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J. G. Richards · G. J. F. Heigenhauser · C. M. Wood

Exercise and recovery metabolism in the pacific spiny dogfish (*Squalus acanthias*)

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Abstract We examined the effects of exhaustive exercise and post-exercise recovery on white muscle substrate depletion and metabolite distribution between white muscle and blood plasma in the Pacific spiny dogfish, both in vivo and in an electrically stimulated perfused tail-trunk preparation. Measurements of arterial-venous lactate, total ammonia, β-hydroxybutyrate, glucose, and L-alanine concentrations in the perfused tail-trunk assessed white muscle metabolite fluxes. Exhaustive exercise was fuelled primarily by creatine phosphate hydrolysis and glycolysis as indicated by 62, 71, and 85% decreases in ATP, creatine phosphate, and glycogen, respectively. White muscle lactate production during exercise caused a sustained increase (~12 h post-exercise) in plasma lactate load and a short-lived increase (~4 h post-exercise) in plasma metabolic acid load during recovery. Exhaustive exercise and recovery did not affect arterial PO_2, PCO_2, or PNH_3 but the metabolic acidosis caused a decrease in arterial HCO_3^- immediately after exercise and during the first 8 h recovery. During recovery, lactate was retained in the white muscle at higher concentrations than in the plasma despite increased lactate efflux from the muscle. Pyruvate dehydrogenase activity was very low in dogfish white muscle at rest and during recovery (0.53 ± 0.15 nmol g wet tissue^{-1} min^{-1}; $n = 40$) indicating that lactate oxidation is not the major fate of lactate during post-exercise recovery. The lack of change in white muscle free-carnitine and variable changes in short-chain fatty acyl-carnitine suggest that dogfish white muscle does not rely on lipid oxidation to fuel exhaustive exercise or recovery. These findings support the notion that extrahepatic tissues cannot utilize fatty acids as an oxidative fuel. Furthermore, our data strongly suggest that ketone body oxidation is important in fuelling recovery metabolism in dogfish white muscle and at least 20% of the ATP required for recovery could be supplied by uptake and oxidation of β-hydroxybutyrate from the plasma.

Keywords Elasmobranch white muscle · β-Hydroxybutyrate · Lipid · Carbohydrate · Lactate

Abbreviations CoA-SH free coenzyme A · CPT-1 carnitine palmitoyltransferase-1 · CrP creatine phosphate · ΔH^+_m metabolic proton load · ΔLac lactate load · PDH pyruvate dehydrogenase · PVP polyvinylpyrrolidone · SCFA-carnitine short-chain fatty acyl-carnitine · TAG triacylglycerol · TENS trancutaneous electrical nerve stimulator

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J. G. Richards · C. M. Wood
Department of Biology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1

J. G. Richards (✉)
Department of Zoology, The University of British Columbia, 6270 University Blvd., Vancouver, British Columbia, Canada, V6T 1Z4
E-mail: jrichard@zoology.ubc.ca
Tel.: +1-604-822 6759
Fax: +1-604-822 2416

G. J. F. Heigenhauser
Department of Medicine, McMaster University, Hamilton, Ontario, Canada, L8S 4K1

Introduction

It has long been recognized that the metabolic organization of marine elasmobranchs differs from that of other fish species (Ballantyne 1997). Elasmobranchs are osmoconformers and thus retain high concentrations of solutes, in particular urea and methylamines, to prevent osmotic water loss from the body fluids to the marine environment. The retention of high concentrations of urea impacts upon protein and membrane structure which further impacts upon the basic mechanisms of lipid, ketone body, and amino acid metabolism (Ballantyne 1997). Despite this deviation from "normal" metabolism, very little research has focused on the

mechanism by which elasmobranchs respond to metabolic perturbations such as exercise.

The patterns of white muscle substrate use during exhaustive exercise and re-synthesis during recovery have been well studied in teleost fish. Creatine phosphate (CrP) and glycogen are the primary substrates used to supply ATP for white muscle contraction during exhaustive exercise (Dobson and Hochachka 1987; Wang et al. 1994; Richards et al. 2002a). Exhaustive exercise results in large decreases in white muscle ATP, CrP, and glycogen with reciprocal and stoichiometric increases in inosine monophosphate, free creatine, and lactate, respectively. During recovery, ATP, CrP, and glycogen must be resynthesized to power another possible bout of exhaustive exercise. In rainbow trout (*Oncorhynchus mykiss*), the costs of ATP, CrP and glycogen synthesis during recovery are met by mitochondrial ATP production and lipid serves as the primary substrate for ATP production (Moyes et al. 1992; Richards et al. 2002b).

There is mounting evidence to suggest that elasmobranchs differ considerably from teleosts in their organization of lipid metabolism. Elasmobranchs lack the ability to oxidize fatty acids in extrahepatic tissue (Ballantyne 1997). Most extrahepatic tissues, including red and white muscle, lack detectable levels of carnitine palmitoyltransferase-1 activity (CPT-1; Crabtree and Newsholme 1972; Zammit and Newsholme 1979; Watson and Dickson 2001) and thus lack the ability to oxidize long-chain fatty acids in the mitochondria. Moyes et al. (1990) detected no oxidation of long-chain fatty acids by mitochondria isolated from red or cardiac muscle of dogfish. Instead, elasmobranchs appear to use the ketone bodies, β-hydroxybutyrate and acetoacetate, as their labile "lipid fuel". Hepatic fatty acid oxidation yields ketone bodies, which are released into circulation for oxidation by extrahepatic tissues. Enzymatic analysis indicates that extrahepatic tissues have the necessary machinery to oxidize ketone bodies at high rates (Zammit and Newsholme 1979) and recent enzymatic evidence supports a role for liver-derived ketone bodies as an aerobic fuel in the muscle of active shark species (Watson and Dickson 2001).

The objectives of the present study were to characterize the effects of exhaustive exercise on substrate utilization by white muscle of the Pacific spiny dogfish (*Squalus acanthias*) and to determine the pattern of metabolite recovery during the post-exercise period. Specifically, we measured changes in phosphogens (ATP, CrP, and creatine), triacylglycerol, glycogen, L-alanine, ammonia, β-hydroxybutyrate, oxidative metabolites (e.g. acetyl-CoA, esterified carnitine), glycolytic products (lactate and pyruvate), and the activity of pyruvate dehydrogenase (PDH) in an attempt to determine whether carbohydrates, lipids, or ketone bodies were oxidized during recovery to support metabolite synthesis. We specifically hypothesized that enhanced ketone body oxidation during the post-exercise period would provide some of the ATP required to fuel metabolite recovery. Furthermore, we examined the movement of lactate, ammonia, β-hydroxybutyrate, glucose, and L-alanine across white muscle membranes to assess the potential contribution of exogenous fuels in supporting recovery metabolism.

Materials and methods

Animal care

Adult, male and female Pacific spiny dogfish (*S. acanthias*, 0.8–3.7 kg) were obtained by angling and long-lining in Bamfield, British Columbia, Canada. After capture, dogfish were held at Bamfield Marine Station in a large tank continually supplied with seawater at 11°C for ~3 days before experimentation. During holding fish were not fed. One day before an experiment, fish were transferred into separate 10-l boxes served with flowing seawater (~500 ml min^{-1}) and vigorous aeration and allowed to settle.

Experimental protocols

Three separate experiments were run. In the first experiment we characterized the in vivo effects of exhaustive exercise and recovery (up to 30 h) on blood acid-base chemistry and plasma metabolites. In the second experiment we terminally sampled white muscle from dogfish at rest, immediately after exhaustive exercise and at various times up to 4 h post-exercise in order to examine the effects of exercise and recovery on white muscle substrate use. In the third experiment an in vitro perfused tail-trunk preparation was used to examine the plasma-to-tissue fluxes of metabolites across the white muscle membranes both at rest and at 30 min post-exercise.

In vivo exercise and recovery: blood and plasma

In order to repeatedly sample blood, dogfish were fitted with caudal artery catheters. Briefly, dogfish were anaesthetized with 0.08 g l^{-1} 3-aminobenzoic acid ethyl ester (MS-222, methanesulfonate salt; adjusted to pH 8 with KOH) and a 2-cm incision was made along the lateral line of the caudal peduncle. The spinal column was exposed, a nick was made in the caudal artery, and the artery was cannulated with a 50-cm length of Clay-Adams PE-50 polyethylene tubing filled with non-heparinized dogfish saline (Part et al. 1998). All surgical procedures were performed while the gills were irrigated with water containing anesthetic. The catheter was fed to the exterior through a PE-160 grommet that was tightly sutured into place. The incision was dusted with oxytetracycline hydrochloride (Sigma Chemical, Mississauga, Ontario) to help prevent infection and the incision sutured closed. Once surgery was complete, dogfish were returned to their individual boxes and revived by forced ventilation of seawater containing no anaesthetic. Fish were allowed to recover from surgery for at least 18 h before experimentation.

Arterial blood samples were taken at rest, immediately after exhaustive exercise (0 h), and at 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h, and 30 h post-exercise. Resting blood samples were taken 1 h before exercise. For exhaustive exercise, fish were transferred from their individual boxes to a 200-l tank filled with seawater and exercised to exhaustion by chasing (5 min; similar protocol to Wright et al. 1988). Upon exhaustion, a blood sample was taken and each dogfish was returned to its individual box, and blood samples were taken at the pre-assigned times. For blood sampling, 2 ml blood was withdrawn from the caudal catheter into an ice-cold gas-tight Hamilton syringe and replaced with non-heparinized dogfish saline. An aliquot of blood (~500 µl) was used for blood gas and acid-base analysis (PaO_2, pH_a, total CO_2; see below). The

remaining blood was centrifuged at 16,000×*g* for 10 s and the plasma separated from the blood samples. An aliquot (300 µl) of plasma was deproteinized in 600 µl of 1 mol l^{-1} $HClO_4$ and used for lactate, pyruvate, and glycerol analysis. The remaining plasma was frozen in liquid N_2 and used for analysis of total ammonia, β-hydroxybutyrate, triacylglycerol, and L-alanine (see below for analytical procedures).

In vivo exercise and recovery: white muscle

White muscle was terminally sampled at rest, immediately after exhaustive exercise (0 h), and at 0.25 h, 0.5 h, 2-h, and 4 h post-exercise. Resting fish were kept in their boxes for at least 20 h before sampling. Fish were exercised in the same manner as described for the blood experiment. Upon exhaustion, dogfish were returned to their individual boxes and sampled at the pre-assigned recovery times. At sampling, each dogfish was terminally anaesthetized by adding 0.5 g l^{-1} MS-222 to the surrounding water from a neutralized stock solution. At complete anaesthesia (~1 min), each fish was removed from the water and a dorsal white muscle sample was excised posterior to the dorsal fin with a scalpel. Muscle samples were immediately freeze-clamped between two aluminum plates cooled in liquid N_2, and all samples were stored at –86°C for later analysis. White muscle sampling took less than 10 s. Muscle samples were analyzed for ATP, total ammonia, CrP, creatine, glycogen, pyruvate, lactate, glucose, β-hydroxybutyrate, L-alanine, acetyl-CoA, free-CoA (CoA-SH), acetyl-, total-, free-, and short-chain fatty acyl-carnitine (SCFA-carnitine) and PDH activity (see below for analytical procedures).

In vitro perfused tail-trunk preparation

To examine the movement of metabolites across white muscle membranes at rest and during recovery from exhaustive exercise we used a modified perfused tail-trunk preparation similar to that used by Wang et al. (1996a; 1996b) for rainbow trout. Briefly, dogfish were anaesthetized with MS-222 and the tail severed at the point of the anus and weighed. Arterial and venous catheters (PE-160) were implanted into the caudal artery and vein of the severed tail, respectively, and secured by ligature around the vertebral column. Carbon transcutaneous electrical nerve stimulators (TENS electrodes; 5 cm×10 cm) were placed on either side of the tail and secured into place with gauze. The trunk was then placed into a temperature-controlled saline bath (11°C) and immediately perfused at a rate of 2 ml min^{-1} 100 g^{-1} tail wet weight with perfusate. Perfusate was delivered to the trunk by a peristaltic pump calibrated gravimetrically at the beginning and end of each experiment. The perfusate consisted of dogfish saline (Part et al. 1998), modified to contain 100 mmol l^{-1} trimethylamine oxide, 2.5 mmol l^{-1} (R)-β-hydroxybutyrate, 1 mmol l^{-1} lactate, 0.15 mmol l^{-1} L-alanine, and 20 g l^{-1} polyvinylpyrrolidone (PVP), pH 7.8. Before the PVP was added to the perfusate, high concentrations of contaminating ammonia were removed by treating a concentrated stock of PVP with a strongly acidic cation-exchange resin (Dowex 50WX8–400; 60 g PVP in 500 ml deionized water with 5 g of Dowex resin; Sigma). The PVP-resin solution was stirred for 20 min and the resin was removed by gravity filtration (10-µm Whatman filter). The concentrated ammonia-free PVP stock was then diluted in concentrated dogfish saline to yield a final concentration of 20 g mol l^{-1} PVP and appropriate ion concentrations. Total ammonia levels in the final perfusate averaged 49.5 ± 5.9 µmol l^{-1} ($n = 16$), a typical in vivo level. Before the perfusate entered the trunk it was equilibrated with 0.25% CO_2, balance O_2 (Wosthoff gas-mixing pump, Bochum, Germany) by passing through 7 m of Silastic tubing (inner diameter 1.47 mm, wall thickness 0.24 mm; Fisher Scientific, Nepean, Ontario) held in a gassing chamber fed with the CO_2/O_2 mixture. After gassing, the perfusate was fed through a heat exchange coil (11°C) and delivered to the tail via a windkessel to dampen pressure oscillations. This gassing and temperature equilibration procedure yielded stable perfusate pH (7.57 ± 0.02; $n = 18$), PO_2 (534 ± 8 torr; $n = 18$), and total CO_2 (4.3 ± 0.1 mmol l^{-1}; $n = 18$) levels. A pressure transducer, attached to the perfusion line, monitored arterial perfusion pressure. All tubing was stainless steel (for more detail see Wang et al. 1996a).

At the time the tail was severed, a muscle sample was taken immediately anterior to the point of severance and freeze-clamped in liquid N_2 for later analysis: this tissue sample represented the resting white muscle sample. The isolated tail was then perfused for 30 min to remove red blood cells. At that time, venous followed by arterial perfusate samples (2 ml) were taken via sampling ports with gas-tight Hamilton syringes and placed on ice for metabolite analysis (see below). These represented the pre-stimulation samples for calculation of resting metabolite fluxes. Preliminary experiments ($n = 4$) demonstrated stable acid-base variables and lactate production in the isolated trunk over 4 h of perfusion. To exercise the trunk, we used a protocol similar to that used by Wang et al. (1998). Briefly, the trunk musculature was stimulated for 5 min at 100 V, 20 ms pulse duration, and 10 Hz frequency using a Grass Stimulator (G88; Grass Instruments, Mass., USA). The polarity was altered at 20-s intervals to equally exercise both sides of the trunk. Muscle fatigue was established by a lack of contractile activity in response to further electrical stimulation. After stimulation, perfusion continued for an additional 30 min at which time venous followed by arterial samples were collected and a white muscle sample was taken and freeze-clamped. These represented the post-stimulation samples for calculation of post-exercise metabolite fluxes. A sampling time of 30 min post-exercise was chosen because preliminary experiments demonstrated that tissue to extracellular fluid gradients were close to maximum at this time for lactate and ammonia. Perfusate samples were analyzed for PO_2, pH, and total CO_2 (see below). An aliquot of perfusate (300 µl) was deproteinized in 600 µl of 1 mol l^{-1} $HClO_4$ for lactate analysis, and the remaining perfusate was frozen in liquid N_2 for later analysis of total ammonia, β-hydroxybutyrate, glucose, and L-alanine. Freeze-clamped muscle was analyzed for lactate, total ammonia, β-hydroxybutyrate, glucose, and L-alanine as described below.

Analytical protocols

Blood and perfusate were handled in a similar manner. Blood and perfusate pHs were determined at experimental temperature with a Radiometer microelectrode (E5021) attached to a PHM72 acid-base analyzer (Radiometer, Copenhagen, Denmark). Blood and perfusate PO_2 was measured at experimental temperature with a Radiometer PO_2 electrode (E5046) connected to a Cameron Instruments (OM-200) O_2 meter. True plasma for total CO_2 analysis was obtained by filling two capillary tubes with arterial blood, centrifuging at 9,000×*g* for 5 min, then removing the plasma anaerobically. Plasma and perfusate total CO_2 was determined using a Corning 965 total CO_2 analyzer.

Lactate and pyruvate were measured enzymatically on deproteinized plasma or perfusate by the reversible L-lactate dehydrogenase method (Bergmeyer 1983). Plasma glycerol was measured enzymatically on deproteinized samples by the coupled glycerol 3-phosphate dehydrogenase/glycerol kinase method (Bergmeyer 1983). Total ammonia was measured enzymatically on the first thaw of non-deproteinized plasma using glutamate dehydrogenase (Sigma kit). L-Alanine was measured enzymatically on non-deproteinized plasma and perfusate using L-alanine dehydrogenase (Bergmeyer 1983). Plasma and perfusate glucose was measured enzymatically by the coupled hexokinase/glucose 6-phosphate dehydrogenase method (Bergmeyer 1983). β-Hydroxybutyrate was measured enzymatically on non-deproteinized plasma and perfusate using β-hydroxybutyrate dehydrogenase (Sigma kit). Plasma TAG was measured enzymatically on non-deproteinized samples by the coupled lipase/glycerol kinase/glycerol phosphate oxidase/peroxidase method (Sigma kit).

An aliquot of frozen white muscle (~200 mg) was broken into small pieces (~50 mg) using an insulated mortar and pestle cooled

with liquid N_2. Several pieces of muscle were stored separately under liquid N_2 for later determination of PDH (see below). The remaining pieces of white muscle were ground into a fine powder under liquid N_2. A portion of the frozen muscle was used for the enzymatic (glutamate dehydrogenase) determination of total ammonia as described by Wang et al. (1994). The remaining frozen powder was then lyophilized for 72 h and stored dry at –86°C for subsequent analysis.

The active fraction of PDH was measured using a technique modified from Richards et al. (2002b). Briefly, muscle was homogenized using a 2-ml glass homogenizer in ten times its wet weight in a buffer containing (mmol l^{-1}): 200 sucrose, 100 KCl, 10 $MgCl_2$, 320 urea, 200 trimethylamine oxide, 5 [ethylenebis-(oxyethylenenitrilo)]-tetraacetic acid, 50 TRIS HCl, 50 NaF, 5 dichloroacetic acid, 0.1% Triton X-100 at pH 7.5. Homogenates were immediately frozen in liquid N_2 and stored until analysis on the same day. To assay for PDH activity, homogenates were thawed on ice, and 60-µl aliquots were incubated in duplicate at 9°C in an assay buffer containing (in mmol l^{-1}) 200 TRIS HCl, 0.72 EDTA, 5 $MgCl_2$, 320 urea, 200 trimethylamine oxide, 3 NAD^+, 1 free coenzyme A, and 1 thiamine pyrophosphate at pH 7.5. The reaction was initiated by the addition of 1 mmol l^{-1} pyruvate, and 200-µl aliquots of incubation media were sampled at 2, 4, and 6 min and added to 40 µl of 0.5 mol $l^{-1}HClO_4$. Tissue blanks were also run with homogenate incubated in the same buffer, but with the addition of deionized water instead of pyruvate. The acidified aliquots were neutralized with K_2CO_3, centrifuged for 3 min at 16,000×*g*, and stored at –80°C until analysis of acetyl-CoA (see below). Pyruvate dehydrogenase activity determined in the presence of pyruvate was corrected for PDH activity in the blank, and a regression between acetyl-CoA production and time was used to calculate reaction rates.

An aliquot of lyophilized muscle was used for the determination of glycogen. Enzymatic digestion of glycogen with amyloglucosidase was not successful in liberating glucose from glycogen in dogfish white muscle; therefore we employed acid hydrolysis to liberate glucose from glycogen. Briefly, ~20 mg of white muscle was digested in 1 ml of 30% KOH at 100°C. Glycogen was isolated as described by Hassid and Abraham (1957), and free glucose was determined in a neutralized sample after digestion with 2 N HCl at 95°C for 2 h.

Aliquots of the lyophilized muscle were used for the extraction of white muscle metabolites. Briefly, ~20 mg of lyophilized muscle was weighed into borosilicate tubes and homogenized with 1 ml of ice-cold, 1 mol l^{-1} $HClO_4$ for 20 s at 0°C using a Virtis hand-held homogenizer set at 30,000 rpm. Homogenates were stored on ice for 5 min, centrifuged for 5 min at 5,000×*g* at 4°C, and the supernatant was neutralized with 3 mol l^{-1} K_2CO_3. ATP and CrP were measured enzymatically on the same extract sample using the coupled creatine kinase, hexokinase, glucose 6-phosphate dehydrogenase assayed outlined by Bergmeyer (1983). Creatine was measured enzymatically in muscle extracts using the coupled creatine kinase, pyruvate kinase, and lactate dehydrogenase assay outlined by Bergmeyer (1983). Muscle extracts were also assayed for pyruvate, lactate, glycerol, β-hydroxybutyrate, and L-alanine using the same assays employed for plasma and perfusate analysis (see above). Acetyl-CoA, CoA-SH, acetyl-, free-, and total-carnitine were assayed on neutralized extracts according to radiometric methods (Cederblad et al. 1990). Short-chain fatty acyl-carnitine was estimated by subtracting acetyl- and free-carnitine from total carnitine.

Calculations

Blood and perfusate PO_2 and $[HCO_3^-]$ were calculated by manipulation of the Henderson-Hasselbalch equation using appropriate constants for dogfish plasma at 9°C (Boutilier et al. 1984).

Blood plasma metabolic acid load (ΔH_m^+) was calculated as described by Milligan and Wood (1986):

$$\Delta H_m^+ = \left[HCO_3^-\right]_1 - \left[HCO_3^-\right]_2 - \beta(pHa_1 - pHa_2),$$

where β, the non-bicarbonate buffer capacity of the true plasma of dogfish (−7.13 mmol pH $unit^{-1}$ l^{-1}) was taken from Gilmour et al. (2002).

Plasma and perfusate $[NH_3]$ were calculated from total ammonia by manipulation of the Henderson-Hasselbalch equation, using pK values from Boutilier et al. (1984) and Cameron and Heisler (1983). The partial pressure of NH_3 (PNH_3) in plasma and perfusate was calculated as described by Wright et al. (1988):

$$P_{NH_3} = \frac{[NH_3]}{\alpha_{NH_3}},$$

where α_{NH_3},the solubility coefficient of NH_3 in plasma, was taken from Boutilier et al. (1984).

Flux rates of lactate, ammonia, β-hydroxybutyrate, glucose, L-alanine, CO_2, and O_2 (MO_2) between white muscle and perfusate in the isolated tail-trunk preparation were calculated according to the Fick principle using the perfusion rate, tissue mass, and the arterial-venous difference for each metabolite. In order to convert PO_2measurements to total O_2 concentration, solubility coefficients for O_2 at 9°C in dogfish perfusate were estimated from Boutilier et al. (1984).

Statistical analysis

All data are reported as mean ± SE. All muscle metabolite concentrations determined on lyophilized tissues were converted back to wet weight by taking into account a wet:dry ratio of 4:1 (Wang et al. 1994). Changes in arterial blood metabolites were analyzed statistically using a repeated measures analysis of variance (ANOVA) followed by a Dunnett's post-hoc test to isolate where significant differences occurred. Changes in white muscle metabolites were analyzed statistically using a one-way ANOVA followed by a Dunnett's post-hoc test. Differences between arterial and venous perfusate, and pre- and post-stimulation muscle samples were analyzed statistically using a Student's paired, two-tailed *t*-test. Significance was accepted at $P < 0.05$.

Results

In vivo exercise and recovery

In response to exhaustive exercise, dogfish thrashed vigorously for the first 2–3 min, then slowed until exhaustion occurred at ~5 min of exercise. Exhaustion was characterized by the lack of response to further handling. There was no correlative relationship between body size and plasma or white muscle lactate or ammonia, thus the large range of body sizes used in the present study did not affect our results.

Arterial PO_2, PCO_2, and acid-base status

Arterial PO_2, approximately 102 torr, did not change significantly after exhaustive exercise or during the post-exercise period (Fig. 1A). Arterial pH decreased 0.18 pH units immediately after exhaustive exercise and continued to decrease by another 0.13 pH units at 30 min post-exercise (Fig. 1B). Arterial pH returned to values that were not significantly different from rest (approximately 7.8) by 4 h post-exercise and then remained stable through 30 h post-exercise. Exhaustive exercise and

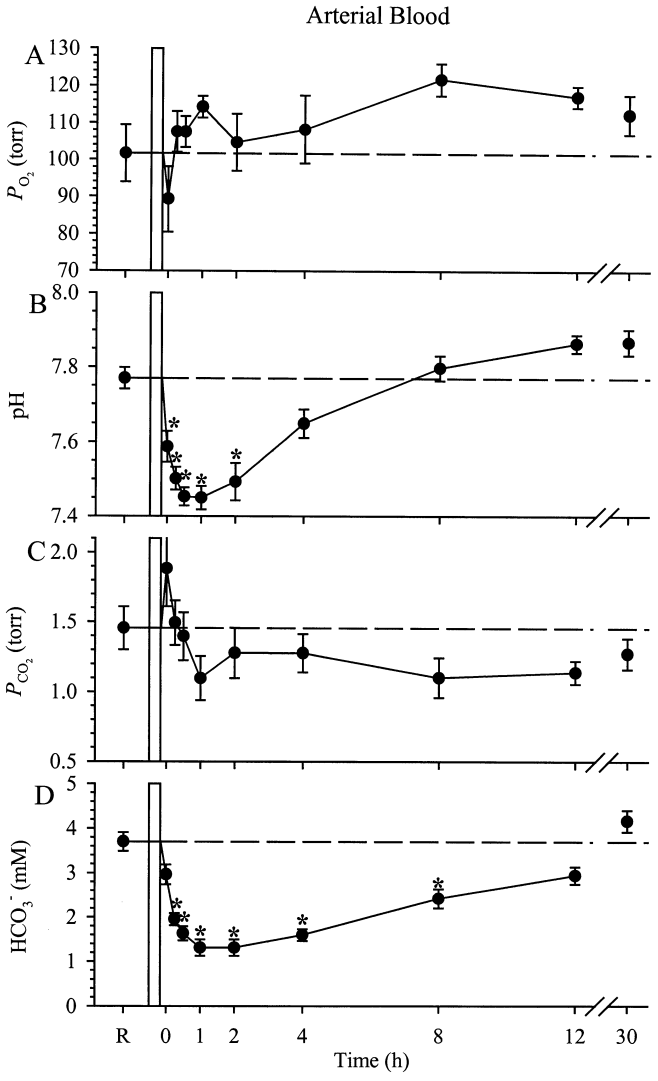

Fig. 1 Arterial PO_2 (**A**), pH (**B**), PCO_2 (**C**), and HCO_3^- (**D**) at rest (*R*) and during 30 h recovery from exhaustive exercise. *Vertical bar* represents 5 min of exhaustive exercise. Data are mean ± SE; $n = 8$ for each point. *Asterisks* represent significant differences from rest

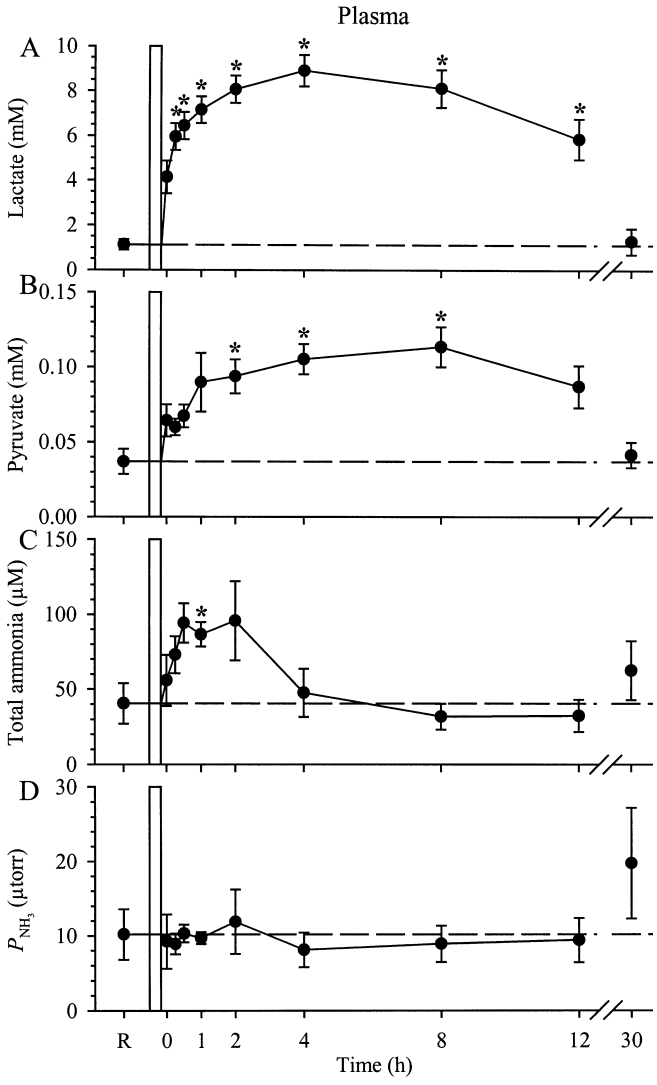

Fig. 2 Plasma lactate (**A**), pyruvate (**B**), total ammonia (**C**), and partial pressure of NH_3 (PNH_3) (**D**) at rest (*R*) and during 30 h recovery from exhaustive exercise. Data are mean ± SE; $n = 8$ for each point. *Asterisks* represent significant differences from rest

recovery had no significant effect on arterial PCO_2 which remained around 1.3 torr (Fig. 1C). Arterial plasma [HCO_3^-] decreased by 20% due to the exercise regime and continued to decrease by an additional 56% during the 1st hour post-exercise (Fig. 1D). Arterial plasma [HCO_3^-] returned to resting values (approximately 3.7 mmol l^{-1}) by 12 h recovery. Plasma total CO_2 exhibited very similar changes to those in plasma [HCO_3^-] (data not shown).

Plasma metabolites

Plasma [lactate] was about 1 mmol l^{-1} at rest, increased 3.7-fold immediately after exhaustive exercise and continued to increase to 7.9-fold resting values by 4 h post-exercise (Fig. 2A). Plasma [lactate] (Fig. 2A) remained elevated compared to resting values for 12 h post-exercise and then returned to resting values by 30 h post-exercise. Plasma [pyruvate], approximately 0.04 mmol l^{-1} at rest, followed a similar trend to plasma lactate: plasma [pyruvate] increased 2.5-fold at 2 h post-exercise, remained elevated for 8 h post-exercise, and thereafter returned to values that were not significantly different from rest by 12 h post-exercise (Fig. 2B). Exhaustive exercise did not affect plasma total [ammonia] which was about 40 μmol l^{-1} at rest; however, there was a transient, 2.1-fold increase in plasma total [ammonia] at 1 h post-exercise, followed by a return to values that were not statistically different from rest by 2 h post-exercise (Fig. 2C). Plasma PNH_3, approximately 10 μtorr, did not change significantly after exhaustive exercise or during the post-exercise period (Fig. 2D). Metabolic proton load (ΔH^+_m) increased immediately after exercise and continued to increase to ~3.6 mmol l^{-1} at 1 h post-exercise (Fig. 3). Plasma ΔH^+_m returned to resting

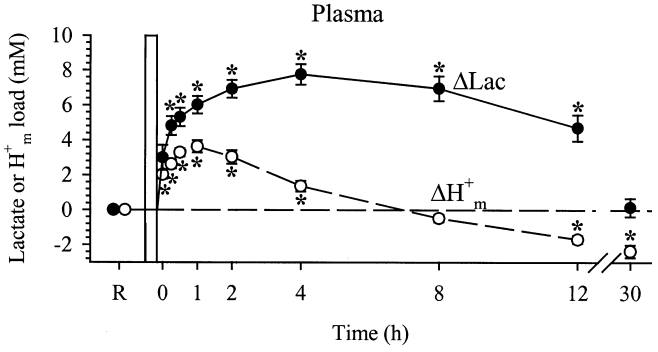

Fig. 3 Plasma lactate (Δlac; *filled circles*) and proton (ΔH^+_m; *open circles*) loads at rest (*R*) and during 30 h recovery from exhaustive exercise. Δlac and ΔH^+_m are zero, by definition, at rest. Data are mean ± SE; $n = 8$ for each point. See text for detailed calculation

values at 8 h post-exercise, then decreased to values below resting. Metabolic lactate load (ΔLac) exceeded ΔH^+_mthroughout recovery (Fig. 3).

Exhaustive exercise and recovery had no significant effect on plasma [*β*-hydroxybutyrate], [TAG], or [L-alanine] (Table 1). Glycerol concentrations were very variable, but tended to be consistently elevated after exercise and during recovery. Compared to resting levels of 0.33 mmol l^{-1}, plasma [glycerol] was 7.9-fold greater at 2 h post-exercise (Table 1).

White muscle metabolites

White muscle [ATP], about 3.5 μmol g^{-1} wet tissue at rest, decreased by 62% during exercise and remained lower than resting values for at least 4 h post-exercise (Fig. 4A). Exhaustive exercise caused a 4.4-fold increase in white muscle total [ammonia] compared to resting levels (0.58 μmol g^{-1} wet tissue; Fig. 4B). White muscle total [ammonia] remained elevated compared to resting values for 0.5 h post-exercise, then returned to values that were not significantly different from resting values by 2 h post-exercise. White muscle [CrP] was about 6-fold greater than [ATP] at rest (cf. Fig. 4A and C). Exhaustive exercise caused a 71% decrease in [CrP] that was restored to resting concentrations by 30 min post-exercise (Fig. 4C). White muscle [free creatine], approximately 19 μmol g^{-1} wet tissue at rest, increased by 1.6-fold immediately after exhaustive exercise and

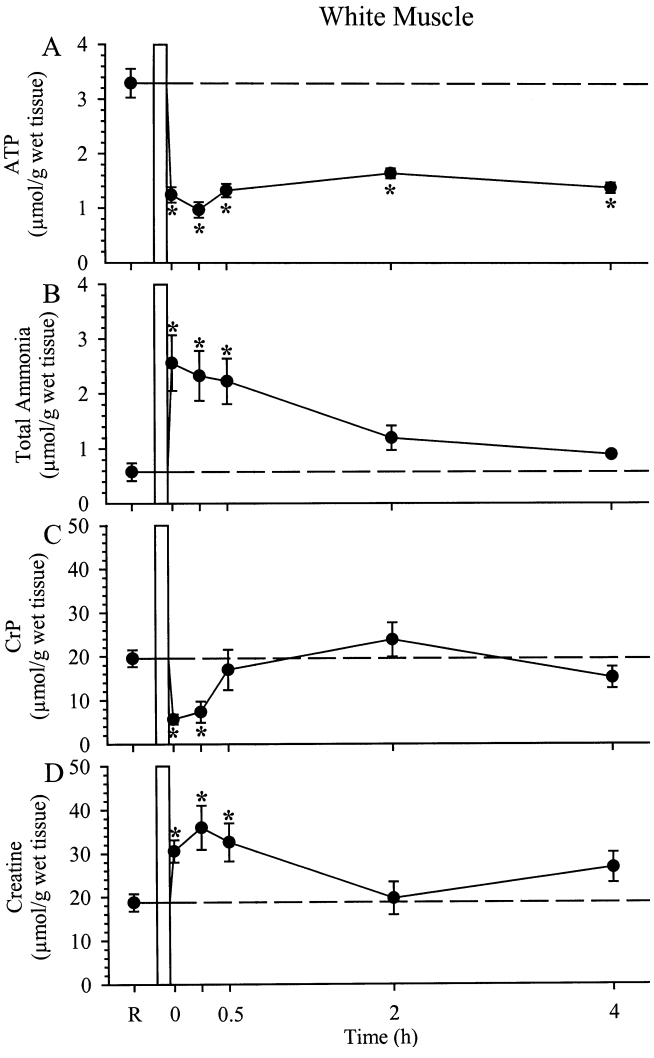

Fig. 4 White muscle ATP (**A**), total ammonia (**B**), creatine phosphate (CrP; **C**), and free creatine (Cr; **D**) at rest (*R*) and during 4 h recovery from exhaustive exercise. Data are mean ± SE. From left to right, $n = 11$, 8, 5, 6, 4, and 10. *Asterisks* represent significant differences from rest

remained elevated compared to resting values for 30 min post-exercise (Fig. 4D). White muscle creatine returned to resting values by 2 h post-exercise. Changes in [CrP] and [creatine] were almost reciprocally stoichiometric.

White muscle [glycogen] was 16 μmol glycosyl units g^{-1} wet tissue at rest (Fig. 5A). Exhaustive exercise

Table 1 Plasma *β*-hydroxybutyrate (*β*-HB), glycerol, triacylglycerol (TAG), and L-alanine content in dogfish at rest and during 30 h of recovery from high-intensity exercise

Measure	Rest	Exhausted	Recovery time (h)							
			0.25	0.5	1	2	4	8	12	30
β-HB	2.64 ± 0.82	2.77 ± 0.82	2.68 ± 0.78	2.53 ± 0.66	2.41 ± 0.69	2.46 ± 0.79	1.88 ± 0.58	1.92 ± 0.67	2.12 ± 0.64	2.72 ± 0.62
Glycerol	0.33 ± 0.14	1.54 ± 0.80	1.92 ± 0.93	1.19 ± 0.61	2.09 ± 0.83	2.66 ± 1.07*	2.36 ± 0.86	1.83 ± 0.78	1.40 ± 0.50	1.18 ± 1.02
TAG	0.63 ± 0.14	0.66 ± 0.15	0.64 ± 0.14	0.67 ± 0.13	0.66 ± 0.15	0.69 ± 0.12	0.72 ± 0.13	0.63 ± 0.11	0.60 ± 0.11	0.60 ± 0.12
L-Alanine	0.25 ± 0.02	0.24 ± 0.03	0.27 ± 0.02	0.26 ± 0.02	0.27 ± 0.02	0.29 ± 0.04	0.29 ± 0.03	0.23 ± 0.02	0.24 ± 0.03	0.17 ± 0.04

Data are mean ± SE (mmol l^{-1}); $n = 8$ for each TAG is expressed in terms of triolein
*Significant difference from rest ($P < 0.05$)

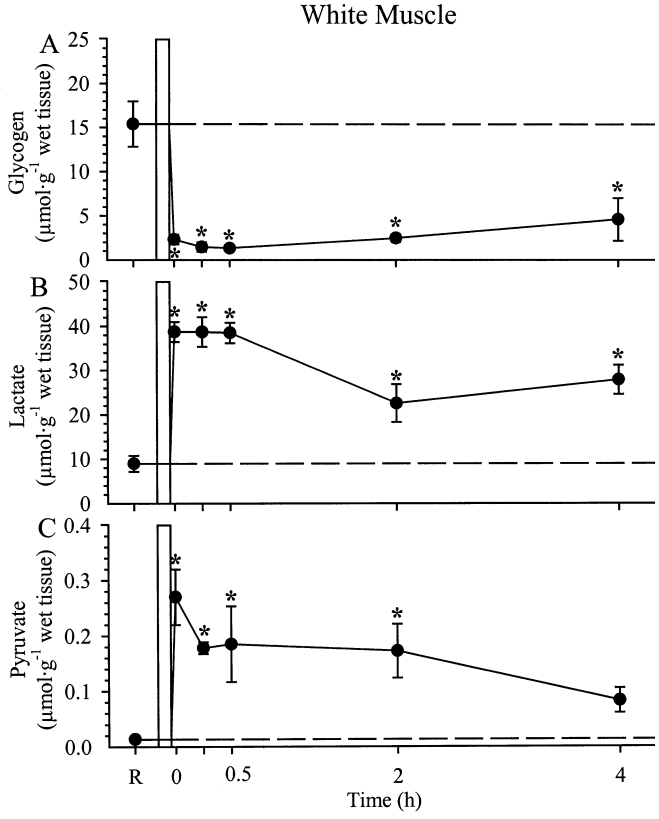

Fig. 5 White muscle glycogen (**A**), lactate (**B**), and pyruvate (**C**) at rest (*R*) and during 4 h recovery from exhaustive exercise. Glycogen is expressed in terms of μmol glucosyl units g^{-1} wet tissue. Data are mean ± SE. From left to right, $n = 11$, 8, 5, 6, 4, and 10. *Asterisks* represent significant differences from rest

caused an 85% decrease in [glycogen], which remained low for at least 4 h post-exercise. White muscle [lactate] was about 9 μmol g^{-1} wet tissue at rest, increased 4.3-fold immediately following exhaustive exercise, and remained elevated compared to resting values for more than 4 h post-exercise (Fig. 5B). The initial changes in [glycogen] and [lactate] were approximately stoichiometric (two lactate per glycosyl unit) in a reciprocal fashion. White muscle [pyruvate] was extremely low (0.01 μmol g^{-1} wet tissue) at rest, increased 27-fold following exhaustive exercise, remained elevated compared to resting fish for 2 h post-exercise, and then returned close to resting values before 4 h post-exercise (Fig. 5C).

Exhaustive exercise and recovery had no effect on white muscle [glucose], [β-hydroxybutyrate], [L-alanine], [acetyl-CoA], or [CoA-SH] (Table 2). Exhaustive exercise caused a 46% decrease in [acetyl-carnitine] which returned to values that were not significantly different from rest by 15 min post-exercise (Table 2). White muscle [acetyl-carnitine] remained at resting values for 2 h post-exercise, but decreased by 44% at 4 h post-exercise. Exhaustive exercise and recovery did not affect white muscle [total carnitine] or [free carnitine] (Table 2). White muscle [SCFA-carnitine] decreased by 50% immediately after exhaustive exercise, then increased to 1.6-fold compared to resting values by 30 min post-exercise, and finally returned to values that were not significantly different from rest by 2 h recovery (Table 2).

White muscle pyruvate dehydrogenase activity

Exhaustive exercise and recovery had no effect on white muscle PDH activity (data not shown). Mean PDH activity was 0.53 ± 0.15 nmol g^{-1} wet tissue min^{-1} ($n = 40$), which is close to the functional detection limit of the assay (~0.3 nmol g^{-1} wet tissue min^{-1}).

In vitro perfused tail-trunk preparation

Perfusion pressure in the tail-trunk preparation was 7 cmH_2O during both the pre- and post-stimulation periods (Table 3). During the pre-stimulation period the dogfish trunk exhibited an arterial-venous PO_2

Table 2 White muscle glucose, β-hydroxybutyrate, L-alanine, acetyl-CoA, free CoA, and carnitine content in dogfish at rest and during 4 h of recovery from exhaustive exercise

Measure	Rest	Exhausted	Recovery time (h)			
			0.25	0.5	2	4
Glucose	1.26 ± 0.30	1.27 ± 0.21	1.16 ± 0.34	0.82 ± 0.20	1.18 ± 0.29	1.82 ± 0.26
β-HB	2.00 ± 0.52	1.91 ± 0.41	0.99 ± 0.62	1.42 ± 0.82	1.08 ± 0.70	0.99 ± 0.29
L-Alanine	1.00 ± 0.32	1.14 ± 0.30	0.91 ± 0.33	1.28 ± 0.34	1.10 ± 0.36	0.97 ± 0.29
Acetyl-CoA	0.54 ± 0.04	0.50 ± 0.05	0.46 ± 0.03	0.66 ± 0.06	0.54 ± 0.07	0.55 ± 0.02
CoA-SH	6.22 ± 0.48	6.65 ± 1.17	5.23 ± 0.44	6.70 ± 0.77	6.74 ± 0.54	5.73 ± 0.53
Acetyl-carn	31.5 ± 3.7	17.1 ± 2.5*	24.0 ± 6.4	43.1 ± 7.5	22.3 ± 5.2	17.8 ± 3.2*
Total-carn	253.8 ± 27.0	211.8 ± 34.1	225.2 ± 66.4	366.6 ± 63.8	255.1 ± 64.1	245.1 ± 35.3
Free-carn	108.6 ± 14.4	137.7 ± 33.3	116.7 ± 31.7	142.6 ± 27.0	162.7 ± 41.1	163.2 ± 24.2
SCFA-carn	113.7 ± 15.4	57.0 ± 5.8*	84.46 ± 28.9	180.9 ± 45.9*	70.0 ± 20.9	64.0 ± 11.5

Data are mean ± SE. From left to right $n = 11$, 8, 5, 6, 4, and 10. Glucose, β-HB, and L-alanine are measured in μmol g^{-1} wet weight and acetyl-CoA, CoA-SH, acetyl-, total-, free-, and SCFA-carnitine are measured in nmol g^{-1} wet weight

Abbreviations: *acetyl-CoA* acetyl-coenzyme A, *CoA-SH* free coenzyme A, *acetyl-carn* acetyl-carnitine, *total-carn* total carnitine, *free-carn* free carnitine, *SCFA-carn* short chain fatty acyl-carnitine
*Significant difference from rest ($P < 0.05$)

Table 3 Perfusion pressure and arterial (*a*) and venous (*v*) perfusate partial pressure of O_2 (PO_2), O_2 consumption (MO_2), pH, total CO_2, CO_2 efflux, partial pressure of CO_2 (PCO_2), HCO_3^- and partial pressure of NH_3 (PNH_3) before and 0.5 h after 5 min of electrical stimulation

	Pre-stimulation	Post-stimulation
Perfusion pressure (cm H_2O)	6.8 ± 0.6	7.6 ± 0.2^{NS}
P_aO_2	539 ± 11	531 ± 11^{NS}
P_vO_2	314 ± 14	248 ± 9*
MO_2 (mmol kg^{-1} h^{-1})	0.48 ± 0.03	0.60 ± 0.04*
pH_a	7.59 ± 0.02	7.55 ± 0.02^{NS}
pH_v	7.39 ± 0.02	7.29 ± 0.03*
Total arterial CO_2 (mmol l^{-1})	4.4 ± 0.2	4.2 ± 0.1^{NS}
Total venous CO_2 (mmol l^{-1})	4.9 ± 0.1	4.8 ± 0.2^{NS}
CO_2 efflux (mmol kg^{-1} h^{-1})	0.5 ± 0.1	0.7 ± 0.1^{NS}
P_aCO_2 (torr)	2.6 ± 0.1	2.8 ± 0.1^{NS}
P_vCO_2 (torr)	4.4 ± 0.2	5.6 ± 0.4*
Arterial HCO_3^- (mmol l^{-1})	4.2 ± 0.2	4.1 ± 0.1^{NS}
Venous HCO_3^- (mmol l^{-1})	4.6 ± 0.1	4.5 ± 0.2^{NS}
P_aNH_3 (μtorr)	7.6 ± 0.9	8.2 ± 1.2^{NS}
P_vNH_3 (μtorr)	8.8 ± 1.1	18.2 ± 1.4*

Values are mean ± SE (n = 9 for each)
*Significant difference from pre-stimulation ($P < 0.05$)
^{NS}Not significant

difference of ~225 torr, which increased to ~283 torr at 0.5 h post-stimulation (Table 3). Trunk MO_2 increased by 1.25-fold at 0.5 h post-stimulation (Table 3). Arterial-venous pH difference increased from 0.20 during the pre-stimulation period to 0.26 at 0.5 h post-stimulation (Table 3). Arterial-venous total CO_2 difference and total CO_2 efflux were not affected by 0.5 h recovery from 5 min of electrical stimulation (Table 3). However, during the post-stimulation period there was an increase in arterial-venous PCO_2 compared to the pre-stimulation period. There were no differences in arterial-venous HCO_3^- between the pre- and post-stimulation period; mean arterial-venous HCO_3^- difference was 0.4 mmol l^{-1}. During the pre-stimulation period the dogfish trunk exhibited an arterial-venous PNH_3 difference of ~1.2 μ torr, which increased to ~10.1 μtorr at 0.5 h post-stimulation (Table 3).

Levels of lactate, total ammonia, β-hydroxybutyrate, glucose, L-alanine in the pre-stimulation samples were similar to those measured at rest in the in vivo series. After 0.5 h recovery from electrical stimulation, there were 3.2- and 7.7-fold increases in white muscle [lactate] and total [ammonia], respectively, at 0.5 h post-stimulation (Fig. 6). These increases in white muscle [lactate] and total [ammonia] observed in vitro closely resembled the increases in white muscle lactate and ammonia observed in vivo after exhaustive exercise and at 0.5 h recovery (cf. Figs. 6, 4B and 5B). White muscle [glucose] increased 1.5-fold at 0.5 h post-stimulation (Fig. 6). There were no differences in white muscle [β-hydroxybutyrate] or [L-alanine] between the pre- and post-stimulation periods (Fig. 6).

After 0.5 h recovery from electrical stimulation, there were 4.1- and 4.3-fold increases in the efflux rates of lactate and total ammonia, respectively, from the white muscle to the perfusate compared to the pre-stimulation

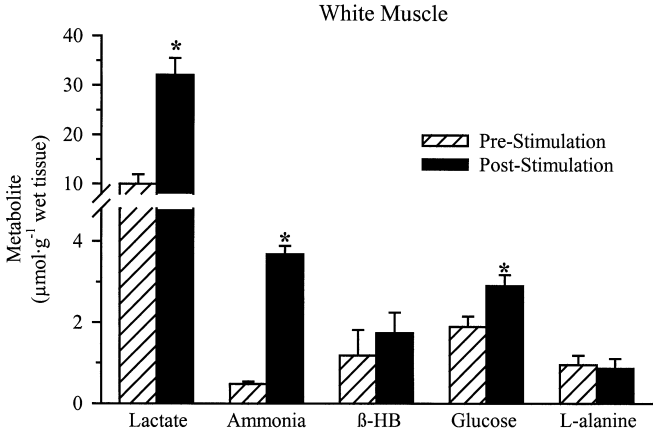

Fig. 6 White muscle lactate, total ammonia, β-hydroxybutyrate (β-HB), glucose, and L-alanine in the perfused tail-trunk preparation before (*striped bars*) and 0.5 h after 5 min of electrical stimulation (*solid bars*). Data are mean ± SE; $n = 9$ for each point. *Asterisks* represent significant differences from pre-stimulation period

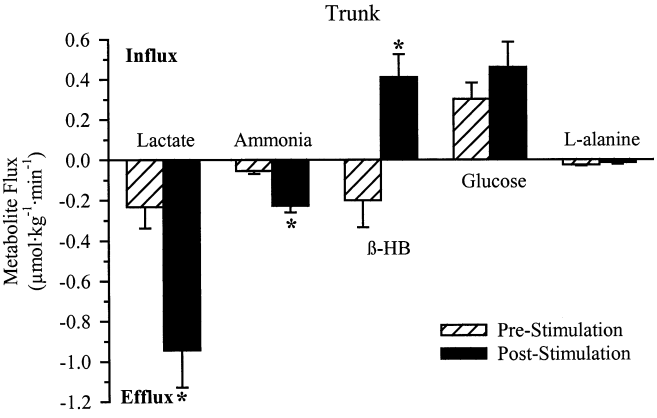

Fig. 7 Fluxes of lactate, total ammonia, β-hydroxybutyrate (β-HB), glucose, and L-alanine across the perfused tail-trunk preparation before (*striped bars*) and 0.5 h after 5 min of electrical simulation (*solid bars*). Data are mean ± SE; $n = 9$ for each point. *Asterisks* represent significant differences from pre-stimulation period

rates (Fig. 7). During the pre-stimulation period, there was a net efflux of β-hydroxybutyrate from the dogfish white muscle to the perfusate. Notably, this flux was reversed during the post-stimulation period as the muscle now took up β-hydroxybutyrate from the perfusate. There were no differences in net glucose flux between the pre- and post-stimulation periods (Fig. 7). There was a very small efflux of L-alanine from the dogfish white muscle during the pre-stimulation period that did not change at 0.5 h of post-stimulation perfusion (Fig. 7).

Discussion

The present study examined the time-course of metabolite recovery after exhaustive exercise in the white muscle and blood of the Pacific spiny dogfish and

specifically quantified white muscle metabolite fluxes at rest and at 0.5 h post-exercise. The metabolite fluxes were measured using an in vitro perfused tail-trunk preparation similar to that used to characterize post-exercise distribution of NH_3 (Wang et al. 1996a), lactate (Wang et al. 1996b, 1997), CO_2, and metabolic protons (Wang et al. 1998) across the white muscle cell membranes of rainbow trout.

Electrical stimulation of the perfused trunk successfully reproduced the metabolic changes observed in vivo in white muscle following exhaustive exercise. At 0.5 h post-exercise, white muscle [lactate] and total [ammonia] were similar in both the electrically stimulated perfused tail-trunk and the intact animal (cf. Figs. 4B, 5B, and 6), indicating that the electrically stimulated muscle had similar metabolic disturbances as the in vivo exercised muscle. Furthermore, unchanged values of white muscle [β-hydroxybutyrate] and [L-alanine] at 0.5 h post-stimulation agree with in vivo observations (cf. Fig. 6 and Table 2). There were small, but significant increases in white muscle glucose at 0.5 h post-stimulation in the perfused tail-trunk preparation that were not observed in vivo (cf. Fig. 6 and Table 2); however, for the most part, the electrically stimulated tail-trunk appears to accurately model the in vivo responses of dogfish white muscle to exhaustive exercise. Thus, measuring arterial-venous metabolite differences during post-electrical stimulation in the perfused tail-trunk provides a tool to examine changes in metabolite flux during recovery from exhaustive exercise.

Blood gases and acid-base balance

Exhaustive exercise and recovery did not cause respiratory disturbances in the dogfish as indicated by the lack of disturbance in arterial PO_2,PCO_2, or PNH_3 (Figs. 1A, B and 2D). However, exhaustive exercise did cause a significant arterial acidosis, which persisted for between 2 h and 4 h post-exercise in vivo (Fig. 1B). Significant increases in white muscle and plasma lactate (Figs. 2A and 5B) and increases in lactate and proton efflux from the white muscle (Fig. 7 and Table 3) during recovery indicate that the acidosis was metabolic in origin. ATP hydrolysis during exhaustive exercise (Fig. 4A) also yielded metabolic protons and probably contributed to the post-exercise acidosis. Plasma ΔLac was higher and took longer to recover than plasma ΔH^+_m (Fig. 3), which indicates that the distribution of lactate and protons across the white muscle membrane is governed by different mechanisms (see Wang et al. 1994, 1996b). Wang et al. (1997) demonstrated that lactate and proton distribution across muscle membranes was governed by multiple transporters (Lac^-/H^+ symporter, Lac^-/HCO_3^-, Cl^- antiporter) and diffusion, thus providing a mechanism to explain the uncoupled distribution of lactate and protons across trout muscle membranes. Similar patterns in extracellular fluid ΔLac and ΔH^+_m have been observed in rainbow trout (Milligan and Wood 1986; Wang et al. 1994) and dogfish (Piiper et al. 1972; Holeton and Heisler 1983). An excess of ΔLac over ΔH^+_m (e.g., Fig. 3) is a commonly observed pattern in active species (reviewed by Wood and Perry 1985) and is explained, in part, by the ability of such fish to rapidly shuttle acid equivalents across the gills into the external environment while lactate is retained in the extracellular fluid. Although perfusate buffer capacity was not measured in the present study, approximate calculations based on the change in acid-base status of the perfusate as it passed through the post-exercise tail-trunk indicate that H^+_m efflux was at least equal to and probably greater than lactate efflux.

No significant increase in arterial PCO_2 was observed in vivo (Fig. 1C) in contrast to most exhaustive exercise studies with other fish species (reviewed by Wood and Perry 1985). Gilmour et al. (2001) demonstrated the presence of extracellular carbonic anhydrase in dogfish that probably facilitates CO_2 clearance at the gills and may explain the lack of increase in arterial PCO_2 during the post-exercise acidosis. These changes in arterial acid-base status observed immediately after exhaustive exercise and during the post-exercise period are similar to those reported by Piiper et al. (1972) and Holeton and Heisler (1983) in the European dogfish (*Scyliorhinus canicula*) except for the lack of a significant respiratory component to the post-exercise acidosis (i.e., no increase in $PaCO_2$) in the present study.

ATP production during exercise

On the basis of substrate depletion, exhaustive exercise in dogfish is fuelled primarily by substrate-level phosphorylation resulting in large decreases in white muscle [ATP] (Fig. 4A), [CrP] (Fig. 4C), and [glycogen] (Fig. 5A). Decreases in [CrP] and [glycogen] were matched by reciprocal and stoichiometric increases in [free Cr] (Fig. 4D) and [lactate] (Fig. 5B and C), respectively. Accumulation of white muscle ammonia (Fig. 4B and Fig. 6) and increases in ammonia efflux (Fig. 7) from the white muscle during post-stimulation recovery explain in vivo accumulation of plasma total ammonia during post-exercise recovery (Fig. 2C). White muscle production and subsequent release of ammonia to the plasma suggests that ATP was deaminated to inosine monophosphate and coincides with the reduction of white muscle ATP (Fig. 4A). These patterns of white muscle substrate utilization and product accumulation in the dogfish are similar to the patterns observed in rainbow trout (Wang et al. 1994; Milligan 1996; Kieffer 2000; Richards et al. 2002a, 2002b) indicating the importance of substrate-level phosphorylation in fuelling exhaustive exercise in both teleosts and elasmobranchs.

Richards et al. (2002a,2002b) demonstrated that during exhaustive exercise, rainbow trout fully transform PDH into its active form indicating that glycolytically derived pyruvate is also utilized by oxidative

phosphorylation for ATP production. However, the transformation of PDH into its active form and subsequent oxidation of pyruvate contributed only 2% of the total ATP utilized during a bout of exhaustive exercise in trout. In dogfish white muscle, there was no transformation of PDH during exhaustive exercise or recovery. Furthermore, the maximum PDH activity detected was ~3% of the resting PDH activity and <1% of the maximum activity of PDH observed in trout white muscle (cf. Richards et al. 2002a). These very low PDH activities observed in dogfish white muscle, and their apparent lack of responsiveness to exercise, suggest that dogfish white muscle has a reduced capacity to oxidize carbohydrate via PDH during exercise and recovery. Although carbohydrate does not appear to be utilized as a substrate for oxidation phosphorylation during exercise and recovery, decreases in white muscle acetyl-carnitine (Table 2) during exhaustive exercise do indicate that oxidative utilization of acetyl groups occurs for ATP production. If the decrease in white muscle acetyl-carnitine represents complete oxidation of the acetyl groups, acetyl-carnitine oxidation would yield 0.17 μmol ATP g^{-1} wet tissue, which represents ≤ 0.3% of the ATP produced by CrP hydrolysis and glycolysis. Further investigations into the organization of oxidative phosphorylation in dogfish muscle are warranted.

Recovery in white muscle

The patterns of white muscle metabolite recovery observed in dogfish are in general agreement with many previously published studies on rainbow trout (Milligan and Wood 1986; Wang et al. 1994; Milligan 1996; Richards et al. 2002b). In general, fish confined during recovery restore resting levels of CrP and ATP within 0.5 and 4 h post-exercise, respectively. In dogfish, white muscle CrP synthesis was rapid and complete by 0.5 h post-exercise (Fig. 5C), but contrary to that observed in rainbow trout, white muscle ATP showed no recovery at 4 h post-exercise in dogfish (Fig. 5A). For the resynthesis of ATP from IMP, there must be the activation of the IMP-AMP conversion arm of the purine-nucleotide cycle that requires the input of nitrogen (as aspartate) and guanosine 5'-triphosphate (Mommsen and Hochachka 1988). White muscle ammonia decreased gradually throughout the 4 h recovery period and appeared in the plasma at relatively low concentrations early in the post-exercise period (Fig. 2C). Ammonia efflux from white muscle during recovery (Fig. 7) can only account for ~3% of the total ammonia disappearance from white muscle during the 4 h post-exercise period (Fig. 7). Thus, ammonia disappearance from the white muscle is likely due to in situ ammonia scavenging (glutamine synthetase) for nitrogen assimilation. Assimilated nitrogen, as amino acids, may then be utilized as a substrate by the purine-nucleotide cycle for the slow recovery of ATP.

Glycogen recovery in dogfish white muscle was slow to occur with only a small, non-significant increase in glycogen observed at 4 h post-exercise (Fig. 5A). However, during the 4 h post-exercise period, there was a 36% reduction in white muscle lactate (Fig. 5B) and a full recovery of white muscle pyruvate (Fig. 5C). At 4 h recovery, 4–6 μmol lactate g^{-1} wet tissue was unaccounted for by the small amount of glycogen synthesized. These results are in general agreement with those observed in rainbow trout where white muscle lactate disappearance tends to be faster than glycogen synthesis (Wang et al. 1994; Milligan 1996; Richards et al. 2002b).

Fate of lactate during recovery

The fate of lactate during recovery remains controversial in fish. The general consensus is that lactate is retained in white muscle of most fish during recovery from exhaustive exercise for in situ glycogenesis (Schulte et al. 1992; Moyes and West 1995; Wang et al. 1997; Labree and Milligan 1999); however, direct evidence in support of in situ glycogenesis as the major fate of lactate has not yet been obtained. In the present study, lactate is retained in dogfish white muscle during the post-exercise period (cf. Fig. 2A, maximum plasma [lactate] ~9 mmol l^{-1}; Fig. 4B, estimated intracellular [lactate] ~50 mmol l^{-1}; calculated using that estimates of Heisler et al. (1980) for intracellular fluid volume). Furthermore, post-exercise lactate efflux from white muscle (0.94 μmol kg^{-1} min^{-1}; Fig. 7) accounts for only 2% of the total lactate clearance over the 4-h post-exercise period (Fig. 5B) and is ~20-fold lower than post-exercise lactate efflux from trout white muscle (Wang et al. 1996b). Taken together, the high concentrations of lactate in the white muscle intracellular compartment and the very low lactate efflux from white muscle clearly indicate that dogfish retain lactate in their white muscle during recovery. Lactate oxidation via the low PDH activity can only account for a maximum of 2% of the white muscle lactate disappearance, thus oxidation is not the major fate of the retained lactate (see Fig. 5B). Likely, the major fate of lactate in dogfish white muscle is in situ glycogen synthesis and the discrepancy between glycogen synthesis and lactate disappearance might be explained by the accumulation of glycolytic or gluconeogenic intermediates.

ATP production during recovery

During recovery, pathways must be activated to generate ATP to fuel ATP, CrP, and glycogen synthesis in preparation for another possible bout of exhaustive exercise. In the rainbow trout, Richards et al. (2002b) demonstrated that enhanced lipid oxidation during the post-exercise period supplied the ATP necessary for recovery metabolism. The increase in fatty acid oxidation was, in part, due to an enhanced transport of fatty

acids into the mitochondria for oxidation via the CPT-1 catalyzed formation of fatty acyl-carnitine.

Marine elasmobranchs lack the ability to utilize fatty acids in extrahepatic tissue (Moyes and West 1995) and as a result have very low circulating fatty acids (Ballantyne et al. 1993) and TAG concentrations ($\sim$0.6 mmol l^{-1}; Table 1, present study versus $\sim$20 mmol l^{-1} in trout (Richards et al. 2002b). During recovery in dogfish white muscle, enhanced β-hydroxybutyrate influx into muscle (Fig. 7) suggests that ketone bodies are an important substrate for recovery metabolism. Despite the increased β-hydroxybutyrate uptake into white muscle during the post-exercise period, there were no changes in plasma or muscle [β-hydroxybutyrate] (Tables 1 and 2, Fig. 5). This balancing of tissue and plasma [β-hydroxybutyrate] suggests a close matching of hepatic ketone body production with muscle utilization at rest and during recovery. If the measured influx of β-hydroxybutyrate was maintained during the 4-h post-exercise period and represented oxidation, β-hydroxybutyrate oxidation could provide $\sim$21% ($\sim$3.3 μmol ATP g^{-1} wet tissue) of the ATP required to fuel the observed recovery ($\sim$16 μmol ATP g^{-1} wet tissue). Thus, exogenous β-hydroxybutyrate is not the only fuel utilized to support ATP production during recovery. Increases in plasma glycerol during the post-exercise period (Table 1) suggests that there was enhanced breakdown of TAG for ketone body production, probably in the liver, and suggests that other ketone bodies (e.g., acetoacetate) may also be release into circulation for uptake by muscle. Future investigations should examine the roles of exogenous acetoacetate and endogenous peroxisomal fatty acid oxidation or ketone body production in supplying ATP for recovery metabolism (Moyes et al. 1990).

During recovery, there is a rapid accumulation and subsequent decrease in white muscle acetyl-carnitine (Table 2). Breakdown of ketone bodies yield acetyl-CoA for oxidation and ATP production *via* oxidative phosphorylation. However, to sustain flux through pathways responsible for ketone body breakdown early in recovery, acetyl-CoA concentrations must kept low and CoA-SH concentrations must be kept high through the formation of acetyl-carnitine. Thus, the rapid synthesis of acetyl-carnitine during the first 0.5 h recovery is probably due to enhanced ketone breakdown early within recovery and the acetyl-groups are subsequently utilized to fuel ATP and glycogen synthesis. The absence of changes in white muscle free-carnitine and the variable changes in SCFA-carnitine (Table 2) during the post-exercise period indicates that long- and short-chain fatty acids are not important substrates for mitochondrial oxidation during recovery, and corroborates the lack of detectable CPT-1 activity in dogfish muscle (Zammit and Newsholme 1979).

Amino acids have also been implicated as important substrates for ATP production in marine elasmobranch muscle (Ballantyne 1997). However, in our studies we observed no change in white muscle L-alanine flux from rest to recovery in the electrically stimulated perfused dogfish trunk (Fig. 7), suggesting that L-alanine is not an important substrate for recovery metabolism. During starvation, Leech et al. (1979) demonstrated that trunk musculature of dogfish released L-alanine into circulation. Clearly, future investigations should focus on determining the role of amino acid metabolism, in particular L-alanine and glutamine oxidation, in fuelling exercise and recovery in dogfish.

In conclusion, exhaustive exercise in dogfish is powered predominately by white muscle CrP hydrolysis and glycolysis. White muscle lactate production and ATP hydrolysis cause a severe post-exercise metabolic acidosis. The pattern of metabolite recovery is similar to that observed in the rainbow trout with rapid synthesis of CrP and slower recovery of ATP, lactate, and glycogen. Lactate is retained in white muscle during recovery probably to act as a substrate for in situ glycogen synthesis. Recovery metabolism in white muscle is probably fuelled by enhanced ketone body oxidation, of which at least 20% is supplied by β-hydroxybutyrate uptake from the plasma. A detailed analysis of intermediates in lipid metabolism provides no evidence for white muscle fatty acid oxidation. These findings therefore support the common belief that extrahepatic tissues of marine elasmobranchs are not able to oxidize fatty acids.

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