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WHITE MUSCLE INTRACELLULAR ACID–BASE AND LACTATE STATUS FOLLOWING EXHAUSTIVE EXERCISE: A COMPARISON BETWEEN FRESHWATER- AND SEAWATER-ADAPTED RAINBOW TROUT

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Summary

The intracellular acid–base status of white muscle of freshwater (FW) and seawater (SW) -adapted rainbow trout was examined before and after exhaustive exercise.

Exhaustive exercise resulted in a pronounced intracellular acidosis with a greater pH drop in SW (0.82 pH units) than in FW (0.66 pH units) trout; this was accompanied by a marked rise in intracellular lactate levels, with more pronounced increases occurring in SW (54.4 mmol l^{-1}) than in FW (45.7 mmol l^{-1}) trout. Despite the more severe acidosis, recovery was faster in the SW animals, as indicated by a more rapid clearance of metabolic H^+ and lactate loads.

Compartmental analysis of the distribution of metabolic H^+ and lactate loads showed that the more rapid recovery of pH in SW trout could be due to (1) their greater facility for excreting H^+ equivalents to the environmental water [e.g. 15.5 % (SW) *vs* 5.0 % (FW) of the initial H^+ load was stored in external water at 250 min post-exercise] and, to a greater extent, (2) the more rapid removal of H^+, facilitated *via* lactate metabolism *in situ* (white muscle) and/or the Cori cycle (e.g. heart, liver). The slower pH recovery in FW trout may also be due in part to greater production of an 'unmeasured acid' [maximum approx. 8.5 mmol kg^{-1} fish (FW) *vs* approx. 6 mmol kg^{-1} fish (SW) at 70–130 min post-exercise] during the recovery period. Furthermore, the analysis revealed that H^+-consuming metabolism is quantitatively the most important mechanism for the correction of an endogenously originating acidosis, and that extracellular pH normalization gains priority over intracellular pH regulation during recovery of acid–base status following exhaustive exercise.

Introduction

Exhaustive exercise in fish generally results in the production of large quantities of H^+ and lactate as the main end-products of anaerobiosis in the working white

Key words: rainbow trout, exercise, acid–base regulation, proton, lactate, muscle, intracellular pH, *Oncorhynchus mykiss*.

muscle. Efflux of anaerobically produced H^+ from the white muscle intracellular space (ICS) often leads to a marked acid–base disturbance in the extracellular space (ECS) and a net H^+ equivalent excretion to the environmental water. Such extracellular acidosis and H^+ excretion have been well characterized in a large number of studies (see reviews by Wood and Perry, 1985; Heisler, 1984, 1986*b*). However, far less is known about the changes in acid–base status within the ICS of white muscle, which represents the largest intracellular space in the fish body, and in which anaerobic glycolysis takes place. Intracellular changes in acid–base status following exhaustive exercise in fish have been indirectly estimated either by model calculations (Heisler, 1986*b*) or by the DMO distribution technique (Milligan and Wood, 1986*a*,*b*). Although the latter technique is suitable for measurements in the steady state, rapid changes are difficult to resolve owing to limitations regarding redistribution of the weak acid, as well as perfusion limitations.

Recently, Pörtner *et al.* (1990) developed a technique for determining intracellular pH by direct measurement of tissue homogenates under metabolic control. The procedure has been shown to be reliable for determining intracellular pH in various tissues from a wide range of animals. Moreover, this technique makes it possible to record fast intracellular transients, since the only delays of the homogenate method are those associated with rapid excision and freezing of the muscle. In the present study, we have applied this technique to examine the acid–base status of white muscle of trout following exhaustive exercise.

Our previous studies show that seawater (SW) -adapted trout are more proficient than freshwater (FW) -adapted trout at correcting extracellular acid–base disturbances. They do this by excreting greater numbers of H^+ equivalents to the environmental water following either exhaustive exercise (Tang *et al.* 1989) or acid infusion (Tang and Boutilier, 1988*a*; Tang *et al.* 1988). As before, the present investigation used FW and SW trout of the same genetic stock. The intracellular data of the present study together with the data on the extracellular compartment and H^+ equivalent efflux to the environmental water (Tang and Boutilier, 1988*b*; Tang *et al.* 1989) were used to analyze the compartmental changes in the pools of H^+ and lactate.

Materials and methods

Experimental animals

Freshwater rainbow trout [*Oncorhynchus mykiss* (Walbaum); formerly *Salmo gairdneri* Richardson], weighing 200–350 g, were obtained from Merlin Farms, Wentworth, Nova Scotia. After 1 week of acclimation to dechlorinated Halifax city tapwater, they were divided randomly into two stocks. One stock remained in fresh water (Na^+ 0.3 mequiv l^{-1}; Cl^- 0.2 mequiv l^{-1}; HCO_3^- 0.5 mmol l^{-1}; pH 7.5–7.7; 6–9°C) as a freshwater-adapted group. The other stock was adapted to filtered sea water (32‰; Na^+ 470 mequiv l^{-1}; Cl^- 540 mequiv l^{-1}; HCO_3^- 2.2 mmol l^{-1}; pH 7.9; 8–10°C) supplied by the Aquatron Laboratory of

Dalhousie University. Fish of both stocks were maintained in 4 m^3 fibreglass tanks supplied with a continuous flow of aerated water for at least 2 months before the experiment. The animals were fed daily with commercially prepared pellets (Canada Packers Inc.). Ten days prior to use, feeding was suspended and the animals were transferred to a 560-l Living Stream tank (Frigid Unit Inc., USA) where they were acclimated to the experimental temperature (10±0.2°C).

Experimental protocol

Fish were exercised to exhaustion by manual chasing in a cylindrical container for 10 min. At this point animals were incapable of further burst performance, but still able to swim slowly around the tank. Fish were then either sampled immediately following exercise (0 min of recovery) or sampled after 10, 40, 70, 130 and 250 min of recovery in flux boxes (see McDonald and Rogano, 1986, for details) supplied with flowing aerated water from a thermostatted Living Stream tank (10±0.2°C). At 2.5 min before sampling (except for the 0 min samples), the flux box was closed and a concentrated solution (100 ml) of MS222 (adjusted to pH 7 by the addition of $NaHCO_3$) was slowly introduced to a final concentration of 0.2 g l^{-1}. The vigorous aeration of an outer chamber in the flux box provided rapid mixing of the anaesthetic solution throughout the inner animal chamber. The animals lost balance after 1–2 min and were usually removed from the animal chamber after 2.5 min. By this time, they were fully anaesthetized. A sample of white epaxial muscle was then quickly excised from beside the spine starting at the middle of the dorsal fin and cutting 3–4 cm backwards. Samples were immediately freeze-clamped by a second investigator, and stored in liquid N_2 prior to analysis. The time between removing the fish from flux box and freeze-clamping the tissue was less than 10 s. The animals were then killed by anaesthetic overdose. For control (pre-exercise) samples, animals were kept individually in the flux boxes for 48 h prior to anaesthetization and muscle sampling.

Analytical techniques and calculations

Muscle samples were ground to a fine powder under liquid N_2 using a pre-cooled mortar and pestle. The powder was kept in liquid N_2 at all times.

The acid–base status of muscle tissue was analysed by direct measurement of the pH of tissue homogenates under metabolic control, i.e. by use of fluoride and nitrilotriacetic acid, as described by Pörtner *et al.* (1990). In brief, about 150 mg of tissue powder (wet with liquid N_2) was transferred to a pre-weighed 0.5 ml Eppendorff tube containing 0.2 ml of ice-cold medium (potassium fluoride 150 mmol l^{-1}; nitrilotriacetic acid 6 mmol l^{-1}). The tube (containing the mixture powder and medium) was quickly weighed, filled with some more medium until almost full, briefly stirred with a needle, capped, and re-weighed. The mixture was then stirred in a Vortex mixer for 3–4 s, and centrifuged for 3–5 s. Samples of the supernatant were immediately taken for measurements of pH and total CO_2 (T_{CO_2}). The pH of the supernatant was determined at 10°C using a microcapillary pH electrode (Radiometer G279/G2) coupled with a PHM84 pH meter. The T_{CO_2}

of the supernatant was measured using a gas chromatography method (Boutilier *et al.* 1985). The white muscle intracellular pH (pHi) was calculated from the pH of the tissue powder taking the estimated influence of extracellular compartments into consideration (see Pörtner *et al.* 1990, for details of the calculation). Correction for extracellular compartments never exceeded 0.01 pH units. Muscle intracellular values of T_{CO_2} on the basis of intracellular fluid (ICF) ($T_{CO_2,i}$, mmol l^{-1} ICF) were calculated from the values of supernatant T_{CO_2}, tissue mass and the extracellular and intracellular volumes of white muscle before and after exercise. The latter were taken from the study by Milligan and Wood (1986*b*). Muscle intracellular concentrations of HCO_3^- ($[HCO_3^-]_i$, mmol l^{-1} ICF) were estimated as:

$$[HCO_3^-]_i = T_{CO_2,i} - (\alpha_{CO_2} \times Pa_{CO_2}),$$

where α_{CO_2} (muscle intracellular CO_2 solubility) was calculated using the equation given by Heisler (1986*a*). Muscle intracellular CO_2 tension was assumed to be in equilibrium with the arterial plasma CO_2 tension (Pa_{CO_2}); the plasma values were determined in parallel studies employing identical exercise protocols (Tang and Boutilier, 1988*b*; Tang *et al.* 1989).

For the measurement of muscle lactate concentration, about 500 mg of the tissue powder was transferred to a pre-weighed vial containing 1 ml of ice-cold 0.6 mol l^{-1} perchloric acid (PCA) and then reweighed. A further 2 ml of PCA was added and the mixture was immediately homogenized on ice for 2×15 s using an Ultra-Turrax homogenizer. The homogenate was then centrifuged for 3 min at 13 000 revs min^{-1} and 4 °C. A known volume of supernatant was immediately neutralized (pH 7.0) with Tris base (Sigma), frozen and kept in liquid N_2 until analysis. The lactate concentrations of the supernatant were analyzed by the L-lactate dehydrogenase/NADH method using Sigma reagents. The values of muscle intracellular lactate concentration ($[lactate]_i$, mmol l^{-1} ICF) were calculated in the same way as for $T_{CO_2,i}$ (see above).

Statistical analysis

Mean values±1 S.E.M. are reported throughout. Differences between groups were analysed statistically using unpaired Student's *t*-test, 5 % being taken as the fiducial limit of significance.

Results

White muscle pHi values (Fig. 1) in the pre-exercise state were the same in SW and in FW trout (7.31±0.01 *vs* 7.29±0.01, respectively). Following exercise, pHi in SW animals decreased to a minimum value of 6.49±0.02 (0.82 pH units drop) at 0 min. It then rapidly rose to 6.69±0.04 at 10 min. During the following recovery period, pHi changes were slow, with a minor decrease at 70 min and gradual increases thereafter. By 250 min post-exercise, pHi of SW animals was still 0.18 units lower than the pre-exercise value. Exhaustive exercise in FW animals

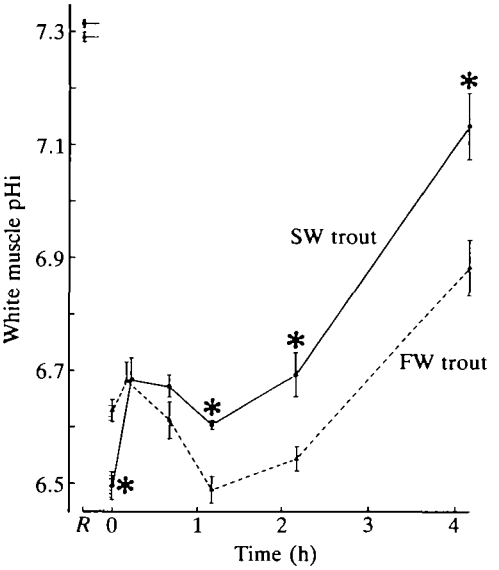

Fig. 1. White muscle intracellular pH (pHi) in freshwater- (FW) and seawater- (SW) adapted rainbow trout prior to and following exhaustive exercise. Means±1 S.E.M. *R*, rest; stippled bar, 10 min of exhaustive exercise, 0, immediately after exercise. FW trout: N=11 (rest), N=10 (0 min), N=6 (10 min), N=9 (40 min), N=11 (70 and 130 min), N=7 (250 min). SW trout: N=12 (rest), N=10 (0 min), N=6 (10 min), N=9 (40 min), N=10 (70 min), N=11 (130 min), N=8 (250 min). * indicates a significant difference (P<0.05) between the corresponding values for FW and SW trout.

caused pHi to change in a similar pattern, but the magnitude of the change was different from that of SW animals. The initial pHi decrease (0.66 pH units) was significantly less than that in SW trout. After a small increase at 10 min, the pHi of the FW animals kept falling to a minimum of 6.49±0.03 at 70 min. During the following post-exercise period, pHi recovered gradually, but much more slowly than in SW trout. This resulted in significantly lower values in FW than in SW trout at 70, 130 and 250 min. Despite these differences between SW and FW trout, an analysis based on the principles outlined in Wood *et al.* (1977) indicated that the post-exercise acidosis in the white muscle of both SW and FW trout was predominantly of metabolic origin. Accompanying the acidosis were exponential decreases in the levels of $[HCO_3^-]_i$ from 3.11±0.29 (SW trout) and 1.45±0.28 (FW trout) mmol l^{-1} ICF to minima of 0.36±0.06 (SW trout) and 0.26±0.12 (FW trout) mmol l^{-1} at 40 min (Fig. 2). Thereafter, $[HCO_3^-]_i$ in SW trout began to increase steadily; however, $[HCO_3^-]_i$ in their FW counterparts showed no signs of recovery. By 250 min, $[HCO_3^-]_i$ in SW trout had recovered to 40 % of its pre-exercise level, and had become significantly higher than that in FW trout.

At rest, white muscle $[lactate]_i$ was 1.66±0.11 and 0.71±0.07 mmol l^{-1} ICF in SW and FW trout, respectively (Fig. 3). Immediately following exercise, $[lactate]_i$ increased markedly, to a greater extent in SW than in FW trout (56.06±2.13 *vs*

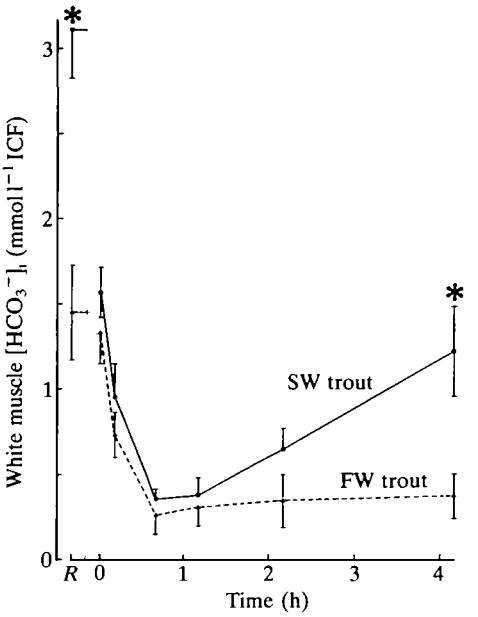

Fig. 2. White muscle intracellular concentration of bicarbonate ($[HCO_3^-]_i$) in FW and SW rainbow trout prior to and following exhaustive exercise. Other details as in Fig. 1.

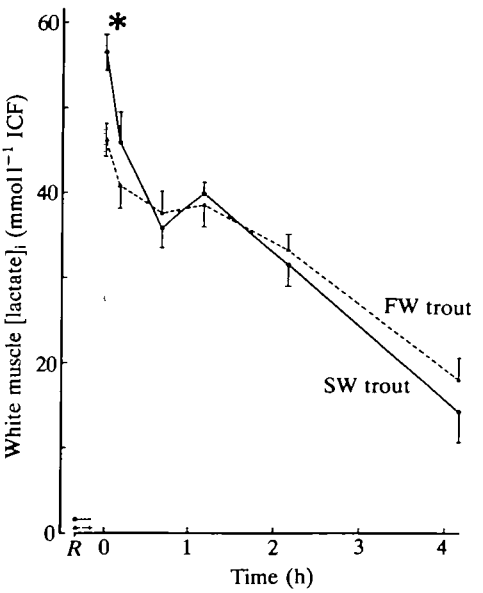

Fig. 3. White muscle intracellular concentration of lactate $[lactate]_i$ in FW and SW rainbow trout prior to and following exhaustive exercise. Other details as in Fig. 1.

46.44±1.89 mmol l^{-1} ICF). These initial lactate loads were quickly cleared from the white muscle by 40 % (SW trout) and 20 % (FW trout) following 40 min of recovery. Thereafter, the lactate loads became gradually reduced in both SW and FW trout.

Discussion

Critique of methods

Muscle sampling technique

Methods such as biopsy needle puncture and decapitation (see Table 1) have often been employed for sampling fish muscle tissue. However, these techniques unavoidably involve handling the live animal. The associated struggling can lead to marked changes in the acid–base and metabolic status of the animal (Heisler, 1986*a*,*b*), especially in active fish such as rainbow trout. For example, 3–4 tail flaps in rainbow trout can cause as much as 70 % and 30 % reductions in the concentrations of white muscle phosphocreatine and ATP, respectively (Dobson and Hochachka, 1987). In the present study, we anaesthetized the fish to minimize such handling stresses. Although MS222 (the anaesthetic used) has been reported to cause metabolic and acid–base changes in favour of anaerobic tissue metabolism (Soivio *et al.* 1977; Cornish and Moon, 1986), we used lower dosages and shorter exposure durations to try to avoid such changes. Indeed, a disturbance towards anaerobiosis seems unlikely given the relatively lower levels of muscle lactate found in the present investigation compared with others without anaesthetization (see Table 1).

Muscle pHi measurement

The DMO distribution technique has been commonly used for determination of steady-state intracellular pHi levels in fish muscle (i.e. eel, *Anguilla rostrata*:

Table 1. *Resting levels of white muscle lactate ([lactate]$_i$) in freshwater-adapted rainbow trout determined by different muscle sampling techniques*

White muscle [lactate]$_i$	Muscle sampling technique	Source
14.79±1.68 (12) (mmol kg^{-1} wet mass)	Plunging whole fish into liquid N_2	Johnston (1975)
13.85±1.70 (9) (mmol l^{-1} ICF)	Biopsy needle puncture	Milligan and Wood (1986*b*)
10.06±1.04 (5) (mmol kg^{-1} wet mass)	Biopsy needle puncture	Turner *et al.* (1983)
6.62±0.95 (6) (mmol kg^{-1} wet mass)	Head stun	Dobson and Hochachka (1987)
5.78±1.39 (7) (mmol kg^{-1} wet mass)	Decapitation	Dunn and Hochachka (1986)
3.33±0.60 (5) (mmol kg^{-1} wet mass)	Decapitation	Boutilier *et al.* (1988)
3.0±0.4 (5) (mmol kg^{-1} wet mass)	Decapitation	Parkhouse *et al.* (1987)
0.71±0.07 (13) (mmol l^{-1} ICF)	Anaesthetization	Present study

Values are means±S.E.M. (*N*).
ICF, intracellular fluid.

Walsh and Moon, 1982; catfish, *Ictalurus punctatus*: Cameron, 1980; Cameron and Kormanik, 1982; rainbow trout: Hōbe *et al.* 1984; Milligan and Wood, 1985). However, the estimation of pHi by this method is dependent upon full equilibration of the DMO between the intra- and extracellular compartments. This time delay effectively limits the application of this technique for recording fast pHi changes associated with various stress situations such as exhaustive exercise. Milligan and Wood (1985) have confirmed that DMO redistribution was complete 15 min after an acute hypercapnic acidosis in the white muscle of rainbow trout. They went on to apply this technique to record pHi transients following exhaustive exercise in the same species (Milligan and Wood, 1986*a*,*b*). However, the immediate changes in pHi that occurred within 0–15 min following exercise were still not known with any certainty, because of the above-mentioned methodological limitations (noted by Milligan and Wood, 1986*a*,*b*). The method used in the present study (i.e. measurement of pH in tissue homogenates under chemical control), has proved to be reliable in determining steady-state pHi in various tissues of a range of animals (Pörtner *et al.* 1990). Moreover, this analysis is independent of any methodological time delay and is, therefore, ideal for recording rapid pHi transients.

Similar amounts of lactate were produced by the exhaustive exercise procedure of the present study and that of Milligan and Wood (1986*b*), as indicated by the similarity in the levels of $[lactate]_i$ following exercise (Fig. 4B). The pHi values measured immediately after exercise were, however, markedly different between

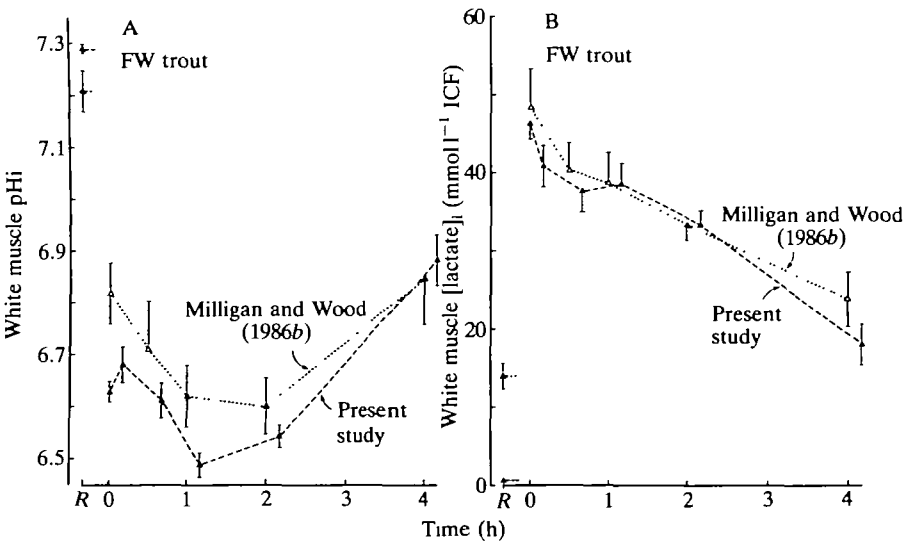

Fig. 4. Comparison of the values of white muscle (A) pHi and (B) $[lactate]_i$ pre- and post-exercise in FW and SW rainbow trout between the present study (broken lines) and the study by Milligan and Wood (1986*b*; dotted lines). The latter study employed 180–420 g FW trout and 6 min of exhaustive exercise at 15±1°C. See text for more details.

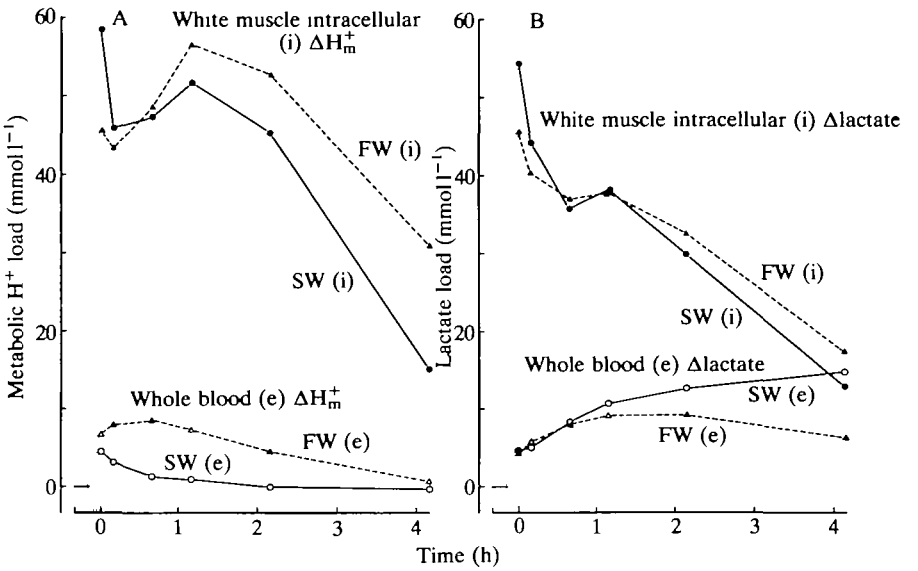

Fig. 5. Post-exercise changes in (A) metabolic H^+ load (ΔH_m^+) and (B) lactate load (Δlactate) within white muscle intracellular compartment (i) and whole blood (e) in FW and SW rainbow trout. Whole-blood values were from Tang and Boutilier (1988*b*; 0 min post-exercise) and Tang *et al.* (1989; 10–250 min post-exercise).

the two investigations (Fig. 4A). The much higher values (0 min post-exercise) determined by the DMO method in Milligan and Wood's study may be caused by the aforementioned methodological time delay. Note also in the present study the close match between metabolic proton load and lactate load in white muscle immediately after exercise (Fig. 5).

White muscle intracellular acid–base and lactate status

Resting status

At rest, a small difference in pHi between SW and FW trout was accompanied by a comparatively large difference in $[HCO_3^-]_i$ (Figs 1 and 2). This, however, could easily be the result of a relative respiratory acidosis in resting FW trout considering the high buffering capacity (β) of these tissues. For example, assuming that white muscle β was the same in both SW and FW trout ($\beta = -73.59$ mmol pH unit^{-1} l^{-1} ICF from Milligan and Wood, 1986*b*), a 0.022 (7.312–7.290) unit difference in pH would cause a 1.62 mmol l^{-1} difference in $[HCO_3^-]$i, which agrees well with the measured 1.66 (3.11–1.45) difference. Similar values of white muscle pHi (7.296) and $[HCO_3^-]_i$ (1.59 mmol l^{-1}) have also been reported in resting FW trout by Hõbe *et al.* (1984). Higher resting pHi (7.56) and $[HCO_3^-]$ (approx. 5.5 mmol l^{-1}) have recently been reported in a SW fish, *Platichthys stellatus* (Milligan and Wood, 1987).

The significantly lower resting $[lactate]_i$ measured in the present study, compared with that in other studies, suggests that $[lactate]_i$ may be sensitive to

sampling disturbances (Table 1). The intracellular lactate concentrations for muscle of FW and SW animals (0.71 and 1.66 mmol l^{-1} ICF, respectively) are very close to those found in blood (0.5–0.6 mmol l^{-1}) (Milligan and Wood, 1986*b*; Tang *et al.* 1989). These findings go against the conventional view that a large lactate gradient exists between white muscle and extracellular fluid in resting fish (see review by Wood and Perry, 1985).

Post-exercise status

Despite incurring a greater acidosis, SW trout appear to be more proficient than FW trout at correcting the intracellular acid–base disturbances caused by exhaustive exercise (Figs 1, 2). The much greater post-exercise depression in the pHi of SW trout and the greater elevation of $[lactate]_i$ indicated that SW animals had done more anaerobic work than their FW counterparts (Fig. 3). The ability to do more work could be related to the acclimation history of the fish; i.e. SW-adapted fish may have a greater anaerobic scope. This could either be related to a greater 'on board' metabolic machinery or to their enhanced ability to correct acidoses through branchial net H^+ excretion (Tang and Boutilier, 1988*a*; Tang *et al.* 1989).

Metabolic H^+ can be cleared from the intracellular compartment in three ways: (1) buffering by intracellular non-bicarbonate buffers; (2) export to the extracellular compartment; (3) H^+-consuming metabolism. The non-bicarbonate buffer value (β) of the white muscle of a wide range of teleost fishes is positively correlated to their potential for anaerobic work (Castellini and Somero, 1981; Hochachka and Somero, 1984). However, such correlations are only significant among groups of fishes with distinct locomotory habits (e.g. warm-bodied fishes; pelagic ectothermic fishes; deep-sea sit-and-wait fishes). There is little variation in white muscle buffering capacity within groups of fishes with similar locomotory habits (see Table 2 in Castellini and Somero, 1981). Any differences in buffering capacity between SW and FW trout are expected to reflect these general trends and, therefore, to be minor. This would mean that differences in buffering capacity are probably not sufficient to account for a major portion of the post-exercise differences in acid–base recovery. Export of protons to the ECS is another important way to clear the metabolic H^+ load. Such H^+ efflux is thought to be rate-limited because of relatively small volume and low buffering capacity of the ECS (e.g. 'equilibrium limitation'; Holeton and Heisler, 1983). In this case, protons can be transferred to the ECS only to the extent that they are removed from the ECS by excretion into the external water (or consumed by metabolism). In our previous study (Tang *et al.* 1989), H^+ equivalent excretion to external water following exercise in SW trout was found to be five times that in FW trout. The present data along with those of our previous study (Tang *et al.* 1989) strongly suggest that the higher H^+ excretion in SW animals was not due primarily to a higher H^+ production, but to the chemical composition of the external medium (e.g. availability of counter-ions) and/or permeability characteristics of the gill, e.g. for H^+ excretion. For example, during the post-exercise recovery period, the

metabolic H^+ load in both white muscle and blood of SW trout was lower than that of FW trout (Fig. 5), while H^+ excretion was five times higher in SW trout (see Fig. 3 in Tang *et al.* 1989). Clearly, the much faster recovery of white muscle pH in SW trout can be attributed, at least in part, to greater net H^+ excretion to the environmental water (see next section for quantitative analysis). In contrast, metabolism of lactate, the main H^+-consuming metabolism following exercise, can also remove H^+ from the ECS *via* the Cori cycle (e.g. in heart and liver), since metabolic conversion of lactate (whether to H_2O and CO_2, or to glycogen) would consume an equivalent amount of H^+. The extent of this process in H^+ clearance is unclear. However, the much higher blood [lactate] during the recovery period in SW trout (Fig. 5) may favour this process owing to greater availability of substrate. Moreover, lactate metabolism *in situ* has been found to be an important mechanism for lactate and H^+ clearance from white muscle in trout (Turner *et al.* 1983; Milligan and Wood, 1986*b*). Indeed, the Cori cycle appears to play only a minor role in the metabolism of lactate in both the salmon, *Oncorhynchus kisutch*, and the flounder, *Platichthys stellatus* (Milligan and McDonald, 1988).

Compartmental analysis of H^+ and lactate loads

The data in the present study, and comparable data on the blood acid–base status and H^+ equivalent flux from animal to environmental water in our previous studies (Tang and Boutilier, 1988*b*; Tang *et al.* 1989), allow analysis of changes in the metabolic H^+ load (ΔH^+_m) and lactate load (Δlactate) in three compartments: intracellular, extracellular and environmental water. This analysis is based on the assumption that the intracellular compartment of white muscle, which makes up 66 % of the body mass (Stevens, 1968), represents the total intracellular compartment. The present experiments on muscle acid–base status, combined with data from our previous studies (Tang and Boutilier, 1986*b*; Tang *et al.* 1989), form the basis of the model calculations that follow. The entire data set is thought to be comparable since, for all experiments, we used the same stock of rainbow trout, acclimated to the same temperature and studied during the same season.

Intracellular (I), extracellular (E), environmental water (W) and total (tot) H^+ load (mmol kg^{-1}) at each time were calculated as:

$$\Delta H^+_{m,I} = \{[HCO_3^-]_{i,1} - [HCO_3^-]_{i,2} - \beta(pH_{i,1} - pH_{i,2})\} \times ICFV\,,$$

$$\Delta H^+_{m,E} = BV \times \Delta H^+_{m,WB} + [(ECFV - PV) \times \Delta H^+_{m,ISF}]\,,$$

$$\Delta H^+_{m,W} = J^H_{net} \times time\,,$$

$$\Delta H^+_{m,tot} = \Delta H^+_{m,I} + \Delta H^+_{m,E} + \Delta H^+_{m,W},$$

where β is the nonbicarbonate buffer value of white muscle taken from Milligan and Wood (1986*b*); ICFV and ECFV are the white muscle intra- and extracellular fluid volumes (ml kg^{-1}), respectively, extrapolated from the values given by Milligan and Wood (1986*b*); BV and PV are blood and plasma volumes (l kg^{-1}), respectively, using the data reported by Milligan and Wood (1982); whole- blood metabolic proton load ($\Delta H^+_{m,WB}$) was calculated from data in Tang and

Boutilier (1988*b*; 0 min post-exercise) and Tang *et al.* (1989; 10–250 min post-exercise). The metabolic proton load of the interstitial fluid ($\Delta H^+_{m,ISF}$) was calculated, using the equation described in Tang *et al.* (1989), in the same way as $\Delta H^+_{m,WB}$ taking $\beta = -2.59$ mmol pH unit^{-1} l^{-1} (i.e. separated plasma β, from Wood *et al.* 1982) and assuming that interstitial pH and [HCO_3^-] were the same as those in plasma. J^H_{net} is the net flux of acidic equivalents across the gills reported in Tang *et al.* (1989). Acidic equivalent excretion to water during the 10 min exercise period was assumed to be at the same rate as that between 0 and 1 h post-exercise.

Intracellular, extracellular and total lactate load (mmol kg^{-1}) at each time were calculated as:

$$\Delta\text{lactate}_I = \{[\text{lactate}]_{i,1} - [\text{lactate}]_{i,2}\} \times \text{ICFV},$$

$$\Delta\text{lactate}_E = \text{BV} \times \Delta\text{lactate}_{WB} + [(\text{ECFV} - \text{PV}) \times \Delta\text{lactate}_{WB}],$$

$$\Delta\text{lactate}_{tot} = \Delta\text{lactate}_I + \Delta\text{lactate}_E,$$

where lactate_{WB} was taken from the same source as for $\Delta H^+_{m,WB}$; $\Delta\text{lactate}_E$ was calculated assuming that interstitial Δlactate was the same as that in whole blood. Lactate excretion to the environmental water has been reported to be insignificant (Milligan and Wood, 1986*a*) and, therefore, was ignored in the above analysis.

Owing to the nature of the experiment (i.e. each animal was sampled only once, and intra- and extracellular data were measured from different groups of animals), the above analysis was restricted to using mean values at each time.

Analysis of the ΔH^+_m distribution (Table 2) clearly showed that most of the H^+ load (>92 %) in FW trout was retained in white muscle ICS at all times during recovery, with only small amounts (3–6 %) transferred to the ECS. A similar situation occurred in SW trout at the early stages of recovery except that less H^+ (0.8–2.8 %) appeared in the ECS. However, SW trout 'stored' three times more H^+ in the external water than did their FW counterparts; e.g. by 250 min post-exercise, 15.5 % of the initial total H^+ load had been excreted to the external water in SW trout, only 5.0 % in FW trout. The amount of H^+ excreted in FW animals was so small (1.173 mmol kg^{-1} at 250 min) that it could only account for the clearance of the initial ECS H^+ load (1.215 mmol kg^{-1} at 0 min). It therefore contributed little to the pHi correction. However, the amount excreted in SW animals was enough to clear the ECS H^+ load and should also have contributed to the more rapid H^+ decline in the ICS. It is clear that the relative amounts of total H^+ removed (see $\Delta H^+_{m,tot}$ at t min/$\Delta H^+_{m,tot}$ at 0 min in Table 2) were far greater than could be accounted for by H^+ excretion to external water alone (see $\Delta H^+_{m,w}$ at t min/$\Delta H^+_{m,tot}$ at 0 min in Table 2). Presumably, proton-consuming metabolism accounts for the remainder of the H^+ removed. For example, at 250 min post-exercise (see Fig. 6), 35.2 % (FW) and 60.9 % (SW) of the initial total H^+ load had been removed, of which 85.7 % (FW) and 74.5 % (SW) was cleared metabolically. The analysis revealed that the amount of H^+ cleared *via* metabolic processes in SW trout was about twice that in FW trout (Fig. 6A). This appears to be the main reason for the faster recovery of white muscle pHi in SW trout. Although the exact

Table 2. *Distribution of metabolic proton load in intracellular space ($\Delta H^+_{m,I}$), extracellular space ($\Delta H^+_{m,E}$) and external water ($\Delta H^+_{m,W}$) following exhaustive exercise in freshwater- (FW) and seawater- (SW) adapted rainbow trout*

Time post-exercise (min)	$\Delta H^+_{m,I}$	$\Delta H^+_{m,E}$	$\Delta H^+_{m,W}$	$\Delta H^+_{m,tot}$	$\frac{\Delta H^+_{m,tot} \text{ at } t \text{ min}}{\Delta H^+_{m,tot} \text{ at } 0 \text{ min}}$	$\frac{\Delta H^+_{m,W} \text{ at } t \text{ min}}{\Delta H^+_{m,tot} \text{ at } 0 \text{ min}}$
Freshwater trout						
0	22.187 (94.6 %)*	1.215 (5.2 %)*	0.048 (0.2 %)*	23.450	100.0 %	0.2 %
10	20.999 (93.6 %)	1.346 (6.0 %)	0.096 (0.4 %)	22.441	95.7 %	0.4 %
40	23.556 (93.2 %)	1.532 (6.1 %)	0.192 (0.7 %)	25.280	107.8 %	0.8 %
70	27.691 (94.2 %)	1.336 (4.5 %)	0.382 (1.3 %)	29.409	125.4 %	1.6 %
130	25.827 (94.5 %)	0.811 (3.0 %)	0.681 (2.5 %)	27.319	116.5 %	2.9 %
250	14.332 (92.9 %)	−0.077 (−0.5 %)†	1.173 (7.6 %)	15.428	65.8 %	5.0 %
Seawater trout						
0	28.374 (96.6 %)	0.824 (2.8 %)	0.189 (0.6 %)	29.387	100.0 %	0.6 %
10	22.225 (95.8 %)	0.585 (2.5 %)	0.378 (1.6 %)	23.188	78.9 %	1.3 %
40	22.980 (95.7 %)	0.286 (1.2 %)	0.756 (3.1 %)	24.022	81.7 %	2.6 %
70	25.349 (93.6 %)	0.213 (0.8 %)	1.513 (5.6 %)	27.075	92.1 %	5.1 %
130	22.207 (90.1 %)	−0.030 (−0.1 %)†	2.482 (10.1 %)	24.659	83.9 %	8.4 %
250	7.015 (61.1 %)	−0.087 (−0.8 %)†	4.561 (39.7 %)	11.489	39.1 %	15.5 %

Values are in mmol kg^{-1} body mass; $\Delta H^+_{m,tot} = \Delta H^+_{m,I} + \Delta H^+_{m,E} + \Delta H^+_{m,W}$.

* The percentage values in brackets represent the proportion of ΔH^+_m in each compartment at the same sampling time.

† Negative values signify a metabolic base load.

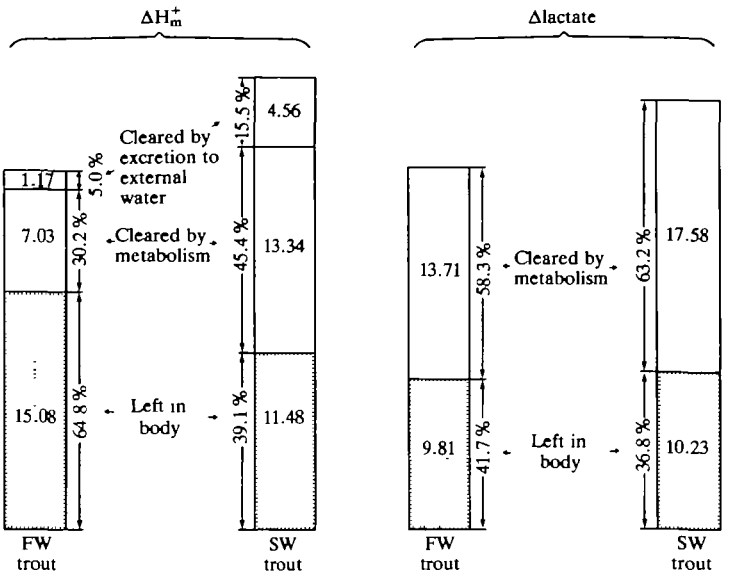

Fig. 6. Diagram of compartmental analysis of the fate of (A) ΔH_m^+ and (B) Δlactate at 250 min post-exercise in FW and SW rainbow trout. Values inside the columns are the concentrations of either ΔH_m^+ or Δlactate (mmol kg^{-1}) in each compartment at 250 min.

amount of metabolically processed H^+ in each site (i.e. *in situ* and other tissues) cannot be assessed quantitatively with the data at hand, the analysis does indicate that H^+-consuming metabolism *in situ* plays a major role in reducing the H^+ load following exercise. This is indicated further by the distribution of lactate (Table 3), which is the main substrate for H^+-consuming metabolism following exercise.

Analysis of the lactate pool (Table 3; Fig. 6B) showed a similar distribution to that of metabolic H^+, with most of the lactate load (>80 %) remaining in the white muscle ICS. However, the total lactate load decreased at a faster rate in SW trout than in FW trout (see $\Delta lactate_{tot}$ at t min/$\Delta lactate_{tot}$ at 0 min in Table 3). The faster clearance of total lactate load in SW animals could be due to a more rapid metabolism of lactate *in situ* or in other tissues.

Comparison of the total metabolic H^+ and lactate loads revealed that the amount of lactate removed was not matched to the amount of metabolic H^+ cleared. For example, at 250 min following exercise (Fig. 6), the amount of lactate removed (FW 13.71 mmol kg^{-1}; SW 17.58 mmol kg^{-1}) was greater than the H^+ cleared by metabolism (FW 7.03 mmol kg^{-1}; SW 13.34 mmol kg^{-1}). Given that lactate metabolism consumes equivalent amounts of H^+, this discrepancy indicates that additional H^+, from sources other than lactate formation, may be produced during the post-exercise recovery period. This $\Delta H_{m,tot}^+ - \Delta lactate_{tot}$ discrepancy (Fig. 7) gradually increased following exercise, reaching peak values at 70–130 min, and gradually declining thereafter. This could occur as a result of an

Table 3. *Distribution of lactate load in intracellular space ($\Delta lactate_I$) and extracellular space ($\Delta lactate_E$) following exhaustive exercise in rainbow trout*

Time post-exercise (min)	$\Delta lactate_I$	$\Delta lactate_E$	$\Delta lactate_{tot}$	$\frac{\Delta lactate_{tot} \text{ at } t \text{ min}}{\Delta lactate_{tot} \text{ at } 0 \text{ min}}$
Freshwater trout				
0	22.19 (94.3 %)*	1.33 (5.7 %)*	23.52	100.0 %
10	19.50 (92.2 %)	1.64 (7.8 %)	21.14	89.9 %
40	17.89 (88.7 %)	2.28 (11.3 %)	20.17	85.8 %
70	18.54 (87.7 %)	2.61 (12.3 %)	21.15	89.9 %
130	15.94 (85.9 %)	2.62 (14.1 %)	18.56	78.9 %
250	8.03 (81.9 %)	1.78 (18.1 %)	9.81	41.7 %
Seawater trout				
0	26.39 (94.9 %)	1.42 (5.1 %)	27.81	100.0 %
10	21.42 (93.6 %)	1.46 (6.4 %)	22.88	82.3 %
40	16.47 (87.5 %)	2.36 (12.5 %)	18.83	67.7 %
70	18.68 (86.0 %)	3.05 (14.0 %)	21.73	78.1 %
130	14.63 (80.0 %)	3.64 (20.0 %)	18.27	65.7 %
250	5.80 (57.8 %)	4.23 (42.2 %)	10.03	36.1 %

Values are in mmol kg^{-1} body mass; $\Delta lactate_{tot} = \Delta lactate_I + \Delta lactate_E$.

* The percentage values in brackets represent the proportion of Δlactate in each compartment at the same sampling time.

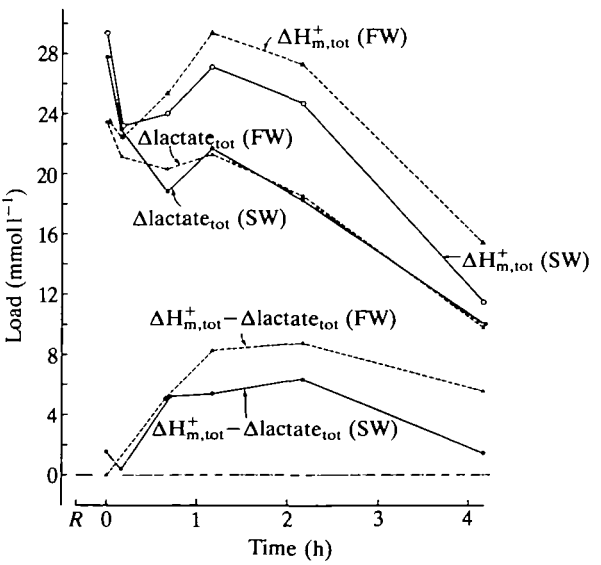

Fig. 7. Post-exercise changes in total metabolic H^+ load ($\Delta H^+_{m,tot}$) and total lactate load ($\Delta lactate_{tot}$), and the discrepancy between them ($\Delta H^+_{m,tot} - \Delta lactate_{tot}$, signs considered) in FW and SW rainbow trout following exhaustive exercise.

imbalance between ATP consumption and production (i.e. consumption>production) due to uncoupling between glycolysis and ATP hydrolysis. Indeed, such a situation has been found in the white muscle of FW trout immediately after exercise. Apparently, however, it is a short-lived phenomenon (e.g. 0–60 min post-exercise, Milligan and Wood, 1986*b*; 0–45 min post-exercise, Dobson and Hochachka, 1987) and, therefore, may not be the main reason for the observed discrepancy (70–130 min post-exercise) between total loads of H^+ and lactate in the present study (Fig. 7). The other probable cause of this discrepancy would be the production of an 'unmeasured organic acid' which would result in accumulation of additional H^+. Indeed, Wood *et al.* (1983) have reported the appearance of an 'unmeasured anion' in the plasma of FW trout post-exercise, changing in a similar pattern to that of the H^+–lactate discrepancy found in the present study (see Fig. 5 in Wood *et al.* 1983). This 'unknown acid' could be an anaerobic end-product produced *via* modified metabolic pathways, since products such as succinate have been found to accumulate in fish during severe hypoxia (Johnston, 1975; Smith and Heath, 1980). Regardless of the nature of the acid, it should be

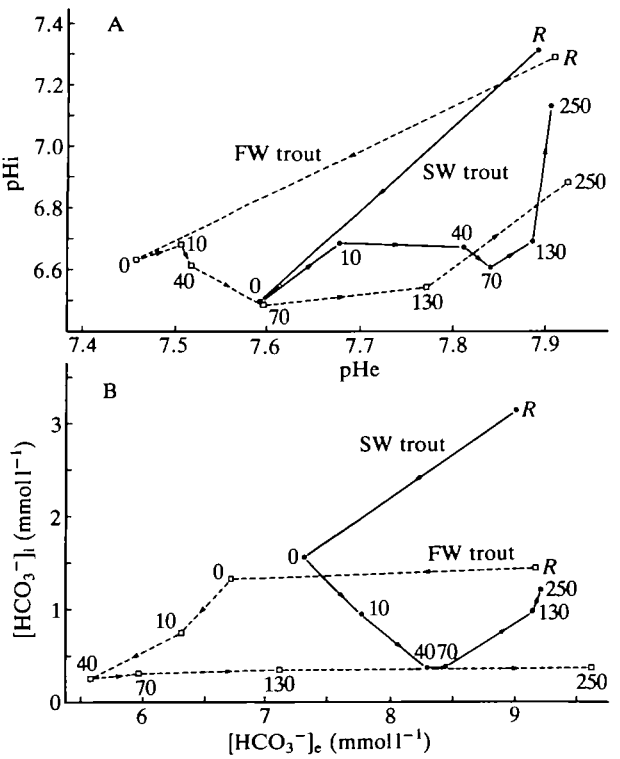

Fig. 8. The relationships between (A) white muscle pHi and plasma pH (pHe), and (B) white muscle $[HCO_3^-]_i$ and plasma $[HCO_3^-]$ ($[HCO_3^-]_e$) in FW and SW rainbow trout prior to and following exhaustive exercise. *R*, rest; times (in min) of post-exercise values are indicated adjacent to each data point. Plasma values were from Tang and Boutilier (1988*b*; 0 min post-exercise) and Tang *et al.* (1989; 10–250 min post-exercise).

noted that the H^+ from this unknown source represented 39–47 % (FW trout) and 25–35 % (SW trout) of the total H^+ load during the 70–130 min recovery periods (Fig. 7). Thus, it could be responsible for the secondary drop of white muscle pHi in the 70–130 min period of recovery (Fig. 1).

In conclusion, it is clear that FW trout and SW trout exhibit marked differences in their intracellular responses to acid–base disturbances. The more rapid recovery from metabolic acidosis in SW trout evidently resides with their greater use of the external environment as a storage depot for metabolic H^+. Even so, the contribution of this mechanism to the correction of the overall acidosis is relatively small (e.g. 15.5 % in SW *vs* 5.0 % in FW trout) over the time period followed. Although the number of proton equivalents excreted to the external medium is not large, compared with the total number cleared during the post-exercise period, this transient 'storage' of H^+ seems to play a crucial role in the restoration of extracellular, and presumably also intracellular, acid–base balance. The normalization of extracellular pH (pHe) before that of muscle pHi (see Fig. 8) may be important for active fish such as rainbow trout, in order to minimize proton loading in the blood and its consequent effects on blood O_2 transport. Mechanisms to offset pH-induced interference with blood O_2-carrying properties can be considered as adaptive in supporting continued performance of aerobic red muscle in active pelagic fish. Metabolic H^+-consuming processes seem to be the main mechanism for the clearance of endogenously produced protons. The faster clearance of metabolic H^+ during post-exercise recovery in SW trout relative to FW trout can be attributed to their greater H^+-consuming metabolism (primarily lactate metabolism) and their comparatively smaller production of an 'unknown acid' during the recovery period.

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