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IMPLICATIONS OF HYPERGLYCEMIA FOR POST-EXERCISE RESYNTHESIS OF GLYCOGEN IN TROUT SKELETAL MUSCLE

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Summary

Rates of whole-body glucose turnover and muscle-specific glucose utilization were determined in rainbow trout (*Oncorhynchus mykiss*) at rest and at intervals during recovery from burst swimming. Plasma glucose level was high in the experimental animals (range 6–38 $mmol\,l^{-1}$), but hyperglycemia was not related specifically to exercise. Estimated glucose turnover, 19.3±2.6 (rest) and 15.8±3.9 $\mu mol\,min^{-1}\,kg^{-1}$ (recovery), was also highly variable, but was linearly associated with plasma glucose concentration (turnover=0.97[glucose]+0.57, r=0.93) in both resting and recovering fish. While utilization of glucose in the whole animal was clearly responsive to plasma glucose availability, estimated total skeletal muscle disposal of glucose accounted for less than 15 % of glucose turnover, indicating that glucose was utilized largely by tissues other than locomotory muscle. Rates of glucose utilization in white muscle (range 0.5–4 $nmol\,min^{-1}\,g^{-1}$) provide direct evidence that glucose, regardless of plasma concentration, accounted for less than 10 % of glycogen repletion during exercise recovery. In red muscle, glucose uptake was influenced by plasma glucose level below 10–12 $mmol\,l^{-1}$ (utilization range 1–15 $nmol\,min^{-1}\,g^{-1}$), but was independent of glucose concentration above about 12 $mmol\,l^{-1}$ (utilization plateaued at 15–20 $nmol\,min^{-1}\,g^{-1}$). Trout red muscle is similar to mammalian white muscle in the sense that glucose is estimated to account incompletely for glycogen restoration (25–60 %), suggesting dependence on both glycogenesis and glyconeogenesis during recovery. It is concluded that hyperglycemia may be important to the pattern of substrate incorporation into red muscle glycogen and to the rate of repletion observed, but glucose availability has, as predicted from earlier indirect studies, little relevance to white muscle glycogen restoration. The regulatory mechanisms that govern apparently very high glucose turnover rates during extreme hyperglycemia, concomitant with low disposal rates in skeletal muscle, require further investigation.

Introduction

Burst swimming in teleosts causes dramatic changes in metabolite levels which can

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leave the white muscle depleted of fuel reserves at the end of an exercise bout (Milligan and Wood, 1986*b*; Mommsen and Hochachka, 1988; Schulte *et al.* 1992). The resulting accumulation of metabolic end-products (e.g. lactate, IMP) occurs in stoichiometries that quantitatively reflect depletion of intracellular fuels (e.g. glycogen and ATP). Subsequent post-exercise clearance/recovery of white muscle metabolites is generally a slow process, with little metabolite exchange occurring between muscle and plasma (Tang and Boutilier, 1991), characteristics which have proved useful in comparative examinations of possible limits to metabolite recovery rates after exercise (Moyes *et al.* 1992, 1993). With respect to glycogen recovery, evidence suggests that the quantitative significance of glycogenesis is minimal because of the predominance of an, as yet undefined, intramuscular glyconeogenic pathway from lactate (Arthur *et al.* 1992; Moyes *et al.* 1992; Schulte *et al.* 1992; Pagnotta and Milligan, 1991; Tang and Boutilier, 1991). (The term glyconeogenesis refers to *in situ* glycogen formation from 3-carbon precursors, while the direct incorporation of glucose into glycogen is called glycogenesis.) However, the importance of white muscle as a site of circulatory glucose disposal cannot be overlooked, if only because of the large relative mass of the tissue.

In contrast to the situation in white muscle, the stoichiometry of lactate accumulation in red muscle does not appear to be matched with glycogen depletion at the end of exercise (Parkhouse *et al.* 1988), which perhaps indicates a greater transfer of metabolites between the plasma and the well-perfused red muscle mass. Post-exercise glycogen repletion in red muscle of other species is very much dependent on the uptake of substrate from the circulation, primarily glucose in mammals (Bonen *et al.* 1990; Johnson and Bagby, 1988; Pagliassotti and Donovan, 1990) and lactate in some terrestrial ectotherms (Gleeson and Dalessio, 1990). While it is clear that glycogen stores of salmonid red muscle are greatly depleted by high-intensity exercise (Parkhouse *et al.* 1988), the relative utility of lactate compared with glucose as a precursor for glycogen synthesis and the rate of fuel repletion are not known for this tissue.

In the present study, radiolabeled 2-deoxyglucose (2-DG) was used to assess rates of glucose utilization in skeletal muscle of rainbow trout during recovery from burst exercise. The advantage of 2-DG uptake is that it provides an *in vivo* rate of tissue-specific glucose phosphorylation. In turn, this measure of glucose utilization can serve as a maximal estimate of *in vivo* skeletal muscle glycogenesis, particularly during a period of net glycogen synthesis (Kusunoki *et al.* 1993). In addition to determinations of muscle glucose uptake, glucose turnover was estimated simultaneously using bolus injections of [6-^{3}H]glucose. This combination of measurements identifies sites of glucose disposal in the whole animal and permits an evaluation of the proportion of glucose released into the circulation that is utilized by the total skeletal muscle mass. The results are discussed in terms of the pertinence of possible changes in post-exercise glucose availability to glycogenesis in different skeletal muscle types.

Materials and methods

Animals

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] of both sexes and weighing

300–600 g were obtained from a local supplier and maintained in the aquarium facility at the Department of Zoology, University of British Columbia. Fish were held in flow-through fresh water (10–15 °C) and fed to satiation three times weekly. Fish were maintained in these conditions for at least 1 month prior to experimentation.

Surgery

Fish that had been deprived of food for 2 days were anesthetized in water containing 0.5 g l^{-1} MS-222 (tricaine methanesulfonate, Sindell Laboratories) buffered with 1 g l^{-1} $NaHCO_3$. Individual fish were positioned in an operating sling and the gills were irrigated with chilled (10 °C), oxygenated water containing 0.1 g l^{-1} MS-222. A single 60 cm cannula (PE-50, Clay–Adams) was placed in the dorsal aorta of each fish. The cannula was filled with Cortland saline (Wolf, 1963) containing 5 i.u. ml^{-1} heparin (Glaxo Canada, Ontario) and was flushed twice daily. Each experimental fish was allowed to recover for 48 h in a black Perspex box supplied with a continuous flow of aerated fresh water (10–15 °C).

Exercise and recovery protocol

Fish were netted from the recovery boxes and transferred to a Brett-type swim tunnel. A protocol similar to the one used by Schulte *et al.* (1992) was followed to ensure recruitment of white muscle and reduction of glycogen stores. Briefly, water speed was increased slowly over a period of 2–3 min to a maximum level, defined as the point where the fish first displayed burst-and-glide swimming behavior. This speed was maintained until the fish started to rest against the rear screen of the swim space. Water velocity was immediately reduced to a point where the fish could be induced to swim again and then once more increased gradually to the maximum. The cycle was repeated for about 15 min until burst swimming could no longer be induced. At this point, the fish was quickly removed to a holding box for recovery and eventual administration of radiolabels.

Studies of white muscle metabolite status in fish following maximal exercise show that complete recovery is a long process, requiring 12–24 h for some metabolites. However, most studies indicate that up to 50 % of glycogen replenishment can be expected between 2 and 8 h post-exercise (Milligan and Wood, 1986*b*; Pagnotta and Milligan, 1991; Pearson *et al.* 1990; Scarabello *et al.* 1991*a*,*b*; Schulte *et al.* 1992). Isotope administrations were designed to bracket a portion of this interval in recovery so that glucose utilization could be related to changes in muscle metabolite levels. Glucose turnover and uptake in muscle were determined in a total of nine exercised fish with bolus injections of isotope given at 2.5, 4 or 5 h (three fish per group) post-exercise. Glucose fluxes and tissue glucose utilization rates were also determined in six unexercised animals.

Isotope injection and tissue sampling

Bolus injections of [6-^{3}H]glucose (0.56 MBq) and 2-deoxy-[^{14}C]glucose (2-DG, 0.19 MBq), purchased from Amersham Canada, were used to determine *in vivo* circulatory glucose turnover and muscle glucose utilization, respectively. For each preparation, the appropriate volume of each isotope was dried under a continuous stream of N_2 gas and these were reconstituted together in a single bolus of Cortland saline (150 μl). A sample

(10 μl) was removed to determine the dose of each radiolabel injected, while the remainder was taken up in a glass Hamilton syringe to verify the bolus volume. The syringe was connected to a three-way valve positioned on the free end of the dorsal aortic cannula and the bolus was administered to the fish. Fresh saline (250 μl) was drawn into the glass syringe and then delivered to the fish in order to flush the cannula. Blood samples (125 μl) were collected through the same cannula at 1, 2, 3, 4, 5, 10, 20, 30 and 60 min post-injection. After sampling, the cannula was flushed with saline (125 μl) to replace the blood volume. Red blood cells were separated immediately from each sample by centrifugation and the plasma retrieved was deproteinized with an equal volume of 0.6 mol l^{-1} perchloric acid (PCA). The samples were neutralized with 3 mol l^{-1} K_2CO_3 in 0.5 mol l^{-1} triethanolamine and kept frozen (−70 °C) until analysed for metabolites and radioactivity.

At the end of the sampling period, a mixture of sodium pentobarbital (1 ml of 65 mg ml^{-1}; MTC Pharmaceuticals, Ontario) and *d*-tubocurarine chloride (0.1 mg ml^{-1} dissolved in 1 ml of saline; Sigma Chemicals, MO) was delivered through the cannula to immobilize the fish. When opercular movements ceased (30–60 s), the spinal cord was severed immediately posterior to the head. Red and white muscle was taken with a single pass of a double-bladed cleaver through the musculature of the tail just posterior to the dorsal fin. The steak was clamped between Wollenberger tongs, pre-chilled in liquid N_2 and immersed in liquid N_2. Only 10–15 s passed from the time the fish was killed to the moment that the tissues were frozen. The samples were kept frozen at −70 °C.

Metabolite assays

Muscle was prepared for homogenization by dissecting away the skin and separating red and white fiber masses with the steak in a liquid N_2 bath. Pieces of tissue (0.5–1 g) were placed in preweighed tubes and quickly reweighed. Ice-cold 0.6 mol l^{-1} PCA (4 vols) was added while the tissues were still frozen. The tissues were immediately homogenized with three 20 s passes of an Ultra-turrax homogenizer. Samples (2×100 μl) of homogenate were removed for glycogen determination as described previously (Arthur *et al.* 1992). The remainder of the homogenate was centrifuged (12 000 ***g*** for 10 min at 4 °C) and the acidic supernatant was neutralized and stored at −70 °C. Assays for tissue lactate and glucosyl units and plasma glucose were adapted for use with microtitration plates (0.3 ml) and a Titertek Multiskan plate reader (Arthur *et al.* 1992).

The tissue content of 2-deoxy-[^{14}C]glucose 6-phosphate (2-[^{14}C]DGP) was determined in neutralized tissue extracts either by separation of 2-[^{14}C]DGP from 2-[^{14}C]DG on columns of DEAE Sephadex A-125 (Sigma Chemicals, MO) as described previously (Suarez *et al.* 1989) or by precipitation with 0.3 mol l^{-1} $Ba(OH)_2$ and 0.3 mol l^{-1} $Zn(SO_4)$ (sample:reagent volumes, 1:1.5:1.5, West *et al.* 1993). Both methods extracted more than 90 % of enzymatically prepared 2-[^{14}C]DGP in prepared fish muscle homogenates. Tissue 2-[^{14}C]DGP was calculated from the difference in radioactivity between samples of neutralized extract (2-DG+2-DGP) and column (or precipitation) effluents (2-DG).

The 2-DG lumped constant

The lumped constant (LC) is a correction factor needed in the calculation of tissue-

specific glucose utilization to account for different rates of 2-DG and glucose phosphorylation in tissues (Sokoloff, 1983). In previous studies with trout red muscle and heart, the LC was estimated to be 0.40 (West *et al.* 1993), a value that was unaffected by changes in tissue glycolytic activity. In the present study, estimation of the LC for trout white muscle was made by determining simultaneous *in vitro* rates of glucose utilization and 2-DGP formation in slices of white muscle as described previously for red muscle (West *et al.* 1993). Briefly, slices of white muscle were prepared and preincubated in saline bubbled with 99 % O_2:1 % CO_2 and containing 0.5 % bovine serum albumin (BSA) and (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 and 5 glucose; pH 7.4. Slices were then incubated individually for 40 min with 0.01 MBq of [5-^{3}H]glucose (Amersham Canada) and 0.006 MBq of 2-[^{14}C]DG in fresh saline (2.5 ml). At the end of the incubation, the slice was removed, weighed and analyzed for 2-[^{14}C]DGP content. ^3H_2O was separated from [5-^{3}H]glucose by distillation of the PCA-deproteinized/neutralized incubation medium as described previously (West *et al.* 1993). The LC was formulated as the rate of 2-[^{14}C]DGP formation divided by the rate of glucose utilized (calculated from ^3H_2O production).

Glucose turnover and muscle uptake

Samples (20 μl) of deproteinized/neutralized plasma were dried under a continuous stream of nitrogen gas for 5 h to remove ^3H_2O. 2-[^{14}C]DG was determined and [^{3}H]glucose was estimated after reconstituting the dried plasma with 1 ml of H_2O and adding 10 ml of aqueous scintillant (ACS II; Amersham Canada). Radioactivity was assayed using a dual counting protocol prepared on an LKB Rackbeta scintillation counter.

For individual fish, the plasma washout of each of the injected radiolabels was plotted over time as the ratio of plasma radioactivity to the steady-state glucose concentration (disints min^{-1} μmol^{-1}). A curve with two exponential terms was fitted to each set of washout data (Jana; Statistical Consultants, Lexington, KY) and extrapolated to time zero and infinity (Katz, 1992). The area under the curve was determined by integration (MATHCAD; Mathsoft Inc., Cambridge, MA). Estimates of glucose turnover (in μmol min^{-1} kg^{-1} body mass) were calculated as the quotient of the injected dose for each fish divided by the area under the [6-^{3}H]glucose specific activity washout curve.

Glucose uptake in specific muscle types (in nmol min^{-1} g^{-1} tissue mass) was determined as the ratio of tissue 2-DGP content at the end of the 60 min blood sampling period to the area under the plasma 2-[^{14}C]DG washout curve over the same period (Ferré *et al.* 1985; West *et al.* 1993). The result for each tissue was divided by the lumped constant to discriminate glucose from 2-DG.

Statistics

Data are presented as means ± S.E.M. The significance of differences between groups was assessed using analysis of variance (ANOVA) and Tukey's HSD test.

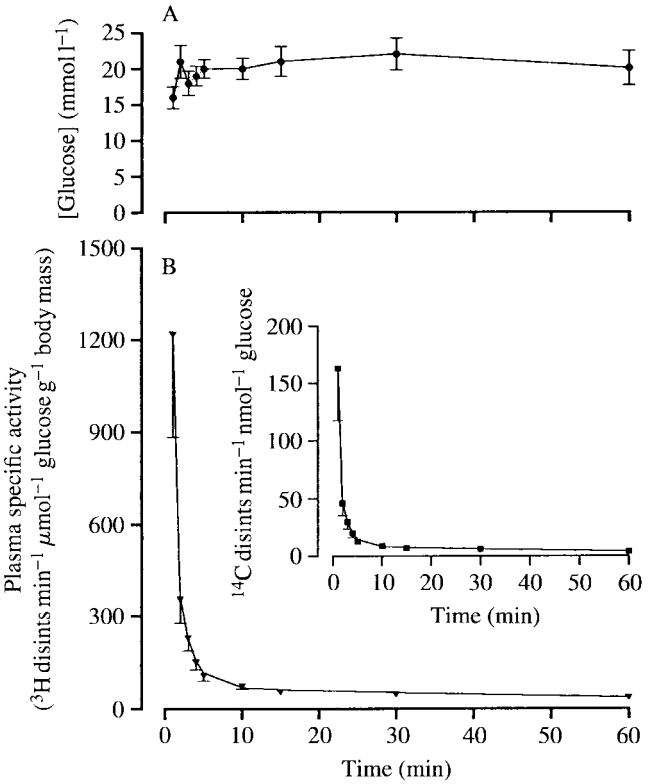

Fig. 1. (A) Steady-state plasma glucose concentration in resting rainbow trout (*N*=6). (B) Estimated plasma specific activity of [6-^{3}H]glucose and plasma ratio of 2-deoxy-[^{14}C]glucose per nmol of glucose (inset axes) in resting fish followed for 60 min post-injection of isotopes (*N*=6). Bars show ±S.E.M.

Results

Glucose concentration and turnover rate

Average plasma glucose concentrations and washout curves for the injected radiolabels are depicted in Fig. 1 (resting fish) and Fig. 2 (recovering fish). Plasma glucose concentration varied considerably among experimental animals (range 6–38 mmol l^{-1}), but individual fish remained in steady state for the period following bolus injection of radiolabels (coefficients of variation for blood glucose in individuals were less than 15 %).

No significant differences in plasma glucose concentration were evident between experimental groups (Table 1). High variability and small sample sizes during recovery probably accounted for the lack of differences between groups, even though plasma glucose at 4–5 h of recovery was comparatively low. Average glucose turnover rate was also depressed during the 4–5 h interval, although there were no statistical differences between recovery groups (Table 1). Turnover rate was linearly associated with steady-state glucose concentration for individual fish at rest and throughout the 2.5–6 h of recovery (Fig. 3). Separate regressions for resting and recovering fish were not

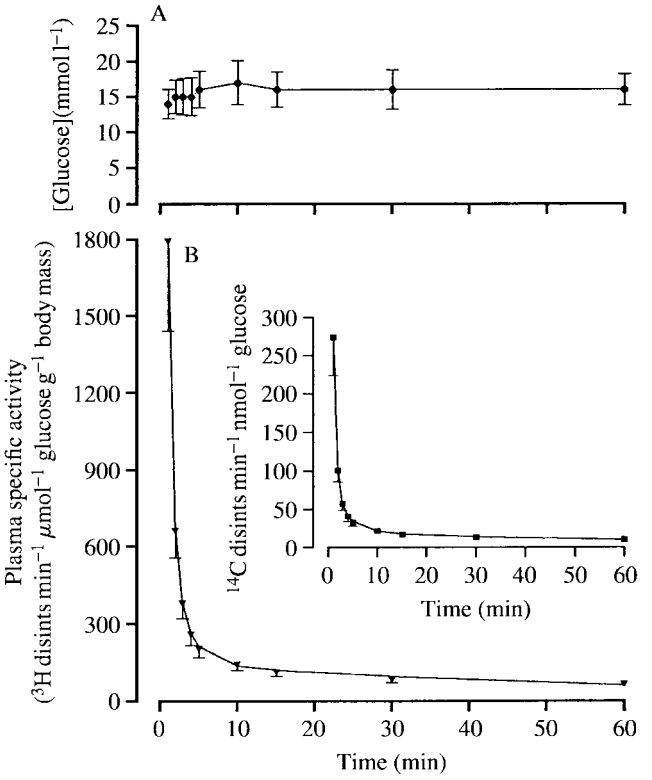

Fig. 2. (A) Plasma glucose concentration in rainbow trout during recovery from burst exercise (*N*=9). (B) Estimated plasma-specific activity of [6-^{3}H]glucose and plasma ratio of 2-deoxy-[^{14}C]glucose per nmol of glucose (inset axes) in exercised fish followed for 60 min after injection of isotopes (*N*=9). Bars show ±S.E.M.

significantly different, suggesting that changes in plasma glucose status had a direct influence on glucose kinetics whereas effects of exercise state of the fish were less important. The plasma clearance of glucose, calculated as turnover rate divided by glucose concentration, was shown to be independent of plasma glucose level (Fig. 3). This means that the volume of plasma cleared of glucose was relatively constant at 0.5–1.5 ml min^{-1} kg^{-1}, regardless of plasma glucose concentration.

Muscle glucose utilization

Pre- and post-exercise rates of glucose utilization in white muscle were calculated using the lumped constant determined from muscle slice incubations (0.51±0.06, *N*=12). There were no significant differences (ANOVA, $P>0.05$, glucose concentration was a significant covariate $P=0.02$) in utilization rate between control and recovering fish (Table 1). Like turnover rate, glucose utilization in white muscle beteen 4–5 h of recovery tended to be one-third to half of the rates estimated at other recovery times and at rest. Glucose uptake in white muscle varied within relatively narrow limits (Fig. 4) and plasma glucose concentration had a less noticeable influence on utilization rate than on

Table 1. *Whole-body glucose turnover and glucose utilization in skeletal muscle during recovery from enforced maximal exercise*

		Recovery interval (h)		
	Pre-exercise	2.5–3.5	4–5	5–6
Plasma glucose (mmol l^{-1})	19.3±2.6	20.2±8.9	8.1±1.2	18.2±5.9
Glucose turnover (μmol kg^{-1} min^{-1})	19.1±2.3	19.7±8.8	7.1±0.6*	20.7±6.8
Glucose utilization (nmol g^{-1} min^{-1})				
Red muscle	18.5±1.7	12.2±3.2	5.2±1.1†	10.9±3.6
White muscle	1.5±0.3	2.1±0.9	0.8±0.6	2.0±0.3

*P=0.07 for comparison with pre-exercise glucose turnover.

†P=0.008 for comparison with pre-exercise red muscle glucose utilization.

Utilization was calculated using lumped constants of 0.4 for red muscle (West *et al*. 1993) and 0.51 for white muscle.

Sample sizes were N=6 for pre-exercise and N=3 for each recovery interval.

turnover rate. As indicated, the significance of a regression through these data was strongly influenced by a single observation which emphasizes that utilization was only marginally dependent on glucose availability (Fig. 4).

As is suggested in Fig. 5, glucose uptake in red muscle of recovering trout changed with plasma glucose concentration only between 6 and 12 mmol l^{-1}. Above 12 mmol l^{-1}, utilization seemed to be independent of concentration. Since all of the resting fish had relatively high plasma glucose levels (>12 mmol l^{-1}), the average resting utilization rate from nine fish with lower plasma glucose levels (<5 mmol l^{-1}, from West *et al*. 1993) is included in Fig. 5 for comparison. The data suggest that the trends in glucose uptake were similar for both resting and recovering trout. There were differences (ANOVA, P=0.04, glucose concentration was an insignificant covariate) among groups and the lower utilization at the end of 5 h of recovery (Table 1) appears to have resulted from the data for this one group falling completely within the range where utilization is sensitive to plasma glucose level (Fig. 5).

Glycogen replenishment in skeletal muscle

Post-exercise recovery of white and red muscle glycogen is illustrated in Figs 4 and 5, respectively. Red muscle glycogen content was still reduced 3.5 h into the recovery period and had recovered to 60 % of the pre-exercise value by the end of 6 h. The data indicate a rate of replenishment of about 20 nmol glucosyl units min^{-1} g^{-1} red muscle over the interval from 3.5 to 6 h post-exercise. Glucose utilization in red muscle during recovery was 5–12 nmol min^{-1} g^{-1} (Table 1), indicating that plasma glucose possibly supported a large portion, 25–60 %, of glycogen replenishment in this tissue. In comparison, although white muscle glycogen content remained significantly reduced 6 h post-exercise, the rate of glycogen replenishment between 2 and 6 h (about 40 nmol min^{-1} g^{-1}) was 20–40 times higher than the rate of glucose phosphorylation (1–2 nmol min^{-1} g^{-1}, Table 1).

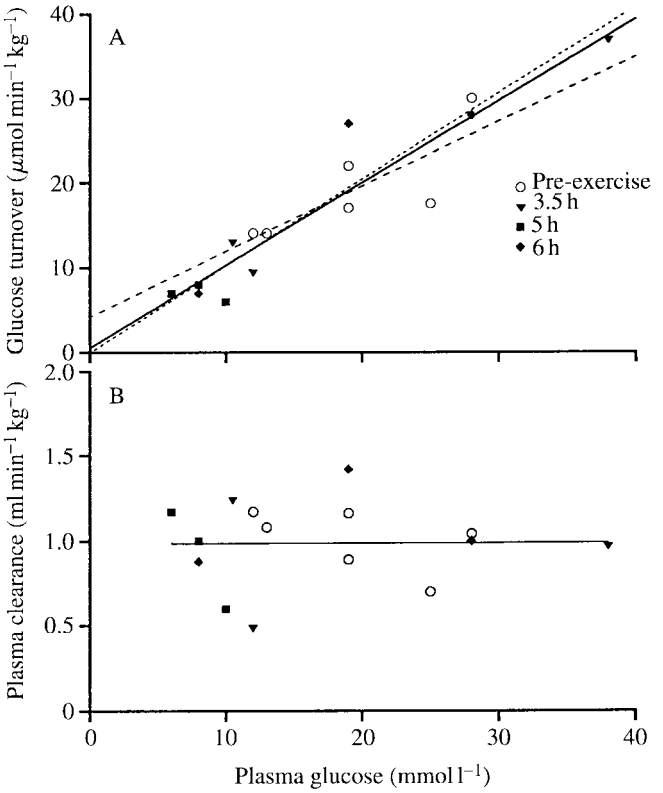

Fig. 3. (A) Glucose turnover in resting and recovering trout as a function of plasma glucose concentration (turnover=0.97[glucose]+0.57, r=0.93; solid line). Broken lines are regressions fitted separately to data for resting (long dashes) and exercised (short dashes) fish. Slopes and intercepts for the two groups were not significantly different. (B) Plasma clearance of glucose in resting and exercised trout was independent of plasma glucose concentration.

Discussion

An unexpected result in this study was the range of plasma glucose concentrations among the experimental animals. This might suggest variable levels of stress (Barton and Schreck, 1987), but identifying the nature of any stress is difficult. In previous studies that used the same pre-experimental care, anesthetization, surgical and handling procedures, there was no hyperglycemia evident (West *et al.* 1993). In the present study, the glucose level was higher in resting fish than in those that were exercised, indicating that hyperglycemia was not a direct result of the exercise protocol. In turn, trends in glucose turnover were explained primarily by plasma glucose status rather than exercise state (rest or recovery). However, while hyperglycemia and concentration-dependent glucose turnover are remarkable from a glucoregulatory perspective, these kinetics seemed to be dissociated from the carbohydrate recovery pattern in trout white muscle. It is important to note that turnover increased proportionately with plasma glucose concentration, suggesting that whole-body utilization of glucose may have occurred predominantly *via* substrate-mediated disposal. Despite this, glucose utilization rate in white muscle, the

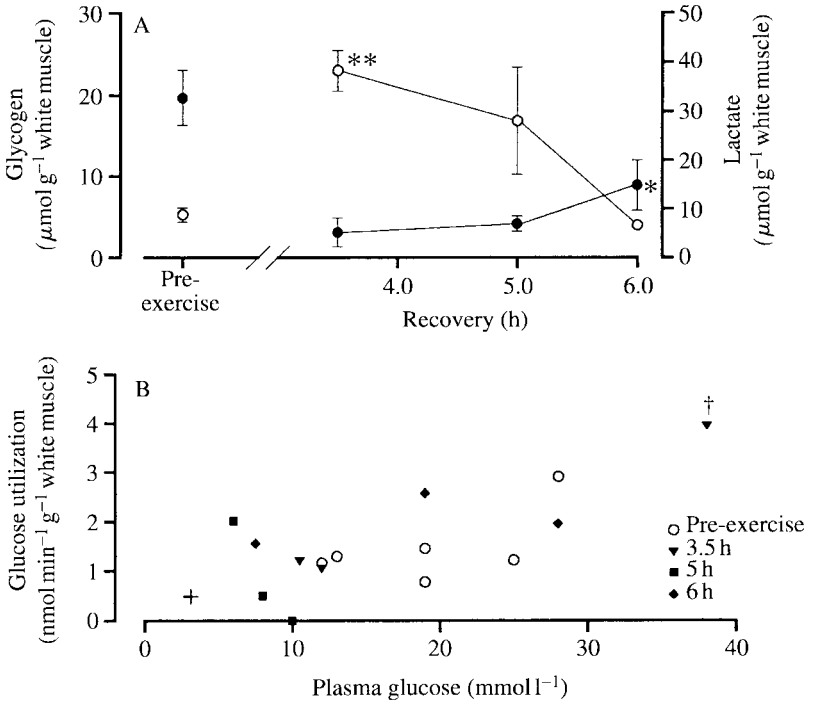

Fig. 4. (A) White muscle glycogen (●) and lactate (○) in resting and recovering trout. *Significantly different from the pre-exercise value (P<0.05), **significantly different from the pre-exercise and 6 h values (P<0.001). (B) Glucose utilization in white muscle of resting and recovering fish as affected by plasma glucose concentration (utilization= 0.07[glucose]+0.31, r=0.68). †An observation with a large influence on a regression (regression slope is not significant, r=0.48, without this datapoint). The average of nine glucose uptake measurements at lower plasma glucose levels (from West *et al.* 1993) is also shown (cross).

largest homogeneous tissue mass in trout, was low and was relatively insensitive to plasma glucose concentration. This situation emphasizes that glucose availability was pertinent to tissues other than white muscle and, further, that white muscle glycogen was resynthesized essentially independently of glucose availability. The interpretation in red muscle is less straightforward, since the pattern of glucose utilization in relation to glucose availability was biphasic, with an initial concentration-dependent phase followed by a concentration-independent phase at higher plasma glucose levels. It would seem that glycogen reformation in this muscle type is dependent on both glucose availability and glyconeogenic flux. Post-exercise blood glucose status, which is moderately hyperglycemic in some studies (discussed later), could therefore have considerable influence on the relative importance of glucose incorporation into red muscle glycogen.

Glucose turnover

Average glucose turnover rates presented for rainbow trout (Table 1) were unusually high for salmonids (see Garin *et al.* 1987). Turnover was on par with rates determined for

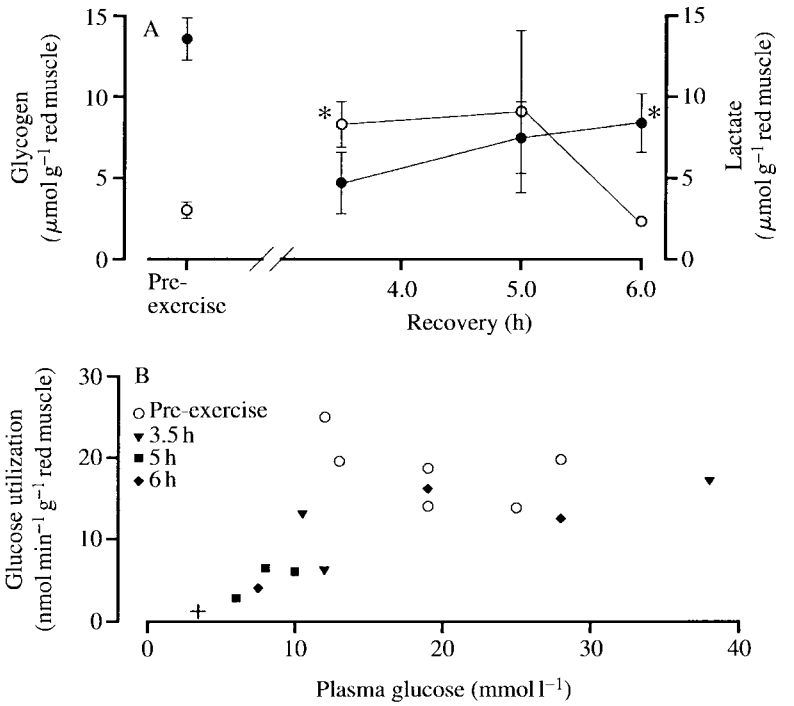

Fig. 5. (B) Red muscle glycogen (●) and lactate (○) in resting and recovering trout. *Significantly different from pre-exercise value (P<0.05). **Significantly different from pre-exercise and 6 h values (P<0.01). (B) Glucose utilization in red muscle is affected by plasma glucose concentration. The average resting glucose utilization rate for nine trout with lower plasma glucose levels is also shown (cross).

warm-bodied skipjack tuna (Weber *et al.* 1986) and the American eel (Cornish and Moon, 1985), both of which may rely on glucose to a large extent for energy provision. In neither of these species was glucose turnover necessarily accompanied by hyperglycemia. In trout, blood glucose status was closely connected to glucose turnover rate in both resting and recovering fish (Fig. 3). Turnover rates in resting salmonids with similar plasma glucose levels are not available for comparison. However, the overall regression does suggest that at plasma glucose levels below 10 mmol l^{-1} turnover rate is likely to fall within 2–10 μmol min^{-1} kg^{-1}. This estimate is in agreement with previous measurements in resting rainbow trout in which plasma glucose was less than 10 mmol l^{-1} (Dunn and Hochachka, 1987; Washburn *et al.* 1992) and demonstrates the apparent utility of plasma glucose concentration as a predictor of whole-body glucose flux.

Beyond its predictive value, the relationship between plasma glucose concentration and turnover rate implies that whole-body glucose disappearance in trout responds to glucose availability over a wide range. Proportional changes in turnover with concentration accounted for the observation that a relatively constant blood volume was cleared of glucose, regardless of glucose concentration (Fig. 3B). Concentration-dependent turnover further suggests that glucose concentration itself may have been an

important regulator of whole-body glucose disposal. This would be consistent with the generalization that glucoregulatory hormones are slow to respond to elevated glucose levels in teleosts (Harmon *et al.* 1991), but it is not known what regulatory characteristics are specifically involved with enhanced glucose production *in vivo*. With respect to glucose disposal, an important point is that, even at relatively low turnover rates (e.g. from 4 to 5 h of recovery; Table 1), disposal of glucose in muscle tissue (calculated from glucose utilization and assuming body mass is 10 % red muscle and 60 % white muscle) is estimated to have been less than 15 % of the total glucose removed from the circulation. This also holds for resting salmonids with lower plasma glucose concentrations (2–4 $mmol\,l^{-1}$), based on total muscle glucose disposal of about 0.5 $\mu mol\,min^{-1}\,kg^{-1}$ body mass (West *et al.* 1993) and turnover rates of 2–5 $\mu mol\,min^{-1}\,kg^{-1}$ (Lin *et al.* 1978; Washburn *et al.* 1992, or predicted from Fig. 3). Therefore, the largest tissue mass in the body utilized only a small portion of the glucose released into the circulation. The suggestion that glucose metabolism is involved largely with mucus production in fish (Bever *et al.* 1981), rather than energy provision (West *et al.* 1993), would be particularly interesting to investigate in relation to glucoregulation during stress-induced hyperglycemia.

White muscle recovery

The present study provides direct evidence that extramuscular pathways of glucose formation are of minor importance to white muscle glycogen recovery. Two observations point to the relative unimportance of changes in glucose availability to the process of glycogen resynthesis in this muscle mass. First, post-exercise changes in white muscle glycogen and lactate levels appeared to be unaffected by hyperglycemia and elevated glucose kinetics since recovery rates were comparable to previous findings (25–50 nmol glucosyl units $min^{-1}\,g^{-1}$ and 40–100 nmol lactate $min^{-1}\,g^{-1}$, Milligan and Wood, 1986*b*; Pagnotta and Milligan, 1991; Tang and Boutilier, 1991; Schulte *et al.* 1992). Second, the rate of glucose utilization in white muscle spanned quite narrow limits, despite the highly variable plasma glucose levels, and utilization was not linked in a discernible way to recovery interval. It can be calculated that, regardless of plasma glucose availability, glucose phosphorylation in white muscle could have potentially accounted for 5–10 % of the glycogen formed during recovery (assuming white muscle is 60 % of body mass and that glucose utilization at 1–2 nmol $min^{-1}\,g^{-1}$ is uniform throughout the tissue). This estimate of white muscle glycogenesis is somewhat higher than one other estimate made previously with different methodology (see Pagnotta and Milligan, 1991). Accounting for the extent to which competing pathways utilize glucose 6-phosphate might be expected to bring these different calculations in closer agreement. However, the likelihood that muscle glucose is directed primarily towards glycogen storage during recovery from exercise (>80 % in rats, Kusunoki *et al.* 1993) suggests that the present 2-DG measurements of glucose utilization rate are reasonable estimates of *in vivo* glycogenesis.

Teleost white muscle can be regarded as almost a self-contained metabolic system with respect to carbohydrate status during burst exercise and recovery. Intramuscular reconversion of lactate to glycogen allows gross lactate/glycogen changes in white muscle to be examined in a reasonably quantitative manner (Arthur *et al.* 1992; Milligan

and Wood, 1986*a*,*b*; Moyes *et al.* 1992; Pagnotta and Milligan, 1991; Schulte *et al.* 1992; Tang and Boutilier, 1991). However, the fine details of lactate clearance and the role of glucose are more difficult to place into a quantitative framework. Terminal sampling studies suggest that some lactate (perhaps 10–20 %, Tang and Boutilier, 1991; Schulte *et al.* 1992) may be oxidized after exercise (discussed elsewhere, Scarabello *et al.* 1991*a*,*b*; Moyes *et al.* 1992) or metabolized extramuscularly. Indeed, oxidation of a carbon source is required to fuel intramuscular glyconeogenesis, although this may well be accomplished by fatty acids rather than lactate (see Moyes *et al.* 1992). Extramuscular metabolism *via* hepatic gluconeogenesis is apparently minimal (Milligan and McDonald, 1988; Weber *et al.* 1986), but *in vivo* rates of oxidation in heart and red muscle and of red muscle glyconeogenesis are unknown. Such 'losses' of lactate might explain minor mismatches between white muscle glycogen replenishment and lactate clearance rates that are sometimes observed and it is likely that glucose is used for small adjustments of post-exercise glycogen restoration. A better understanding of the role of glucose uptake in white muscle recovery metabolism may emerge from studies that include specific examinations of mismatches in lactate/glycogen stoichiometries and of apparent deficits in glycogen recovery (range 0–50 % in trout, Milligan and Wood, 1986*b*; Mommsen and Hochachka, 1988; Pagnotta and Milligan 1991; Pearson *et al.* 1990; Schulte *et al.* 1992, and this study). It is expected, however, that circulatory glucose serves mainly as a supplement to the normal turnover of intramuscular glycogen reserves and perhaps in an energetic role for resting muscle metabolism.

Red muscle recovery

Unlike glucose utilization in white muscle, utilization rate with respect to plasma glucose concentration was apparently biphasic in red muscle and could account for a relatively large proportion of glycogen repletion (25–60 %). The difference in the rate and concentration-dependence of glucose utilization between the muscle types may be related to the larger number of glucose transporters in red muscle, as in mammalian skeletal muscle (Marette *et al.* 1992). Although the properties of glucose transporters in trout skeletal muscles are not known, it might be expected that transporter abundance corresponds to muscle-type differences in hexokinase activity, which is higher in red muscle of trout (Johnston, 1977). In any case, the significance of an increased capacity to phosphorylate glucose in red muscle is the greater potential use of glucose for glycogen synthesis compared with white muscle. Furthermore, the concentration-dependence of glucose uptake suggests that post-exercise blood glucose status could have influenced the rate of glucose incorporation into red muscle glycogen. The present data indicate that glucose incorporation into red muscle glycogen is potentially adjustable over a limited range of plasma glucose concentration, up to 10–12 $mmol\,l^{-1}$. While hyperglycemia in the present study was not specifically related to exercise state, it is known from previous studies that moderate, yet sustained, hyperglycemia is sometimes seen in recovering trout (Milligan and Wood, 1986*a*; Mommsen and Hochachka, 1988; Pagnotta and Milligan, 1991; Scarabello *et al.* 1991*b*; T. G. West, unpublished observations). Typically post-exercise plasma glucose level is within the range where concentration-dependent utilization in red muscle was observed in the present study. We can conclude that glucose

availability is likely to influence the rate of glucose incorporation into red muscle glycogen but, given that glycogen synthesis occurred at a faster rate than could be accounted for by glucose utilization alone, it is probable that both of the processes of glycogenesis and glygoneogensis contributed to carbohydrate recovery after exercise.

Trout are similar to rats in the sense that both rely on glucose to a greater extent for glycogen synthesis in red muscle than in white muscle (Bonen *et al.* 1990; Kusunoki *et al.* 1993). In mammals, however, both red and white muscles use relatively more glucose for glycogen synthesis, >90 % and 30–50 % respectively (Bonen *et al.* 1990; Johnson and Bagby, 1988; Pagliassotti and Donovan, 1990). Lactate is expected to be the major glyconeogenic precursor in vertebrate muscle, and greater oxidative losses of lactate in mammals (Brooks, 1986) than in fish may partly explain the greater dependence on glycogenesis in rat muscle types. It is evident, however, that rats use glucose almost exclusively in red muscle, even when lactate is made available for glyconeogenesis (Bonen *et al.* 1990), whereas trout red muscle resembles mammalian white muscle in that glucose accounts substantially, yet incompletely, for glycogen synthesis.

Glyconeogenesis is therefore probably involved in trout red muscle glycogen restoration, as it is in mammalian white muscle (Bonen *et al.* 1990), but the specific role for lactate is unknown. Changes in red muscle lactate content after exercise (Fig. 5) provide few insights. Nevertheless, plasma lactate concentration increases measurably in salmonids between 2 and 8 h post-exercise (Milligan and Wood, 1986*a*,*b*; Mommsen and Hochachka, 1988; Pagnotta and Milligan, 1991; Schulte *et al.* 1992; T. G. West, unpublished observations), as does lactate turnover (Milligan and McDonald, 1988). It could be that the relative availability of lactate, as well as glucose, affects substrate incorporation into trout red muscle glycogen throughout a period of recovery from exercise. Minor efflux of lactate from the white muscle may be relevant to red muscle glyconeogenesis, as has been suggested for lizards (Gleeson and Dalessio, 1990). Similarly, oxidation of lactate is expected to be a reasonable means of energy provision for the process of red muscle glyconeogenesis. Regulation of these processes requires further study in trout, particularly in view of the fact that 10–20 % of the white muscle lactate load represents a massive glyconeogenic substrate pool for the relatively small red muscle mass. The pathway of glyconeogenesis in muscle is not known, but all muscle types in mammals appear to use an extramitochondrial route, independent of phosphoenolpyruvate carboxykinase activity (Pagliassotti and Donovan, 1990). Reversal of pyruvate kinase has been suggested in trout white muscle (Moyes *et al.* 1992), but extension of the same arguments to red muscle requires further examination of metabolite profiles during recovery.

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