The oxygen debt hypothesis in juvenile rainbow trout after exhaustive exercise

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Abstract. A 5 min bout of exhaustive exercise in 2–3 g rainbow trout resulted in a 2.0–2.5 fold increase in oxygen consumption ($\dot{M}_{O_2}$), a 5-fold increase in whole-body lactate (LAC) levels and a near depletion in whole-body glycogen (GLY), ATP and creatine phosphate (CP) stores; glucose, ADP and AMP did not change. Recovery of $\dot{M}_{O_2}$ and LAC was complete by 6 h, by which time GLY had stabilized at about 65% resting levels without further recovery through 24 h. Complete recovery of ATP required 1.0–1.5 h, whereas restoration of CP required only 5 min. The $\dot{M}_{O_2}$ recovery curve was resolved into an initial fast component ($t_{1/2} = 0.23$ h) and a second slower component ($t_{1/2} = 2.1$ h), comprising approximately 20% and 80% respectively of the excess post-exercise oxygen consumption (EPOC). The fast component was satisfactorily accounted for by the standard components of the 'alactacid O_2 debt'. However, the slow component could not be completely explained by changes in whole body LAC and GLY during recovery based on scenarios of either oxidation or GLY resynthesis as the primary fate of LAC. The classical 'O_2 debt hypothesis' (Hill and Lupton, Quart. J. Med. 16: 135–171, 1923; Margaria *et al.*, Am. J. Physiol. 106: 689–715, 1933) cannot be the complete explanation of EPOC in the trout.

The 'oxygen debt' hypothesis originated by Hill and Lupton (1923) was based on the observation that during strenuous exercise, muscle apparently required a greater oxygen (O_2) utilization than the amount of O_2 supplied during the period of exercise itself. Most of this extra energy was derived from anaerobic metabolism with the formation of lactic acid. Thus, the muscle went into an 'O_2 debt' which would subsequently be repaid during recovery by maintenance of an elevated O_2 consumption ($\dot{M}_{O_2}$). The excess post-exercise O_2 consumption (EPOC) above rest, *i.e.*, the oxygen debt, was defined as the total amount of O_2 used to recover from exercise. The lactate (LAC) accumulated during exercise acted as 'security', which was oxidized during recovery in order to repay

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this O_2 debt (Hill and Lupton, 1923). In 1933, Margaria *et al.* modified this hypothesis slightly to include an initial fast component ('alactacid oxygen debt'), which was attributed to recovery of adenosine triphosphate (ATP), creatine phosphate (CP), and internal O_2 stores. The second, slower component ('lactacid oxygen debt') was used in LAC recovery, oxidation of some of the LAC providing sufficient ATP to convert the remainder back to glucose or glycogen (GLY).

There is good evidence in mammals that the O_2 debt hypothesis cannot be the sole explanation of EPOC (see Gaesser and Brooks, 1984, for review). Factors other than LAC accumulation, such as intensity and duration of exercise (Segal and Brooks, 1979; Bahr *et al.*, 1987), have been shown to affect EPOC; these changes occurred independent of LAC accumulation. Furthermore, elevations in both body temperature and catecholamine levels in mammals have been found to significantly contribute to EPOC (Barnard and Foss, 1969; Brooks *et al.*, 1971).

Although the O_2 debt hypothesis was originally based on experiments with a poikilothermic preparation (isolated frog muscle; Hill and Lupton, 1923), to our knowledge, there has only been one quantitative examination of this concept in an *intact* poikilotherm. Wieser *et al.* (1985), using juvenile rainbow trout, reported that post-exercise energy generation exceeded apparent energy demand. However, their calculation of energy demand considered only the cost of replenishing the internal phosphagen and O_2 stores (alactacid debt), and not the fate of LAC. Our goal was to extend this analysis to both fast (alactacid) and slow (lactacid) components of the debt in juvenile trout.

The present study evaluates the classical O_2 debt hypothesis by means of a detailed quantitative examination of the cost of recovery from exhaustive exercise, in terms of both O_2 and metabolic (ATP) equivalents in juvenile rainbow trout. Small fish offer certain advantages for such studies. First, the low body temperature results in a slow time course of changes, which improves the accuracy of assessment. Second, body temperature elevation as a result of the exercise, which is a complication in mammals, is minimal in poikilothermic fish because of efficient heat transfer at the gills. Third, the small body size of trout fry is ideal for rapid whole body freeze-clamping (*e.g.* Wieser *et al.*, 1985; Van den Thillart *et al.*, 1990), quickly halting metabolic reactions, as well as avoiding the problem of compartmentalization of metabolites.

To this end, $\dot{M}_{O_2}$ was measured simultaneously with a number of metabolites (lactate, glycogen, glucose, creatine phosphate, ATP, ADP, AMP) in the whole body at rest, and over a 24 h period following a 5 min bout of exhaustive exercise. In order to better understand the basis of EPOC, the data were compared with theoretical scenarios based on either glycogen resynthesis or oxidation as the primary fate of the post-exercise LAC burden.

Materials and methods

Animals. Juvenile rainbow trout ($n = 170$) were obtained from Spring Valley Trout Farm (Petersburg, Ontario) and held in large 400 L circular tanks with well-aerated

fresh dechlorinated Hamilton tap water (composition as in Milligan and Wood, 1986) 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 other day for the 2 weeks prior to experimentation and over the course of the experiments, which took 4 weeks to complete. Fish were fasted for 2–3 days prior to experiments and then were weighed. A small weight range (2–3 g) was used in order to minimize variation in exercise capacity and metabolite levels.

Respirometry.   Fish were allowed to acclimate overnight to respirometers (blackened 20 ml syringe barrels fitted with 3-way stopcocks), which were connected to a Gilson Minipuls peristaltic pump to regulate flow (0.7–0.8 L/h). Samples of inflowing and outflowing water were taken in 1 ml glass syringes for measurement of P_{O_2} (Radiometer E5046 P_{O_2} electrode thermostatted to $15\,°C$, connected to a Cameron Instruments oxygen meter, and calibrated with precision gas/water mixtures). O_2 consumption ($\dot{M}_{O_2}$) in terms of $\mu mol \cdot g^{-1} \cdot h^{-1}$ was calculated by the Fick principle:

$$\dot{M}_{O_2} = [\Delta P_{O_2}\,(Torr) \cdot \alpha_{O_2}\,(\mu mol \cdot Torr^{-1} \cdot L^{-1}) \cdot flow\,(L \cdot h^{-1})]/[weight\,(g)]$$

where α_{O_2} is the solubility coefficient of O_2 in water at the experimental temperature. Inflow P_{O_2} was 145–160 Torr; typically ΔP_{O_2} at rest was 15–20 Torr, which increased to 30–45 Torr after exercise. Tests demonstrated that the minimum mixing time to yield a representative ΔP_{O_2} was 5 min; thus the first post-exercise $\dot{M}_{O_2}$ measurement was made at 5 min after the end of the exercise (when the fish was replaced in the chamber).

$\dot{M}_{O_2}$ *study.*   After 12 h acclimation, three resting $\dot{M}_{O_2}$ measurements were taken at 10 min intervals just prior to exercise. Fish ($n = 10$) were then exercised to exhaustion by severely chasing an individual fish for 3 min by hand around a 10 L bucket containing 1.5 L of water and then for another 2 min by applying a 9 V battery to the tail. By the end of the 5 min, fish no longer struggled or responded when handled and were returned to the respirometers. No animals died as a result of the experimental exercise treatment.

Inflow and outflow water samples were taken at 5 min post-exercise, then every 10 min up to 90 min, and then every subsequent 30 min up to 270 min (4.5 h). Coincident with these measurements, samples from control fish were taken every 30 min for the full 4.5 h 'recovery' period but the fish were not exercised. These $\dot{M}_{O_2}$ data were subsequently analyzed by curve stripping (Riggs, 1963), to determine the fast and slow component of the O_2 debt. For comparison, another method was used to independently measure the total EPOC in the same data set. The total area under the mean $\dot{M}_{O_2}$ recovery curve, relative to pre-exercise levels, was directly measured using a GTCO Digi-Pad digitizer connected to a Zenith Data Systems microcomputer.

Values have been reported as means ± 1 standard error ($n = 10$). Significance was tested using Student's two-tailed t-test, paired design, at $P < 0.05$.

Metabolite study.   Fish were acclimated to the respirometer, exercised as described, and allowed to recover for 24 h. $\dot{M}_{O_2}$ was measured on 8–10 separate fish at each of various times during the recovery (5 min, 0.5 h, 1.0, 1.5, 2, 3, 4, 6, 8, 12, 16, 20 and 24 h). The same fish were then immediately terminated at these times for whole body metabolite determination (*i. e.*, simultaneous $\dot{M}_{O_2}$ and metabolite measurements). In addition, a group was terminated immediately after the end of exercise (0 h) for metabolite measurement only ($\dot{M}_{O_2}$ could not be measured at this time). Two control $\dot{M}_{O_2}$ and metabolite groups (non-exercised fish placed in the respirometers for the appropriate duration) were also sampled at the start of the experiment (C1) and at around 14 h recovery (C2). The total EPOC was measured as the area under the mean $\dot{M}_{O_2}$ recovery curve, relative to C1, using the digitizer pad.

Sampling of fish was accomplished within 5 s by quickly emptying the syringe into a small net, stunning the fish on the head, and then freeze-clamping it in liquid nitrogen with pre-cooled ($-196\,°C$) aluminium tongs. Fish that struggled excessively (more than 3 tail flaps) were discarded. 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 desiccator at $-60\,°C$ until metabolites could be measured.

Freeze-dried tissue was assayed fluorometrically (Fluoro-micro-photometer, American Instrument Co., Maryland) for ATP, ADP, AMP, CP, LAC, GLY, and glucose by the enzymatic methods of Bergmeyer (1965). All values have been reported as means $\pm$ 1 S.E.M. (*n* between 8–10), unless otherwise stated. Significance was tested using Student's two-tailed *t*-test at $P < 0.05$.

Results

$\dot{M}_{O_2}$ study. $\dot{M}_{O_2}$ increased from $10.5 \pm 0.8\ \mu mol \cdot g^{-1} \cdot h^{-1}$ (mean of three samples taken prior to exercise) to $22.1 \pm 1.9\ \mu mol \cdot g^{-1} \cdot h^{-1}$ at 5 min after the cessation of exercise (fig. 1A) and returned to resting levels between 3.5–4.5 h. Control $\dot{M}_{O_2}$ did not vary significantly over time. This study was undertaken to measure $\dot{M}_{O_2}$ continuously over time, both before and after exercise, in the same fish so that variability in the measurements was kept to a minimum. This would serve as a reference response to ensure that when terminal samples were used in the metabolite study (different individual fish at each sample time), the pattern of $\dot{M}_{O_2}$ recovery remained consistent.

The $\dot{M}_{O_2}$ recovery curve can be described by two exponential relations plus a constant C, which is the baseline level for resting $\dot{M}_{O_2}$. The equation has the form:

$$Y = Ae^{-k_1 t} + Be^{-k_2 t} + C.$$

Thus, for the data in fig. 1A, the following equation was obtained:
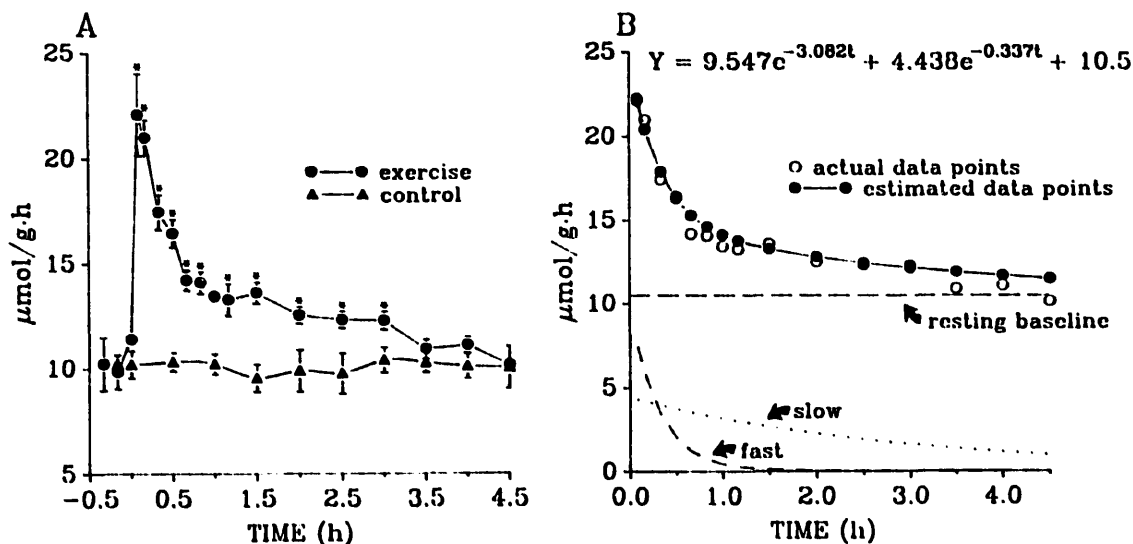
$$Y = 9.547e^{-3.082t} + 4.438e^{-0.337t} + 10.5.$$

Fig. 1. (A) Effect of a 5 min bout of exhaustive exercise on O_2 consumption ($\dot{M}_{O_2}$) in juvenile rainbow trout, from the $\dot{M}_{O_2}$ study. Pre-exercise values are a mean of 3 measurements per fish. First post-exercise sample was taken at 5 min post-exercise. (▲) Indicates non-exercised controls. (●) Indicates exercised fish. Means ± 1 SE, $n = 10$. The same 10 fish were followed throughout. (*) Indicates significantly different ($P < 0.05$) from controls. (B) Curve-stripping analysis of the data from A: mean post-exercise $\dot{M}_{O_2}$ values with estimated equation of the curve. (O) Actual data points. (●) Estimated data points. The straight dashed line indicates the pre-exercise baseline value. The fast (dashed) and slow (dotted) components are also plotted separately.

Subtracting the baseline and calculating the integral of the separate components (Riggs, 1963), the EPOCs for the fast and slow components were determined (fig. 2B). The EPOC of the fast component was 2.4 μmol O_2/g wet wt. or 12.0 nmol O_2/mg dry wt. The half-time of the reaction was 0.23 h (based on $t_{1/2} = \ln 2/k$). The EPOC of the slow component was 8.75 μmol O_2/g wet wt. or 43.75 nmol O_2/mg dry wt., with $t_{1/2} = 2.1$ h. Thus, the total EPOC was 55.75 nmol O_2/mg dry wt. The total EPOC directly measured as the area under the $\dot{M}_{O_2}$ recovery curve was 45.5 nmol O_2/mg dry wt. This small discrepancy largely resulted from uncertainty in the 'tail' of the $\dot{M}_{O_2}$ recovery curve; the area measurements terminated at 3.5 h post-exercise, when $\dot{M}_{O_2}$ was no longer significantly elevated (fig. 1A), whereas the curve-stripping analysis extrapolated to the pre-exercise baseline (fig. 1B). A slight elevation of the fitted line relative to the actual data points was evident beyond 3.0 h (fig. 1B). An additional possible source of error is the fact that the first $\dot{M}_{O_2}$ measurements were not taken until 5 min after the cessation of exercise. However, the curve-stripping technique takes the first 5 min into account by extrapolating back to time 0 using exponentials based on the later data points (fig. 1B).

Metabolite study.   The corresponding $\dot{M}_{O_2}$ measurements (results not shown) were very similar to the $\dot{M}_{O_2}$ study, though with greater variability as a different group of fish was employed at each sample time. $\dot{M}_{O_2}$ increased about 2.5 times (from 7.7 ± 0.6 to 19.2 ± 0.7 μmol·g^{-1}·h^{-1} wet wt.), required between 3–6 h to return to resting levels
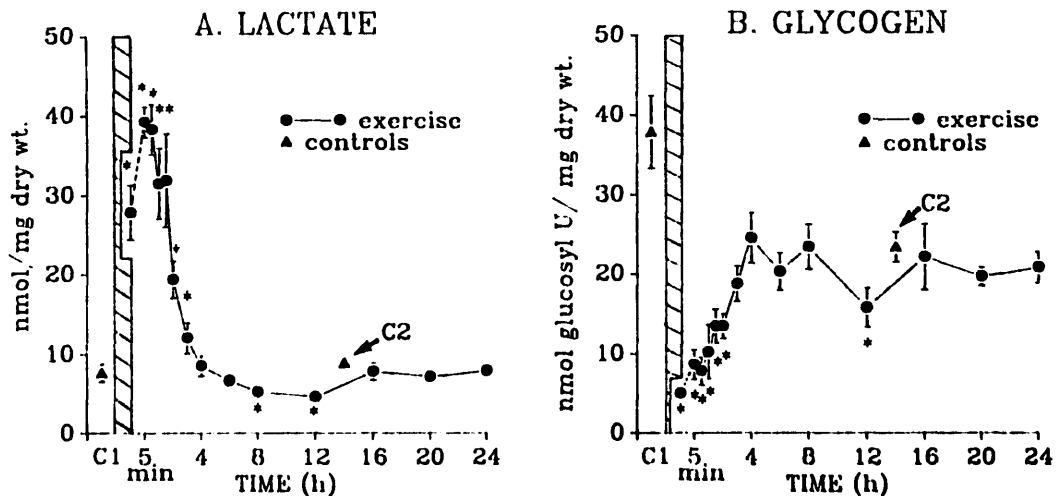
Fig. 2. Changes in whole-body (A) lactate levels and (B) glycogen levels after exhaustive exercise in juvenile rainbow trout. (▲) Denoted as C1 and C2, indicates non-exercised controls. (●) Indicates exercised fish. Cross-hatched bar indicates 5 min exercise bout. Note the expanded time scale separating the zero (immediately post-exercise) sample and the 5 min sample. Means ($n = 10$) $\pm$ 1 SE. Error bars not seen are contained within the sample points. In A, * found above sample indicates significantly elevated ($P < 0.05$) above C1, while * found below sample indicates significantly lower ($P < 0.05$) than C1. In B, since all sample points are significantly less than C1, * indicates significantly less than C2.

and thereafter remained relatively constant through 24 h. The total EPOC, estimated as the area under the mean $\dot{M}_{O_2}$ recovery curve, was 12.5 μmol/g wet wt. or 62.5 nmol/mg dry wt.

For all whole body metabolites, except GLY (see below), the two control values (C1 and C2) were not significantly different from one another. LAC in the whole body (fig. 2A) increased from 7.7 $\pm$ 1.1 nmol/mg dry wt. at rest to 27.8 $\pm$ 3.4 nmol/mg dry wt. immediately post-exercise, and continued to increase to 39.2 $\pm$ 2.0 nmol/mg dry wt. at 5 min post-exercise. After 0.5 h, disappearance of LAC occurred at a more or less exponential rate and there was no longer a significant difference relative to pre-exercise values by 4 h. At 8 and 12 h, whole-body LAC dropped significantly below resting levels and then returned to pre-exercise levels. Thereafter, LAC levels remained relatively constant for the remainder of the recovery period.

Whole-body GLY (fig. 2B) decreased to 13% of resting levels immediately post-exercise (37.8 $\pm$ 4.6 to 5.0 $\pm$ 0.7 nmol glucosyl U/mg dry wt.) and began to recover immediately. By 4 h, levels had stabilized at about 65% of the C1 resting levels (24.5 $\pm$ 3.2 nmol glucosyl U/mg dry wt.). However, this was not significantly different from the second control (C2, 23.4 $\pm$ 1.9 nmol glucosyl U/mg dry wt.), which had also declined over time. Very little change occurred for the remainder of the 24 h recovery period. Glucose levels (not shown) in the whole body averaged about 7 nmol/mg dry wt. and exhibited no significant change throughout the entire experimental period.

ATP levels in the whole body (fig. 3A) decreased to 24% resting levels after exercise (7.9 $\pm$ 1.1 to 1.9 $\pm$ 0.5 nmol/mg dry wt.), and had recovered by 1.0–1.5 h. There was no significant change thereafter. Whole-body CP (fig. 3B) decreased to 35% resting
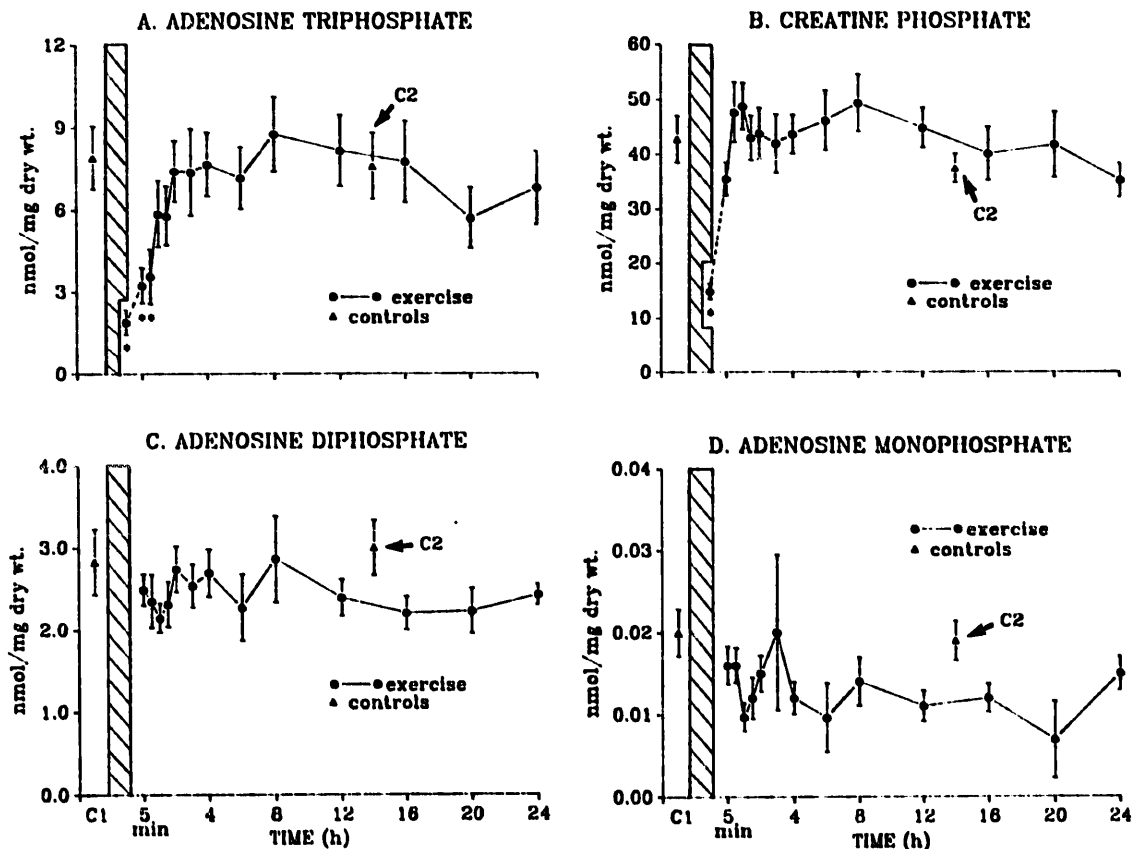
Fig. 3. Changes in whole-body (A) adenosine triphosphate (ATP) levels, (B) creatine phosphate (CP) levels, (C) adenosine diphosphate (ADP) levels and (D) adenosine monophosphate (AMP) levels after exhaustive exercise in juvenile rainbow trout. Means (n = 10) ± 1 SE. Other details as in legend of Fig. 2A.

levels immediately post-exercise (42.7 ± 2.7 to 14.8 ± 1.4 nmol/mg dry wt.), but by 5 min had recovered to 81% resting levels (35.5 ± 3.1 nmol/mg dry wt.), a non-significant difference from controls. A tendency for overshoot during the next few hours was observed, but this was also non-significant. ADP (2.8 ± 0.4 nmol/mg dry wt; fig. 3C) and AMP (0.020 ± 0.003 nmol/mg dry wt; fig. 3D) levels did not vary significantly at any time after exercise.

Theoretical analysis.   The budget analysis is based on the first 6 h of recovery, by which time $\dot{M}_{O_2}$ and all metabolites had returned to resting and/or stable levels. The values used in the following calculations are based on the measured mean changes in metabolite levels as tabulated in table 1. All values have been expressed in terms of nmol/mg dry weight. Since the overall pattern and magnitude of $\dot{M}_{O_2}$ changes were very similar in the two studies, we have chosen to use the EPOC values estimated from the $\dot{M}_{O_2}$ study, rather than from the metabolite study where they were slightly more variable; none of our conclusions would have been substantially altered had we chosen the latter instead.

(1) The *fast component* of EPOC was 12.0 nmol O_2/mg dry wt. (table 2). Assuming

TABLE 1

Changes in metabolite levels over the period 0–6 h after a burst of exhaustive exercise.

	Resting levels	Post-exercise levels	At 6 h recovery	Difference (0–6 h)
LAC (nmol/mg dry wt.)	7.7	39.2	6.7	32.5
GLY (nmol glucosyl U/mg dry wt.)	37.8	7.9	20.3	12.4
ATP (nmol/mg dry wt.)	7.9	1.9	7.2	5.3
CP (nmol/mg dry wt.)	42.7	14.8	46.1	31.3
Glucose (nmol/mg dry wt.)	6.1	7.1	8.2	–[a]
ADP (nmol/mg dry wt.)	2.8	2.5	2.3	–[a]
AMP (nmol/mg dry wt.)	0.02 ·	0.02	0.02	–[a]

GLY, glycogen; CP, creatine phosphate; LAC, lactate.

[a] As these metabolites failed to show any significant changes due to exercise, these values were not included in the cost of recovery budget.

TABLE 2

A budget analysis of the fast component of EPOC.

	ATP equivalents (nmol/mg dry wt.)	O_2 equivalents (nmol/mg dry wt.)
Measured restoration of ATP[a]	15.9	2.7
Measured restoration of CP	31.3	5.2
Estimated restoration of blood and tissue O_2 stores	–	0.7
Estimated increased cost of ventilatory work[b]	7.2	1.2
Estimated increased cost of cardiac work[b]	1.4	0.2
Total	55.8	10.0
Measured fast component		12.0
Percentage accounted for		83%

See text for details.

[a] As there were no changes in ADP and AMP, *de novo* synthesis of ATP is assumed (*i.e.* 3 ∼P/ATP resynthesized).

[b] Based on Farrell and Steffensen (1987).

this largely represents the classic alactic component (Margaria *et al.*, 1933), it could be satisfactorily accounted for by the measured resynthesis of ATP and CP, reasonable assumptions about the restoration of body O_2 stores, and increased ventilatory and cardiac energy expenditure over this period (table 2). High energy phosphate restoration was converted into O_2 equivalents based on a standard $P : O_2$ ratio of 6 (Hultman *et al.*, 1967). The body O_2 repletion was estimated as 50% of the blood capacity plus 10% of the dissolved component. If significant myoglobin O_2 stores are also involved, this may have been slightly underestimated, but would, in any event, be a small component of the total. Ventilatory (10% of EPOC) and cardiac (1.9% of EPOC) expenditures were taken from the analysis of Farrell and Steffensen (1987). Since ADP and AMP levels were unchanged, IMP reamination was assumed for ATP synthesis.

(2) The *slow component* of EPOC was 43.8 nmol/mg dry wt. Two different scenarios were tested to account for this component.

The first scenario (A) assumed that all of the GLY resynthesized (12.4 nmol/mg) came from LAC (24.8 nmol/mg), while the remainder of the LAC (7.7 nmol/mg) would be oxidized (table 3). GLY resynthesis consumed about 75% of the total LAC cleared, leaving about 25% for oxidation. A portion of the oxidized LAC would likely have contributed to the fast component. Assuming that all of the fast component (12 nmol/mg) was fueled by oxidation of LAC (4 nmol/mg), this would leave only 3.7 nmol LAC/mg (= 11.1 nmol O_2/mg) to contribute to the slow component. Only 41% of the *total* EPOC would be accounted for. On the other hand, this scenario would account for all of the ATP produced.

The second scenario (B) assumed that the entire slow component of the EPOC was devoted to oxidizing LAC, as was the entire fast component, and that the remainder of the LAC was converted to GLY (table 4). Oxidation of LAC by the slow component

TABLE 3

Budget analysis of the total EPOC based on assumption that all GLY resynthesis was from LAC, and the remainder of the LAC was oxidized (scenario A).

	O_2 (nmol/mg)	LAC (nmol/mg)	GLY (nmol/mg)	ATP (nmol/mg)
GLY resynthesis	–	– 24.8	+ 12.4	– 80.6
LAC oxidation due to slow component	– 11.1	– 3.7	–	+ 66.6
LAC oxidation due to fast component	– 12.0	– 4.0	–	+ 72.0
Total	– 23.1	– 32.5	+ 12.4	+ 58.0
Measured changes	– 55.8	– 32.5	+ 12.4	+ 55.8[a]
Percent accounted for	41%	100%	100%	104%

Restoration of the fast component has been assumed as having come from LAC oxidation. See text for details. All units are in nmol/mg dry weight. For abbreviations, see table 1.

[a] From table 2.

TABLE 4

Budget analysis of the total EPOC based on assumption that the entire EPOC oxidized LAC and that the remaining LAC was converted to GLY (scenario B).

	O_2 (nmol/mg)	LAC (nmol/mg)	GLY (nmol/mg)	ATP (nmol/mg)
GLY resynthesis	–	– 13.9	+ 6.9	– 45.2
LAC oxidation due to slow component	– 43.8	– 14.6	–	+ 262.8
LAC oxidation due to fast component	– 12.0	– 4.0	–	+ 72.0
Total	– 55.8	– 32.5	+ 6.9	+ 289.6
Measured changes	– 55.8	– 32.5	+ 12.4	+ 55.8[a]
Percent accounted for	100%	100%	56%	519%

Restoration of the fast component has been assumed as having come from LAC oxidation. See text for details. All units are in nmol/mg dry weight. For abbreviations, see table 1.
[a] From table 2.

accounted for 45% (14.6 nmol/mg) of the total LAC burden (32.5 nmol/mg), while the fast component accounted for a further 12% (4 nmol/mg). Thus only 43% (13.9 nmol/mg) of the LAC burden remained for GLY resynthesis, accounting for 56% (6.9 nmol/mg) of the actual amount of GLY resynthesized (12.4 nmol/mg). However, a much greater quantity (> 500%) of ATP was produced by this scenario than could be accounted for by the measured changes (table 4). Presumably, part of this excess could be used to resynthesize GLY from other sources.

Clearly, whichever scenario is chosen, the measured slow component of EPOC was much greater than could be accounted for by the standard pathways of LAC oxidation and GLY resynthesis from LAC.

Discussion

Resting levels. Resting $\dot{M}_{O_2}$ measurements (7.7–10.5 μmol·g^{-1}·h^{-1}; fig. 1) in the present study on 2–3 g rainbow trout agree well with the findings of Wieser *et al.* (1985) in similar sized rainbow trout, as do resting LAC (7.7 nmol/mg dry wt; fig. 2A) and ATP levels (7.9 nmol/mg; fig. 3A). However, CP levels in the present study (42.7 nmol/mg; fig. 3B) were about 40% higher. Pearson *et al.* (1990) have recently used the nervous depressant diazepam to reduce sampling disturbance when freeze clamping 50 g rainbow trout. Assuming that white muscle constitutes about 60% of the body weight, their resting LAC levels were 50% lower and resting ATP levels 100% higher than in the present study. While this difference suggests that their fish were closer to 'absolute rest', their CP levels were only 55% of CP in the present study. Resting CP, ADP, GLY and LAC levels in the present study were also comparable to those reported by Milligan

and Wood (1986) and Dobson and Hochachka (1987). While variability in resting metabolite levels may result from differences in size, seasonal variation, sampling methods and analytical methods (Van den Thillart *et al.*, 1990), the present resting levels are in general agreement with previous determinations. CP levels, which are usually considered the most sensitive index of disturbance, seemed to be slightly higher than those reported in the literature, suggesting that the freeze drying technique used in the present study may have helped prevent degradation due to thawing prior to analysis (Van den Thillart *et al.*, 1990).

The effect of exercise. $\dot{M}_{O_2}$ increased about 2–2.5 times after exhaustive exercise (fig. 1), comparable to the increase in $\dot{M}_{O_2}$ in 3–10 g rainbow trout (11.3 to 22.2 μmol·g^{-1}·h^{-1} wet wt.) after 60 s of electrical stimulation (Wieser *et al.*, 1985). However the increase was much less than that seen in 50 g salmon, burst swimming at 4 BL/s (about 4.5 to 56.3 μmol·g^{-1}·h^{-1} wet wt.), although recovery time was similar (Brett, 1964). The relative increase in $\dot{M}_{O_2}$ after exhaustive exercise is greater in larger fish than in smaller fish (Goolish, 1989). Thus, the discrepancies between studies may be size-related or due to differences in exercise protocol.

Whole-body GLY levels decreased to about 13% resting levels after the 5 min exercise bout (fig. 2B), indicating that the fish were thoroughly exhausted. Recovery of GLY reserves was slow, as has been shown in several previous studies (*e.g.*, Black *et al.*, 1962; Milligan and Wood, 1986; Pearson *et al.*, 1990). Maximum restoration, amounting to only 65% resting GLY levels, was achieved by 4 h. The significantly lower GLY seen in the second control sample taken at around 14 h into the experiment (C2 relative to C1) suggests that the fish had recovered as much GLY as was possible without another source of fuel (*i.e.*, food).

Whole-body LAC levels were elevated about 3.5 times resting levels immediately after exercise, but continued to increase significantly up to 30 min (fig. 2A), even though neither GLY (fig. 2B) nor glucose (not shown) levels decreased. Glycolytic intermediates, such as glucose 6-phosphate and fructose 6-phosphate, are elevated in the muscle immediately after exercise (Pearson *et al.*, 1990), at levels which could easily account for the extra 11.4 nmol LAC/mg generated after the cessation of exercise in the present study. The later reduction in LAC levels below resting values (at 8–12 h; fig. 2B) has also been demonstrated previously (Milligan and Wood, 1986), and suggests that the fish are able to tap their normal LAC reserve as an additional energy source at this time.

About 30 nmol GLY/mg dry wt. (in glucosyl units) were depleted in the 5 min exercise bout, whereas a post-exercise maximum of only 32 nmol LAC/mg dry wt. had accumulated. This is far from the expected stoichiometry of 1:2 GLY depletion:LAC accumulation, had the GLY utilization been entirely anaerobic, as in the studies of Black *et al.* (1962) and Dobson and Hochachka (1987). The present fish probably also utilized GLY as an aerobic fuel during exhaustive exercise, so that the rate of pyruvate oxidation increased along with the increase in glycolytic flux, resulting in a smaller accumulation of LAC than depletion of GLY. Indeed, the fact that half of the GLY was apparently

oxidized during exercise, rather than transformed to LAC, may have been an additional factor contributing to the incomplete restoration of GLY during the post-exercise period (fig. 2B).

The near depletion of both ATP and CP stores after exhaustive exercise (fig. 3A and B) was consistent with the findings of others (Milligan and Wood, 1986; Dobson and Hochachka, 1987; Pearson et al., 1990). Since neither ADP nor AMP levels changed significantly after exercise (fig. 3C and D), all the ATP hydrolyzed presumably formed IMP and NH_4^+ (Dobson and Hochachka, 1987). IMP and NH_4^+ were not measured in the present study.

A major point of variation amongst exercise studies on salmonids is the rate of ATP and CP resynthesis. In the present study, both ATP and CP were repleted rapidly (< 1 h), with CP resynthesis clearly preceding ATP restoration, as in mammals. Wieser et al. (1985) also reported fast phosphagen repletion (within 10 min). On the other hand, Milligan and Wood (1986) and Dobson and Hochachka (1987) found CP recovery was much slower than ATP recovery, requiring up to 4 h, but with comparable ATP repletion times to the present study. Pearson et al. (1990) reported 1 h repletion times for CP and 3 h for ATP. The reason(s) for these substantial discrepancies amongst different studies is unknown. However, one possibility is the potential for error in accurately measuring true resting levels, due either to degradation of substrate during extraction and assay (Van den Thillart et al., 1990), or to phosphagen depletion caused by sampling disturbance (Dobson and Hochachka, 1987). Clearly, there is a need for further detailed studies on the pathways and controls of ATP and CP resynthesis in salmonids.

Interpretation of the theoretical analysis. A primary goal of this study was to construct a detailed quantitative analysis of the relationship(s) between EPOC and metabolic changes during post-exercise recovery. As in mammals, EPOC in the trout exhibited two components, with fast and slow rate constants (fig. 1B). The relative sizes of the two components (fast = 20%; slow = 80%) were comparable to the situation in mammals (fast = 10% in humans; Bangsbo et al., 1990), but the time constants were much slower (fast component $t_{1/2}$ = 0.23 h *versus* ≈ 30 s in man; slow component $t_{1/2}$ = 2.1 h *versus* < 0.5 h in man – Margaria et al., 1933). This difference was at least partially attributable to the Q_{10} effect on respiration and metabolism (trout = 15 °C, mammal = 37 °C). Furthermore, body temperature increases after exercise in mammals (Brooks et al., 1971), but stays more or less constant in the poikilothermic fish.

The fast alactic component in the trout (table 2) was satisfactorily accounted for by the same factors as in mammals (Bangsbo et al., 1990). About 65% was due to the measured repletion of ATP and CP stores, and the remainder accounted for by reasonable estimates of the refilling of body O_2 stores and increased ventilatory and cardiac work loads. The fuel source which was oxidized by the fast component did not appear to be glucose (which was stable) nor GLY (which actually increased during this period; fig. 2B). LAC is certainly a possibility, and so in the subsequent analyses we have assumed LAC was the fuel utilized by the fast component. However, even if this assumption is not true, it would reinforce the basic conclusion of the analyses – *i.e.* the non-agreement between LAC metabolism and EPOC.

The original explanation of the slow component by the classical 'oxygen debt' hypothesis was the cost of LAC removal, the majority of which was resynthesized to GLY. In humans, it now appears that oxidation is the major fate of LAC; only 25% of the O_2 debt could be attributed to GLY resynthesis (Bangsbo *et al.*, 1990). In fish, the fate of LAC remains controversial, though an increasing body of evidence suggests that the bulk of the post-exercise LAC load remains in the white muscle, to be metabolized *in situ* by gluconeogenesis or oxidation, rather than being released into the blood stream for processing by the liver (*i.e.* Cori cycle) and other tissues as in mammals (*e.g.* Milligan and Wood, 1986; Milligan and McDonald, 1988). However, the relative contributions of oxidation *versus* GLY resynthesis remains unknown. On the one hand, the oxidative capacity of teleost white muscle appears to be low, at least in comparison to more aerobic tissues such as heart, liver, and red muscle (Bilinski and Jonas, 1972). On the other hand, to our knowledge there is yet no definitive evidence that the necessary enzymes for GLY resynthesis from LAC are present in teleost white muscle.

In light of this uncertainty, we investigated scenarios representing the two possible extremes – *i.e.* that all GLY resynthesis was from LAC so that only the 'left over' LAC was oxidized (table 3), or that all of the slow component of EPOC represented LAC oxidation, so that only the left over LAC was resynthesized to GLY (table 4). The first scenario would clearly represent the more energy efficient strategy. However, by this scenario, for every molecule of LAC which was oxidized, only about 3.2 molecules of LAC were converted into GLY (table 3), in contrast to Hill and Lupton's (1923) ratio of 1 : 5 in frog muscle. Even at this lower efficiency, this scenario explained only 25% of the measured slow component of EPOC (11.1 nmol O_2/mg used for LAC oxidation *versus* 43.8 nmol O_2/mg estimated from the curve stripping analysis).

The alternative scenario resulted in a vast amount of ATP which could not be accounted for by the measured data, and failed to account for all the GLY resynthesized due to the 'wastage' of LAC in oxidation (table 4). The true fate of the LAC burden may lie somewhere between these two extremes. However, a conclusion common to both scenarios was that the measured slow component of EPOC was much greater than could be explained by standard pathways of LAC oxidation and GLY resynthesis from LAC. We conclude that as in mammals, the classical O_2 debt hypothesis cannot be the sole explanation of EPOC in trout.

A potential source of error in either scenario would be storage of ATP equivalents in glycolytic intermediates (*e.g.* glucose 6-phosphate, fructose 6-phosphate) or the production of alternate end-products of anaerobic metabolism (*e.g.* alanine, malate, pyruvate) which were not measured in the present study. The data of Dobson *et al.* (1987) on exhaustively exercised trout indicate that the latter are very minor end-products relative to LAC, and would have had negligible quantitative influence on the calculations. However, the results of Pearson *et al.* (1990) indicate that the production of glycolytic intermediates can account for up to 20% of the measured GLY depletion in the first hour after exercise. This potential error, while significant, would not change our basic conclusion that the EPOC is too large to be explained satisfactorily by either of the scenarios.

In mammals, post-exercise elevation of body temperature is thought to play a major role in the discrepancy between EPOC and LAC metabolism (Brooks et al., 1971). This cannot be the explanation in poikilothermic fish, so other explanations must be sought. Two factors which are thought to be significant contributors to the discrepancy in mammals are worthy of future investigation in fish. The first is the possible role of increased 'futile' substrate cycling after exercise, which would increase ATP turnover (Newsholme, 1978). For example, increased cycling between triacylglycerol and fatty acids during recovery from exercise can account for up to 14% of the O_2 debt in humans (Wolfe et al., 1990). The second is the mobilization of catecholamines by the combined exercise plus stress protocol. Catecholamine mobilization is known to stimulate both metabolic rate (Barnard and Foss, 1969) and futile substrate cycling (Bahr et al., 1987). Plasma adrenaline and noradrenaline have been reported to increase up to 100-fold after a similar exhaustive exercise-stress protocol in trout (Butler et al., 1986).

References

Bahr, R., I. Ingnes, O. Vaage, O. Sejersted and E. Newsholme (1987). Effect of duration of exercise on postexercise oxygen consumption. *J. Appl. Physiol.* 62: 485–490.

Bangsbo, J., P.D. Gollnick, T.E. Graham, C. Juel, B. Kiens, M. Mizuno and B. Saltin (1990). Anaerobic energy production and O_2 deficit-debt relationship during exhaustive exercise in humans. *J. Physiol.* 422: 539–559.

Barnard, R.J. and M.L. Foss (1969). Oxygen debt: effect of beta-adrenergic blockade on the lactacid and alactacid components. *J. Appl. Physiol.* 27: 813–816.

Bergmeyer, H.V. (1965). Methods in enzymatic analysis. New York, Academic Press.

Bilinski, E. and R.E.E. Jonas (1972). Oxidation of lactate to carbon dioxide by rainbow trout *(Salmo gairdneri)* tissues. *J. Fish. Res. Board Can.* 29: 1467–1471.

Black, E.C., A.R. Conner, K.C. Lane and W.G. Chiu (1962). Changes in glycogen, pyruvate and lactate in rainbow trout *(Salmo gairdneri)* during and following muscular activity. *J. Fish. Res. Board Can.* 19: 409–436.

Brett, J.R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Board Can.* 21: 1183–1226.

Brooks, G.A., K.J. Hittelman, J.A. Faulkner and R.E. Beyer (1971). Tissue temperatures and whole-animal oxygen consumption after exercise. *Am. J. Physiol.* 221: 427–431.

Butler, P.J., J.D. Metcalfe and S.A. Ginley (1986). Plasma catecholamines in the lesser spotted dogfish and rainbow trout at rest and during different levels of exercise. *J. Exp. Biol.* 123: 409–421.

Dobson, G.P. and P.W. Hochachka (1987). Role of glycolysis in ε `nylate depletion and repletion during work and recovery in teleost white muscle. *J. Exp. Biol.* 129: 125–140.

Farrell, A.P. and J.F. Steffensen (1987). An analysis of the energetic cost of the branchial and cardiac pumps during sustained swimming in trout. *Fish Physiol. Biochem.* 4: 73–79.

Gaesser, G.A. and G.A. Brooks (1984). Metabolic basis of excess post-exercise oxygen consumption: a review. *Med. Sci. Sports Exerc.* 16: 29–43.

Goolish, E.M. (1989). A comparison of oxygen debt in small and large rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.* 35: 597–598.

Hill, A. V. and H. Lupton (1923). Muscular exercise, lactic acid and the supply and the utilization of oxygen. *Quart. J. Med.* 16: 135–171.

Hultman, E., J. Bergstrom and N. McLennan Anderson (1967). Breakdown and resynthesis of phosphoryl creatine and adenosine triphosphate in connection with muscular work in man. *Scand. J. Clin. Lab. Invest.* 19: 56–66.

Margaria, R., H. T. Edwards and D. B. Dill (1933). The possible mechanisms of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. *Am. J. Physiol.* 106: 689–715.

Milligan, C. L. and C. M. Wood (1986). Tissue intracellular acid-base status and the fate of lactate after exhaustive exercise in the rainbow trout. *J. Exp. Biol.* 123: 123–144.

Milligan, C. L. and D. G. McDonald (1988). *In vivo* lactate kinetics at rest and during recovery from exhaustive exercise in coho salmon *(Oncorhynchus kisutch)* and starry flounder *(Platichthys stellatus). J. Exp. Biol.* 135: 119–131.

Newsholme, E. A. (1978). Substrate cycles: their metabolic, energetic and thermic consequences in man. *Biochem. Soc. Symp.* 43: 183–205.

Pearson, M. P., L. L. Spriet and E. D. Stevens (1990). Effect of sprint training on swim performance and white muscle metabolism during exercise and recovery in rainbow trout *(Salmo gairdneri* Richardson). *J. Exp. Biol.* 149: 45–60.

Riggs, D. S. (1963). The mathematical approach to physiological problems. New York, Williams and Wilkins Co., pp. 146–161.

Segal, S. S. and G. A. Brooks (1979). Effects of glycogen depletion and workload on post-exercise O_2 consumption and blood lactate. *J. Appl. Physiol.* 47: 514–521.

Van den Thillart, S., A. Van Waarde, H. J. Mullar, C. Erkelens and J. Lugtenburg (1990). Determination of high energy phosphate compounds in fish muscle: ^{31}P-NMR spectroscopy and enzymatic methods. *Comp. Biochem. Physiol.* 95B: 789–795.

Wieser, W., U. Platzer and S. Hinterleitner (1985). Anaerobic and aerobic energy production of young rainbow trout *(Salmo gairdneri)* during and after burst of activity. *J. Comp. Physiol.* 155B: 485–492.

Wolfe, R. R., S. Klein, F. Carraro and J.-M. Weber (1990). Role of triglyceride-fatty acid cycle in controlling fat metabolism in humans during and after exercise. *Am. J. Physiol.* 258: E382–E389.