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Effects of swimming on metabolic recovery from anoxia in the painted turtle

Daniel E. Warren* and Donald C. Jackson

Department of Molecular Pharmacology, Physiology and Biotechnology, Brown University, Box G, Providence, RI 02912, USA

*Author for correspondence (e-mail: Daniel_E_Warren@brown.edu)

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Summary

Anoxic submergence in the Western painted turtle results in a severe metabolic acidosis characterized by high plasma lactate and depressed arterial pH, a response similar to that seen in other vertebrates following exhaustive exercise. We tested the hypothesis that 1 or 2 h of aerobic swimming following anoxic submergence would enhance the rate of lactate disappearance from the blood just as sustained aerobic exercise does in mammals and fishes following strenuous exercise. Following 2 h of anoxic submergence at 25°C and 1 h of recovery, the pattern of plasma lactate disappearance in turtles previously trained to swim in a flume and swum aerobically (2–3× resting $\dot{V}_{O_2}$) for 1 or 2 h did not differ significantly from that in trained and untrained non-swimming turtles. Turtles were fully recovered by 7–10 h post-anoxia. The response patterns also did not differ between treatments for arterial P_{O_2}, P_{CO_2}, pH, and plasma glucose and HCO_3^-. Blood pH and plasma HCO_3^- recovered by 1 and 4 h, respectively. Despite the large lactate load, painted turtles are able to sustain periods of continuous swimming for at least 2 h without compromising metabolic recovery. Although this activity did not consistently enhance recovery, the rate of lactate disappearance was positively correlated with oxygen consumption rate in actively and passively recovering turtles. We suggest that active recovery was not a more important enhancer of recovery either because swimming may have had an inhibitory effect on hepatic gluconeogenesis or that there is variation in fuel utilization during the swimming period.

Key words: active recovery, anoxia, *Chrysemys picta bellii*, lactate, reptile, swimming, turtle.

Introduction

Anoxic submergence in turtles causes a severe metabolic acidosis characterized by increases in plasma lactate and decreases in blood HCO_3^- and pH (Jackson and Silverblatt, 1974). This acid load is buffered by the mobilization of shell minerals and by the high buffering capacity of the turtle's body fluids in general, the details of which are reviewed elsewhere (Jackson, 2000a,b, 2002; Jackson et al., 2000a,b). With the exception of a few studies (Herbert and Jackson, 1985; Jackson et al., 1996; Keiver et al., 1992), little is known about the metabolic recovery from anoxia induced lactic acidosis in turtles.

Vertebrate lactate metabolism is best understood under conditions following exhaustive exercise. Within vertebrates, the fate of lactate varies across class. The traditional view is that lactate is primarily oxidized in mammals (Brooks and Gaesser, 1980), although recent evidence indicates this might not be clearcut (Fournier et al., 2002). Fishes and reptiles (Gleeson, 1996), including turtles following anoxic submergence (Jackson et al., 1996), convert most of the lactate load back to glycogen in muscle and liver. It has been suggested that any difference in lactate clearance strategy is due to the marked differences in metabolism between ectotherms and endotherms, and, specifically, that the lower metabolic rate of ectotherms limits the proportion of the lactate load that can be oxidized (Gleeson and Dalessio, 1989).

Within mammals, moderate aerobic exercise after a bout of exhaustive exercise, termed active recovery, results in an enhanced rate of lactate disappearance from blood (Ahmaidi et al., 1996; Bangsbo et al., 1994). The mechanism for enhanced lactate clearance during active recovery is thought to be increased lactate oxidation, which generates ATP to help sustain the exercise. Active recovery following lactic acid accumulation by strenuous exercise has also been shown to enhance metabolic recovery in trout (Milligan et al., 2000), although the suggested mechanism is thought to be related to the inhibitory effects of cortisol on glycogen repletion in muscle (Milligan, 2003). The effect of active recovery has yet to investigated in reptiles recovering from lactic acidosis or any animal recovering from hypoxia or anoxia.

In this experiment, we tested the hypothesis that elevated recovery metabolism, through 1–2 h of continuous aerobic exercise at 2–3× resting oxygen consumption rate ($\dot{V}_{O_2}$), would increase the rate of lactate disappearance from plasma following 2 h of anoxic submergence in the Western painted turtle *Chrysemys picta*. Oxygen consumption and carbon dioxide production rates as well as blood acid–base status were

monitored at rest and throughout the recovery period. The results of these experiments reveal the relative importance of active recovery in modulating metabolic processes in turtles following anoxic submergence and demonstrate how recovery from exhaustive exercise and anoxic submergence are affected by a similar alteration in metabolic state.

Materials and methods

Animals

Western painted turtles, *Chrysemys picta bellii* Gray (body mass 397.9–1142.1 g), of both sexes were obtained from Lemberger (Oshkosh, WI, USA) and maintained at Brown University under natural photoperiod in water at 22–26°C with access to a platform. Air temperature ranged from 19–25°C. Turtles were fed Turtle Brittle (Nasco, Fort Atkinson, WI, USA) *ad libitum* and their water was changed daily during the experiment. All experiments were performed between June and October in 2001 and 2002.

Training protocol

Prior to experiments (Days 1–14), turtles were divided into two groups: trained and untrained. Trained animals were swum in a flume similar in design to that used by Prange (1976) (see Fig. 1) for 40 min each day for 2 weeks at speeds ranging from 10.6–13.7 cm s^{-1}. During this time, they learned to swim in the flume and to breathe from a chamber built at the front of a lid covering the surface of the swimming area (see Fig. 1). This range of speeds was chosen to ensure that the animal would swim throughout the entire training session. The lid prevented the animal from breathing anywhere except in the chamber, where expired gases could be collected for determination of oxygen consumption and carbon dioxide rates during recovery from anoxia as described below. The untrained animals were placed in the flume for the same amount of time and learned to find the breathing chamber, but were not made to swim against a current.

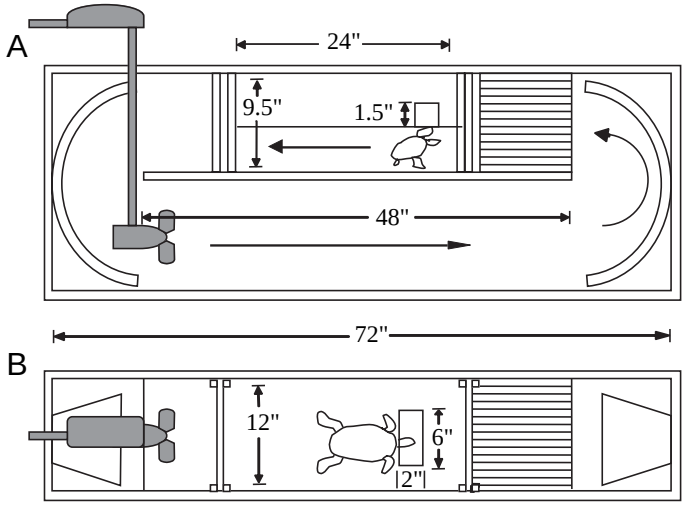

Fig. 1. Schematic of flume used to train turtles for 14 days prior to and during the experiment. Turtles were transferred to the working chamber of the apparatus after 1 h of recovery from 2 h of anoxic submergence and then swum for 0, 1 or 2 h. (A) Side view; (B) top view.

Catheterization

On day 15, all turtles were anesthetized with isoflurane and catheterized (PE50, Intramedic) occlusively in the right carotid artery through a 2.5 cm hole cut through the right pectoral scute of the plastron using a trephine. The catheter was led out the side of the neck through a small hole and filled with 20 IU ml^{-1} heparinized 0.8% NaCl solution. An acrylic plug was placed in the plastron hole and sealed with dental acrylic (Bosworth Original Truliner, Stokie, IL, USA). The animals were fully recovered 1–3 h after plug sealing, placed in 20 liter buckets with approximately 2–5 cm of water and allowed to recover overnight. The following day (Day 16), the turtle was fixed with adhesive tape to a brick and placed in a water bath at 25°C filled just high enough to completely cover the carapace without preventing access to air. The fixing of the animal served two functions: first, to prevent the animal from swimming around the water bath. Second, to allow for a chamber to be lowered over the area on the water's surface above the turtle's head so that when it raised its head to breathe, it exchanged gases with a bias flow that passed through the chamber. The bias flow was analyzed for O_2 and CO_2 (see further details below). The animal was allowed to acclimate to the temperature and chamber overnight.

Experimental protocol

The experimental protocol was approved by the Brown University Institutional Animal Care and Use Committee (IACUC). Resting oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) rates were measured for at least 1 h, after which a 0.6 ml blood sample was obtained for analysis of arterial blood P_{O_2}, P_{CO_2}, pH and plasma glucose and lactate. The brick and turtle were then tipped onto their sides, the weight of the brick anchoring the turtle to the bottom of the chamber and preventing it from reaching the surface to breathe. Previous work in red-eared sliders *Trachemys scripta* (Belkin, 1968) has established that cutaneous oxygen consumption at 22°C is a negligible fraction of the total oxygen demand, making it unnecessary to bubble the surrounding water with nitrogen to induce anoxia. After 2 h, the turtle and brick were righted so that the animals could resume breathing from the chamber to begin the recovery period. At this point, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ measurements were also resumed. The arterial blood sample was taken after the first breath to obtain the most accurate measurement of plasma lactate at the start of recovery. As a consequence, an end anoxia arterial P_{O_2} measurement was not obtained. After the 1 h recovery blood sample, the turtle was transferred to the flume and either swum for 1 or 2 h (1 h active and 2 h active, respectively) at 12.1–12.9 cm s^{-1} or did not (passive/trained and passive/untrained groups). The passive/trained group was used to determine if the 14 days of training affected any of the recovery parameters. The 1–2 h

swimming period was chosen because we determined from a preliminary experiment, in which a turtle was swum for 6 h after 2 h of anoxia and 1 h of recovery, that 1–2 h would be adequate time to see a potential difference while cutting down on the already heavy logistical demands associated with the continuous attending to the turtle during the swimming period. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ measurements were recorded throughout the remainder of the recovery period (10 h total, 9 h in the flume). Blood samples were withdrawn and analyzed 1, 3, 6 and 9 h after transfer to the flume. An additional blood sample was taken at 2 h after transfer to the flume in the 2 h active group, but was only analyzed for plasma lactate. The flume temperature remained at 25.0±0.3°C throughout recovery.

Blood analysis

All blood samples were drawn into glass syringes and placed on ice for no more than 1 h prior to analysis. Approximately 0.2 ml of the blood was used to measure P_{O_2}, P_{CO_2} and pH (Radiometer PHM 73 pH/Blood Gas Monitor and BMS3 Mk2 Blood Microsystem thermostatted to 25°C; Copenhagen, Denmark). The remaining blood was placed in a 1.5 ml microcentrifuge tube, centrifuged at 9300 ***g*** for 3 min and lactate and glucose levels measured on the plasma (YSI 2300 Statplus, Yellow Springs, OH, USA). Blood HCO_3^- concentrations were calculated using the Henderson–Hasselbach equation using pK′=6.153 as interpolated from Reeves (1976) and αCO_2=0.0404 from Severinghaus (1965).

Metabolic rate measurements

$\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were measured using standard flow-through respirometry techniques (Withers, 1977). As indicated above, for the resting and first recovery hour measurements, the turtles breathed from a chamber that had been lowered over the surface of the water directly above the turtle's head, through which a bias flow passed. Once the turtles were transferred to the flume, the bias flow was diverted through the chamber, from which the turtles had learned to breathe during the training sessions. Chamber bias outflows passed through Drierite and a portion was pumped (AEI model R-2; Pittsburgh, PA, USA) through a carbon dioxide analyzer (AEI model CD-3A) and an oxygen analyzer (AEI model S-3A). Gas meter outputs were recorded and analyzed using the BIOPAC MP100 and Acknowledge data acquisition software (Goleta, CA, USA). Bias flows were measured at the end of each experiment (Vol-u-meter, Brooks Instruments, Hatfield, PA, USA) and ranged from 210–350 ml min^{-1}. The average gas fractions were determined from the integral divided by the time integrated for each gas at each time point and a minimum of three breathing episodes was used for each of the calculations. The whole of each of the first 3 h recovery time was integrated while the integrated signals for the resting, 4, 7 and 10 h recovery times ranged from 4.5–89.3 min. The following equations from (Otis, 1964) were used to calculate $\dot{V}_{CO_2}$, respiratory exchange ratio (RE) and $\dot{V}_{O_2}$:

$$\dot{V}_{CO_2} = \dot{V}_E (F_{ECO_2} - F_{ICO_2}) , \quad (1)$$

$$RE = [(1–F_{IO_2})F_{ECO_2}] / [(1–F_{ECO_2})F_{IO_2} – F_{EO_2})] \quad (2)$$

and

$$\dot{V}_{O_2} = \dot{V}_{CO_2} / RE , \quad (3)$$

where $\dot{V}_E$ is the bias flow, F_{ECO_2} the quotient of the integral divided by the time integrated from the CO_2 channel, F_{ICO_2} was assumed to be 0.03%, F_{IO_2} was assumed to be 21.00% and F_{EO_2} the quotient of the integral divided by the time integrated from the O_2 channel. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are expressed as ml (STPD) kg^{-1} min^{-1}.

Statistical analysis

Data were analyzed by two-way repeated measures multivariate analysis of variance (RM-MANOVA) to determine if swimming or training affected each parameter over time. Student's *t*-tests were used to elucidate any time effects or treatment × time interactions. A difference was considered significant if $P<0.05$. All statistical computations were carried out using JMP 4.0.4 (SAS Institute).

Results

Expired gases

Oxygen consumption rate ($\dot{V}_{O_2}$) doubled from resting during the first hour of recovery in all groups and returned to resting in passive recovery groups by the start of the second hour, and was not different from resting in these groups for the remainder of the recovery period (Fig. 2A). Swimming increased $\dot{V}_{O_2}$ to 3× the resting value in the 1 h active group and 2.5× the resting value in the 2 h active group. $\dot{V}_{O_2}$ in the 1 h active group was significantly higher than in the 2 h active group during the first swimming hour. There was no difference in $\dot{V}_{O_2}$ between the first and second swimming hours in the 2 h active group. $\dot{V}_{O_2}$ values in both active groups returned to resting levels after swimming periods had ended and were not different from passive groups for the remainder of the experiment.

Carbon dioxide production rate ($\dot{V}_{CO_2}$) was elevated 5–6× than the resting value during the first hour of recovery in all animals (Fig. 2B) and returned to the resting value in passive animals by the end of the second hour. $\dot{V}_{CO_2}$ remained elevated during the swimming periods in both the 1 and 2 h active groups, although it was only 2–3× the resting value. $\dot{V}_{CO_2}$ returned to resting levels soon after the end of the swimming periods in both active groups.

Respiratory exchange ratio (RE) (Fig. 2C) was significantly elevated after the first swimming hour in all groups. The trained/passive recovery group had a significantly higher RE than both the 2 h active and passive/untrained groups during the first recovery hour and was significantly lower than resting values during the second recovery hour. RE returned to resting levels in all other groups during the second recovery hour and was unaffected by swimming or training.

Blood

Swimming and training did not affect the recovery of arterial P_{CO_2}, pH or plasma [HCO_3^-] (Fig. 3). They also had no effect on plasma lactate and glucose or arterial P_{O_2} (Table 1).

Arterial P_{CO_2} (Fig. 3A) was significantly higher than resting values at the start of the recovery period and then fell

significantly below them after 1 h. It gradually increased during the remainder of the recovery period and was higher than resting values by 7 h.

Arterial pH (Fig. 3B) was significantly lower than resting at the start of recovery, was restored to resting values after 1 h, and fell slightly but significantly in all groups below resting until 10 h.

Plasma [HCO_3^-] (Fig. 3C) was significantly lower at the start of recovery than at rest and was fully restored by 4 h. By 10 h, blood [HCO_3^-] was slightly but significantly greater than resting values. Apparent differences between the 2 h active group and the other treatments at various time points results from this group having a generally higher plasma [HCO_3^-] (a treatment effect) and is not due to an effect of the swimming (treatment × time interaction).

Arterial P_{O_2} (Table 1A) was variable, tended to increase until 1 h, and then steadily decreased until it became significantly lower than resting values at 7 and 10 h.

Plasma lactate and glucose (Table 1B,C, respectively) peaked at the start of recovery and steadily declined until returning to resting values at 10 h. Because of the variability in the lactate accumulation after submergence (mean=21.0 mmol l^{-1}; S.D.=4.5 range=13.7–30.1) and because the rate of lactate disappearance for a given time period is dependent on the lactate concentration at the start of the period in question, we also expressed the recovery plasma lactate concentrations as a % change per hour from the previous time point (Fig. 4). This rate was highest during the first 2 h recovery than during the subsequent 6 h in all treatments except the passive recovery/trained group, indicating a biphasic pattern to the recovery process with respect to plasma lactate. The 2 h active group had the fastest rate of decrease during the second hour, and was significantly higher than the other treatments at this time point and remained at these levels until the animals stopped swimming.

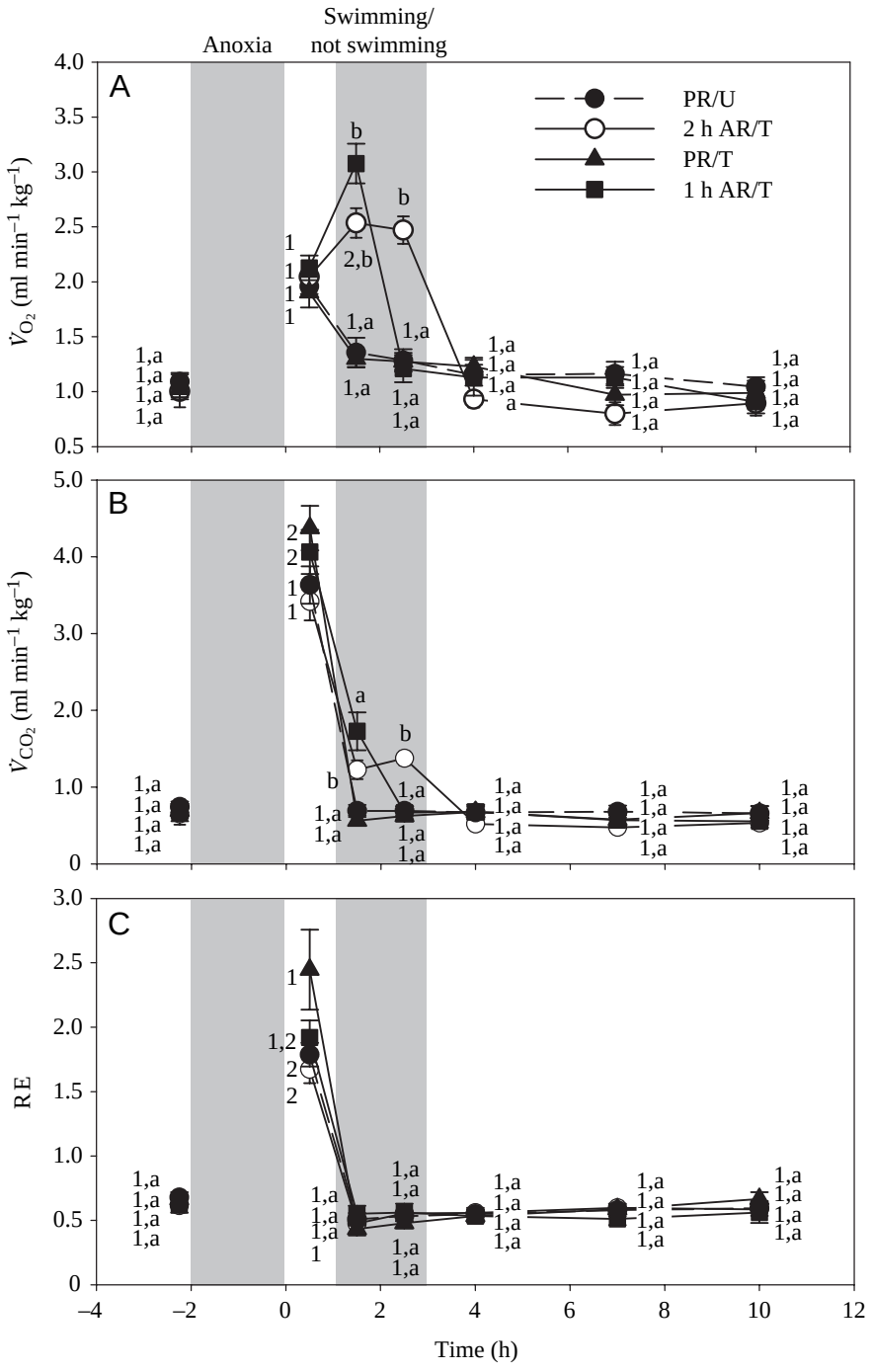

Fig. 2. (A) Oxygen consumption rate ($\dot{V}_{O_2}$), (B) carbon dioxide production rate ($\dot{V}_{CO_2}$) and (C) respiratory exchange ratio (RE) in trained and untrained turtles swum from 0 to 2 h during recovery from anoxic submergence. Values are mean ± S.E.M. (N=6–9). Differing letters indicate significant differences between time points within a treatment. Differing numbers indicate significant differences between treatments at a specific time point (two-way RM-MANOVA, Student's t-tests, P<0.05). PR, passive recovery; AR, active recovery; U, untrained; T, trained.

Oxygen consumption versus *lactate disappearance*

To determine if there was any relationship between metabolic rate and the rate of lactate disappearance, we plotted the percentage change in plasma lactate concentration against $\dot{V}_{O_2}$ for the second recovery hour (first hour of swimming or not swimming) and for the third and fourth recovery hours (second hour of swimming or not swimming + one hour of not swimming) (Fig. 5). The third and fourth recovery hours were pooled in this way

because plasma lactate data were not collected at the end of the third recovery hour in the 1 h active group or either of the passive groups. There was a significant correlation between these two rates (P<0.001, r=0.52), indicating that the rate of lactate disappearance is positively correlated with metabolic rate.

Discussion

These experiments yielded several important findings. Painted turtles are able to fully restore their blood pH by the end of the first recovery hour following 2 h of anoxic submergence at 25°C. Complete recovery from this metabolic insult requires 7–10 h. Despite a large circulating lactate load (~15 mmol l^{-1}), painted turtles can sustain moderate swimming intensities for at least 2 h without compromising metabolic recovery. Lastly, the rate of lactate disappearance from plasma is correlated with the rate of oxygen consumption during hours 2–4 of the recovery period, although this did not result in consistently faster restoration of plasma lactate in the active recovery groups.

Two hours of anoxic submergence at 25°C in painted turtles leads to a significant acidemia, similar to that seen in the closely related red-eared slider turtle in an anoxia experiment under similar conditions (Jackson and Silverblatt, 1974). This acidemia is caused by a combined respiratory acidosis, with mean arterial P_{CO_2}=47 torr (1 torr=133.3 Pa), and a metabolic acidosis, with mean plasma lactate elevated to 21 mmol l^{-1} and plasma [HCO_3^-] depleted to 18 mmol l^{-1} from 31 mmol l^{-1}. These lactate and HCO_3^- changes are equivalent to those in iguanas and varanid lizards after exhaustive exercise at 35°C (Gleeson and Bennett, 1982). Although the duration of anoxia in these experiments was long (2 h) compared to the anoxia tolerance other vertebrates, turtles are known to recover from up to 4 and even 7 h of anoxia at similar temperatures (Jackson, 1968).

Upon re-emergence, painted turtles hyperventilated for the first hour, eliminating the respiratory acidosis and compensating for the lactic acidosis, thereby restoring blood pH. These responses mirrored those of other reptiles after exhaustive exercise (Gleeson, 1982, 1996; Gleeson and Dalessio, 1989), in which the RE values reach similar values for a similar duration. Both varanids and iguanas recover much faster with respect to plasma lactate and blood HCO_3^-, requiring less than 1 h to return to resting values, a difference we attribute partly to the higher recovery

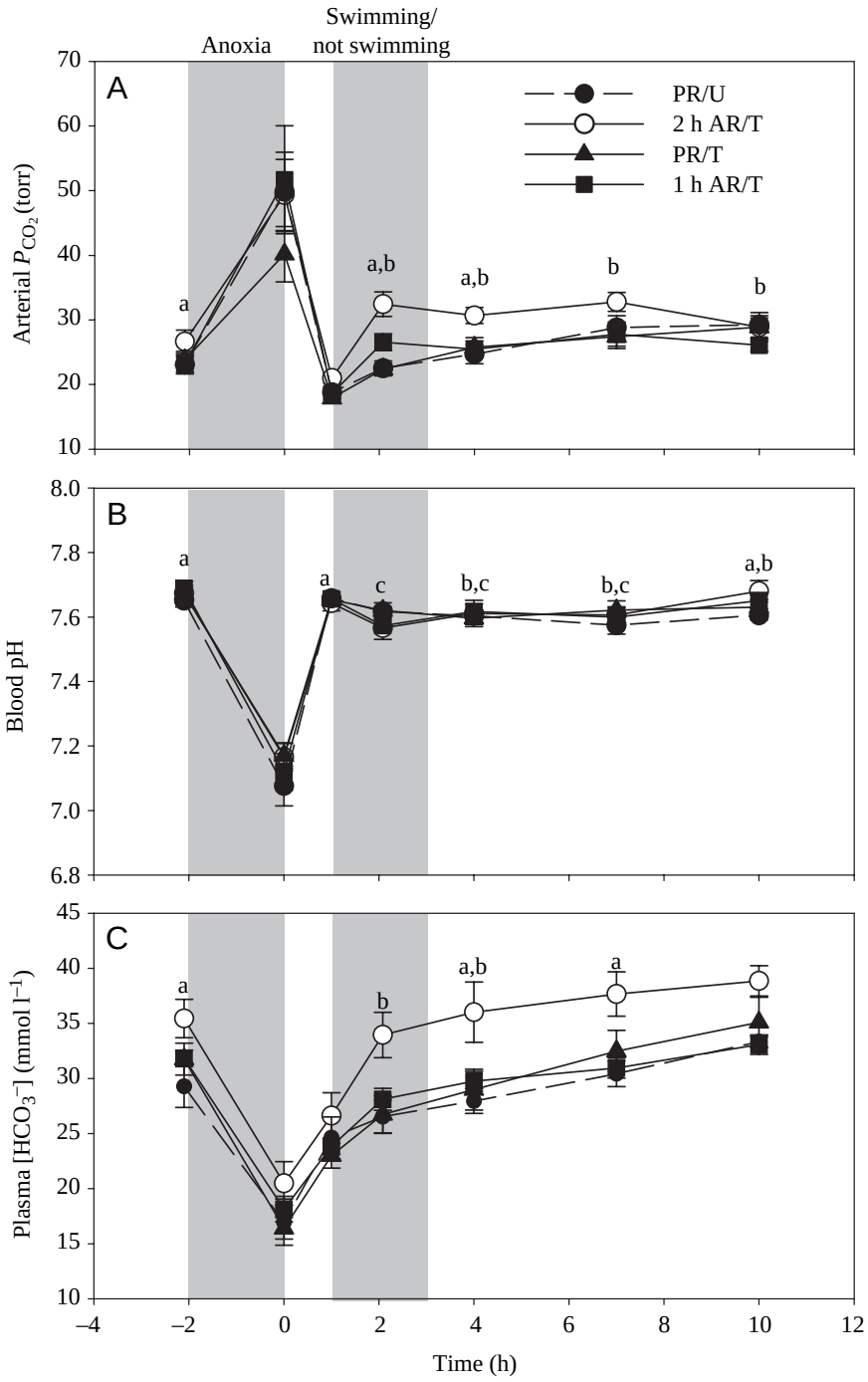

Fig. 3. (A) Arterial P_{CO_2}, (B) blood pH and (C) plasma bicarbonate concentration in trained and untrained turtles swum from 0 to 2 h during recovery from anoxic submergence. There was no significant interaction between time and treatment for any of the parameters. Values are mean ± S.E.M. (N=6–9). The pooled means of the 2 h active recovery/trained treatment were significantly higher than those from the other treatments for arterial P_{CO_2} and blood pH. Differing letters indicate significant differences between time points for pooled means (two-way RM-MANOVA, Student's t-test, P<0.05). PR, passive recovery; AR, active recovery; U, untrained; T, trained. 1 torr=133.3 Pa.

Table 1. *Arterial* P_{O_2}*, plasma lactate and glucose concentrations in trained and untrained turtles swum for 0, 1 or 2 h after 1 h of recovery from anoxic submergence*

A Arterial P_{O_2} (torr)

Recovery time (h)	2 h Active/Trained (*N*=6–7)	1 h Active/Trained (*N*=9)	Passive/Trained (*N*=9)	Passive/Untrained (*N*= 8)	Mean across treatments (*N*=32–33)
Rest	76.3±6.6	74.6±7.4	82.1±9.6	71.5±7.5	76.2±3.9[a,b]
0	78.9±8.2	64.1±8.8	76.0±9.5	65.2±5.8	70.8±4.1[a,c]
1	79.0±6.6	82.4±9.2	78.8±6.7	94.6±4.8	83.7±3.6[b]
2	58.4±7.0	69.3±4.6	71.4±7.1	68.2±6.2	67.3±3.5[a,c]
4	64.5±5.9	72.1±5.6	72.5±8.2	66.1±8.4	69.1±4.3[a,c]
7	53.2±5.3	67.6±9.6	72.4±8.7	63.9±9.5	64.9±4.2[c]
10	70.0±8.6	73.1±9.6	73.1±7.5	77.5±8.7	73.6±3.1

B Plasma [lactate] (mmol l^{-1})

Rest	1.6±0.2	2.0±0.5	1.9± 0.6	2.1±0.5	1.9±0.2[a]
0	21.0±1.7	21.7±1.9	21.2±1.2	20.3±1.6	21.0±0.8
1	15.0±1.5	16.3±1.6	15.4±1.0	15.3±1.1	15.5±0.6
2	9.3±1.1	11.3±1.1	11.7±0.7	11.3±1.0	11.0±0.4
3	6.8±1.0	–	–	–	–
4	5.4±0.8	7.8±1.0	7.8±0.6	7.9±0.8	7.3±0.3
7	3.12±0.5	4.0±0.7	3.8±0.5	4.6±0.6	3.9±0.2
10	2.0±0.4	2.0±0.4	2.0±0.4	2.8±0.5	2.2±0.5[a]

C Plasma [glucose] (mmol l^{-1})

Rest	7.91 ±1.41	9.3±2.0	6.7±1.2	7.0±1.4	7.7±0.8[a]
0	22.1±3.0	24.6±2.2	21.6±2.2	23.8±2.1	23.1±1.1[b]
1	21.3±2.6	23.0±2.3	20.5±1.9	22.8±1.8	21.9±1.0[b,c]
2	19.2±2.4	21.5±2.5	18.6±1.9	20.3±1.4	19.9±1.1[c]
4	14.8±2.0	18.3±2.9	14.2±2.2	15.8±1.2	15.8±1.2
7	9.82±1.76	14.4±3.2	8.7±2.2	10.7±1.1	11.0±1.2[d]
10	7.86±1.68	2.1±3.3	5.8±1.9	7.4±1.0	8.2±1.0[a,d]

Values are means ± S.E.M. 1 torr=133.3 Pa.

There was no significant interaction between time and treatment (two-way RM-MANOVA, $P<0.05$). Differing letters indicate significant differences between time points for combined data across all treatments (Student's *t*-test, $P<0.05$).

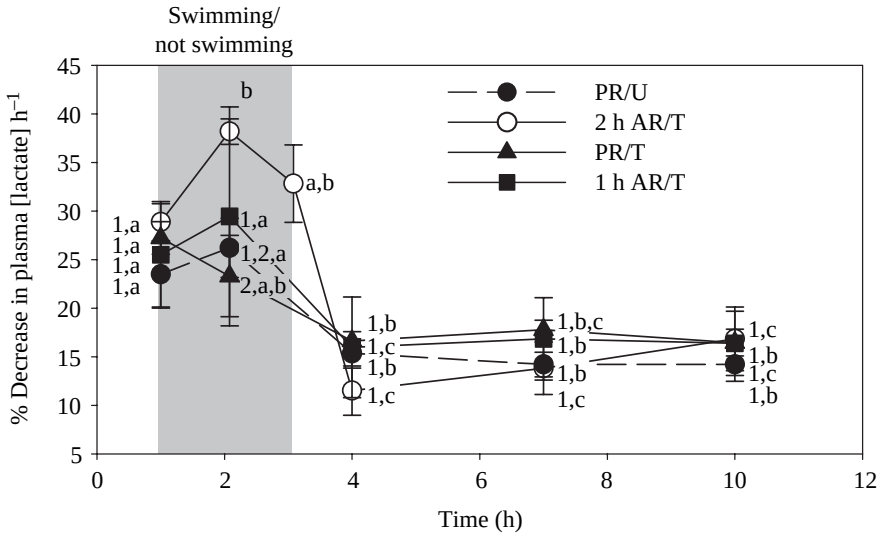

Fig. 4. Percent decrease in plasma lactate per hour in trained and untrained turtles swum from 0 to 2 h during recovery from anoxic submergence. Each point is the % decrease in plasma lactate from the preceding time point divided by the time between the samples. Values are mean ± S.E.M. (*N*=6–9). Differing letters indicate significant differences between time points within a treatment. Differing numbers indicate significant differences between treatments at a specific time point (two-way RM-MANOVA, Student's *t*-tests, $P<0.05$). PR, passive recovery; AR, active recovery; U, untrained; T, trained.

temperatures in the lizard experiments. In addition, because anoxia is a condition that affects all tissues whereas exercise involves primarily the locomotory muscles, the anoxic turtles experienced a greater overall lactate load that may also explain the prolonged elevations in plasma lactate concentration. This lactate would continue to distribute to the extracellular fluid from these tissues until, after conversion to pyruvate, it is either converted back to glycogen or oxidized in the TCA cycle.

At the end of the first recovery hour, elevated plasma lactate levels did not compromise the animal's ability to sustain moderate exercise, which elevated $\dot{V}_{O_2}$ 2–3× over the resting value during the swimming period. We cannot state in absolute terms whether the anoxia diminished swimming capacity because the variables in question were not measured during swimming in animals that were not made anoxic. However, the purpose of the study was to determine the effects of swimming on recovery processes, not the effects of anoxia on swimming capacity.

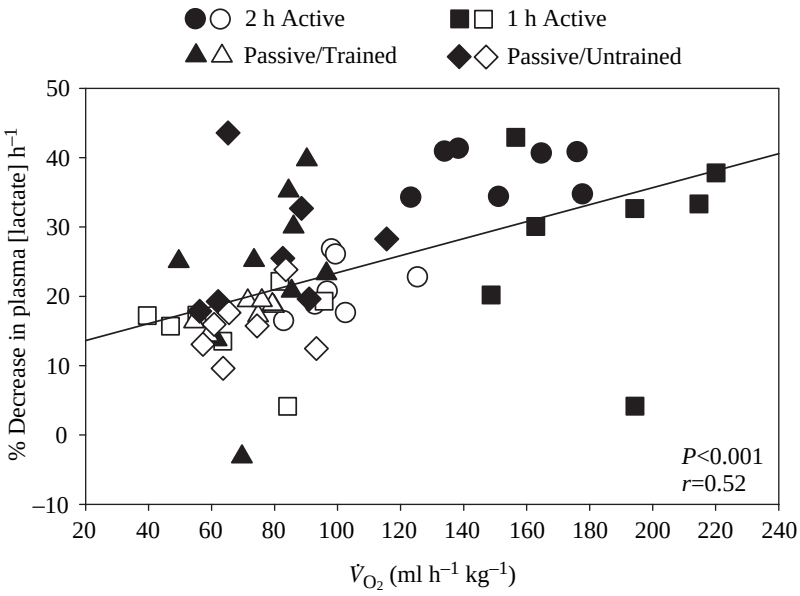

Fig. 5. Percent change in plasma lactate per hour *versus* oxygen consumption rate ($\dot{V}_{O_2}$) for all turtles during the second hour of recovery (first hour of swimming or not swimming; closed symbols) and the third and fourth hours of recovery (second hour of swimming or not swimming + one hour of not swimming; open symbols) following 2 h of anoxia at 25°C. There was a significant correlation between these two rates (P<0.001, r=0.52).

This is the first study in reptiles where animals were exercised during recovery from metabolic acidosis of any kind and the first in any animal following hypoxia or anoxia. Swimming during recovery from anoxia-induced metabolic acidosis did not consistently affect the rate of plasma lactate disappearance (Fig. 4). Although the 2 h active group appeared to recover faster than both passive groups by the second and fourth hours of recovery, the 1 h active group was unaffected at the second hour. If swimming were a major modulator of the lactate recovery kinetics in painted turtles, we would have seen similar responses after the first hour of swimming between both active groups.

Because the metabolic recovery from exercise-induced lactic acidosis in rainbow trout (Milligan et al., 2000) and mammals (Bangsbo et al., 1994; Choi et al., 1994; Peters Futre et al., 1987) is enhanced by light to moderate exercise, we predicted a more pronounced effect on plasma lactate disappearance in our turtles than the one we observed. Despite a similar effect of exercise on the recovery processes of fish and mammals after exercise, the mechanisms for the enhanced recovery are very different between the two classes. In trout, swimming during recovery decreases circulating cortisol, a hormone that inhibits glycogen repletion from lactate in muscle, thereby hastening overall lactate clearance (Milligan, 2003). It is unlikely, however, that corticosteroids play a major role in lactate metabolism during recovery from anoxic submergence in turtles. Corticosterone decreases during anoxia at 5°C in painted turtles and does not exceed normoxic levels during recovery (Keiver et al., 1992) and lactate metabolism by lizard muscle is unaffected by corticosterone (Gleeson et al., 1993).

The faster recovery in mammals is due to a combination of greater lactate oxidation by moderately exercising muscle and enhanced clearance of lactate from the sites of production by increased blood flow (Gladden, 2000). The correlation between the rates of lactate disappearance and oxygen consumption (Fig. 5) suggests that this mechanism might be working similarly in turtles. However, the weak correlation between the two parameters and relatively high variability in the rate of lactate disappearance from plasma potentially reflect the overlap of the two complex processes, exercise and recovery from anoxia.

A number of factors could account for this variable recovery response of the turtles to swimming. First, and simplest, is that lactate could have been generated during the swimming period in some of the animals. We consider this unlikely because all RE values were below 0.8, the $\dot{V}_{O_2}$ values reached during exercise were only 20–30% of aerobic scope (Gatten, 1974), and the exercise was sustainable for several hours.

A second explanation is that $\dot{V}_{O_2}$ was not significantly elevated long enough or high enough to have had a large effect on lactate metabolism. We do not think that swimming the animals faster, thereby elevating $\dot{V}_{O_2}$, would have had much of an effect because the 1 h active group, which had a slightly but significantly higher $\dot{V}_{O_2}$ than the 2 h active group during the first hour of exercise, seemed to be affected less (Fig. 4). Swimming the turtles for a longer period, thereby maintaining elevated $\dot{V}_{O_2}$ for a longer duration, might have affected recovery more. The pattern of lactate decrease in Fig. 4 supports this hypothesis, although it appeared that the effectiveness of swimming at maintaining a high rate of plasma

lactate disappearance was beginning to decrease, casting some doubt on how much a longer swimming period would have enhanced recovery.

Third, it is possible that, in some animals, increased lactate oxidation was offset by decreased lactate used for glycogen repletion in liver and other tissues. This explanation is plausible because exercise decreases gut blood flow in some fishes (Axelsson and Fritsche, 1991; Farrell et al., 2001), and if it occurred in our experiments, it could have reduced hepatic gluconeogenesis from lactate, an important source of lactate removal in turtles recovering from anoxia (Jackson et al., 1996).

A fourth explanation is that in some of the animals, the exercise altered the proportions of the lactate load that were oxidized or used as a gluconeogenic substrate, which would occur if the turtles were also using fuels other than lactate to support aerobic ATP production during the swimming, specifically fatty acids. Mammals use primarily fats during continuous, low intensity exercise and carbohydrates during high intensity exercise (Christmass et al., 1999). In rainbow trout, fuel use is speed- and temperature-dependent, with fats being the favored substrate at warmer temperatures and at lower speeds (Kieffer et al., 1998). Ecological evidence supports this scenario as painted turtles emerging from winter hibernation have been found to have 70% of their glycogen stores depleted while only depleting 40% of their fat (Crawford, 1994). From this, it is logical to think that turtles might be adapted to use fat to sustain activity during recovery from anoxia without compromising replenishment of precious glycogen stores. The low RE values observed in these experiments support this hypothesis, although these are difficult to interpret because of the high probability that the animals were retaining CO_2 during recovery. This is supported by the observation that the passive/trained group had a RE significantly lower than resting values during the second recovery hour (Fig. 3C).

These experiments also found that training had no effect on recovery. This is not surprising as reptiles do not increase their aerobic capacity in response to 8 weeks of endurance training (Garland et al., 1987) and, because our turtles were trained for only 2 weeks, there was probably not sufficient time for any physiological changes to occur even if the potential existed. In contrast, endurance-trained mammals recover more quickly than untrained mammals from exercise-induced lactic acidosis (Gladden, 2000).

In conclusion, recovery metabolism in painted turtles after 2 h of anoxic submergence at 25°C is not affected by 1 or 2 h of moderate exercise. The rate of lactate disappearance is correlated with the metabolic state of the animal, but this correlation does not translate into a consistently faster recovery when metabolic rate is increased through swimming in these experiments. Future studies should investigate fuel utilization during recovery from anoxia in resting and elevated metabolic states and whether swimming can limit the liver's capacity for gluconeogenesis in turtles.

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