.4±0.0	11 640±468	13 058±115	12.5±0.5	11.1±0.1
5	523	25	51.8±0.6	5.2±0.2	5.9±0.1	20 733±994	15 530±242	10.8±0.6	14.4±0.2
6	632	27	41.2±0.5	9.5±0.1	7.7±0.1	9 279±295	9 812±484	16.4±0.5	15.5±0.5
7	656	31	38.6±2.7	11.5±0.3	9.5±0.1	5 736±326	5 694±330	28.3±1.6	28.6±1.7
8	616	17	49.3±1.7	4.4±0.1	4.1±0.1	12 232±625	12 910±150	14.1±0.7	13.3±0.1
Mean	706	27	46.9	6.8	5.8	11 603	11 236	16.9	17.0
S.E.M.	41	1.9	2.3	1.0	0.8	2 026	1 693	3.8	3.8

Body mass ( $M_b$ ), haematocrit (Hct) and oxygen consumption ( $\dot{M}O_2$ ) are also presented.

For each fish, values are means  $\pm$  S.E.M. ( $N=4$  blood samples, except for  $\dot{M}O_2$ , where  $N=5$  and measurements were made at 10, 25, 40, 50 and 70 min of infusion).

rates of approximately  $4 \mu\text{mol kg}^{-1} \text{min}^{-1}$ , but higher values are found after adding catecholamines ( $6 \mu\text{mol kg}^{-1} \text{min}^{-1}$ ) (Mommsen *et al.* 1988) or when using liver slices ( $8 \mu\text{mol kg}^{-1} \text{min}^{-1}$ ) (Morata *et al.* 1982).

One notable limitation of the validation technique used in the present study was our inability to eliminate all endogenous glucose sources. As in mammals, the liver is the most important glucose-producing organ, and it was surgically removed. The kidney is the only other source, but it had to be left intact in our experiments because nephrectomy cannot be performed in trout without seriously damaging the circulation and the surrounding tissues. However, the contribution of the kidney to total glucose production is minor compared with that of the liver, as indicated by the large differences in gluconeogenic enzyme activities between the two organs (Knox *et al.* 1980; Suarez and Mommsen, 1987). Here, glucose production by rainbow trout kidney was estimated for the first time in experiments in which no unlabelled glucose was infused in hepatectomized animals (group NR). Under these conditions, turnover rate did not fall to zero, but was maintained at  $1.1 \pm 0.1 \mu\text{mol kg}^{-1} \text{min}^{-1}$  by the kidney (Fig. 1). In experiments in which hepatic glucose production was replaced (group R), the contribution of the kidney explains why the uncorrected percentage error between measured turnover rate and known glucose entry rate from the pump was consistently positive, averaging +17 to +22% (Table 1). Furthermore, subtracting renal glucose production from the measured turnover rates in group R decreased this average error to -0.7 to -5.7% for plasma and whole-blood values, respectively (Table 1). It is important to note that the rate of renal glucose production reported here is only an estimate and may not represent *in vivo* kidney function very accurately. Measurements were carried out in anaesthetized animals in which renal gluconeogenesis was probably depressed. Alternatively, renal glucose production may have been stimulated to compensate for the decrease in circulating glucose levels resulting from the removal of the liver. Assuming that these limitations have no significant effect on our estimate, the trout kidney would be responsible for approximately 10% of total glucose production at rest, a value similar to that in mammals (Wolfe, 1992).

Contrary to expectations, our results show that differences in glucose specific activity between plasma and whole blood, if they exist, are too small to be measured and do not influence estimates of glucose flux (Tables 1, 2). Therefore, the glucose turnover rate of rainbow trout can be quantified equally well, and interchangeably, from plasma or whole-blood samples. The absence of detectable differences in specific activity is probably not due to rapid label equilibration across the red cell membrane, because glucose translocation is known to be particularly slow in this species (Tse and Young, 1990). However, our results may be explainable even if partial isotope equilibration across the membrane were taking place because intracellular glucose represents a small proportion of the total blood glucose in trout (Sephton and Driedzic, 1994). The fact that glucose concentration and glucose activity were proportionately lower in whole blood than in plasma, leaving

specific activity constant, supports this interpretation. The mean rate of glucose production reported here ( $17 \mu\text{mol kg}^{-1} \text{min}^{-1}$ ) is much higher than that measured previously in our laboratory ( $9 \mu\text{mol kg}^{-1} \text{min}^{-1}$ ) (Haman and Weber, 1996). Because the purpose of these experiments was to determine whether the specific activity of glucose was the same in whole blood and in plasma, all the data were included in our results even though some animals were clearly hyperglycaemic and not representative of normal resting trout. Removing the three animals with glucose concentrations above  $8 \text{mmol l}^{-1}$  from Table 2 (i.e. fish 3, 6 and 7) would bring mean glucose production to  $10.5 \mu\text{mol kg}^{-1} \text{min}^{-1}$ , a value equivalent to previously published rates.

In conclusion, this study provides the first experimental validation of continuous tracer infusion in fish, and it shows that this method quantifies glucose kinetics accurately from plasma and whole-blood measurements of specific activity. Consequently, the continuous infusion of labelled glucose could become a very powerful tool for future research on the hormonal regulation of fish glucose metabolism at the whole organism level.

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