Effects of temperature on anoxic submergence: skeletal buffering, lactate distribution, and glycogen utilization in the turtle, *Trachemys scripta*

Daniel E. Warren and Donald C. Jackson
Department of Molecular Pharmacology, Physiology, and Biotechnology, Brown University, Providence, Rhode Island

Submitted 13 March 2006; accepted in final form 19 March 2007

Warren DE, Jackson DC. Effects of temperature on anoxic submergence: skeletal buffering, lactate distribution, and glycogen utilization in the turtle, *Trachemys scripta*. Am J Physiol Regul Integr Comp Physiol 293: R458–R467, 2007. First published March 29, 2007; doi:10.1152/ajpregu.00174.2006.—To test the hypothesis that submergence temperature affects the distribution of the lactate load and glycogen utilization during anoxia in turtles, we sampled a variety of tissues after 7 days, 24 h, and 4 h of anoxic submergence at 5, 15, and 25°C, respectively. These anoxic durations were chosen because we found that they produced similar decreases in plasma HCO$_3^-$ (~18–22 meq/l). The sampled tissues included ventricle, liver, small intestine, carapace, and the following muscles: flexor digitorum longus, retrahens capitis, iliofibularis, and pectoralis. Shell and skeleton sequestered 41.9, 34.1, and 26.1% of the estimated lactate load at 5, 15, and 25°C. The changes in plasma Ca$^{2+}$ and Mg$^{2+}$, relative to the estimated lactate load, decreased with increased temperature, indicating greater buffer release from bone at colder temperatures. Tissue lactate contents, relative to plasma lactate, increased with the temperature of the submergence. Glucose mobilization and tissue glycogen utilization were more pronounced at 15 and 25°C than at 5°C. We conclude that, in slider turtles, the ability of the mineralized tissue to participate in the buffering of lactate acid during anoxia is inversely related to temperature, causing the lactate burden to shift to the tissues at warmer temperatures. Muscles utilize glycogen during anoxia more at warmer temperatures.

anoxia; lactic acidosis

Turtles are the most anoxia-tolerant air-breathing vertebrates because of their abilities to enter a hypometabolic state, exploit their large glycogen stores, and buffer lactic acidosis (14). An extreme example is the painted turtle, *Chrysemys picta*, which can recover from 13 wk of anoxic submergence at 3°C, during which time plasma lactate can reach nearly 100 mmol/l (13). The turtle’s large skeleton, ~38% of the turtle body mass, contributes significantly to the buffering of this extreme lactic acidosis by releasing Ca$^{2+}$ and Mg$^{2+}$ carbonates, which buffer H$^+$ to form CO$_2$ that diffuses across the integument into the surrounding water, and by sequestering and buffering the lactic acid in the bone itself through a mechanism that probably involves the complexing of Ca$^{2+}$ to HCO$_3^-$ and lactate (15), resulting in lactate accumulations greater than expected from wet weight alone.

Other animals without large skeletons utilize a variety of strategies to buffer large lactate loads incurred through exhaustive exercise (11). For example, rainbow trout retain 80–85% of the lactate load within the muscle (29), a strategy believed to minimize the extracellular acid-base consequences of the large lactate load generated during burst exercise. Mammalian muscles, in contrast, “shuttle” lactate from exercising muscle to other tissues where it can be metabolized (2). The strategy of exercising lizards falls between these two extremes; lizards can transport some lactate into the extracellular fluid only to take it back up during recovery to replenish muscle glycogen stores (11).

Less understood is the effect of temperature on the buffering of lactic acid in vertebrate ectotherms. Temperature is a critically important environmental determinant of most physiological processes in turtles and affects metabolic rate, acid-base status, and cardiorespiratory function. Past studies (16, 19) have shown that, at low temperature, bone accumulates more lactic acid than at warmer temperatures, but plasma lactate levels also typically reach higher values. A focus of this study is to determine whether this higher shell lactate reflects a shift in the lactate-buffering strategy or if it is simply a consequence of a larger lactate load. We hypothesized that, if turtles use their shell as a buffer less at warmer temperatures, they would retain more lactate in the tissues to minimize the extracellular acid-base consequences of lactic acid accumulation.

Accompanying lactate accumulation during anoxia in turtles is the utilization of tissue glycogen, which has been investigated mostly in liver, heart, and brain (4, 5, 20, 21, 23, 32, 41). Glycogen utilization during anoxia has not been studied in the context of varied submergence temperature. Because temperature directly determines metabolic rate and the rates of enzymatic processes associated with glucose mobilization, we expect there to be a differential effect on the pattern of glycogen utilization. Knowledge of glycogen utilization is important in gauging tissue-specific metabolic demands and, because most of the lactate load is used to replenish glycogen (19), in identifying the tissues that are most important in the recovery process postanoxia. Because tissue glycogen utilization has been shown to be an important factor in determining anoxia tolerance in turtles (40), knowing how temperature affects its utilization will help elucidate which factors limit anoxia tolerance at warmer vs. colder temperatures.

In this study, we tested the hypothesis that anoxic turtles use their shell less as a buffer at warmer temperatures and, as a consequence, rely more on tissue buffers for this purpose. We also investigated the effects of submergence temperature on glycogen utilization, an understudied but critically important determinant of anoxia tolerance in turtles. Submergence times were based on the time required to cause similar decreases in extracellular HCO$_3^-$, determined from preliminary experiments. Our results show that anoxic turtles maintain a larger...
fraction of the lactate load in the tissues at warmer temperatures, where bone contributes less. Our results also indicate that muscle glycogen is utilized more in turtles that are anoxic at warmer temperatures.

**MATERIALS AND METHODS**

**Animals.** Red-eared slider turtles, *Trachemys scripta* (body mass of 309.9–625.8 g), were obtained from Lemberger (Oshkosh, WI) and maintained at Brown University under a 12:12-h day-night photoperiod at temperatures ranging from 20 to 25°C with access to clean Providence tap water, heating lamps, and a warming platform. Turtles were fed Turtle Brittle (Nasco, Fort Atkinson, WI) ad libitum every other day until they were used in experiments. Experiments were completed between May and October.

The experimental protocol used in this study was approved by the Brown University Institutional Animal Care and Use Committee.

**General protocol.** The general protocol used in these experiments consisted of cannulation and recovery of turtles, followed by acclimation to 5, 15, or 25°C. Turtles were attached to bricks (19 × 9 × 30 cm) with adhesive tape and placed in plastic containers (13 × 28 × 11 cm, width × length × height) filled with 2.5 liters of water, which still allowed the turtle access to air. Half of the turtles at each temperature were prevented from having access to air by raising the water level to begin the anoxic submergence. The remaining half was left undisturbed with access to air. The normoxic or anoxic periods lasted 7 days, 24 h, and 4 h at 5, 15, or 25°C, respectively. These times were chosen because they resulted in similar degrees of plasma HCO₃⁻ depletion and, therefore, extracellular metabolic acidosis. Arterial pH could not be used for this purpose because it shows large changes with temperature and P₂O₃. Plasma lactate was not used because it does not necessarily relate to the total lactate load of the animal. Additional details concerning cannulations, acclimations, and sampling are described below.

**Cannulation.** All turtles were fasted for 3 days before cannulation. The cannulation procedure was similar to that described in a previous study (19). While the turtle was under isoflurane anesthesia, a 2.5-cm hole was cut in the plastron through the right pectoral scute with a trephine and the right subclavian artery was isolated and cannulated occlusively with an Intramedic PE90 catheter (Becton Dickinson, Sparks, MD). The catheter was flushed and filled with 20 IU/ml heparinized 0.8% NaCl solution and led out of a hole pierced in the skin of the neck. An acrylic plug, machined to match the diameter of the trephine, was placed in the hole that was cut in the plastron and sealed with dental acrylic (Bosworth Original Truliner, Stokie, IL). The animals were ventilated with oxygen until recovery (1–3 h) and placed on a diaper in covered plastic containers with the dimensions 13 × 38 × 79 cm (height × width × length) until the following day.

**Temperature acclimation.** Turtles were acclimated to 5, 15, and 25°C as described in a previous study of anoxic turtles (13). Experiments were performed in groups of three or four turtles at 5°C and one or two turtles at 15 and 25°C. For the 5°C group, turtles were acclimated starting the day after surgery by placing them in a 36-liter aquarium at 15°C and lowering the temperature 2°C every day for 5 days. After 1 full day at 5°C, the turtles were attached to the bricks and placed in the plastic containers as described above. For the 15 and 25°C groups, turtles were attached to bricks, placed in tanks regulated at 15 and 25°C with access to air, and acclimated to the experimental situation overnight. Turtles were considered acclimated to each temperature after these times because their blood acid-base status in control samples was consistent with what has been seen in studies of alpha-stat regulation in turtles (28). Food was withheld from all turtles because turtles acclimated to 15 and 25°C do not eat. Pond turtles can fast for 18–22 wk without a noticeable effect on tissue glycogen and metabolic enzyme activity (31).

**Blood sampling.** After 1 full day in the experimental apparatus, turtles from each temperature were randomly assigned to the anoxic group or the normoxic group (n = 9, 6, and 7 turtles per group at 5, 15, and 25°C, respectively). A 1-ml control blood sample was drawn anaerobically from all turtles into a syringe and was analyzed for arterial PO₂, PCO₂, and pH and plasma lactate, glucose, Mg²⁺, and Ca²⁺. For anoxic turtles at 5°C, a plastic sheet with exit ports for catheters and gas-line tubing was placed over the containers. An additional 1.5 liters of water was added to each turtle’s container to prevent access to air, and four bubbling stones were threaded through ports into the water surrounding the animal. Nitrogen gas was bubbled throughout the submergence to displace oxygen from the water and reduce or eliminate transcutaneous oxygen consumption. The PO₂ of the water was ~20 Torr or less throughout the experiment. Additional 0.3-ml blood samples were taken at 1, 4, 5, and 6 days and analyzed only for ions and metabolites. A final 1-ml blood sample was taken on day 7 and was analyzed for blood acid-base status, ions, and metabolites. Normoxic turtles at 5°C were left undisturbed with air access and were sampled similarly after 4, 5, and 6 days.

Turtles were also made anoxic at 15 and 25°C by raising the water level high enough to prevent access to air except that the water was not bubbled with nitrogen. As a result, the PO₂ of the water was presumed to be similar to that of ambient air. At these higher temperatures, transcutaneous oxygen consumption has been found to be insignificant (1). Blood was sampled after 3, 6, 16, and 24 h from anoxic 15°C turtles and after 1, 2, 3, and 4 h from anoxic 25°C turtles. The middle 0.3-ml samples were analyzed only for ions and metabolites, and the final 1-ml samples were analyzed for ions, metabolites, and blood acid-base status. Normoxic turtles at 15 and 25°C were left undisturbed and were euthanized and sampled as described below.

**Tissue sampling.** Immediately after the final blood sample, the turtles were euthanized with an overdose of Beuthanasia-D Special (Schering-Plough, Millisborough, DE) via the cannula. The turtles were quickly removed from their chambers, the plastron was removed with a bone saw, and the following tissues were sampled and quickly flash frozen in clamps cooled with liquid nitrogen: ventricle, liver, small intestine, carapace, pectoralis muscle, retractor carpopatagialis muscle (RC), flexor digitorum longus muscle (henceforth FD or simply flexor digitorum), and iliofibularis muscle. FD is composed primarily of oxidative muscle fibers [49% slow-oxidative (SO), 41% fast-oxidative glycolytic (FOG)], whereas iliofibularis and RC are composed primarily of fast glycolytic (FG) fibers (iliofibularis: 35.2% FOG, 49.3% FG (3, 24)). To our knowledge, the composition of pectoralis muscle primarily of fast glycolytic (FG) fibers (iliofibularis: 35.2% FOG, 49.3% FG (3, 24)). To our knowledge, the composition of pectoralis muscle is not known. All tissues were stored at −75°C until analyzed for lactate. Soft tissues were also analyzed for glucose and glycogen content.

**Blood and plasma analyses.** The 1-ml samples taken at the start and finish of the experiment were analyzed for blood acid-base status. Approximately 0.3 ml was immediately used to measure arterial PO₂ and PCO₂ with the use of a Radiometer PHM73 pH/blood-gas monitor and BMS3 Mk2 blood microsystem (Radiometer, Copenhagen, Denmark) thermostatted to 5, 15, or 25°C. The remainder (~0.7 ml) was immediately injected through a 4-cm segment of Intramedic polyethylene (PE)-60 into the bottom of a 10-mm-diameter round-bottom test tube containing an Orion pH electrode (8103BN; Thermo Orion, Beverly, MA) with a 6.3-mm diameter. With the blood sample, PE tubing, and pH electrode in the tube, the surface area for gas exchange was 46.2 mm² with 58 mm between the air-blood interface and the opening of the tube. The pH electrode and test tube were thermostated to the temperature of the BMS3 Mk2 by storing them in one of its ampoule wells before and during each measurement. This technique is similar to the one used in a previous study (17) and gives pH values similar to those that used other methods. Plasma HCO₃⁻ was calculated with the Henderson-Hasselbach equation at each temperature with pK values of 6.217, 6.189, and 6.1295 (30) and the carbon...
dioxide solubility coefficient \( \Delta \text{CO}_2 \) of 0.0748, 0.0539, and 0.0404 (34) at 5, 15, and 25°C, respectively.

The recovered blood from the pH measurements and the 0.3-ml samples from the middle time points were analyzed for their plasma concentrations of lactate and glucose with the use of an automated analyzer (2300 STAT, Yellow Springs Instruments, Yellow Springs, OH). Plasma \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \) were measured by atomic absorption spectrophotometry (model 280; Perkin-Elmer, Boston, MA).

**Tissue analyses.** All soft tissues were analyzed for lactate, glucose, and glycogen. Carapace was analyzed for lactate only. Frozen samples of all tissues (100–200 mg), except small intestine, were homogenized in 1 ml of ice-cold 0.6 N perchloric acid by using a Mini-Beadbeater 3110BX (Biospec Products, Bartlesville, OK) with 1-mm glass beads for 4 min as described previously (40). Frozen small intestine was first ground to a fine powder with a mortar and pestle under liquid nitrogen before it was homogenized with the Mini-Beadbeater. A 150-μl sample of this homogenate was buffered with 75 μl of 1 M KHCO₃, centrifuged for 3 min at 9,300 g, and analyzed for glucose and lactate with standard kits (glucose assay reagent: Sigma, St. Louis, MO; lactate reagent: Trinity Biotech, St. Louis, MO). A 50-μl sample of the homogenate was buffered with 25 μl of 1 M KHCO₃ and analyzed for glycogen using the amyloglucosidase method of Keppler and Decker (23a). This homogenate was analyzed for glucose as described above. The tissue glycogen content was calculated as the difference between the glucose content after glycogen hydrolysis and the free glucose content from the tissue homogenate.

Frozen carapace (~1 g) was pulverized to a powder under liquid nitrogen (Spex 6700 Freezer Mill). The powder was incubated in 5 vol of 0.6 N perchloric acid for 2 h, vortexing every 15 min. A sample of this homogenate was centrifuged at 9,300 g, and lactate was measured on the supernatant by a standard kit (lactate reagent; Sigma, St. Louis, MO). A 50-μl sample of this homogenate was centrifuged at 9,300 g, and analyzed for glucose and lactate with standard kits (glucose assay reagent: Sigma, St. Louis, MO; lactate reagent: Trinity Biotech, St. Louis, MO). A 50-μl sample of the homogenate was buffered with 25 μl of 1 M KHCO₃ and analyzed for glycogen using the amyloglucosidase method of Keppler and Decker (23a). This homogenate was analyzed for glucose as described above. The tissue glycogen content was calculated as the difference between the glucose content after glycogen hydrolysis and the free glucose content from the tissue homogenate.

**Statistical analysis.** Two-factor repeated-measures multivariate ANOVA was used to determine whether tissue type affected tissue lactate, glucose, and glycogen contents, also at each temperature. When distributions were nonnormal, the data sets were log transformed. Raw data were not directly compared statistically across temperatures. However, one-factor ANOVA was used to determine whether temperature affected the ratio of tissue lactate to plasma lactate for each tissue. Student’s t-tests were used to elucidate significant interactions. The significance value was \( P < 0.05 \). All computations were carried out with JMP 5.0 (SAS Institute, Cary, NC).

### RESULTS

#### Blood acid-base status.

The effects of 7 days, 24 h, and 4 h of anoxia at 5, 15, and 25°C, respectively, on the blood acid-base status are summarized in Table 1. Mean arterial PO₂ at the end of anoxic submergence was <1 Torr at all temperatures, indicating that the experimental setup was sufficient to induce extremely severe hypoxia such that we refer to it as anoxia. From a previous study (27), we estimated that the arterial PO₂ results of anoxic turtles in the present study, which ranged from 0.4 to 0.6 Torr, translated into hemoglobin saturations that ranged from 4% at 5°C to 0.2% at 25°C.

Although there were differences in mean arterial PO₂ between controls for the groups at each temperature, we do not believe these are physiologically important because they reflect the variability stemming from the turtle’s capacity for intracardiac shunting and because the PO₂ results were well above the \( \text{O}_2 \) half-saturation pressure results for turtles at these temperatures (26).

Mean arterial PCO₂ was elevated from controls at all temperatures in anoxic animals, indicating a respiratory acidosis. This contributed to a significant acidemia in anoxic turtles at all temperatures. All anoxic turtles incurred a metabolic acidosis as indicated by reduced plasma HCO₃⁻ concentration. The total change in plasma HCO₃⁻ concentration across temperatures was similar (18–22 meq/l), indicating a similar degree of extracellular metabolic acidosis. With the exception of a very small decrease in arterial pH at 5°C, compared with anoxia-induced changes, none of the acid-base variables changed at any temperature during the simultaneous normoxic sampling period.

#### Plasma glucose.

The changes in plasma glucose during the 7 days, 24 h, and 4 h at 5, 15, and 25°C, respectively, are summarized in Table 2. Plasma glucose decreased in normoxic turtles at 5°C and increased in anoxic turtles at 15 and 25°C. At 5°C, plasma tended to increase but did not reach statistical significance. In anoxic turtles at 15 and 25°C, plasma glucose was elevated after 6 and 1 h, respectively, and continued to increase steadily throughout the remainder of the submergence. Qualitatively, the highest plasma glucose occurred at the end of anoxia at 15°C and was higher than all of the tissues.

#### Tissue-specific glycogen utilization and glucose concentrations.

The effects of 7 days, 24 h, and 4 h of anoxia at 15, and 25°C, respectively, on tissue glycogen are summarized in

### Table 1. Blood acid-base data from anoxic red-eared slider turtles

<table>
<thead>
<tr>
<th>Arterial PO₂, Torr</th>
<th>Arterial PO₂, Torr</th>
<th>Arterial pH</th>
<th>Plasma HCO₃⁻, meq/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>End Exp</td>
<td>Control</td>
</tr>
<tr>
<td>5°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>33.3 ± 9.9</td>
<td>15.5 ± 6.5</td>
<td>11.4 ± 0.4</td>
</tr>
<tr>
<td>7-day Anoxia</td>
<td>16.9 ± 4.1</td>
<td>0.4 ± 0.1*</td>
<td>11.4 ± 0.4</td>
</tr>
<tr>
<td>15°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>36.9 ± 1.7</td>
<td>52.6 ± 7.3</td>
<td>19.2 ± 1.4</td>
</tr>
<tr>
<td>24-h Anoxia</td>
<td>41.8 ± 10.0</td>
<td>0.6 ± 0.2*</td>
<td>18.3 ± 0.9</td>
</tr>
<tr>
<td>25°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>50.0 ± 7.5</td>
<td>49.9 ± 10.7</td>
<td>29.7 ± 2.3</td>
</tr>
<tr>
<td>4-h Anoxia</td>
<td>65.7 ± 9.4</td>
<td>0.5 ± 0.3*</td>
<td>29.3 ± 2.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 6–9 \) turtles for each treatment at each temperature. End Exp, sample taken at the end of the anoxic submergence. *Significant difference between control and End Exp samples for each treatment and temperature, \( P < 0.05 \).
Fig. 1. Anoxia did not affect the glycogen content of liver at any temperature but decreased glycogen at all temperatures in ventricles and FD muscle. The glycogen content of iliofibularis was reduced only at 15°C. The other muscles, pectoralis and RC, had reduced glycogen contents only at 25°C. Small intestine had reduced glycogen only at 5°C.

The effects of the anoxia bouts on tissue glucose concentrations are summarized in Table 3. Anoxia increased tissue glucose at 15 and 25°C in all tissues except small intestine. At 5°C, glucose was elevated only in liver during anoxia. At 15 and 25°C, liver had the highest glucose compared with the other tissues. Glucose was highest in the plasma during anoxia at 15 and 25°C but was similar to all muscles except FD at 5°C. At 15 and 25°C, small intestine had the lowest glucose concentrations during anoxia, whereas ventricle and small intestine had the lowest glucose at 5°C.

**Tissue and plasma lactate accumulation.** The effects of 7 days, 24 h, and 4 h of anoxia at 5, 15, and 25°C, respectively, on plasma lactate are summarized in Fig. 2. Plasma lactate increased steadily in anoxic turtles at all three temperatures. Qualitatively, plasma Ca²⁺ was highest in 5°C turtles but was similar at 15 and 25°C, whereas peak plasma Mg²⁺ was higher at 5 and 15°C relative to 25°C. There were no changes in the plasma concentrations of Ca²⁺ or Mg²⁺ in the normoxic turtles at any temperature.

**DISCUSSION**

These experiments examined lactate distribution, glycogen utilization, and the importance of bone buffering in anoxic turtles.
turtles experiencing similar depletions in plasma \( \text{HCO}_3^- \) at 5, 15, and 25°C. Results have shown that tissue lactate accumulation and glycogen utilization are dependent on the tissue sampled and on the temperature of the anoxic bout. Muscle glycogen was utilized more at warmer temperatures (three muscles at 25°C, two at 15°C, and one at 5°C), whereas liver glycogen did not change at any temperature. Qualitatively, tissue and plasma glucose and plasma \( \text{Ca}^{2+} \) responses tended to be more similar to one another at 15 and 25°C than to those at 5°C. The contribution of the bone buffering mechanisms decreased with temperature. In contrast, the nonskeletal tissue lactate concentrations, relative to plasma, generally increased with temperature. These results indicate that the ability of the mineralized tissue to participate in the buffering of lactic acid during anoxia is inversely related to temperature and suggests that the burden of lactic acid buffering is transferred to soft tissues at warmer temperatures. Glycogen is probably an important energy source in muscle during anoxia at warmer temperatures.

**Pattern of glycogen utilization.** The rapid and nearly complete depletion of ventricular glycogen (Fig. 1) at all temperatures has been observed in previous studies and shows that the heart quickly becomes reliant on glucose to support ATP production during anoxia. Ventricular glycogen has been shown to be depleted in painted turtles after 28 days of anoxia at 5°C (23) and in red-eared slider turtles after 13 days at 3°C (40).

Despite increases in plasma glucose (Table 2), liver glycogen did not decrease; however, as discussed later, it is most likely because the anoxia duration was too short and the sample size too small (\( n = 6-9 \)) for changes to be detectable. In painted turtles, liver glycogen decreases after 4 h of anoxia at 22°C (21) but not after 6 h at 20°C (41), showing that this parameter is usually variable after anoxic dives of relatively short duration.

This study differs from previous studies in that muscle glycogen was utilized in at least one muscle at each temperature, with more muscles utilizing glycogen as submergence temperature increased. Previous studies of muscle glycogen found very little change during anoxia in painted turtles at 20°C (41) or in musk turtles at 10°C (20). In all of these previous experiments, however, pectoralis was sampled as the representative muscle. In the present study, pectoralis glycogen decreased only at 25°C, indicating future investigations into muscle glycogen utilization in anoxic turtles should sample a variety of muscles.

It is notable that FD, a highly oxidative muscle consisting of 49% SO and 41% FOG fibers, had the highest resting glycogen content of the muscles sampled (Fig. 1). This contrasts with the

### Table 3. Tissue glucose concentrations from normoxic and anoxic red-eared slider turtles

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normoxia</th>
<th>5°C</th>
<th>15°C</th>
<th>25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (μmol/l)</td>
<td>4.02</td>
<td>4.02</td>
<td>4.02</td>
<td>4.02</td>
</tr>
<tr>
<td>Liver glucose (μmol/g)</td>
<td>0.83</td>
<td>0.83</td>
<td>0.83</td>
<td>0.83</td>
</tr>
<tr>
<td>Small intestine glucose (μmol/l)</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td>Ventricle glucose (μmol/l)</td>
<td>1.81</td>
<td>1.81</td>
<td>1.81</td>
<td>1.81</td>
</tr>
<tr>
<td>Flexor Digitorum glucose (μmol/l)</td>
<td>1.98</td>
<td>1.98</td>
<td>1.98</td>
<td>1.98</td>
</tr>
<tr>
<td>Iliofibularis glucose (μmol/l)</td>
<td>2.69</td>
<td>2.69</td>
<td>2.69</td>
<td>2.69</td>
</tr>
<tr>
<td>Pectoralis glucose (μmol/l)</td>
<td>2.78</td>
<td>2.78</td>
<td>2.78</td>
<td>2.78</td>
</tr>
<tr>
<td>Retrahens Capitis glucose (μmol/l)</td>
<td>1.04</td>
<td>1.04</td>
<td>1.04</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Values are means ± SE in μmol/l or μmol/g; \( n = 6-9 \) for each treatment and temperature. a,b,c,d Differing letters indicate a significant difference between tissues for each treatment at each temperature, \( P < 0.05 \). *Significant difference between anoxia and normoxia for a specific temperature and tissue, \( P < 0.05 \).
Tissue lactate concentrations from normoxic and anoxic red-eared slider turtles

Table 4.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normoxia</th>
<th>15°C</th>
<th>25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal Muscles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricles</td>
<td>1.28±0.28</td>
<td>1.42±0.23</td>
<td>1.28±0.28</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.93±0.09*</td>
<td>0.93±0.09*</td>
<td>1.03±0.10</td>
</tr>
<tr>
<td>Liver</td>
<td>1.86±0.26</td>
<td>1.86±0.26</td>
<td>1.86±0.26</td>
</tr>
<tr>
<td>Plasma</td>
<td>1.28±0.28</td>
<td>1.42±0.23</td>
<td>1.28±0.28</td>
</tr>
<tr>
<td>Normoxia</td>
<td>3.94±0.02</td>
<td>4.92±0.09</td>
<td>4.92±0.09</td>
</tr>
<tr>
<td>7-day Anoxia</td>
<td>3.39±0.06</td>
<td>3.39±0.06</td>
<td>3.39±0.06</td>
</tr>
<tr>
<td>15°C Anoxia</td>
<td>3.39±0.06</td>
<td>3.39±0.06</td>
<td>3.39±0.06</td>
</tr>
<tr>
<td>24h Anoxia</td>
<td>1.34±0.55</td>
<td>1.34±0.55</td>
<td>1.34±0.55</td>
</tr>
<tr>
<td>25°C Anoxia</td>
<td>3.06±1.17</td>
<td>3.06±1.17</td>
<td>3.06±1.17</td>
</tr>
</tbody>
</table>

Values are means ± SE (animal or turtle; n = 6–9 for each treatment and temperature). *Significant difference between normoxic and anoxic for a specific tissue and temperature. **Significant difference between normoxic and anoxic for a specific tissue and temperature. ***Significant difference between normoxic for a specific tissue and temperature. #Significant difference between normoxic and anoxic for a specific tissue and temperature. P < 0.05.

The most likely explanation for the higher muscle glycogen utilization at warmer temperatures is a larger catecholamine response than that shown at colder temperatures. It has been previously shown that the catecholamine response in anoxic turtles is much larger at 22°C than at 5°C (21, 22). Catecholamines have been shown to stimulate muscle glycogenolysis in mammals (12, 33, 42) and rainbow trout (9).

The hyperglycemia observed at 15 and 25°C (Table 2) is most likely caused by catecholamine-induced hepatic glycogenolysis (22), as liver glycogen concentrations exceeded plasma glucose in anoxic animals at all temperatures. Arguing against this suggestion is the absence of differences in liver glycogen between normoxic and anoxic turtles. However, if one assumes an extracellular fluid volume of 25% and liver mass of 6% (40), then the change in liver glycogen required to account for the most extreme hyperglycemia in anoxic 15°C turtles was just 113 µmol/g. For comparison, the standard deviation for liver glycogen for 15°C anoxic turtles was 251 µmol/g (n = 6). Therefore, the magnitude of the changes observed in plasma glucose could have occurred without a significant decrease in liver glycogen.

Skeletal muscle, the major site of glycogen storage in addition to liver, is not a likely source of the increased plasma glucose for two reasons: first, skeletal muscle has low glucose 6-phosphatase activity, the enzyme required to produce free glucose, and, second, glucose concentrations in plasma were greater than those in muscle at all temperatures (Table 3).

Postexercise frogs, in contrast, are known to release glucose from skeletal muscle via glucosidic pathways that produce free glucose (7, 8). In the frogs (8), however, muscle glucose levels were greater than plasma levels.

A notable observation is that normoxic turtles at 5°C showed a progressive decrease in plasma glucose during the course of the experiment (Table 2). To our knowledge, this has not been previously observed in turtles, although it has been seen in the leopard frog (37) after 9 days of acclimation to 6°C. The mechanism for this is unclear but could reflect cold-induced changes in glucose availability that might also contribute to the profound differences in intra- and extracellular glucose concentrations observed at the different temperatures in the present experiment. However, a previous study of unanesthetized red-eared slider turtles that were cold acclimated by using nearly the same protocol showed no difference in plasma glucose after 46 days at 3°C (40), suggesting that the plasma glucose may have been elevated from the surgery and simply returned to normal values over time.

Lactate distribution during anoxia. Turtle bone (shell and skeleton) contributes to lactic acid buffering in two ways: by

mammalian condition in which it is the more glycolytic muscles, composed of FOG and FG muscles, that usually have higher glycogen contents (25). The same paradox exists in the sampled portion of the turtle neck muscle RC, which is composed mostly of FG fibers (3) but had the lowest resting glycogen contents of the four muscles sampled (Fig. 1). These observations are in agreement with a previous histochemical study of turtle muscle, which also showed that the metabolic profiles of turtle muscle are different from those of mammalian muscle for a given fiber type (3). Thus the commonly accepted paradigm relating muscle fiber type and resting glycogen content in mammals does not apply to turtles.

The most likely explanation for the higher muscle glycogen utilization at warmer temperatures is a larger catecholamine response than that shown at colder temperatures. It has been previously shown that the catecholamine response in anoxic turtles is much larger at 22°C than at 5°C (21, 22). Catecholamines have been shown to stimulate muscle glycogenolysis in mammals (12, 33, 42) and rainbow trout (9).

The hyperglycemia observed at 15 and 25°C (Table 2) is most likely caused by catecholamine-induced hepatic glycogenolysis (22), as liver glycogen concentrations exceeded plasma glucose in anoxic animals at all temperatures. Arguing against this suggestion is the absence of differences in liver glycogen between normoxic and anoxic turtles. However, if one assumes an extracellular fluid volume of 25% and liver mass of 6% (40), then the change in liver glycogen required to account for the most extreme hyperglycemia in anoxic 15°C turtles was just 113 µmol/g. For comparison, the standard deviation for liver glycogen for 15°C anoxic turtles was 251 µmol/g (n = 6). Therefore, the magnitude of the changes observed in plasma glucose could have occurred without a significant decrease in liver glycogen.

Skeletal muscle, the major site of glycogen storage in addition to liver, is not a likely source of the increased plasma glucose for two reasons: first, skeletal muscle has low glucose 6-phosphatase activity, the enzyme required to produce free glucose, and, second, glucose concentrations in plasma were greater than those in muscle at all temperatures (Table 3).

Postexercise frogs, in contrast, are known to release glucose from skeletal muscle via glucosidic pathways that produce free glucose (7, 8). In the frogs (8), however, muscle glucose levels were greater than plasma levels.

A notable observation is that normoxic turtles at 5°C showed a progressive decrease in plasma glucose during the course of the experiment (Table 2). To our knowledge, this has not been previously observed in turtles, although it has been seen in the leopard frog (37) after 9 days of acclimation to 6°C. The mechanism for this is unclear but could reflect cold-induced changes in glucose availability that might also contribute to the profound differences in intra- and extracellular glucose concentrations observed at the different temperatures in the present experiment. However, a previous study of unanesthetized red-eared slider turtles that were cold acclimated by using nearly the same protocol showed no difference in plasma glucose after 46 days at 3°C (40), suggesting that the plasma glucose may have been elevated from the surgery and simply returned to normal values over time.

Lactate distribution during anoxia. Turtle bone (shell and skeleton) contributes to lactic acid buffering in two ways: by
releasing Ca\(^{2+}\) and Mg\(^{2+}\) carbonates into the extracellular fluid and by buffering and sequestering lactate within the bone matrix (14, 15). As a consequence, increases in the plasma lactate load will not necessarily cause an equal decrease in extracellular HCO\(_3\)\(^{-}\). In the present study, the increases in plasma Ca\(^{2+}\) and Mg\(^{2+}\) (Fig. 4) and in shell lactate content (Table 4) confirm that anoxic red-eared slider turtles utilize both mechanisms but that they do so more at low temperatures than at high temperatures. Accumulation of Ca\(^{2+}\) and Mg\(^{2+}\) occurs because minimal renal function prevents the excretion of these ions in urine (38). The magnitude of the Ca\(^{2+}\) and Mg\(^{2+}\) increases is, therefore, an indicator of the amount of buffer released from the bone. As shown in Fig. 4, the release of both ions, and therefore the release of buffer, tended to be greater at low temperature.

Lactate uptake into bone is believed to occur by the complexing of lactate to a component of bone, most likely Ca\(^{2+}\), and the buffering of the accompanying H\(^{+}\) by bone carbonate (18). To estimate the bone’s contribution to the buffering of lactic acid at each temperature, we calculated the percentage of total body lactate that resided in bone at the end of the anoxic periods. The total body lactate was determined by multiplying the concentrations of lactate in the various tissues (including bone) and in the extracellular fluid volume times their weights. We assumed that unmeasured muscles and other organs had lactate concentrations equal to the average lactate of the four muscles that we sampled. From the estimates, presented in Table 5, bone sequestration is an important mechanism for buffering lactic acid at all temperatures, but its importance increases as temperature falls. The percentage of total body lactate in bone increased as temperature fell, from 26.1% of the total at 25°C to 41.9% of the total at 5°C. The estimated percentage at 5°C is similar to that shown in painted turtles (Chrysemys picta bellii) at 3 and 10°C, where 44% and 43%, respectively, of the lactate loads were estimated to reside in bone (16).

It is evident from these results that at higher temperatures, where the skeleton is less utilized, lactate is retained within the tissues, particularly in skeletal muscle and liver (Fig. 3). A limitation on lactate washout from muscle is not a likely cause because muscle blood flow increases as a proportion of systemic blood flow in anoxic turtles at 21°C compared with at 5°C (36). Instead, it is more likely that there is a transport limitation in the muscle that occurs along with the higher glycolytic fluxes during anoxia at warm temperatures. Such a transport limitation occurs in trout, which maintain the majority of the lactate load produced during an exercise bout in muscle in part because its white muscle is deficient in lactate uptake.

---

**Fig. 3.** Mean ± SE ratio of tissue lactate (mmol/kg) to plasma lactate (mmol/l) from anoxic animals as a function of temperature; n = 6–9 turtles for each treatment at each temperature. A: small intestine. B: ventricle. C: liver. D: carapace. E: rethraens capitis muscle. F: flexor digitum (digit) muscle. G: iliofibularis muscle. H: pectoralis muscle. Differing letter indicate significant differences between temperatures for each tissue (two-factor ANOVA, Student’s t-test, P < 0.05).
transporters to export the lactate (35). There is evidence that lizard muscle (6) and turtle erythrocytes (39) possess lactate transporters, but, in general, the mechanisms of lactate transport in reptile tissues are not well studied.

The two strategies of lactate sequestration during anoxic submergence, in the soft tissues or in bone, have key similarities and differences with respect to their effects on metabolism and acid-base status. Both strategies minimize the loss of lactate in urine during recovery when the kidney is reperfused, which has been observed in turtles recovering from anoxia at 20°C (19). They also preserve extracellular buffer stores and slow the rate at which extracellular pH falls. The strategies differ in that the total buffering capacity of the skeleton is much higher than that of soft tissue, so a turtle that exploits the shell as a buffer should be able to tolerate larger lactate loads, such as those that occur during overwintering. However, because lactate clearance from bone is a slow process (19), lactate sequestration in the soft tissues instead of bone would enhance metabolic recovery, which might be more important at warmer temperatures, when turtles are likely to accumulate smaller lactate loads resulting from burst exercise or short anaerobic escape dives.

These experiments also show that muscle fiber-type composition does not affect how lactate is distributed during anoxia at any temperature (Table 4). For example, FD, a muscle composed mostly of SO and FOG fibers, had lactate accumulations similar to those of iliofibularis and RC, muscles with predominantly FG fiber types. It has been shown in exercising mammal...
LACTATE AND GLYCOGEN METABOLISM IN ANOXIC TURTLES


Steck JA, Overgaard J, Farrell AP, Wang T. Alpha-adrenergic regu- lation of systemic peripheral resistance and blood flow distribution in the


