Muscle glucose utilization during sustained swimming in the carp (Cyprinus carpio)

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West, Timothy G., Colin J. Brauner, and Peter W. Hochachka. Muscle glucose utilization during sustained swimming in the carp (Cyprinus carpio). Am. J. Physiol. 267 (Regulatory Integrative Comp. Physiol. 36): R1226-R1234, 1994.—The involvement of circulatory glucose in the energy provision of skeletal muscle and heart of swimming carp was examined. Plasma glucose concentration varied from 3 to 17 mmol·min⁻¹·kg⁻¹ across individual carp, and estimates of glucose turnover rate (R_G) were positively correlated with plasma glucose level in resting fish (range 1.6–6.3 μmol·min⁻¹·kg⁻¹) and in swimming fish (range 4.2–10.7 μmol·min⁻¹·kg⁻¹). Carp that were exercised at 80% of their critical swimming speed displayed a twofold higher R_G at any given plasma glucose concentration. Metabolic clearance rate also doubled in swimming carp (1.0 ± 0.1 ml·min⁻¹·kg⁻¹) relative to resting controls (0.5 ± 0.1 ml·min⁻¹·kg⁻¹). Differences in plasma glucose availability can be explained by changes in muscle glucose uptake, measured with 2-deoxy-D-[14C]glucose. Glucose utilization in red muscle was not dependent on plasma glucose concentration; however, glucose utilization in the red muscle mass was threefold higher in swimming fish than in resting control fish. On the basis of whole body aerobic scope measurements in carp, it was estimated that circulatory glucose flux was 25–30% of the total fuel oxidation in the active red muscle mass. GUI in heart was positively correlated with plasma glucose concentration, and it is possible that glucose availability had considerable influence on the pattern of myocardial substrate oxidation in resting and active carp. Furthermore, measurement of tissue 2-deoxy-D-glucose (2-DG) uptake provides a means of detecting exercise stimulation of glucose disposal that may occur in small muscle masses via redistribution of blood flow or changes in muscle transporters but in the absence of direct effects on whole body glucose flux. In concert with estimates of the in vivo metabolic cost of exercise, these measurements permit estimates of the potential contribution of glucose to the increased oxygen demand of active muscle. Because the fate of glucose in working muscle need not be oxidation, 2-DG uptake in the present study is therefore used as an indicator of the maximum potential for oxidative glucose disposal during rest and exercise. A similar study of rainbow trout (32) swimming at the same relative swimming speed as carp in the present study (80% of U_crit) was used for comparison of the energetic role of glucose in the red muscle and heart of species that display different aerobic swimming capacities.

In vivo substrate utilization is poorly understood in teleosts exercising at submaximal aerobic intensities. Recent studies (30, 32) indicate that carbohydrate, in the form of blood glucose and lactate, contributes minimally to the oxidative fuel demands of locomotor muscle in rainbow trout (Oncorhynchus mykiss) swimming at a critical or fatigue speed (U_crit) of 80–85%. Muscle glycogen may be utilized to some extent (15), but its supply is limited and it is more likely to be spared for bursts of swimming at higher speed. With respect to circulatory glucose, low in vivo glucose turnover rates at rest (7), along with the relatively poor glucoregulatory characteristics of many fish species (7, 10, 17), appear consistent with the likelihood of limited reliance on glucose for swimming energetics. Nevertheless, in the majority of species for which resting glucose fluxes have been determined, it is not known whether glucose availability and flux are altered during sustained aerobic swimming when the demand for substrate in oxidative muscle is expected to be maximal. Measurements of in vivo glucose utilization in concert with changes in whole body activity metabolism will undoubtedly be useful in describing the relative dependence on glucose for muscle energy provision across species.

The carp (Cyprinus carpio) is typical of teleosts in the sense that estimates of in vivo glucose kinetics at rest are relatively low (7). However, in vitro enzyme profiles and mitochondrial substrate preferences do suggest the capacity for red muscle and heart muscle to utilize carbohydrate-based fuels (13, 14, 18). In addition, a comparison of the fat-utilizing capacity of carp with that of the fast-swimming skipjack tuna (Katsuwonus pelamis) suggests that the general hypothesis that better aerobic performers depend to a greater extent on fat-based fuels (e.g., see Ref. 31) may also hold for teleosts with different aerobic swimming capacities (19). The relative small mass of heart and red muscle in conjunction with the relatively sluggish swimming characteristics of carp may mean that glucose disposal is significant in oxidative muscle despite its comparatively low in vivo glucose kinetics.

In the present study, we investigated the importance of circulatory glucose to the swimming energetics of carp (Cyprinus carpio) by examining in vivo glucose kinetics and tissue-specific glucose uptake, determined with bolus injections of [6-3H]glucose and 2-deoxy-D-[14C]glucose (2-[14C]DG). Simultaneous determination of flux and uptake is useful for evaluating the importance of skeletal muscle in whole body glucose disposal. Furthermore, measurement of tissue 2-deoxy-D-glucose (2-DG) uptake provides a means of detecting exercise stimulation of glucose disposal that may occur in small tissue masses via redistribution of blood flow or changes in muscle transporters but in the absence of direct effects on whole body glucose flux. In concert with estimates of the in vivo metabolic cost of exercise, these measurements permit estimates of the potential contribution of glucose to the increased oxygen demand of active muscle. Because the fate of glucose in working muscle need not be oxidation, 2-DG uptake in the present study is therefore used as an indicator of the maximum potential for oxidative glucose disposal during rest and exercise. A similar study of rainbow trout (32) swimming at the same relative swimming speed as carp in the present study (∼80% of U_crit) was used for comparison of the energetic role of glucose in the red muscle and heart of species that display different aerobic swimming capacities.

MATERIALS AND METHODS

Animals. Carp (986–1,790 g, 35- to 45-cm total length) of both sexes were purchased from a local supplier. Fish were held in dechlorinated fresh water (10–13°C) for 1 mo before...
experiments and were fed twice weekly with trout pellets (Moore-Clark, Vancouver).

Estimation of \( U_{\text{crit}} \). The maximum swim speed sustainable for 30 min, was determined for carp using a Brett-type swim tunnel respirometer that is described thoroughly elsewhere (8). Individual fish were placed in the swim tunnel, and water velocity was adjusted to between 0.6 and 0.7 body length/s. Exercise intensity was maintained at this level for 2 h; then the respirometer was closed to inflowing water for 20 min, and \( O_2 \) consumption was determined by monitoring changes in water \( P_{O_2} \) (Radiometer, Copenhagen). Swimming speed was increased by 0.3 body length/s every 30 min, and \( O_2 \) uptake was determined at each new speed unless the fish fatigued within the first 10–15 min. Between estimates of \( O_2 \) uptake, \( O_2 \) level in the water was readjusted to 156 mmHg. The time to fatigue, when a mild electric shock (5 V) no longer able for 30 min, was determined for carp using a Brett-type Perspex boxes.

Sustained swimming in cannulated carp. Carp were anesthetized before surgery in buffered tricine methanesulfonate (MS-222, 0.2 g/l with 0.4 g/l NaHCO\(_3\)). A cannula (PE-90; Clay Adams, Parsippany, NJ) was implanted in the dorsal aorta, sutured to the roof of the mouth, and passed through a piece of PE-200 that was secured through the membrane of the distensible upper lip. Throughout surgery the gills were irrigated with oxygenated water containing a light level of MS-222 (0.08 g/l). Cannulas were filled with Cortland saline (35) that contained 5 U/ml heparin (Glaxo, Montreal), and the fish were allowed to recover from anesthetization for 24–36 h.

Carp were brought to a high sustainable swim speed in a manner similar to that described previously (32). A fish was placed in the swim tunnel and forced to swim at a low intensity (about one-third body length/s) for 2 h. Speed was then increased by 0.5 min (in 0.5 body length/s increments) until the fish displayed obvious burst-and-glide swimming. At this point, water velocity was reduced until the fish could maintain its position in the swim tunnel. This exercise intensity was maintained for a total of 120 min, with radiolabeled metabolites being administered after the first 60 min. During the interval 30–60 min postinjection of radiotracers, \( O_2 \) uptake was determined as described above.

Bolus injections, tissue sampling, and analysis. Appropriate volumes of [\(^6\)H\(^2\)]glucose (15 \( \mu \)Ci) and 2-\[^{14}\]C\(^2\)DG (5 \( \mu \)Ci) were dried under \( N_2 \) gas. Tracers were reconstituted together in saline (140–180 \( \mu \)l), and radioactivity in the mixture was determined in aliquots (10 \( \mu \)l) that were counted in aqueous scintillant [10 ml of antireticular cytotoxic serum (ACS II), Amersham] using a dual-label assay on an LKB Rackbeta. The remaining solution was drawn into a glass Hamilton syringe to determine the volume of the tracer bolus. Tracers were delivered to fish through one port of a three-way stopcock that was attached to the free end of the implanted cannula. Another syringe connected to the second port of the stopcock served as a saline rinse (2 volumes) to flush solution from the glass syringe and cannula into the animal. Blood (125–150 \( \mu \)l) was sampled from carp at 1, 2, 3, 4, 5, 10, 20, 30, and 60 min after injection of tracers. Plasma was separated immediately by centrifugation. Blood was replaced with an equal volume of saline until the final sample, at which time 2 ml of pentobarbital sodium (65 mg/ml) were injected through the cannula. When opercular movements ceased, the spinal cord was severed just posterior to the head. Samples of heart and skeletal muscle were dissected, as described previously (32), and were freeze-clamped between liquid nitrogen-cooled aluminum tongs. Less than 30 s elapsed from the time the fish was killed to the moment the last tissue was immersed in liquid nitrogen.

The frozen skeletal muscle was immersed in a shallow bath of liquid nitrogen, and samples of the lateral red muscle were easily dissected free from the larger white muscle mass. Red and white muscle samples (0.5–1.0 g) were chopped into fragments under liquid nitrogen and transferred to preweighed tubes that contained ~4 volumes of ice-cold 0.6 N perchloric acid (PCA). The tubes were reweighed, and samples were homogenized with two 20-s passes of an Ultra-turrax tissue homogenizer. Two aliquots (100 \( \mu \)l) of this homogenate were removed for glycogen analysis. After centrifugation (10 min, 7,500 \( g \)), the tissue extracts were neutralized with 3 M K\(_2\)CO\(_3\) in 0.5 M triethanolamine HCl and spun again, and the resulting supernatant was frozen at ~80°C. Cardiac muscle was treated in the same manner, but homogenates were not sampled for glycogen analysis. Glycogen in red and white muscle homogenates was hydrolyzed with amyloglucosidase (Boehringer Mannheim, Quebec City, Canada) as described previously (1). Red and white muscle glycoprotein units, as well as muscle lactate, were determined with standard enzymatic assays that were modified for use with microtitration plates (0.3 ml) and a Tittertek Multiskan plate reader (1).

Plasma was deproteinized with one volume of 0.6 N PCA, and protein was precipitated by centrifugation. Samples were neutralized with 3 M K\(_2\)CO\(_3\) in 0.5 M triethanolamine hydrochloride. Aliquots (50 \( \mu \)l) of the final supernatant were placed in 20-ml scintillation vials and evaporated to dryness under streams of \( N_2 \) gas to remove any \(^3\)H\(_2\)O. Residues were reconstituted in 1 ml of H\(_2\)O and assayed using a dual-label protocol on a LKB Rackbeta scintillation counter to estimate radioactivity in plasma [\(^6\)H\(^2\)]glucose and 2-\[^{14}\]C\(^2\)DG. Plasma glucose was determined using a kit (Sigma Chemical, St. Louis, MO) modified for assays in microtitration plates (1).

Radioactivity in dried plasma samples was used to estimate ratios of [\(^6\)H\(^2\)]glucose to glucose and 2-\[^{14}\]C\(^2\)DG to glucose (in disintegrations·min\(^{-1}\)·dpm\(^{-1}\))·mol\(^{-1}\)). The change in each ratio was plotted against time (min postinjection) from 1 to 60 min and fit to a double exponential function. To estimate glucose turnover rate \( (R_g) \), the appropriate curve was extrapolated to \( time \) 0 and infinity, and the area under the curve was calculated. The ratio of the injected dose of [\(^6\)H\(^2\)]glucose (dpm/kg body mass) to the area under the curve (dpm·min\(^{-1}\)·\( \mu \)mol\(^{-1}\)) was formulated to estimate \( R_g \) \( (\mu \)mol·min\(^{-1}\)·kg\(^{-1}\)). Metabolic clearance rate of glucose (MCR, \( ml·min\(^{-1}\)·kg\(^{-1}\)) was calculated as \( R_g \) divided by plasma glucose concentration.

The 2-\[^{14}\]C\(^2\)DG washout curve was integrated from \( time \) 0 to 60 min, and an index of muscle glucose utilization (GUT; see Ref. 28) was calculated as tissue 2-\[^{14}\]C\(^2\)DG (dpm/g tissue) divided by the area under the plasma 2-\[^{14}\]C\(^2\)DG washout curve (dpm·min\(^{-1}\)·\( \mu \)mol\(^{-1}\)). Tissue 2-\[^{14}\]C\(^2\)DG content was calculated as total \(^1\)C radioactivity (2-DG + 2-DGP) – 2-\[^{14}\]C\(^2\)DG in equal volumes of tissue extract (500 \( \mu \)l). Ion exchange separation of 2-\[^{14}\]C\(^2\)DG from extracts was conducted precisely as described in a previous study of trout swimming energetics (32). Calculation of glucose utilization rate (GUR) in specific tissues requires that GUT be corrected with a lumped constant (LC) to account for slower 2 DG phosphorylation compared with glucose (23). For discussion of the energetic importance of glucose in carp muscle, values of GUR in carp tissues were estimated by dividing GUT by LC values determined previously for rainbow trout (32, 33).

Statistics. The significance \( (P < 0.05) \) of differences between group means was assessed by analysis of variance (ANOVA) or by the Mann-Whitney \( U \)-test when differences in variances among means could not be eliminated by a data
transformation. Regression analysis was used to examine relationships between glucose kinetics and plasma glucose concentration.

RESULTS

Carp swimming performance. There was considerable variability in determinations of O₂ uptake among carp, but individuals showed similar rates of change in log O₂ consumption with increasing exercise intensity (Fig. 1). Maximal O₂ consumption of the four fish that attained a speed of 1.5 body lengths/s averaged 175 ± 36 mg O₂·h⁻¹·kg⁻¹. Extrapolation of the general regression to the y-axis indicates that the O₂ consumption of resting carp at 15°C should average about 43 mg O₂·h⁻¹·kg⁻¹ (95% confidence limits indicate a range of 25–65 mg O₂·h⁻¹·kg⁻¹).

U₉ for the six exercised carp ranged from 1.00 to 1.68 body lengths/s, averaging 1.38 ± 0.11 body lengths/s (61 cm/s for the average body length of 44 cm). Two fish had U₉ values of ~1 body length/s, and these were eliminated from further swimming tests to minimize variability in U₉ of the fish used subsequently in glucose kinetics experiments. The four remaining carp, with one new fish added to the group, were cannulated and reexercised 24–36 h later at an average speed of 1.26 ± 0.10 body lengths/s (54 cm/s). This corresponded to ~80% of the mean U₉ determined for the 4 reexercised fish (1.56 ± 0.06 body lengths/s or 69 cm/s). As indicated (Fig. 1), the measured rate of O₂ uptake for the cannulated fish (130 ± 10 mg O₂·h⁻¹·kg⁻¹) was in agreement with the rate expected for uncannulated carp. Given that cannulated carp exercised at the test speed for 120 min and that average O₂ consumption could be predicted from the velocity-dependent metabolic rate in uncannulated carp, it would seem that the implanted cannulas had minimal effect on swimming performance. Because burst-and-glide activity was observed in carp at one velocity increment (0.30 body length/s) above the final test speed of 1.26 ± 0.10, U₉ was probably similar in carp before and after placement of the cannulas.

Glucose turnover. Plasma glucose ranged 3–17 mM among individual carp at rest and 5–10 mM among swimming fish. Nevertheless, plasma glucose remained in steady state in individual fish from both groups (Fig. 2); the coefficient of variation of plasma glucose was <15% for individual fish throughout the blood-sampling period. Mean plasma glucose concentrations for individual fish were used in the formulation of isotope washout curves used for estimating RT (Fig. 2). An effect of exercise on RT was not revealed from a comparison of group means (Table 1). However, it was apparent that much of the variance among resting and swimming carp could be explained by the dependence of RT on plasma glucose concentration (Fig. 3). Slopes of these relationships were significantly different (P = 0.05), although intercepts of the two lines were not. Interestingly, RT in both swimming and resting carp changed proportionally with concentration. This meant that at any given concentration, at least within the

![Fig. 1. O₂ consumption of 6 carp during a stepwise increase in swimming speed. Fatigue speed (U₉) was determined (see text), and 4 of these same fish, plus 1 additional fish, were cannulated and exercised again at 80% U₉. Arrows denote average speed and O₂ consumption of swimming, cannulated carp.](http://ajpregu.physiology.org/)

![Fig. 2. Plasma glucose concentration (A) and changes in plasma [6-³H]glucose-to-glucose (B) and 2-deoxy-P-[¹⁴C]glucose-to-glucose (B, inset) ratios (means ± SE) in resting and exercising carp after bolus injection of radiolabeled tracers.](http://ajpregu.physiology.org/)
range of 5–10 mM, the effect of exercise was evident as a roughly constant relative stimulation of Rₜ, or about twofold. The effect of exercise on glucose kinetics, independent of plasma glucose level, was more clearly represented by the plasma clearance of glucose. MCR ranged from 0.3 to 1.3 ml·min⁻¹·kg⁻¹ (Fig. 3), and regression slopes of MCR vs. glucose concentration in resting and swimming carp were not different from zero. However, MCR was on average twofold higher in swimming fish than in those at rest (Table 1).

**Muscle glucose utilization.** Muscle GUI was examined as a function of plasma glucose concentration in individual fish to determine whether the pattern of glucose uptake was influenced by glucose availability and exercise (Fig. 4). GUI in white muscle was low and varied over a relatively narrow range (0.1–3.5 nmol·min⁻¹·g⁻¹) in both swimming and resting fish. Although a significant regression was found for white muscle GUI against plasma glucose concentration in swimming fish, significant separation of white muscle GUI from the resting control fish was not evident. White muscle GUI was 1.1 ± 0.4 nmol·min⁻¹·g⁻¹ at rest and 1.7 ± 0.6 nmol·min⁻¹·g⁻¹ during exercise. In red muscle, GUI was independent of plasma glucose level in both resting and swimming carp, as indicated by regression slopes that were not different from zero. At rest, red muscle GUI averaged 3.3 ± 1.5 nmol·min⁻¹·g⁻¹. During exercise, there was one high estimate of glucose uptake (50 nmol·min⁻¹·g⁻¹) in a fish that exhibited a low postexercise level of red muscle glycogen (< 1 μmol glucosyl unit/g; see Fig. 5). Excluding this one outlier (value is > 2.5 SDs higher than the group mean), GUI in the four other exercised carp displayed no tendency to change with increasing plasma glucose concentration, although the mean GUI (10.7 ±
GLUCOSE UPTAKE IN ACTIVE CARP

Fig. 4. Muscle glucose utilization index (GUI) in resting and swimming carp in relation to plasma glucose concentration. There was a significant difference between regression slopes of resting and swimming carp in only the myocardial GUI (P = 0.05).

2.6 nmol·min⁻¹·g⁻¹ was significantly higher (P < 0.05) than that of resting controls (3.3 ± 1.5 nmol·min⁻¹·g⁻¹).

In heart muscle, significant regressions were calculated for data from resting (P = 0.04) and swimming (P = 0.05) carp (excluding the outlier). Slopes of these concentration-dependent relationships were significantly different (P = 0.05), displaying an effect of exercise that was similar to the pattern observed for glucose turnover rate.

Glycogen and lactate levels were not different in postexercise carp (Table 1) because of the large degree of variability in the levels of these metabolites among resting and exercised fish (e.g., see Fig. 5). However, it did seem that GUI was associated with the terminal glycogen content of muscle. Figure 5 illustrates that at very low glycogen levels, red muscle GUI could have been elevated by more than four times above the average rate for fish that had red muscle glycogen levels in excess of 10 µmol glucosyl units/g. Low glycogen content was observed in red and white muscle of just this one exercised carp. Although myocardial glycogen was not determined in this fish, the elevated cardiac GUI (see Fig. 4) suggests that glycogen level was possibly reduced in all muscles of this one animal.

DISCUSSION

The present study examined the role of circulatory glucose in the swimming energetics of carp. Although glucose RT increased with exercise, the absolute difference in flux between resting and swimming fish was dependent on plasma glucose concentration. Despite this, glucose availability was apparently of less importance to the active locomotory muscle than to other tissues, like myocardium, inasmuch as GUI in the active lateral red muscle was concentration independent. Nevertheless, GUI in red muscle increased threefold with exercise, and it is possible that glucose disposal accounted for 25–30% of the fuel demands of this muscle mass, with the assumption that red muscle accounted for the bulk of the estimated aerobic scope of carp. It would seem that carp were more reliant on circulatory glucose than were better aerobic performers like rainbow trout, with the latter using glucose for < 10% of total fuel utilization during exercise at 80% Ucrit (32).

O₂ consumption. Carp O₂ uptake in the present study compares well with previous measurements. The extrapolation of active O₂ uptake to zero activity concurred with other such procedures and with determinations of O₂ consumption in inactive carp at 15°C (~50 mg O₂·h⁻¹·kg⁻¹ in 0.8- to 2.0-kg animals) (4, 11, 24). Maximum active O₂ consumption in the present study was about fourfold higher than the estimated resting rate, resulting in a metabolic scope of 132 mg O₂·h⁻¹·kg⁻¹. This is less than one-half the metabolic scope of salmonids of similar mass (2). Although resting O₂ consumption is similar for 1-kg carp and salmonids, metabolic scope in the latter is higher because of a superior swimming capacity (e.g., Ucrit of trout is 2 body lengths/s). O₂ consumption in carp swimming at 80% Ucrit was 130 mg O₂·h⁻¹·kg⁻¹, threefold higher than the predicted resting rate. This difference was similar to the aerobic scope for spontaneous activity observed previously in carp (24).

Glucose turnover. In resting carp, the mean glucose RT was somewhat higher than had been measured...
previously for other teleosts (7). This resulted largely from higher glucose levels in carp in the present study. The regression describing proportional changes in resting glucose turnover with glucose concentration allows us to predict that, at 2–4 mM glucose, whole body glucose utilization would be within the range estimated in most species of teleosts that have been studied (1.4–2.2 μmol·min⁻¹·kg⁻¹) (7).

It is interesting that blood glucose level varied among resting carp (3–17 mM) and that Rₜ increased with plasma glucose concentration. This suggests, first, that carp are typical of teleosts in not regulating resting blood glucose concentration to within rigid in vivo limits (10, 17) and, second, that glucose availability itself may have been important in mediating uptake in peripheral tissues. Substrate-mediated glucose disposal is evident in mammalian models that display ineffective (diabetics) or underdeveloped (neonate dogs) hormonal glucoregulation (5, 12, 26). Regulatory hormones, like insulin, undoubtedly help to reduce plasma glucose level in teleosts (17), but the response to elevated glucose is slow and may be suppressed by other hormonal interactions (10). Proportional changes in glucose Rₜ with increasing glucose concentration tend to support a possibly dominant role for glucose availability in regulating tissue disposal rate in carp, regardless of activity state.

The increase in regression slope for Rₜ vs. glucose concentration in swimming carp suggests that it could be effective for individual fish to “become hyperglycemic” during a rest-to-work transition to enhance delivery of glucose to those tissues with increased demand for oxidative substrate (i.e., note the absolute difference between Rₜ of resting and swimming fish at higher plasma glucose concentrations). We have no direct evidence for a kinetic pattern that involves a change in plasma glucose concentration during exercise in carp. However, another study (25) has shown that plasma glucose in carp swimming at a high relative speed for 25 h was unchanged from the preexercise level throughout exercise. This implies that stimulation of glucose flux in the present study most likely occurred in the absence of a direct effect of exercise on plasma glucose concentration, supposedly via parallel upregulation of the rates of glucose appearance and disappearance (29). Thus potential expansion of glucose use in active carp would seem to be largely fortuitous, depending very much on the prevailing blood glucose status of individual fish before exercise. This situation is consistent with having poor control over plasma glucose concentration and with possible substrate mediation of glucose uptake. However, the regulatory features that would allow maintenance of steady-state glucose concentration as a result of the coordinated upregulation of hepatic glucose release and glucose disposal require further investigation.

When Rₜ changes approximately in proportion with plasma glucose concentration, the formulation of MCR (Rₜ divided by glucose concentration) affords a means of examining perturbations in glucose kinetics that occur independently of glucose concentration. In both resting and swimming carp, clearance of glucose from the blood is constant. The twofold increase in glucose flux during exercise is emphasized by the higher MCR in swimming fish (1.0 ml·min⁻¹·kg⁻¹) compared with resting controls (0.5 ml·min⁻¹·kg⁻¹). The relative constancy in the volume of blood cleared of glucose in both swimming and resting fish supports the possibility that peripheral uptake of glucose is dominated by substrate-mediated disposal.

Muscle glucose uptake. Muscle tissue comprises close to 70% of the body mass of carp and might therefore be expected to be a major site for the disposal of circulatory glucose. As indicated in Table 2, heart and skeletal muscle are estimated to account for 50–70% of glucose disappearance in resting and swimming carp. The relative importance of each skeletal muscle type is similar because of the differences in relative mass and relative glucose uptake between the two tissues. However, glucose disposal in red muscle was somewhat higher when turnover was low and increased in both low and high turnover conditions during exercise. Note that the one glycogen-depleted fish (discussed later) was eliminated from this analysis because of the apparent dependency of glucose uptake on muscle glycogen below levels of 10 μmol glucosyl units/g (Fig. 5). In the more general situation, red muscle GUI was stimulated to a relatively constant level in swimming carp (Fig. 4). Given that glucose concentration had no effect on red muscle GUI, it would seem that elevation of Rₜ in swimming carp with high plasma glucose levels had more relevance to tissues other than the active red muscle. White muscle might be expected to be one such tissue, given its preference for carbohydrate fuels (18). A positive trend was noted in white muscle GUI of swimming fish, but the range of values was relatively narrow and not significantly different from that of resting fish. Only the heart showed changes in GUI that paralleled effects of exercise and glucose concentration on Rₜ (Fig. 4). However, its small relative mass makes it an insignificant disposal site for circulatory glucose (Table 2). Pink skeletal muscle was not sampled in the present study.

Table 2. Total glucose disposal in cardiac and skeletal muscle of carp

<table>
<thead>
<tr>
<th>Muscle Type</th>
<th>Weight, g</th>
<th>Total Glucose Disposal, mmol g⁻¹</th>
<th>Glucose Uptake, nmol min⁻¹ kg⁻¹</th>
<th>%Turnover Accounted For</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1,360–1,830</td>
<td>3,000–11,400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1,600–5,200</td>
<td>28–34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red</td>
<td>2,300–2,700</td>
<td>40–17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>34–60</td>
<td>&lt;1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It was assumed that carp body mass comprised 66% white muscle and 7% red muscle and that ventricle mass was 0.76 g/kg (3, 22). Contribution of specific muscles to whole body disappearance of glucose assumes a 2-deoxy-D-glucose lumped constant (see text) of 0.4 for red muscle and heart (32) and 0.51 for white muscle (33) for calculations of glucose utilization rate (GUR). To produce data ranges, resting and swimming carp were subdivided on basis of glucose Rₜ: carp with Rₜ < 5 μmol·min⁻¹·kg⁻¹ were arbitrarily designated as having low Rₜ, and those with ≥ 5 μmol·min⁻¹·kg⁻¹ as having high Rₜ. The 1 glycogen-depleted fish was excluded.
but it is ~10% of the carp myotome (13, 14) and could well have accounted for a large portion of glucose disposal.

If, as a starting assumption, the metabolic scope for sustained swimming in carp (87 mg O₂·h⁻¹·kg⁻¹) is attributed solely to the increased aerobic functioning of the lateral red muscle, then oxidative demand for glucose would have been equivalent to ~7,400 mmol·min⁻¹·kg⁻¹. In turn, assuming ~70 g of red muscle per kg body mass, the oxidative demand for glucose in active red muscle per gram of tissue mass is estimated at just over 100 nmol·min⁻¹·g⁻¹. With an absolute rate of glucose uptake (GUR) of ~28 nmol·min⁻¹·g⁻¹ [calculated with a red muscle LC of 0.40, (32)], it is estimated that circulatory glucose could have accounted for 25–30% of the oxidative energy production in swimming carp. This estimate would be higher in the event that other aerobic metabolic costs also contributed to the whole body metabolic scope. For instance, electromyograms from previous work on swimming carp (40 cm in length and acclimated to 15°C) indicated that intermediate pink muscle was recruited at 1.3 body lengths/s (14), a speed that is comparable to 80% U₉ in the present study. Given that sustained swimming was established for individual carp by visual inspection of swimming behavior (i.e., water speed was slowed until burst-and-glide swimming ceased), we cannot rule out the possibility of aerobic involvement of intermediate fibers in the present study. White muscle recruitment, however, was probably minimal inasmuch as such recruitment in carp is generally accompanied by rapid fatigue (21).

Intramuscular glycogen could have augmented, or even dominated, any carbohydrate demands of red muscle in swimming carp, as suggested in a previous study (15). Changes in glycogen and lactate content in the present study were inconclusive because of the variability in resting glycogen levels and because the protocol used did not account for potential intermittent use of muscle glycogen during step changes in swimming speed. Nevertheless, it is noteworthy that GUI increased markedly when red muscle glycogen had been depleted to <10 μmol glucosyl units/g in swimming fish (Fig. 5). At the moment, it is not known what factors (e.g., hormones, contractions) influence glucose transport in fish muscle, but at least the capacity to expand uptake is apparent from the correlation between GUI and intramuscular carbohydrate level. Clearly, this relationship requires further direct investigation. A cautious interpretation that favors carbohydrate as the preferred substrate in red muscle of active carp is that this situation reflected reversal of the inhibitory effects of glycogenolysis on hexokinase activity (34) as glycogen depletion progressed. In humans with McArdle’s disease, the absence of muscle phosphorylase, and thus of glycogenolysis, is in a sense analogous because glucose utilization is enhanced in patients exercising at intensities that normally induce muscle glycogen mobilization (27). As has been argued for swimming trout, however, similar inhibition of red muscle glucose utilization could also occur if lipid-based fuels were oxidized (32). In any case, it would seem that red muscle glucose utilization was dependent less on the availability of glucose than on the availability of other competing substrates. Given that the endurance of carp at relatively high swim speeds [≥25 h can be sustained (25)] would overburden typical red muscle glycogen stores, the possibility of shifts in preferred red muscle fuel sources (intramuscular to extramuscular) and types (carbohydrate vs. lipid) throughout an endurance swim should be examined thoroughly.

Unlike skeletal muscle, GUI in cardiac muscle displayed concentration-dependent uptake and an effect of exercise that paralleled the responses seen in glucose K₁. Complete oxidation of glucose at the utilization rates estimated in this study would have accounted for 10–40% of total myocardial fuel consumption in resting and swimming carp (assumes resting power output of ~1 mW/g for slow swimming species (6); exercising power output 4 × resting (A. P. Farrell, personal communication); 20% cardiac efficiency (9); caloric equivalent of 20.1 J/ml and a myocardial LC of 0.4 (32)). The range (10–40%) in this calculation reflects the dependence of heart GUI on glucose availability, indicating that glucose concentration may have greatly influenced the pattern of substrate use for myocardial energy provision. Fuels other than glucose would appear to dominate cardiac energetics at low glucose levels. The possibility of shifts in glucose dependence in carp myocardium is consistent with previous analyses suggesting that different substrate types (e.g., carbohydrate or lipid) can support heart energetics in fish (22).

Synopsis: carp vs. trout. A comparison of glucose utilization in swimming carp and rainbow trout (see Ref. 32) suggests that carp utilize glucose to a somewhat greater extent than do trout. A direct comparison of in vivo cardiac energetics is difficult because of the variability in plasma glucose level and GUI among individual carp. The in vivo response of myocardial GUI to changes in glucose concentration in trout is not known because glucose utilization was previously determined at plasma glucose levels of 2–4 mM. Unlike carp, an exercise effect on myocardial glucose uptake was not detected in trout (32). However, if the regressions for GUI against glucose concentration in carp (Fig. 4) are extrapolated to low plasma glucose levels, then it is apparent that an effect of exercise on glucose utilization would also have been difficult to detect in carp. At this level of glucose availability, it was estimated that glucose could have accounted for <10% of the myocardial O₂ consumption in swimming carp. In comparison, the same calculation for swimming trout was <1% (32). Confirmation that teleosts may increase glucose use for myocardial energetics at elevated plasma glucose levels awaits direct determination of glucose oxidation in relation to glucose availability. That carp may show such concentration-dependent glucose utilization is at least suggested from the dependence of GUI on glucose availability.

Estimated glucose utilization in red muscle was stimulated to a similar absolute level in swimming carp and trout (20–30 nmol·min⁻¹·g⁻¹, which assumes a 2-DG LC of 0.40 for both species). However, the smaller
metabolic scope for sustained swimming in the less active carp results in a higher estimate of maximal glucose use in red muscle of carp (25–30%) than of trout (< 10% (32)) while swimming at 80% \( U_{\text{crit}} \). A key assumption in these calculations was that red muscle accounted for much of the change in whole animal \( O_2 \) uptake during the transition from rest to exercise at 80% \( U_{\text{crit}} \). Calculations of glucose oxidation in this manner are very likely to be underestimates because other tissues undoubtedly contribute to the whole animal metabolic scope. As was discussed earlier, white muscle is unlikely to have been recruited in carp, although aerobic recruitment of pink muscle could well have occurred at 80% \( U_{\text{crit}} \). However, the higher white muscle aerobic capacities of faster swimming species (see Refs. 18 and 19) may allow for aerobic recruitment of white muscle during sustained exercise in highly active fish and could partially explain differences in swimming capacity of trout and carp. Nevertheless, glucose utilization in red muscle of trout would still account for only 10% of energy production, even if one-half of the whole body \( O_2 \) uptake was accounted for by white muscle (32), whereas the distribution of \( O_2 \) uptake among different muscle types (e.g., red and pink) in carp could increase the importance of glucose to one-half the total substrate needs in active red muscle.

On the one hand, the lower aerobic demands of carp swimming musculature may well favor the use of blood glucose in red muscle compared with the situation in the fast-swimming trout, where alternative fuel types seem to be exploited. This would be consistent with the hypothesis that highly aerobically adapted species are increasingly dependent on lipid oxidation for exercise. On the other hand, differences in glucose utilization may not strictly reflect differences in the endurance characteristics of carp and trout. For instance, the omnivorous carp takes up dietary glucose at four times the rate of carnivorous species like trout (16). This correlates with the tendency for carp to have four- to tenfold higher glycogen stores in liver and red muscle than the rainbow trout (13–15) and suggests that dependency on glucose could foremost reflect species differences in the tolerance of dietary carbohydrate. Further comparisons of tissue glucose uptake across species should reveal the relevance of swimming capacity, muscle recruitment pattern, diet, glycogen storage sites, and changes in plasma glucose availability to the in vivo substrate preference of fish muscle.

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