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Lipid oxidation fuels recovery from exhaustive exercise in white muscle of rainbow trout

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Richards, Jeff G., George J. F. Heigenhauser, and Chris M. Wood. Lipid oxidation fuels recovery from exhaustive exercise in white muscle of rainbow trout. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R89–R99, 2002; 10.1152/ajpregu.00238.2001.—The oxidative utilization of lipid and carbohydrate was examined in white muscle of rainbow trout (*Oncorhynchus mykiss*) at rest, immediately after exhaustive exercise, and for 32-h recovery. In addition to creatine phosphate and glycolysis fueling exhaustive exercise, near maximal activation of pyruvate dehydrogenase (PDH) at the end of exercise points to oxidative phosphorylation of carbohydrate as an additional source of ATP during exercise. Within 15 min postexercise, PDH activation returned to resting values, thus sparing accumulated lactate from oxidation. Glycogen synthase activity matched the rate of glycogen resynthesis and represented near maximal activation. Decreases in white muscle free carnitine, increases in long-chain fatty acyl carnitine, and sustained elevations of acetyl-CoA and acetyl carnitine indicate a rapid utilization of lipid to supply ATP for recovery. Increases in malonyl-CoA during recovery suggest that malonyl-CoA may not regulate carnitine palmitoyltransferase-1 in trout muscle during recovery, but instead it may act to elongate short-chain fatty acids for mitochondrial oxidation. In addition, decreases in intramuscular triacylglycerol and in plasma nonesterified fatty acids indicate that both endogenous and exogenous lipid fuels may be oxidized during recovery.

pyruvate dehydrogenase; glycogen synthase; carbohydrate; lactate; metabolism; malonyl-coenzyme A; nonesterified fatty acids

OVER THE PAST several decades many studies have examined the metabolic responses of fish white muscle to high-intensity, exhaustive exercise together with the pattern of metabolite recovery (16). These studies have led to the development of a model of fuel selection during exhaustive exercise based on hydrolysis of high-energy phosphates [i.e., creatine phosphate (CrP) and ATP] and "anaerobic" glycolysis leading to lactate accumulation. Furthermore, it has been demonstrated that there is a temporal shift in fuel selection during exhaustive exercise from an initial hydrolysis of CrP (7, 8) to an activation of glycogenolysis and glycolysis (25). As a result, exhaustion in rainbow trout is characterized by a 40 to 60% decrease in white muscle ATP and CrP concentrations and up to a 90% decrease in muscle glycogen concentrations with reciprocal and stoichiometric increases in inosine monophosphate (IMP), free creatine (Cr), and lactate, respectively (e.g., Ref. 39).

During recovery, pathways must be activated to resynthesize ATP, CrP, and glycogen in preparation for another possible bout of exercise. To this end, trout experience an excess postexercise O_2 consumption (EPOC) (34), in part, representing a stimulation of oxidative phosphorylation for ATP production during recovery. The tricarboxcylic acid (TCA) cycle supplies reducing equivalents for mitochondrial oxidative phosphorylation through the utilization of acetyl-CoA. Acetyl-CoA can be produced either from the decarboxylation of pyruvate via pyruvate dehydrogenase (PDH) or through β-oxidation of lipid fuels. Amino acids can also supply substrate for the TCA cycle and support ATP production, but it is believed that the contributions of protein oxidation to metabolism are low and can be ignored, particularly during exercise (41). Therefore, the two major fuel sources available to trout white muscle during recovery are the accumulated lactate from glycolysis and lipid fuels. The complete oxidation of a small amount of lactate (4 to 6 μmol/g wet tissue), through the activation of PDH, could yield adequate ATP to support recovery in white muscle. However, there is accumulating circumstantial evidence that suggests the majority of accumulated lactate in trout white muscle is spared from an oxidative fate (20, 45) and retained as the substrate for in situ glyconeogenesis (25, 35).

Traditionally, lipids have not been considered an important fuel during exhaustive exercise and recovery; however, there is mounting evidence that suggests many species rely on lipid oxidation in muscle to fuel recovery (15, 38). In the rainbow trout, Wang et al. (39) showed that immediately after exhaustive exercise, there were decreases in free carnitine and increases in acetyl-carnitine and acetyl-CoA concentrations in white muscle. Accumulation of acetyl groups during recovery points to an activation of oxidative phosphorylation during recovery. Decreases in free carnitine, accompanied by the accumulation of short-chain acyl-carnitine, suggested that lipid was the source of acetyl

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groups. Decreases in white muscle total lipid concentrations (20) and decreases in plasma nonesterified fatty acids (NEFA) (7) during postexercise recovery in trout further support the use of lipids in fueling recovery from exhaustive exercise.

If we accept this scenario that β-oxidation may contribute to ATP production during postexercise resynthesis of CrP, ATP, and glycogen, then the question arises as to the underlying mechanism that regulates fuel selection during recovery. Moyes et al. (24) demonstrated that NEFA oxidation inhibited pyruvate oxidation when isolated trout white muscle mitochondria were incubated simultaneously with pyruvate and NEFA. These workers speculated that this inhibition of carbohydrate oxidation in the presence of NEFA was due to allosteric inhibition of PDH, providing a mechanism by which carbohydrate can be spared at the expense of lipid oxidation. Furthermore, in higher vertebrates, recent evidence has implicated malonyl-CoA in regulating lipid oxidation in skeletal muscle (32). Malonyl-CoA is the first committed step in the de novo synthesis of fatty acids and has been shown in muscle to allosterically regulate carnitine palmitoyltransferase-1 (CPT 1), the enzyme responsible for catalyzing the rate-limiting transfer of fatty acids to carnitine for uptake by mitochondria (1). There remains considerable debate surrounding the regulatory role of malonyl-CoA in fuel selection in muscles of different species (32, 43).

The objectives of the present research were to determine the metabolic fuels oxidized during recovery in trout white muscle to support synthesis of CrP, ATP, and glycogen. Specifically, we measured the activation state of PDH and glycogen synthase (GS) and changes in oxidative metabolites (e.g., acetyl-CoA) and glycolytic intermediates in an attempt to isolate whether lipid or carbohydrate was oxidized during recovery in trout white muscle. Furthermore, we measured changes in intramuscular triacylglycerol (IMTG) and plasma NEFA in an attempt to determine whether endogenous or exogenous lipids were oxidized during recovery. Insights into the control of lipid and carbohydrate oxidation were gained through measurements of malonyl-CoA and estimates of free ADP and AMP (ADP_f and AMP_f, respectively).

METHODS

Animal Care

Adult rainbow trout (*Oncorhynchus mykiss*, Walbaum; 240–350 g) were purchased from Humber Springs Trout Hatchery, Orangeville, Ontario, Canada, and held under flow through conditions in 800-liter tanks supplied with aerated, dechlorinated city of Hamilton tap water [composition as described by Milligan and Wood (22); 10°C] for at least 1 mo before experimentation. During holding, fish were fed daily with commercial trout pellets. Three days before an experiment, fish were transferred into a separate tank and feeding ceased.

Experimental Protocol

Fish were anesthetized with 0.08 g/l 3-aminobenzoic acid ethyl ester (methanesulfonate salt; neutralized to pH 8.0 with KOH) and fitted with dorsal aortic (DA) catheters using Clay-Adams PE-50 polyethylene tubing while their gills were irrigated with water containing anesthetic (37). Heparin was not used during surgery or blood sampling due to its stimulation of lipoprotein lipase (31). Once surgery was complete, trout were revived in fresh water containing no anesthetic and allowed to recover for ~48 h in dark, aerated 2.5-liter acrylic boxes supplied with ~100 ml/min freshwater at 10°C. During recovery, catheters were flushed daily with Cortland saline (44).

Arterial blood and white muscle were terminally sampled at rest, immediately after exhaustive exercise, and at 0.25-, 0.5-, 1-, 2-, 4-, 8-, 16-, and 32-h recovery. Resting fish were kept in the acrylic boxes for at least 48 h before sampling. For exhaustive exercise, individual fish were transferred from their acrylic box to a 150-liter circular tank filled with water at experimental temperature and manually chased to exhaustion [5 min; similar protocol to Wang et al. (39)]. Upon exhaustion, identified by no further response to manual stimulation, trout were returned to their individual boxes and sampled at the preassigned recovery times. At sampling, trout were terminally anesthetized by adding 0.5 g/l MS-222 to their surrounding water from a neutralized stock solution. During the onset of anesthesia, 3 ml of arterial blood were drawn into an ice-cold gas-tight Hamilton syringe through the DA catheter. Plasma was immediately separated from blood cells by centrifugation at 16,000 *g* for 10 s. A portion (300 μl) of the plasma was deproteinized in 600 μl of 1 M $HClO_4$, and the remaining plasma (~1.5 ml) was frozen in liquid nitrogen.

At complete anesthesia (~1 min), the fish were removed from the water, and a white muscle sample was excised from between the lateral line and dorsal fin with a scalpel. The muscle samples were immediately freeze-clamped between two aluminum blocks cooled in liquid N_2, and all samples were stored under liquid N_2 for later analysis. White muscle sampling took less than 10 s.

Analytic Techniques

An aliquot of frozen white muscle was broken into small pieces (50 to 100 mg) in an insulated mortar and pestle cooled with liquid N_2. Several pieces of white muscle were stored separately in liquid N_2 for determination of PDH activity. The remaining broken muscle was lyophilized for 72 h, dissected free of connective tissue, powdered, and stored dry at −80°C for subsequent analysis.

The active fraction of PDH (PDH_a) was measured in muscle homogenates using a modified technique of Putman et al. (29). Briefly, muscle (30 to 50 mg) was homogenized in 15 times its wet weight in a buffer containing (in mM) 200 sucrose, 50 KCl, 5 $MgCl_2$, 5 EGTA, 50 Tris·HCl, 50 NaF, 5 dichloroacetic acid, and 0.1% Triton X-100 at pH 7.5. Homogenates were immediately frozen in liquid N_2 until analysis on the same day. To assay for PDH activity, homogenates were thawed on ice, and 60-μl aliquots of homogenate were incubated in duplicate at 10°C in an assay buffer containing (in mM) 144 Tris·HCl, 0.72 EDTA, 1.44 $MgCl_2$, 3 NAD^+, 1 CoA-SH, and 1 thiamine pyrophosphate at pH 7.5. The reaction was initiated by the addition of 1 mM pyruvate, and 200-μl aliquots of the incubation media were sampled at 2, 4, and 6 min, except those tissues from the exhausted fish that were sampled at 1, 2, and 3 min because of the high-PDH activity. Tissue blanks were also run with homogenates in-

cubated in the same buffer, but with the addition of deionized water instead of pyruvate. The reaction was stopped by the addition of each aliquot to 40 μl of 0.5 M $HClO_4$. After 5 min at room temperature, each aliquot was neutralized with 1 M K_2CO_3, centrifuged for 3 min at 16,000 *g*, and stored at −80°C until analysis of acetyl-CoA. PDH 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.

Total PDH activities (PDH_{tot}) were assayed on a separate group of fish taken from the same stock. Briefly, muscle was homogenized in a similar buffer as described for PDH_a with the addition of 10 mM D-glucose, 10 mM $CaCl_2$, and 4 U/ml sulfate-free hexokinase. Homogenates were immediately frozen in liquid N_2, thawed on ice, and incubated at 10°C for 30 min before samples were incubated in assay buffer as described above for PDH_a. The percent mole fraction of PDH transformation was determined by dividing PDH_a by PDH_{tot}.

An aliquot of lyophilized muscle was used for the determination of GS activity. Briefly, 5 to 10 mg of dry muscle were homogenized at −25°C in 200 μl of buffer containing (in mM) 50 imidazole·HCl, 100 KF, 10 EDTA, and 60% (vol/vol) glycerol at pH 7.5. Homogenates were then diluted with 800 μl of the above buffer without glycerol and homogenized further at 0°C. Total GS (GS_{tot}) and the active fraction (GS_a) were determined at saturating and physiological concentrations of glucose 6-phosphate (glu 6-P), respectively. The GS assay measured the incorporation of glucose from UDP-glucose into glycogen with the subsequent analysis of liberated UDP. For GS_{tot} activity (high glu 6-P), 100-μl aliquots of homogenate were incubated with 450 μl of buffer containing 50 mM imidazole·HCl, 2 mM EDTA, 0.2% (wt/vol) glycogen, 0.02% (wt/vol) BSA, 0.5 mM dithiothreitol, and 10 mM glu 6-P at pH 7.5. For GS_a, 100-μl aliquots of homogenate were incubated with 450 μl of buffers of the same composition as for GS_{tot}, except glu 6-P concentrations were adjusted to reflect those measured in vivo in white muscle (see Table 2 in RESULTS). The reactions for GS_a and GS_{tot} were initiated by the addition of 8 mM UDP-glucose and incubated at 10°C for 45 min. The reaction was stopped by the addition of 60 μl 0.5 M HCl, and after 10 min on ice, samples were neutralized with 60 μl 0.5 M KOH, centrifuged at 20,000 *g* for 5 min at 4°C, and the supernatant was assayed for free UDP. Free UDP was assayed in a buffer containing (in mM) 20 Tris·HCl, 30 KCl, 4 $MgCl_2$, 0.02% (wt/vol) BSA, 0.4 phospho(*enol*)pyruvate, 0.2 NADH, and 5 U/ml lactate dehydrogenase following the oxidation of NADH after the addition of 3 U/ml pyruvate kinase. The percent mole fraction of GS activation was determined by dividing GS_a by GS_{tot}.

For the determination of muscle glycogen, ~20 mg of lyophilized muscle were digested in 1 ml of 30% KOH at 100°C. Glycogen was isolated as described by Hassid and Abraham (12), and free glucose was determined after digestion with amyloglucosidase (2). IMTG was determined by measuring total glycerol spectrophotometrically after transmethylation with tetraethylammonium hydroxide (20% aqueous solution) (15).

For the extraction of metabolites from white muscle, aliquots of lyophilized muscle (~20 mg) were weighed into borosilicate tubes; 1 ml of 1 M $HClO_4$ was added and homogenized for 20 s at 0°C using a Virtis handishear homogenizer at the highest speed. Homogenates were transferred to 1.5-ml centrifuge tubes, centrifuged for 5 min at 20,000 *g* at 4°C, and the supernatant was neutralized with 3 M K_2CO_3. These extracts were assayed spectrophotometrically for ATP, CrP, Cr, pyruvate, lactate, glu 6-P, fructose 6-P (fru 6-P), glycerol 3-phosphate (gly 3-P), and glycerol (2). Acetyl-CoA, free CoA (CoA-SH), acetyl-, free-, and total carnitine were assayed on neutralized extracts according to radiometric methods (4). Short-chain fatty acyl carnitine was estimated by subtracting acetyl-carnitine and free carnitine from total carnitine. Long-chain fatty acid carnitines were determined on digested white muscle pellets after $HClO_4$ extraction. Briefly, white muscle pellets were suspended in 1 M $HClO_4$, vortexed, and centrifuged at 4,800 *g* for 5 min at 4°C. The washed pellet was then digested in 0.5 ml of 0.5 M KOH for 1.5 h at 50°C, neutralized with 0.25 ml of 1 M HCl, and centrifuged for 10 min at 20,000 *g* at 4°C. The supernatant was then assayed for free carnitine as described above.

Malonyl-CoA was extracted from lyophilized white muscle in 15 times its weight of 0.5 M $HClO_4$ containing 50 μM dithioerythritol and 10 μg/ml isobutyl-CoA as an internal standard. After homogenization for 20 s at 0°C at the highest speed of the Virtis homogenizer, homogenates were centrifuged at 20,000 *g* for 10 min at 4°C, and 200 μl of supernatant were transferred to a borosilicate vial. Extract pH was adjusted to 4 or 5 with 17.5 μl of 4 M NaOH while being vortexed. Supernatants were transferred to autosample vials containing 20 μl of 1 M MOPS (pH 6.8), and final pH of the sample was determined using pH paper: pH was always <5. Autosample vials containing the tissue extracts were immediately placed into a refrigerated autosampler (4°C; WISP 601; Waters, Mississauga, Ontario, Canada). Malonyl-CoA was separated by reverse-phase HPLC using a modified method originally described by Demoz et al. (6). Briefly, 50-μl aliquots of extract were automatically injected onto a Kromasil-octadecyl silane (ODS) column [25 × 0.46 cm; 100 Å ODS, 5 μm; Chromatography (CSC) Sciences, Montreal, Quebec, Canada] fitted with a guard column packed with the same material. An elution gradient, set up by a Waters 660 controller, was used to separate the CoA esters. *Solvent A* was 100 mM sodium phosphate and 75 mM sodium acetate in ultrapure deionized water (pH 4.2), and *solvent B* was the same as *A* except in 30% CH_3CN. The gradient was as follows: 0 min, 90% *A*; 10 min, 60% *A*; 17.6 min, 10% *A*. Baseline condition was established again after 8 min of washing with 90% *A*. The elution was carried out at ambient temperature, and the flow rate was 1.5 ml/min. Absorbance measurements were made at 254 nm on a photodiode array detector (Waters). Resulting peaks were manually identified by comparison of retention times to standards of known composition, and peaks were quantified by comparison with the internal standard.

Plasma lactate and glycerol were analyzed on deproteinized plasma, and triacylglycerol (TAG) was analyzed on plasma using spectrophotometric methods (2). Plasma total NEFA was analyzed using a Wako NEFA C assay kit (WAKO Chemicals, Osaka, Japan). To determine the fatty acid profiles, plasma NEFA samples were methylated using a modification of the methods of Lepage and Roy (18) and separated using gas chromatography. Briefly, 150 μl of frozen plasma, along with 15 μg of heptadecanoic acid (internal standard), were added to 5 ml methanol-acetyl chloride mixture (50:1) in siliconized vials with tight-fitting teflon-lined caps. Vials were maintained at 25–26°C in a Reacti-Therm dry block and placed on a rotating stir plate for 45 min. Methylation was stopped by the addition of 3 ml of 6% K_2CO_3 followed by the addition of 400 μl of hexane. Tubes were shaken and centrifuged at 2,000 *g* for 10 min, and 300 μl of the upper hexane layer, containing the methyl esters, were removed and placed into 2-ml borosilicate vials with tight-fitting teflon-lined caps. Hexane was then evaporated under N_2 gas, and the methyl esters were redissolved in 50 μl of CS_2. Methyl esters dissolved in CS_2 were stored under N_2 at

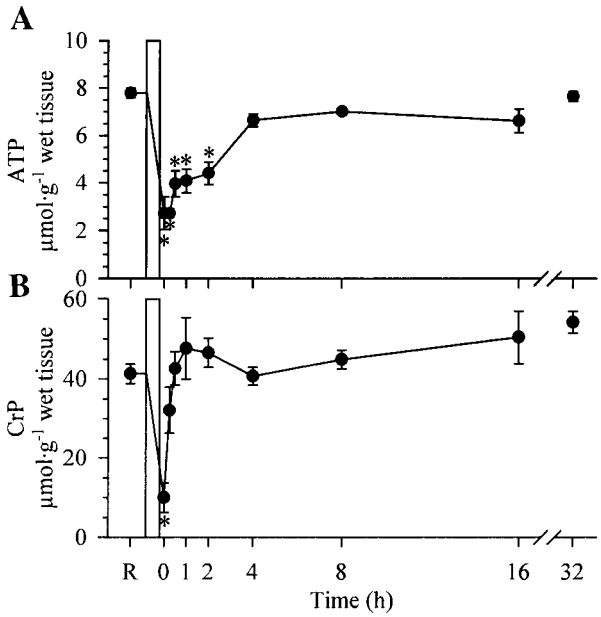

Fig. 1. White muscle ATP (*A*) and creatine phosphate (CrP; *B*) concentrations at rest (R) and during 32-h recovery from exhaustive exercise. Vertical bar represents 5 min of exhaustive exercise. Data are means ± SE; $n = 8$ for each point except at *time 0* where $n = 7$. *Significant differences from rest.

−25°C until analysis. One microliter of CS_2 containing methyl esters was injected into a gas chromatograph (3400 star gas chromatograph; Varian, Mississauga, Canada) fitted with a flame-ionization detector. Fatty acid methyl esters were separated on a DB-1 capillary column (30-m × 0.25-mm ID, 0.25 μm film; Chromatographic Specialities, Brockville, Ontario, Canada) using a temperature gradient from 100 to 300°C increasing at a rate of 5°C/min and H_2 carrier gas. Unknown fatty acid methyl esters were identified by comparing their retention times with those of a known standard, and the fatty acid methyl esters were quantified by comparison with the internal standard.

Calculations

The concentrations of ADP_f and AMP_f were calculated from the near-equilibrium reactions of Cr kinase and adenylate kinase, respectively (9), and constants were calculated from Schulte et al. (35). Intracellular pH (pH_i) values used for the calculation of ADP_f and AMP_f were taken from a similar study from our laboratory (39) that demonstrated similar changes in muscle CrP and lactate immediately after exhaustive exercise and during 4-h recovery. Changes in muscle pH_i after manual exhaustive exercise are very similar across different studies (e.g., Refs. 22, 39).

Data Presentation and Statistical Analysis

All data are presented as means ± SE (n). All muscle metabolite concentrations determined on lyophilized tissues were converted back to wet weights by taking into account a wet:dry ratio of 4:1 (39). Exercise and recovery data were tested against resting data by a one-way ANOVA. Significance was set at $\alpha = 0.05$, and, when obtained, Tukey's honestly significant difference post hoc test was used to identify where significant differences occurred.

RESULTS

In response to manual chasing, trout swam vigorously for the first 1–2 min; thereafter swimming slowed for the remaining 5 min of exercise. Complete exhaustion was characterized by the lack of an avoidance response to >20 s of handling.

Muscle Metabolites

Adenylates and CrP. Muscle [ATP] decreased by ~65% due to the exercise regime and remained lower than resting values for greater than 2 h postexercise (Fig. 1). Exhaustive exercise caused a 75% decrease in [CrP] that was restored to resting concentrations within 15 min (Fig. 1). Decreases in [CrP] were mirrored by stoichiometric increases in [Cr] that remained higher than resting values for >1 h (Table 1). The calculated [ADP_f] and [AMP_f] increased immediately after exhaustive exercise, but they returned to resting values or lower by 15 min postexercise. The ATP/ADP_f ratio followed the same pattern as [ADP_f] and [AMP_f], decreasing immediately after exhaustive exercise and then recovering to resting values by 15 min (Table 1).

GS. The maximal GS_{tot} activity was similar at rest and throughout the recovery period at 15.1 ± 0.3 nmol·g wet tissue^{-1}·min^{-1} ($n = 72$), except at *time 0* where GS_{tot} was significantly lower at 11.8 ± 0.6 nmol·g wet tissue^{-1}·min^{-1} ($n = 7$). The activation state of GS (% of GS in the "a" form) increased from ~40% at rest to almost 90% during the bout of exhaustive exercise and remained transformed for >8 h recovery (Fig. 2).

Table 1. *White muscle creatine concentrations and adenylate status at rest and during 32-h recovery from exhaustive exercise*

	Time									
Measure	Rest	Exhausted	0.25 h	0.5 h	1 h	2 h	4 h	8 h	16 h	32 h
Cr	19.8 ± 2.3	57.6 ± 4.2*	44.8 ± 5.3*	33.1 ± 2.9*	29.9 ± 2.5	17.6 ± 2.5	20.8 ± 3.6	16.3 ± 1.6	18.4 ± 3.9	18.6 ± 2.3
pH_i†	7.20	6.80	6.75	6.79	6.82	7.05	7.05	7.2‡	7.2‡	7.2‡
ADP_f	7.9 ± 1.2	21.1 ± 5.6*	4.0 ± 1.4	2.5 ± 0.4	2.5 ± 0.5	2.8 ± 0.7	5.2 ± 1.2	5.2 ± 0.6	5.6 ± 1.4	5.5 ± 0.8
AMP_f	0.23 ± 0.06	9.31 ± 4.61*	0.30 ± 0.22	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.15 ± 0.08	0.10 ± 0.02	0.21 ± 0.13	0.11 ± 0.03
ATP/ ADP_f	1,201 ± 248	237 ± 100	1,165 ± 268	1,720 ± 208	1,942 ± 259	2,554 ± 774	1,710 ± 323	1,469 ± 161	2,051 ± 716	1,960 ± 330

Data are means ± SE ($n = 8$ for each, except exhausted where $n = 7$). Cr, free creatine; pH_i, intracellular pH; ADP_f, free ADP; AMP_f, free AMP. Cr measurements are in μmol/g wet tissue; ADP_f and AMP_f are in nmol/g wet tissue. *Significant difference from rest. †Data are taken from a similar study by Wang et al. (39). ‡These time points were not measured by Wang et al. (39); thus they are assumed to be equal resting values.

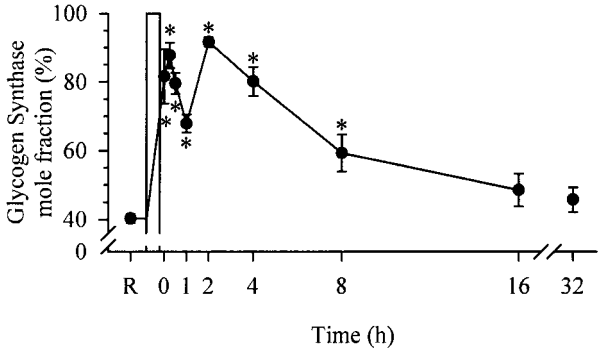

Fig. 2. White muscle glycogen synthase activation state at rest and during 32-h recovery from exhaustive exercise. *Significant differences from rest. See Fig. 1 legend for other details.

Glycogen, glycolytic intermediates, and IMTG. White muscle [glycogen] decreased by 85% during exhaustive exercise, and this decrease was mirrored by stoichiometric (1:2) increases in muscle [lactate] (Fig. 3). Muscle glycogen took between 8 and 16 h to return to values that were not statistically different from resting concentrations, although they were still nonsignificantly lower at 32 h. [Lactate] recovered to resting values within 4 h.

Exhaustive exercise in trout caused large increases in the glycolytic intermediates, glu 6-P and fru 6-P, which remained elevated compared with resting values for 2 h and 15 min, respectively, thereafter returning to resting values (Table 2). Muscle [gly 3-P] increased by 85% due to the exercise and remained elevated for >2 h; these changes in [gly 3-P] were matched by nonsignificant decreases in glycerol (Table 2). White muscle [pyruvate] increased due to exhaustive exercise and returned to resting values by 30 min (Table 2). [IMTG] did not change during exercise, but it decreased to a value that was significantly lower than resting concentrations at 1-h recovery (Table 2).

PDH. Total PDH activity was 167.6 ± 8.9 nmol·g wet tissue^{-1}·min^{-1} (n = 7). Exhaustive exercise caused a 50-fold increase in PDH_a in trout white muscle and fully transformed PDH into the active state (Fig. 4). After the activation of PDH at exhaustion, there was a dramatic decrease in PDH_a transformation and activity, back to resting values, within the first 15 min postexercise.

Acetyl group accumulation and carnitine. Muscle [CoA-SH] did not change significantly after exercise and throughout the postexercise period (Fig. 5) and constituted ~90% of the total CoA pool within the muscle. Muscle [acetyl-CoA] increased by 1.6-fold at 15-min recovery and remained elevated compared with resting values for >2 h (Fig. 5).

[Acetyl-carnitine] increased by fivefold during exhaustive exercise and continued to increase by another 1.3 times the resting value during the first 15 min of the postexercise period (Fig. 6). Acetyl-carnitine concentrations remained elevated for up to 4 h and then returned to resting values.

Total and short-chain fatty acyl carnitine concentrations remained constant throughout the exercise regime and during recovery (Fig. 6). Muscle [free carnitine] was not affected by the exercise regime, but it decreased by 35% during the first 15 min of the postexercise period. Free carnitine concentrations remained low for 1-h recovery and then returned to resting values. Long-chain fatty acyl carnitine concentrations increased 1.4-fold over the first 15 min (Fig. 6) and then returned to resting values.

Malonyl-CoA. Muscle [malonyl-CoA] did not change due to the exhaustive exercise, but it increased gradually to approximately twice the resting levels at 2 and 4 h (Fig. 7). Subsequently, [malonyl-CoA] returned to resting values by 8 h.

Plasma Metabolites

Plasma [lactate] increased about fivefold due to exhaustive exercise, and the level reached 10-fold during the first 1 h of the postexercise period (Table 3). Plasma lactate concentrations returned to resting values by 8 h. Plasma [glycerol] increased due to the exercise regime, but it returned to resting concentrations within 15 min (Table 3). Plasma TAG remained constant compared with resting concentrations throughout the exercise regime and during the postexercise period (Table 3).

Total [NEFA], measured by enzymatic analysis, was not affected by exhaustive exercise, but it decreased within the first 15 min postexercise, remained depressed for 1 h, and then returned to resting values (Table 4). Analysis of plasma NEFA by gas chromatography (GC) (Table 4) yielded changes in total [NEFA] that followed a similar trend to that observed by enzymatic analysis, but the concentrations were four- to sixfold higher by GC. At rest, palmitic acid (16:0) accounted for ~24% of the total [NEFA], whereas unsat-

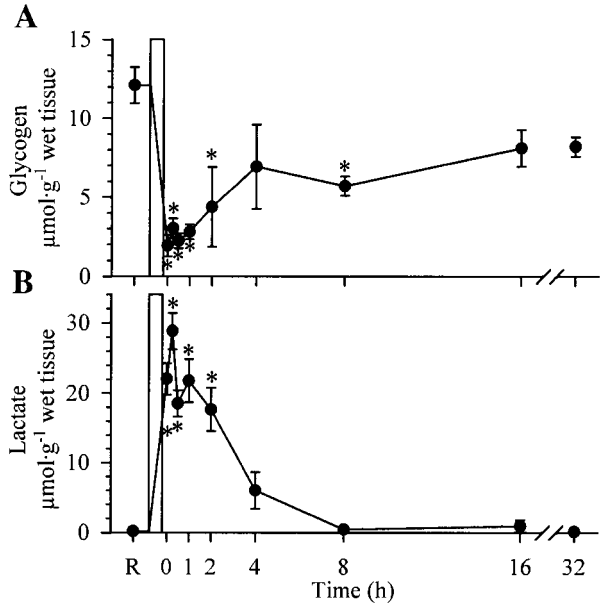

Fig. 3. White muscle glycogen (*A*) and lactate (*B*) concentrations at rest and during 32-h recovery from exhaustive exercise. *Significant differences from rest. See Fig. 1 legend for other details.

Table 2. *White muscle metabolite content at rest and during 32-h recovery from exhaustive exercise*

Measure	Time									
	Rest	Exhausted	0.25 h	0.5 h	1 h	2 h	4 h	8 h	16 h	32 h
Glu 6-P	0.02 ± 0.01	0.51 ± 0.24*	0.57 ± 0.16*	0.23 ± 0.09	0.11 ± 0.04*	0.49 ± 0.13*	0.28 ± 0.10	0.10 ± 0.05	0.05 ± 0.03	0.12 ± 0.09
Fru 6-P	<0.01	0.19 ± 0.12*	0.24 ± 0.10*	0.05 ± 0.04	0.03 ± 0.01	0.22 ± 0.10	0.09 ± 0.07	0.07 ± 0.03	0.03 ± 0.02	<0.01
Gly 3-P	0.13 ± 0.02	0.82 ± 0.09*	0.94 ± 0.05*	0.81 ± 0.66*	0.85 ± 0.15*	0.84 ± 0.12*	0.39 ± 0.13	0.07 ± 0.02	0.21 ± 0.11	0.08 ± 0.02
Pyruvate	0.05 ± 0.02	0.43 ± 0.07*	0.30 ± 0.04*	0.13 ± 0.03	0.25 ± 0.05	0.18 ± 0.04	0.04 ± 0.01	0.03 ± 0.01	0.14 ± 0.12	0.03 ± 0.01
Glycerol	1.42 ± 0.21	0.51 ± 0.21	0.61 ± 0.31	0.54 ± 0.69	0.71 ± 0.27	0.65 ± 0.18	1.00 ± 0.17	1.34 ± 0.18	3.51 ± 1.93	2.04 ± 0.51
IMTG	19.2 ± 1.1	16.5 ± 0.9	15.0 ± 2.6	18.0 ± 2.5	12 ± 1.2*	15.7 ± 1.2	16.9 ± 2.4	14.6 ± 1.3	15.0 ± 0.8	18.4 ± 1.2

Data are means ± SE in μmol/g wet tissue (n = 8 for each, except exhausted where n = 7). Glu 6-P, glucose 6-phosphate; Fru 6-P, fructose 6-phosphate; Gly 3-P, glycerol 3-phosphate; IMTG, intramuscular triacylglycerol. *Significant difference from rest.

urated 18, 20, and 22 carbon NEFA comprised 20, 15, and 26%, respectively, of the total [NEFA]. The decreases in total [NEFA] observed by enzymatic and GC analysis during recovery were made up of significant decreases in palmitoleic acid (16:1) and unsaturated 18 carbon fatty acids, plus nonsignificant decreases in many of the others (Table 4).

DISCUSSION

The present study examined the effects of 5-min exhaustive exercise on white muscle metabolism in trout and monitored the recovery of muscle metabolites for up to 32 h. On the basis of substrate depletion and enzyme activities, we have demonstrated that CrP hydrolysis, glycolysis, and oxidative phosphorylation of carbohydrate fuels are responsible for ATP production during exhaustive exercise in trout white muscle. Immediately postexercise, there is a dramatic shift in substrate utilization from phosphagen and carbohydrate during exercise to lipid during recovery. Furthermore, this substrate shift from carbohydrate to lipid oxidation during recovery occurs in the presence of a high concentration of carbohydrate substrate (lactate). Our data argue against lactate oxidation during recovery [classical O_2 debt hypothesis (13)] and add further evidence to the growing idea that recovery metabolism is supported by lipid oxidation (15, 24, 39).

ATP Production During Exercise

It has been well established in the literature [reviewed by Kieffer (16)] that the high-ATP turnover rates observed during exhaustive exercise in fish are sustained primarily by substrate-level phosphorylation (CrP) and "anaerobic" glycolysis yielding lactate production. The large changes in white muscle ATP and CrP concentrations (Fig. 1) and the large decreases in white muscle glycogen and accumulation of lactate (Fig. 3) observed immediately after exercise in the present study further add credence to the notion of substrate phosphorylation and glycolytic supply of ATP to support exercise. However, the maximal transformation of PDH to PDH_a observed at the end of exercise (Fig. 4) also clearly implicates oxidative phosphorylation of carbohydrate-based fuels as an additional pathway supplying ATP for muscle contraction during exercise.

PDH is the rate-limiting enzyme that regulates the entry of glycolytically derived pyruvate into the TCA cycle and oxidative metabolism (42); therefore, PDH controls the oxidative utilization of carbohydrate fuels. PDH activity is regulated by both product inhibition (NADH and acetyl-CoA) and by reversible covalent modification (phosphorylation and dephosphorylation). The transformation of PDH between active PDH_a and inactive PDH_b is regulated by the relative activities of PDH kinase, which phosphorylates and thus deactivates PDH, and the activity of PDH phosphatase, which dephosphorylates and thus activates PDH (42). PDH kinase is stimulated by elevated ratios of acetyl-CoA to CoA-SH, ATP to ADP_f, and NADH to NAD^+ and

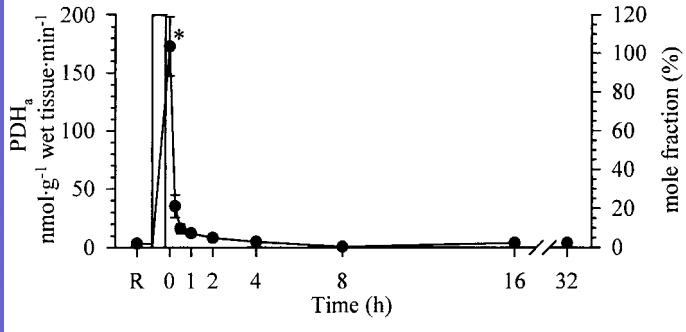

Fig. 4. White muscle pyruvate dehydrogenase (PDH) activity and active (PDH_a) mole fraction at rest and during 32-h recovery from exhaustive exercise. *Significant differences from rest. See Fig. 1 legend for other details.

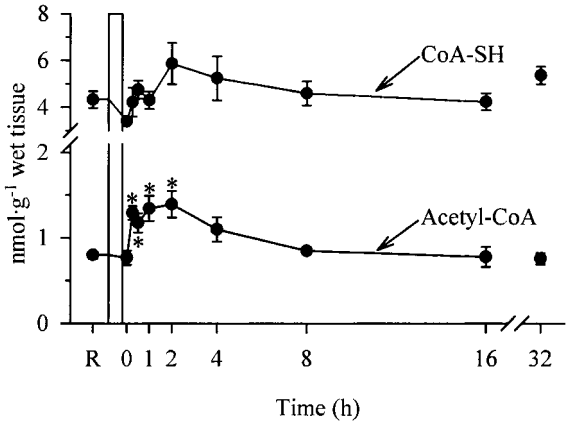

Fig. 5. White muscle acetyl-CoA and free CoA (CoA-SH) concentrations at rest and during 32-h recovery from exhaustive exercise. *Significant differences from rest. See Fig. 1 legend for other details.

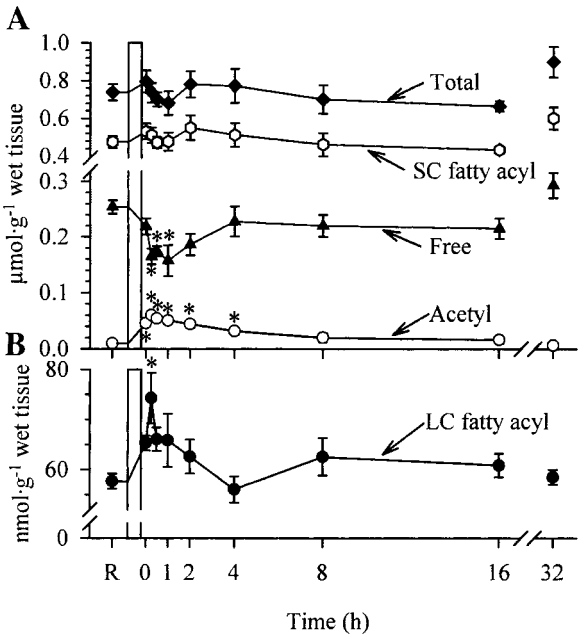

Fig. 6. White muscle acetyl-carnitine (Acetyl), free carnitine (Free), short-chain (SC) fatty acyl carnitine, and total carnitine (Total) concentrations (*A*) and long-chain (LC) fatty acyl carnitine (*B*) at rest and during 32-h recovery from exhaustive exercise. *Significant differences from rest. See Fig. 1 legend for other details.

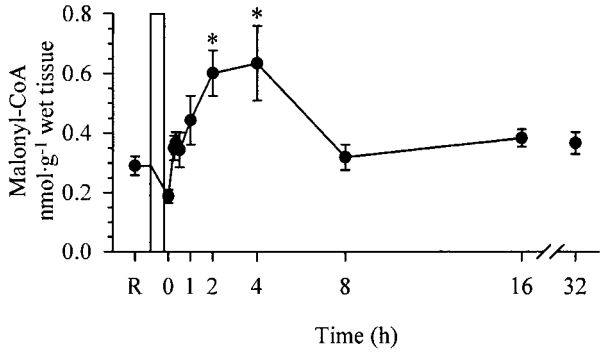

Fig. 7. White muscle malonyl-CoA concentrations at rest and during 32-h recovery from exhaustive exercise. *Significant differences from rest. See Fig. 1 legend for other details.

is inhibited by elevated pyruvate concentrations (38). PDH phosphatase is stimulated by elevated Ca^{2+} concentration (42).

At the onset of exercise, Ca^{2+} release from the sarcoplasmic reticulum probably acted as the initial cue to activate PDH in the trout white muscle. Subsequently, an accumulation of pyruvate (Table 2) from high glycolytic flux and a decrease in ATP/ADP_f (Table 1) likely acted to maximally stimulate PDH during exhaustive exercise. There was no change in acetyl-CoA/CoA-SH ratio at exhaustion (Fig. 5), and in a very similar study in our laboratory, the redox potential (NADH/NAD^+) of white muscle cytoplasm did not change during a comparable exercise regime (39). The constant acetyl-CoA/CoA-SH ratio and redox state at exhaustion indicate that there were no strong inhibitory forces acting on PDH kinase and thus PDH transformation. If PDH was fully transformed into PDH_a for the 5 min of exhaustive exercise, oxidative phosphorylation of pyruvate could contribute up to 13 μmol ATP/g wet tissue in addition to the ~80 μmol ATP/g wet tissue supplied by ATP, CrP, and glycogen.

The activation of PDH at exhaustion also challenges the dogma that lactate accumulation during high-intensity exercise is due to "anaerobiosis" (8). In fact, accumulating evidence in human muscle suggests that lactate accumulation is due to a mismatched balance between the activities of glycogen phosphorylase, which sets the upper limit for glycogen entry into glycolysis, and the activity of PDH (28). The same, yet accentuated, explanation of lactate production can be applied to trout white muscle. White muscle of fish has a very low mitochondrial content (<2% volume density) (14) and very likely has a low copy number of PDH. Maximal activation of PDH in trout white muscle is not sufficient to accommodate the rate of pyruvate production by glycolysis during exhaustive exercise, thus resulting in lactate accumulation. Clearly, more research is needed to clarify the role of PDH in ATP and lactate production in trout white muscle during exhaustive exercise.

Recovery Metabolism in White Muscle

The pattern of white muscle metabolite recovery observed in the present study is in general agreement with many previously published studies (35, 39, 45). In general, trout confined in acrylic black boxes during recovery show a rapid recovery of CrP (within 15 min), slower recovery of ATP and lactate (2–4 h), and very slow recovery of glycogen (>8 h). The rapid recovery of CrP indicates an immediate activation of ATP-generating pathways, which results in the phosphorylation of accumulated free Cr. The reason for the slower recovery of ATP seems elusive, but several possibilities exist. First, decreases in white muscle ATP concentrations are mirrored by a stoichiometric increase in IMP

Table 3. *Plasma metabolite content at rest and during 32-h recovery from exhaustive exercise*

	Time									
Measure	Rest	Exhausted	0.25 h	0.5 h	1 h	2 h	4 h	8 h	16 h	32 h
Lactate	0.6 ± 0.2	3.1 ± 0.3*	4.4 ± 0.5*	3.9 ± 0.6*	6.6 ± 0.6*	5.6 ± 0.7*	5.5 ± 1.1*	1.4 ± 0.4	0.5 ± 0.2	0.43 ± 0.12
Glycerol	0.03 ± 0.03	0.16 ± 0.02*	0.06 ± 0.03	0.11 ± 0.03	0.09 ± 0.01	0.06 ± 0.01	0.05 ± 0.02	0.06 ± 0.02	0.11 ± 0.02	0.04 ± 0.02
TAG	18.1 ± 3.0	22.9 ± 4	15.0 ± 2.9	21.7 ± 4.5	18.8 ± 3.4	17.8 ± 3.4	16.4 ± 4.0	13.8 ± 3.1	18.2 ± 2.0	18.6 ± 4.4

Data are means ± SE in μmol/ml; n = 8 for each, except at rest where n = 7. *Significant difference from rest.

Table 4. *Plasma NEFA content at rest and during 32-h recovery from exhaustive exercise*

	Time									
Measure	Rest	Exhausted	0.25 h	0.5 h	1 h	2 h	4 h	8 h	16 h	32 h
14:0	39 ± 9	39 ± 13	17 ± 10	25 ± 9	21 ± 13	43 ± 1	56 ± 25	17 ± 13	48 ± 20	4 ± 4
16:0	359 ± 64	306 ± 39	241 ± 40	246 ± 21	256 ± 21	317 ± 40	276 ± 40	229 ± 46	277 ± 20	254 ± 45
16:1	67 ± 16	41 ± 14	14 ± 7*	36 ± 11	39 ± 4	57 ± 1	46 ± 11	19 ± 10	49 ± 19	28 ± 6
18:0	100 ± 14	120 ± 37	81 ± 17	115 ± 34	96 ± 12	118 ± 32	93 ± 21	66 ± 27	81 ± 26	79 ± 30
18 Unsat	304 ± 76	168 ± 23	91 ± 36*	169 ± 32	149 ± 10*	187 ± 40	194 ± 32	152 ± 18	178 ± 17	197 ± 64
20:0	8 ± 5	28 ± 15	12 ± 8	18 ± 12	11 ± 6	19 ± 19	9 ± 7	N/D	N/D	4 ± 4
20 Unsat	185 ± 24	178 ± 59	129 ± 26	141 ± 33	123 ± 16	258 ± 96	145 ± 25	72 ± 25	112 ± 24	121 ± 5
22:0	N/D	2 ± 2	N/D	4 ± 4	N/D	19 ± 19	N/D	N/D	N/D	N/D
22 Unsat	406 ± 66	392 ± 110	210 ± 79	326 ± 50	303 ± 37	510 ± 214	343 ± 60	260 ± 108	298 ± 52	297 ± 36
24:0	11 ± 5	17 ± 8	6 ± 6	6 ± 4	13 ± 7	37 ± 2	13 ± 7	9 ± 5	11 ± 142	19 ± 8
Total GC	1,479 ± 221	1,292 ± 296	802 ± 187	1,087 ± 152	1,010 ± 84	1,565 ± 458	1,174 ± 189	860 ± 145	1,053 ± 142	1,000 ± 140
Total Enz	320 ± 30	260 ± 50	140 ± 40*	130 ± 20*	110 ± 20*	220 ± 70	240 ± 90	200 ± 40	200 ± 30	260 ± 50

Data are means ± SE in nmol/ml. From left, n = 8, 7, 5, 7, 8, 2, 8, 6, 6, and 4. All plasma nonesterified fatty acid (NEFA) data were determined by gas chromatography (GC) except Total Enz, which was determined by enzymatic analysis (see METHODS). N/D, nondetectable; Unsat, unsaturated. *Significant difference from rest.

(35, 39). For the resynthesis of ATP from IMP, there must be activation of the IMP-AMP conversion arm of the purine-nucleotide cycle that requires the input of nitrogen and guanosine 5′-triphosphate (23). In addition, utilization of ATP during recovery of CrP and glycogen may delay the recovery of endogenous ATP.

Glycogen synthesis is regulated, in part, by the activation of the rate-limiting enzyme GS, which catalyzes the addition of glucose from UDP-glucose to glycogen (36). The transformation of GS between active GS_a and inactive GS_b is regulated by the relative activities of a GS kinase and a GS phosphatase. Phosphorylation by a kinase inactivates GS, and dephosphorylation by a phosphatase activates GS. In human muscle, GS phosphatase is primarily stimulated by an increase in glu 6-P and inhibited by high ATP and glycogen concentrations (27).

The large decreases in white muscle glycogen observed at the end of exercise (Fig. 3) and the accumulation of glu 6-P during the first 4 h of recovery were probably the main allosteric regulators for the stimulation of GS phosphatase activity and therefore activation of GS. As a result, within 30 min postexercise, ~90% of the GS was transformed into GS_a (relative to a resting level of ~40%) and remained elevated compared with resting values, for >8 h postexercise (Fig. 2). During this period, there was a 53% increase in white muscle glycogen and a return to resting values for white muscle lactate (Fig. 3). However, our data suggest further that the maximal activity of GS_a (14.4 nmol·g wet tissue^{-1}·min^{-1}) may limit the rate of recovery in trout white muscle. At the maximal GS activities measured during recovery in trout muscle, it would take >12 h for glycogen to be resynthesized, similar to the pattern of glycogen recovery observed (Fig. 3).

The slow recovery observed in the present study is probably due to the confinement of the trout immediately after exercise. Milligan et al. (21) have shown that rainbow trout swum at 0.9 body lengths/s during recovery restore white muscle glycogen and lactate concentrations to resting values within 2 h postexercise vs. >4 h needed in the present study and others (7, 20, 39). There is mounting evidence that cortisol release during the postexercise period in confined trout may be responsible for prolonged recovery times. If cortisol levels are kept low, either by allowing the fish to swim slowly during the postexercise period (21) or by pharmacological blockade of cortisol synthesis or release (10), trout white muscle carbohydrate and acid-base status recovers at an accelerated rate. However, the precise mechanism behind the action of cortisol during recovery remains elusive. Given the apparent limiting activity of GS in trout white muscle demonstrated in the present study, there might be a yet uninvestigated link between cortisol release and GS activity.

At the onset of recovery, the main purpose of ATP production shifts from providing energy for actin-myosin cycling during the exercise to providing energy for the resynthesis of metabolites. During recovery, ATP must be rapidly generated to resynthesize CrP (~31 μmol ATP/g wet tissue needed within 15-min recovery) followed by a slower synthesis of ATP and glycogen (>2 and >8 h, respectively). From the present study, ATP synthesis from IMP (39) would require ~10 μmol ATP/g wet tissue, and glycogen synthesis from lactate would require between 19 and 30 μmol ATP/g wet tissue. The total ATP required to fuel recovery in the present study was calculated to be between 60 and 70 μmol ATP/g wet tissue.

Fate of Lactate During Recovery

Over the past several decades, numerous studies have aimed to determine the fate of accumulated lactate during recovery. Although it is generally well accepted that lactate is retained in trout white muscle during recovery for in situ glycogen synthesis (35), no study has been able to conclusively rule out oxidation as a minor end-point for the accumulated lactate. In fact, the complete oxidation of only 4 to 6 μmol lactate/g wet tissue could supply enough ATP (60 to 70 μmol/g wet tissue) to support the complete recovery of

white muscle CrP, ATP, and glycogen and represents only 15 to 20% of the accumulated lactate. Lactate disappearance in trout white muscle is faster than glycogen resynthesis (Fig. 3), and this discrepancy has been taken as evidence to support the contention that a portion of lactate is oxidized by muscle during recovery to supply the ATP for glycogen synthesis (13). However, the discrepancy between lactate and glycogen recovery can, in part, be explained by lactate appearance in the plasma (see Table 3) and slow oxidation or carboxylation of pyruvate by pyruvate carboxylase in other tissues such as the liver (20).

Lactate oxidation during recovery would require the sustained transformation of PDH into PDH_a as well as a maintained catalytic rate. Within 15 min postexercise, PDH is nearly fully transformed into its inactive form (PDH_b). This rapid transformation into PDH_b is probably due to increases in acetyl-CoA/CoA-SH (see Fig. 5) and ATP/ADP_f (Table 1) ratios acting to increase PDH kinase activity, resulting in greater PDH phosphorylation and inactivation of PDH. On the sole basis of the transformation state of PDH, ~6 μmol·g wet $tissue^{-1}\cdot min^{-1}$ of pyruvate could be decarboxylated by PDH during the first 4 h of recovery, allowing enough lactate oxidation to provide ATP for recovery. Under most exercise conditions (e.g., Refs. 28, 29, 42), the catalytic rate of PDH is equal to the transformation state. However, in the present study, it seems likely that the catalytic rate of PDH would be far lower than indicated by transformation because of significant product inhibition (acetyl-CoA from lipid oxidation; Fig. 5) and the low substrate concentration (pyruvate) observed during recovery (Table 2). Therefore, the rate of lactate oxidation in vivo would be far less than required to supply ATP for recovery. However, these regulatory effects of low pyruvate and elevated acetyl-CoA on PDH have only been documented in mammalian muscle (e.g., Ref. 42) and await confirmation in fish muscle.

Lipid Oxidation During Recovery

The present research contributes significantly to the proposed idea that the majority of ATP needed for recovery is generated by an activation of β-oxidation using lipid as a substrate (24, 39). Fish maintain large labile lipid stores both within their muscle, as IMTG, and in adipose tissue, also as TAG (25), both of which can release NEFA for oxidation. For long-chain NEFA to be oxidized by β-oxidation, they must first be bound to carnitine by CPT 1 for transport into the mitochondria (38). During the first hour postexercise, there was a rapid binding of long-chain NEFA to carnitine, resulting in a decrease in muscle free carnitine (Fig. 6). The subsequent action of β-oxidation yielded acetyl-CoA, which contributed to the significant elevation in white muscle acetyl-CoA concentrations for >2 h (Fig. 5). However, to sustain flux through β-oxidation during the initial portion of recovery, intramitochondrial acetyl-CoA concentrations were kept relatively low through the formation of acetylcarnitine (Fig. 6). These results are in general agreement with those of Wang et al. (39), except the decreases in free carnitine in that study were due to an increase in binding of short-chain NEFA to carnitine rather than long-chain NEFA alone as observed in the present study. This is a subtle difference, and the preferential binding of long-chain NEFA to carnitine as observed in the present study makes sense in that short-chain fatty acids can pass freely through mitochondrial membranes and do not necessarily require carnitine for mitochondrial transport (38).

The rate-limiting step in muscle lipid oxidation is the CPT 1-catalyzed binding of NEFA, especially long-chain NEFA, to carnitine for the subsequent transfer of fatty acyl carnitines into the mitochondria. Recent evidence implicates CPT 1 as the main point of regulation of lipid oxidation through the interactions with malonyl-CoA. Malonyl-CoA is an intermediate in the de novo synthesis of fatty acids and has been demonstrated in rat muscle to negatively regulate CPT 1 and thus lipid metabolism (33) and to further contribute to the regulation of the glucose-fatty acid cycle (30). However, malonyl-CoA does not equally regulate CPT 1 in all organisms. In human muscle, malonyl-CoA participates in the regulation of fuel selection at rest, but it does not appear to be important for fuel selection during exercise (26). In the trout white muscle, malonyl-CoA concentrations were low at rest and increased between 2 and 4 h postexercise (Fig. 7). It is paradoxical that there were increases in malonyl-CoA during a period characterized by an increase in lipid oxidation. Two possibilities exist to explain these increases in malonyl-CoA during recovery. First, malonyl-CoA may not be an important modulator of CPT 1 in trout white muscle during recovery. Elevated malonyl-CoA may represent an increased elongation of short-chain fatty acids in an attempt to maintain elevated concentrations of long-chain fatty acids for mitochondrial oxidation (32). Second, the delayed increase in malonyl-CoA may indicate that the majority of the costs of recovery are met within the 2-h recovery and that subsequently lipid oxidation is allosterically inhibited by increasing malonyl-CoA. Further research is needed to clarify the role of malonyl-CoA in trout muscle during recovery.

Further support for lipid oxidation during the early stages of recovery is provided by the general decreases in IMTG that were significant at 1 h postexercise (38% reduction; Table 2). IMTG hydrolysis yields three NEFA for β-oxidation and one for glycerol. If the decrease in IMTG represents complete oxidation of TAG containing palmitic acid (16:0), it could supply 1.8 mmol ATP/g wet tissue, 21-fold more ATP than required for recovery metabolism. Thus it is likely that in addition to increased oxidation of fatty acids during recovery, there is probably an increase in TAG-NEFA cycling between the muscle and other tissues, both contributing to the EPOC observed in juvenile rainbow trout (34). Utilization of endogenous lipid during recovery is supported further by the results of Milligan and Girard (20) who showed large, highly variable decreases in trout white muscle total lipid concentrations after exhaustive exercise that persisted through 6-h recovery. The significant accumulation of gly

3-P and generally depressed white muscle glycerol concentration (Table 2) suggest that glycerol liberated by TAG breakdown enters glycolysis and may contribute to glycogen resynthesis.

NEFA released into the bloodstream from adipose tissue may also be used during recovery for ATP synthesis. Plasma total NEFA concentration, determined using enzymatic analysis, decreased during the first 15 min and remained depressed for up to 1 h postexercise. These decreases in plasma NEFA concentration were primarily due to significant decreases in palmitoleic (16:1) and unsaturated 18 carbon fatty acids, although many of the others also tended to decrease (Table 4). Furthermore, these decreases in plasma NEFA were not associated with a change in plasma TAG (Table 3), indicating that esterification of NEFA into TAG does not occur in the extracellular fluid during recovery. The decrease in plasma NEFA observed during recovery was probably due to the combined effects of increased NEFA uptake from the plasma in addition to decreased NEFA release from adipose tissue.

The mobilization of NEFA from adipose tissue is determined by the relative activities of two opposing nonequilibrium reactions: lipolysis of stored TAG and reesterification of NEFA into TAG. This substrate cycling between NEFA and TAG constitutes a means of rapidly adjusting substrate flux without extreme activation or inactivation of any one reaction (40). Stimulation of hormone-sensitive lipase (HSL), by the characteristic mobilization of catecholamines into trout plasma after exhaustive exercise (e.g., Ref. 21), would be expected to shift NEFA-TAG cycling toward NEFA production, thus resulting in a release of NEFA into circulation (40). However, high plasma lactate concentrations, such as those observed during the postexercise period in trout (Table 3), have been demonstrated to inhibit HSL in human adipose tissue (3, 40). Inhibition of HSL would result in reduced NEFA release from adipose tissue. Increased muscle uptake of NEFA coupled with decreased NEFA release from adipose tissue may explain the reduction in plasma NEFA concentrations.

The distribution of NEFA within plasma, as determined by GC, is similar to distributions observed by other researchers who employed the same methylation technique (11); however, there are unresolved differences in plasma NEFA concentrations when analyzed using enzymatic analysis vs. GC. Trout, similar to many teleost fish, are unique in that they have high concentrations of high-density lipoprotein in their plasma [HDL; 15 mg/ml (5) vs. ~2 mg/ml in mammals (19)], and in addition to albumin, they use HDL as a fatty acid transport protein. The chemical methylation process involved in GC may liberate NEFA from HDL and therefore yield higher plasma [NEFA], whereas HDL-bound NEFA may be undetectable by the enzymatic method. If this analytic possibility is true, it suggests that HDL may be the major fatty acid binding protein in trout plasma (similar to carp) (17), accounting for ~80% of the total NEFA carrying capacity of the plasma. This analytic possibility deserves further attention.

The present study provides evidence that during recovery, the majority of the ATP needed to synthesize CrP, ATP, and glycogen is provided for by lipid oxidation. NEFA, especially long-chain NEFA, from both exogenous and endogenous sources are taken up by the mitochondria of white muscle via a carnitine-dependent transport mechanism (CPT 1) and oxidized by β-oxidation yielding acetyl-CoA. Acetyl groups are accumulated in the muscle postexercise and support ATP production through increased TCA cycle flux and oxidative phosphorylation. Increases in malonyl-CoA during recovery do not appear to limit fatty acid oxidation, but they may represent elongation of fatty acids for mitochondrial oxidation. Lactate is saved from an oxidative fate during recovery by a rapid transformation of PDH into its inactive form, in addition to product inhibition, thus providing further evidence that glycogen synthesis is likely the major fate of lactate during recovery.

Perspectives

The notion that lipid oxidation provides ATP to support recovery from exercise has been in the literature for about a decade (e.g., Ref. 24), but this hypothesis has remained relatively untested in most organisms. Recently, Keins and Richter (15) have demonstrated lipid utilization during recovery from high-intensity exercise in the human. This represents a major shift from the classical O_2 debt hypothesis where lactate oxidation was thought to fuel recovery metabolism. By measuring the activities of flux-generating enzymes (GS and PDH), their allosteric modulators, and changes in substrate concentrations, we are able to provide insight into the mechanisms that regulate lipid vs. carbohydrate oxidation. Our data strongly suggest that lipid oxidation prevails during recovery. Comprehensive studies that examine the regulation of substrate selection such as presented herein will prove to be a powerful tool for elucidating how substrate selection occurs during high-intensity exercise and during graded exercise intensities. The elusive role of malonyl-CoA in regulating CPT 1 also deserves further attention.

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