Reductions in systemic oxygen delivery induce a hypometabolic state in the turtle *Trachemys scripta*

BJÖRN PLATZACK AND JAMES W. HICKS

*Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92697-2525*

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Platzack, Björn, and James W. Hicks. Reductions in systemic oxygen delivery induce a hypometabolic state in the turtle *Trachemys scripta*. Am J Physiol Regulatory Integrative Comp Physiol 281: R1295–R1301, 2001.—We investigated the effects of vagal reductions in O₂ delivery on oxygen consumption (VO₂) in the anesthetized freshwater turtle *Trachemys scripta*. Specifically, these experiments tested the hypothesis that reductions in arterial oxygen partial pressure (PO₂) and/or systemic oxygen transport (SOT) trigger a metabolic downregulation. During electric stimulation of the efferent branch of the sectioned right vagus nerve (RVEF), systemic cardiac output decreased 60–70%, systemic PO₂ fell by ~30%, and SOT decreased by 60–70%. During RVEF simulation, VO₂ dropped ~35%. During control conditions, injection of the metabolic uncoupler 2,4-dinitrophenol (DNP) more than doubled VO₂, reflecting an increase in ATP turnover. RVEF stimulation after DNP injection produced similar cardiovascular and blood gas changes as before DNP, but VO₂ was higher than the VO₂ measured in untreated control animals, indicating that oxygen availability during RVEF stimulation is still sufficient to support VO₂ rates that are even higher than resting rates. We conclude that vagal stimulation triggers metabolic downregulation, primarily through the effects on oxygen transport, although the factor(s) that trigger the hypometabolism remain unknown. The PO₂ may still be an important messenger in metabolic control, but our results suggest that changes in SOT to the metabolically active tissues, rather than changes in PO₂ per se, play an important role in triggering hypometabolism in the freshwater turtle.

**Oxygen consumption; metabolic rate; vagal control; 2,4-dinitrophenol**

**Most vertebrates have** the ability to reduce metabolic rate in response to reduction in oxygen availability (36). In endothermic vertebrates (i.e., mammals and birds) this metabolic downregulation can be achieved by reducing metabolic heat production resulting in a decrease in oxygen demands (2, 14, 36). In addition, endothermic and ectothermic vertebrates also alter thermoregulatory behavior in heterothermic environments to downregulate body temperature and reduce O₂ demands (36). Although ectothermic animals lack mammalian-like physiological regulatory mechanisms for thermoregulation and rely on altered thermoregulatory behavior for thermoregulatory control in response to lower O₂ levels, they still have the ability to downregulate cellular metabolism and heat production in response to anoxia (3, 11, 21, 28, 37). This metabolic downregulation (the hypometabolic state) is a regulated response that involves changes in active membrane transport and membrane permeability and downregulation of various synthetic pathways, such as protein synthesis (4, 22, 25, 26).

Many reptiles and amphibians exhibit intermittent breathing patterns with brief periods of ventilation interspersed among periods of apnea of variable duration. In turtles, large right-to-left (R-L) intracardiac shunts often develop during apnea, which causes internal hypoaxia (17, 33, 34). This R-L shunt can occur rapidly (s), reducing arterial oxygen levels, increasing arterial partial pressure of carbon dioxide (PCO₂) levels and decreasing pH, as a consequence of recirculated metabolically produced CO₂. In a recent study by Hicks and Wang (20), it was hypothesized that the development of an R-L shunt with its ensuing reductions in arterial PO₂ would induce hypometabolism. They demonstrated that progressive hypoaxia induces hypometabolism in the anesthetized turtle *Trachemys scripta*. These authors suggest that the hypometabolic state may be an important means of oxygen conservation resulting in prolonged aerobic dive times in freshwater turtles.

In *T. scripta*, the rapid onset of a R-L shunt is under vagal control (19). Therefore, the aim of the present study was to test the hypothesis that vagally induced R-L shunts with the subsequent reductions in arterial oxygen partial pressure (PO₂) and/or systemic oxygen transport (SOT) trigger a metabolic downregulation.

**MATERIALS AND METHODS**

**Animals**

The study was performed on freshwater red-eared sliders, *T. scripta* (body mass, 1.0–2.2 kg; mean 1.55 kg; n = 18), obtained from Lemberger (Oshkosh, WI). Animals were housed at room temperature (24–26°C) in a 0.8 × 1.5-m plastic tank containing fresh water and free access to dry and heated areas, allowing for behavioral thermoregulation. Animals were fasted for at least 1 wk before the experiments.

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Animal Preparation

On the day of experimentation, turtles were anesthetized with an intramuscular injection of 50 mg/kg pentobarbital sodium (Veterinary Laboratories). In cases where the pedal withdrawal response did not disappear within 60 min, an additional dose of pentobarbital (25 mg/kg) was injected to abolish the withdrawal response. Animals were placed in a supine position, tracheostomized, and ventilated with room air (3–4 breaths/min) at a tidal volume of 30–40 ml/kg (SAR-830 ventilator, CWE).

An occlusive saline-filled PE-50 or PE-10 polyethylene catheter was inserted in the left femoral artery and advanced toward the heart ~2–3 cm. The catheter was used for blood sampling and infusion of drugs. In seven of the animals (group 2), an additional occlusive saline-filled PE-50 polyethylene catheter was inserted in the left femoral vein for infusion of plasma, saline, and drugs.

To access the central blood vessels, a 3 × 3-cm portion of the ventral plastron was removed with a bone saw. The pectoral muscles were gently loosened from the excised piece, and bleeding from small superficial vessels was stopped by cauterization. For measurements of pulmonary blood flow (Qpul), a 1-cm section of the left pulmonary artery (LPA) was freed from connective tissue for placement of a 28 transit-time ultrasonic blood flow probe (Transonic Systems, Ithaca, NY). The total Qpul was calculated as 2 × LPA flow. For measurements of systemic blood flow (Qsys), a flow probe was placed on the left aortic arch (LAo), a flow probe was placed on the far right branch (RRAo) of the right aortic arch, and finally, a flow probe was placed around both the right subclavian (RSC) and right common carotid (Rcc). The total Qsys was calculated as the sum of LAo, RRAo, and 2 × (RSC + Rcc) flows.

The left cervical vagus was isolated by separating the muscles located laterally to the trachea. The nerve was carefully dissected free from the carotid artery. Two loops of silk suture (3–0) were tied around the vagus, and the nerve was sectioned between the loops so that either the afferent (RVAF) or the efferent (RVEF) part of the vagus nerve could be independently manipulated onto a stimulating electrode during the experiments.

After instrumentation the turtles were left unmanipulated for ~60 min before any measurements were conducted.

Measurements

All measurements were conducted on anesthetized animals at an ambient temperature of 25 ± 2°C.

Rate of oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were computed by measuring static samples of inflow-outflow gas concentration multiplied with the lung ventilation over a given period of time. Inflow gas concentrations were sampled from ambient air, and outflow gas concentrations were sampled from a mixing chamber attached to the excurrent line of the ventilator. O₂ and CO₂ concentrations were measured with an oxygen analyzer (S-3A, Applied Electrochemistry, Sunnyvale, CA) and a CO₂ electrode (E5037), and a capillary pH electrode (G299A), respectively. All electrodes were maintained at 25°C in a BMS Mk2 electrode assembly. Hematocrit (Hct) was measured using a standard capillary tube method. Total amount of VO₂ content (O₂ct) was estimated using the partial pressure of oxygen (PO₂), Hct, and pH values and from known values of the Bohr effect and Hill’s n, reported in previous studies in T. scripta (6, 27). Blood plasma lactate content was analyzed by adding 300 μl of blood to an Eppendorf tube containing 1,200 μl of a 6% perchloric acid solution kept on ice. Plasma lactate was measured enzymatically with a Sigma kit (no. 826-UV).

Signals from the O₂, CO₂, and Transonic meters were continuously collected onto a computer using a commercial data acquisition system (AcqKnowledge MP 100, Goleta, CA). Beat-to-beat heart rate (HR) was derived from the LAo flow signal.

Experimental Protocol

Dose/response. The animals were divided into three different experimental groups. Group 1 (n = 5) was used to find the optimal dose/response for the metabolic uncoupler 2,4-dinitrophenol (DNP) and VO₂. Values of Qpul, Qsys, HR, VO₂, and VCO₂ were recorded at rest and 3 min after the onset of the RVEF stimulation (4 Hz, 8 V, 2–30 μA, and 200 ms duration). Stimulation current was adjusted to produce a 60–70% reduction in HR. After a recovery period of 30 min, animals were intra-arterially injected with a dose of 5 mg/kg DNP (10 mg DNP and 5 mg NaHCO₃/ml H₂O), and control and RVEF stimulation recordings were repeated, starting 15 min after DNP injection. The same procedures were repeated for accumulative doses of 5 mg/kg DNP every 30 min to a total of 30 mg/kg injected DNP.

Vagal stimulation. In group 2 (n = 6), control values of Qpul, Qsys, HR, VO₂, and VCO₂ were recorded at rest, 3 min after the onset of RVAF stimulation (4 Hz, 8 V, 2–30 μA, and 200 ms duration), 3 min after the onset of RVEF stimulation (4 Hz, 8 V, 2–30 μA, and 200 ms duration), and 30 min after RVAF stimulation. Stimulation currents were adjusted to produce a visually detectable change in Qpul (RVAF) and a 60–70% reduction in HR (RVEF) [according to Hicks and Comeau (19)].

A dose of 5 mg/kg NaHCO₃ (used as control of the vehicle) was injected 30 min after RVEF stimulation, and DNP (10 mg/kg) was injected after an additional 30 min. Cardiovascular values and VO₂ were recorded 25 min after each injection and repeated RVAF and RVEF stimulations, following the same protocol as preinjections.

A 450-μl blood sample was withdrawn during the period of each one of the abovementioned data recordings for analysis of blood gases, pH, Hct, and lactate. In addition, recordings of cardiovascular and VO₂ “control” values were made just prior to each RVEF stimulation.

Reduced Hct. This part of the study was performed in an attempt to mimic the 60–70% reduction in SOT seen during the RVEF stimulations (for further information see RESULTS and DISCUSSION). In group 3 (n = 7), an initial blood sample was taken and Hct was measured. Control values of Qpul, Qsys, HR, VO₂, and VCO₂ were recorded at rest and 3 min after the onset of RVEF stimulation (4 Hz, 8 V, 2–30 μA, and 200 ms duration). After the vagal stimulation, 10 ml of blood was withdrawn from the arterial catheter and 10 ml of saline was infused simultaneously through the venous catheter. The blood was centrifuged, and the plasma was recovered and mixed with saline to a total volume of 20 ml. Hct was measured 10 min after withdrawal/infusion. Withdrawals/infusions of 20 ml blood/plasma and saline mixtures followed by Hct measurements were repeated until Hct was reduced to 30–40% of the control value. VO₂ was recorded before and...
10 min after an injection of DNP (10 mg/kg). Thirty minutes after the DNP injection, an additional RVEF stimulation was conducted, and $V_O_2$ was measured 3 min after the onset of the stimulation.

Data Analysis and Statistics

All recordings of blood flows, HR, $V_O_2$, and $V_CO_2$ were analyzed using AcqKnowledge data analysis software (version 3.2.3; Biopac). For control measurements, 30 min after DNP injection and 30 min after NaHCO$_3$ injection, mean values of Qsys, Qpul, HR, $V_O_2$, and $V_CO_2$ were created. For each vagal stimulation, mean values of Qsys, Qpul, HR, $V_O_2$, and $V_CO_2$ were determined for a 1-min period starting 3 min after the onset of stimulation. Blood samples for analysis of $P_{O_2}$, $P_{CO_2}$, pH, Hct, lactate, and calculation of $O_2$ct were withdrawn 3 min after the onset of vagal stimulation. All data are presented as mean values ± SE for $n$ animals.

Evaluation of statistically significant differences ($P \leq 0.05$) in the observations was made using the Wilcoxon signed-ranks test. A sequentially rejective Bonferroni test (23) was used to reduce, as far as possible, the possibility of discarding any true null hypothesis.

RESULTS

Group 1

Accumulative doses of 5 mg/kg DNP (5–30 mg/kg) were used to establish a dose-response curve for the effect of DNP on $V_O_2$ during rest and RVEF stimulation. DNP induced significant ($P \leq 0.05$) increases in resting $V_O_2$ at all doses used, with a maximum increase in $V_O_2$ after 15 mg/kg (Fig. 1). At rest and after each of the DNP doses, $V_O_2$ was significantly depressed (~25–40%) during RVEF stimulation. In addition, the $V_O_2$ recorded during RVEF stimulation was significantly higher after 10–30 mg/kg compared with control values of $V_O_2$ recorded at rest. A DNP dose of 10 mg/kg was chosen for all experiments in group 2.

Group 2

RVAF stimulation before injection of DNP produced a significant increase in Qpul and HR (~30 and 6%, respectively) (Fig. 2) but did not change $V_O_2$ or any of the blood parameters measured (Table 1). RVEF stim-
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Table 1. Metabolic rate, blood gas composition, Hct, pH, and lactate concentration in arterial blood from anesthetized and artificially ventilated Trachemys scripta at various treatments

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>RVAF (DNP)</th>
<th>RVEF (DNP)</th>
<th>NaHCO₃</th>
<th>DNP</th>
<th>RVAF (DNP)</th>
<th>RVEF (DNP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂, ml·min⁻¹·kg⁻¹</td>
<td>1.28 ± 0.11</td>
<td>1.28 ± 0.09</td>
<td>1.14 ± 0.08</td>
<td>1.44± 0.19</td>
<td>1.98 ± 0.51</td>
<td>1.15 ± 0.32</td>
<td>1.65 ± 0.32</td>
</tr>
<tr>
<td>P0₂, mmHg</td>
<td>69.3 ± 6.27</td>
<td>77.0 ± 10.0</td>
<td>47.9 ± 4.44</td>
<td>58.5 ± 5.48</td>
<td>45.2 ± 4.48</td>
<td>55.8 ± 6.96</td>
<td>46.7 ± 6.47</td>
</tr>
<tr>
<td>O₂ct, mmol/l</td>
<td>4.04 ± 0.27</td>
<td>4.01 ± 0.32</td>
<td>3.69 ± 0.38</td>
<td>4.14 ± 0.22</td>
<td>3.60 ± 0.19</td>
<td>3.57 ± 0.08</td>
<td>3.62 ± 0.21</td>
</tr>
<tr>
<td>Hct</td>
<td>0.31 ± 0.02</td>
<td>0.29 ± 0.02</td>
<td>0.30 ± 0.02</td>
<td>0.31 ± 0.01</td>
<td>0.31 ± 0.01</td>
<td>0.30 ± 0.01</td>
<td>0.32 ± 0.02</td>
</tr>
<tr>
<td>pH</td>
<td>7.99 ± 0.05</td>
<td>8.06 ± 0.06</td>
<td>7.91 ± 0.14</td>
<td>7.94 ± 0.10</td>
<td>7.60 ± 0.10</td>
<td>7.46 ± 0.11</td>
<td>7.38 ± 0.10</td>
</tr>
<tr>
<td>[Lactate], mmol/l</td>
<td>5.33 ± 0.83</td>
<td>5.35 ± 0.81</td>
<td>5.96 ± 0.90</td>
<td>6.19 ± 0.74</td>
<td>14.2 ± 1.95</td>
<td>16.3 ± 2.04</td>
<td>17.6 ± 2.28</td>
</tr>
<tr>
<td>VCO₂, ml·min⁻¹·kg⁻¹</td>
<td>1.03 ± 0.14</td>
<td>1.02 ± 0.12</td>
<td>0.75 ± 0.08</td>
<td>0.87 ± 0.07</td>
<td>2.21 ± 0.31</td>
<td>2.36 ± 0.30</td>
<td>1.64 ± 0.29</td>
</tr>
<tr>
<td>PCO₂, mmHg</td>
<td>6.34 ± 0.99</td>
<td>5.96 ± 0.85</td>
<td>5.16 ± 1.03</td>
<td>5.84 ± 0.62</td>
<td>15.4 ± 2.63</td>
<td>16.6 ± 2.53</td>
<td>15.7 ± 1.35</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 (except for PO₂, O₂ct, and PCO₂, where n = 5). RVAF, afferent branch of right vagus nerve; RVEF, efferent branch of right vagus nerve; DNP, 2,4-dinitrophenol; VO₂, rate of oxygen consumption; PO₂, oxygen partial pressure; O₂ct, oxygen content; Hct, hematocrit; [Lactate], lactate concentration; VCO₂, carbon dioxide production; PCO₂, carbon dioxide partial pressure. *Significant difference (P < 0.05) from individual control. †Significant difference from control conditions at rest.

ulation in untreated animals produced significant reductions in Qpul, Qsys, and HR (60–70%) (Figs. 2 and 3), and a 60–70% decrease in SOT (Fig. 3) accompanied by significant decreases in VO₂, PO₂, VCO₂, and PCO₂ (Table 1 and Fig. 3). Injection of DNP induced a drop in total cardiac output (Qtot = Qpul + Qsys) caused by a drop in Qpul during rest (Fig. 2). VO₂, VCO₂, and PCO₂ were significantly increased and PO₂ was significantly decreased during resting conditions after DNP injection. DNP also induced a drop in pH and an increase in plasma lactate levels, changes that were further expressed in the two after vagal stimulations (Table 1 and Fig. 3). In addition, post-DNP RVAF stimulation produced increases in Qtot, Qpul, VCO₂, and PCO₂ (Fig. 2 and Table 1). Post-DNP RVEF stimulation induced significant decreases in VO₂, PO₂, VCO₂, PCO₂, and SOT (Table 1 and Fig. 3). In comparison of pre-DNP RVEF stimulation and post-DNP RVEF stimulation, there were no differences in any of the flows measured, but VO₂ was significantly higher after DNP injection. VO₂ was significantly higher during RVEF stimulation after DNP injection than in resting controls (Figs. 2 and 3, Table 1). No changes in plasma lactate levels could be correlated to the stimulations per se, but a gradual two- to threefold increase was observed after the DNP injection (Table 1). Finally, injection of NaHCO₃ (used as control of DNP vehicle) had no effects on any of the cardiovascular or blood parameters measured (Table 1). O₂ct did not significantly change during the experiment (Fig. 3).

**Group 3**

A 60–70% reduction of Hct produced a significant decrease in VO₂, without any significant changes in Qsys or Qpul (Fig. 4). VO₂ after Hct reduction was not significantly different from the VO₂ measured during the control RVEF stimulation. Injection of DNP reversed the VO₂ to control values. The RVEF stimulation after DNP produced decreases of ~50% in VO₂ and 60–70% in Qsys and Qpul. The VCO₂ values in this group followed those of VO₂, and HR was not significantly different from groups 1 and 2 (data not shown).

**DISCUSSION**

This study shows that vagal nerve stimulation triggers a decrease in VO₂ in anesthetized turtles. This
hypometabolic response is correlated with the reductions in arterial $\text{PO}_2$ and SOT and was not associated with significant increases in plasma lactate. In all animals, injections of DNP during vagal nerve stimulation increased $\dot{V}_O_2$ to levels greater than those measured during control periods. We conclude that the reduction in $\dot{V}_O_2$ associated with vagal nerve stimulation and the following reduction in SOT most likely reflect downregulation of ATP turnover rates.

Comparison with Data on Conscious Turtles

The gas exchange, cardiovascular, acid-base and blood gas data measured in our in situ preparation (Table 1) were in close agreement with values measured in previous studies in turtles. The $\dot{V}_O_2$ reported from previous studies on metabolism in turtles ranges from 0.4 to 1.4 ml/min (5, 24, 33). Although pulmonary and systemic blood flows in our study were high compared with previously reported data, both were still within the range of blood flows previously measured in $T. \text{scripta}$ ([28–75 ml/min]; Refs. 7, 8, 16, 32, 33). Finally, our measured values for arterial $\text{PO}_2$, pH, and plasma lactate were similar to the values reported in anesthetized and conscious resting turtles at a similar body temperature (8, 20, 34).

Hypometabolism Induced by Vagal Stimulation

In the following discussion, $\dot{V}_O_2$ will be used as an indicator of metabolic rate. This study demonstrates that RVEF stimulation produces a decrease in $\dot{V}_O_2$ of $\sim$35%, reflecting a reduction in aerobic metabolic rate to the same extent (Table 1). Associated with RVEF stimulation were reductions in both HR and cardiac output, which decreased by as much as 70% (Fig. 2). This vagally induced reduction in cardiac output clearly contributes to the reduction in the overall $\dot{V}_O_2$, and the obvious question arises: How much of the overall reduction in $\dot{V}_O_2$ can be accounted for by the reduction in cardiac work? Although we did not directly measure the $\dot{V}_O_2$ of the heart, the contribution of the $\dot{V}_O_2$ of the heart to the total $\dot{V}_O_2$ can be estimated from previous studies on heart metabolism in the turtle (1, 5, 9, 10, 12) and extrapolated to the conditions during our experiments. From this extrapolation, we estimate that the $\dot{V}_O_2$ of the heart is $<$10% of the total $\dot{V}_O_2$. Thus, if the $\dot{V}_O_2$ of the heart is $<$10% of total $\dot{V}_O_2$, the reduction in the work of the heart, theoretically, cannot be responsible for more than 20% of the $\dot{V}_O_2$ reduction measured during RVEF. The remaining 80% of the metabolic decrease must therefore be attributed to a general decrease in metabolic rate, i.e., the onset of a hypometabolic state.

Delivery vs. Controlled Response: DNP

The reduction in $\dot{V}_O_2$ during RVEF stimulation may be caused by either an inability of the cardiopulmonary system to supply a sufficient amount of oxygen to the tissues or a downregulation of metabolism, i.e., reduction in ATP demand. To distinguish between these two possibilities, we used injections of the metabolic uncoupler DNP. We found that the reduction in $\dot{V}_O_2$, associated with RVEF and reduced SOT, reversed after DNP injection. This result indicates that during RVEF stimulation, the lower SOT was still sufficient to support tissue respiration, because $\dot{V}_O_2$ after DNP exceeded even the $\dot{V}_O_2$ measured in untreated control animals (Table 1 and Fig. 3). Pharmacological stimulation of $\dot{V}_O_2$ during periods of tissue hypoxia have been previously reported for isolated cells and whole animals. In the aquatic turtle Chrysemys scripta, isolated hepatocytes exhibit rapid metabolic depression during anoxia, which is reversed by treatment with DNP (4). In the freshwater turtle $T. \text{scripta}$, hypoxia-induced hypometabolism is reversed by a single injection of DNP (20). Finally, in rats, an intra-
venous injection of DNP (17 mg/kg) reversed the hypoxic induced reduction in Vo2 (30).

Reduced Hct

The approach of mimicking the reduced SOT seen during RVEF stimulation by reducing Hct further supports the hypothesis that SOT is at least one of the components that triggers the hypometabolic state. A 60–70% reduction in SOT caused by the reducing the Hct results in a decrease in Vo2 comparable to the 60–70% reduction in SOT caused by reduced Qsys (Figs. 3 and 4). When DNP was injected in animals with reduced Hct, the Vo2 increased to a level not significantly different from control values, without any detectable change in SOT (Fig. 4). In addition, RVEF stimulation after DNP injection produced a decrease in Vo2 similar to that during the RVEF stimulation under control conditions (Fig. 4).

We conclude that the reduction in Vo2 during vagal nerve stimulation reflects a downregulation of ATP turnover rate. This conclusion is based on the observations that 1) plasma lactate did not significantly increase during periods of vagal reductions in SOT, indicating that net ATP production derived from anaerobic glycolysis was not increased; and 2) Vo2 could be pharmacologically stimulated by injection of DNP during periods of reduced SOT, suggesting that oxygen delivery alone did not limit aerobic metabolism. Similar conclusions have been previously reached with comparable data in both ectotherms and endothermic vertebrates (20, 30). In endothermic vertebrates (mammals), the reduction of metabolism during periods of hypoxia has been suggested to result from inhibition of the pathways involved in thermogenesis (13). However, as previously suggested (20), significant thermogenic processes are absent in ectotherms, and, therefore, the reduction in Vo2 at the organismal level may result from mechanisms that are similar to those that depress metabolism during anoxia.

Role of the R-L Shunt in Control of the Hypometabolic State

In freshwater turtles, diving is often associated with bradycardia, decreased Qpul, and the development of a large R-L intracardiac shunt (17, 32–35). A recent study by Hicks and Wang (20) in the anesthetized turtle demonstrated that severe hypoxia (FIO2 = 0.05), which reduced arterial oxygen levels to values observed during diving, induced a 30% reduction in Vo2. Consequently, Hicks and Wang (20) hypothesized that the development of an R-L shunt may trigger the rapid onset of a hypometabolic state in the tissues and therefore contribute to the prolongation of aerobic dive times.

In the present study, we could not see from the flow data alone evidence for the development of a large net R-L intracardiac shunting during RVEF stimulation. However, in turtles, the net blood flows are not necessarily precise indicators of R-L shunt (17, 18). Intracardiac shunts can be defined as either anatomic or effective (18). An anatomic shunt is defined as a shift of net blood flow from the pulmonary to the systemic circulation or vice versa. The effective shunt is the relative amount of systemic blood that mixes with pulmonary blood and is detected by measurements of blood oxygen levels (18). An effective R-L shunt (i.e., reduction in arterial Po2) has been demonstrated to occur during RVEF stimulation in the turtle (19). An effective R-L shunt most likely occurs in our experiments, because arterial Po2 drops during RVEF stimulation. During RVEF stimulation, arterial O2ct did not change but arterial Po2 was significantly reduced (Table 1), suggesting that Po2 may be the mediator for the metabolic downregulation. However, this is unlikely in the present study, because the Po2 changes measured were only 25–30% of the changes reported by Hicks and Wang (20) to produce hypometabolism. Whether the changes in Po2 are the consequences of an R-L shunt cannot be confirmed by our data.

The present study supports the hypothesis that reduction in oxygen transport induced by vagal stimulation triggers a downregulation of metabolism, which reflects a reduction in ATP turnover in the tissues of the freshwater turtle. Reduced arterial Po2 is a possible mediator but is not necessary for the metabolic downregulation. Another, perhaps more likely, candidate is the combination of Qsys and arterial O2ct, the SOT. In the study by Hicks and Wang (20), hypoxia induced a decrease in O2ct without any changes in blood flow, resulting in a reduced SOT and an induction of a hypometabolic state. This agrees with our findings, where SOT was reduced by the decrease in Qsys and by the reduction in Hct. Obviously, further investigations are needed to extend these findings to in vivo studies on diving turtles.

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