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IN VIVO UTILIZATION OF GLUCOSE BY HEART AND LOCOMOTORY MUSCLES OF EXERCISING RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

TIMOTHY G. WEST, PETER G. ARTHUR, RAUL K. SUAREZ, CHRISTOPHER J. DOLL and PETER W. HOCHACHKA

Department of Zoology, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4

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Summary

In vivo rates of glucose utilization were estimated in red muscle and heart of rainbow trout using the radiolabeled glucose analogue, [^{14}C]-2-deoxyglucose ([^{14}C]-2-DG). The 2-DG 'lumped constant' (LC), representing the uptake ratio between 2-DG and glucose for each tissue, was determined *in vitro* using tissue slices. For both red muscle and heart, the LC was approximately 0.40. In addition, changes in circulatory glucose utilization induced in an isolated trout heart preparation had no effect on the LC. The LC was applied to calculations of *in vivo* rates of circulatory glucose disposal in muscle tissue of resting and swimming trout. Utilization was estimated at 0.87±0.15 and 5.31±1.04nmol g^{-1} min^{-1} in red muscle and heart of resting fish, respectively. In trout swimming at 80 % U_{crit}, glucose utilization in heart was unchanged compared to resting controls, while red muscle utilization increased by 28-fold. Estimates of the oxidative demand of heart and locomotory muscle of swimming trout indicate that glucose utilization accounted for less than 10% of the energy production in both tissues. In trout heart, *in vitro* measures of glucose flux suggest an apparent excess capacity to use circulatory glucose. Preference for other intra- and extramuscular fuels may partially explain limited glucose utilization, but it remains unclear to what degree, and under what conditions, glucose utilization may be increased *in vivo.*

Introduction

Skeletal muscle is the principal peripheral site of circulatory glucose disposal in mammals during exercise and, along with fatty acids and lactate, its uptake contributes substantially to energy provision for endurance activity (Weber, 1988). In contrast, less is known about the kinetics of glucose and its oxidative role in fish tissues. Plasma turnover and oxidation are slower in most teleosts than in mammals (Van den Thillart, 1986; Garin *et al.* 1987; Machado *et al.* 1989) and long-term perturbations like starvation/migration suggest limited reliance on plasma glucose as an oxidizable substrate (Mommsen *et al.* 1980; Black and Love, 1986). *In vivo* measurements of the circulatory turnover of

Key words: rainbow trout, exercise, heart, red muscle, glucose, 2-deoxyglucose, *Oncorhynchus mykiss.*

glucose, while undoubtedly indicative of overall steady-state glucose disposal, are unsatisfactory for estimating utilization by specific fish tissues.

Fuel use in specific tissues is implied from *in vitro* measurement of maximal enzyme activity (V_{max}) and mitochondrial oxidation rates, both of which provide maximal capacities to utilize particular substrates. It is understood that these *in vitro* estimates of fuel utilization may not resemble flux capacity in tissues of the whole animal (Moyes *et al.* 1990; Wright and Albe, 1990), but in comparative analyses such observations have provided clues about which carbon sources may be used *in vivo* under conditions of maximal aerobic demand. In endotherms for instance, cardiac enzyme profiles suggest that maximal aerobic metabolism is supported by fat utilization to a greater extent in animals adapted for aerobic endurance than in more sedentary animals (Driedzic *et al.* 1987). Mammalian models of substrate preference based on *in vivo* metabolite kinetics also suggest a greater reliance on fat in more aerobic species during exercise (Weber, 1992). Unfortunately, similar enzyme analyses of fish muscle from different species suggest either that highly aerobic tissues rely more on fat (Moyes *et al.* 1992*a*) or that there is no association between fat-utilizing capacity and increasing tissue aerobic capacity (Sidell *et al.* 1987). Within fish species, the adaptive effects of endurance training include small shifts in activity of some key muscle and heart enzymes (Farrell *et al.* 1991; Johnston and Moon, 1980), but modified substrate flux through a single pathway is usually not implied.

Based on fish red muscle and heart enzymes and observations with isolated hearts, there seems to be enough capacity for oxidative muscle to utilize glucose for aerobic contractions *in vitro* (Crabtree and Newsholme, 1972; Lanctin *et al.* 1980; Driedzic and Hart, 1984). However, verification of the contribution of glucose to tissue oxidation *in vivo* is more difficult because of the complexities of substrate storage, mobilization, transport and pathway interaction processes in the whole animal (see Weber, 1988, 1992). In addition, exercise intensity is an important consideration when evaluating muscle fuel use *in vivo* since fuel types or stores utilized at high aerobic intensites may be different from that at low intensity. In salmonids, swimming speeds of 80–85% of fatigue or critical speed (U_{crit}) can be maintained indefinitely and are expected to place close to maximum steady-state aerobic demands on oxidative muscle. In the present study, the glucose analogue 2-deoxyglucose (2-DG) was used to assess *in vivo* glucose uptake in rainbow trout (*Oncorhynchus mykiss*) red muscle, heart and white muscle during this type of intense aerobic exercise. Direct determinations of glucose utilization in specific tissues give us the opportunity to test the degree to which the circulatory delivery of glucose supports estimated maximal rates of tissue oxidative metabolism *in vivo.*

Materials and methods

Animals

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] of both sexes (300–1200g) were purchased from local suppliers. Smaller fish (<600g) were used for *in vitro* isolated heart studies, conducted at the University of British Columbia. *In vivo* glucose utilization was

determined in larger fish at the Bamfield Marine Station, Bamfield, BC. All fish were held in fresh water (15–17˚C) and were fed to satiation twice weekly.

2-DG and the lumped constant

Glucose utilization was calculated from the tissue content (disintegrations per minute, disintsmin^{-1}) of [^{14}C]-2-DG phosphate (2-DGP) and areas under plasma [^{14}C]-2-DG washout curves according to the equation of Ferré *et al.* (1985):

$$\text{Utilization } (\mu\text{molmin}^{-1})_t = \frac{\text{Tissuedisintsmin}^{-1}\ \text{2-DG}}{\text{LC}\int_0^t (\text{disintsmin}^{-1}\ \text{2-DG}/\mu\text{molglucose})\text{d}t}.$$

Utilization was corrected for tissue mass and is presented as nanomoles per gram of muscle per minute. The term LC is a dimensionless 'lumped' constant, which is necessary to correct for differences between glucose and 2-DG phosphorylation rates *in vivo* (see Sokoloff, 1983). Simultaneous determinations of glucose and 2-DG uptake were made *in vitro* from rates of $^{3}H_2O$ (Ashcroft *et al.* 1972) and [^{14}C]-2-DGP formed from [5-^{3}H]glucose and [^{14}C]-2-DG, respectively. The LC was calculated using the equation defined by Ferré *et al.* (1985):

$$\text{LC} = \frac{\text{tissue2-DGP/2-DG in medium}}{\text{glucose utilized/glucose in medium}}.$$

Tissue 2-DGP and 2-DG in the incubation or perfusion medium are presented as disintsmin^{-1} ^{14}C, while the glucose in the medium and total glucose utilized during the tissue incubation or heart perfusion period are in units of nmol.

LC determination

Tissue slices

Fish were netted from the holding tank and killed quickly with a sharp blow on the head. A rectangular incision that encompassed the lateral line was made in the musculature between the dorsal and adipose fins. The skin was peeled back to expose the muscle and the area was flooded with ice-cold saline (containing in mmol l^{-1}: 127 NaCl, 4.9 KCl, 1.0 $CaCl_2$, 3.7 $NaHCO_3$, 1.2 $MgSO_4$, 2.9 NaH_2PO_4, 1.2 KH_2PO_4, 11.5 Na_2HPO_4; pH7.4). A strip of skeletal muscle was dissected free by gently maneuvering a razor blade through the exposed muscle along the long axis of the fish. The strips were trimmed of white muscle and the red muscle slices (0.5–1.0cm^2, 1–2mm thick) were transferred to a vessel of fresh saline (100ml, bubbled with 99% O_2:1% CO_2) containing 5 mmol l^{-1} glucose. The vessel was placed in a shaking water bath (15±1˚C) and the tissues were preincubated for 20min. Ventricular strips were similarly prepared from hearts that had been immersed in saline and cut in half from the base to the apex. Strips were made by slicing parallel to the freshly exposed surface of spongy myocardium. Two to four slices of each tissue were prepared from every fish.

Following the preincubation, individual slices were transferred to scintillation vials

containing 2.5ml of fresh saline which contained [5-^{3}H]glucose (11.1kBq) and [^{14}C]-2-deoxyglucose (55.6kBq) (Amersham) and 5mmol l^{-1} unlabelled glucose. The incubation vials were sealed with rubber plugs through which plastic access tubes were affixed for gentle gas infusion. The vials were positioned in the shaker and incubations were run for no longer than 45min. Preliminary trials with [5-^{3}H]glucose indicated that glucose utilization (^3H_2O production) remained constant over this period. Two to three blanks containing only saline and radioisotope were performed with every run to assess background levels of ^3H_2O. Specific activity (SA) of [5-^{3}H]glucose and (disintsmin^{-1}) [^{14}C]-2-DG in the medium were determined by counting 200 μl samples mixed with 10ml of ACS II (Amersham) on an LKB Rackbeta scintillation counter.

Samples of saline (200 μl) retrieved from the vials following incubation were placed on columns of Dowex-1-borate (1ml bed volume) to separate glucose from ^3H_2O produced (Hammerstedt, 1973). The samples were allowed to percolate into the column bed by gravity flow before washing the resin with water (5×1 ml washes). The washes were collected separately into 20ml glass scintillation vials and assayed for (disintsmin^{-1}) ^{3}H. Total (disintsmin^{-1}) ^{3}H for each sample was corrected for background (disintsmin^{-1}) and adjusted to the total incubation volume to calculate ^3H_2O accumulated. Levels of [^{14}C]-2-DGP were determined in tissues that had been homogenized (3×30s bursts with an Ultra-turrax tissue homogenizer) immediately post-incubation in 1.5ml of ice-cold 7% perchloric acid (PCA). Homogenates were centrifuged (10000revsmin^{-1} for 10min) and the supernatants were neutralized with 3 mol l^{-1} K_2CO_3 in 0.5mol l^{-1} triethanolamine. A sample of neutralized supernatant was counted to determine total [^{14}C] (2-DG+2-DGP) present. Phosphorylated DG was removed from an equal volume either by precipitation with 0.3mol l^{-1} $Ba(OH)_2$ and 0.3mol l^{-1} $ZnSO_4$ (sample:reagents, 1:1.5:1.5) or by anion exchange on a column of DEAE–Sephadex A-125 (1ml bed volume) equilibrated with 20mmol l^{-1} imidazole chloride, pH7.2. When adjusted to the total neutralized supernatant volume, the difference in (disintsmin^{-1}) between the two samples was the (disintsmin^{-1}) of tissue 2-DGP. Lumped constants were calculated from the amount of glucose utilized and 2-DGP produced using the equation presented earlier.

Isolated trout hearts

The effect of glucose utilization rate on the LC was investigated using isolated perfused trout hearts. Isolation and cannulation procedures were as outlined by Farrell *et al.* (1989). Isolated hearts were suspended in a saline bath and air-equilibrated perfusate was introduced from water-jacketed reservoirs (15°C). Output flow from the ventral aorta was monitored continuously as outlined by Graham and Farrell (1989) and oxygen consumption was calculated from the difference in oxygen content, measured with a Radiometer electrode and meter, of perfusate entering and leaving the heart. Additionally, an inlet branch was placed at the level of the preload pressure head for infusing sodium cyanide (NaCN). Electrodes attached to the input and output cannulae allowed for the electrical control of heart rate.

Changes in circulatory glucose utilization were invoked by manipulation of heart rate

and input and output pressure heads, to reduce or increase cardiac power output, and by infusion of NaCN (1mmol l^{-1}) at low power output. Power output, in mW g^{-1} ventriclemass (mW g^{-1}), was calculated according to Graham and Farrell (1989). Isotope perfusions were initiated only after output flow recordings became steady under the desired conditions with 'unlabeled perfusate'. Low-power perfusions with and without NaCN were run for 40–60min, while the higher-power output perfusions were stopped after 15–20min.

Glucose utilization and 2-DGP formation were determined in hearts with single-pass perfusions of [5-^{3}H]glucose (3.7kBq) and [^{14}C]-2-DG (0.79kBq). Following perfusion, hearts were removed from the saline bath, blotted, and immersed in pre-weighed vials of 7% PCA (2ml). Tissues were homogenized, then neutralized and assayed for [^{14}C]-2-DGP. Perfusate collected at the afterload outlet was assayed for lactate according to Bergmeyer (1985) and for $^{3}H_2O$ by vacuum distillation. For the latter, a flask containing outflow perfusate (3ml) was attached to a micro condenser with an 8cm still head, then immersed in a water bath (60–65°C) where the perfusate was brought to boiling under constant vacuum (approximately 80kPa). All of the distillate was collected and samples (1ml) were counted for ^{3}H and ^{14}C. The lack of detectable ^{14}C above background level indicated no contamination of raw perfusate in the distillate. Perfusion times and flow rates from the afterload outlet were monitored to calculate cardiac output and to determine total $^{3}H_2O$ produced and total amounts of isotope and substrate to pass each heart to calculate LCs.

Glucose utilization in swimming trout

Rainbow trout were anesthetized (MS-222, 0.5 g l^{-1} buffered with $NaHCO_3$, 1 g l^{-1}) and placed in a standard fish operating sling. Oxygenated water containing a light level of buffered MS-222 (0.1 g l^{-1}) was circulated over the gills, and dorsal aortic cannulae (PE-50, Clay Adams) were implanted. Fish recovered from anesthesia (12–18h) in dark plastic tubes (15cm diameter) which were submerged in a flow-through water (15–17°C) reservoir at the downstream end of a Brett-type swim tunnel. Once recovered, each fish was handled separately. A fish was guided to the entrance of the tunnel and each swam of its own accord from the recovery tube into the swim space. Water speed was adjusted to about 0.5bodylengths(BL) s^{-1} (15–20cm s^{-1}) and the fish was left for approximately 10 h before the experiment was started. Fish used to determine resting rates of glucose utilization recovered from anesthesia in black Perspex boxes, supplied with fresh flow-through water (15°C).

A high sustainable swimming speed was found by increasing water velocity every 15min by 20cm s^{-1} increments until the fish began to demonstrate 'burst and glide' swimming. Water velocity was then reduced until the fish held its position against the flow of water. This velocity was maintained for 1h prior to isotope injection.

Bolus injections consisted of [^{14}C]-2-DG (185kBq) that had been pre-dried under nitrogen and reconstituted in 250 μl of Cortland saline (Wolf, 1963). A sample (10 μl) was taken to determine the (disintsmin $^{-1}$) of ^{14}C injected and the remaining volume was injected into the dorsal aorta *via* the cannula. The cannula was flushed with two volumes of saline. Blood (150 μl) was drawn through the same cannula at 1, 2, 3, 4, 5, 10, 20, 40,

60, 90 and 120min post-injection. Neutralized PCA (1:1, 7% PCA:sample) extracts were assayed for (disintsmin^{-1}) ^{14}C in glucose and plasma glucose concentration (Sigma glucose assay kit). Plasma washout kinetics were determined using a curve-stripping program (JANA; Statistical Consultants, Lexington, KY) and areas under curves were determined (MATHCAD; Mathsoft Inc., Cambridge, MA). When blood sampling had been completed in each fish, the spinal cord was severed just posterior to the head and tissues were removed and freeze-clamped between Wollenberger tongs pre-cooled on dry ice. A double-bladed cleaver was used to cut a steak rapidly from the musculature immediately posterior to the dorsal fin. The heart was removed and the ventricle was quickly cleared of blood and freeze-clamped. The delay between death of the fish and freeze-clamping of tissues was less than 20s. Tissues were stored at −80°C until assayed for the content of 2-DGP.

Statistics

The significance (P<0.05) of differences between mean (± S.E.) LCs and glucose utilization rates was determined using unpaired *t*-tests or analysis of variance (ANOVA) with Tukey's multiple-comparison test.

Results

Tissue LCs and the effect of glycolytic rate

Table 1 lists LCs for red muscle and heart ventricle from tissue incubations. The LCs calculated were quite variable, although most values were between 0.2 and 0.6. Box plots in Table 1 provide an indication of the range and clustering of data points around the median LC for each tissue. LCs calculated for red muscle and heart were not significantly different.

The isolated trout heart preparation provided a means for assessing whether changes in perfusate glucose uptake affected the 2-DG LC (Table 2). The use of perfusate glucose

Table 1. *Lumped constants (LC) calculated from* in vitro *incubations of trout red muscle slices and heart ventricular strips with [^{14}C]-2-DG and [5-^{3}H]glucose*

	Red muscle (N=15)	Heart (N=18)
Tissue 2-DGP (disintsmin^{-1})	760±160	1130±240
Glucose utilized (nmol)	48.1±9.7	76.2±13.8
LC	0.43±0.06	0.38±0.05
	0 0.2 0.4 0.6 0.8 1.0 LC	0 0.2 0.4 0.6 0.8 1.0 LC

Total [^{14}C]-2-DG in 2.5 ml of saline averaged 459800±57800disintsmin^{-1} and 421900± 48300(disintsmin^{-1}) for the red muscle and heart incubations, respectively.

Box plots are presented for red muscle and heart LCs with data outside the fifth and ninety-fifth percentiles marked ■.

Table 2. *The effects of cardiac power output and NaCN (1mmol l^{-1}) perfusion on glucose utilization rate and the 2-DG lumped constant (LC)*

	Cardiac output (ml kg^{-1} min^{-1})	Power output (mW g^{-1})	Oxygen consumption (nmol g^{-1} min^{-1})	Lactate production (nmol g^{-1} min^{-1})	Glucose utilization (nmol g^{-1} min^{-1})	LC
Perfusate 5mmol l^{-1} glucose						
Low work (N=4)	4.5±0.8	0.07±0.02	212±34	49±13	29±6	0.41±0.03
High work (N=4)	24.8±2.0	1.69±0.09	1468±139	118±28[a]	121±22[a]	0.41±0.06
Cyanide (N=6)	6.6±1.3	0.10±0.02	–	1012±129[a,d]	253±57[a,b]	0.40±0.03
Glucose-free perfusion					(disints g^{-1})	
High work (N=3)	22.7±2.3	1.22±0.11	1296±178*	182±59*	15400±500	0.39±0.04
Cyanide (N=3)	5.8±0.7	0.10±0.02	–	791±72*	43500±2400[c]	0.41±0.03

Cardiac output was normalized per kilogram body mass, while power output was normalized per gram ventricle mass.

Oxygen consumption and lactate production per gram of ventricle mass were also determined.

[a]Significantly different from low work load (P<0.05).

[b]Not significantly different from glucose-perfused high work load (P=0.17).

[c]Significantly different from glucose-free, high work load (P<0.001).

[d]Significantly different from glucose-perfused high work load (P<0.01).

*Not significantly different from oxygen and lactate in the corresponding glucose-perfused hearts.

ranged from trace levels in the substrate-free perfusions to 250nmol g^{-1} min^{-1} in the NaCN perfusions. However, there was no effect of utilization rate on the LCs calculated from the various perfusions.

Interestingly, power output of the NaCN hearts spanned a narrow subphysiological range (0.04–0.17mW g^{-1}), yet potent enhancement of perfusate glucose utilization occurred within this group in response to small increases in power output (Fig. 1). The variability is probably the reason for the glucose utilization not being significantly different from that of the high-work-rate hearts (Table 2). We did not attempt experiments to confirm that power output was in fact the principal factor affecting glucose utilization during NaCN perfusions. As presented in Fig. 1, the regression of glucose utilization against cardiac power output is y=2003x+49, r^2=0.70. Individual LCs for these hearts are also depicted, but there is no indication that the use of glucose and the accumulation of [^{14}C]-2-DGP changed disproportionately with increased perfusate glucose utilization.

All *in vivo* red muscle and heart glucose utilization rates were calculated using the average LC determined from these *in vitro* experiments (LC=0.40).

Glucose utilization in swimming trout

Plasma profiles of disintsmin^{-1} [^{14}C]-2-DG μmol^{-1} glucose and of glucose

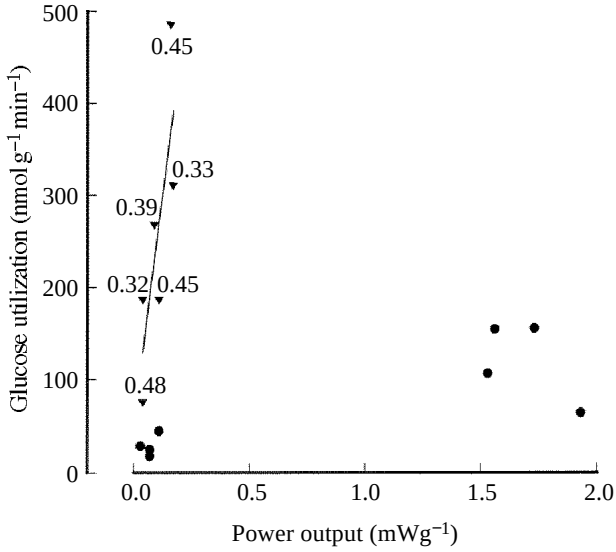

Fig. 1. Glucose utilization in relation to power output in normoxic (circles) and NaCN-treated (triangles) hearts. Lumped constants calculated for individual NaCN perfusions are also depicted. The line shows the regression of glucose utilization on power output in NaCN-treated hearts: y=2003x+49, r^2=0.70.

concentration from exercised and unexercised trout are shown in Fig. 2. Small fluctuations in plasma glucose level were typical of individual resting and exercising fish. Dynamic steady state was assumed if during the sampling period the coefficient of variation (CV) for the mean plasma glucose level was less than 15%. The ^{14}C activity washout curves were all fitted to equations described by two exponential terms.

Table 3 lists data for individual resting and swimming trout. Plasma glucose ranged from 2 to 5mmol l^{-1} among both control and exercised fish. Relative swimming speed was very reproducible with the exercise protocol used. The average relative speed, in BL s^{-1}, corresponded to an absolute velocity of 71.5±0.78cm s^{-1}. The *in vivo* rate of muscle glucose utilization in unexercised trout was highest in heart ventricle, being about sixfold higher than in red muscle. However, during sustained steady-state exercise, cardiac glucose utilization did not change significantly compared to the values observed in resting fish. Conversely, red muscle utilization increased from less than 1 nmol g^{-1} min^{-1} at rest to about 21nmol g^{-1} min^{-1}, representing a 24-fold change in the use of circulatory glucose. White muscle utilization, estimated assuming that the LC was 0.4 for this tissue, averaged less than 0.5nmol g^{-1} min^{-1} at rest and during exercise.

Discussion

The 2-DG lumped constant

The LC as described by Sokoloff (1983) for normal brain tissue of mammals differs according to the species studied (ranging from 0.3 to 0.6), but seems to be invariable within a species when determined under a variety of physiological conditions. Similarly,

the LC calculated for rat skeletal muscle is influenced minimally by treatment-induced changes in glucose utilization (Mészáros *et al.* 1987; Furhler *et al.* 1991). Despite the apparent stability of the LC with respect to tissue treatment, it is still difficult to generalize, even within a species. The LC chosen as characteristic for rat skeletal muscle in different studies ranges from 0.4 to 1.0 (Ferré *et al.* 1985; Furhler *et al.* 1991; Mészáros

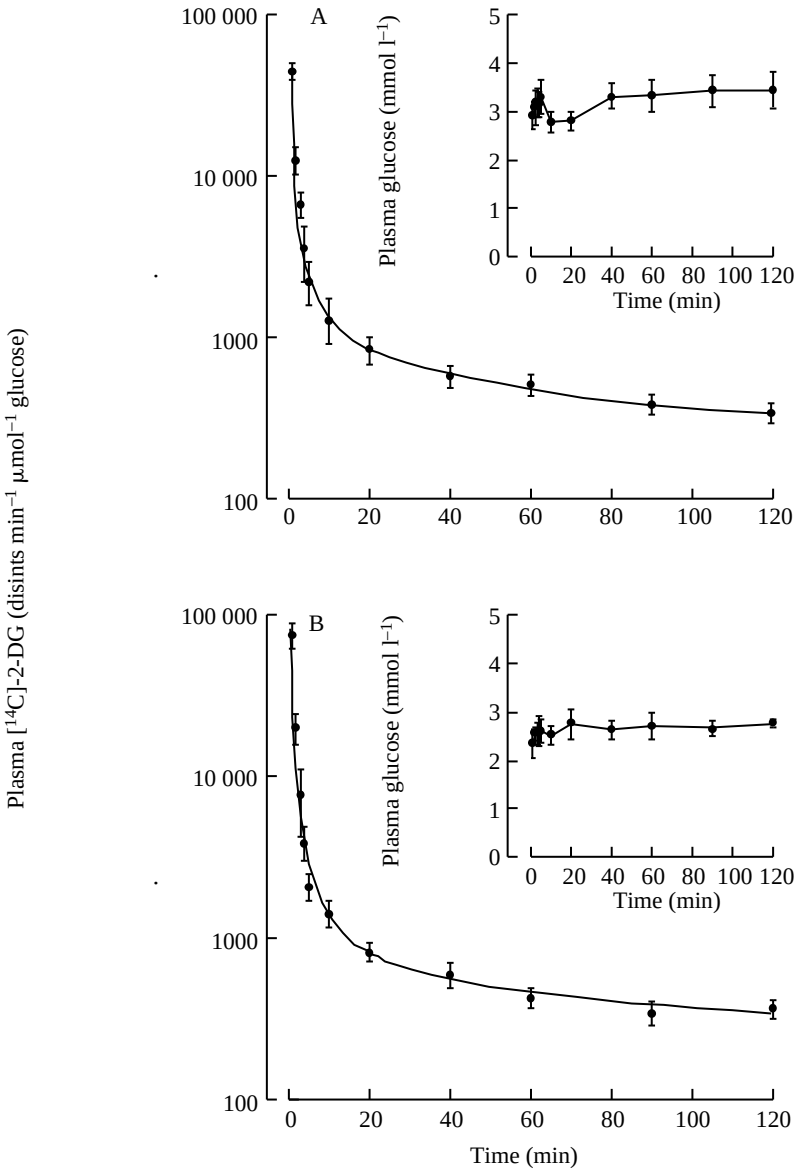

Fig. 2. Plasma [^{14}C]-2-DG washout curves for resting (A) and exercising (B) rainbow trout. Inset axes show profiles of plasma glucose concentration during isotope washout period. Values are mean ± S.E. (N=9, resting; N=7, exercised).

Table 3. In vivo *glucose utilization in red muscle and heart of resting and swimming rainbow trout*

				Glucose utilization		
Trout number	Body mass (g)	Swimming speed ($BL\ s^{-1}$)	Plasma [glucose] ($mmol\ l^{-1}$)	Red muscle ($nmol\ g^{-1}\ min^{-1}$)	Heart ($nmol\ g^{-1}\ min^{-1}$)	White muscle ($nmol\ g^{-1}\ min^{-1}$)
Resting						
1	983	–	2.32±0.10	–	6.63	–
2	650	–	2.77±0.09	1.39	6.65	0.20
3	875	–	2.83±0.10	0.83	–	0.40
4	601	–	2.51±0.07	1.34	8.91	1.04
5	944	–	3.69±0.14	0.37	8.85	0.20
6	1120	–	4.61±0.20	1.11	2.50	0.29
7	607	–	2.49±0.10	0.20	1.78	0.20
8	675	–	3.93±0.15	0.75	1.94	0.23
9	776	–	3.29±0.11	0.99	5.22	0.31
Mean±S.E.	803±62			0.87±0.15	5.31±1.04	0.36±0.10
Exercised						
1	1066	1.68	2.11±0.17	18.91	1.81	0.60
2	898	1.61	2.32±0.08	4.93	1.85	0.40
3	1079	1.62	2.43±0.07	30.93	6.31	0.96
4	1096	1.67	2.35±0.10	11.17	4.46	0.12
5	798	1.76	2.71±0.09	48.44	13.05	0.83
6	805	1.76	3.52±0.14	12.96	0.90	0.20
7	740	1.66	2.78±0.11	18.63	4.58	0.22
Mean±S.E.	926±57	1.68±0.02		20.85±5.52*	4.62±1.60	0.48±0.12

An *in vivo* 2-deoxyglucose lumped constant of 0.40 was used for calculations of tissue glucose uptake.
*Significantly different from resting control, $P<0.001$.

et al. 1987), which perhaps indicates that attributes of individual muscle groups, such as fiber type distribution and glucose transporter type or density, can affect LC determination. In addition, mammalian tissues other than brain and skeletal muscle may be more sensitive to changes in experimental conditions. Indeed, the LC for rabbit myocardium averages 0.6, but extremes of flow rate and contraction frequency, albeit beyond the range of physiological relevance, were shown to have significant effects on the LC in perfusions of isolated interventricular septa (Krivokapich *et al.* 1987). The use of 2-DG methodology will clearly produce fewer ambiguities when extrapolating LCs to the whole animal if *in vitro* determinations are made in the tissues of interest in concert with the *in vivo* experimental design.

One point to make about the *in vitro* analysis of trout tissues is that the LC determined with isolated heart preparations was generally more reproducible than with the slices. Despite the fact that slices were preincubated for 20min, the broad range of LCs may in part reflect a varying degree of cellular damage since disruption of the plasma membrane could have altered the importance of a possible transport component of the LC.

Determinations of the LC with the more intact isolated heart preparation were always between 0.3 and 0.6 (see Fig. 1, for example). Nevertheless, the LC values produced from the two protocols are similar and the indication is that an LC of 0.4 is a reasonable application to both heart and red muscle calculations of *in vivo* glucose utilization.

The additional utility of the isolated trout heart was in the determination of LCs during variable rates of perfusate glucose uptake. The high power output hearts used in the present study matched basal power output levels set normally in isolated and *in situ* trout hearts (1.0–2.0mW g^{-1}, Farrell *et al.* 1989; Milligan and Farrell, 1991). Maximal work loads in our isolated preparations were not stable long enough to coordinate steady-state cardiac variables reliably with isotope perfusions. For the same reason, the NaCN-perfused hearts had to be set at subphysiological work levels. However, the advantage of this protocol was that a broad range of glucose utilization could be induced. The results indicate that, with stimulation of glycolytic rate by anoxia or changes in power output, the rate of 2-DG uptake and phosphorylation should remain a constant proportion of glucose utilization. Furthermore, both the aerobic and NaCN perfusions lacking unlabeled glucose show that, even if cardiac function is dominated by endogenous fuel utilization, the LC calculated from tracer uptake is unaffected.

Glucose utilization in vivo *during aerobic swimming*

Red muscle glucose utilization

For rainbow trout of the size used in this study, an average steady-state swimming speed of 1.7bodylengths s^{-1} corresponds to approximately 80 % U_{crit} (Kiceniuk and Jones, 1977). From rest to this high level of sustained swimming, rates of 2-DGP accumulation indicate that glucose utilization increased by 28-fold in the lateral red muscle. What proportion of the *in vivo* energy production in red muscle is accounted for by glucose?

The red muscle of trout constitutes about 5–10% of the total muscle mass (Webb, 1971). A 1kg trout, possessing 30–60g of red muscle, consumes about 25 μmol O_2 kg^{-1} min^{-1} at rest and increases this by about sevenfold when swimming aerobically at close to 80 % U_{crit} (Kiceniuk and Jones, 1977). Swimming at this intensity results mainly from the power output of the active red muscles (reviewed by Jones, 1982) and, given the concomitant redistribution of blood flow to these fibers (Randall and Daxboeck, 1982), it is likely that the cost of swimming, above routine maintenance costs, is associated largely with the increased aerobic demand of this muscle mass. Assuming that glucose is fully oxidized in red muscle (6nmol O_2 per nmol glucose), it is then apparent that, given an uptake rate of 21nmolglucose g^{-1} min^{-1}, circulatory glucose is maximally 5% of the total red muscle substrate oxidation *in vivo*. It is an oversimplification to attribute 100% of the cost of swimming to red muscle and we obviously underestimate red muscle glucose oxidation *in vivo* by not being able to account directly for other exercise-induced changes in whole-body metabolic costs. However, combined oxidation in other muscle types, for example heart, because of its small mass, and white muscle, because of its low aerobic demand (Moyes *et al.* 1992*b*), is probably minor compared to red muscle oxidative demand during exercise. In addition, osmoregulatory costs do not appear to change with increased aerobic exercise, although

this is probably species-dependent (Pérez-Pinzón and Lutz, 1991). Activity-related changes in costs of ion regulation and ventilation are difficult to quantify *in vivo*, but it is apparent that if such costs diverted half of the whole-animal oxygen consumption at 80 % U_{crit}, then our estimation of glucose oxidation in red muscle would change only slightly, to 10% of total substrate oxidation.

It is interesting that a large increase in glucose utilization in exercising red muscle (28-fold above resting) is seemingly unimportant to red muscle energetics. It may be that a major determinant of red muscle uptake of circulatory glucose is simply the redistribution of blood flow during exercise. Most of the cardiac output is delivered to the active red fibers in swimming trout (Randall and Daxboeck, 1982) and it is evident that muscle glucose uptake correlates positively with blood flow (Chaliss *et al.* 1986). Although the metabolic demand of red muscle increases, the use of glucose might be limited by the availability of plasma glucose or by potent glycolytic inhibition brought on by the use of fatty acids in highly oxidative muscle (Kobayashi and Neely, 1979). As a result, the potential contribution of glucose to overall red muscle substrate oxidation would be small, as calculated, despite a several-fold increase in uptake over resting rates.

It may be that lipid-based substrates are utilized for sustained aerobic swimming in trout. This possibility is strengthened by the likelihood that carbohydrates from all *in vivo* sources are a small component of red muscle oxidation. Blood-borne lactate is a minor fuel in trout during an endurance-type swim (Weber, 1991) and red muscle glycogen, although possibly important during submaximal swimming in some species (Johnston and Goldspink, 1973), is available in limited supply in trout (20 μmol g^{-1}; T. G. West, unpublished observation) and cannot solely furnish fuel for intense aerobic swimming. The use of fat fuels has not been quantified *in vivo*, but the potential for reliance on fatty acids in teleosts is suggested at least indirectly from intramuscular storage and mobilization capacities, from triglyceride depletion studies in migrating/starving species and from *in vitro* measurements of flux capacity (Black and Love, 1986; Mommsen *et al.* 1980; Sheridan, 1988; Moyes *et al.* 1992*a*).

White muscle glucose uptake during exercise

Recruitment of white fibers in rainbow trout is initiated at swimming speeds beyond about 80 % U_{crit} (Webb, 1971). However, the lack of glucose utilization in the epaxial muscle is not unexpected since circulatory substrates are probably of minimal importance in this tissue during exercise. A reduction in blood flow to the white fibers at 80 % U_{crit} (Randall and Daxboeck, 1982) is consistent with the view that any recruitment is fueled mostly by glycogenolysis. About 95% of the blood flow to the total mosaic musculature of rainbow trout at 80 % U_{crit} is probably received by the small proportion of 'red' fibers dispersed throughout the white muscle mass (Randall and Daxboeck, 1982). Glucose kinetics in these deeper red fibers might well behave as in the lateral red muscle during exercise, but changes would probably always be masked by glucose uptake, or a lack of glucose uptake, in the larger white fiber population.

Disposal of glucose in muscle tissue

Glucose uptake in the total red muscle and heart masses (about 60g and 1 g

respectively) of a resting 1kg trout requires a rate of circulatory glucose delivery of about $70 nmol min^{-1}$. The total white muscle mass (about $660 g kg^{-1}$ bodymass) is estimated to use considerably more of the circulatory turnover of glucose at rest (about $300 nmol min^{-1}$, based on utilization rates in Table 3). However, we cannot be certain what proportion of resting whole-body glucose disposal is accounted for by combined utilization in these tissues. Estimates of glucose turnover range from 1 to $10 \mu mol min^{-1} kg^{-1}$ in most teleosts (Dunn and Hochachka, 1987; Garin *et al.* 1987). Uptake in muscle tissue at rest would therefore seem to account for 3–30% of circulatory glucose turnover, suggesting that the bulk of disposal in plasma steady-state conditions occurs in other tissues. In resting trout at least, possible preference for fatty acids in oxidative muscle, and possibly white muscle (Moyes *et al.* 1992*b*), may spare circulatory glucose for tissues like brain. An evaluation of the significance of red muscle as a site of glucose disposal during exercise awaits reliable determinations of steady-state glucose turnover in swimming fish. Whether glucose is used preferentially by other trout tissues or whether glucose becomes quantitatively more significant as a red muscle substrate in species with limited or exhausted lipid stores should also be investigated.

Cardiac glucose utilization

In vivo glucose utilization in trout heart was not affected by increased aerobic swimming. Before estimating the contribution of glucose to cardiac energy production, it should be noted that cardiac power output in isolated trout hearts varies from 1 to $2 mW g^{-1}$ ventricle mass to maximally 6–$8 mW g^{-1}$ (see Milligan and Farrell, 1991). Interpreting this as an *in vivo* scope for cardiac power output means that realistic limits of oxygen consumption range from 1 (rest) to 4 (exercise) $\mu mol g^{-1} min^{-1}$ (assuming a cardiac efficiency of 20%, Graham and Farrell, 1989, and a caloric equivalent of O_2 of about $20.1 J ml^{-1}$). Oxygen consumption by isolated hearts in this study agrees with the lower end of this estimated *in vivo* range (see Table 2). An *in vivo* rate of glucose utilization of approximately $5 nmol g^{-1} min^{-1}$ indicates that circulatory glucose accounted for about 6% of the expected cardiac oxygen uptake at rest and less than 1% during exercise.

As with red muscle metabolism, there are still some inadequacies in our understanding of the importance of various circulatory and endogenous fuels in fish hearts. Evidence that fat is utilized in the absence of any glycolytic flux comes from *in vitro* observations that iodoacetate-treated hearts remain functional when provided with palmitate in the perfusate (Driedzic and Hart, 1984) and possibly *via* endogenous triglyceride mobilization (see Milligan and Farrell, 1991). However, the effects of fatty acid utilization on glycolytic flux in fish hearts is not known. It may be that the trout heart is similar to mammalian heart models in which fatty acids restrict glucose oxidation, possibly through combined effects on transport and glycolytic enzymes, and enable sustained cardiac function over a broad work range (Kobayashi and Neely, 1979; Saddik and Lopaschuk, 1991). This agrees with calculations of low rates of glucose oxidation *in vivo* and with observations that glucose in the absence of other substrates accounts for up to 50% of the oxygen consumption of isolated trout hearts operating at 1–$2 mW g^{-1}$ (calculated from Table 2 values). Endogenous fuel stores presumably account for a

substantial portion of the oxygen consumed in trout hearts, but quantification of the specific role of myocardial glycogen and triglyceride in relation to work intensity requires more study.

Myocardial fuel utilization *in vivo* could also switch from fat preference during low cardiac work to lactate during elevated aerobic activity, as speculated for skipjack tuna *Katsuwonus pelamis* (Moyes *et al.* 1992*a*). High concentrations (10mmol l^{-1}) of circulatory lactate can serve as the sole oxidative substrate in isolated trout hearts over a range of power output (Milligan and Farrell, 1991). Indeed, even at concentrations observed in plasma of trout performing endurance-type exercise (2–3mmol l^{-1}; Weber, 1991), lactate will inhibit cardiac glucose oxidation *in vitro* (Lanctin *et al.* 1980) and can partially alleviate trends of diminishing performance seen in glycolytically inhibited hearts (Driedzic and Hart, 1984). While it is doubtful that lactate is the preferred fuel for whole-body metabolism in trout (Weber, 1991), the doubling of *in vivo* lactate turnover from rest to 85 % U_{crit} (Weber, 1991) may be pertinent to the oxidative needs of the relatively small cardiac tissue mass. Either case of a fat-to-lactate transition in relation to increased myocardial energy demand or of an overall dependence on fatty acids is compatible with a low rate of glucose oxidation *in vivo* and prompts the generalization that cardiac glucose metabolism in teleosts is directed mainly towards biosynthesis of storage substrates (glycogen and triglyceride), with a potential oxidative role only during periods of fatty acid limitation.

Maximum myocardial glucose flux

Measurements of trout heart hexokinase *in vitro* indicate a potential glucose flux of up to 8300nmol g^{-1} min^{-1} (see Driedzic *et al.* 1987), more than 1000 times greater than observed *in vivo* fluxes. It is very likely, however, that actual maximal *in vivo* fluxes are lower since cellular substrate concentrations are usually less than saturating and possible structural roles for enzymes may influence their availability for maximal flux compared to *in vitro* estimates (Wright and Albe, 1990). It may be that the peak glucose utilization seen in the NaCN heart perfusions (i.e. approximately 500nmol g^{-1} min^{-1}) more closely resembles flux capacity *in vivo*. What is surprising is that *in vivo* rates of glucose utilization are so low (approximately 5 nmol g^{-1} min^{-1}) – considerably lower, in fact, than even rates of uptake in normoxic isolated hearts (30–120nmol g^{-1} min^{-1}). Seemingly, both endogenous (e.g. preference for intracellular fat) and circulatory (hormone effects and circulatory fuel supply) factors contribute to nearly complete suppression of glucose utilization in normal post-absorptive trout. From another perspective, however, the *in vitro* results suggest at least the potential to expand myocardial reliance on glucose *in vivo*. It is relevant to ask under what conditions the apparent capacity for glucose flux might be approached in the whole animal.

The trout heart during exercise clearly does not require glucose flux of the magnitude seen *in vitro*, although it is not known how kinetics change in salmonids when fat fuels become limited. A more suitable *in vivo* analogy to the NaCN perfusions is probably hypoxia, in which reduced oxygen availability may be compensated for by elevated glycolytic flux. In sea raven hearts, power output remains steady through the early stages of progressive hypoxia even though oxygen consumption declines steadily (Farrell *et al.*

1985). Cardiac performance may be protected to some extent by glycolytic stimulation until the oxygen level falls below a critical point. The responses of trout heart to hypoxia are less clear, although there is an indication that performance drops off gradually with progressive hypoxia (Farrell *et al.* 1989) and this roughly coincides with a drop in whole-body oxygen consumption (see Boutilier *et al.* 1988). A decline in heart metabolism in this case may mean that the heart is able to function aerobically and shows enhanced glycolytic activity only at very low arterial oxygen levels. Support for this alternative possibility comes from whole-animal responses to hypoxia, in which metabolic depression occurs earlier in a hypoxic challenge than does stimulation of glycolysis (Boutilier *et al.* 1988). In any case, eventual increases in cardiac glycolytic flux during progressive hypoxia might be one instance where *in vivo* flux accounts for more of the apparent excess glycolytic capacity seen in *in vitro.*

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