O_2 CONSUMPTION AND METABOLISM OF TROUT AFTER EXERCISE

EFFECTS OF EXHAUSTIVE EXERCISE ON OXYGEN CONSUMPTION AND METABOLISM OF JUVENILE RAINBOW TROUT: AN EVALUATION OF THE O_2 DEBT HYPOTHESIS

By

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ABSTRACT

This thesis examined the effects of exhaustive exercise and post-exercise recovery on metabolism and respiratory gas exchange in juvenile rainbow trout, in relation to the classical oxygen (O_2) debt hypothesis (Hill & Lupton, 1923).

The initial study was a detailed quantitative analysis of the metabolic cost of post-exercise recovery in terms of O_2 and ATP equivalents. A 5 min bout of exhaustive exercise resulted in about a 2.0-2.5 fold increase in O_2 consumption (MO_2), a 6-8 fold increase in whole-body lactate (LAC) levels and a near depletion in whole-body glycogen (GLY), adenosine triphosphate (ATP) and creatine phosphate (CP) stores. Recovery of MO_2, LAC and GLY was usually complete by 6 h, though GLY did not always return to resting levels. Recovery of resting whole-body ATP required 1.0-1.5 h, whereas restoration of CP required only 5 min. Quantitative budgets of the cost of recovery were prepared, based on two opposite assumptions. The first scenario (A) assumed that all the GLY resynthesized came from LAC, and that the remaining 25% of the total LAC cleared was oxidized. This scenario accounted for 48% of the excess post-exercise O_2 consumption (EPOC) and 94% of the ATP budget. The alternative scenario (B), attributed 100% of the EPOC to LAC oxidation (64% of the total LAC cleared), while the remaining LAC (36%) was resynthesized into GLY. As scenario

B accounted for only 35% of the ATP budget, scenario (A) appeared more probable.

Subsequent experiments attempted to experimentally dissociate LAC disappearance from EPOC, via repetitive exercise bouts and prior GLY depletion. A 2nd bout of exhaustive exercise, given 6 h after the 1st, significantly reduced EPOC by 40%, whereas metabolite status (LAC disappearance and GLY, ATP, and CP restoration) remained essentially unchanged. In contrast, prior GLY depletion by 5 days starvation significantly lowered the LAC burden while EPOC remained unchanged.

Neither the two scenarios of the theoretical analysis could adequately explain the relationships between EPOC and the various metabolites. It is likely that some blend of the two approaches may be more realistic. Nevertheless, these approaches all point to the conclusion that the EPOC is not directly related to the metabolism of LAC after exercise in the rainbow trout. Thus, in contrast to the classical O_2 debt hypothesis, LAC disappearance does not determine the magnitude EPOC after exhaustive exercise in rainbow trout.

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LIST OF ABBREVIATIONS

ADP:	adenosine diphosphate
AMP:	adenosine monophosphate
ATP:	adenosine triphosphate
NH_4^+:	ammonium ion
BL/s:	body length per second
Ca^{++}:	calcium ion
CO_2:	carbon dioxide
^{14}C:	carbon-14, a radioactive isotope of carbon
cm/s:	centimeter per second
CP:	creatine phosphate
U_{crit}:	critical swimming velocity
°C:	degree Celcius
EPOC:	excess post-exercise oxygen consumption
R.E.:	gas exchange ratio
GLY:	glycogen
g:	gram
h:	hour
P_i:	inorganic phosphate
IMP:	inosine monophosphate
LAC:	lactate
l:	liter
MO_2max:	maximum O_2 consumption rate
µm:	micro-meter
µmol:	micromole
mg:	milligram
mmol:	millimole
min:	minute
$C_6H_{12}O_6$:	molecular formula of glucose
mATPase:	myosine adenosine triphosphatase
nmol:	nanomole
NH_3:	non-ionized ammonia
O_2:	oxygen
PO_2:	partial pressure of O_2
K^+:	potassium ion
M_{amm}:	rate of ammonia excretion
MCO_2:	rate of CO_2 excretion
MO_2:	rate of O_2 consumption
R.Q.:	respiratory quotient
s:	second
Na^+:	sodium ion
αO_2:	solubility constant of O_2
H_2O:	water

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CHAPTER 1

GENERAL INTRODUCTION

Exercise Metabolism

Exercise, in all vertebrates, can be defined as an increase in the work performed by the locomotory muscles, and is accompanied by an increase in energy demand (Jones & Randall, 1978). During sustained activity at low work levels, the increase in oxygen consumption (MO_2) is adequate to meet these energy demands aerobically through stimulation of glycolysis and O_2 phosphorylation via activation of phosphorylase and pyruvate dehydrogenase, two key regulatory enzymes (Newsholme & Start, 1976). During high intensity work, energy demands are in excess of what can be supplied aerobically. Thus there is an accumulation of metabolic end-products, such as lactate, produced via glycolysis (McGilvery, 1983).

Though it was once thought that these two processes were mutually exclusive in mammals (Margaria *et al.*, 1963), it is now known that lactate (LAC) can be produced by muscles performing low intensity work under essentially "aerobic" conditions (Jobsis & Stainsby, 1968; Brooks, 1971; Connett *et al.*, 1984). However, LAC oxidation by non-working muscles is also stimulated, such that there is no net LAC accumulation (Hubbard, 1973; Ryan *et al.*, 1979). During maximum intensity work, LAC production exceeds LAC

utilization, since a greater percentage of the total energy demand is supplied by glycolytic metabolism.

Oxygen Debt - The Classical Theory

The importance of O_2 for the maintenance of bodily processes, even during the resting state, has long been recognized. Excised tissues cannot survive long periods of O_2 deprivation without loss of activity. However, muscle tissue can survive considerable periods of anoxia during the resting state, especially when kept under lower temperatures. Furthermore, for a limited time, a stimulated muscle can survive in the complete absence of O_2. This was the basis for Hill & Lupton's classical work in 1923 on isolated amphibian muscle, in which they examined the interaction of muscular exercise and the utilization of O_2.

The "oxygen debt" hypothesis originated by Hill & Lupton (1923) was based on data obtained from Hill's earlier studies on isolated frog sartorius muscle, as well as work by Fletcher & Hopkins in 1907 and Meyerhoff in 1920. The muscle was able to tolerate a large difference between the O_2 demand and the O_2 supplied during exercise, such that the muscle went into an "O_2 debt" which was subsequently repaid during recovery by maintenance of an elevated MO_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.

Hill & Lupton (1923) also found that LAC accumulated in the muscle either in the presence of O_2 while being

stimulated, or in the absence of O_2. They felt that the LAC accumulated during this time acted as "security", which was oxidized during recovery in order to repay "the O_2 debt" (Hill & Lupton, 1923).

Glycogen (GLY) was identified as the precursor of LAC during muscular contraction (Hill & Lupton, 1923). GLY disappeared in corresponding amounts with LAC accumulation and reappeared with LAC disappearance, except for a small quantity which was oxidized, as calculated from the O_2 consumption. They estimated that for every 6 molecules of LAC which disappeared, 5 molecules were converted into GLY and 1 molecule of LAC was oxidized to drive the conversion of LAC into GLY.

In 1933, Margaria *et al.* modified this hypothesis slightly due to a lag observed in LAC disappearance after the cessation of exercise. The change in the O_2 consumption curve after exercise was found to be a sum of two exponential functions of time. The "oxygen debt" was then divided into an initial fast component, which was attributed to adenosine triphosphate (ATP) and creatine phosphate (CP) recovery, and the second, slower component, which was used in LAC recovery. These were termed the "alactacid" and "lactacid oxygen debts", respectively (Margaria *et al.*, 1933). The "alactacid oxygen debt" was also observed at low work levels even when LAC did not significantly accumulate.

Evaluation of the O_2 Debt Hypothesis in Mammals

In mammals, the oxygen debt hypothesis may be a far too simplistic explanation of EPOC, as many authors (Harris, 1969; Stainsby & Barclay, 1970; Gaesser & Brooks, 1984) have pointed out. Considerable controversy has arisen at least partially due to differences in the type and degree of exercise employed, the muscle group (fiber types) sampled, and the species examined.

Studies in mammals have indicated that LAC accumulation during exercise in no way determines the magnitude of EPOC. In fact, intensity and duration of exercise appear to be the major determining factors of EPOC (Knuttgen, 1970; Segal & Brooks, 1979). In humans, an O_2 debt or EPOC was present at work intensities as low as 20% MO_2max (maximum O_2 consumption rate), whereas significant changes to resting muscle and blood LAC levels did not occur until 60% MO_2max (Knuttgen & Saltin, 1972). Furthermore, the O_2 consumption recovery curve always consisted of an initial, fast component and a second slower component, regardless of exercise duration (Hagberg *et al.*, 1980), in humans exercised at intensities between 50-80% MO_2max. Thus, both components of EPOC can be present at low work intensities when LAC does not significantly accumulate. This is in contrast to Margaria and co-workers' suggestion (1933) that only the alactacid component is present at these low work intensities.

Furthermore, humans, pre-treated by appropriate diet and exercise so as to be depleted in muscle GLY, exhibited smaller blood LAC accumulations after both moderate (55% MO_2max) and heavy (95% MO_2max) exercise than control subjects. However, the same values of EPOC were measured at both exercise intensities in normal GLY and GLY-depleted subjects (Segal & Brooks, 1979).

Other studies have shown that LAC and MO_2 have dissimilar kinetics and time courses of recovery (Gaesser & Brooks, 1984) and that this may be species dependant. For example, in rats exercised to exhaustion, MO_2 required up to 2 h for recovery, whereas liver, blood and muscle LAC levels were restored in 15 minutes (Gaesser & Brooks, 1980). In contrast, humans exercised at 95% MO_2max showed elevated blood LAC up to 30 minutes after cessation of exercise whereas MO_2 had returned to resting levels much more quickly (Segal & Brooks, 1979).

Furthermore, the classical "O_2 debt hypothesis" is not supported by the results of LAC infusion studies in mammals. During LAC infusion at rest, there was no correlation between LAC disappearance and MO_2. In fact, MO_2 increased with both sodium-LAC infusion and saline infusion (Alpert & Root, 1954) as well as sodium-pyruvate injection (Bertram *et al.*, 1967). This suggests an increase in substrate cycling (eg. Newsholme, 1978), which was not due to the LAC *per se*. LAC infusion during exercise, on the other hand, was associated with a smaller increase in MO_2 compared to

resting subjects (Ryan *et al.*, 1979). Again, there was no correlation between LAC levels and MO_2 recovery.

The Fate of Lactate in Mammals

The fate of LAC after exercise is also still highly controversial. Three opposing views have been presented in the past. The first is the classical Cori cycle theory which proposes that about 85% of the LAC produced from GLY in the muscle during exercise is taken up by the liver, where it is reconverted to GLY. The liver subsequently breaks down the GLY into glucose, which is transported by the blood back to the muscle and stored as GLY (Cori & Cori, 1929). Rowell *et al.* (1966) have since shown that of the total LAC produced in humans after moderate exercise, about 46% was removed by the liver in 60 minutes. Oxidation was estimated to account for about 20% of the total LAC delivered, suggesting that gluconeogenesis was the primary fate of LAC taken up by the liver.

In contrast, Gaesser & Brooks (1980) state that the primary fate of LAC is oxidation. Rats injected with [U-^{14}C] LAC in the resting state incorporated very little (<2%) of the label into GLY, whereas 75% was recovered as $^{14}CO_2$. After exercise to exhaustion, 45-50% of the ^{14}C-labelled LAC was recovered as $^{14}CO_2$ and 20% was recovered as labelled GLY. The major portion of the GLY restored during this time was thought to be resynthesized from blood-borne glucose. Hubbard (1973) found a somewhat different pattern in humans. About 3-7% of a bolus injection of ^{14}C-LAC was recovered as

$^{14}CO_2$ during rest, which increased to 35-68% during exercise. This discrepancy in recovery of the radioactive label may be due to technical differences *i.e.* which of the three carbons of LAC is labelled. Nevertheless, the two studies are in agreement that the largest portion of the accumulated LAC after exercise was oxidized to CO_2 and water and not converted into GLY.

The third viewpoint is in support of the O_2 debt hypothesis and holds that the primary fate of LAC is GLY resynthesis occurring directly in the muscle (Hermansen & Vaage, 1977). Based on rates of LAC disappearance and GLY resynthesis in quadriceps of men exercised intermittently to exhaustion, the production and transfer of an intermediate product such as glucose was improbable. Furthermore, very little LAC efflux or glucose influx occurred, supporting a direct pathway for the resynthesis of GLY in the muscle. More recently, Astrand *et al.* (1986) found that about 50% of the total LAC produced during exercise in humans was utilized for GLY resynthesis. Only a small portion (10%) was taken up by the liver, about 17% was accounted for in the total body water and the remainder (23%) was oxidized.

Much of the evidence provided in support of an *in situ* gluconeogenic pathway has been indirect, leading to controversy regarding its existence and/or function at physiologically significant rates. Crabtree *et al.* (1972) could not detect any pyruvate carboxylase activity in rat quadriceps, and found only low activities of

phosphoenolpyruvate carboxykinase and fructose diphosphatase (FDPase). They suggested that a direct gluconeogenic pathway was theoretically possible; however, due to the low activities of these critical gluconeogenic enzymes, this was unlikely. In contrast, McLane & Holloszy (1979) found significant rates of phosphoenolpyruvate carboxykinase and FDPase in rat hindlimb.

Perhaps some of the differences seen in the past, about whether muscles have the enzymatic capacity to resynthesize GLY may be due to muscle fiber type differences. Fast-twitch muscle fibers in rats were found to convert LAC to GLY at highly significant rates, whereas slow-twitch fibers did not (McLane & Holloszy, 1979). They attributed the slow rate of GLY resynthesis in slow-twitch red fibers to the low activity of FDPase observed. Fell *et al.* (1980) have suggested another possible explanation. Rats exercised to exhaustion and subsequently fed a carbohydrate-rich (65%) diet accumulated GLY in the liver at a faster rate than in the muscle. In contrast, fasted rats exercised to exhaustion preferentially restored muscle GLY at the expense of liver GLY.

Alternative Explanations of EPOC

In summary, studies to date have been unable to show any consistent relationship between the amount of LAC accumulated during exercise and the excess post-exercise O_2 consumption (EPOC). Any such relationships appear to be coincidental at best without temporal or causal association.

Alternative explanations of EPOC have therefore been proposed.

The fast component of EPOC, as originally proposed by Margaria *et al.* (1933), has been repeatedly confirmed as mainly attributable to the repayment (*i.e.* resynthesis) of high-energy phosphates such as CP and ATP, across species and in different muscle groups (Hultman *et al*, 1967; quadriceps of man; Piiper & Spiller, 1970; gastrocnemius of dog). Other factors involved are the restoration of O_2 stores in myoglobin, hemoglobin and body fluids (such as tissue water) and the cost of increased ventilatory and circulatory work associated with exercise (Knuttgen, 1970; Stainsby & Barclay, 1976). After moderate exercise in humans, about 10% of EPOC is required to replace myoglobin stores and 2-5% for blood O_2 stores. After exhaustive exercise, these factors are thought to contribute only a little to the total EPOC (Stainsby & Barclay, 1976).

Recent studies have indicated that a number of factors may significantly contribute to the slow component of EPOC in mammals. Temperature is perhaps the most important of these factors. Prolonged muscular work in mammals results in an increase in core body temperature despite activation of heat loss mechanisms. An increase in temperature will cause an increase in the rates of biochemical reactions and therefore, an increase in cellular respiration (McGilvery, 1983). In rats, for example, Brooks *et al.* (1971) found that the time course of body temperature elevation after exercise

roughly paralleled the time course of EPOC. The authors suggested that a large portion (though not quantified) of EPOC must be attributed to the Q_{10} effect on biochemical reactions. In humans, Hagberg *et al.* (1980) estimated that 60-70% of the slow component of EPOC was attributed to the increase in body temperature after moderate exercise.

Another portion of EPOC is thought to be caused by elevated levels of catecholamines released during exercise (von Euler & Hellner, 1952). The increase in plasma noradrenaline has also been correlated with work intensity in man (Haggendal *et al.*, 1970). Administration of propranolol (a ß-blocking agent) to exercising dogs was found to decrease EPOC, by as much as 30% (Barnard & Foss, 1969). More recently, Gladden *et al.* (1982) found that noradrenaline infusion caused about a 40% increase in EPOC of canine muscle recovering from stimulated contractions.

Substrate cycling or futile cycling is also thought to contribute to EPOC (Newsholme, 1978). A substrate cycle occurs when the forward reaction and the reverse reaction are simultaneously active. Since this would break down ATP without a net change in substrate levels, the cycle is thought to be futile. During exercise, the rate of cycling may be increased, improving the sensitivity of biochemical reactions. This would result in an increase in MO_2 directly, as well as indirectly by the thermogenic effect.

Other minor factors contributing to EPOC are the cost of electrolyte redistribution after muscle contraction (for

example, sodium (Na^+), potassium (K^+) and calcium (Ca^{++})) and tissue repair. The magnitude of these contributions is yet unknown, but it is thought to be small (Stainsby & Barclay, 1976).

The Swimming Musculature of Fish

Muscular tissue of the fish comprises about 40-60% of the total body mass, the greatest portion of which is in the myotomal musculature used for swimming and locomotion. Several types of skeletal muscle can be characterized; the distinction is primarily based upon adenosine triphosphatase (ATPase) activity and fiber diameter but differences in other characteristics also exist.

Red muscle is localized along the lateral line on either side of the fish and makes up about 5-20% of the muscle mass. It is suited for O_2 phosphorylation, showing high myoglobin, lipid and glycogen levels. Red muscle also exhibits high activity of oxidative enzymes such as succinic dehydrogenase and cytochrome oxidase, and low activity of ATPases, and creatine phosphokinase and glycolytic enzymes (Johnston *et al.*, 1975; Johnston *et al.*, 1977). It is made up of small diameter fibers, ranging from 10-50 µm, but the majority of fibers lie between 20-30 µm in diameter (Nag, 1972; Johnston *et al.*, 1975).

Pink muscle, in rainbow trout, comprises less than 1-2% of the total muscle mass and is not a discrete region (Johnston, 1982). It consists of intermediate sized fibers (25-45 µm), usually only found as a few scattered cells.

Pink muscle is partly glycolytic and partly oxidative in nature, showing intermediate activities of both types of enzymes (Johnston *et al.*, 1975; Johnston *et al.*, 1977).

White muscle makes up the rest of the body musculature. In rainbow trout, the white muscle is heterogeneous and often referred to as mosaic muscle for it contains both large and small diameter fibers. They range in diameter from 15 to 95 µm, though most are around 50-75 µm (Nag, 1972; Johnston *et al.*, 1975). The properties of the small white fibers are similar to those of the large white fibers, and dissimilar to those of small red fibers. They are thought to be either small fibers split off from larger white fibers or developing stages of larger white fibers (Proctor *et al.*, 1980). White muscle fibers are generally glycolytic in nature and show high activity of ATPase and creatine phosphokinase but low activity of succinic dehydrogenase and cytochrome oxidase (Johnston *et al.*, 1975; Johnston *et al.*, 1977). They also have low mitochondrial, lipid, myoglobin and glycogen content as compared to red muscle fibers.

The different muscle types are adapted for different types of activities. The red muscle functions during slow sustained swimming, *i.e.* low intensity but high duration exercise (generally less than 2 body lengths per second (BL/s)). This speed can be maintained almost indefinitely, without resulting in fatigue (Beamish, 1978). On the other hand, the white muscle is used during burst activity as when catching prey or escaping from a predator, *i.e.* the highest

speeds possible, which can be maintained for only a short time. However, this division of labour between muscle types is not as clear-cut as it may appear (Mosse & Hudson, 1977).

Electromyographical recordings have in fact shown that fish utilize white muscle to support sustained swimming and there is a threshold speed for white muscle recruitment which is species-specific (Johnston *et al.*, 1977; Johnston & Moon, 1980). In rainbow trout, this threshold is quite low, about 1 BL/s (Hudson, 1973; Greer-Walker & Emerson, 1978). Significant hypertrophy (48% increase in fiber diameter) in rainbow trout white (*i.e.* mosaic) muscle was also observed after 3-4 weeks of swimming at 1 BL/s (Hudson, 1973), supporting the idea that mosaic muscle is in fact used at this low speed. It is thought that the mosaic muscle of salmonids is better suited for sustained swimming than white muscle of other species (Greer-Walker & Emerson, 1978).

Respiratory Adjustments to Exercise in Fish

The primary site for gas exchange in fish is the branchial epithelium; the counter-current structure of the gills transfers O_2 and CO_2 between the environmental water and the blood with high efficiency. During rest and sustained steady state swimming, O_2 uptake via the gills and delivery via the bloodstream are sufficient to meet all energy demands aerobically. However, during burst swimming, most of the energy demand is met glycolytically (Randall, 1970).

The increase in MO_2 is achieved by increased cardiac output and ventilation volume, adaptive changes in the area and thickness of the branchial diffusion barrier and perhaps in its O_2 permeability, and increases in the driving force (*i.e.* mean O_2 partial pressure gradient between water and blood - reviewed in detail by Jones & Randall, 1978; Wood & Perry, 1985). The magnitude of this increase in MO_2 is quite variable, however, dependant on the species and severity of exercise used.

Brett (1964) showed that MO_2 and swimming speed were exponentially related in coho salmon. As swimming speed was increased, the energy supply, *i.e.* MO_2, closely followed the energy demand eventually reaching an MO_2max level 9 times greater than resting levels. At speeds greater than about 4 BL/s, burst swimming occurred. The energy demand exceeded the aerobic energy supply, *i.e.* the maximal rate of MO_2 was reached. The fish ultimately fatigued and collapsed, such that the energy demand dropped instantaneously, but the MO_2 remained elevated for 4-5 hours (Brett, 1964).

In a recent article by Steffensen *et al.* (1987), MO_2 was found to increase by 71% after "burst swimming to exhaustion". Rainbow trout (300-700 g) were forced to "burst swim" for 10 minutes when swimming speed was increased from 40 cm/s to 80-85 cm/s. This is roughly (actual length of fish used not given in the paper) 1-2 BL/s at the control speed and 2-4 BL/s at the "burst speed". It would appear, however, that this exercise protocol was not very strenuous

or fatiguing, and is comparable to sustained type swimming. Increases in MO_2 of this magnitude are in fact comparable to those found by Brett (1964, 1972) for sockeye salmon swimming at 3-4 BL/s and by van den Thillart (1986) for rainbow trout swimming at 80% U_{crit}. U_{crit} (critical velocity) is the maximum sustained swimming speed, which is dependant on the size and species of fish.

In comparison, Rao (1968) found a five-fold increase in MO_2 in 100 g rainbow trout swimming at U_{crit}. Dickson & Kramer (1971) found similar increases in maximum sustainable O_2 consumption in trout. Bennett (1978), in an extensive review, found anywhere between 5-15 fold increase depending on the species (*i.e.* large-mouth bass compared to salmon).

In contrast, burst swimming by chasing, resulting in fatigue, elevated MO_2 only 2.6x in 12 g rainbow trout (Weiser *et al.*, 1985). Similar to the mammalian situation, MO_2 recovery in these fish was biphasic, although over-all, the rates of recovery were much slower. The fast component of MO_2 recovery was about 0.5 h and the slow component about 2.5 h.

A number of factors are now known to affect both resting and active MO_2 rates in fish. Perhaps the most important is temperature. Increasing temperatures (5-25°C) increased resting MO_2 but maximum sustained O_2 consumption did not increase beyond 15-20°C (Rao, 1968; Dickson & Kramer, 1971; Brett, 1972). Size and age of fish are also determining factors. Weiser *et al.* (1985) found that weight-

specific maximum sustained MO_2 increased with increasing age and size of rainbow trout fry whereas the routine rate was unaffected. Exposure to high salinities (*i.e.* greater osmoregulatory costs) increased both resting and maximum MO_2 (Rao, 1968). Starvation lowered standard metabolic rate but not active metabolic rate (Dickson & Kramer, 1971).

There is very little information about CO_2 excretion rates (MCO_2) in resting and swimming fish, partly due to the difficulty in measuring CO_2 in water (Kutty, 1968; Wood & Perry, 1985). CO_2 excretion doubled when resting rainbow trout (about 330 g) were forced to swim at 80% U_{crit} (van den Thillart, 1986). Steffensen *et al.* (1987) found an increase of 104% in MCO_2 in rainbow trout swimming at roughly 3 BL/s but MCO_2 had returned to resting levels in just over an hour.

The ratio of CO_2 excreted and O_2 consumed is known as the respiratory quotient (R.Q.). If this ratio is measured during a steady-state condition such as rest or sustained swimming, the R.Q. may provide an indication of the fuel that is burned at the time (Eckert & Randall, 1983). For example, if carbohydrates such as glucose are the main source of energy in an animal, the R.Q. would be 1, as reflected in the following reaction.

$$C_6H_{12}O_6 + 6\ O_2 \text{ ---> } 6\ CO_2 + 6\ H_2O$$

An R.Q. value of about 0.71 is indicative of fats as the primary fuel source, while 0.81 reflects protein metabolism and/or a mixed fuel supply. R.E. (ratio of

respiratory exchange) values, on the other hand, represent instantaneous changes in the MCO_2/MO_2 ratio and may be markedly different from true R.Q. values due to factors such as metabolic acidosis, which results in "excess" MCO_2 due to titration of bicarbonate stores.

Rainbow trout typically have resting R.Q. values of around 0.85-0.91 (Kutty, 1968; van den Thillart, 1986; Playle *et al.*, 1989). During non-steady-state swimming to reach 1.6 BL/s, rainbow trout showed elevated R.E. values about 1.2 for the first hour, which then started to drop and reached a steady state 2-3 hours later with R.Q. values around 0.96 (Kutty, 1968). During exhaustive swimming, R.E. values approach and exceed 1, due to the metabolic acidosis resulting from the exercise (Milligan & McDonald, 1988).

Metabolic Adjustments to Exercise in Fish

High energy compounds, such as ATP and CP, are the common currency of chemical energy which the muscle utilizes to do mechanical work (Astrand & Rodahl, 1970). Upon initiation of exercise, ATP, stored directly in the muscle, is hydrolyzed by myosin ATPase (mATPase), resulting in the formation of adenosine diphosphate (ADP) and inorganic phosphate (P_i) (Driedzic & Hochachka, 1978).

$$ATP \xrightarrow{\text{mATPase}} ADP + P_i \qquad (1)$$

The creatine phosphate pool is a high energy buffer which serves to quickly regenerate ATP, via the reaction:

$$CP + ADP \xrightarrow{\text{creatine kinase}} \text{creatine} + ATP \qquad (2)$$

ATP and CP are stored in high concentrations directly in the muscle but are used relatively quickly (within seconds) after the start of exercise (Driedzic & Hochachka, 1978). As the CP pool begins to deplete, ADP levels begin to rise and ATP is resynthesized via the adenylate kinase reaction due to a mass-action effect. This results in an increase in adenosine monophosphate (AMP) levels.

$$2\ ADP \xrightarrow{\text{adenylate kinase}} ATP + AMP \qquad (3)$$

Elevated AMP levels activate the deaminase reaction, resulting in a depletion of the total adenylate pool and a corresponding increase in inosine monophosphate (IMP) and ammonia (NH_3). Adenylate pool depletion and IMP accumulation, in fact, occur in a 1:1 stoichiometry (Driedzic & Hochachka, 1976; 1978).

$$AMP^{2-} + H_2O \xrightarrow{\text{AMP deaminase}} IMP^{2-} + NH_3 \qquad (4)$$

At physiological pH, however, NH_3 is immediately converted to ammonium ion (NH_4^+) (Mommsen & Hochachka, 1988).

The deaminase reaction is one step in the purine nucleotide cycle. As mentioned, this reaction (4) is activated during exercise. Two other reactions, catalyzed by adenylosuccinate synthetase and adenylosuccinate lyase, are functional only during the recovery phase, when the deaminase reaction is effectively shut off (Mommsen & Hochachka, 1988). These other enzymes function to replete the adenylate pool, such that an inverse relationship of ATP and IMP levels is always maintained.

Elevated NH_4^+ muscle levels result in a slight elevation in blood NH_4^+ levels (Wood, 1988; Wright & Wood, 1988). This is reflected as an increase in NH_4^+ excretion at the gills (Milligan & Wood, 1986), some of which may also arise from protein metabolism. However, most of the NH_4^+ is retained in the white muscle where it is formed. This serves as a nitrogen source to form aspartate required for the resynthesis of AMP from IMP (Mommsen & Hochachka, 1988).

Since CP depletion occurs rapidly after the onset of exercise, a much larger energy store (carbohydrates such as glycogen) is needed to ensure ATP turnover. If the work intensity is low, aerobic glycolysis and the Krebs cycle will serve to resynthesize the ATP during the course of the exercise. If the intensity of exercise is high, the rate of ATP utilization exceeds the rate of ATP repletion. Thus, there is an over-all reduction in the adenylate pool, which may require up to 2 h for recovery (Dobson & Hochachka, 1987).

Once exercise is initiated, glycogenolysis is one of the primary energy yielding pathways. Through the breakdown of glycogen, up to 36 ATP per glucosyl unit can be generated via glycolysis (and/or glycogenolysis), the Krebs cycle and the electron transport chain. The breakdown of glycogen to pyruvate does not require O_2. Pyruvate, however, can enter either the Kreb's cycle, which does require O_2, or an alternative route to form LAC, which does not. During high intensity work, the Kreb's cycle can become limiting such

that there is an accumulation of pyruvate. This drives the conversion into LAC, via the enzyme lactate dehydrogenase, at a very fast rate (McGilvery, 1983). The conversion to LAC from pyruvate can generate sufficient ATP to sustain muscular contraction but consumes considerably more glycogen per ATP generated (0.5 versus 0.03 glucose per ATP generated). Thus in comparison to the complete oxidation to CO_2 and water this pathway is relatively inefficient. If the activity is prolonged, GLY stores are depleted and LAC accumulates in large quantities (Dobson *et al.*, 1987).

In adult rainbow trout, white muscle GLY decreased by 80% (1.39 to 0.28 nmol/100 g wet wt., as glucosyl units) after 15 minutes of strenuous exercise (Black *et al.*, 1962), while muscle LAC increased 4-fold by an almost equimolar amount (1 GLY = 2 LAC) from 0.83 to 3.33 mmol/100 g wet weight. Immediately after exercise LAC levels started to decline slowly, but required up to 8 h for recovery. Muscle GLY on the other hand, had recovered to about one-third resting values by 30 minutes and thereafter remained constant.

These findings have been generally confirmed by Milligan and Wood (1986). White muscle LAC levels increased 3.5x (13.8 to 48.3 nmol/l intracellular water) after exhaustive exercise in adult rainbow trout, but had returned to pre-exercise levels by 8 h. However, muscle GLY levels remained near depleted up to 4 h after the cessation of

exercise. Thereafter, GLY recovered to 50% resting levels by 8 h and were fully restored by 24 h (Milligan & Wood, 1986).

Both ATP and CP levels in white muscle of severely exercised rainbow trout were near zero (Milligan & Wood, 1986). Muscle ATP required 1 h for recovery but continued to rise to twice the original resting levels. Muscle CP levels on the other hand, were not fully restored until 4 h post-exercise and also tended to over-shoot during the remainder of the recovery period.

These findings are consistent with Dobson and Hochachka's work (1987) in 60-100 g trout. They also found a depletion in the total adenylate pool entirely explained by the large drop in ATP. ADP levels did not change significantly and AMP levels actually rose four-fold. Repletion of the adenylate pool required up to 2 h post-exercise.

In contrast, CP levels in the whole body of very small (<10 g) rainbow trout recovered to pre-exercise levels in only 5-10 minutes after 60 s of electrical stimulation (Weiser *et al.*, 1985). Similarly, whole-body ATP levels were nearly recovered in 10 minutes. Whole-body ADP and AMP levels did not change significantly. It would appear, however, that the resting levels of the adenylates and CP were low in comparison to other work (Milligan & Wood, 1986; Dobson & Hochachka, 1987). This may reflect a difference in sampling protocol since it is known that only a couple of tail flaps can diminish CP by 45% and ATP by 10% in white

muscle (Dobson & Hochachka, 1987). However, the differences between the studies may be alternately attributable simply to the difference in the size of fish used.

Sustained swimming (at 3.5 BL/s) increased whole-body LAC levels two-fold in trout at the start of exercise, which then declined to control levels after 1 h (Wokoma & Johnston, 1981). This supports the idea that anaerobic metabolism is utilized at the start of exercise until aerobic metabolism can take over. It was proposed that more aerobic tissues, such as red muscle, heart and liver (Bilinski & Jonas, 1972), were involved in oxidizing the accumulated LAC. However, swimming at greater speeds (5 BL/s) resulted in an overall increase in whole-body LAC levels after 4 h. This indicates that LAC production rate was greater than LAC catabolic rate at this higher swimming speed.

In summary, from the available literature on salmonid fish, LAC recovery appears to be considerably slower than the return of MO_2 to control levels, requiring up to 12 h (Black *et al.*, 1962) as compared to 4 h (Brett, 1964). However, it must be emphasized that metabolic and MO_2 measurements have not yet been performed in the same study. The considerably faster rate of recovery of MO_2 appears to parallel that of ATP and CP repletion, about 1-4 h in white muscle (Milligan & Wood, 1986; Dobson & Hochachka, 1987). This would suggest that the EPOC is used initially to replenish the high energy phosphate stores, presumably via

LAC oxidation. Additional phosphagen synthesis from LAC oxidation could be used to convert the remaining LAC into GLY, directly in the muscle. Since very little of the total LAC produced actually enters the blood (10%; Turner & Wood, 1983), and GLY recovery is slow (Black *et al.*, 1962; Milligan & Wood, 1986), this would support the conclusion that the primary fate of LAC is GLY resynthesis *in situ* (Milligan & McDonald, 1988). There is a clear need for simultaneous measurements of MO_2 and metabolites after exercise in fish to confirm or disprove these hypotheses.

Objectives of the Study

In summary, there is good evidence in mammals that the O_2 debt hypothesis cannot be the sole explanation of the elevated post-exercise MO_2. Factors, other than LAC accumulation, such as intensity and duration of exercise (Knuttgen, 1970; Segal & Brooks, 1979), have been shown to affect EPOC, and that these changes occurred independent of LAC accumulation. Furthermore, elevations in both body temperature and catecholamine levels have been found to significantly contribute to EPOC (Barnard & Foss, 1969; Brooks *et al.*, 1971; Hagberg *et al.*, 1980).

To date, the O_2 debt hypothesis has yet to be rigorously tested in poikilotherms, where it was originally proposed by Hill & Lupton (1923) based on an *in situ* preparation. The possible relationship between EPOC, LAC recovery and the restoration of other metabolites has yet to be examined in a quantitative fashion in fish. The

advantages of fish, as poikilotherms, are that low body temperatures result in a slow time course of changes, which improves the accuracy of assessment. Furthermore, body temperature elevation as a result of the exercise, which is a complication in mammals, does not occur in fish. Furthermore, the small body size of juvenile fish, in particular, is ideal for whole body freeze-clamping (eg. Weiser *et al.*, 1986), thereby avoiding the problem of compartmentalization of metabolites.

The objectives of my thesis, therefore, were to examine the temporal relationships between O_2 consumption, LAC recovery, GLY restoration and the repletion of high energy phosphate stores, following exhaustive exercise in the whole body of juvenile (2-6 g) rainbow trout. The hypothesis that the disappearance of LAC after exercise determines the magnitude of EPOC was specifically tested (*i.e.* the "classical" O_2 debt hypothesis). Whole body samples have been taken simultaneously with MO_2 measurements, and the quantitative relationships have been evaluated.

In the first series of experiments (Chapter 2), whole-body LAC, GLY, ATP and CP, as well as ADP, AMP and glucose have been measured at various times during recovery from exhaustive exercise. A thorough quantitative analysis was undertaken by preparing a "budget" of the cost of recovery, in terms of ATP and O_2 equivalents, in order to evaluate the classical O_2 debt hypothesis.

As the results of the first series suggested some association between LAC restoration and EPOC, the objectives of the second and third series of experiments were to attempt to experimentally dissociate the EPOC from the disappearance of the accumulated LAC. In humans, both repetitive exercise and changes in diet (low carbohydrate) have been shown to decrease muscle GLY (Segal & Brooks, 1979). Both moderate and severe exercise resulted in identical EPOC in the GLY-depleted subjects compared to controls despite different LAC accumulations. These types of experiments have not yet been conducted in fish. Therefore, these two experimental protocols (repetitive exercise bouts and GLY-depletion) were employed in an attempt to generate smaller post-exercise LAC burdens in rainbow trout, and thus examine the effect on the pattern and magnitude of post-exercise MO_2.

In the second series of experiments (Chapter 3), a second exercise bout was given at the 6^{th} hour of recovery from the first bout. Once again, LAC recovery and GLY, ATP, CP, and glucose restoration were measured simultaneously with MO_2; CO_2 and ammonia excretion were also measured over the 24 h recovery period. In the third series of experiments (Chapter 4), a 50% depletion in whole body GLY was achieved after 5 days starvation. MO_2 was measured simultaneously with whole body LAC, GLY, ATP, CP and glucose over a 12 h recovery period from exhaustive exercise.

CHAPTER 2

OXYGEN CONSUMPTION AND BIOCHEMICAL CHANGES IN JUVENILE RAINBOW TROUT AFTER EXHAUSTIVE EXERCISE

INTRODUCTION

Exhaustive exercise (high intensity and low duration) in salmonid fish leads to an accumulation of LAC, an elevation in MO_2 and a number of other metabolic and systemic disturbances (Wood & Perry, 1985). Hill & Lupton (1923) proposed that the excess MO_2 seen after exercise was dependant on the oxidation of 1 mole of LAC to reconvert 5 moles of GLY. In mammals, there is evidence that the relationship between LAC recovery and EPOC is only coincidental, and a number of factors other than LAC have been shown to contribute to EPOC as discussed in Chapter 1 (see Gaesser & Brooks, 1984, for review).

In fish, the relationship between LAC accumulation and EPOC has not yet undergone rigorous testing. The purpose of this study, therefore, was to evaluate the classical O_2 debt hypothesis in rainbow trout, by detailed quantitative examination of the cost of recovery from exhaustive exercise, in terms of both O_2 and metabolic (ATP) equivalents. MO_2 recovery after a 5 min bout of exhaustive exercise was measured simultaneously with a number of metabolites (LAC, GLY, CP, ATP, ADP, AMP, glucose) in the whole body over a 24 h period. Furthermore, a budget of the costs of all factors known to contribute to EPOC, such as the O_2 consumed by the branchial and cardiac pumps, the

restoration of tissue and blood O_2 stores and the O_2 used for LAC oxidation, was prepared. The goal was to test the hypothesis that the disappearance of the LAC accumulated after exercise played a significant role in the EPOC, as proposed by the classical O_2 debt hypothesis.

METHODS & MATERIALS

Animals

Juvenile rainbow trout were obtained from Spring Valley Trout Farm in Petersburg, Ontario. Fish were held in large 400 l circular tanks with fresh dechlorinated Hamilton tap water. Water was well aerated and averaged about 15 ± 1°C during the summer months. Fish were fed *ad libitum* with 1.5 Gr. trout pellets (Martin Feed Mills, Don Mills, Ontario) every day for the first two weeks, then every other day for the next two weeks until experimentation began, and over the course of the experiments, which took four weeks to complete. Fish were fasted for 2-3 days prior to experiments and then were weighed just before placing them in the respirometers for acclimation. A small range in weight (2-3 g) was used in order to minimize variation in exercise capacity.

Respirometry

Fish were placed in blackened 20ml syringe barrels, which were connected to a Gilson Minipuls peristaltic pump regulating flow. These respirometers were fitted with inflow and outflow sampling ports (3-way stopcocks). Flow averaged about 0.7-0.8 l/h. Water temperature was 15 ± 1°C. Fish were allowed to acclimate overnight to the respirometers.

Water samples were taken in 1 ml glass syringes and measured with a Radiometer E5046 PO_2 electrode connected to a Cameron Instruments oxygen meter. Inflow water samples gave a measure of the PO_2 in water flowing into the

respirometer whereas outflow samples were of water immediately leaving the chamber. The following formula was used to calculate O_2 consumption (MO_2).

$$MO_2\ (\mu mol/g.h) = \frac{\Delta PO_2\ (torr) * \alpha O_2\ (\mu mol/torr.l) * flow\ (l/h)}{weight\ (g)}$$

where αO_2 is the solubility coefficient of O_2 in water at the experimental temperature. PO_2 of inflow water samples (P_iO_2) ranged between 145-160 torr. Typically, the ΔPO_2 at rest was 15-20 torr, which doubled after exercise to around 30-40 torr. Five min was determined as the minimum time required for adequate mixing of the water present in the chamber to give a representative ΔPO_2. Thus the first post-exercise MO_2 measurement was made at 5 min after the fish was replaced in the chamber.

Experimental Protocol

MO_2 Study

After 12 h acclimation, three resting MO_2 measurements were taken at 10 min intervals just prior to exercise. Fish 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 further stimulation for 2 min with a 9V battery by directly touching the tail with the battery. By the end of the 5 minutes, fish no longer struggled or responded when handled and were returned to the experimental chamber.

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.

The area under the mean MO_2 recovery curve (EPOC) relative to pre-exercise levels (5 min - 3.5 h) was measured with 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

For the metabolite portion of the study, fish were acclimated to the respirometer and exercised as described, and allowed to recover for up to 24 h. MO_2 was measured at various times during the recovery (at 5 min, 0.5 h, 1.0, 1.5, 2, 3, 4, 6, 8, 12, 16, 20 and 24 h), and the fish immediately terminated at these times for whole body metabolite measurement. Thus for each sample time, the MO_2 and metabolite measurements were made on the same group of fish. In addition, a group was terminated immediately after the end of exercise (0 h) for metabolite measurement only (MO_2 could not be measured at this time). Two control MO_2 and metabolite samples (non-exercised fish placed in the respirometers for the appropriate duration) were taken at the start of the experiment (C1) and at around 14 h "recovery" (C2). The area under the mean MO_2 recovery curve, relative to C1 over the period 0-6 h (see Results), was

measured using a digitizer pad as in the initial MO_2 study. This area was used for the budget calculations.

Sampling of fish was accomplished 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) aluminum tongs. About 5 s elapsed between emptying the syringe and submerging the fish in liquid nitrogen, and a further 30 s were required until the fish stopped bubbling, indicating it had reached liquid nitrogen temperature throughout. Controls were sampled in the same manner. Fish that struggled excessively (more than 3 tailflaps) or had jumped out of the small net were discarded.

The whole fish was subsequently powdered in a mortar and pestle under liquid nitrogen and weighed in a pre-cooled (-196°C) polyethylene tube. The samples were then freeze-dried at -60°C under vacuum for 48 h, re-weighed, and transferred to plastic vials with a screw cap. Once freeze-dried, the samples could be kept at room temperature for several hours (unavoidable when performing assays) as long as they were kept in a desiccator (M. Ganagarajah, personal communication). At all other times, tissue samples were stored in an evacuated desiccator at -60°C.

Analytical Techniques

Freeze-dried tissue was assayed flourometrically (Fluoro-micro-photometer, American Instrument Co., Maryland) for ATP, ADP, AMP, CP, LAC, GLY and glucose by the enzymatic

methods of Bergmeyer (1963). For the determination of the adenylates, CP and LAC, about 30 mg of tissue were extracted in 1 mL of 6% perchloric acid (PCA) for 20 min at room temperature and then centrifuged at 10 000 g for 10 min at 4°C. A volume of supernatant (750 µl) was removed and neutralized with 165 µl K_2CO_3 (2.5 M, pH>7) for 10 min at room temperature. This was recentrifuged at 10 000 g for 20 min (4°C). An aliquot (50 µl for CP and ATP, 300 µl for AMP and ADP, 200 µl for resting LAC samples, 100 µl for exercised LAC samples) was used in the enzymatic reaction, using substrates (from Sigma) and enzymes (from Boeringer-Mannheim) as required for the particular assay. Buffer for each assay was made up fresh each day, and added to unknowns and standards to make up a total volume of 2 ml per tube.

ATP and CP were assayed via the hexokinase and creatine kinase reactions, respectively, measuring increases in fluorescence of NADPH with the glucose-6-phosphate (G-6-P) dehydrogenase reaction.

$$\text{CP} + \text{ADP} \xrightarrow{\text{creatine kinase}} \text{creatine} + \text{ATP}$$

$$\text{ATP} + \text{glucose} \xrightarrow{\text{hexokinase}} \text{G-6-P} + \text{ADP}$$

$$\text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{G-6-P dehydrogenase}} \text{gluconate-6-phosphate} + \text{NADPH} + \text{H}^+$$

AMP and ADP were assayed via the myokinase and pyruvate kinase reactions, respectively, following increases in NAD^+ fluorescence after the lactate dehydrogenase reaction (Bergmeyer, 1963).

$$\tfrac{1}{2}\ AMP + \tfrac{1}{2}\ ADP \xrightarrow{\text{myokinase}} ADP + \text{phosphoenol pyruvate (PEP)}$$

$$ADP + PEP \xrightarrow{\text{pyruvate kinase}} \text{pyruvate} + ATP + NADH$$

$$\text{pyruvate} + ATP + NADH \xrightarrow{\text{lactate dehydrogenase}} NAD^{+} + H^{+} + \text{lactate}$$

GLY was broken down into glucose with amyloglucosidase and assayed as glucose via the hexokinase reaction (Bergmeyer, 1963).

$$\text{glycogen} + (n-1)\ H_2O \xrightarrow{\text{amyloglucosidase}} n\ \text{glucose}$$

$$\text{glucose} + ATP \xrightarrow{\text{hexokinase}} G\text{-}6\text{-}P + ADP$$

$$G\text{-}6\text{-}P + NADP^{+} \xrightarrow{\text{G-6-P dehydrogenase}} \text{gluconate-6-phosphate} + NADPH + H^{+}$$

A volume of 1.1 ml of acetate buffer (0.2 M, pH 4.8) was added to about 15 mg (exact weight determined) of dried tissue and then centrifuged at 10,000 g (4°C) for 10 min. An aliquot of 50 µl was removed for background determination of glucose. To the remainder, 25 µl of amyloglucosidase were added to break down glycogen into glucose. This was incubated in a shaking water bath at 37°C for 2 h, then neutralized with 400 µl of Trizma base (0.3 M; Sigma). The samples were recentrifuged at 10,000 g (4°C) for 10 min. A 50 µl aliquot was removed from the supernatant and assayed for glucose (hexokinase reaction).

Actual concentrations were obtained using standard curves. Substrates for standards were obtained from Sigma and prepared fresh each day and then read as unknowns.

Values read against the standard curve were corrected for tissue weight and dilution. All values have been reported as means ± 1 standard error (n between 8-10), unless otherwise stated. Significance was tested using Student's two-tailed t-test at $p<0.05$.

RESULTS

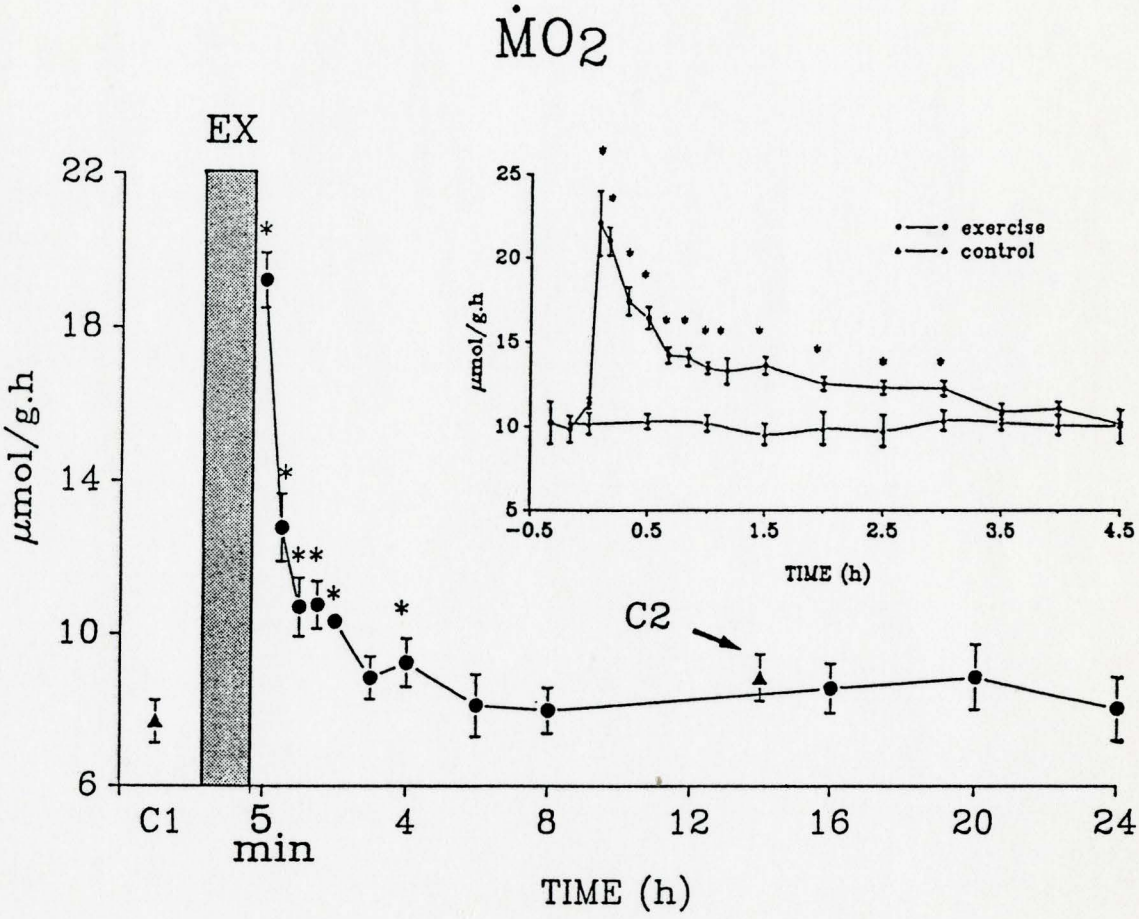
MO_2 Study

In the first study (see insert of Fig.2.1), MO_2 increased from 10.5 µmol/g.h (mean of three samples taken prior to exercise) to 22.1 µmol/g.h (measured 5 min after the cessation of exercise). There was an approximately exponential decrease in MO_2 after exercise and resting levels were re-established between 3.5-4.5 h. Control MO_2 did not vary significantly over time. This study was undertaken to measure MO_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 ensure that when terminal samples for individual fish (different at each sample time) were used in the metabolite study, the pattern of MO_2 recovery remained consistent. The area under the mean curve between 0-3.5 h, relative to pre-exercise levels, was measured to be 9.1 µmol/g wet wt. or 45.5 nmol/mg dry wt. This value is known as the "O_2 debt" or EPOC (excess post-exercise O_2 consumption).

Metabolite Study

For the metabolite portion of the study, the corresponding MO_2 measurements were similar to the MO_2 study (Fig.2.1). MO_2 increased about 2.5 times above resting levels (from 7.7 to 19.2 µmol/g.h wet wt) and required between 3-6 h to return to resting levels. At the 12 h point after exercise, MO_2 was significantly elevated above resting levels (11.2 ± 0.7 µmol/g.h wet wt., n=10). However, this

Figure 2.1. Effect of exhaustive exercise on O_2 consumption (MO_2) in juvenile rainbow trout. Insert shows MO_2 changes in individual fish prior to and following a 5 min exercise bout, and MO_2 in non-exercised controls. Pre-exercise values are a mean of 3 measurements per fish. First post-exercise sample was taken at 5 min. Main figure shows MO_2 changes in exercised and non-exercised fish, using terminal samples from the metabolite study. (▲), labelled C1 and C2, indicates non-exercised controls. (●) indicates exercised fish. Grey bar indicates the 5 min exercise bout. Means ± 1 S.E.M., $n \geq 8$. * indicates significantly different ($p<0.05$) from controls (inset) or from C1 (main figure). Error bars not shown are contained within the sample points.


$\dot{M}O_2$
EX
22
18
14
10
6
µmol/g.h
C1
5
min
4
8
12
16
20
24
TIME (h)
C2
25
20
15
10
5
µmol/g.h
−0.5
0.5
1.5
2.5
3.5
4.5
TIME (h)
exercise
control

point was considered to be an outlier and has been omitted from the graph (Fig.2.1). The two controls (labelled C1 and C2) were not significantly different. The area under the curve relative to C1 over the period 0-6 h, *i.e.* EPOC, indicating the total extra O_2 consumed during recovery, was 12.5 µmol/g wet wt. or 62.5 nmol/mg dry weight. The area under the curve up to 6 h, rather than 3.5 h, was used in this study because of the significant elevation of the 4 h point (Fig.2.1).

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.2.2) increased from 7.7 nmol/mg dry wt. at rest to 27.8 nmol/mg dry wt. immediately post-exercise, and continued to increase to 39.2 nmol/mg dry wt. at 5 min post-exercise. Thereafter, disappearance of LAC occurred at more or less an exponential rate. There was no longer a significant difference relative to pre-exercise values at 4 h, but the mean remained slightly elevated until 6 h. At 8 and 12 h, whole-body LAC dropped significantly below resting levels and then returned to pre-exercise conditions. Thereafter, very little change occurred for the rest of the recovery period. The LAC recovery curve (Fig.2.2) approximately paralleled that of MO_2 recovery (Fig.2.1).

Whole-body GLY (Fig.2.3) decreased to 13% resting levels immediately post-exercise (37.8 to 5.0 nmol glucosyl

Figure 2.2. Changes in whole-body lactate levels after exhaustive exercise in juvenile rainbow trout. (▲), denoted as C1 and C2, indicates non-exercised controls. (●) indicates exercised samples. Grey 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) ± 1 S.E.M. Error bars not seen are contained within the sample points. * found above sample indicates significantly elevated ($p<0.05$) above C1, * found below sample indicates significantly lower ($p<0.05$) than C1.

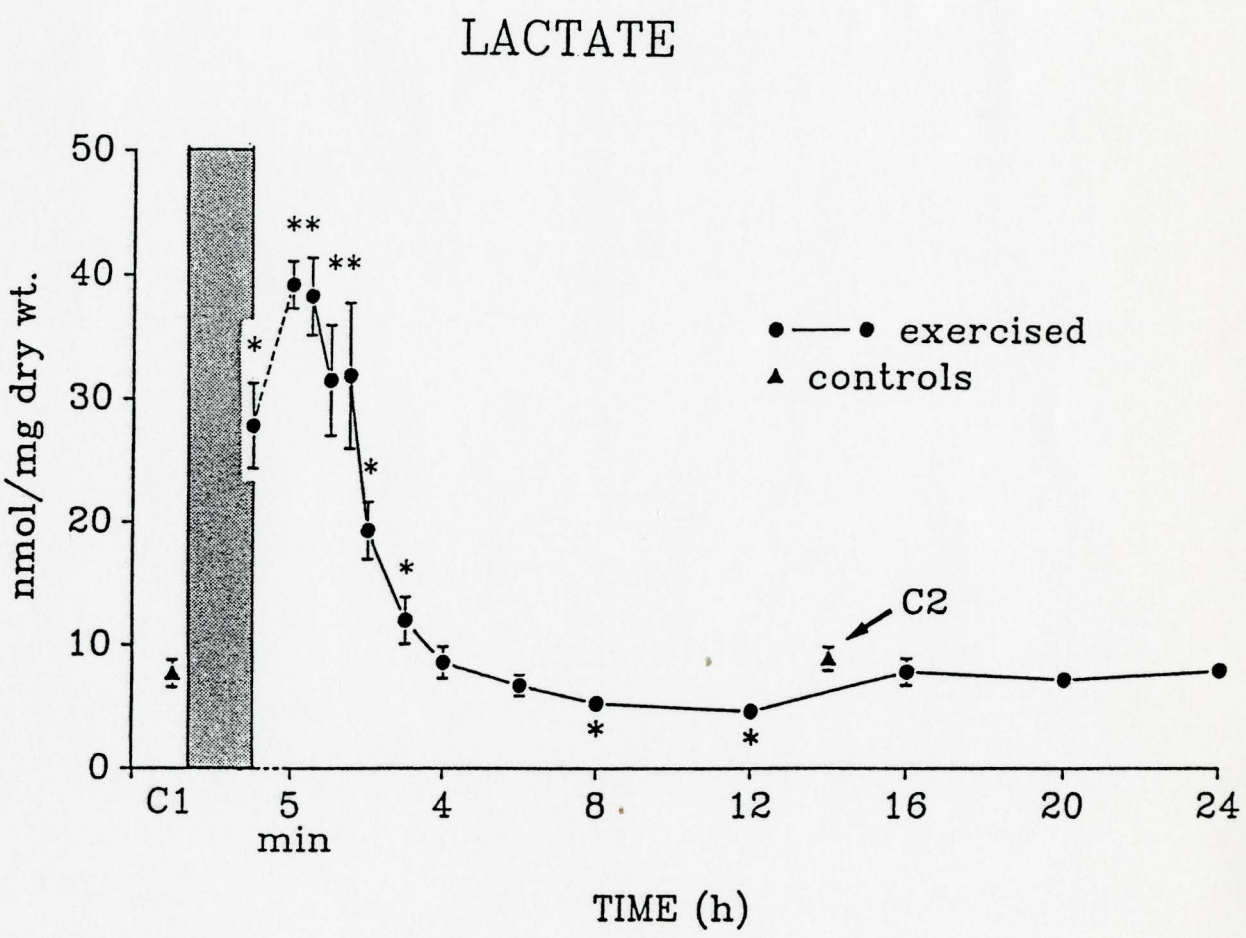
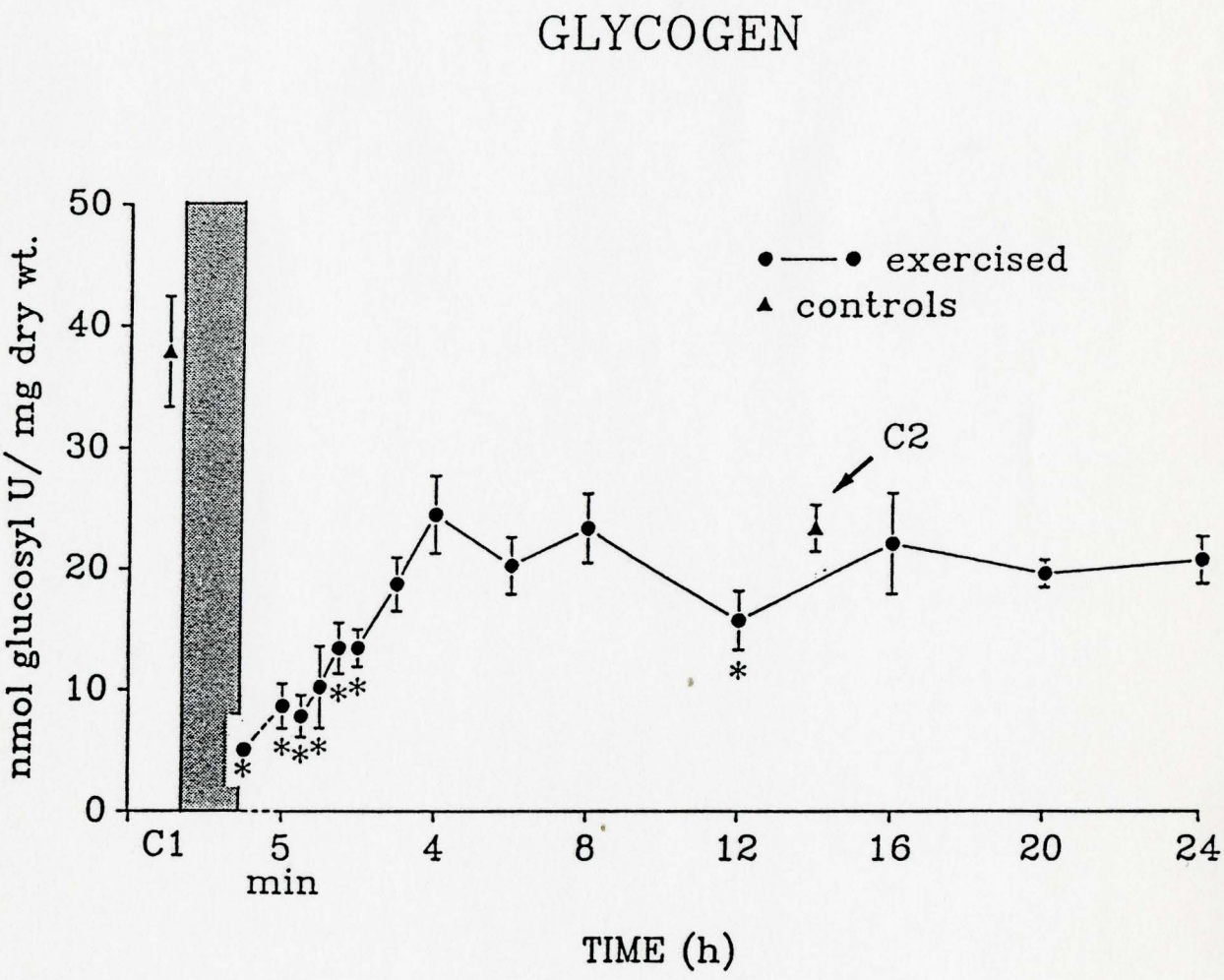

LACTATE
nmol/mg dry wt.
50
40
30
20
10
0
C1
5
min
4
8
12
16
20
24
TIME (h)
exercised
controls
C2

Figure 2.3. Changes in whole-body glycogen levels after exhaustive exercise in juvenile rainbow trout. (▲), denoted as C1 and C2, indicates non-exercised controls. (●) indicates exercised samples. Means (n=10) ± 1 S.E.M. * indicates significantly different from C2, since all points are significantly less than C1. Other details as in legend of Fig.2.2.


GLYCOGEN
nmol glucosyl U/ mg dry wt.
50
40
30
20
10
0
exercised
controls
C2
C1
5
min
4
8
12
16
20
24
TIME (h)

U/mg dry wt.) and began to recover immediately. By 4 h, levels had reached about 65% resting levels (24.5 nmol glucosyl U/mg dry wt.). Although this was below resting levels at the start of the experiment, there was no significant difference from the second control (C2, 23.4 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 in the whole body averaged about 7 nmol/mg dry wt. throughout the entire recovery period after exhaustive exercise (Fig.2.4).

ATP levels in the whole body (Fig.2.5) decreased to 24% resting levels after exercise (7.9 to 1.9 nmol/mg dry wt.), and had recovered by 1.0-1.5 h. There was no significant change thereafter. Both ADP (2.8 nmol/mg dry wt; Fig.2.6A) and AMP (0.02 nmol/mg dry wt; Fig.2.6B) levels appeared to decrease somewhat after exercise, but these changes were not significant.

Whole-body CP decreased to 35% resting levels immediately post-exercise (42.7 to 14.8 nmol/mg dry wt.), but by 5 min had recovered to 81% resting levels (35.5 nmol/mg dry wt.), a non-significant difference from controls (Fig.2.7). A tendency for overshoot during the next few hours was observed, but this was also non-significant.

Theoretical Analysis

The analysis is based on the first 6 h of recovery, by which time MO_2 and all metabolites had returned to resting and/or stable levels. Two possible scenarios are presented.

Figure 2.4. Changes in whole-body glucose levels after exhaustive exercise in juvenile rainbow trout. (▲), denoted as C1 and C2, indicates non-exercised controls. (●) indicates exercised samples. Means (n=10) ± 1 S.E.M. Other details as in legend of Fig.2.2.

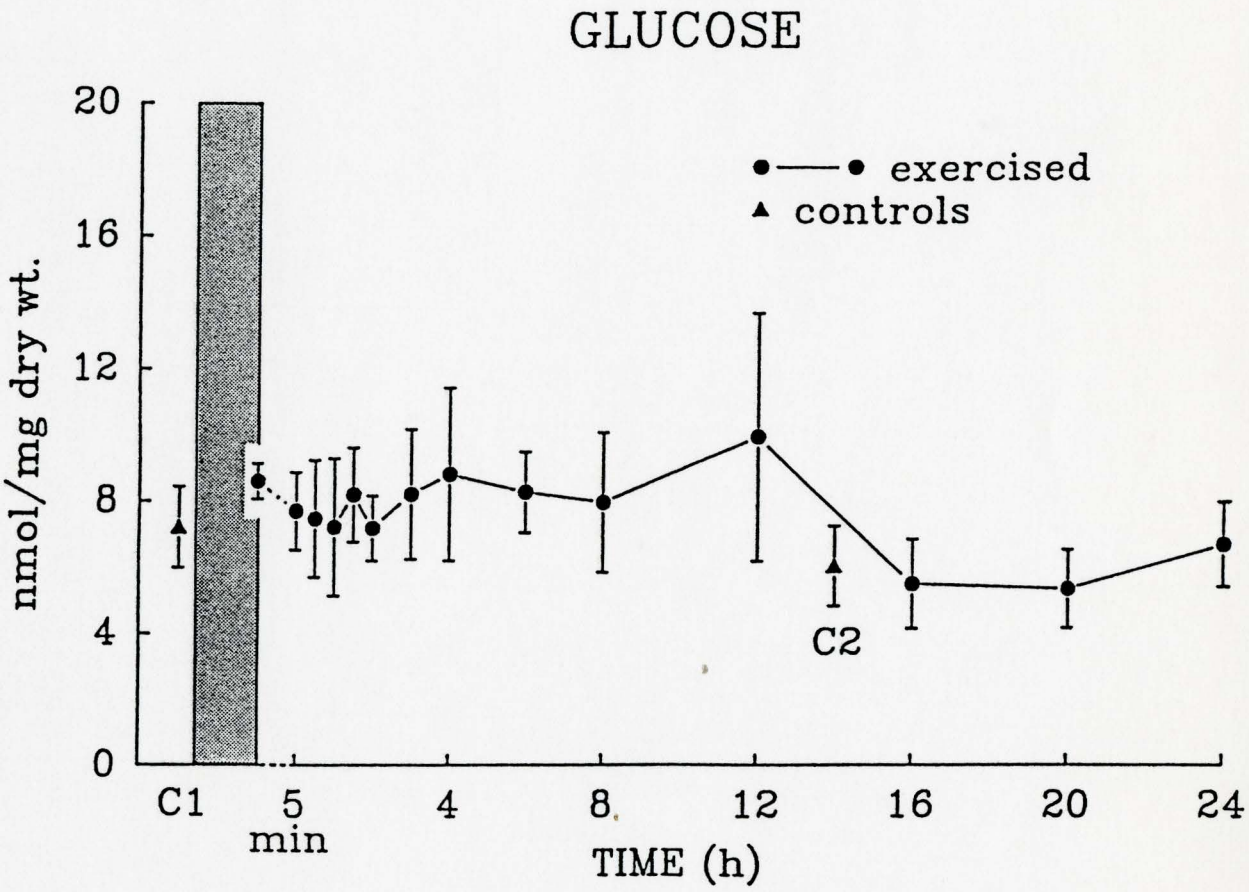

GLUCOSE
exercised
controls
nmol/mg dry wt.
20
16
12
8
4
0
C1
C2
5
min
4
8
12
16
20
24
TIME (h)

Figure 2.5. Changes in whole-body adenosine triphosphate (ATP) levels after exhaustive exercise in juvenile rainbow trout. (▲), denoted as C1 and C2, indicates non-exercised controls. (●) indicates exercised samples. Means (n=10) ± 1 S.E.M. Other details as in legend of Fig.2.2.

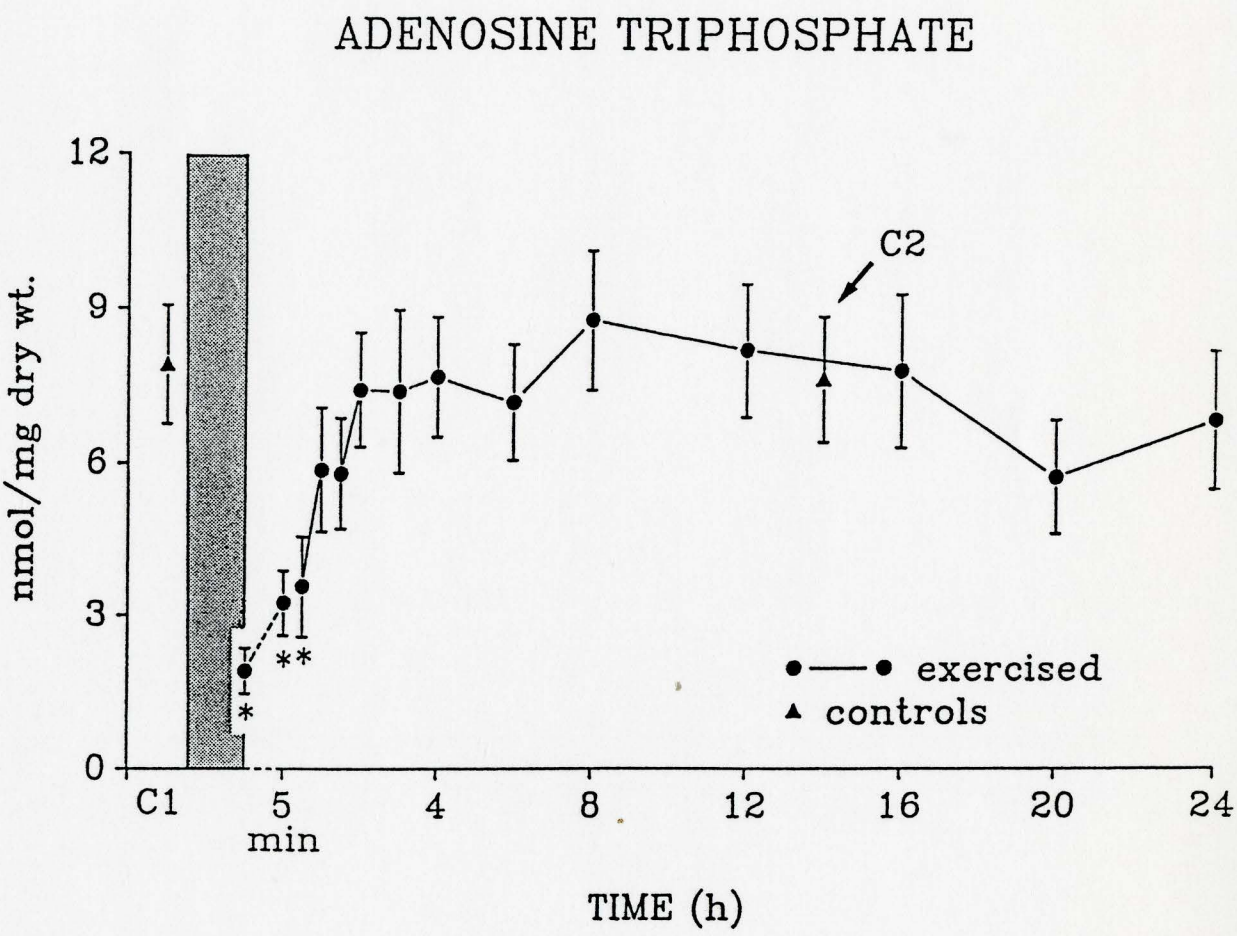

ADENOSINE TRIPHOSPHATE
nmol/mg dry wt.
12
9
6
3
0
C1
C2
5
min
4
8
12
16
20
24
TIME (h)
*
**
exercised
controls

Figure 2.6. Changes in whole-body (A) adenosine diphosphate (ADP) levels and (B) adenosine monophosphate (AMP) levels after exhaustive exercise in juvenile rainbow trout. (▲), denoted as C1 and C2, indicates non-exercised controls. (●) indicates exercised samples. Means (n=10) ± 1 S.E.M. There are no significant differences due to exercise. Other details as in legend of Fig.2.2.

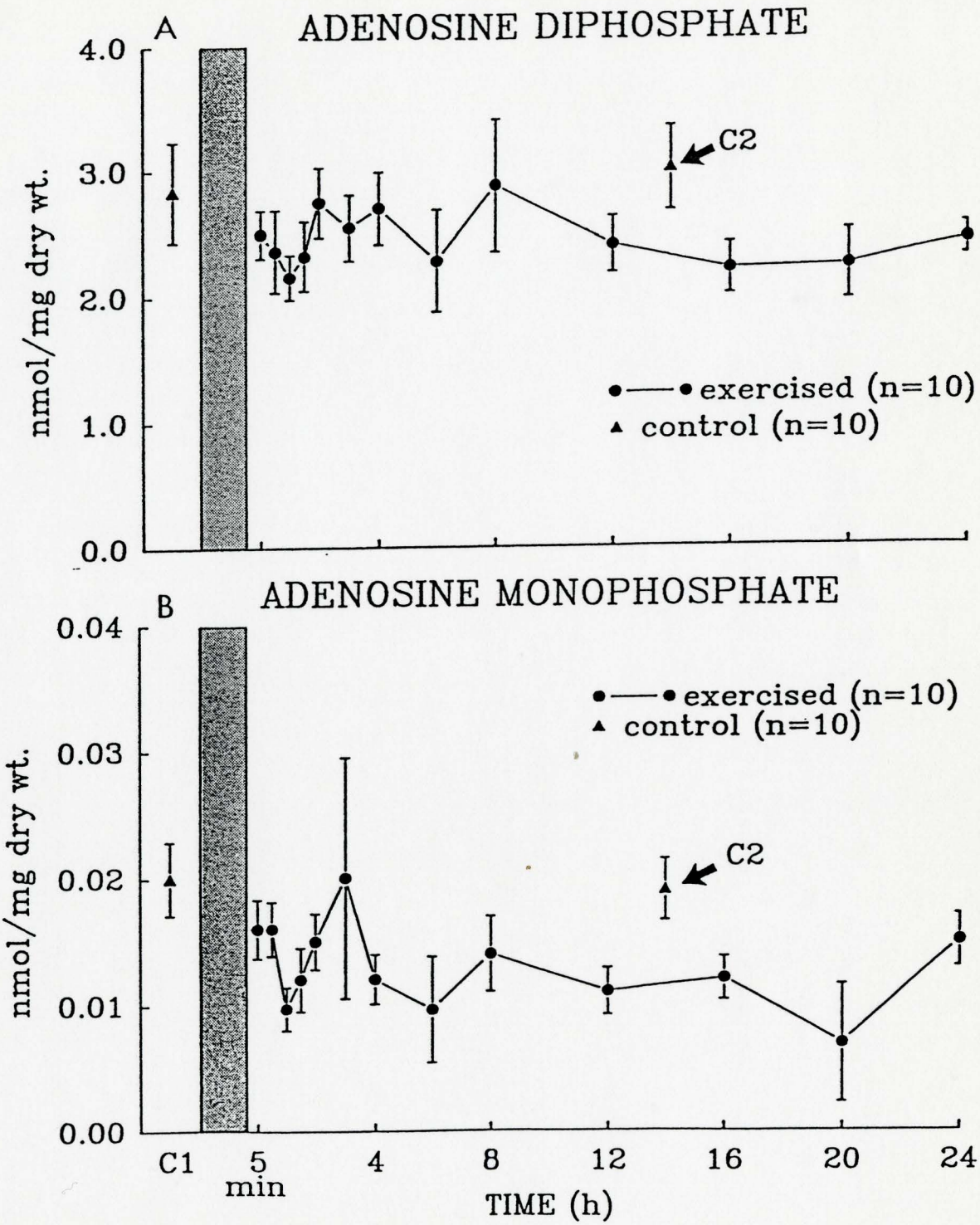
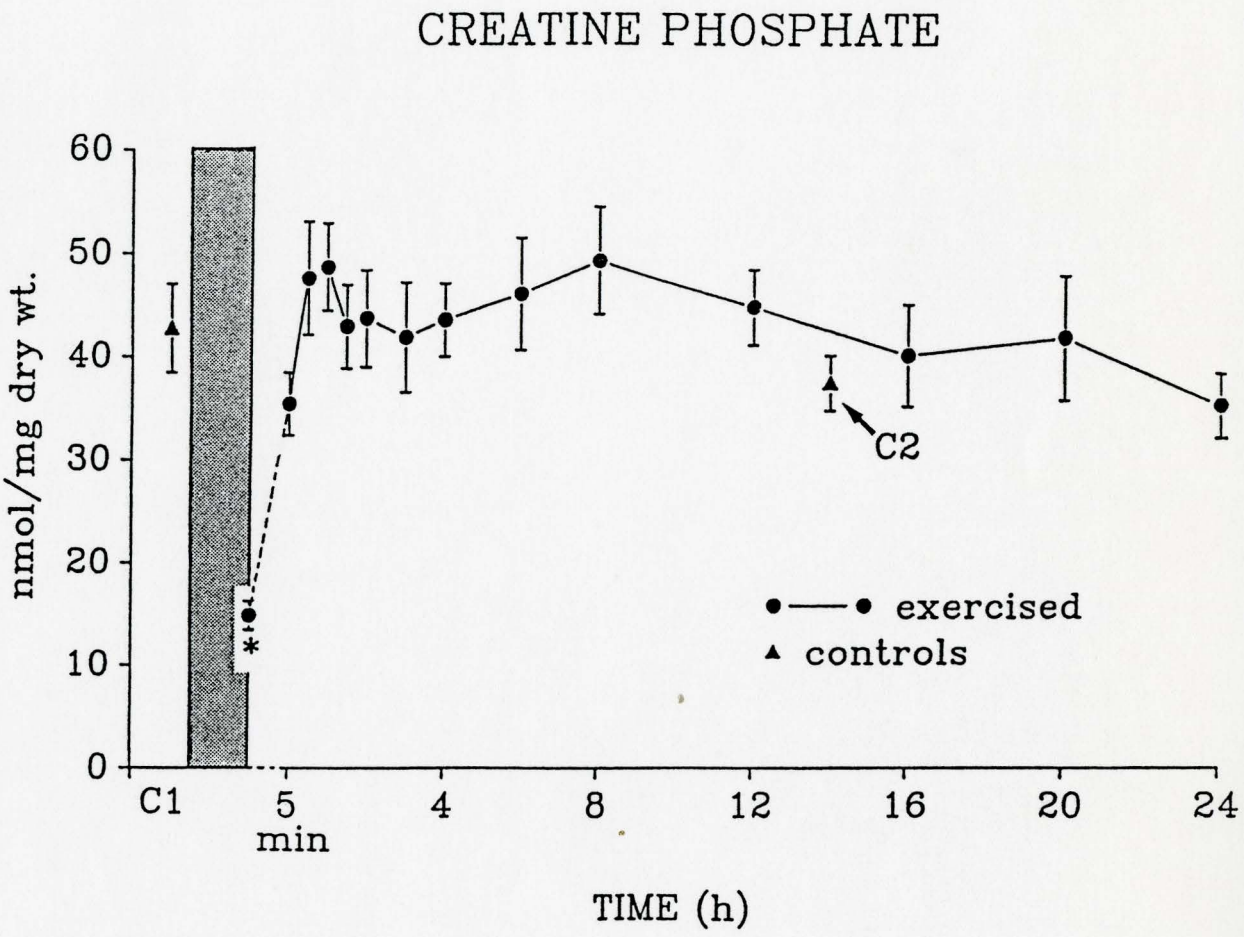

A
ADENOSINE DIPHOSPHATE
4.0
3.0
2.0
1.0
0.0
nmol/mg dry wt.
C2
exercised (n=10)
control (n=10)
B
ADENOSINE MONOPHOSPHATE
0.04
0.03
0.02
0.01
0.00
nmol/mg dry wt.
exercised (n=10)
control (n=10)
C2
C1
5
min
4
8
12
16
20
24
TIME (h)

Figure 2.7. Changes in whole-body creatine phosphate (CP) levels after exhaustive exercise in juvenile rainbow trout. (▲), denoted as C1 and C2, indicates non-exercised controls. (●) indicates exercised samples. Means (n=10) ± 1 S.E.M. Other details as in legend of Fig.2.2.


CREATINE PHOSPHATE
nmol/mg dry wt.
60
50
40
30
20
10
0
C1
5
min
4
8
12
16
20
24
TIME (h)
C2
exercised
controls

The first (A) was based on the assumption that all of the GLY resynthesized came from LAC. The remainder of the LAC would be oxidized, which would account for the EPOC. The second "alternative" scenario (B) was based on a very different assumption, *i.e.* the opposite extreme. It was assumed that the entire EPOC was devoted to oxidizing LAC, generating large amounts of ATP, while the remainder of the LAC (*i.e.* non-oxidized LAC) was converted back into GLY.

(A) The changes in metabolite levels are quantified in Table 2.1. All values have been expressed in terms of nmol/mg dry wt. Based on those changes, the following assumptions have been made.

(1) All GLY resynthesis was from LAC:

2 LAC + 6.5 ATP --> 1 GLY. Therefore:

Restoration of 12.4 nmol glucosyl U of GLY/mg consumed 24.8 nmol LAC/mg (about 75% of the total (32.5 nmol/mg) removed) and 80.6 nmol ATP/mg.

(2) The rest of the LAC cleared (about 25%) was oxidized:

LAC + 3 O_2 --> 3 CO_2 + 3 H_2O + 17.5 ATP

Therefore: 7.7 nmol LAC/mg was oxidized,

consuming 23. nmol O_2/mg and producing 134.8 nmol ATP/mg.

A budget of the cost of recovery in terms of O_2 and ATP equivalents is shown in Tables 2.2A and 2.2B. Of the 30.1 nmol O_2 equivalents accounted for, 5 nmol O_2 (7.5% of the

Table 2.1. Changes in MO_2 and measurement of EPOC, with corresponding metabolite changes over the period 0-6 h after a burst of exhaustive exercise.

	resting levels	immediately * post-exercise	at 6 h recovery	difference (0-6 h)
MO_2 (µmol/g.h wet wt.)	7.7	19.2	8.1	see EPOC
EPOC (nmol/mg dry wt.)	N/A	N/A	N/A	62.5
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	---#
ADP (nmol/mg dry wt.)	2.8	2.5	2.3	---#
AMP (nmol/mg dry wt.)	0.02	0.02	0.02	---#

* The measurement used in the calculation was 5 min post-exercise. In the case of LAC, this was when peak levels were reached; thus the 5 min GLY sample was also used. In the case of MO_2, ADP and AMP, this was the first sample taken.

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

Table 2.2 Budget of the cost of recovery from exhaustive exercise based on scenario A (that all GLY resynthesized came from LAC), in terms of (A) O_2 equivalents and (B) ATP equivalents in the whole body juvenile rainbow trout.

(A) OXYGEN COST OF RECOVERY (nmol/mg)

23 O_2	used for lactate oxidation and phosphate energy charge resynthesis
5 O_2	used for increased ventilatory work
2 O_2	used for increased cardiac work
0.01 O_2	used for restoration of tissue and blood stores
30 O_2	accounted for versus:
63 O_2	measured as EPOC ("O_2 debt")

≈52% unaccounted for

(B) ATP equiv. COST OF RECOVERY (nmol/mg)

5 ATP	were restored
10 ATP	equiv. needed in ATP resynthesis
31	creatine phosphate restored
81 ATP	used in GLY resynthesis from LAC
127 ATP	accounted for versus:
135 ATP	produced from LAC oxidation

≈6% ATP unaccounted for

Values are rounded figures based on results in nmol/mg dry weight. See Table 2.1 and Theoretical Analysis for more detail. Estimates for increased ventilatory work (5-10%) and increased cardiac work (0-5%) were based from the estimates of Farrel & Steffensen (1987).

total EPOC) were required to maintain the branchial pumps and 2 nmol O_2 (2.5% of the total EPOC) were required for the increased work done by the cardiac pump, as estimated from the work of Farrel & Steffensen (1987). Only a very small portion (0.02%) was estimated to replenish blood and tissue O_2 stores. The majority of the EPOC (23.1 nmol/mg) was used for the oxidation of about 25% of the accumulated LAC. The remainder of the EPOC, about 52%, is still unaccounted for by this analysis.

Of the total ATP generated from the oxidation of LAC (135 nmol/mg dry wt.; Table 2.2B), the majority was used to drive the conversion of the remaining LAC into GLY (80.6 nmol/mg dry wt.). About 31 and 5 nmol/mg were utilized for the replenishment of CP and ATP stores, respectively. An additional 10 nmol ATP/mg were needed to resynthesize this ATP directly from IMP, since ADP and AMP levels were unchanged. Thus in terms of ATP equivalents, approximately 6% cannot be accounted for by this analysis. Note that the cost of increased ventilatory and cardiac work does not enter the ATP budget, which is solely for the fate of ATP generated by LAC oxidation.

(B) An alternative analysis was carried out on the basis of a very different assumption, mainly that all of the EPOC reflected LAC oxidation.

(1) Since total EPOC = 62.5 nmol/mg dry wt.,

$$62.4\ O_2 + 20.8\ LAC \rightarrow 364.0\ ATP + 62.4\ H_2O + 62.4\ CO_2$$

based on $3\ O_2 + LAC \rightarrow 17.5\ ATP + 3\ H_2O + 3\ CO_2$

This accounts for about 64% of the total LAC burden (32.5 nmol/mg).

(2) The remainder of the LAC (36%; 11.7 nmol/mg) was converted into GLY.

Thus: 5.85 GLY <-- 11.7 LAC + 38.0 ATP

based on 1 GLY <-- 2 LAC + 6.5 ATP

(3) Of the total GLY resynthesized during this time (12.4 nmol glucosyl U/mg), 48% came from LAC and the remainder (6.5 nmol glucosyl U/mg; 52%) came from other sources.

A budget of the total ATP equivalents utilized during the first 6 h of recovery from exercise is shown in Table 2.3. The increased work done by the branchial and cardiac pumps was converted into metabolic (ATP) equivalents from the estimates of O_2 utilization. Of the total ATP generated from LAC oxidation (364.0 nmol/mg), only about 35% (126.1 nmol/mg) can be accounted for by this analysis. However, the extra ATP generated could have been utilized in the resynthesis of GLY from sources other than LAC, which was not taken into account in the budget.

Table 2.3. Budget of the cost of recovery from exhaustive exercise based on the "alternative" scenario B (that the entire EPOC was devoted to oxidizing LAC), in terms of ATP equivalents.

29	ATP used for increased ventilatory work
12	ATP used for increased cardiac work
5	ATP were restored
11	ATP equiv. needed in ATP resynthesis
31	creatine phosphate were restored
38	ATP used in GLY resynthesis from LAC

126	ATP accounted for versus:
364	ATP produced from LAC oxidation
	≈65% unaccounted for

Values were taken from Table 2.1. See Theoretical Analysis for more detail. All values are in nmol/mg dry wt. Estimates for increased ventilatory work (5-10%) and increased cardiac work (0-5%) were based from the estimates of Farrel & Steffensen (1987).

DISCUSSION

Resting Levels

Resting MO_2 measurements in this study (6.5-10.0 µmol/g.h; Fig.2.1) agree well with the findings of Weiser *et al.* (1985) in similar sized rainbow trout, as well as resting LAC (7.6 nmol/mg dry wt.; Fig.2.2) and ATP levels (7.9 nmol/mg; Fig.2.5). However, CP levels (42.7 nmol/mg; Fig.2.7) are about 40% higher, ADP levels (2.8 nmol/mg; Fig.2.6A) about 2-fold higher, and AMP levels (0.02 nmol/mg; Fig.2.6B) only about 1/30th of those found by Weiser and colleagues (1985). The reasons for this discrepancy are unclear, though one possibility may be a lower degree of sampling disturbance (*i.e.* struggling) in this study compared to the study of Weiser *et al.* (1985). In contrast, Lackner *et al.* (1988) found considerably lower LAC levels (around 2 nmol/mg dry wt.) in whole-body chub (1.5-2.5 g). These authors suggest that LAC levels greater than 5 nmol/mg dry wt. are an indication that some struggling has occurred. It is possible, however, that this discrepancy is due to species differences, and is not indicative of excessive struggling in the present study, since the CP levels reported by Lackner et al. (1988) in chub (51.0 nmol/mg dry wt.) were fairly similar to the present levels (42.7 nmol/mg dry wt.) in rainbow trout.

The present study also shows the same resting GLY, CP, and ADP levels, and only slight differences in ATP, AMP and glucose levels, in comparison to levels based on white

muscle analysis in 50 g rainbow trout (Dobson & Hochachka, 1987). (White muscle was considered to constitute 55-65% of the whole body weight (Stevens, 1968)). However, resting LAC levels were almost 3 times lower in the present study than the values reported by Dobson & Hochachka. The present resting LAC levels, as well GLY, CP and ATP, were comparable to the findings of Milligan & Wood (1986) in 200-400 g rainbow trout. In summary, it is evident that there is a great deal of variability in the literature, which may be due to differences in the sizes and species of fish sampled. Nevertheless, the present resting levels are in broad general agreement with previous determinations.

Post-exercise Changes

MO_2 increased about 2-2.5 times after exhaustive exercise in both the MO_2 study and the metabolite study (Fig.2.1). However, in the MO_2 study, recovery time was slightly faster (3.5-4.5 h) than in the metabolite study (3-6 h). The EPOC appears different (45.5 vs 62.5 nmol/mg dry wt.) between the studies, but it cannot be tested statistically. The increase in MO_2 in the present study is comparable to the increase in MO_2 in 3-10 g rainbow trout stimulated electrically for 60 s at 20°C (11.3 to 22.2 µmol/g.h wet wt.) but slightly less than at 12°C (6.3 to 30.1 µmol/g.h wet wt.; Wieser *et al.*, 1985). The increase in MO_2 was also less than that seen in 50 g salmon, burst swimming at 4 BL/s (about 4.5 to 56.3 µmol/g.h wet wt.), although recovery time was similar (Brett, 1964).

Whole-body GLY levels decreased to about 13% resting levels after severe exercise. Recovery was gradual, and after 4 h, 10.8 nmol glucosyl U/mg dry wt were resynthesized, bringing the whole body GLY levels to about 20 nmol/mg dry wt. This was about 50% resting levels, which was maintained for the remainder of the 24 h recovery period (Fig.2.3). The low GLY levels after exercise are a good indication that the fish were thoroughly exhausted after the 5 min exercise bout. The slow recovery process of GLY stores has been shown in previous studies (Black *et al.*, 1962; Milligan & Wood, 1986), where white muscle GLY was only partially restored at 12 h and required up to 24 h for full recovery.

Since MO_2 and LAC levels had returned to normal (Fig.2.1 & Fig.2.2) at 4-6 h and glucose levels did not vary significantly throughout the entire period (Fig.2.4), it is likely that the fish were no longer able to replenish their GLY stores. This may have been due in part to the fact that the fish were not fed throughout the entire experiment. The significantly lower GLY seen in the control sample taken at around 14 h into the experiment (C2 relative to C1) supports the contention that the fish had recovered as much GLY as was possible without another source of fuel (*i.e.*food).

Whole-body LAC levels continued to increase even after the cessation of exercise (Fig.2.2), even though neither GLY (Fig.2.3) nor glucose (Fig.2.4) levels continued to drop. This continued rise in LAC after the end of exercise has

been shown previously in rainbow trout white muscle (Dobson & Hochachka, 1987). The authors attributed the increase in muscle LAC to liver-derived plasma glucose, since a rise in muscle glucose was measured post-exercise. The purpose of the continued "glycolysis", as proposed by Dobson & Hochachka (1987), was to replenish ATP stores. From my results, this does not appear to be the appropriate explanation. Whole-body measurements would not pick up a transfer of glucose from the liver to the muscle; however, whole-body glucose levels would probably have dropped if glucose were being used to generate more LAC. Continued production of LAC from glycolytic intermediates after the cessation of exercise is a more likely explanation. Glycolytic intermediates, such as glucose-6-phosphate and fructose-6-phosphate, are elevated after exercise in rainbow trout white muscle (Pearson *et al.*, 1989). These authors suggested that extra glucosyl units were "stored" within the glycolysis pathway. Levels of these intermediates had decreased by about 7 nmol/mg dry wt. in 1 h, which could easily account for the extra 11.4 nmol LAC/mg generated in 5 min in the present study (Fig.2.2). Elevated glycolytic intermediates have also been measured in the whole-body of juvenile roach, *Rutilus rutilus* (Dalla Via *et al.*, 1989).

The results also showed that LAC levels dropped below resting levels at 8-12 h (Fig.2.2). This has been demonstrated previously (Milligan & Wood, 1986) and these authors proposed that the fish could dip into its LAC

reserve in order to fully replenish its GLY stores. However, in the present study, GLY levels did not continue to increase at this time (Fig.2.3). The reason for the continued drop in LAC is therefore unclear.

About 30 nmol GLY/mg dry wt. (in glucosyl units) were depleted in the 5 min exercise bout, whereas only 32 nmol LAC/mg dry wt. had accumulated. This is far from the stoichiometry of 1:2 GLY depletion:LAC accumulation, as expected from this type of exhaustive exercise (Dobson & Hochachka, 1987). This suggests that only about half of the energy derived from GLY breakdown during exercise came from anaerobic means. Another possibility is that the rate of LAC metabolism increased along with the increase in the rate of LAC production, *i.e.* increased turnover (Milligan & McDonald, 1988), resulting in a smaller accumulation of LAC than expected.

Whole-body CP levels were recovered within 10 minutes, findings similar to those of Weiser *et al.* (1985). However, in that study, resting CP levels were lower than those found in the present study, although the magnitude of the decrease after exercise was comparable (23 nmol/mg dry wt., Weiser *et al.*, 1985 versus 28 nmol/mg dry wt., Fig.2.7). Weiser *et al.* also found that ATP levels had returned to pre-exercise levels within 10 min whereas the process took 1.0-1.5 h in the present study. This difference may have been due to a less severe exercise protocol (30-60 s electrical stimulation, in contrast to 5 min severe chasing in the

present study) as their post-exercise whole-body ATP levels were not as depleted (6.7 nmol/mg dry wt., Weiser *et al.*, 1986 versus 1.9 nmol/mg dry wt.; Fig.2.5) as in the present study.

Dobson & Hochachka (1987) found similar recovery times for white muscle ATP in 50 g rainbow trout swum to exhaustion in a swim tunnel compared to the present study. However, white muscle CP levels took over 2 h for complete restoration. They argued that ATP replenishment must necessarily precede CP restoration because the conversion of IMP to ATP, via the purine nucleotide cycle, generates protons. In the acidotic state following severe exercise, this further prevents the conversion of ATP to CP (see reaction 2 in General Introduction).

The findings of Milligan & Wood (1986) were in agreement with Dobson & Hochachka's work on this point. The resynthesis of ATP in white muscle of 200-400 g rainbow trout preceded CP resynthesis by 3 h. The findings of the present study (Fig.2.6) were more consistent with the rapid rates of both ATP and CP resynthesis often found in mammals. In quadriceps of man, ATP decreased to 60% (from 2.4 to 1.6 nmol/mg dry wt.) and CP decreased to 5-10% (from 6.8 to 0.7 nmol/mg dry wt.) pre-exercise levels after 5 min of heavy exercise (Hultman *et al.*, 1967), but required only 5-10 min to fully return to resting conditions.

Since neither ADP nor AMP levels changed significantly after exercise (Fig.2.6A and Fig.2.6B), all the ATP

hydrolyzed should have formed IMP and NH_4^+. This is again consistent with the whole body measurements of Weiser *et al.* (1985) but not with the white muscle measurements of Dobson & Hochachka (1987). IMP and NH_4^+ were not measured in the present study.

Interpretation of the Theoretical Analysis

It appears that the time courses of LAC disappearance and MO_2 recovery were similar, requiring up to 6 h. As all the metabolites had reached resting and/or stable levels by this time, this period was used as the basis for the quantitative analysis of the changes occurring post-exercise.

(A) Primary fate of LAC is GLY resynthesis.

Assuming that the initial recovery of GLY in the 6 h period was due to LAC conversion, about 25 nmol LAC/mg dry wt. were resynthesized to GLY (see Theoretical Analysis) and the remainder (7.7 nmol/mg dry wt.) of the LAC was oxidized. Thus for every molecule of LAC which was oxidized, about 3.2 molecules of LAC were converted into GLY. This is considerably less than the original estimate of Hill & Lupton (1923) of 5 converted for 1 oxidized.

These budget calculations also demonstrated that about half of the EPOC was utilized to oxidize the accumulated LAC and replenish ATP and CP stores. A small portion of the EPOC was probably used in the very first stages of recovery to restore tissue and blood O_2 stores and maintain the branchial and cardiac pumps (Table 2.2A). The costs of

increased ventilatory work (7.5%) and cardiac work (2.5%) were estimated from the theoretical calculations of Farrel & Steffensen (1987) for rainbow trout, using interpolated values midway between their estimates for costs under resting and maximal exercise conditions. These values are now considered more reasonable than the earlier much higher estimates (ventilatory work = 7.5-40%; cardiac work = 20-40%) by Jones (1971). From the total EPOC measured, 52% was unaccounted for (Table 2.1A). A portion of this may be due to simple variation in the data. For example, if the first control sample were identical to the 6 h sample (Fig.2.1, a non-significant difference), the area under the curve would be reduced by 20%. This idea is supported by the smaller EPOC measured in the initial MO_2 study. The remainder of the EPOC unaccounted for then, must be used in reactions other than the disappearance of LAC and restoration of phosphagens and O_2 stores.

Other factors known to contribute to EPOC in mammals, such as elevated catecholamine levels, substrate cycling and ion regulation, as discussed in Chapter 1, have not yet been quantified in terms of cost of recovery from exercise. It is likely that these factors are also important in fish. Further study into these aspects would allow a more complete picture of the situation in either fish or mammals.

The oxidation of 2 LAC is almost as efficient as complete oxidation of 1 glucose molecule, producing only 2 less ATP (McGilvery, 1983). The energy derived from the

oxidation of LAC (7.7 nmol LAC/mg dry wt. produced 135 nmol ATP/mg dry wt.) is transferred to numerous sinks (Table 2.1B). Part of the ATP, or phosphagen bond equivalent, is used to replenish both ATP stores (Fig.2.6) which occurred in about 1h, and CP stores (Fig.2.8) which occurred within the first 5 minutes of recovery. An additional 10 nmol ATP were required in the ATP resynthesis process from IMP.

Of the total ATP equivalents generated from LAC oxidation by this scenario, about 6% were unaccounted for. Part of the discrepancy may have been due to the difficulty in measuring true resting ATP and CP levels. Struggling fish may reduce ATP levels by 10% in white muscle and CP levels by as much as 45% after only 2 tail flaps (Dobson & Hochachka, 1987).

The assumption that LAC is metabolized *in situ* after severe exercise is not unreasonable, at least, in rainbow trout. First, based on work with an isolated-perfused trunk muscle preparation, only about 10% of the LAC produced actually entered the blood (Turner & Wood, 1983). Second, peak levels in the blood are reached about 2 h after the cessation of exercise, accounting for only about 20% of the whole body LAC burden at this time (Milligan & Wood, 1986). Third, the predicted clearance time of LAC from blood turn-over rates in a similar salmonid, the coho salmon, is much greater than actual clearance time suggesting that the majority of the LAC was metabolized *in situ* (Milligan & McDonald, 1988). All these observations suggest that LAC

transfer to the blood is not an effective method of clearance. Furthermore, since white muscle shows limited oxidative capacity, in comparison to more aerobic tissues such as heart, liver, and red muscle (Bilinski & Jonas, 1972), it seems likely that the primary fate of LAC was GLY resynthesis.

The LAC which entered the blood was probably oxidized by the other more aerobic tissues (Bilinski & Jonas, 1972), thereby supplying the energy necessary for the restoration of GLY (McGilvery, 1983). Furthermore, whole-body glucose levels (Fig.2.4) did not change significantly after exercise in this study, indicating that indeed, the Cori cycle was unimportant in contrast to the situation in mammals (Gaesser & Brooks, 1984).

(B) Primary fate of LAC is oxidation.

Since it has not yet been concretely determined whether white muscle contains the necessary enzymes to resynthesize GLY, a second "alternative" scenario was proposed. This opposite case scenario presents a situation where the primary fate of LAC is oxidation (as in mammals) as opposed to GLY resynthesis. This may not be totally unreasonable since white muscle in rainbow trout does have some oxidative capacity, albeit less than that of red muscle or heart (Bilinski & Jonas, 1972). Also, white muscle constitutes the single largest tissue of the body (55-65% of total body weight), thus white muscle oxidation may contribute significantly to total body MO_2.

The budget calculations using this alternative scenario attributes the entire EPOC to LAC oxidation. Thus, 62.5 nmol O_2/mg dry wt. oxidized 20.8 nmol LAC/mg. This accounts for 64% of the LAC burden; the remainder (36%) was converted into GLY. By this analysis, only half of the GLY resynthesized came from LAC. The rest would have come from other sources, possible stored glycolytic intermediates.

Of the total ATP generated from LAC oxidation (364 nmol/mg dry wt.), only 35% can be accounted for. The O_2 needed to maintain the increased work of the branchial and cardiac pumps was converted into ATP equivalents, and factored into the budget. Some of the ATP was used to replenish ATP and CP stores, and the rest was utilized to drive the conversion of LAC back into GLY. Elevated catecholamine levels, increased rate of substrate cycling and ion regulation were thought to contribute to EPOC, and thus would utilize ATP equivalents. However, as the exact contribution is not known, it could not be factored into the budget. Furthermore, some of the excess (65%) ATP measured would also be utilized to resynthesize GLY from sources other than LAC. Again, the exact quantity is not known.

Conclusions

The results seem to indicate a correlation between EPOC and LAC recovery in their similar time courses of recovery. It also appears that there was reasonable agreement, based on Analysis A, between the O_2 consumed after exercise and restoration of the major metabolites. That is, the

quantitative discrepancy was relatively small, as most of the ATP produced via LAC oxidation was accounted for. Although there was a portion of EPOC not accounted for, it seems reasonable to assume that elevated catecholamines, increased substrate cycling and ion regulation play a role. Also, about 75% of the total accumulated LAC was converted into GLY and the rest (25%) was oxidized to CO_2 and water. This is again a reasonable explanation, at least in rainbow trout, as little LAC is thought to leave the white muscle where it is formed.

In contrast, the analysis based on the entire EPOC being utilized for LAC oxidation revealed large quantities of ATP which were not accounted for. It is likely that some ATP would be utilized due to elevated catecholamines and increased substrate cycling, as well as to resynthesize GLY from sources other than LAC. However, it seems doubtful that these processes could account for all the "extra" ATP. Only 50% of the total GLY resynthesized could be accounted for as having come from LAC. Although GLY resynthesis from other sources is possible, the more classical view is that LAC is the primary source of GLY resynthesis after exercise. Thus, it seems that the first analysis is the more reasonable explanation for the primary fate of LAC, *i.e.* GLY resynthesis rather than oxidation.

However, at this point, firm conclusions cannot be drawn. There are portions of the budget which cannot be accounted for. Based on the evidence available at this time,

the classical O_2 debt hypothesis of Hill & Lupton (1923) can neither be rejected nor accepted. The subsequent studies of this thesis (Chapters 3 & 4) will attempt to experimentally dissociate EPOC from LAC disappearance, via repetitive exercise bouts and prior GLY depletion, in order to examine their relationship.

CHAPTER 3

THE EFFECT OF REPETITIVE EXHAUSTIVE EXERCISE ON EPOC AND BIOCHEMICAL CHANGES OF JUVENILE RAINBOW TROUT

INTRODUCTION

Chapter 2 described the metabolic changes in the whole body of rainbow trout after a single severe exercise bout. MO_2 recovery and LAC disappearance after exercise had similar patterns and time courses of recovery (4-6 h), although only about 50% of the EPOC could be accounted for quantitatively on the assumption that the primary fate of LAC was GLY resynthesis. On the alternative assumption that the primary fate of LAC was oxidation, the EPOC was not large enough to account for all LAC removal. These results were not sufficiently clear to either accept or reject the classical O_2 debt hypothesis as the explanation for EPOC in the rainbow trout. It was evident that experimental manipulations would be required to further characterize the relationship(s) between EPOC and LAC disappearance after exercise. Therefore, a second series of experiments was undertaken, with the goal of comparing .

The results of Chapter 2 demonstrated that after 6 h recovery from exercise, GLY levels were still depressed whereas all other metabolites had returned to normal. This suggested, therefore, that if a second exercise bout were to be given at this time, a smaller quantity of LAC would accumulate because of the decreased availability of glycolytic substrate.

The effect of repetitive, low duration, exercise bouts, given at 1 h intervals, have been shown to be additive in rainbow trout (Stevens & Black, 1966). Exercise bouts of 15-30 s given at 1 h intervals resulted in further increases in white muscle LAC levels and greater white muscle GLY depletion since GLY stores had not been thoroughly exhausted. After a 5 min exercise bout, muscle GLY stores were already near zero (Stevens & Black, 1966); thus, a second exercise bout given at 1 h did not result in any change in white muscle LAC or GLy levels. This implied that if two successive 5 min exercise bouts were given sufficiently far enough apart for recovery to occur, as proposed for the present study, different LAC accumulations could occur, dependant only on initial GLY levels.

The purpose of the present series of experiments was to experimentally dissociate LAC disappearance and EPOC by sequentially submitting the same fish to separate bouts of exhaustive exercise in which two different LAC burdens could be created. The hypothesis that a direct relationship existed between EPOC and LAC disappearance after exercise, as originally proposed by Hill & Lupton (1923), was tested. This severe, repeated "handling stress" is similar to that which fish might normally experience in the wild, as a result of predator-prey relations or "catch-and-release" from angling, and the implications were examined.

METHODS & MATERIALS

Animals

Juvenile rainbow trout were obtained from Aqua Farms Ltd. (Feversham, Ontario). Note that these fish were from a different stock than those used in Chapter 2. The fish were acclimated for 3-4 weeks in either dechlorinated Hamilton tapwater (hard water) for the metabolite study or synthetic soft water for the gas exchange study. Holding conditions, feeding, and temperature control (15 ± 1°C) were as described in Chapter 2. The use of synthetic softwater facilitated measurements of CO_2 excretion in water, which are normally quite difficult in regular Hamilton hard water due to high background CO_2 levels. Hard water conditions were Ca^{++}≈2 meq/l, Na^{+}≈0.6 meq/l, Cl^{-}≈0.8 meq/l, titratable alkalinity to pH 4.0 ≈1.8 meq/l, pH 8.0. Soft water conditions were Ca^{++}≈47 µeq/l, Na^{+}≈68 µeq/l, Cl^{-}≈95 µeq/l, titratable alkalinity ≈130 µeq/l. Soft water pH levels were reduced with 0.1 M HCl to an average pH = 6.2-6.3 to maintain lower background CO_2 levels (10-30 µM).

Gas Exchange Study

Fish weighed an average of 6 g and were fasted for 1 day prior to experimentation. Fish were acclimated overnight to the respirometers (synthetic softwater) and exercised (5 min exercise bout by chasing) the following morning (see Chapter 2 for details). At 6 h recovery after the first exercise bout, a second exercise bout (5 min) was

given and recovery monitored over the next 18 h. Control fish were treated in the same manner but were not exercised.

Rates of O_2 consumption (MO_2), CO_2 excretion (MCO_2) and ammonia excretion (M_{amm}) were measured at 5 min (0.08 h), 0.5 h, 1, 1.5, 2, 3, 4, 6, 6.08, 7, 7.5, 8, 9, 10, 12, 14, 16 and 24 h. Gas exchange rates were measured as the product of flow rate through the respirometer and the difference in gas content of inflow and outflow water samples, as described for O_2 in Chapter 2. O_2 content of water samples was determined with a Radiometer E5046 PO_2 electrode, total CO_2 content was determined by gas chromatography (Shimadzu GC-8A gas chromatograph with Shimadzu C-R3A integrator) and total ammonia content by a modified salicylate-hypochlorite method of Verdouw *et al.* (1978).

The areas under the curve for all three rates of gas exchange after both exercise bouts, relative to pre-exercise levels, were measured on an individual fish basis using a digitizer pad (GTCO Digi-pad) and a Zenith Data Systems microcomputer.

Values reported are means ± 1 S.E.M. ($n>6$). Significant differences between pre-exercise and post-exercise values, and between corresponding sample times after each exercise bout, were determined with a two-tailed, Student's t-test, paired design ($p<0.05$).

Metabolite Study

For the metabolite study, fish were held in hard water at 15°C. Fish were acclimated to the respirometers over-

night and exercised as in the gas exchange study. However, at a particular time in the recovery (0.08, 1, 3, 6, 6.08, 7, 9 and 12 h), MO_2 was measured and immediately thereafter, fish were freeze-clamped in liquid nitrogen as described in Chapter 2. Samples were subsequently freeze-dried and assayed enzymatically for LAC, GLY, ATP, CP and glucose. Fish were also sampled immediately post-exercise (0 h), for which a corresponding MO_2 measurement could not be obtained. Two control (non-exercised) samples were taken, one at the start (C1) of the experiment and one at the end (C2; *i.e.* after 12 h "recovery").

The analytical techniques used in the present study were identical to those of Chapter 2. All values were reported as means ± 1 S.E.M. (n=8) unless otherwise stated. Significance was tested via a two-tailed Student's t-test, unpaired design at $p<0.05$.

In contrast to the gas exchange study, which measured MO_2 on the same fish throughout the recovery period, MO_2 was measured on different fish for each sample point. Thus EPOC could not be quantified on an individual fish basis. EPOC, however, was measured on the mean MO_2 recovery curve (using a digitizer pad and microcomputer as above) for the purpose of comparison. In the theoretical analysis section, EPOC from the gas exchange study was used in the budget calculations, for reasons presented in Results.

RESULTS

Gas Exchange Study

MO_2 increased to about 3x resting levels from 6.3 to 20.1 µmol/g.h wet wt. after the first exercise bout (Fig.3.1), started to decline immediately thereafter and required 4 h to return to pre-exercise levels. This was similar to the response found in the first series of experiments (Chapter 2, Fig.2.1). The area under the curve, *i.e.* the EPOC, after the first exercise bout was measured to be 14.5 ± 1.9 (16) µmol/g wet wt. or 72.5 nmol/mg dry wt, based on individual fish. This was larger than the EPOC from the MO_2 study in Chapter 2 (9.1 µmol/g wet wt.) but was similar to the EPOC from the metabolite study of Chapter 2 (12.5 µmol/g wet wt.).

At 6 h, the second exercise bout resulted in the same elevation of MO_2; however, only 2-3 h were required for recovery. The EPOC was found to be significantly smaller ($p<0.05$) after the second exercise bout (8.8 ± 0.9 (16) µmol/g wet wt. or 44.0 nmol/mg dry wt.) compared to the first by about 40% (Fig.3.2).

MCO_2 increased almost 6-fold (from 4.1 to 23.6 µmol/g.h wet wt.) and required 4-6 h for return to pre-exercise levels (Fig.3.3). After the second exercise bout, MCO_2 increased to the same degree but required only 3 h for recovery. The areas under the curve after the two exercise bouts were not significantly different (22.5 ± 4.9 (6) versus 12.0 ± 1.5 (6) µmol/g wet wt.) due to the larger

Figure 3.1. Changes in the rates of O_2 consumption (MO_2) following two successive 5 min bouts of exhaustive exercise in juvenile rainbow trout. (▲) indicates non-exercised controls, n=9. (●) indicates exercised samples, n=16. Means ± 1 S.E.M. Resting samples immediately prior to exercise were a mean of 3 per fish. First sample after exercise is at 5 min. The two exercise bouts are labelled EX1 and EX2, with arrows. * indicates significantly elevated relative to the respective pre-exercise level for each bout ($p<0.05$).

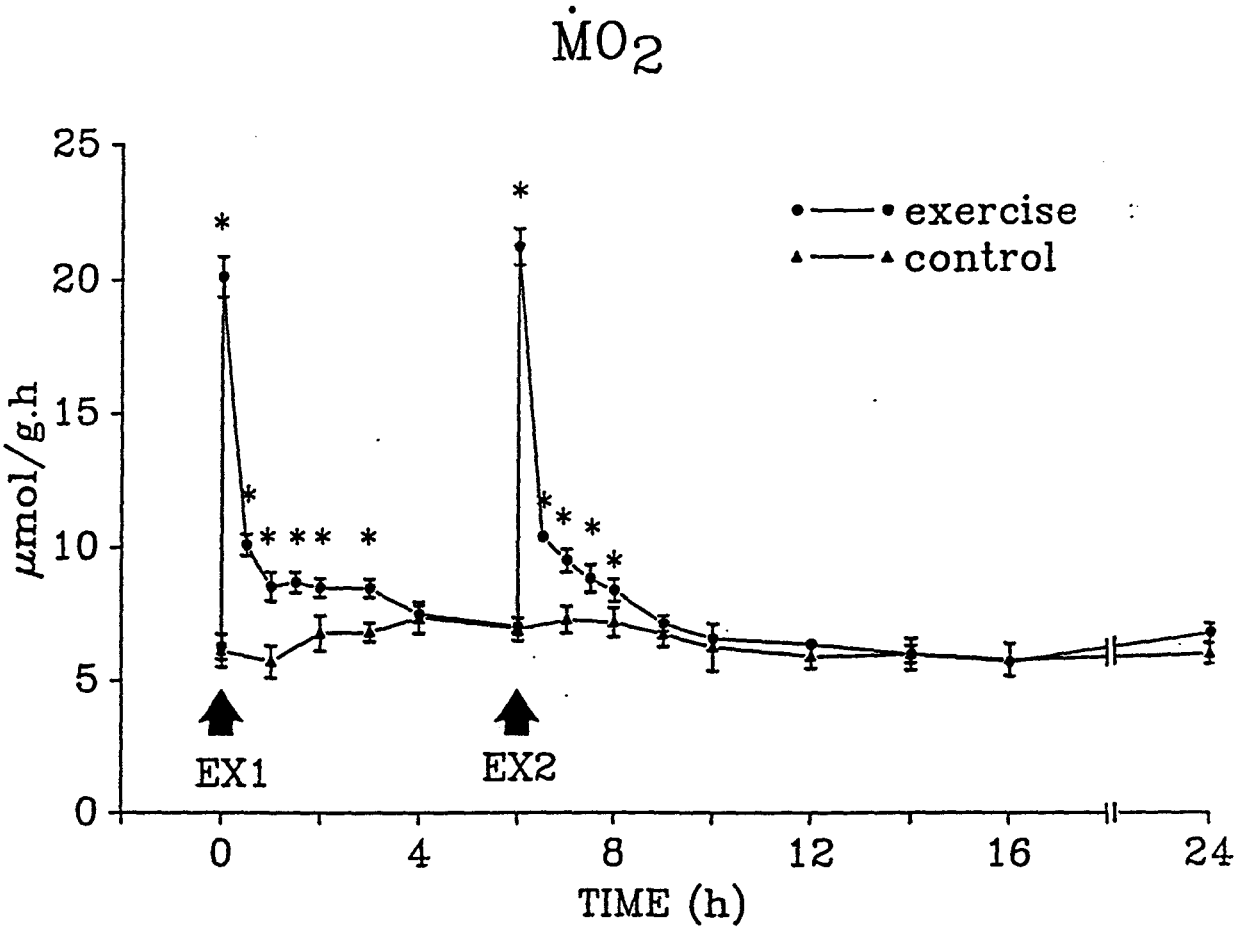

$\dot{M}O_2$
25
20
15
10
5
0
μmol/g.h
exercise
control
EX1
EX2
0
4
8
12
16
24
TIME (h)

Figure 3.2. The excess post-exercise O_2 consumption (EPOC) of two successive bouts of exhaustive exercise in individual rainbow trout, as calculated from the data of Fig.3.1. Mean (n=16) ± 1 S.E.M. * indicates EPOC of 2nd exercise bout is significantly less than ($p<0.05$) EPOC after the 1st bout.

EXCESS POST–EXERCISE OXYGEN CONSUMPTION

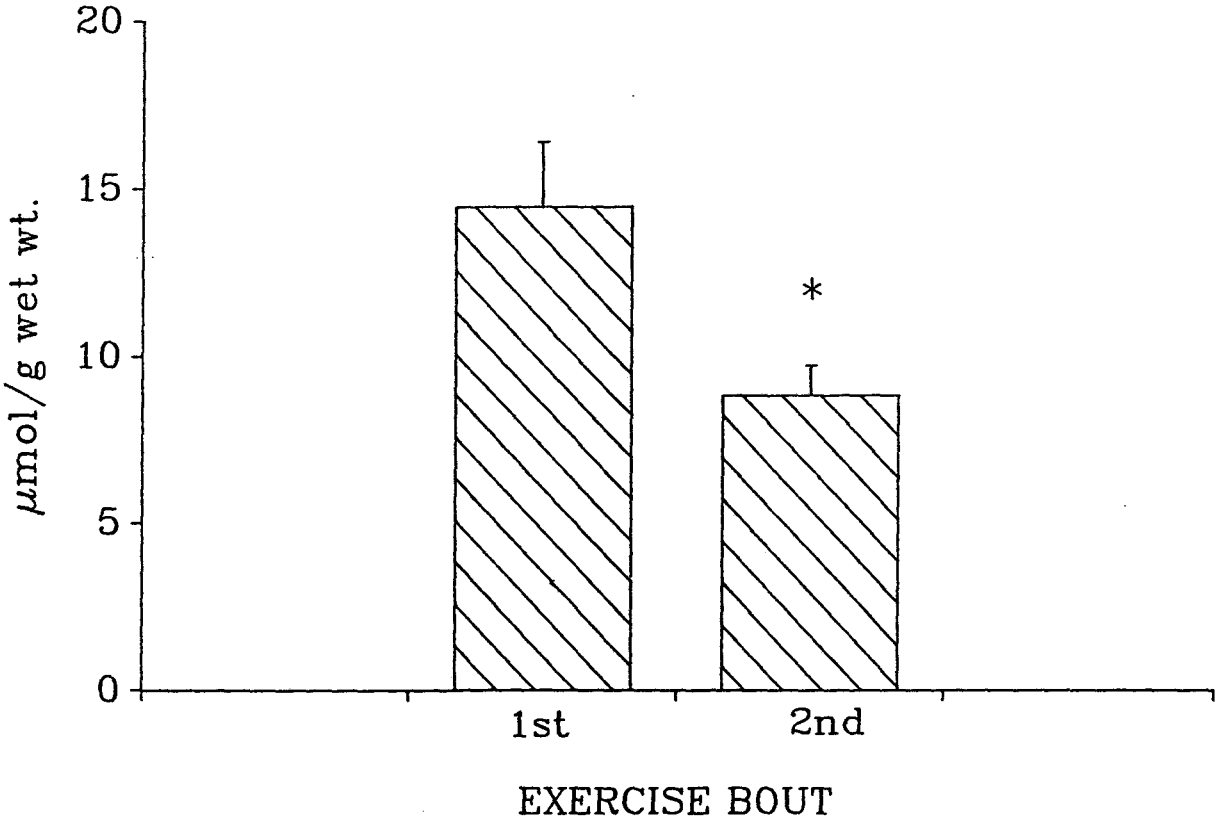
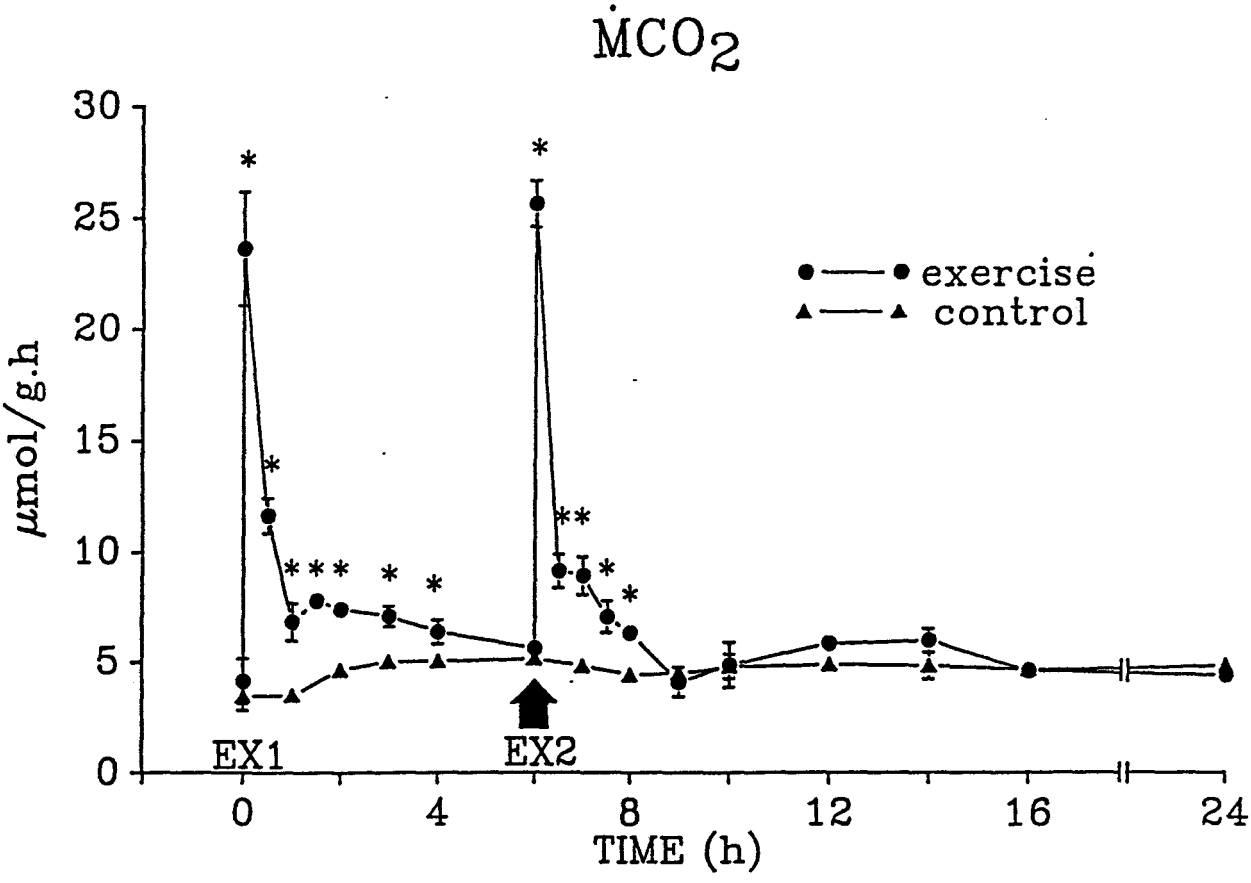


Figure 3.3. Changes in the rates of CO_2 excretion (MCO_2) following two successive 5 min bouts of exhaustive exercise in juvenile rainbow trout. (▲) indicates non-exercised controls, n=6. (●) indicates exercised samples, n=6. Means ± 1 S.E.M. Other details as in Fig.3.1.


$\dot{M}CO_2$
30
25
20
15
10
5
0
μmol/g.h
exercise
control
EX1
EX2
0
4
8
12
16
24
TIME (h)

degree of variability in CO_2 measurements and the small number of fish tested.

The instantaneous respiratory gas exchange ratios (R.E.) are reported in Table 3.1, and were calculated as mean MCO_2/mean MO_2 for exercised and non-exercised rainbow trout. The increase in MCO_2 was greater than the increase in MO_2 post-exercise, as reflected by the increase in R.E. from 0.66 to 1.17. R.E. values returned to control levels around 4-6 h in parallel to the recovery of both MO_2 and MCO_2 levels. A similar increase in R.E. was seen after the second exercise bout. In control, non-exercised fish, R.E. tended to increase very slightly with time, with an overall average R.E. value of 0.71.

The rate of ammonia excretion (M_{amm}) increased about 3x resting levels (from 0.6 to 1.8 µmol/g.h and from 0.7 to 2.0 µmol/g.h wet wt. after the 1st and 2nd exercise bouts, respectively); however, recovery was again more rapid after the 2nd exercise bout than the 1st (3 h vs 1.5 h; Fig.3.4). The areas under the curve, however, were not significantly different (2.3 ± 0.3 (8) versus 2.3 ± 0.6 (8) µmol/g wet wt.).

A summary of the changes in the rates of gas exchange (MO_2, MCO_2 and M_{amm}) following the two successive exercise bouts are shown plotted together in Fig.3.5. The M_{amm} measurements were about 1 order of magnitude smaller than either MO_2 or MCO_2.

Table 3.1. Measurements of the respiratory gas exchange ratio (R.E.) in exhaustively exercised (two subsequent 5 min bouts) and control (non-exercised) rainbow trout.

Exercised		Control	
Time (h)	R.E.	Time (h)	R.E.
Rest	0.66	Rest	0.56
EX1	--	--	--
0.08	1.17	--	--
0.5	1.15	--	--
1	0.80	1	0.61
1.5	0.89	--	--
2	0.87	2	0.69
3	0.83	3	0.74
4	0.85	4	0.68
6	0.80	6	0.74
EX2	--	--	--
6.08	1.20	--	--
6.5	0.88	--	--
7	0.94	--	--
7.5	0.80	--	--
8	0.75	8	0.66
9	0.57	9	0.67
10	0.74	10	0.77
12	0.92	12	0.83
14	1.00	14	0.80
16	0.80	16	0.80
24	0.64	24	0.81

R.E. calculated based on mean MCO_2 / mean MO_2 ; therefore there are no measures of standard error and no significance tests. Mean MCO_2 n=6. Mean MO_2 n=16.

Figure 3.4. Changes in the rates of ammonia excretion (M_{amm}) following two successive 5 min bouts of exhaustive exercise in juvenile rainbow trout. (▲) indicates non-exercised controls,n=9. (●) indicates exercised samples, n=8. Means ± 1 S.E.M. Other details as in Fig.3.1.

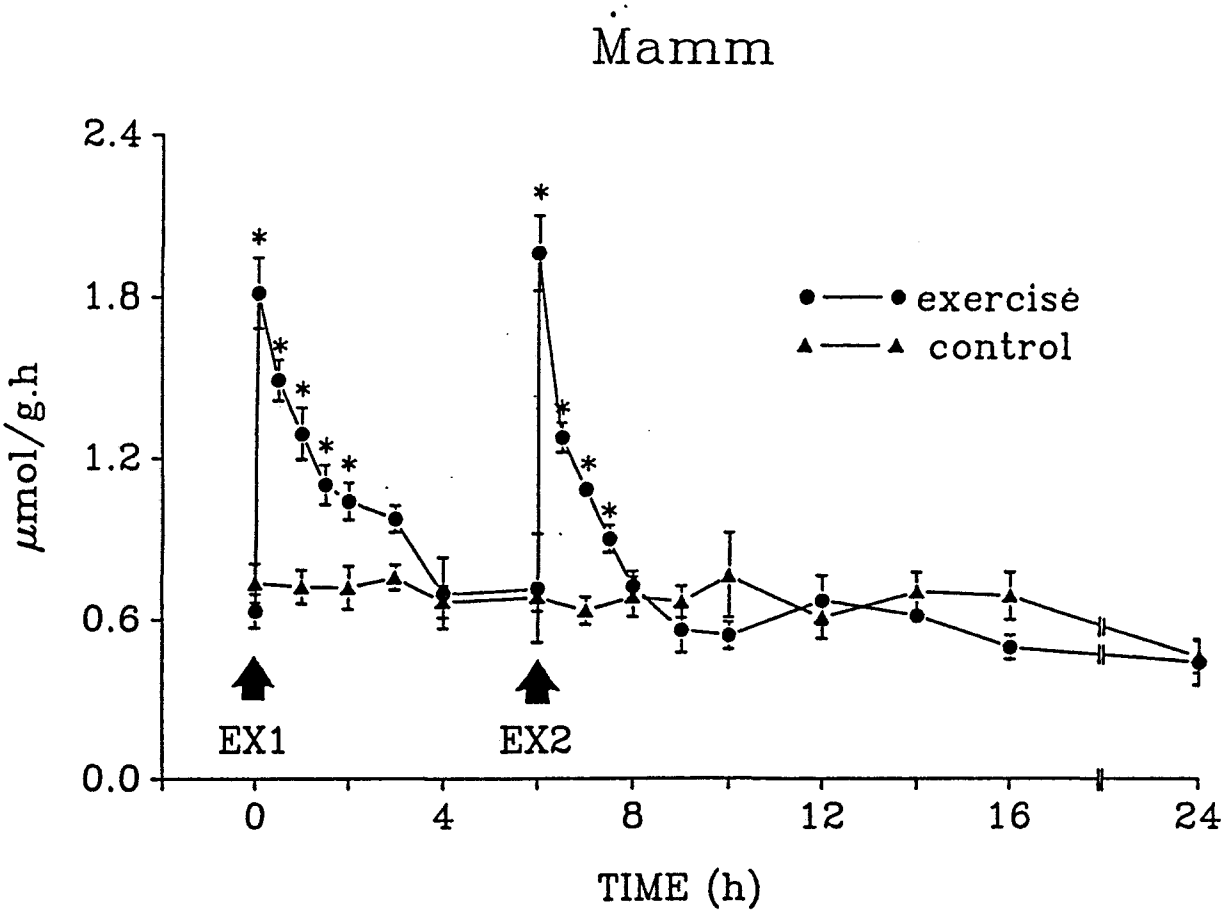
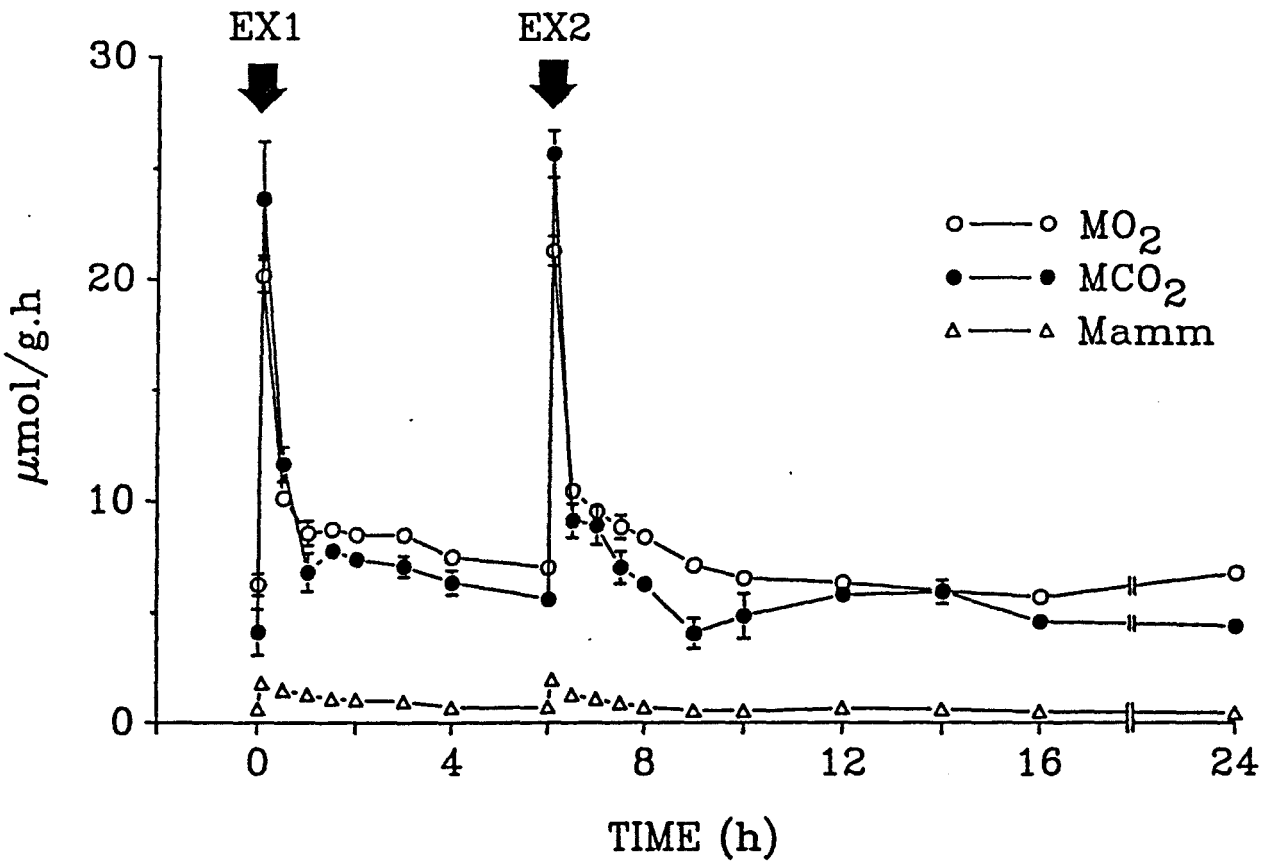

Mamm
2.4
1.8
1.2
0.6
0.0
μmol/g.h
exercisé
control
EX1
EX2
0
4
8
12
16
24
TIME (h)

Figure 3.5. Summary of the rates of gas exchange ($\dot{M}O_2$, $\dot{M}CO_2$, $\dot{M}_{amm}$) following two successive bouts of exhaustive exercise in juvenile rainbow trout. (O) indicates $\dot{M}O_2$, n=16. (●) indicates $\dot{M}CO_2$, n=6. (△) indicates $\dot{M}_{amm}$, n=8. Means ± 1 S.E.M. Significant differences are not indicated in order to avoid confusion. Refer back to Fig.3.1, 3.3, 3.4 for individual plots.

SUMMARY OF RATES OF GAS EXCHANGE

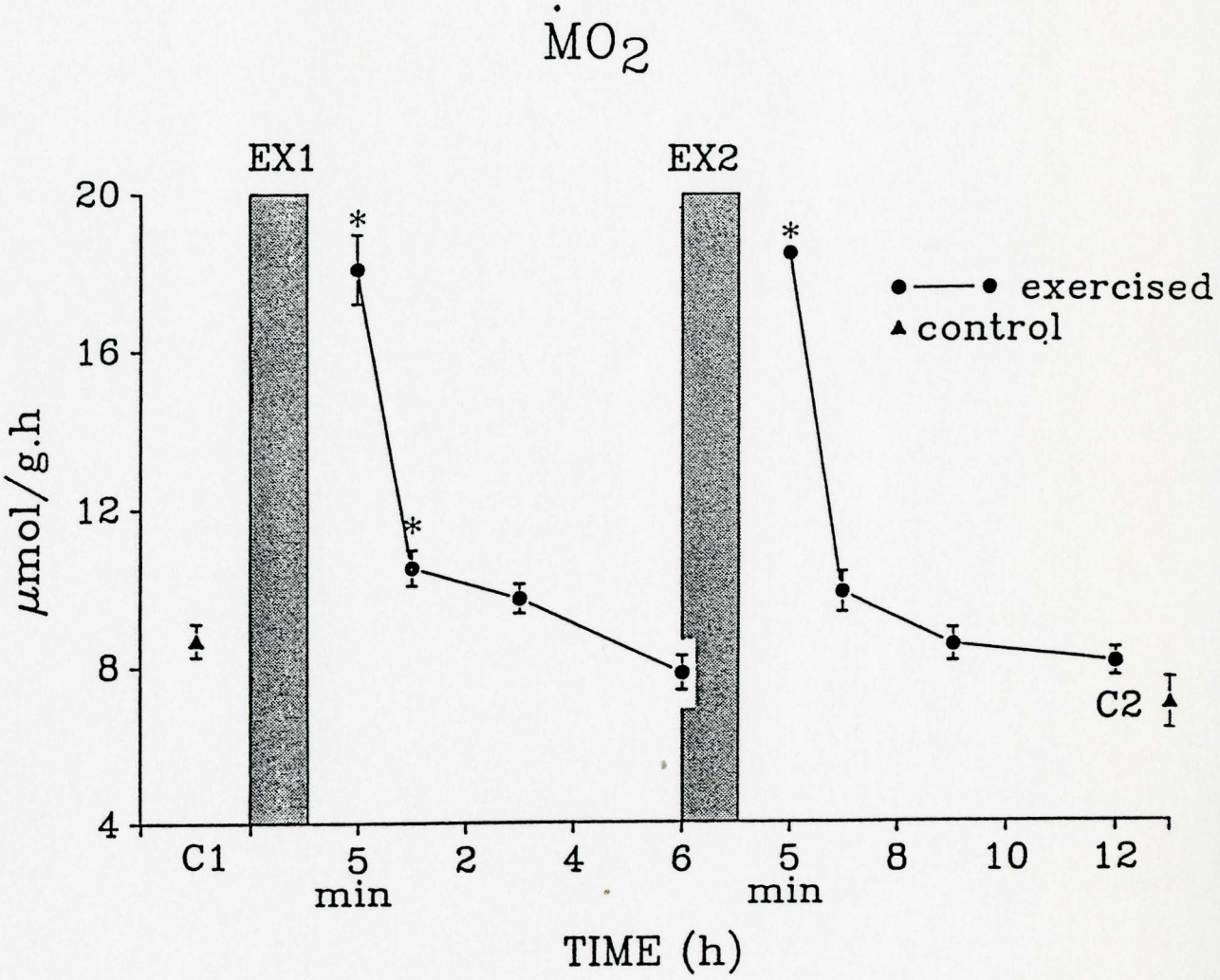


Metabolite Study

For the metabolite portion of the study, both resting and 5 min post-exercise MO_2 levels (8.7 and 18.1 µmol/g.h wet wt.; Fig.3.6) agreed well with the findings of the gas exchange study. Return to pre-exercise MO_2 levels (Fig.3.6) required about 3 h after the first (1st) exercise bout and about 1-3 h after the second (2nd) exercise bout. Again, this was similar to that measured in the gas exchange study (Fig.3.1), indicating that the soft water treatment did not affect gas exchange. However, the time course of recovery after the 1st bout was slightly faster than that found in the previous study (Chapter 2, Fig.2.1, 2.2).

EPOC, based on the mean curve (Fig.3.6), was 10.8 and 7.5 µmol/mg wet wt. for the 1st and 2nd bouts, respectively. While the significance of this difference cannot be assessed statistically, it follows the same trends as in the gas exchange study, where the difference was significant. The absolute values of EPOC were smaller than those measured in the gas exchange study on individual fish, especially after the 1st exercise bout. This may have been due to the small number of fish tested in the present study (n=8 compared to n=16 for the gas exchange study) or slightly more variable data. Despite this discrepancy, it is evident nevertheless, that MO_2 recovers more quickly after the 2nd bout (within 1 h) compared to the 1st (about 3 h). In this study, the two controls were not significantly different.

Figure 3.6. Changes in the rates of O_2 consumption (MO_2) following two successive bouts of exhaustive exercise in juvenile rainbow trout, corresponding to the metabolite portion of the study. (▲), labelled C1 and C2, indicates two control samples taken at the start and end of the experiment, respectively. (●) indicates exercised samples. Means (n=8) ± 1 S.E.M. Grey bars, labelled EX1 and EX2, are the two 5 min exercise bouts, respectively. First sample taken is at 5 min post-exercise. * indicates significantly different from C1 ($p<0.05$). There were no significant differences between corresponding sample times after the 1st and 2nd exercise bouts.


$\dot{M}O_2$
EX1
EX2
20
16
12
8
4
μmol/g.h
exercised
control
C1
C2
5
min
2
4
6
5
min
8
10
12
TIME (h)

Whole-body LAC levels (Fig.3.7) increased from 6.1 to 45.1 nmol/mg dry wt. immediately after the 1st exercise bout. Similar to the findings of the first series of experiments (Chapter 2, Fig.2.2), LAC levels continued to increase to 51.4 nmol/mg dry wt. at 5 min post-exercise. Thereafter, levels began to drop and at 6 h were not significantly elevated above controls levels. After the second exercise bout, LAC levels increased to 45.5 nmol/mg, the same as after the 1st exercise bout in contrast to the original predictions of this study. However, LAC recovery appeared to be faster after the 2nd exercise bout since there was no continued accumulation post-exercise, and by 5 min, levels had already dropped by 6 nmol/mg. Furthermore, the 9 and 12 h samples were significantly lower than the corresponding 3 and 6 h samples indicating a faster rate of recovery.

Disappearance of LAC required about 6 h after both exercise bouts (Fig.3.7), whereas MO_2 recovery required only 3 h and 1 h after the two bouts respectively (Fig.3.6). Thus, in contrast to the previous study (Chapter 2), the time courses of LAC disappearance and MO_2 recovery were not similar.

Whole-body GLY levels (Fig.3.8) dropped by about 20 nmol/mg dry wt. to 30% of rest values (from 28.5 to 8.3 nmol glucosyl U/mg dry wt.) after exhaustive exercise. Resting levels in the first series of experiments (Chapter 2, Fig.2.3), on the other hand, were slightly higher (37.8 nmol

Figure 3.7. Changes in whole-body lactate levels following two successive bouts of exhaustive exercise in juvenile rainbow trout. (▲), labelled C1 and C2, are non-exercised controls. (●) indicates exercised samples. Means (n=8) ± 1 S.E.M. Grey bars indicates the two 5 min exercise bouts. Note the expanded time scale between the zero and 5 min sample points. * indicates significantly different ($p<0.05$) from C1. + indicates significantly different ($p<0.05$) from corresponding sample time after the 1st exercise bout.

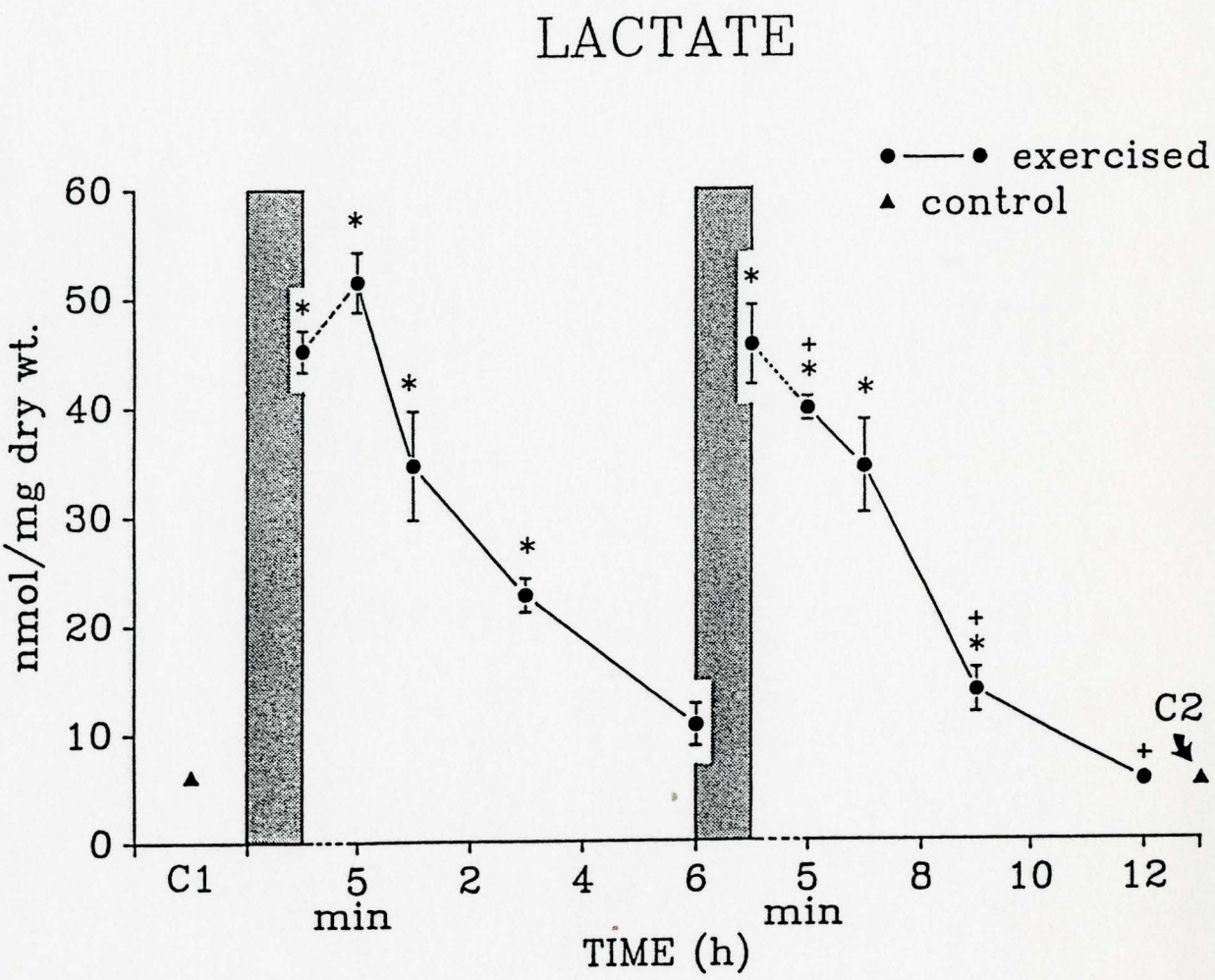
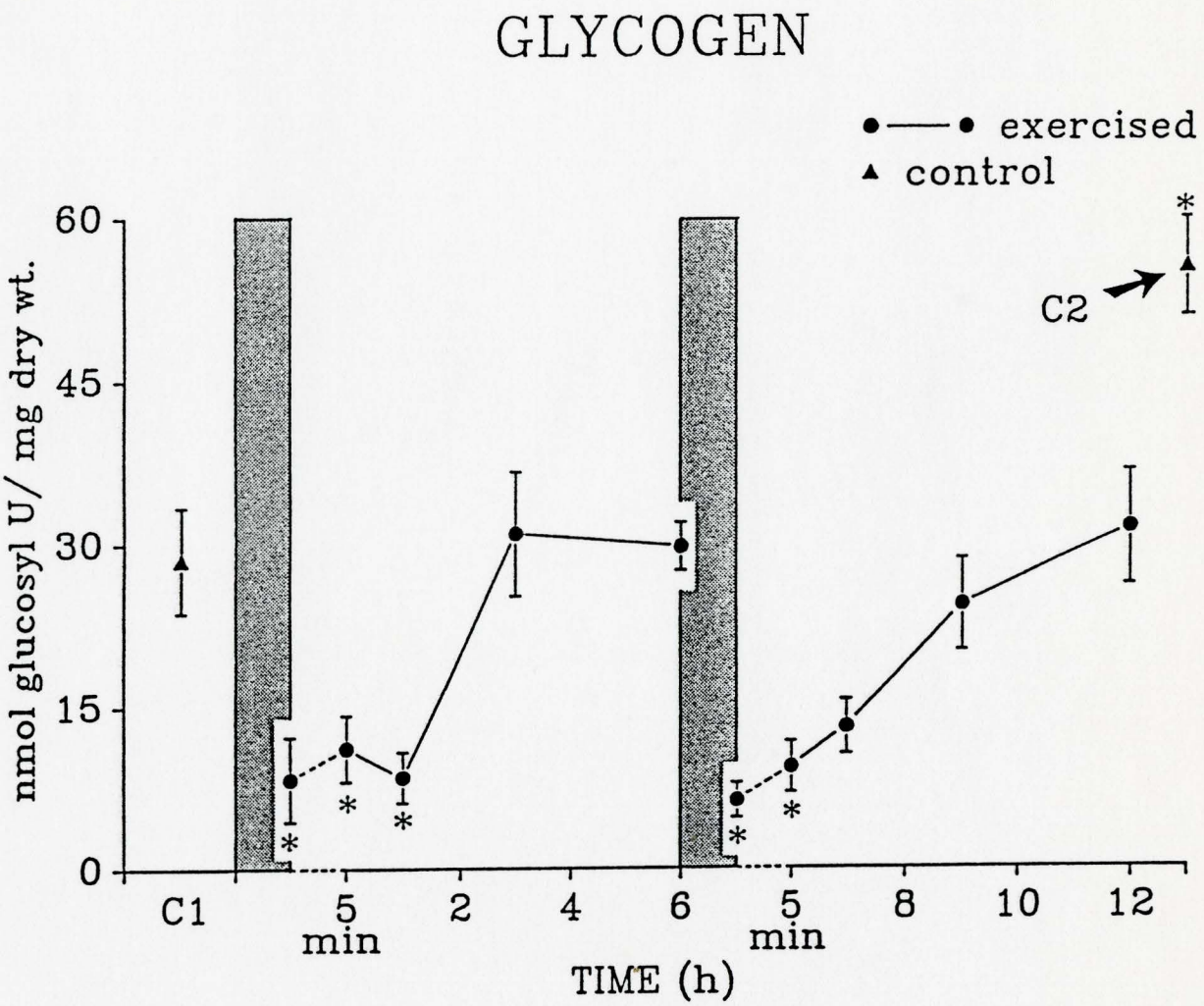

LACTATE
exercised
control
nmol/mg dry wt.
60
50
40
30
20
10
0
C1
5
min
2
4
6
5
min
8
10
12
C2
TIME (h)

Figure 3.8. Changes in whole-body glycogen levels following two successive bouts of exhaustive exercise in juvenile rainbow trout. (▲), labelled C1 and C2, are non-exercised controls. (●) indicates exercised samples. Means (n=8) ± 1 S.E.M. Other details as in Fig.3.7. There were no significant differences between corresponding sample times after the 1st and 2nd exercise bouts.

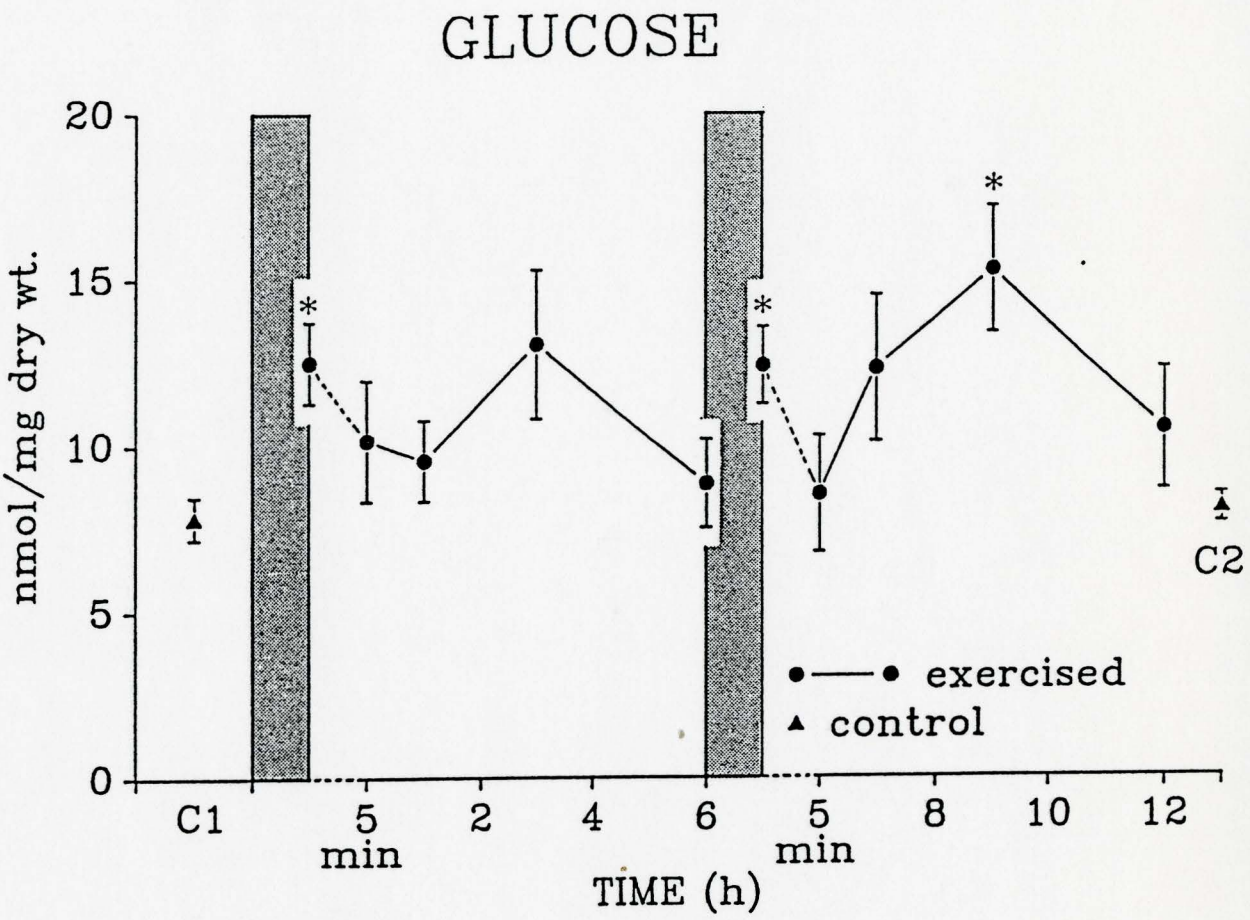

GLYCOGEN
exercised
control
C2
nmol glucosyl U/ mg dry wt.
60
45
30
15
0
C1
5
min
2
4
6
5
min
8
10
12
TIME (h)

glucosyl U/mg dry wt.) than in this study. Furthermore, in contrast to the results of Chapter 2, and in contrast to prediction, GLY was restored to resting levels after 6 h recovery. Therefore, when the 2nd exercise bout was given, the same proportion of GLY was depleted (to about 21% resting levels). This explained the similar LAC burdens after the two exercise bouts (Fig.3.7). The rate of GLY recovery was not significantly different between the two bouts, despite their slightly different pattern (Fig.3.8).

The second control GLY sample was significantly higher than the first control sample (Fig.3.8). However, this control group was run about 2 weeks after all the other groups. It is likely that the weight-specific GLY reserves of the fish had changed by that time. Since all other variables measured showed no difference between C1 and C2, it has been assumed that this point was not a true representation of GLY levels at the end of the experiment.

Whole-body glucose levels increased significantly after both exercise bouts (from 7.8 to 12.5 nmol/mg dry wt. and from 8.8 to 12.4 nmol/mg dry wt., Fig.3.9), in contrast to the first series (Chapter 2) in which no change was observed as a result of exercise (7.1 nmol/mg dry wt., Fig.2.4). However, glucose returned to resting levels by 5 min post-exercise. There was a tendency for a secondary rise in whole-body glucose at 3 h post-exercise, but this was significant only after the 2nd exercise bout (*i.e.* 9 h).

Figure 3.9. Changes in whole-body glucose levels following two successive bouts of exhaustive exercise in juvenile rainbow trout. (▲), labelled C1 and C2, are non-exercised controls. (●) indicates exercised samples. Means (n=8) ± 1 S.E.M. Other details as in Fig.3.7. There were no significant differences between corresponding sample times after the 1st and 2nd exercise bouts.


GLUCOSE
nmol/mg dry wt.
20
15
10
5
0
C1
5
min
2
4
6
5
min
8
10
12
C2
TIME (h)
exercised
control

ATP changes in the whole body were similar after both exercise bouts (Fig.3.10). ATP dropped to 25% resting levels (from 8.0 to 2.1 nmol/mg dry wt. and from 8.6 to 2.3 nmol/mg dry wt. after the 1st and 2nd exercise bouts, respectively). Thereafter, ATP started to recover immediately, requiring about 1 h to return to pre-exercise levels. These findings are similar to that of the first series of experiments (Chapter 2; Fig.2.5).

Whole-body CP decreased to 30% resting levels after the 1st exercise bout (from 30.7 to 8.9 nmol/mg dry wt., Fig.3.11) and had almost recovered in 5 min. Contrary to the first series (Chapter 2, Fig.2.7), in which only a slight tendency to over-shoot was observed, at 1 h, CP levels were significantly elevated (43.9 nmol/mg dry wt.) compared to resting levels (Fig.3.11). By 6 h, however, CP had returned to pre-exercise levels. A similar pattern was observed after the 2nd exercise bout (levels decreased from 28.5 to 5.9 nmol/mg dry wt.). Once again pre-exercise levels were restored by 5 min, however, the subsequent over-shoot was slightly greater, and at 12 h, levels were still slightly higher than the corresponding 6 h sample.

Theoretical Analysis

(A) The assumption that all GLY resynthesized came from LAC in the first 6 h, as proposed in the first series of experiments (Chapter 2), does not hold true in the present study. Values used in the theoretical analysis are shown in Table 3.2.

Figure 3.10. Changes in whole-body adenosine triphosphate (ATP) levels following two successive bouts of exhaustive exercise in juvenile rainbow trout. (▲), labelled C1 and C2, are non-exercised controls. (●) indicates exercised samples. Means (n=8) ± 1 S.E.M. Other details as in Fig.3.7. There were no significant differences between corresponding sample times after the 1st and 2nd exercise bouts.

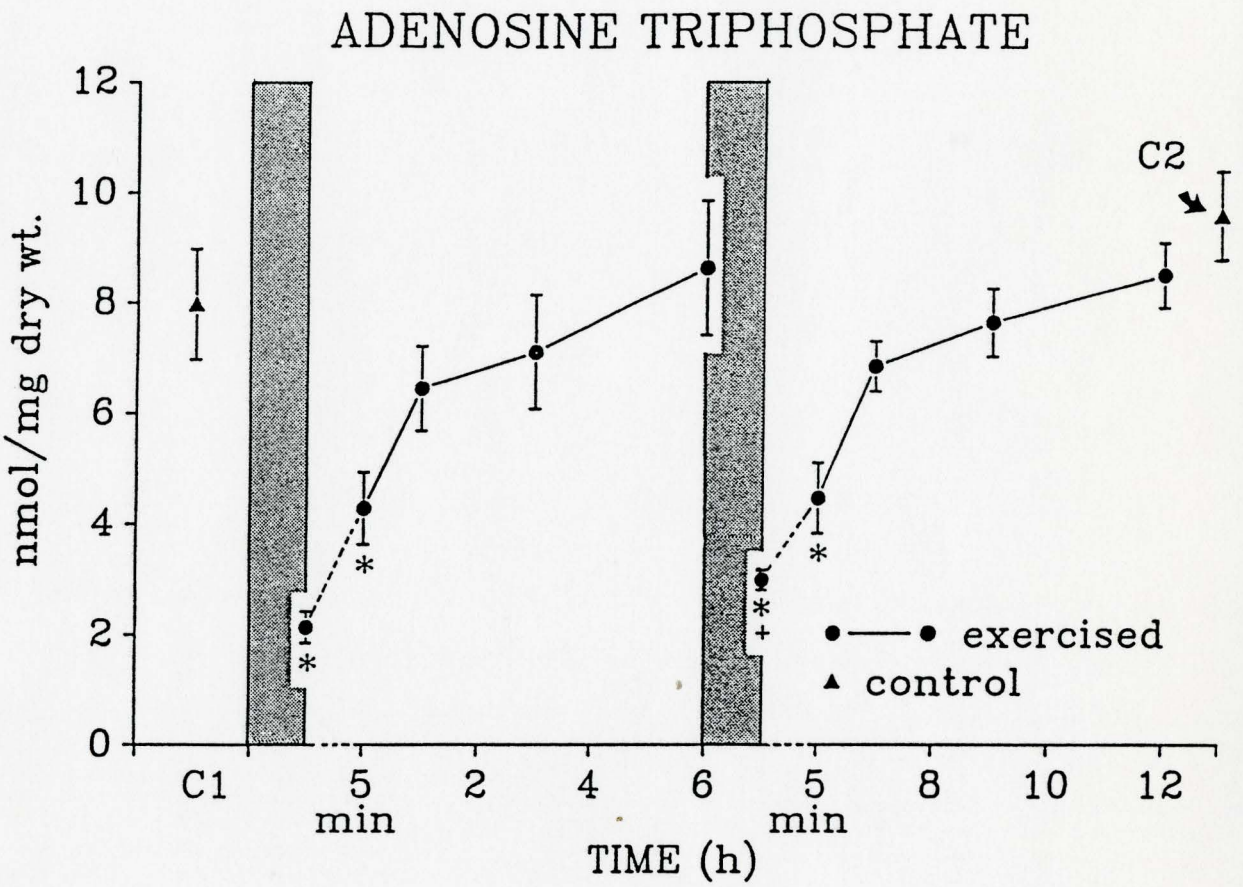

ADENOSINE TRIPHOSPHATE
nmol/mg dry wt.
12
10
8
6
4
2
0
C1
C2
5
min
2
4
6
5
min
8
10
12
TIME (h)
exercised
control

Figure 3.11. Changes in whole-body creatine phosphate (CP) levels following two successive bouts of exhaustive exercise in juvenile rainbow trout. (▲), labelled C1 and C2, are non-exercised controls. (●) indicates exercised samples. Means (n=8) ± 1 S.E.M. * indicates significantly different from C1. + indicates significantly different from the corresponding sample time after the 1st exercise bout ($p<0.05$). Other details as in Fig.3.7.

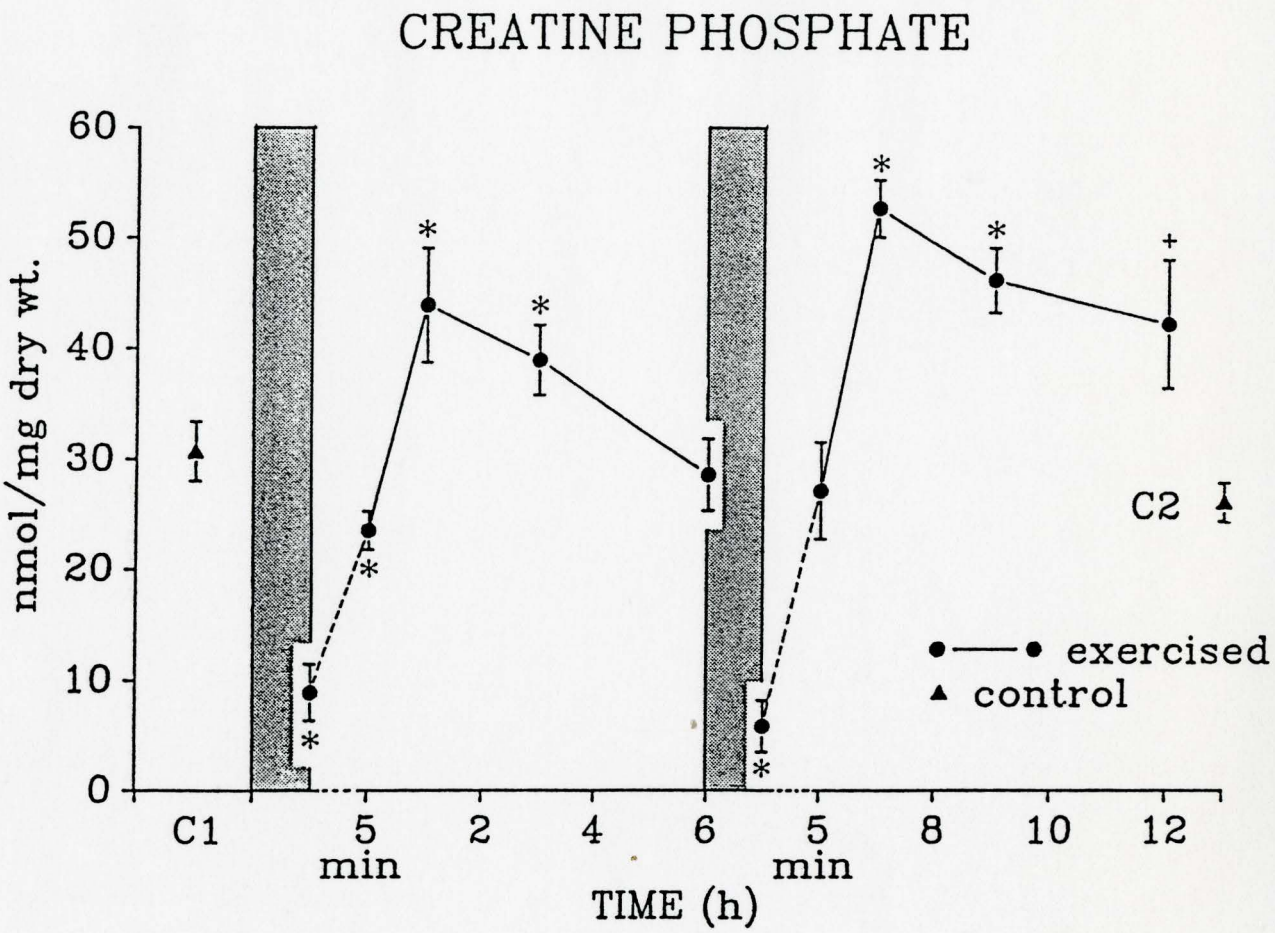

CREATINE PHOSPHATE
nmol/mg dry wt.
60
50
40
30
20
10
0
C1
5
min
2
4
6
5
min
8
10
12
TIME (h)
C2
exercised
control

Table 3.2. Changes in MO_2 and measurement of EPOC, with corresponding metabolite changes over the period 0-6 h after the 1st bout of exhaustive exercise and the period 6-12 h after the 2nd bout.

	resting levels/ at 6 h	immediately * post-exercise	at 6 h/ at 12 h recovery	difference (0-6 h)/ (6-12 h)
MO_2	8.7	18.1	7.8	see EPOC
	7.8	18.5	8.1	see EPOC
EPOC	N/A	N/A	N/A	72.5
	N/A	N/A	N/A	44.0
LAC	6.1	51.4	10.7	40.7
	10.7	45.5	5.5	40.0
GLY	28.5	8.3	29.7	21.4
	29.7	6.3	31.4	25.1
ATP	8.0	2.1	8.6	6.5
	8.6	2.3	8.5	6.2
CP	30.7	8.9	28.5	19.6
	28.5	5.9	42.2	36.3
glucose	7.8	12.5	8.8	3.7
	8.8	12.4	10.4	2.0

* The measurement used in the calculation was 5 min post-exercise. In the case of LAC, this was when peak levels were reached. In the case of MO_2, this was the first sample taken.

MO_2 is in µmol/g.h wet weight. EPOC is in nmol/mg dry wt.
GLY is in nmol glucosyl U/mg dry wt.
All other metabolites are in nmol/mg dry wt.

(1) After the 1st exercise bout, 21.4 nmol GLY/mg (as glucosyl units) were resynthesized in 6 h.
This required 42.8 nmol LAC/mg and 139.1 nmol ATP/mg based on 2 LAC + 6.5 ATP --> 1 GLY.
However, only 40.7 nmol LAC/mg were actually cleared during this time.

Similarly, after the 2nd exercise bout,
25.1 nmol glucosyl U/mg were resynthesized,
needing 50.2 nmol LAC/mg and 163.2 nmol ATP/mg.
Again, only 40.0 nmol LAC/mg were actually cleared during this time.

While allowing a small margin of error, if all the LAC cleared during this time was actually utilized to resynthesize GLY, other questions are raised. Primarily, the fuel source which is burned by the excess MO_2 could not be LAC and its identity is not known. In the first series (Chapter 2), only 75% of the LAC cleared could be accounted for by GLY resynthesis, thus leaving 25% for oxidation. Clearly, in the present study, this type of analysis cannot provide the full explanation.

(B) Based on the "alternative" analysis first mentioned in Chapter 2, another possible explanation is explored. This was based on the opposite assumption that the entire EPOC was utilized to oxidize LAC.

(1) Since total EPOC = 72.5 nmol/mg dry wt.
$72.6\ O_2 + 24.2$ LAC --> 423.5 ATP $+ 72.6\ CO_2 + 72.6\ H_2O$
based on $3\ O_2$ + LAC --> 17.5 ATP $+ 3\ CO_2 + 3\ H_2O$

This accounts for about 59% of the total (40.7 nmol/mg) LAC burden cleared during this time.

(2) The remainder of the LAC (41%; 16.5 nmol/mg) was converted into GLY.

Thus: 8.2 GLY <-- 16.5 LAC + 53.3 ATP

based on 1 GLY <-- 2 LAC + 6.5 ATP.

(3) Of the total GLY resynthesized during this time (21.4 nmol glucosyl U/mg dry wt.), only 38% came from LAC and the majority (62%; 13.2 nmol/mg) came from other sources.

Similar calculations were done for the 2nd exercise bout.

(1) Total EPOC = 44.0 nmol/mg dry wt. Thus:

44.1 O_2 + 14.7 LAC --> 257.3 ATP + 44.1 CO_2 + 44.1 H_2O

This accounts for only 37% of the total LAC burden cleared during this time (40.0 nmol LAC/mg in 6 h).

(2) The remainder (63%) was converted into GLY.

Thus: 12.7 GLY <-- 25.3 LAC + 82.2 ATP.

(3) Of the total GLY resynthesized (25.1 nmol glucosyl U/mg dry wt.) during this time, 51% (12.7 nmol/mg) came from LAC and 49% (12.4 nmol/mg) came from other sources.

Assuming the entire EPOC was utilized to oxidize LAC, the budgets of the cost of recovery, in terms of ATP equivalents, are shown in Table 3.3A and 3.3B for the 1st and 2nd exercise bouts, respectively. Of the total ATP generated via LAC oxidation (423.5 nmol/mg), only 134.7 nmol ATP/mg can be accounted for after the 1st exercise bout (Table 3.3A). However, as most of the GLY resynthesized

Table 3.3. Budget of the cost of recovery based on the "alternative" scenario in terms of ATP equivalents after (A) a bout of exhaustive exercise and (B) after a second, subsequent bout in juvenile rainbow trout.

(A) ATP COST OF RECOVERY AFTER THE FIRST BOUT (nmol/mg)

32	ATP used for increased ventilatory work
11	ATP used for increased cardiac work
7	ATP were restored
14	ATP equiv. needed in ATP resynthesis
20	creatine phosphate were restored
53	ATP used in GLY resynthesis from LAC
137	ATP accounted for versus:
423	ATP produced from LAC oxidation

≈68% unaccounted for

(B) ATP COST OF RECOVERY AFTER THE SECOND BOUT (nmol/mg)

19	ATP used for increased ventilatory work
6	ATP used for increased cardiac work
6	ATP were restored
12	ATP equiv. needed in ATP resynthesis
36	creatine phosphate were restored
87	ATP used in GLY resynthesis from LAC
166	ATP accounted for versus:
257	ATP produced from LAC oxidation

≈35% unaccounted for

Values were taken from Table 3.2. See Theoretical Analysis for more detail. All values are in nmol/mg dry wt. Estimates for increased ventilatory work (5-10%) and increased cardiac work (0-5%) were based from the estimates of Farrel & Steffensen (1987).

during this time did not come from LAC, the exact quantity of ATP required is not known and thus was not taken into account in the budget.

After the 2nd exercise bout, the EPOC was significantly reduced by 40%, therefore accounting for only a small portion of the LAC cleared. This not only generated less ATP (compared to the 1st bout) but also more of the GLY resynthesis was accounted for by the extra LAC. The end result is that only 35% of the ATP generated via LAC oxidation cannot be accounted for by this budget (Table 3.3B).

DISCUSSION

Effects On Gas Exchange

The time course of MO_2 recovery (Fig.3.1) after the 1st exercise bout was similar to that seen in Chapter 2 (Fig.2.1). However, although MO_2 increased to the same degree after the 2nd exercise bout (Fig.3.1), recovery was considerably faster and EPOC significantly reduced by 40% (Fig.3.2). This suggests that most of the EPOC after the second exercise bout in the present study was used for the replenishment of GLY and phosphagen stores and LAC recovery, and very little was required for other processes. It appears that the fish devote less energy to "unnecessary" reactions, resulting in an ability to recover more quickly, suggesting that fish are better capable of handling a repeat stress.

A number of factors known to affect EPOC in mammals, such as elevated catecholamine levels and substrate cycling, may play a role in speeding up recovery. In 400 g rainbow trout, plasma adrenaline and noradrenaline increased from 1.4 to 212 x 10^{-9} mol/l and from 10.2 to 85 x 10^{-9} mol/l respectively, following repeated burst swimming (Butler *et al.*, 1986). The elevated circulating levels of catecholamines may stimulate MO_2 via many different actions, including increases in the number and volume of red blood cells, regulation of erythrocyte pH and increases in cardiac output and ventilation (Wood & Perry, 1985; Primmett *et al.*, 1986). Blood O_2 content may actually rise, despite increased demand by working tissues and decreased blood transit time

through gills. Plasma catecholamines were elevated even after 4 h recovery from burst swimming, while swimming at 80% Ucrit (Primmett *et al.*, 1986). Sustained swimming is not thought to elevate plasma catecholamine levels (Ristori & Laurent, 1985; Butler *et al.*, 1986). It is possible, therefore, that when the 2nd exercise bout was given, plasma catecholamine levels were still elevated, thus maintaining increased blood O_2 transport capacities during the exercise bout itself, so that less post-exercise EPOC is required, thereby speeding up MO_2 recovery.

An alternate possibility is that the fish have been familiarized to the procedure by this time such that the fear and stress component associated with the exercise is removed. Thus, the elevation of catecholamines may actually be less after the 2nd exercise bout than the 1st. This would mean that the return to resting levels of catecholamines is faster. There is less "non-specific" MO_2 and concomitantly, MO_2 recovery is also faster.

Plasma catecholamines also regulate bicarbonate ion entry into fish red blood cells, the rate limiting step in CO_2 excretion (Wood & Perry, 1985). CO_2 excretion rates greatly increased after both exercise bouts (Fig.3.3), with recovery being considerably faster after the 2nd exercise bout. Since the rise in MCO_2 was greater than the rise in MO_2, an increase in the respiratory gas exchange (R.E.) ratio was also observed (Table 3.1). R.E. values greater than 1.0 are an indication of metabolic acidosis due to

anaerobic metabolism, which titrates blood and tissue bicarbonate stores, resulting in very high CO_2 production rates after severe exercise. The resting R.E. values in this study (about 0.71) are slightly lower than those reported by Steffensen *et al.* (1987) for rainbow trout swimming slowly at around 1 BL/s (0.73).

Resting M*amm* measurements in the present study (0.63 µmol/g.h wet wt.; Fig.3.4) agree well with the findings of Brett and Zala (1975) in resting 29 g sockeye salmon (0.5-0.6 µmol/g.h wet wt.). After burst swimming at velocities greater than 4 BL/s, 30-55 g rainbow trout, blood and white muscle ammonium levels increased significantly (Mommsen & Hochachka, 1988). The same response is observed in adult trout (Wright & Wood, 1988). Ammonium increase after exercise is thought to occur as a result of adenylate pool depletion (see General Introduction, reaction 4), as well as possible increased protein metabolism in the white muscle. The majority is retained in the white muscle; ammonium is required in the purine nucleotide cycle to aid the conversion of IMP back into AMP to restore the adenylate pool. However, a small amount is released into the blood and either metabolized in the liver or excreted at the gills. The relative increases in M*amm* after exercise seen in the small fish seen in the present study were similar to those recorded in adult trout (Milligan & Wood, 1986; Wood, 1988).

Metabolite Status

In contrast to the prediction based on the first series of experiments (Chapter 2), the 2nd exercise bout did not result in a smaller LAC burden (Fig.3.7). This is presumably because by 6 h, whole body GLY levels had already returned to control levels (29.7 nmol glucosyl U/mg dry wt., Fig.3.7). This complete repletion of GLY in only 6 h contrasts with both the whole body results of Chapter 2 (see Fig.2.3; only 65% repletion in 24 h), and several previous studies on white muscle GLY levels (Black *et al.*, 1962; Milligan & Wood, 1986; Pearson *et al.*, 1989; all less than 40% repletion in 6 h).

The reason(s) for this discrepancy is unknown. Relative to earlier studies, tissue variation (*i.e.* whole body GLY versus white muscle concentrations) or differences in the size of fish used (greater than 200 g; Black *et al.*, 1962; Milligan & Wood, 1986; Dobson & Hochachka, 1987; 50 g, Pearson *et al.*, 1989; 6 g, present study) may be important. However, these cannot explain the difference from the results of Chapter 2, where the fish weighed 2-3 g. One possibility is the somewhat lower starting GLY levels in the present fish relative to those of Chapter 2 (28.5 versus 37.8 nmol glucosyl U/mg dry wt.) Initial GLY reserves are dependant on numerous factors, such as previous exercise and feeding/fasting (Miller *et al.*, 1959). It may be easier for fish to replete low resting GLY levels than high resting GLY levels.

Since GLY had recovered within 6 h, the second exercise bout resulted in a similar decrease in whole body GLY and increase in LAC. It is known that during strenuous exercise, most of the GLY depleted occurs anaerobically, resulting in a direct relationship with LAC accumulation (Dobson & Hochachka, 1987). About 39 nmol/mg dry wt. of LAC accumulated during the first 5 min exercise bout and 20 nmol glucosyl U/mg dry wt. of GLY were depleted. Thus, in this study, a ratio of 1:2 GLY:LAC does exist, in contrast to the results of Chapter 2, indicating that essentially all of the energy from GLY required for exercise was derived by anaerobic means. In contrast, after the second exercise bout, 23 nmol/mg dry wt. GLY generated only 35 nmol/mg dry wt. LAC, indicating that some of the GLY was metabolized aerobically. This is a more efficient means of generating energy.

One possible explanation for the difference between the results of the present study and those of Chapter 2 with respect to GLY repletion may be due to glucose levels. Whole-body glucose levels in the present study were significantly elevated after exercise (Fig.3.9). It is possible that the fish in the present study were able to resynthesize some of their GLY from glucose.

The time course and pattern of LAC changes (Fig.3.7) after the first exercise bout was similar to that seen in the experiments of Chapter 2 (Fig.2.2). However, the continued post-exercise glycolysis was not observed after

the second exercise bout. Indeed, LAC levels peaked immediately post-exercise, and by 5 min, 17% of the LAC burden had already been removed. Dobson & Hochachka (1987) have speculated that the continued post-exercise glycolysis (lasting 1-2 h) was required to replenish ATP stores. Since the post-exercise glycolysis in the present study lasted only 5 min, it is more likely that glycolytic intermediates, "stored" in the glycolysis pathway, simply continued to run through the pathway (Pearson *et al.*, 1989) as already discussed in Chapter 2.

LAC recovery was faster after the 2nd exercise bout (Fig.3.7). This is the first time that improved recovery ability has been demonstrated after a single exercise bout. However, faster rates of recovery for a number of glycolytic intermediates, as well as GLY and LAC, have been shown in studies involving long-term exercise training in fish (Hochachka, 1961; Hammond & Hickman, 1966; Lackner *et al.*, 1988; Pearson *et al.*, 1989). In contrast to the findings of the present study, intermittently-trained chub exhibited post-exercise glycolysis, which was thought to be the driving force for the accelerated rate of recovery observed (Lackner *et al.*, 1988). Since the opposite result was found in the present study, this cannot be the explanation for the accelerated rates of recovery observed.

The faster rates of recovery, in particular the more rapid return of the rates of gas exchange to resting levels, suggest that, juvenile rainbow trout are capable of handling

severe repetitive exercise bouts. This is not surprising as similar responses in exercise performance and rate of recovery are observed in both sprint trained and endurance trained rainbow trout (Hammond & Hickman, 1966; Lackner *et al.*, 1988). Recently, Pearson *et al.*(1989) found that rainbow trout, sprint trained for 30 s every other day for 9 weeks, showed greater resistance to fatigue by swimming farther and faster than untrained trout. Trained trout were also able to tolerate 32% greater LAC accumulations and showed a faster rate of LAC recovery.

Endurance training in fish resulted in significant hypertrophy of red muscles at speeds in excess of 1 BL/s (Greer Walker & Emerson, 1978). However, it is not likely that these structural and morphological-type changes as a result of training are the explanation for the changes occurring after only 2 successive exercise bouts as in the present study. A much faster mechanism must be in effect, such as elevated catecholamines.

Whole-body ATP (Fig.3.10) and CP (Fig.3.11) showed very similar time courses of recovery after both exercise bouts, ATP recovering in about 1 h and CP requiring only 5 min. Similar rapid rates have been observed by others (Weiser *et al.*, 1985; Pearson *et al.*, 1989). In contrast to the first study (Chapter 2), however, CP significantly overshot resting levels at 1 h post-exercise. This has been shown before in white muscle (Milligan & Wood, 1986), although the reasons have not yet been determined. It is speculated that

the fish quickly build up a large store of CP via LAC oxidation, which is then utilized during the remainder of the recovery period to drive the conversion of LAC to GLY.

Interpretation of the Theoretical Analysis

(A) Primary fate of LAC is GLY resynthesis.

The experiments of Chapter 2 demonstrated that 75% of the total accumulated LAC was utilized for GLY resynthesis while only 25% was oxidized, based on the analysis that all GLY restored was attributed as having come from LAC (see Tables 2.2A & 2.2B). In contrast, in the present study, far more GLY was restored during the first 6 h of recovery than in the previous studies, while approximately the same amount of LAC was removed. LAC removal was not adequate to fully explain all of GLY resynthesis. The discrepancy between the two studies is the GLY levels. Despite the fact that initial GLY levels were lower in this study (Fig.3.8) than in the previous study (Fig.2.3), more GLY was actually resynthesized in the first 6 h of recovery. Since LAC accumulation and disappearance was similar in both studies, clearly some of the GLY resynthesized must have come from sources other than LAC, but the actual contribution is not known. While the primary fate of LAC may still be GLY resynthesis, the quantitative approach used in the previous study (Chapter 2) cannot be used in the present study.

Nevertheless, despite this discrepancy, the present study yields one important conclusion. Regardless of the fate of LAC (*i.e.* contribution to GLY resynthesis or

oxidation), the quantity of LAC cleared remains unchanged after the 2nd exercise bout (Table 3.2), whereas EPOC was significantly reduced (Fig.3.2). Thus, in fact, repetitive exercise bouts, if given sufficiently far enough apart for metabolic recovery, result in a similar response of metabolite status while gas exchange recovery is greatly accelerated.

(B) Primary fate of LAC is oxidation.

Although evidence in the literature (Milligan & Wood, 1986; Dobson & Hochachka, 1987; Milligan & McDonald, 1988) points to the conclusion that the primary fate of LAC is GLY resynthesis *in situ*, the results of the present study do not support it entirely. As the first analysis could not be followed through, a second alternative approach was attempted. By this analysis, the entire EPOC is utilized to oxidize some of the accumulated LAC. After the 1st exercise bout, 59% of the total LAC cleared during the first 6 h was oxidized while only 41% was utilized for GLY resynthesis. As discussed in Chapter 2, this may not be totally unreasonable since some (10%) LAC is known to leave the white muscle (Turner & Wood, 1983) and white muscle itself has some oxidative capacity (Bilinski & Jonas, 1972).

After the 2nd exercise bout, since the LAC burden was essentially unchanged and the EPOC was reduced by 40%, LAC oxidation could only account for 37% of the total LAC cleared after 6 h. Thus, the remainder of the LAC (63%), the majority, was resynthesized into GLY. Despite the fact that

this accounts for only half of the GLY restored, these figures are more comparable to the findings of the previous study (Chapter 2), based on the first analysis and the literature.

Clearly, the metabolism of the accumulated LAC is not the sole explanation of EPOC, since the relation observed after the 1st exercise bout based on the analysis was not maintained after the 2nd exercise bout. The EPOC accounted for 20% less of the LAC disappearance after the 2nd bout, since EPOC was reduced but the LAC burden remained the same.

Conclusions

In summary, for most metabolites measured, the first exercise bout resulted in time courses very similar to that observed in the first study. GLY showed a faster rate of recovery than originally expected, recovery being complete within 6 h, in contrast to the findings of Chapter 2. Except in the case of LAC, which showed an accelerated rate of recovery, all other metabolites showed similar time courses of recovery after the 2nd exercise bout. However, a repetitive exercise bout resulted in considerably faster rates of recovery of MO_2, MCO_2 and M_{amm}. More significantly, EPOC was reduced by 40%. The reasons for this are not yet known, and requires further study.

It is hypothesized that after recovery from the first exercise bout, the fish are in a state of "readiness", possibly dependant on catecholamine levels, such that they are better capable of handling a repeat stress. This has

distinct advantages in the wild while escaping predators or catching prey, when the requirement for burst swimming at maximum effort may be frequent. This also has implications in sport fishing, since fish struggle to exhaustion when "hooked", are later released and may be recaptured. The present study suggests that repetitive exercise bouts may actually increase resistance to distress. Indeed, angling-induced exercise in northern pike, fish well adapted for burst swimming, resulted in similar patterns and time courses in GLY depletion and LAC accumulation (Schwalme & Mackay, 1985).

Thus, a relationship between the removal of the LAC burden and EPOC was not established, even though the time course data and budget analysis of Chapter 2 originally suggested some correlation. Although some of the EPOC is indeed utilized to oxidize a portion of the accumulated LAC, this does not determine the magnitude of EPOC, because the quantity of LAC cleared post-exercise was the same after the repetitive bouts, whereas EPOC was significantly reduced.

CHAPTER 4

THE EFFECT OF PRIOR GLYCOGEN DEPLETION ON EPOC AND BIOCHEMICAL CHANGES AFTER EXHAUSTIVE EXERCISE IN JUVENILE RAINBOW TROUT

INTRODUCTION

While the protocol of Chapter 3 was unsuccessful in creating a smaller post-exercise LAC burden, it was successful in separating EPOC from LAC burden, because the 2nd EPOC was lowered while the LAC burden was unchanged. To confirm this conclusion, another treatment was undertaken, to achieve the original goals of Chapter 3 (*i.e.*lower LAC burden).

In humans, glycogen depletion was found to significantly alter metabolism both prior to and following moderate to heavy exercise (Segal & Brooks, 1979). Glycogen depletion, via changes in diet and prior exercise, did not affect resting MO_2, but lowered blood LAC and glucose levels, and presumably initial muscle GLY levels, though this was not measured. After both heavy and moderate exercise, MO_2 was similar for normal glycogen (NG) and glycogen depleted (GD) subjects, whereas blood LAC was greater in the NG state. Segal and Brooks (1978) concluded that work load, rather than blood LAC levels, was the major determining factor of EPOC, results inconsistent with the O_2 debt hypothesis of Hill & Lupton (1923).

In fish, these conditions have not yet been tested to determine if indeed the accumulated LAC regulates post-

exercise MO_2. It is known, however, that the state of GLY reserves profoundly affects resistance to fatigue and exercise performance (Miller *et al.*, 1959). Fish with lower GLY reserves due to diet manipulations, showed poor resistance to fatigue and slower recovery after both prolonged and burst swimming.

The purpose of this study, therefore, was to manipulate initial GLY stores and concomitantly alter the accumulation of LAC after exercise. The ensuing effects on the pattern of post-exercise MO_2 and more specifically, on EPOC, were examined. The goal was to again experimentally dissociate EPOC and LAC disappearance during recovery from exhaustive exercise, and thereby provide further evidence that the O_2 debt hypothesis of Hill & Lupton (1923) is not the appropriate explanation for the relationship(s) between EPOC and LAC recovery.

METHODS & MATERIALS

Preliminary Experiment

It is known that some fish tend to defend carbohydrate stores during starvation at the expense of either lipid or protein (see Moon & Johnston, 1980). Thus preliminary testing using fish (weight ≈ 6 g) left over from the experiments of Chapter 2, were performed to determine the experimental protocol which would deplete a significant amount of whole-body GLY. It was found that 5 days starvation reduced GLY levels by 52% (from 56.3 to 27.2 nmol glucosyl U/mg dry wt., n=8), 9 days starvation only caused a further decrease by 1% (to 26.5 nmol glucosyl U/mg, n=8), and 3 days of continuous swimming at 2 BL/s without food dropped GLY stores by 37% (to 35.6 nmol glucosyl U/mg dry wt., n=8). It was concluded that 5 days starvation would sufficiently reduce GLY stores for the purpose of this study and thus alter the LAC accumulation after a burst of exhaustive exercise.

Animals

Juvenile rainbow trout were obtained from Rainbow Springs Trout Farm in Thamesford, Ontario and held in large 400 l circular tanks, with fresh inflowing dechlorinated Hamilton tap water. Note that these fish were from a different stock of fish than either of those used in Chapters 2 and 3. The fish weighed approximately 6 g. Temperature of the water was 15 ± 1°C. Fish were fed 1.5 Gr. trout pellets (Martin Feed Mills, Don Mills, Ontario) every

day to satiety. Fish used for the GLY depleted group (GD) were removed from the holding tank 5 days before the experiment and held in square wash tubs, under similar conditions, but were not fed. This duration of starvation was determined to be sufficient to drop GLY levels by about 50%, based on the findings of the preliminary study. Both groups (NG and GD) were weighed 1 day prior to experiments and then were acclimated overnight (10-12 h) to the respirometers, as was done in the previous studies.

Experimental Protocol

MO_2 Study

In this study, only MO_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 3 measurements was taken prior to exercise. The first post-exercise sample was taken at 5 min, then at 0.5 h, 1, 1.5, 2, 3, 4, 6, 8 and 12 h. Control fish were treated in the same manner but were not exercised.

The previous studies demonstrated that MO_2 measurements were less variable in these types of studies since individual fish were followed for the duration of the experiment, in contrast to the use of single terminal samples for the metabolite study. Therefore, although MO_2 was also measured in the metabolite portion of the study, calculations in the budget were based on EPOC measurements from the "MO_2 alone" study. EPOC was measured on an individual fish basis (0-6 h) relative to pre-exercise

RESULTS

MO_2 Study

Resting MO_2 levels in NG fish were about 7 µmol/g.h wet wt., which increased 2.5-fold after the 5 min exercise bout, and required about 4 h for a complete return to resting levels. The increase in MO_2 in NG fish and the time course of recovery (Fig.4.1A) is consistent with the results of the previous studies (Fig.2.1, Fig.3.1).

GD fish had similar resting and immediately post-exercise levels of MO_2 (Fig.4.1B). The time course of recovery after exercise, however, was slightly different. Mean MO_2 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 (Fig.4.2). There were no significant changes in MO_2 in non-exercised controls for either group over the entire experimental period.

Metabolite Study

MO_2 changed as in the MO_2 study, however, the slight differences noted between the NG and GD groups were not observed (Fig.4.3). Except for a small but significant difference at the 5 min post-exercise sample, the two MO_2 curves (in NG and GD samples) were very similar, increasing 2-3 fold after exercise and requiring 3-6 h for a return to resting levels. EPOC was only slightly greater than the EPOC

Figure 4.1. Changes in the rates of O_2 consumption (MO_2) after exhaustive exercise in (A) normal glycogen (NG) and (B) glycogen depleted (GD) juvenile rainbow trout. (▲) indicates non-exercised control fish. (●) indicates exercised samples. Means (n=8) ± 1 S.E.M. Exercise bouts of 5 min are indicated at arrows. Pre-exercise values are means of 3 measurements per fish. The first post-exercise sample is taken at 5 min. * indicates significantly different ($p<0.05$) from pre-exercise values. + indicates significantly different ($p<0.05$) from corresponding NG sample point.

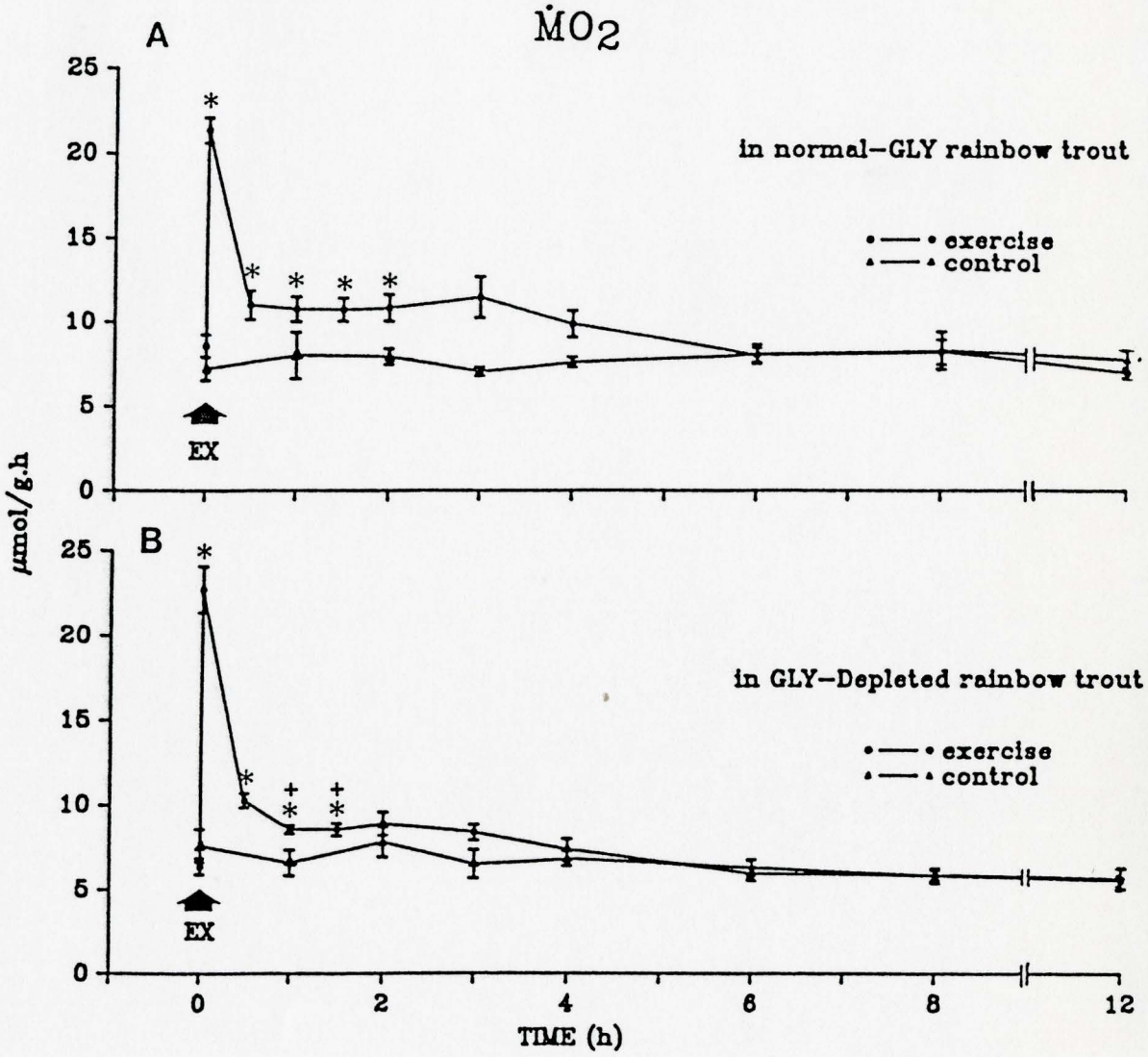

A
$\dot{M}O_2$
in normal–GLY rainbow trout
exercise
control
EX
B
in GLY–Depleted rainbow trout
exercise
control
EX
μmol/g.h
TIME (h)
0
2
4
6
8
12
5
10
15
20
25

Figure 4.2. The excess post-exercise O_2 consumption (EPOC) after exhaustive exercise in individual NG and GD juvenile rainbow trout, as calculated from the data in Fig.4.1. Mean (n=8) ± 1 S.E.M. There was no significant difference between EPOC of NG and GD fish.

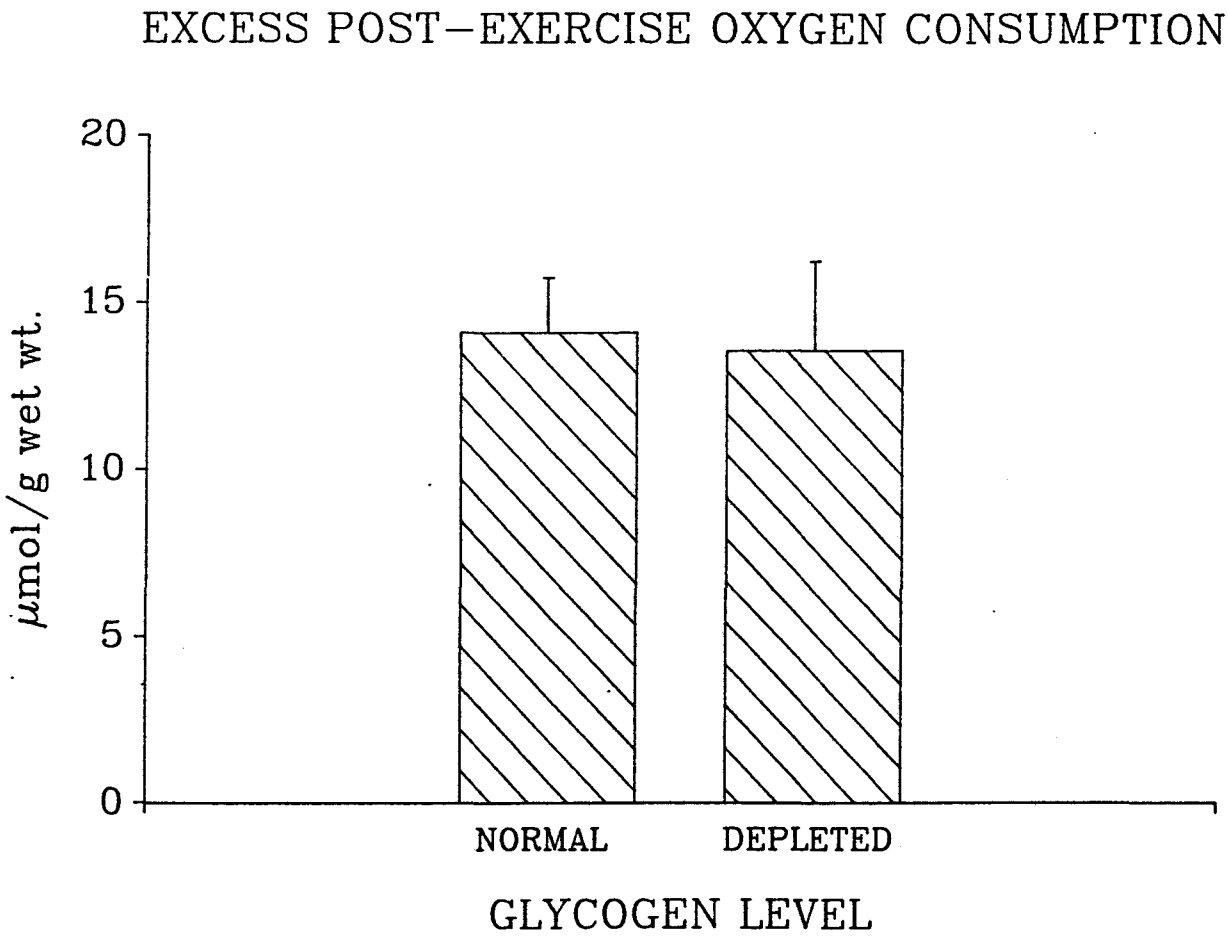
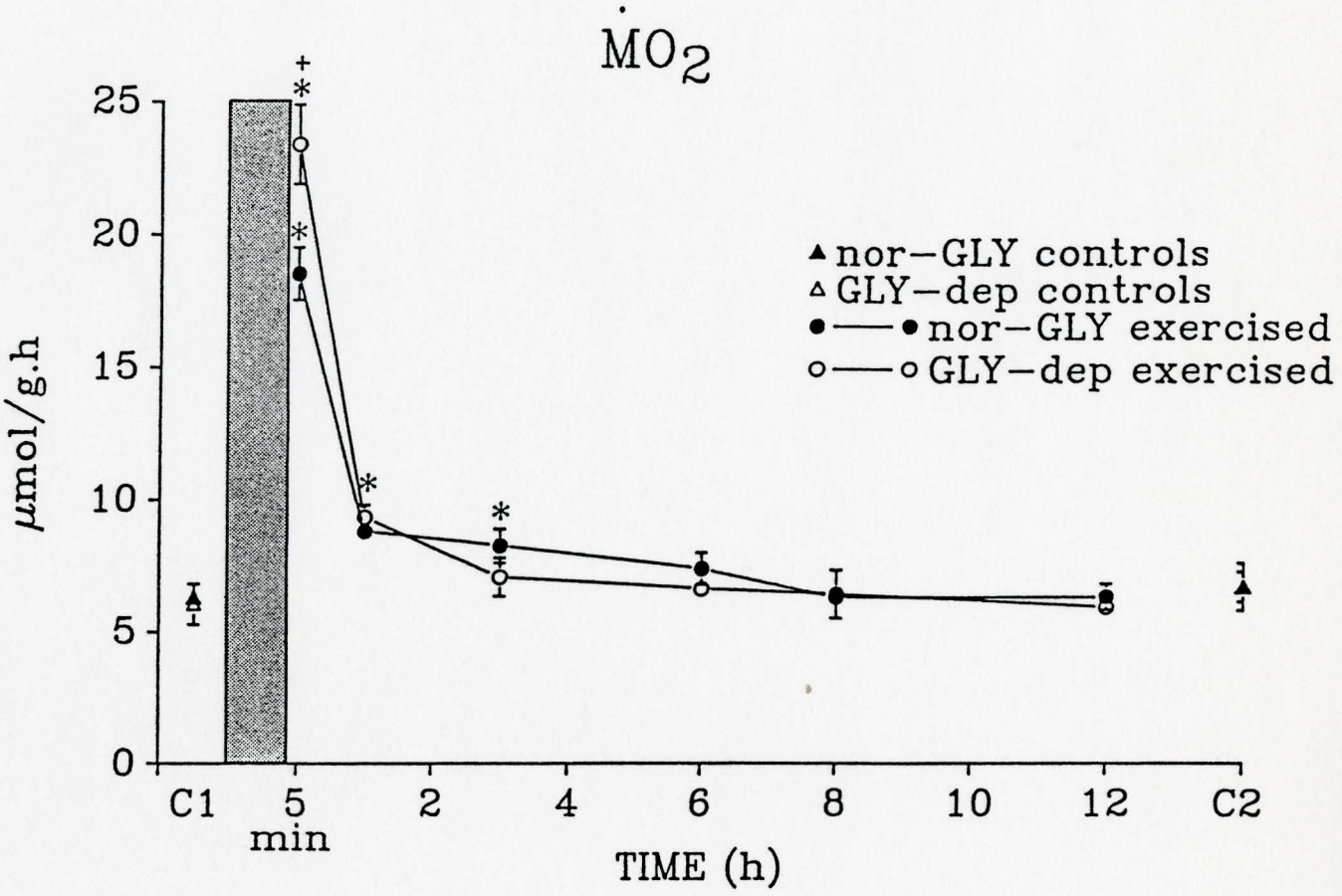

EXCESS POST-EXERCISE OXYGEN CONSUMPTION
20
15
10
5
0
μmol/g wet wt.
NORMAL
DEPLETED
GLYCOGEN LEVEL

Figure 4.3. Changes in the rates of O_2 consumption (MO_2) after exhaustive exercise in NG and GD juvenile rainbow trout, corresponding to the metabolite portion of the study. Two control samples were taken, labelled C1 and C2. (▲, △) indicates NG and GD non-exercised controls, respectively. (●, ○) indicates NG and GD exercised samples, respectively. Means (n=8)± 1 S.E.M. Grey bar indicates exercise bout of 5 min. The first post-exercise sample is taken at 5 min. * indicates significantly different ($p<0.05$) from respective C1 for each treatment group. + indicates significantly different ($p<0.05$) from corresponding NG sample point.


$\dot{M}O_2$
25
20
15
10
5
0
μmol/g.h
C1
5
min
2
4
6
8
10
12
C2
TIME (h)
nor–GLY controls
GLY–dep controls
nor–GLY exercised
GLY–dep exercised

measured from the MO_2 study (NG - 16.7 µmol/g wet wt.; GD - 17.0 µmol/g wet wt.).

Resting GLY levels in NG fish (81.4 nmol/mg dry wt., Fig.4.4) were twice as high as those found in the previous chapters (Fig.2.3, Fig.3.8), which may be attributed to stock differences. Resting GLY levels in GD fish were about half that of NG fish (39.8 nmol/mg dry wt., Fig.4.4). Although this indicated that the experimental protocol (5 days starvation) was successful in reducing GLY reserves, these levels are more comparable to the resting GLY levels of the previous studies.

Exercise caused a severe reduction in GLY levels in both groups. GLY in NG fish dropped to 40% resting levels, a decrease of 49.1 nmol/mg dry weight. 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% 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 LAC metabolism. Resting LAC levels were lower in GD fish, both at the start (C1) and at the end (C2) of the experiment (Fig.4.5). LAC

Figure 4.4. Changes in whole-body glycogen levels after exhaustive exercise in NG and GD juvenile rainbow trout. Two control samples were taken, labelled C1 and C2. (▲,△) indicates NG and GD non-exercised controls, respectively. (●,○) indicates NG and GD exercised samples, respectively. Means (n=8) ± 1 S.E.M. Grey bar indicates exercise bout of 5 min. The first post-exercise sample is taken at 5 min. * indicates significantly different ($p<0.05$) from C1. + indicates significantly different ($p<0.05$) from corresponding NG sample point.

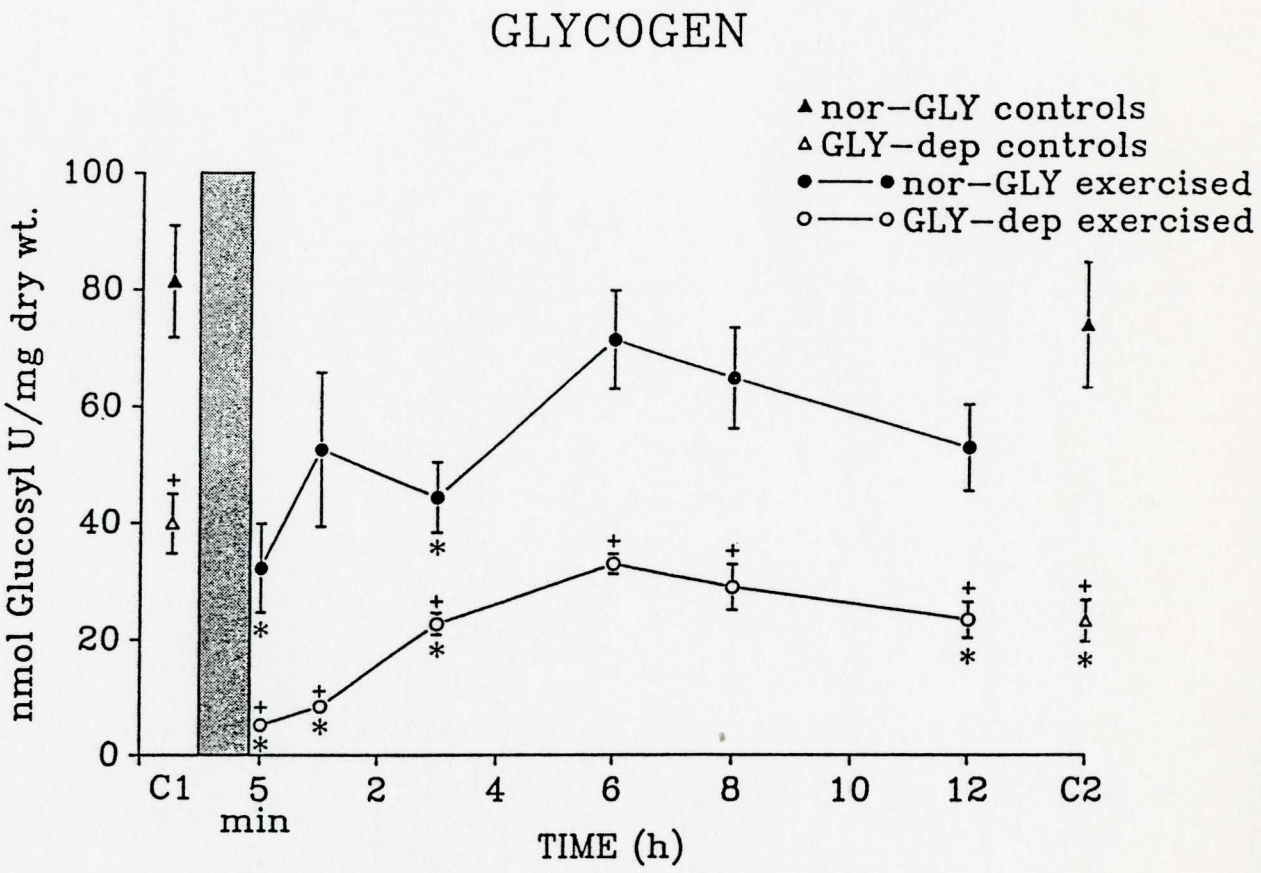
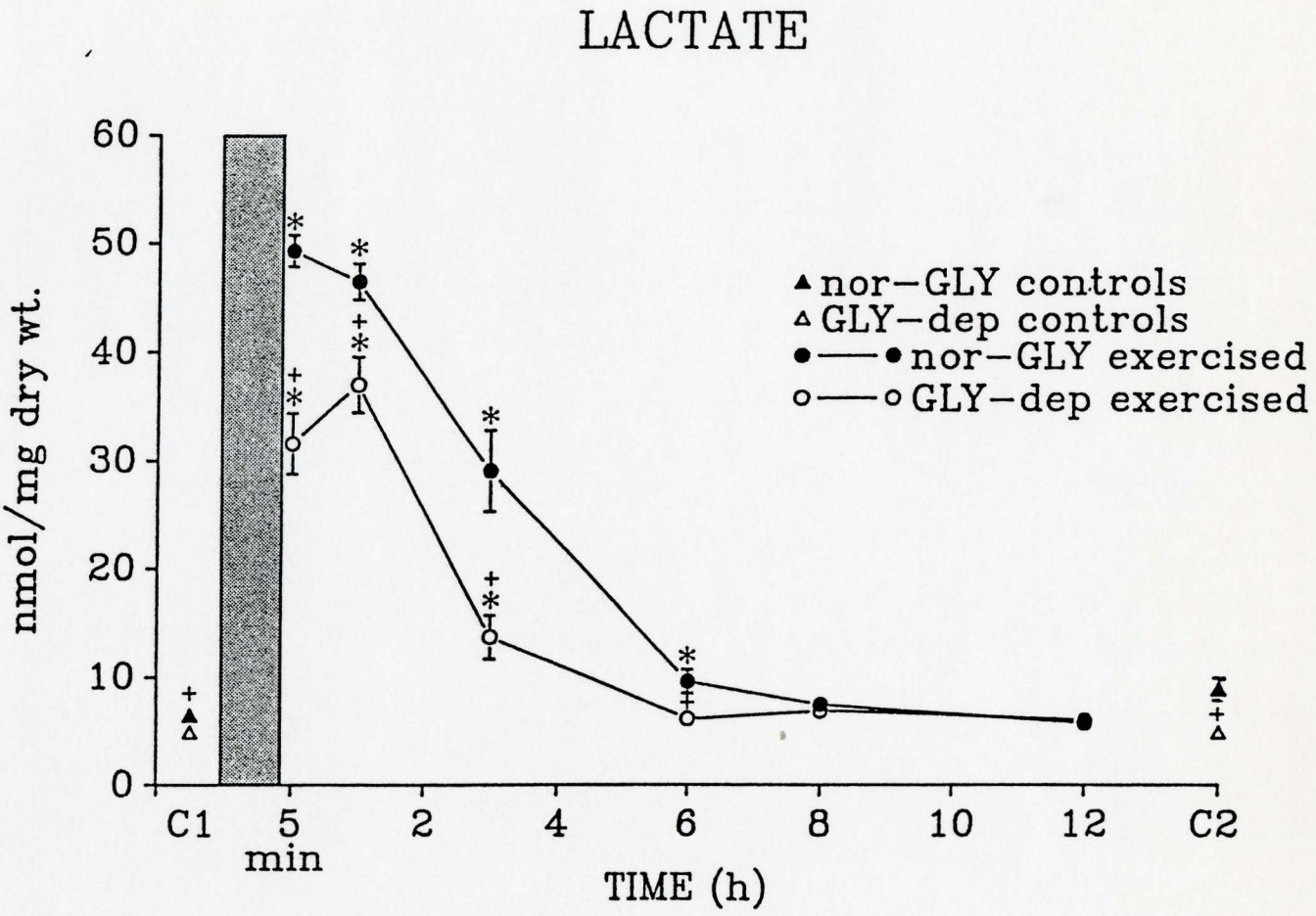

GLYCOGEN
▲nor–GLY controls
△GLY–dep controls
●——●nor–GLY exercised
○——○GLY–dep exercised
nmol Glucosyl U/mg dry wt.
100
80
60
40
20
0
C1
5
min
2
4
6
8
10
12
C2
TIME (h)

Figure 4.5. Changes in whole-body lactate levels after exhaustive exercise in NG and GD juvenile rainbow trout. Two control samples were taken, labelled C1 and C2. (▲,△) indicates NG and GD non-exercised controls, respectively. (●,○) indicates NG and GD exercised samples, respectively. Means (n=8) ± 1 S.E.M. Other details as in Fig.4.4.

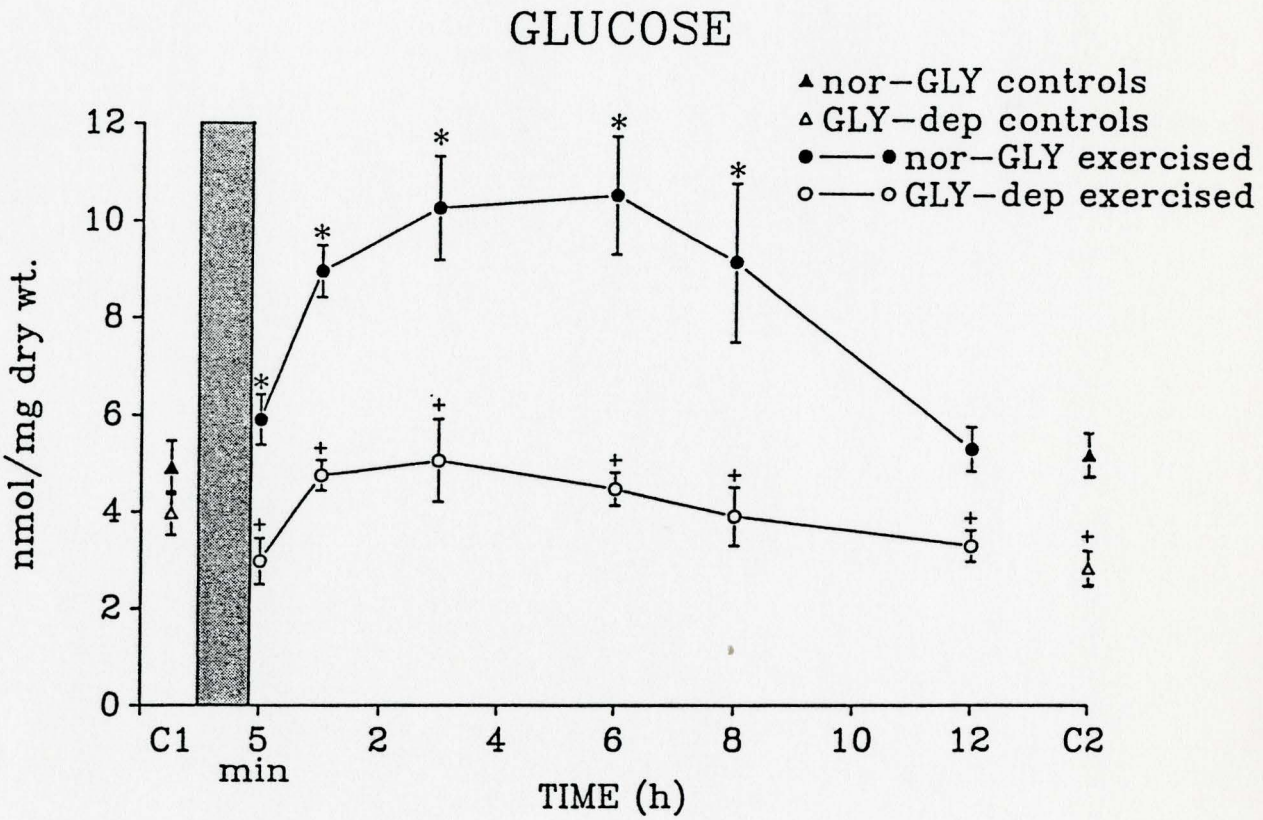

LACTATE
nmol/mg dry wt.
60
50
40
30
20
10
0
C1
5
min
2
4
6
8
10
12
C2
TIME (h)
nor–GLY controls
GLY–dep controls
nor–GLY exercised
GLY–dep exercised

increased by 42.8 nmol/mg dry wt. in NG fish but only by 26.7 nmol/mg dry wt. in GD fish in the first 6 h. After the 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 between both groups (6-8 h). These patterns are similar to those of the previous studies (Fig.2.2, Fig.3.7).

Clearly, the experimental protocol (GLY depletion) affected the total accumulated LAC after exercise. *i.e.* NG fish had a much larger LAC burden than GD fish. However, GLY depletion in no way affected MO_2, thus resulting in identical EPOC between NG and GD groups. Furthermore, in the present study, MO2 required less than 4 h to return to pre-exercise levels, whereas LAC required 6-8 h to return to resting levels.

Resting whole-body glucose levels were not significantly altered by GLY depletion at the start of the experiment (C1; 4.9 nmol/mg in NG fish and 4.0 nmol/mg in GD fish), but were reduced at the end of the period (C2; Fig.4.6). These values are slightly lower than those of the previous studies (Chapters 2 and 3). Glucose levels in GD fish did not vary after exercise similar to the results of Chapter 2 (Fig.2.4). However, glucose was elevated after exercise in NG fish, and continued to increase, reaching peak levels between 3-6 h. This was more similar to the

Figure 4.6. Changes in whole-body glucose levels after exhaustive exercise in NG and GD juvenile rainbow trout. Two control samples were taken, labelled C1 and C2. (▲,△) indicates NG and GD non-exercised controls, respectively. (●,○) indicates NG and GD exercised samples, respectively. Means (n=8) ± 1 S.E.M. Other details as in Fig.4.4.


GLUCOSE
nor–GLY controls
GLY–dep controls
nor–GLY exercised
GLY–dep exercised
nmol/mg dry wt.
0
2
4
6
8
10
12
C1
5
min
2
4
6
8
10
12
C2
TIME (h)

results of Chapter 3, in which glucose levels were more variable, increasing slightly post-exercise (Fig.3.8).

Whole-body ATP levels (Fig.4.7) were only slightly 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 and 1 h after exercise, but had returned to levels not significantly different from pre-exercise values by 3 h. This was similar to the findings of the previous studies (Fig.2.5, Fig.3.10).

Resting whole-body CP levels (C1 and C2; Fig.4.8) were not significantly different between groups, averaging almost 30 nmol/mg dry wt., similar to the findings of Chapter 3 (Fig.3.11), but slightly lower than the results of Chapter 2 (Fig.2.7). CP levels (Fig.4.8) were only slightly affected by GLY depletion after exercise. At 5 min, NG fish still showed depressed CP levels (62% resting levels), whereas GD fish did not (92% resting levels). Note that in contrast to the two previous studies, samples were not taken immediately at the end of the exercise. It is known that CP levels are near depleted immediately after exercise (Fig.2.7, Fig.3.11). CP levels in both groups continue to rise at 1 h, overshooting resting levels, although to a greater extent in GD fish. Thereafter, CP returns to resting levels in both NG and GD fish.

Figure 4.7. Changes in whole-body adenosine triphosphate (ATP) levels after exhaustive exercise in NG and GD juvenile rainbow trout. Two control samples were taken, labelled C1 and C2. (▲,△) indicates NG and GD non-exercised controls, respectively. (●,○) indicates NG and GD exercised samples, respectively. Means (n=8) ± 1 S.E.M. Other details as in Fig.4.4.

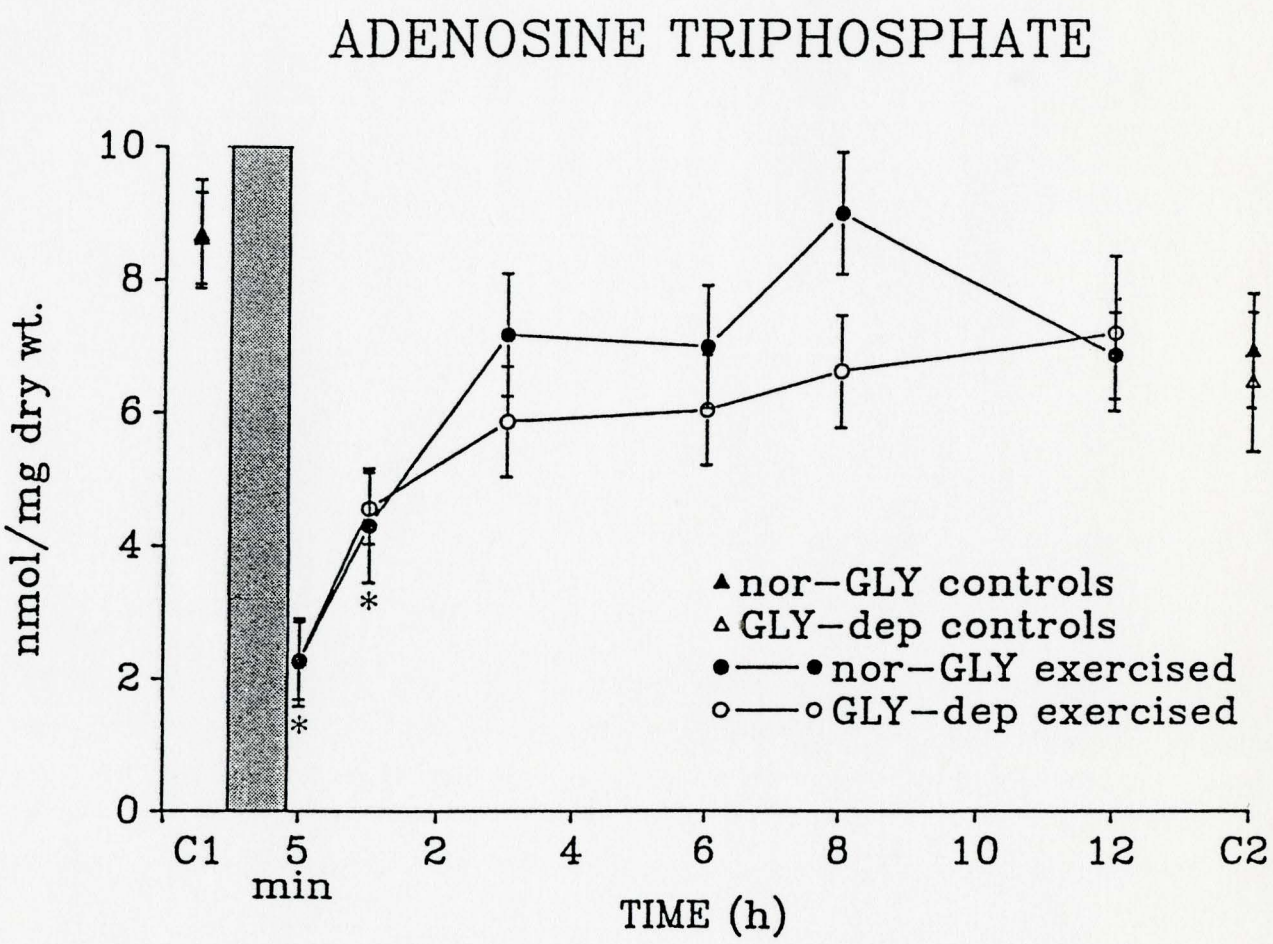
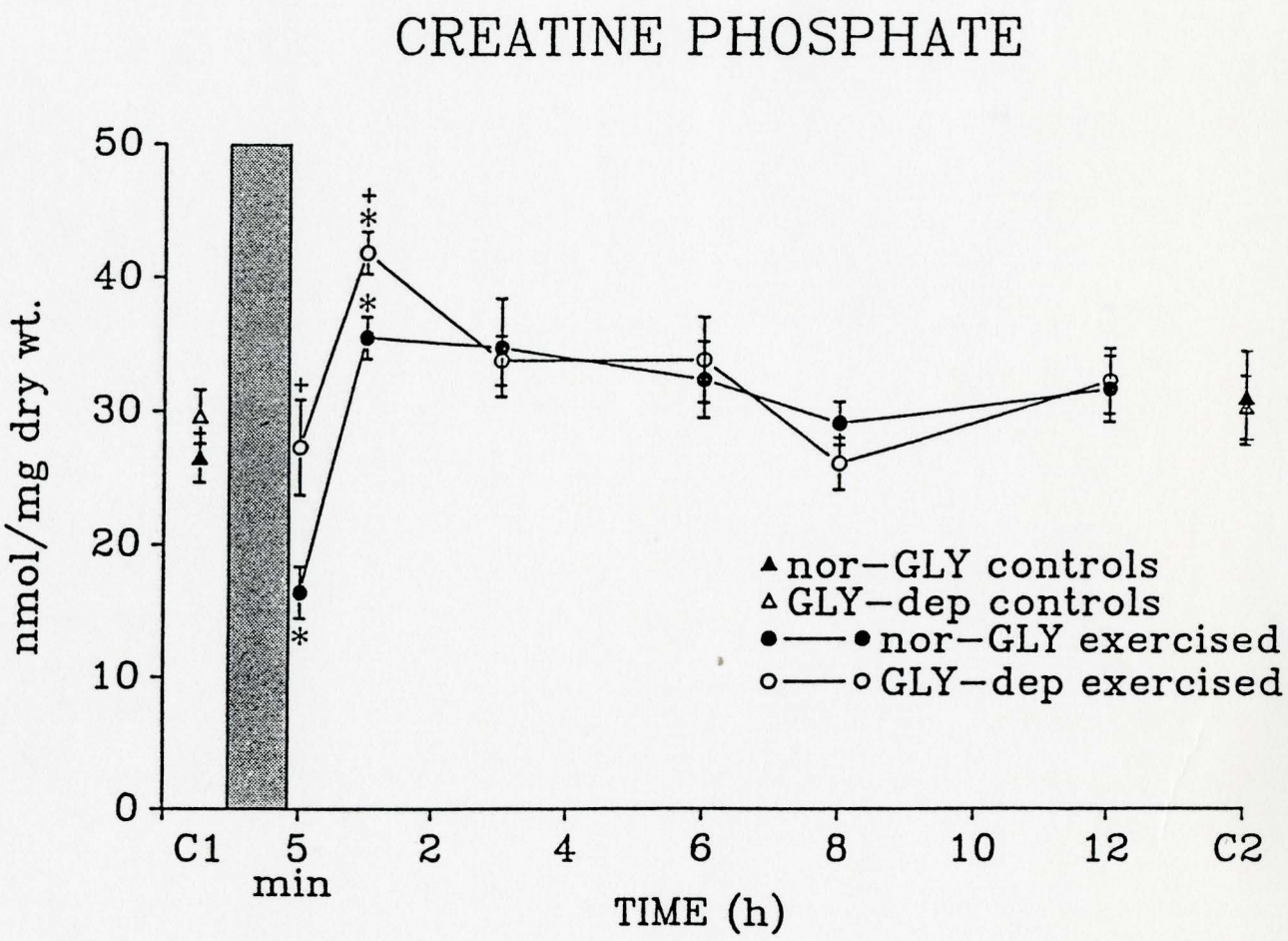

ADENOSINE TRIPHOSPHATE
nmol/mg dry wt.
10
8
6
4
2
0
C1
5
min
2
4
6
8
10
12
C2
TIME (h)
nor–GLY controls
GLY–dep controls
nor–GLY exercised
GLY–dep exercised
*
*

Figure 4.8. Changes in whole-body creatine phosphate (CP) levels after exhaustive exercise in NG and GD juvenile rainbow trout. Two control samples were taken, labelled C1 and C2. (▲,△) indicates NG and GD non-exercised controls, respectively. (●,○) indicates NG and GD exercised samples, respectively. Means (n=8) ± 1 S.E.M. Other details as in Fig.4.4.


CREATINE PHOSPHATE
nmol/mg dry wt.
50
40
30
20
10
0
C1
5
min
2
4
6
8
10
12
C2
TIME (h)
nor–GLY controls
GLY–dep controls
nor–GLY exercised
GLY–dep exercised

Theoretical Analysis

If the exercise was entirely anaerobic, LAC accumulation would have been approximately twice the GLY utilized (in glucosyl units), as in the study of Chapter 3. This was not the case, as indicated by the low LAC accumulations relative to the amount of GLY depleted.

NG fish: used 49 nmol GLY/mg generating 43 nmol LAC/mg (1 GLY:0.88 LAC). (See Table 4.1).

GD fish: used 35 nmol GLY/mg generating 27 nmol LAC/mg (1 GLY:0.77 LAC).

This may have been due to considerable recovery of LAC in the first 5 min after exercise, or more likely to a significant portion of energy for the exercise being derived via aerobic metabolism. In the previous studies, similar LAC accumulations were experienced (Fig.2.2, Fig.3.7), but much smaller amounts of GLY were depleted with exercise, possibly due to lower resting GLY levels. While this situation is different from the situation in Chapter 3 after the 1st exercise bout where 1 GLY:2 LAC ratio was found, it is similar to the findings of Chapter 2 (1 GLY:1 LAC). The reasons for this inconsistency are unclear.

(A) Once again, the analysis utilized in Chapter 2, based on the assumption that all the GLY resynthesized during the first 6 h of recovery came from LAC, does not hold true in the present study.

(1) In NG fish, 39.2 nmol glucosyl U/mg dry wt. of GLY were resynthesized, requiring 78.4 nmol LAC/mg dry wt. and 254.8

Table 4.1. Changes in MO_2 and measurement of EPOC, with corresponding metabolite changes over the period 0-6 h after a burst of exhaustive exercise in normal GLY (NG) and GLY depleted (GD) juvenile rainbow trout.

		resting levels	at 5 min * post-exercise	at 6 h recovery	difference (0-6 h)
MO2	NG	8.7	18.1	7.8	see EPOC
	GD	7.8	18.5	8.1	see EPOC
EPOC	NG	N/A	N/A	N/A	71.0
	GD	N/A	N/A	N/A	67.5
LAC	NG	6.5	49.3	9.6	39.7
	GD	4.9	31.6	6.2	27.7
GLY	NG	81.4	32.2	71.4	39.2
	GD	39.8	5.2	32.9	27.7
ATP	NG	8.7	2.0	7.0	5.0
	GD	8.6	2.3	6.0	3.7
CP	NG	26.5	6.4 (5.5)	32.3	15.9 (26.8)
	GD	28.5	27.3 (6.1)	33.8	6.5 (27.7)
glucose	NG	4.9	5.9	10.5	4.6
	GD	4.0	3.0	4.5	1.5

* The first sample taken in the present study was 5 min post-exercise. Thus, an estimate of CP immediately after exercise was made, based on comparable values from series 2 (CP at rest = 28.5 nmol/mg dry wt. and CP at 0 h = 5.9 nmol/mg dry wt.).

MO_2 is in *f*mol/g.h wet weight. EPOC is in nmol/mg dry wt.
GLY is in nmol glucosyl U/mg dry wt.
All other metabolites are in nmol/mg dry wt.

nmol ATP/mg dry weight. However, only 39.7 nmol LAC/mg were actually cleared during this time. Similarly, in GD fish, 27.7 nmol glucosyl U/mg dry wt. were resynthesized, requiring 55.4 nmol LAC/mg and 180.1 nmol ATP/mg. However, again only 27.7 nmol LAC/mg were actually cleared during this time.

Clearly, the precursor for GLY resynthesis cannot be entirely LAC and other sources must exist. Once again (as in Chapter 3), a budget of the cost of recovery in terms of O_2 equivalents cannot be prepared, since the contribution of LAC oxidation to EPOC is not known.

(B) This analysis assumes that the entire EPOC is utilized to oxidize part of the accumulated LAC. Values used in this analysis were taken from Table 4.1.

(1) In NG fish, total EPOC = 71.0 nmol/mg dry wt.

$71.1\ O_2 + 23.7\ LAC \rightarrow 414.7\ ATP + 71.1\ CO_2 + 71.1\ H_2O$

based on $3\ O_2 + LAC \rightarrow 17.5\ ATP + 3\ CO_2 + 3\ H_2O$.

This accounts for 60% of the total LAC burden (39.7 nmol/mg dry wt.) cleared during this time.

(2) The remainder of the LAC (40%; 16.0 nmol/mg dry wt.) was converted into GLY.

Thus, $8.0\ GLY \leftarrow 16.0\ LAC + 52.0\ ATP$

based on $1\ GLY \leftarrow 2\ LAC + 6.5\ ATP$

(3) Of the total GLY resynthesized (39.2 nmol glucosyl U/mg dry wt.), only 20% came from LAC and the majority came from other precursors.

Similar calculations were done for GD fish.

(1) Total EPOC = 67.5 nmol/mg dry wt. Thus:

$67.5\ O_2 + 22.5$ LAC --> 393.7 ATP + $67.5\ CO_2 + 67.5\ H_2O$.

This accounts for 81% of the total LAC cleared during this time (27.7 nmol LAC/mg dry wt.).

(2) The remainder (19%) was converted into GLY.

Thus, 2.6 GLY <-- 5.2 LAC + 16.9 ATP

(3) Of the total GLY resynthesized (27.7 nmol glucosyl U/mg dry wt.) during this time only 9% came from LAC and the majority (91%) came from other unknown sources.

The cost of the excess work done by the branchial and cardiac pumps was converted into metabolic (ATP) equivalents and tabulated into the budget of the cost of recovery, shown in Tables 4.2A and 4.2B for NG and GD fish, respectively. As a 0 h sample was not taken, and CP is known to recover considerably in 5 min, an estimated value was factored into the budget for CP levels at 0 h, based on similar resting values.

Of the total ATP generated via LAC oxidation in NG fish (414.7 nmol/mg dry wt.), only 33% can be accounted for by this analysis (Table 4.2A). However, as only 20% of the GLY resynthesized was attributed as having come from LAC, a large portion of the excess ATP would be utilized to resynthesize GLY from other sources. Similarly, in GD fish, only 22% of the ATP generated via LAC oxidation (393.7 nmol/mg dry wt.) can be accounted for by this analysis (Table 4.2B). Once again, a large portion of the excess ATP

Table 4.2. Budget of the cost of recovery from exhaustive exercise based on the "alternative" scenario B (that the entire EPOC was devoted to oxidizing LAC) in terms of ATP equivalents after a bout of exhaustive exercise in the whole body of (A) normal glycogen (NG) and (B) glycogen depleted (GD) juvenile rainbow trout.

(A) ATP COST OF RECOVERY IN NG RAINBOW TROUT (nmol/mg)

31	ATP used for increased ventilatory work
11	ATP used for increased cardiac work
5	ATP were restored
10	ATP equiv. needed in ATP resynthesis
27	creatine phosphate were restored
52	ATP used in GLY resynthesis from LAC

136	ATP accounted for versus:
415	ATP produced from LAC oxidation

≈67% unaccounted for

(B) ATP COST OF RECOVERY IN GD RAINBOW TROUT (nmol/mg)

30	ATP used for increased ventilatory work
10	ATP used for increased cardiac work
4	ATP were restored
7	ATP equiv. needed in ATP resynthesis
28	creatine phosphate were restored
9	ATP used in GLY resynthesis from LAC

88	ATP accounted for versus:
394	ATP produced from LAC oxidation

≈78% unaccounted for

Values were taken from Table 4.1. See Theoretical Analysis for more detail. All values are in nmol/mg dry wt. Estimates for increased ventilatory work (5-10%) and increased cardiac work (0-5%) were based from the estimates of Farrel & Steffensen (1987).

would be needed for the resynthesis of GLY from sources other than LAC.

DISCUSSION

MO_2 Study

The experimental manipulation (*i.e.* GLY depletion) did not affect resting MO_2 between the two groups, NG and GD, although slight differences in MO_2 were observed between groups at certain post-exercise sample times (1 and 1.5 h; Fig.4.1A and Fig.4.1B). Return to resting levels was fairly similar (about 2-3 h), and thus there was no significant difference between EPOC of NG and GD fish (Fig.4.2). These EPOCs were similar to that of Chapter 3 (after the 1st exercise bout only), but slightly greater than that of Chapter 2. In general, similar patterns are seen in NG and GD human subjects, though recovery time is less than 20 min (Segal & Brooks, 1979). There is no previous comparable information in fish.

Metabolite Study

The results show that the period of starvation (5 days) successfully manipulated initial GLY reserves. GD fish showed a 50% reduction in resting GLY stores (Fig.4.4), a difference which was roughly maintained at all sample times after exhaustive exercise. A similar GLY depletion has been shown before in plaice, *Pleuronectes platessa*, in which white muscle GLY decreased rapidly in the first 2 weeks of starvation (Johnston & Goldspink, 1973). The authors suggest that plaice preferentially utilize energy reserves from white muscle during starvation as it constitutes the largest

source of energy, while red muscle, needed for propulsion at all swimming speeds, is spared.

The decrease in GLY due to exercise in the present study was greater in NG fish than GD fish (49 vs 35 nmol/mg dry wt.) even though this only amounted to a reduction to 40% resting levels (Fig.4.3). In humans, prior GLY depletion has been associated with a decrease in glycolysis, suggesting that initial GLY reserves dictate the maximum power output that can be achieved during short-term exercise (Heigenhauser *et al.*, 1983). Reports in rainbow trout also confirm this finding (Miller *et al.*, 1959). Trout, with lower initial GLY reserves fatigued more quickly than trout with higher GLY reserves, and mobilized less GLY after a severe exercise bout.

A relatively rapid rate of GLY recovery was observed in both groups (about 6 h), though the repletion did not appear to be 100%. The possible reasons have already been discussed in some detail in Chapter 3. Whole-body glucose levels (Fig.4.6) rose significantly after exercise in NG fish but not GD fish. It is possible that this glucose was utilized to resynthesize some of the GLY.

After 6 h recovery, GD fish continued to lose GLY, and by 12 h, levels were significantly reduced compared to the start of the experiment (C1). The decline observed in C2 indicates that the conditions of the experiment (*i.e.* 12 h acclimation to respirometers plus 12 h "recovery") were probably responsible for this further reduction and not the

exercise bout *per se*. It is apparent that the experimental conditions adversely affected only the fish in the GD state, with already low initial GLY levels, as the decrease at C2 was not observed in NG fish (Fig.4.4). This trend was similar to that found in the fish of Chapter 2, where initial GLY levels (37-40 nmol glucosyl U/mg dry wt.) were the same and where C2 also decreased over time.

GLY depletion was also shown to affect LAC metabolism. Whole-body LAC levels were only slightly, but significantly, higher in NG than in GD fish under resting conditions (Fig.4.5), both at the start and end of the experiment (C1 and C2). This may be due to reduced rates of glycolysis as a result of low GLY reserves, even under aerobic conditions. Similar findings in blood LAC have been observed in humans with reduced GLY stores from manipulations of diet and previous exercise (Segal & Brooks, 1979).

The increase in LAC after exercise (Fig.4.5) was also less in GD fish, again indicating reduced rates of glycolysis, as has been found in other studies (Miller *et al.*, 1959). However, the proportions (GLY:LAC) were far from indicating complete anaerobic metabolism in either group, as expected from this type of exercise (Dobson & Hochachka, 1987). Part of this discrepancy may be due to the fact that samples were not taken immediately post-exercise. By 5 min, small but significant changes may have occurred in LAC and/or GLY levels. Though this is different from the findings of Chapter 3, in which almost perfect 1 GLY: 2 LAC

stoichiometry was observed (*i.e.* anaerobic metabolism), this situation is similar to that of Chapter 2, with an almost 1:1 ratio for GLY disappearance and LAC accumulation.

Recovery of the high energy phosphates, ATP (Fig.4.7) and CP (Fig.4.8), occur extremely rapidly in both humans (Harris *et al.*, 1969) and fish (Weiser *et al.*, 1985). Based on the findings of Chapters 2 and 3, both ATP and especially CP, had recovered by significant amounts in 5 min. In the present study, CP levels were not significantly different from rest at 5 min in GD fish and were only 62% resting levels in NG fish, suggesting that indeed a significant amount of recovery had occurred by this time.

Interpretation of the Theoretical Analysis

(A) Primary fate of LAC is GLY resynthesis.

The results of the present study are similar to those of Chapter 3, in as much as more GLY was restored during the first 6 h of recovery after exhaustive exercise than could be accounted by LAC disappearance. Although the literature available in fish (Milligan & Wood, 1986; Dobson & Hochachka, 1987) suggest that the primary fate of LAC is GLY resynthesis, the proportion of LAC utilized is not known. Thus, one cannot quantitatively assess the results with this scenario. Qualitatively, however, an important conclusion can be drawn from this study. The experimental manipulation (*i.e.* GLY depletion) resulted in smaller LAC accumulations after exhaustive exercise. Thus, the quantity of LAC cleared post-exercise was also smaller. roughly by 30% in GD fish

(see Table 4.1). However, EPOC was not significantly different between groups (Fig.4.2). Thus, LAC disappearance after exercise in no way determines the magnitude of EPOC.

(B) Primary fate of LAC is oxidation.

By this analysis, 60% of the total accumulated LAC was oxidized by EPOC in NG fish, while 40% was converted into GLY. This is similar to the findings of Chapter 3 after the 1st exercise bout only. However, this relation does not hold true in GD fish, since the LAC burden was reduced. In GD fish, 81% of the LAC burden could be accounted for by the EPOC. Thus, once again, the relation between EPOC and LAC disappearance is not consistent, suggesting that they are not directly correlated.

The experimental protocol (GLY depletion) was successful in altering the LAC accumulation after exhaustive exercise (Fig.4.5). However, GLY depletion did not alter MO_2 (Fig.4.1 and 4.3), thus resulting in the same EPOC between NG and GD groups (Fig.4.2). Furthermore, MO_2 recovery was faster (less than 4 h) in both GLY states than LAC recovery (6-8 h). This indicates that the similar time courses of MO_2 and LAC recovery observed in the first study (about 6 h, Chapter 2) were only coincidental.

Once again, no direct quantitative correlation can be drawn between LAC accumulation (and the quantity cleared during recovery) and EPOC. In the present study, GLY depletion significantly reduced the total LAC cleared during the first 6 h of recovery from a bout of exhaustive

exercise, while EPOC remained unchanged. In the previous study (Chapter 3), repetitive bouts of exhaustive exercise significantly reduced EPOC by 40% after the second bout, while the quantity of LAC cleared remained unchanged. Both studies clearly show that the LAC accumulated during exercise does not determine the magnitude of EPOC, despite the fact that some portion may be utilized to oxidize LAC post-exercise.

Conclusions

The effect of 5 days starvation resulted in similar metabolic disturbances as has been found previously in both fish and mammals. Resting and post-exercise whole-body GLY levels were considerably reduced by 50% at all sample times measured. Anaerobic glycolysis associated with exhaustive exercise was also reduced in GD fish, as shown by smaller decreases in GLY and increases in LAC. EPOC was not affected by this treatment, as the pattern and time courses were similar between groups. These findings are not consistent with the classical O_2 debt hypothesis but corroborate studies in mammals. Exercise is a far more complex process than originally proposed by Hill & Lupton (1923).

CHAPTER 5

SUMMARY AND CONCLUSIONS

INTRODUCTION

The classical O_2 debt hypothesis stipulates that a muscle can incur an "O_2 debt" during strenuous exercise (Hill & Lupton, 1923). The O_2 "missed" during exercise results in an accumulation of LAC in the muscle, which acts as "security", such that during recovery, the excess post-exercise O_2 consumption (EPOC) is primarily utilized to oxidize back the LAC. In the amphibian muscle studied, only 20% of the LAC which disappeared was oxidized, the remainder being converted back into GLY. Hill & Lupton (1923) maintained that the EPOC was used almost entirely to remove LAC oxidatively.

Mammalian studies have recently concluded that the EPOC is dependant on work intensity and duration, rather than LAC accumulation (Gaesser & Brooks, 1984). However, complications arise in mammals due to the elevation in body temperature after exercise and the very fast rate of recovery, which makes quantitative analysis difficult. Rainbow trout, being poikilotherms, assume the temperature of the aqueous environment. Thus, rates of reaction are much slower. Furthermore, they do not exhibit increases in body temperature after exercise, nor do they implement energetically costly mechanisms to regulate body temperature. This greatly simplifies the situation, with the

advantage that these processes can be studied in greater detail. This thesis, therefore, examined the relationship between LAC accumulation and EPOC in rainbow trout, since the O_2 debt hypothesis had not yet been rigorously tested in poikilotherms where it was originally proposed.

SUMMARY OF RESULTS

In these studies, severe exercise resulted in an increase in LAC levels by 6-8-fold, GLY levels were near depleted (usually less than 20% resting levels), ATP decreased to about 25% resting levels and CP decreased to 30% resting levels. MO_2 at 5 min post-exercise was elevated about 2-3 times resting levels and required about 4 h for recovery.

Of the total O_2 consumed during recovery from exercise, only a small portion (≈10%) is used for increased work of the branchial and cardiac pumps (Farrel & Steffensen, 1987) and replenishing blood and tissue O_2 stores (Stainsby & Barclay, 1970). Based on the first analysis (scenario A) of the results of Chapter 2, a substantial portion (around 40%) contributed to oxidation of around 25% of the total accumulated LAC. This oxidation generated considerable amounts of ATP, which were utilized to replenish ATP and CP stores, as well as drive the conversion of the remaining LAC (75%) back into GLY. Almost all of the ATP generated could be accounted for.

The remaining portion of EPOC (about 50%) could not be accounted for by any of the above reasons; it was speculated that elevated catecholamine levels, increased rates of substrate cycling and the elevated costs of post-exercise ion regulation may play a role. These factors are known to contribute to EPOC in mammals, but the magnitude of their effects in fish has not yet been determined.

The alternative analysis (scenario B), based the entire EPOC on LAC oxidation, which accounted for 64% of the total LAC cleared during 6 h. The remainder was presumably converted into GLY, however, this accounted for only 48% of the total GLY resynthesized during this time. While other precursors of GLY are possible, it is generally considered that LAC is the primary source of GLY resynthesis after burst exercise *in situ* (Milligan & Wood, 1986; Dobson & Hochachka, 1987; Milligan & McDonald, 1988). This analysis also resulted in a very large portion of ATP unaccounted for, although presumably some would be required to resynthesize GLY from these other sources. It appears that scenario A, based on these findings, is the appropriate explanation.

A second bout of exhaustive exercise, 6 h after the first (Chapter 3), was found to significantly affect EPOC, causing a reduction by 40%. Rates of recovery of MO_2, MCO_2 and M_{amm} were accelerated. Metabolite status, however, was not affected by the successive exercise bout, in that the magnitudes of response (LAC accumulation, GLY depletion, ATP and CP changes) and the time courses of correction were similar after both bouts. The successive exercise bout, however, did result in a slightly faster rate of recovery of LAC, although the quantity accumulated and cleared was the same after both bouts. This suggested that the magnitude of the LAC disappearance did not regulate EPOC *per se*. In mammals, intensity and duration of exercise are the major

determining factors of EPOC (Knuttgen, 1970; Hagberg *et al.*, 1980).

The theoretical analysis of the results, based on scenario A, could not be followed through in the data of Chapter 3. This was because far more GLY was resynthesized in the first 6 h after exercise compared to the previous chapter, whereas comparable LAC accumulations occurred. Thus, the quantity of GLY resynthesized could not be entirely explained as having come from LAC. Some of the GLY resynthesized may have come from glucose, which was significantly elevated after exercise. The alternative analysis of scenario B, although it could be followed through, suggested that the primary fate of LAC is oxidation and not GLY resynthesis. Again, this is in contrast to evidence in the literature in fish. Nevertheless, it suggests that the proportion of LAC oxidized after the 1st exercise bout was not the same after the second exercise bout. Although, this approach may not be the appropriate explanation, it reveals that EPOC and LAC disappearance are not strictly correlated.

Starvation for 5 days lowered GLY levels both pre- and post-exercise, thus reducing LAC accumulation after exercise (Chapter 4). However, GLY depletion did not significantly affect EPOC. Once again, the theoretical analysis of scenario A could not be carried out due to much greater quantities of GLY resynthesized compared to the amount of LAC cleared after exercise. The analysis of scenario B

showed that EPOC and the quantity of LAC cleared were not strictly correlated, as there were large differences between the proportion of LAC oxidized between the NG and GD groups.

CONCLUSIONS

The assumptions used in the two theoretical analyses (scenario A and B) of necessity, represented "extremes", whereas some blend of the two approaches may be more realistic. Nevertheless, in the studies of all three chapters, these analyses point to one important conclusion. The EPOC is not directly related to the metabolism of LAC after exercise in the rainbow trout. The studies failed to show any direct quantitative correlation between the magnitude of EPOC and the quantity of LAC disappearance after exhaustive exercise (Fig.5.1). There was also no correlation between EPOC and the quantity of GLY resynthesized (Fig.5.2) nor between the portion of the LAC burden not oxidized and the quantity of GLY resynthesized (Fig.5.3).

Thus, the classical O_2 debt hypothesis (Hill & Lupton, 1923) does not fully explain EPOC in the rainbow trout; LAC disappearance does not directly determine the magnitude of EPOC. Studies in mammals (Segal & Brooks, 1979; Gaesser & Brooks, 1984) demonstrated that indeed EPOC was largely determined by exercise intensity and duration rather than LAC levels. In this thesis, EPOC was affected only by repetitive exercise bouts whereas LAC disappearance was affected only by prior GLY depletion. The appearance of

Figure 5.1. The relationship between EPOC and the total LAC burden cleared during the first 6 h of recovery after a bout of exhaustive exercise. Data taken from the results of Chapters 2 (■), 3 (○ - 1st bout; ● -2nd bout) and 4 (△-NG; ▲-GD), shown tabulated in Tables 2.1, 3.2 and 4.1. The correlation coefficient (r=-0.14) indicates a lack of significant correlation.

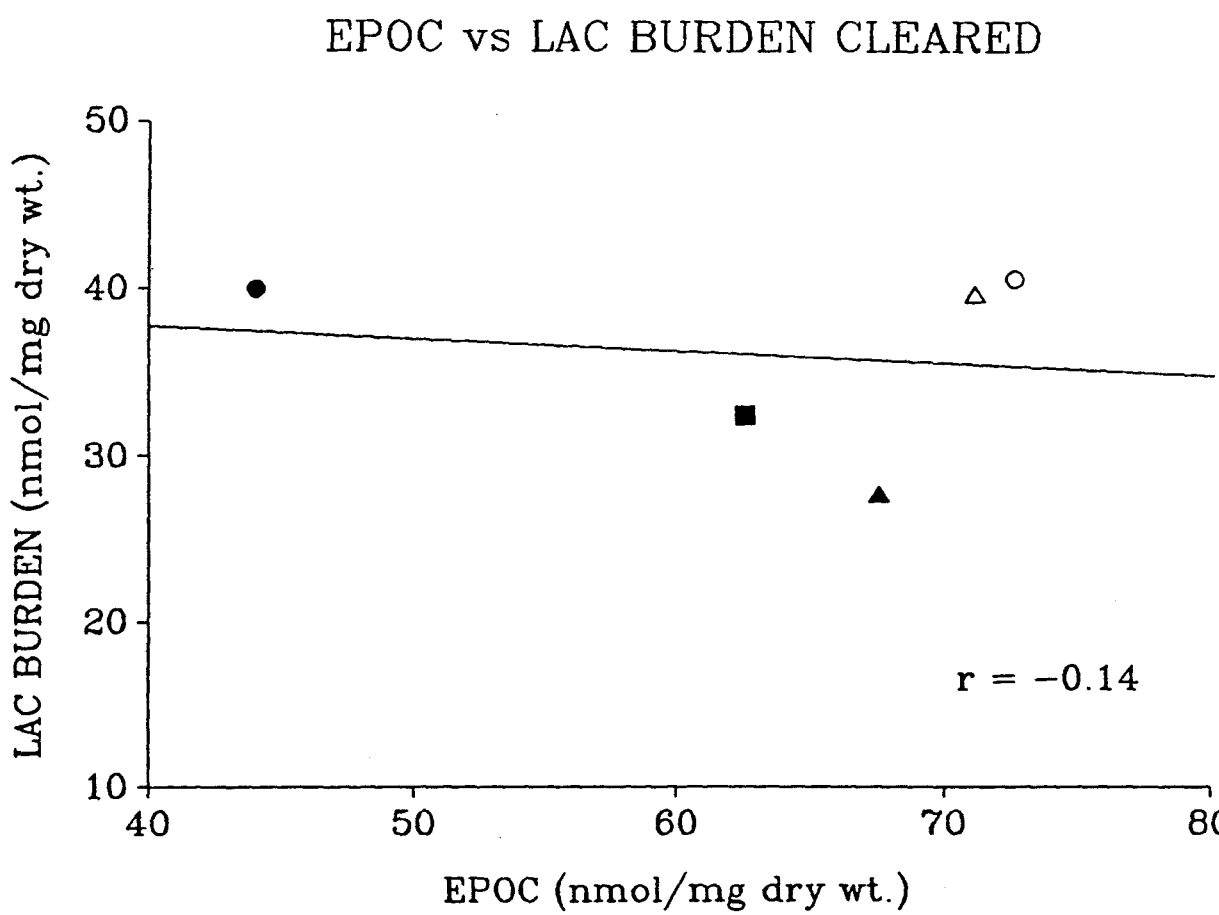

EPOC vs LAC BURDEN CLEARED
LAC BURDEN (nmol/mg dry wt.)
50
40
30
20
10
40
50
60
70
80
EPOC (nmol/mg dry wt.)
r = −0.14

Figure 5.2. The relationship between EPOC and the total quantity of GLY resynthesized during the first 6 h of recovery after a bout of exhaustive exercise. Data taken from the results of Chapters 2 (■), 3 (○ -1st bout; ● -2nd bout) and 4 (△ -NG; ▲-GD), shown tabulated in Tables 2.1, 3.2 and 4.1. The correlation coefficient (r=0.21) indicates a lack of significant correlation.

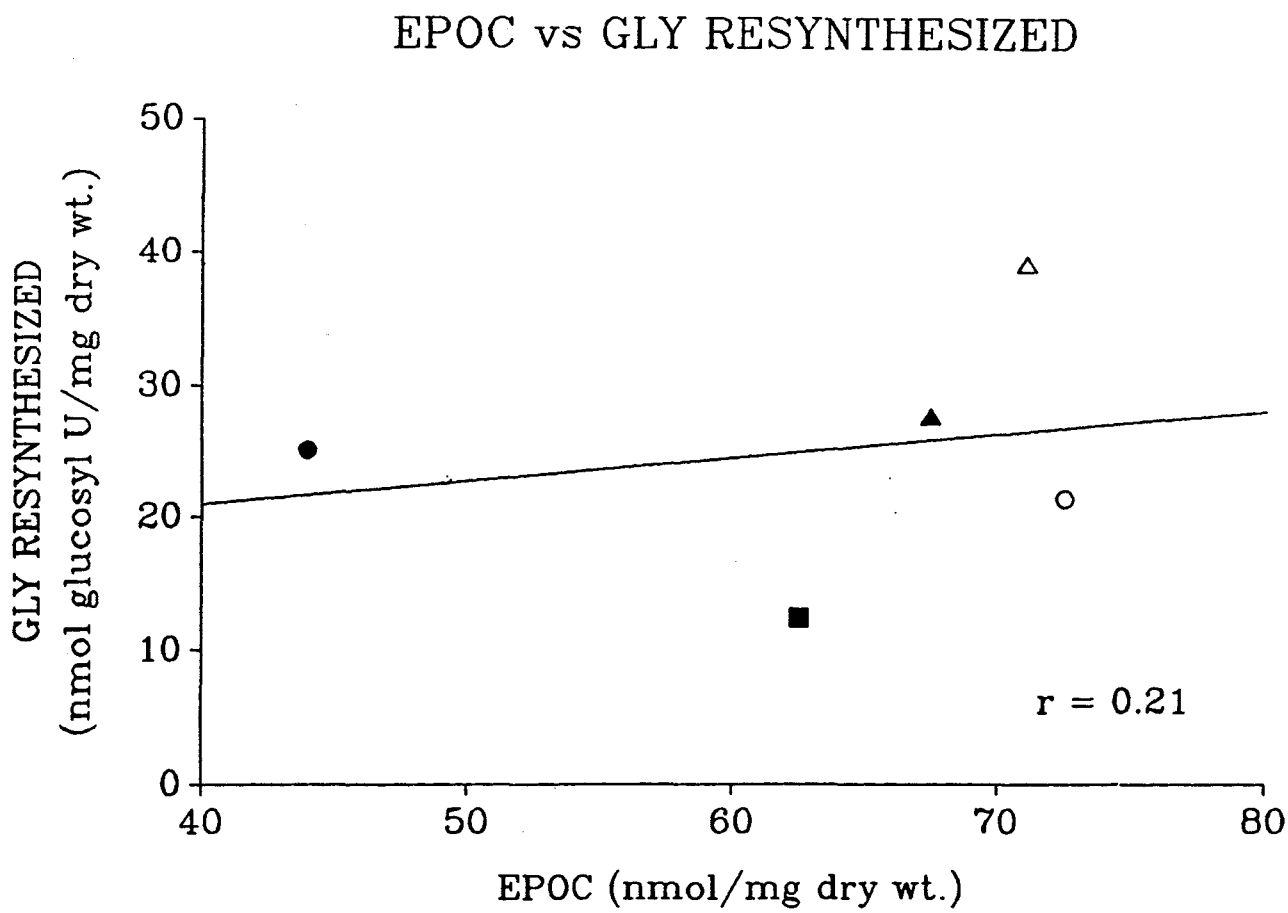
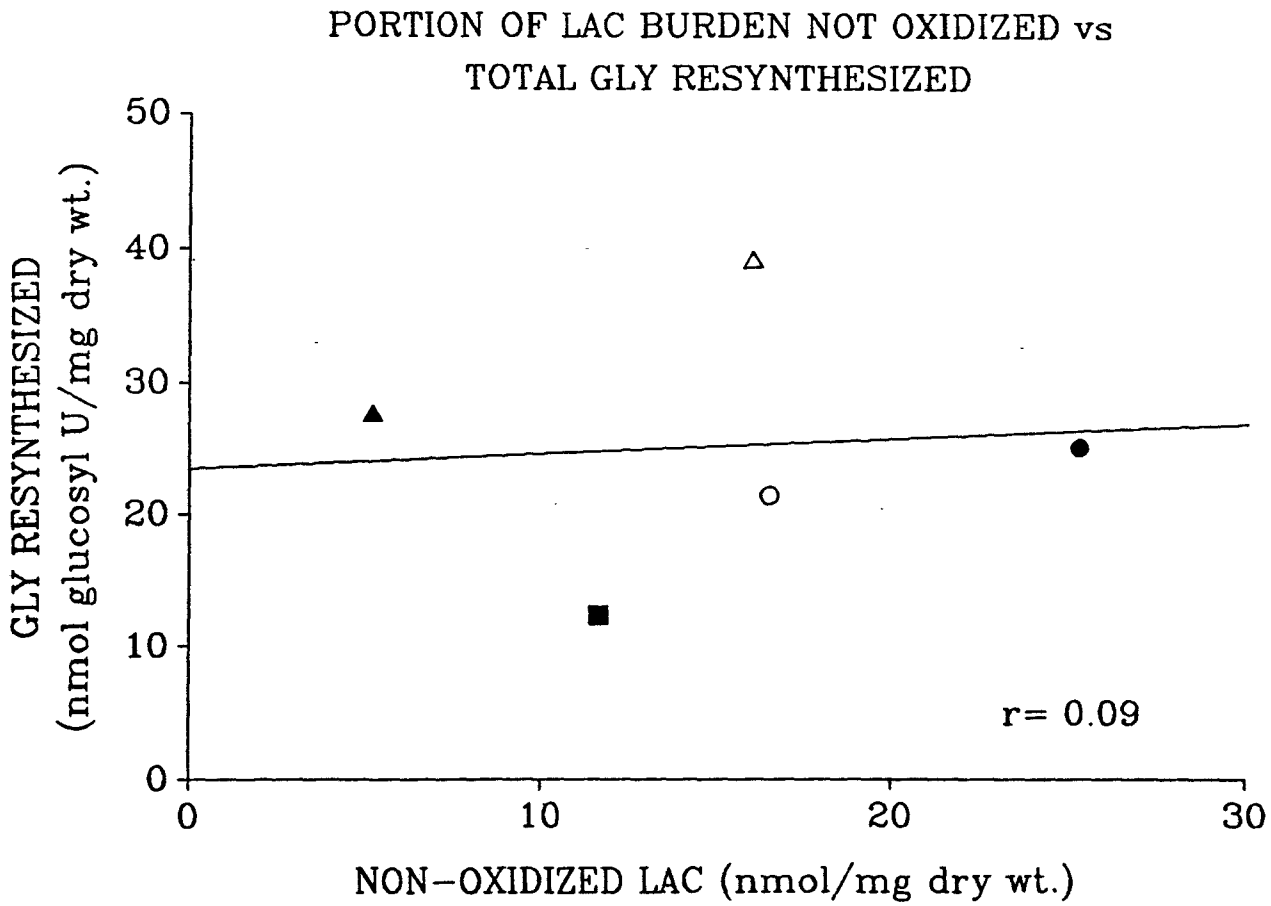

EPOC vs GLY RESYNTHESIZED
GLY RESYNTHESIZED
(nmol glucosyl U/mg dry wt.)
50
40
30
20
10
0
40
50
60
70
80
EPOC (nmol/mg dry wt.)
r = 0.21

Figure 5.3. The relationship between the portion of the LAC burden which was not oxidized and the total quantity of GLY resynthesized during the first 6 h of recovery after a bout of exhaustive exercise. Data taken from the results of Chapters 2 (■), 3 (O -1st bout; ● -2nd bout) and 4 (△ -NG; ▲-GD), shown tabulated in Tables 2.1, 3.2 and 4.1. The correlation coefficient (r=0.14) indicates a lack of significant correlation.


PORTION OF LAC BURDEN NOT OXIDIZED vs
TOTAL GLY RESYNTHESIZED
GLY RESYNTHESIZED
(nmol glucosyl U/mg dry wt.)
50
40
30
20
10
0
0
10
20
30
r= 0.09
NON-OXIDIZED LAC (nmol/mg dry wt.)

similar time courses and patterns of recovery between EPOC and LAC disappearance may only be coincidental, as part of the EPOC is utilized to oxidize a portion of the accumulated LAC. There is a clear need for more detailed biochemical studies on the pathways, relative rates, and regulatory mechanisms for LAC metabolism and GLY resynthesis in fish.

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