# Glycogen depletion in juvenile rainbow trout as an experimental test of the oxygen debt hypothesis

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Glycogen depletion was used as an experimental tool to examine the relationship between excess post-exercise oxygen consumption and lactate metabolism in 6-g rainbow trout. A 5-day starvation period reduced whole-body glycogen stores by 50% and slightly lowered resting lactate levels; resting oxygen consumption, glucose, ATP, and creatine phosphate levels were not affected. After a 5-min bout of exhaustive exercise, significantly less glycogen was utilized by the glycogen-depleted fish, 40% less lactate was accumulated, and glucose levels did not rise in comparison with the control group. Creatine phosphate recovered more quickly in the glycogen-depleted fish, whereas ATP was unaffected. Recovery from excess post-exercise oxygen consumption was not significantly different despite the large absolute differences in lactate removed and glycogen resynthesized. This experimental test demonstrates that the classical oxygen debt hypothesis does not completely explain the excess post-exercise oxygen consumption in the trout.

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Une chute de la concentration de glycogène a servi d'outil expérimental pour examiner la relation entre la consommation d'oxygène excessive après un exercice et le métabolisme du lactate chez des Truites arc-en-ciel de 6 g. Un jeûne de 5 jours a réduit les réserves de glycogène du corps de 50% et diminué légèrement les concentrations de lactate au repos; la consommation d'oxygène au repos de même que les concentrations de glucose, d'ATP et de phosphate de créatine n'ont pas été affectés. Comparativement à des poissons témoins, les poissons à réserves de glycogène réduites soumis à 5 minutes d'exercice exhaustif utilisaient une quantité significativement moindre de glycogène, accumulaient 40% moins de lactate et ne subissaient pas d'augmentation de leurs concentrations de glucose. La concentration de phosphate de créatine est retournée à sa valeur initiale plus rapidement chez les poissons à réserves de glycogène amoindries, et les concentrations d'ATP n'ont pas été affectées. La récupération après la consommation d'oxygène excessive enregistrée après l'exercice ne différait pas significativement chez les deux groupes de poissons, malgré les différences absolues considérables dans la quantité de lactate disparue et la quantité de glycogène resynthétisé. Les résultats de cette expérience démontrent que l'"hypothèse de la dette d'oxygène" ne fournit pas une explication complète de la consommation d'oxygène excessive après l'exercice chez la truite.

[Traduit par la rédaction]

## Introduction

The classical oxygen debt hypothesis (Hill and Lupton 1924; Margaria *et al.* 1933) states that after a period of intense exercise the elevated post-exercise oxygen consumption (EPOC), i.e., the oxygen debt, is utilized to oxidize a small portion of the accumulated lactate (LAC) burden, providing energy to resynthesize the remainder into glycogen (GLY). However, in mammals, accumulated evidence now suggests that EPOC may depend on factors other than LAC accumulation, such as intensity and duration of exercise, body temperature elevation and catecholamine mobilization (Barnard and Foss 1969; Brooks *et al.* 1971; Bahr *et al.* 1987). Segal and Brooks (1979) demonstrated that GLY depletion significantly altered metabolism both before and after moderate to heavy exercise. Both resting and post-exercise blood LAC levels were lowered in glycogen-depleted (GD) subjects, whereas oxygen consumption ($\dot{M}O_2$) levels were not significantly different from normal-glycogen (NG) subjects.

These conditions have not yet been tested in fish. In a recent study we have evaluated the classical oxygen debt hypothesis by preparing theoretical budgets of the cost of recovery based on measured changes in $\dot{M}O_2$ and metabolite status in the whole body of juvenile rainbow trout after a single severe

exercise bout (Scarabello *et al.* 1991). The sum of the factors contributing to the "alactacid debt" (Margaria *et al.* 1933), including adenosine triphosphate (ATP) and creatine phosphate (CP) restoration, agreed well with the measured fast component of the $\dot{M}O_2$ recovery curve. However, the measured slow component ("lactacid debt") was much greater than could be explained on the basis of metabolic scenarios of the classical oxygen debt hypothesis. LAC disposal by itself did not appear to account for the measured EPOC.

The present study was designed as an empirical test of this theoretical conclusion, by experimentally manipulating the post-exercise LAC burden through prior GLY depletion. It is known that dietary status affects GLY stores in trout, and that in turn the state of the GLY reserves profoundly affects exercise performance, resistance to fatigue, and post-exercise recovery (Miller *et al.* 1959; Hochachka and Sinclair 1962; Black *et al.* 1966). We anticipated that a short period of starvation would lower the GLY stores and thereby reduce the post-exercise LAC burden. We predicted that if EPOC and LAC recovery were directly linked, as suggested by the classical oxygen debt hypothesis, then EPOC would be lowered in approximate proportion to the reduction of the post-exercise LAC burden. If, on the other hand, EPOC and LAC recovery were not directly linked, in accord with the theoretical analysis of Scarabello *et al.* (1991), then this proportional relationship would not be seen. Therefore, our goal was to attempt to experimentally dissociate EPOC and LAC disappearance in the

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rainbow trout, during recovery from exhaustive exercise, and thereby provide evidence to support or reject the classical oxygen debt hypothesis of Hill and Lupton (1923).

## Materials and methods

### Animals

Juvenile rainbow trout ($n = 173$) were obtained from Rainbow Springs Trout Farm, Thamesford, Ontario, and held in large, 400-L circular tanks, with well-aerated fresh dechlorinated Hamilton tap water at the experimental temperature ($15 \pm 1°C$). Fish were fed *ad libitum* with 1.5-Gr. trout pellets (Martin Feed Mills, Don Mills, Ontario) every day.

### Glycogen depletion experiment

It is known that during starvation, some fish tend to defend carbohydrate stores at the expense of either lipid or protein (see Moon and Johnston 1980). Thus, initial tests (fish weight $\approx 6$ g) were performed to determine the experimental protocol that would deplete a significant amount of whole-body GLY. Fish were divided into four groups and sampled for whole-body GLY and glucose (GLU) as described below. A control group (fed) was sampled immediately to determine initial levels. Other groups were subjected to 5 or 9 days of starvation, or 3 days of starvation combined with continuous swimming at 2 body lengths/s (BL/s) in a current, and then sampled.

### Experimental treatment

On the basis of the initial experiment (Table 1), 5 days of starvation was selected as the appropriate procedure for GLY depletion. Therefore, the final experimental protocols compared two groups. In the case of the normal GLY group (NG), the regular daily feeding regime was continued until 1 day prior to experimentation. In the case of the GLY depleted group (GD), fish were removed from the holding tank 5 days before the experiment and held in square 50-L tubs under similar conditions, but were not fed. Fish from both groups were removed from the holding tanks 1 day prior to experiments, weighed (about 6 g), and were then acclimated overnight to individual respirometers. The following morning, fish were removed from the respirometers and chased in a bucket for 5 min until thoroughly exhausted. They were then returned to the respirometer and allowed to recover for up to 12 h. Control fish for each group (NG and GD) were not exercised and were left in the respirometers throughout the experimental period.

### Respirometry

The respirometers were darkened 20-mL syringe barrels fitted with three-way stopcocks for inflow and outflow sampling. A Gilson Minipuls peristaltic pump provided a constant flow of approximately 1.5–2 L/h to each respirometer. Samples of inflowing and outflowing water were taken in 1-mL glass syringes for measurement of $P_{O_2}$ via a Radiometer E5046 oxygen electrode, thermostatted to 15°C, and connected to a Cameron Instruments OM-200 meter. Inflow $P_{O_2}$ was about 155 torr; typically the drop ($\Delta P_{O_2}$) at the outflow was 15–20 torr, increasing to as high as 45 torr after exercise. Tests demonstrated that the minimum mixing time to yield a representative $\Delta P_{O_2}$ was 5 min; thus, the first post-exercise determination was made at 5 min after the end of exercise (when the fish was replaced in the chamber). Oxygen consumption ($\dot{M}_{O_2}$; μmol $O_2$/(g wet wt.·h)) was calculated by the Fick principle:

$$\dot{M}_{O_2} = [\Delta P_{O_2} \text{ (torr)} \times \alpha_{O_2} \text{ (μmol/(torr} \cdot \text{L))}$$
$$\times \text{ flow (L/h)]/[weight (g)]}$$

where $\alpha_{O_2}$ is the solubility coefficient of $O_2$ in water at 15°C (2.0111 μmol/(torr·L); Boutlier *et al.* 1984). (Note that 1 torr = $1.33 \times 10^2$ Pa.) As no difference in percent water content was observed between groups or as a result of exercise (results not shown), values were then converted to nmol/(mg dry wt.·h) (mean water content $76.7 \pm 0.44\%$).

### $\dot{M}_{O_2}$ study

In this study, only $\dot{M}_{O_2}$ was measured both before and after a 5-min bout of exhaustive exercise in the same fish for each group ($n = 8$). A mean of three measurements was taken prior to exercise. Post-exercise samples were taken first at 5 min, then at 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 h. Control fish were sampled at the same time but were not exercised. The area under the $\dot{M}_{O_2}$ recovery curve (i.e., until pre-exercise levels were re-established at a maximum of 6 h) corresponds to the EPOC or "oxygen debt." This area was measured on an individual fish basis (0–6 h), relative to pre-exercise levels, using a GTCO Digi-Pad digitizer pad and a Zenith Data Systems microcomputer.

### Metabolite study

Fish from both groups (NG and GD) were acclimated to the respirometer (12 h), exercised for 5 min, and allowed to recover. At 5 min and 1, 3, 6, 8 and 12 h post-exercise, $\dot{M}_{O_2}$ was measured, and immediately thereafter, fish ($n = 8$ at each sample time) were freeze-clamped for simultaneous metabolite measurements by means of Wollenberger aluminum tongs precooled to $-196°C$ in liquid nitrogen. Two control samples (C1 and C2) from nonexercised fish were also taken at the start and end of the experiment. EPOC was measured from the mean $\dot{M}_{O_2}$ recovery curve between 0 and 6 h, relative to C1.

Scarabello *et al.* (1991) demonstrated that $\dot{M}_{O_2}$ measurements were less variable when individual fish were followed for the duration of the experiment, in contrast to the use of single terminal samples required for the metabolite study. The purpose of repeating the $\dot{M}_{O_2}$ measurements in the metabolite study was to ensure that the two treatments were consistent. Calculations in the budget, therefore, were based on EPOC measurements from the study using $\dot{M}_{O_2}$ only.

For whole-body freeze-clamping, the fish was removed from the respirometer by quickly emptying the syringe barrel into a small net, and then stunned by a blow to the head. Fish that struggled were discarded. The delay between removal and freeze-clamping was 5 s. The whole fish was subsequently powdered in a mortar and pestle under liquid nitrogen, weighed, freeze-dried for 48 h, reweighed, and stored in an evacuated dessicator at $-60°C$ until assay of metabolites. LAC, GLY, ATP, CP, and GLU were enzymatically assayed on the freeze-dried powder by standard fluorometric techniques (Bergmeyer 1965).

All values are reported in nmol/mg dry wt., except for $\dot{M}_{O_2}$, which is given in nmol/(mg dry wt.·h). All values are reported as the mean $\pm 1$ SE ($n = 8$). For the study of $\dot{M}_{O_2}$ only, significant differences due to exercise were determined via Student's two-tailed $t$-test ($P < 0.05$), paired design. A Bonferroni procedure for multiple comparison was also employed. For the metabolite study, significant differences from C1 (i.e., effect of exercise) and between groups at the same sample times (i.e., effect of prior GLY depletion) were determined via Student's two-tailed $t$-test at $P < 0.05$, unpaired design.

## Results

### Glycogen depletion experiment

Relative to the fed control group, 5 days of starvation reduced GLY levels by 52%, 9 days of starvation caused no further significant change, and 3 days of continuous swimming at 2 BL/s without food dropped GLY stores by 37% (Table 1). GLU levels, however, were significantly elevated for all three experimental groups. We concluded that 5 days of starvation would have sufficiently reduced GLY stores for the purpose of this study. Note that the initial GLY depletion test was performed on a different batch of trout and at a different time of year than the $\dot{M}_{O_2}$ and metabolite studies, which presumably explains the differences in control GLY levels from those in the later studies (cf. Fig. 2B).

TABLE 1. Whole-body glycogen and glucose levels in rainbow trout after various periods of starvation

| Group | Glycogen (nmol glucosyl U/mg dry wt.) | Glucose (nmol/ mg dry wt.) | N |
|---|---|---|---|
| Control | 56.33±2.95 | 5.78±0.95 | 8 |
| Five days of starvation | 27.18±2.97* | 13.30±1.63* | 8 |
| Nine days of starvation | 26.54±2.74* | 10.15±1.79* | 7 |
| Three days of starvation while swimming at 2 BL/s | 35.61±5.52* | 20.33±2.51* | 6 |

NOTE: Values are given as the mean ± SE.
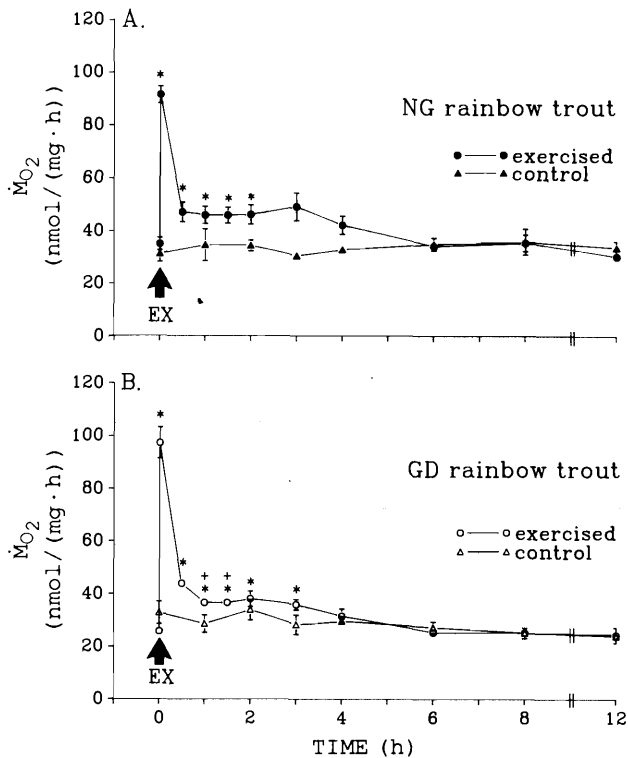*Significantly different ($P < 0.05$) from control group.



FIG. 1. Changes in oxygen consumption rates, from the $\dot{M}O_2$ study, after exhaustive exercise in normal-glycogen (NG) (A) and glycogen-depleted (GD) (B) juvenile rainbow trout. ▲, △, nonexercised controls; ●, ○, exercised fish. Data are shown as the mean ± 1 SE ($n = 8$). Arrows indicate exercise bouts of 5 min. Pre-exercise values are a mean of three measurements per fish. The first post-exercise sample was taken at 5 min. Values are expressed in nmol/(mg dry wt. · h). *, significantly different ($P < 0.05$) from pre-exercise values; +, significantly different ($P < 0.05$) from corresponding NG sample point.
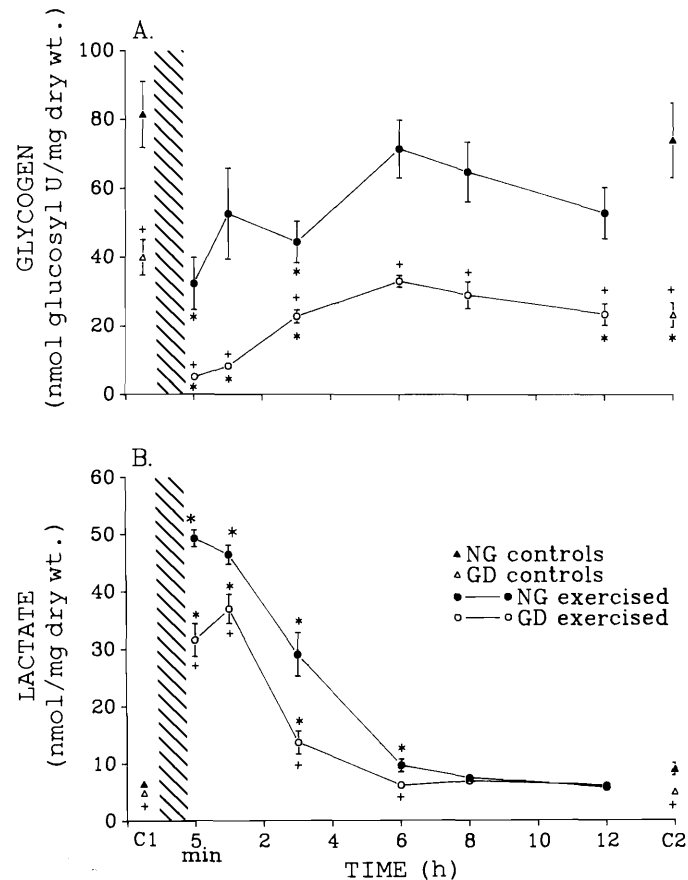


FIG. 2. Changes in whole-body glycogen levels (A) and lactate levels (B) after exhaustive exercise in NG and GD juvenile rainbow trout. Two control samples were taken (C1 and C2). ▲, △, nonexercised NG and GD controls, respectively; ●, ○, exercised NG and GD fish, respectively. Data are shown as the mean ± 1 SE ($n = 8$). The hatched bar indicates a 5-min exercise bout. The first post-exercise sample was taken at 5 min. *, significantly different ($P < 0.05$) from C1. +, significantly different ($P < 0.05$) from corresponding NG sample point.

## $\dot{M}O_2$ study

Resting $\dot{M}O_2$ levels in NG fish were about 30 nmol/(mg dry wt. · h), which increased 2.5-fold after the 5-min exercise bout, and required 4–6 h for a complete return to resting levels (Fig. 1A). GD fish had similar resting and immediately post-exercise $\dot{M}O_2$ levels (Fig. 1B), but the time course of recovery after exercise was slightly different. Mean $\dot{M}O_2$ level at a few sample times (1 and 1.5 h) was significantly lower than the corresponding measurements in NG fish, giving the appearance of a faster rate of recovery. However, when the area under the curve was measured on an individual fish basis, there was no significant difference in EPOC between the two groups. NG fish had an EPOC of 70.8 ± 8.5 nmol/mg dry wt., while GD fish had an EPOC of 67.6 ± 13.5 nmol/mg dry wt. There were no significant changes in $\dot{M}O_2$ in nonexercised controls for either group over the entire experimental period.
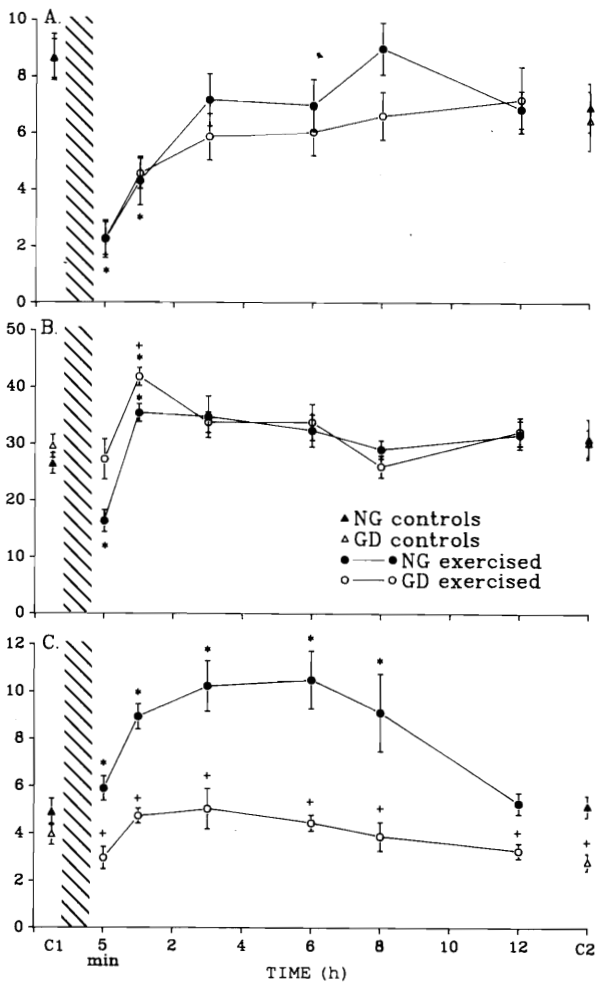
## Metabolite study

The pattern of post-exercise $\dot{M}O_2$ (results not shown) was very similar to that in the $\dot{M}O_2$ study. Except for a small but significant difference at 5-min post-exercise (GD higher than NG), the two $\dot{M}O_2$ curves (in NG and GD samples) were virtually identical, increasing 2- to 3-fold after exercise and requiring 3–6 h for a return to resting levels. EPOC was only slightly greater than the value measured in the $\dot{M}O_2$ study, and the same in the two groups (NG: 83.5 nmol/mg dry wt.; GD: 85.0 nmol/mg dry wt.).

Resting GLY levels in GD fish (39.8 ± 5.2 nmol glucosyl U/mg dry wt.) were about half those in NG fish (81.4 ±9.6 nmol/mg dry wt.; Fig. 2A), indicating that the experimental protocol (5 days of starvation) was again successful in reduc-

TABLE 2. A comparison, in normal (NG) and glycogen-depleted (GD) fish, of measured changes in EPOC and whole-body metabolites, with a budget analysis based on the assumption that the entire EPOC oxidized LAC, and that the remaining LAC was converted to GLY (scenario B) (see text for details)

| | $O_2$ (nmol/mg dry wt.) | LAC (nmol/mg dry wt.) | | GLY (nmol/mg dry wt.) |
|---|---|---|---|---|
| **NG fish** | | | | |
| Measured change | −71.1 | −39.7 ↙ ↘ | | +39.2 |
| Scenario B | −71.1 | −23.7 (oxidized) | −16.0 (to GLY) | +8.0 |
| Percentage accounted for | 100 | 60 | 40 | 20 |
| **GD fish** | | | | |
| Measured change | −67.5 | −27.7 ↙ ↘ | | +27.7 |
| Scenario B | −67.5 | −22.5 (oxidized) | −5.2 (to GLY) | +2.6 |
| Percentage accounted for | 100 | 81 | 19 | 9 |

NOTE: It is assumed that 3 moles $O_2$ per mole LAC were oxidized, and that 2 moles LAC were required per mole GLY resynthesized.

FIG. 3. Changes in whole-body adenosine triphosphate (A), creatine phosphate (B), glucose levels (C) after exhaustive exercise in NG and GD juvenile rainbow trout. Data are shown as the mean ± 1 SE ($n = 8$). For other details see Fig. 2.

ing GLY reserves even though the absolute levels differed from those in the initial GLY depletion test (cf. Table 1). Exhaustive exercise caused a severe reduction in GLY levels in both groups. GLY in NG fish dropped to 40% of resting levels, a decrease of 49.1 nmol/mg dry wt. GLY in GD fish dropped by only 34.7 nmol/mg dry wt.; however, on a relative basis this was a much more severe depletion, to 13% of resting levels. Peak restoration of GLY stores in both groups occurred at 6 h. At this time, GLY levels were not significantly different from pre-exercise values (C1), though somewhat depressed on an absolute basis. By 12 h, GLY in GD fish was once again significantly lower than C1. The second control sample (C2), taken at the end of the experimental period, also showed a decline in GLY levels in GD fish but not in NG fish.

GLY depletion significantly affected both pre- and post-exercise LAC levels. Resting LAC levels were higher in NG fish (6.5 ± 0.6 nmol/mg dry wt.) than in GD fish (4.9 ± 0.3 nmol/mg dry wt.; Fig. 2B). After exercise, LAC increased by 42.8 nmol/mg dry wt. in NG fish but by only 26.7 nmol/mg dry wt. in GD fish at 5 min post-exercise. After cessation of exercise, LAC levels continued to rise in GD fish, and peaked 1 h later. This was not observed in NG fish, in which LAC started to decline after the 5-min sample. Recovery time was similar in the two groups (6–8 h).

Whole-body ATP levels (Fig. 3A) were not significantly affected by GLY depletion, both groups showing similar levels and time courses of recovery. Resting ATP levels averaged around 8.5 nmol/mg dry wt., were significantly depleted at 5 min (by about 75%) and 1 h after exercise, but had returned to levels not significantly different from pre-exercise values by 3 h.

Resting whole-body CP levels (C1 and C2; Fig. 3B) were not significantly different between groups, averaging about 28 nmol/mg dry wt. Samples were not taken immediately at the end of the exercise (i.e., time 0), but we have shown elsewhere that this exercise protocol significantly depletes CP stores immediately after exercise, with a rapid recovery within 5 min (Scarabello et al. 1991). In the present study, NG fish

still showed depressed CP levels (62% of resting levels) at 5 min, whereas GD fish did not (92% of resting levels). CP levels in both groups continued to rise at 1 h, overshooting resting levels, although to a greater extent in GD fish. Thereafter, CP returned to resting levels in both NG and GD fish (Fig. 3B).

Resting whole-body GLU levels were not significantly altered by GLY depletion at the start of the experiment (C1), averaging about 4.5 nmol/mg dry wt., but were reduced at the end of the period to about 3 nmol/mg dry wt. in the GD group (C2; Fig. 3C). GLU levels in GD fish did not vary significantly after exercise but were elevated in NG fish to about 2-fold resting values over the period 1–8 h post-exercise, and returned to control levels by 12 h.

## Discussion

A 5-day starvation regime was used to attempt to experimentally dissociate EPOC and LAC disappearance during recovery from a bout of exhaustive exercise. The starvation regime reduced the glycogen content in GD fish by approximately 50% (Fig. 2B). Following exercise, the total LAC burden in GD fish was reduced by 40% compared with NG fish (Fig. 2A). However, GLY depletion did not alter post-exercise $\dot{M}o_2$ (Fig. 1), reflected in identical EPOC between NG and GD groups in both the $\dot{M}o_2$ and metabolite studies. Clearly, the experimental protocol (GLY depletion) was successful in dissociating the total LAC accumulated after exercise from EPOC. Furthermore, $\dot{M}o_2$ required only 4 h to return to pre-exercise levels (Fig. 1), whereas LAC recovery required 6–8 h (Fig. 2A).

### Effects of starvation

The various starvation tests confirm the known sensitivity of GLY reserves to feeding regime in trout (Miller et al. 1959; Hochachka and Sinclair 1962; Black et al. 1966). The 5-day starvation period was successful in inducing a reduction of about 50% GLY in juvenile trout (Table 1, Fig. 2A), a difference that was roughly the same after 9 days of starvation, and which was also maintained on a relative basis at all sampling times after exhaustive exercise. In older trout, white muscle GLY fell by 10–40% and liver GLY by 60–80% after starvation periods of 3.5 (Black et al. 1966), 7, and 14 days (Hochachka and Sinclair 1962). As in the present study, the results of these earlier investigations suggested that most of the decline occurs in the first few days of starvation, and that GLY reserves are thereafter well defended at lower levels. Hochachka and Sinclair (1962) also reported that intensive feeding was an even more effective mechanism for manipulating GLY reserves, resulting in 4-fold elevations in liver and 9-fold elevations in white muscle.

The 5-day period of starvation did not alter resting ATP and CP reserves (Figs. 3A and 3B) or resting $\dot{M}o_2$ (Fig. 1) in the GD fish relative to the NG fish. The latter finding contrasts with earlier reports that routine $\dot{M}o_2$ declines markedly over the first few days of starvation in trout, owing to a decreased $O_2$ requirement for the assimilation of food (Beamish 1964). However, Dickson and Kramer (1971) reported a slight increase in standard metabolism after starvation periods longer than 3 days. Furthermore, since our fish were confined in small respirometers during the measurements, the reduction in $\dot{M}o_2$ that is routinely associated with the decrease in spontaneous activity during starvation would not be seen.

### Exercise metabolism in normal and GLY-depleted fish

The decrease in whole-body GLY caused by exhaustive exercise was greater in fed (NG) fish than in starved (GD) fish (49 vs. 35 nmol/mg dry wt.), although the relative depletion was greater in the latter (60 vs. 87%). Earlier reports on adult trout are in accord with this pattern (Miller et al. 1959; Black et al. 1966). Trout with lower initial GLY reserves fatigued more quickly than trout with higher reserves, and mobilized less GLY after a severe exercise bout.

GLY depletion clearly altered pre- and post-exercise LAC levels. Whole-body LAC levels were slightly but significantly lower in GD than in NG fish under resting conditions, at both the start (C1) and end (C2) of the experiment (Fig. 2B). This may have been due to reduced rates of glycolysis as a result of lowered GLY reserves, even under aerobic conditions. Similar findings on blood LAC have been observed in humans in whom GLY stores have been reduced by manipulations of diet and previous exercise (Segal and Brooks 1979).

The post-exercise LAC burden in GD trout was only about 60% of that in NG trout (Fig. 2A). This difference was roughly proportional to the difference in post-exercise GLY depletion between the two groups, and was the result that the experiment was designed to achieve. Interestingly, both groups showed a 1:1 ratio of GLY depletion : LAC accumulation, in contrast to the expected stoichiometry of 1:2 had the utilization of GLY been entirely anaerobic, as in some other studies (e.g., Black et al. 1962; Dobson and Hochachka 1987). In our protocol (see also Scarabello et al. 1991), the type of exercise probably involved GLY utilization as an aerobic fuel as well as an anaerobic one. The rate of pyruvate oxidation would increase along with the rate of glycolytic flux, resulting in a smaller accumulation of LAC than depletion of GLY.

In humans, prior GLY depletion has been associated with a decrease in glycolysis during exercise, suggesting that initial GLY reserves dictate the maximum power output that can be sustained during short-term exercise (Heigenhauser et al. 1983). The same may well be true in fish. Interestingly, whole-body GLU levels did not rise significantly after exercise in GD fish (Fig. 3C), perhaps as an indirect consequence of reduced glycolytic rates (i.e., reduced glycogenolysis of gluconeogenesis from LAC). Another possibility is that changes in hormonal levels as a result of starvation are responsible for the reduced GLU levels. For example, it has been shown that 3–4 days' fasting in trout alters the plasma glucagon/insulin ratio (Plisetskaya 1989).

GLY depletion had no effect on post-exercise ATP metabolism (Fig. 3A), though CP repletion with "overshoot" occurred more quickly in the GD group (Fig. 3B). In view of the lability of CP levels to sampling disturbance (Dobson and Hochachka 1987) and degradation during analysis (van den Thillart et al. 1990), the meaning of this difference, and especially the overshoot phenomenon, is unclear. A significant post-exercise overshoot in CP levels has been reported in some studies on older trout (e.g., Milligan and Wood 1986; Pearson et al. 1990), but in our own recent study on very small trout (2–3 g), the overshoot was small and insignificant (Scarabello et al. 1991). Notably, resting CP levels in that study were about 50% higher than the present control values, and therefore similar to the present overshoot values.

### Relationship between LAC burden and EPOC

Despite the substantial difference in LAC burden between

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