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SHORT COMMUNICATION

Oxygen transport is not compromised at high temperature in pythons

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ABSTRACT

To evaluate whether the 'oxygen and capacity limited thermal tolerance' model (OCLTT) applies to an air-breathing ectothermic vertebrate, we measured oxygen uptake ($\dot{V}_{O_2}$), cardiac performance and arterial blood gases during a progressive rise of temperature from 30 to 40°C in the snake *Python regius*. $\dot{V}_{O_2}$ of fasting snakes increased exponentially with temperature whereas $\dot{V}_{O_2}$ of digesting snakes at high temperatures plateaued at a level 3- to 4-fold above fasting. The high and sustained aerobic metabolism over the entire temperature range was supported by pronounced tachycardia at all temperatures, and both fasting and digesting snakes maintained a normal acid–base balance without any indication of anaerobic metabolism. All snakes also maintained high arterial P_{O_2}, even at temperatures close to the upper lethal temperature. Thus, there is no evidence of a reduced capacity for oxygen transport at high temperatures in either fasting or digesting snakes, suggesting that the upper thermal tolerance of this species is limited by other factors.

KEY WORDS: Cardiovascular, Digestion, Metabolism, Reptile, Temperature

INTRODUCTION

Despite considerable scope for behavioural thermoregulation, the body temperature of terrestrial ectotherms is ultimately determined by the available environmental conditions, and temperature is one of the most important abiotic factors that determine the geographical distribution of ectothermic vertebrates (Cossins and Bowler, 1987; Angilletta, 2009). This is because body temperature affects virtually all physiological and biochemical processes, and although the temperature sensitivity of many bodily functions have been studied in considerable detail in ectotherms, the physiological functions that determine thermal tolerance and performance at different temperatures remain debated (e.g. Pörtner, 2002; Pörtner, 2010; Clark et al., 2013). Recently, much emphasis has been placed on the apparent inability of cardiovascular and respiratory systems to deliver sufficient amounts of oxygen as body temperature approaches the upper lethal limit (e.g. Pörtner, 2002; Pörtner, 2010). Thus, the observation that maximal rates of oxygen consumption decrease at high temperatures (which is particularly well-documented in stenothermal crustaceans and salmonid fishes) while resting metabolic rate continues to increase, has led to the proposal that limited oxygen delivery is the most important determinant of the upper critical temperatures of ectothermic animals (e.g. Pörtner, 2002; Pörtner, 2010; Eliason et al., 2011; Eliason et al., 2013). The generality of the 'oxygen- and capacity-limited thermal tolerance' (OCLTT) model has, however, been questioned (see Clark et al., 2013) and for air-breathing ectothermic animals in particular, there is limited support for this model (Clark et al., 2005; Stevens et al., 2010; Seebacher and Franklin, 2011; Overgaard et al., 2012).

The OCLTT model predicts that increased metabolism and the associated demands on the cardiorespiratory systems renders animals more sensitive to elevated temperatures; many studies, therefore, have been devoted to measurement of the oxygen uptake and the attending cardiorespiratory responses during intense exercise at various temperatures. However, intense muscular exercise where metabolism approaches maximal oxygen uptake inadvertently involves some anaerobic component and is rarely sustainable for prolonged periods of time. The marked postprandial rise in metabolism in snakes therefore offers an interesting and alternative opportunity to investigate the OCLTT model because the postprandial state in gorge-feeding snakes represents a long-lasting and substantial rise in aerobic metabolism without the anaerobic contribution typical for exercise (Overgaard et al., 1999; Wang et al., 2001). Furthermore, many snakes and other reptiles behaviourally select a higher body temperature during digestion (Slip and Shine, 1988; Dorcas et al., 1997; Sievert et al., 2005; Bruton et al., 2012). This postprandial thermophilic response primarily acts to increase the rate of digestion and may also optimise digestive efficiency (Dorcas et al., 1997; Wang et al., 2002; Sievert et al., 2005). An improved understanding of the possibility that oxygen transport is constrained at high temperatures may provide insight into why some species of snakes do not elevate body temperature during digestion (see Sievert et al., 2005).

In the present study we provide the first detailed measurements of oxygen transport in fasting and digesting snakes at high temperatures. Based on measurements of oxygen uptake ($\dot{V}_{O_2}$), mean arterial blood pressure (MAP), heart rate (f_H) and arterial blood gases during a gradual rise in temperature from 30 to 40°C over the course of 10 h, we investigate whether oxygen transport is compromised at high temperatures in digesting ball pythons (*Python regius* Shaw 1802).

RESULTS AND DISCUSSION

The preferred body temperature of most snakes, including pythons, is around 30°C (e.g. Slip and Shine, 1988; Bedford and Christian, 1998) and upper lethal temperatures (CT_{max}) of snakes are normally below 42°C (Brattstrom, 1965). We are not aware of previous studies reporting the metabolic responses of pythons exposed to temperatures above 35°C, but the exponential rise in resting $\dot{V}_{O_2}$ as temperature rose from 30 to 40°C (Fig. 1; $P<0.001$) is consistent with previous measurements within lower temperature ranges (Ellis and Chappell, 1987). The almost fivefold elevation of $\dot{V}_{O_2}$ during digestion at 30°C also resembles the well-established specific dynamic action (SDA) response of pythons (Overgaard et al., 1999; Wang et al., 2002; Secor, 2008). The OCLTT hypothesis predicts that maximal oxygen transport capacity is compromised at critically

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Received 10 March 2014; Accepted 12 September 2014

The Journal of Experimental Biology

The Journal of Experimental Biology (2014) doi:10.1242/jeb.105148

List of symbols and abbreviations

CT_{max}	upper lethal temperature
f_H	heart rate
Hct	haematocrit
MAP	mean arterial blood pressure
OCLTT	oxygen- and capacity-limited thermal tolerance
Pa_{CO_2}	partial pressure of CO_2 in arterial blood
Pa_{O_2}	partial pressure of O_2 in arterial blood
SDA	specific dynamic action
$\dot{V}_{O_2}$	rate of oxygen uptake

high temperatures. In our study, the postprandial $\dot{V}_{O_2}$ increased with temperature (P<0.001) to reach a plateau above 34°C, with a non-significant tendency for a reduction at 40°C. Thus, the $\dot{V}_{O_2}$ of digesting snakes was 'only' 3.5 times greater than the fasting $\dot{V}_{O_2}$ at 40°C, but the absolute rise in $\dot{V}_{O_2}$ remained larger at 40 compared with 30°C (3.2 versus 2.3 ml min^{-1} kg^{-1}, respectively). Thus, the fasting pythons clearly continued to possess the capacity for a several-fold rise in $\dot{V}_{O_2}$ at temperatures immediately below the CT_{max}, and it is therefore highly unlikely that the CT_{max} in fasting snakes is related to a reduced capacity for oxygen transport. However, the non-significant decrease in $\dot{V}_{O_2}$ of postprandial snakes suggests that oxygen transport may have been compromised at the highest temperatures, which could also explain our observation that two of the six digesting snakes regurgitated after exposure to 40°C. Because body temperature might have increased by ~1°C during digestion (Tattersall et al., 2004), we may slightly overestimate the effects of temperature on the reported variables, but we would also slightly underestimate the critical body temperatures where oxygen transport is compromised in digesting snakes.

The rise in metabolism with increased temperature was attended by the typical rise in f_H (P<0.001) and a fairly stable MAP (P=0.595), indicative of a general systemic vasodilatation (Fig. 2A,B) (Lillywhite and Seymour, 1978). However, there was a significant interaction between digestive state and temperature on MAP (P<0.001), such that the MAP of digesting snakes increased slightly at high temperatures, while there was a (non-significant) tendency for MAP to decrease at high temperatures in fasting snakes. Similarly, the SDA response was attended by a pronounced tachycardia (P<0.001), which has been shown to be driven by a release of vagal tone and the presence of a non-adrenergic non-cholinergic stimulus of the heart (e.g. Skovgaard et al., 2009; Enok et al., 2012). The ability to increase f_H during digestion, even at high temperatures, is consistent with the increased postprandial $\dot{V}_{O_2}$ at these temperatures, and the postprandial metabolic and cardiac responses of the pythons therefore resemble the exercise-mediated responses at high temperatures in toads (Overgaard et al., 2012) and varanid lizards (Clark et al., 2005). It thus seems that amphibians and reptiles do not 'conform' to the OCLTT predictions, although this has been investigated explicitly in only very few species. Our conclusion that oxygen transport is not limiting thermal tolerance in pythons is consistent with the much earlier studies by Hicks and Wood (Hicks and Wood, 1985), who found that reptiles only exhibit behavioural reductions in preferred body temperature when exposed to inspired oxygen levels below 10%.

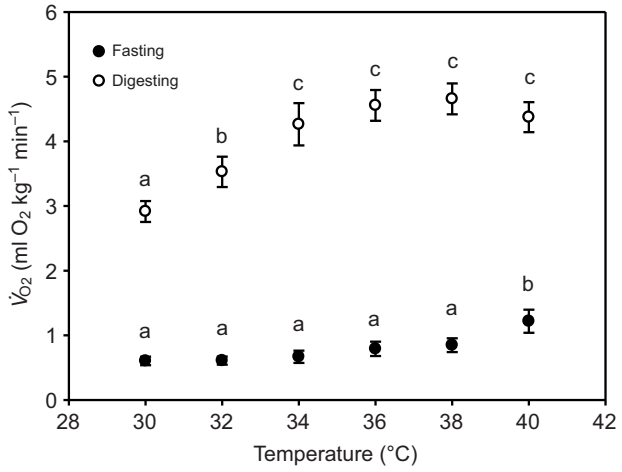

Fig. 1. Metabolic rate estimated from rate of oxygen consumption in fasting and digesting ball pythons exposed to temperatures ranging from 30 to 40°C. Dissimilar letters indicate a significant difference within fasting and digesting snakes, respectively (one-way ANOVA). All data are means ± s.e.m.

The arterial blood gases, even at the highest temperatures, also continued to exhibit the characteristic changes typical for ectothermic vertebrates (e.g. Wang et al., 1998; Stinner et al., 1998). Arterial P_{CO_2} (Pa_{CO_2}) rose (P<0.001) with elevated temperature, causing a decline in arterial pH (P<0.001) (Fig. 2C,D) consistent with the general pattern of alphastat regulation, such that plasma ions remain fairly stable (e.g. Wang et al., 1998; Stinner et al., 1998). In our study, plasma sodium remained between 145±4 and 153±4 mmol l^{-1} independent of temperature (P=0.695) and digestive status (P=0.137), whereas there was small but significant (P<0.001 for effect of temperature) elevation of plasma potassium (from 2.6±0.2 at 30°C to 3.6±0.3 mmol l^{-1} at 40°C) in both fasting and digesting snakes. Plasma calcium ranged between 1.2 and 2.1 mmol l^{-1} independent of temperature (P=0.25 for effect of temperature), but was slightly higher (average 1.8 mmol l^{-1}) in fasting snakes than in digesting snakes (average 1.6 mmol l^{-1}) (P=0.008 for effect of digestive status). The postprandial rise in Pa_{CO_2} of ~6 mmHg at constant pH is indicative of the typical respiratory compensation of the metabolic alkalosis associated with digestion (Overgaard et al., 1999; Wang et al., 2001) (Fig. 2D; P=0.35 for effect of digestive status). In the present study we did not measure plasma lactate levels; however, since Pa_{CO_2} and pH resembled previous measurements in pythons, we conclude that the snakes remained in aerobic balance at all temperatures. As an additional indication that oxygen transport is not limiting at high temperatures, we observed that the digesting snakes continued to hypoventilate (i.e. ventilation did not increase proportionally to CO_2 production) even at the highest temperatures and even though this hypoventilation decreases alveolar P_{O_2} (Overgaard et al., 1999; Secor et al., 2000). Arterial P_{O_2} (Pa_{O_2}) varied differently with temperature between fasting and digesting snakes (P=0.009 for interaction term) such that arterial Pa_{O_2} rose from ~60 mmHg to 100 mmHg when temperature was increased for fasting snakes. In contrast, Pa_{O_2} was constantly high (close to 100 mmHg) in digesting snakes, such that there was a significant difference between fasting and digesting snakes at 30–34°C, but not at 36–40°C (Fig. 2D). The rise in Pa_{O_2} is most likely due to the right-shifted oxygen dissociation curve and possibly also to a reduced intracardiac shunt (Stinner, 1987; Wood and Hicks, 1985).

The apparent reduction in haematocrit (Hct) as temperature rose (P=0.018 for temperature) is undoubtedly due to the repeated blood sampling (see Materials and methods) and blood sampling also explains the apparent lowering of Hct during digestion (Fig. 2E; P=0.009 for digestive status). However, according to the predictions of the OCLTT model, lowered Hct would only render the digesting snakes more susceptible to the limitations of oxygen transport at high temperatures. Given that this is not the case, all metabolic and

The Journal of Experimental Biology (2014) doi:10.1242/jeb.105148

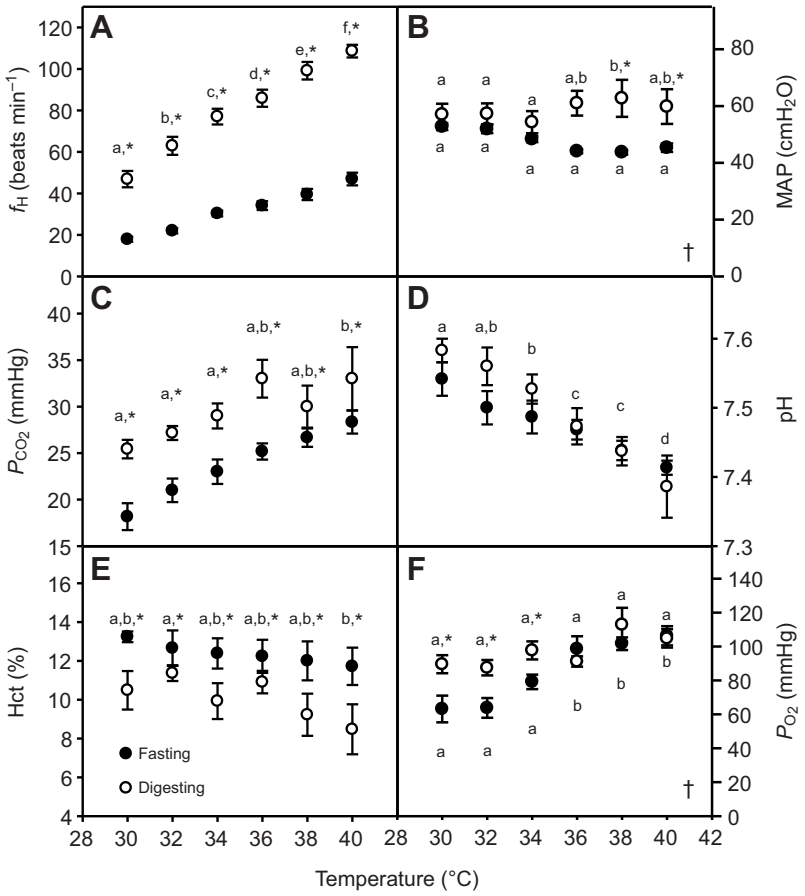

Fig. 2. Cardiovascular and blood parameters in fasting and digesting ball pythons exposed to temperatures ranging from 30 to 40°C. (A) Heart rate (f_H). (B) Mean arterial blood pressure (MAP). (C) Arterial P_{CO_2}. (D) Arterial pH. (E) Haematocrit (Hct). (F) Arterial P_{O_2}. Dissimilar letters indicate a significant difference with temperature and asterisks indicate a significant effect of digestive state (two-way RM ANOVA). In cases of significant interactions (shown by †), the test results are reported for individual comparisons within each temperature or digestive state. All data are means ± s.e.m.

cardiovascular parameters measured in the present study demonstrate that the oxygen transport capacity is well defended in digesting snakes exposed to critically high temperatures, and impaired oxygen delivery is unlikely to determine CT_{max} in these animals. Most snakes exhibit a mild postprandial thermophilic response where they increase preferred body temperatures above 30°C (Dorcas et al., 1997; Sievert et al., 2005), and our study indicates that the magnitude of this thermophilic response is not limited by oxygen transport capacity. Instead, it is likely that the rise in preferred body temperature serves to optimise other aspects of the digestive responses (Dorcas et al., 1997; Wang et al., 2002; Sievert et al., 2005).

MATERIALS AND METHODS

Experimental animals

Ball pythons (*Python regius* Shaw 1802) of undetermined sex were purchased from a commercial supplier and kept in vivaria at Aarhus University for at least 4 weeks. The vivaria contained a heating system to ensure temperatures between 25 and 32°C and the snakes always had free access to a shelter and water. Snakes were fasted for minimum 10 days prior to experiments. Six snakes (506±68 g) were used to determine the effects of the acute temperature rise on oxygen uptake, while blood pressure and arterial blood gases were measured in another eight snakes (432±42 g). Experiments were performed in accordance with Danish Federal Regulations.

Determination of oxygen uptake at increasing temperatures

$\dot{V}_{O_2}$ was measured by closed respirometry (e.g. Overgaard et al., 1999). Briefly, snakes were enclosed in respirometers (3090 ml) shielded from visual and auditory disturbances within a climate chamber. The snakes were allowed to acclimate for ~24 h at 30°C and $\dot{V}_{O_2}$ was then measured in undisturbed resting animals. Two air samples from the containers were taken before and after a closed period of 30 min.

After measurement of $\dot{V}_{O_2}$ at 30°C, the temperature of the climatic chamber was increased by 2°C and the system (chamber, snake and cabinet) was allowed 1.5 hours to reach the new temperature whereupon $\dot{V}_{O_2}$ was measured over 30 min. This procedure was repeated every second hour until reaching a final temperature of 40°C. Temperature was then returned to 30°C, and the animals were fed 20% of body mass. To alleviate handling stress of the snakes during the heating protocol, we did not measure body temperature, but express all data as a function of the temperature in the climate chamber. On the following day, 24 h after feeding, the measurements of gas exchange were repeated using a similar protocol.

Catheterisation to measure blood pressure, heart rate and blood gases

The snakes were anaesthetised by inhalation of a saturated atmosphere of isoflorane until reflexes and muscle tonus disappeared and then intubated for mechanical ventilation with 2% isoflorane mixed with air (tidal volume of 30 ml kg^{-1} at 2 breaths min^{-1}). When the surgical field had been cleaned and soaked with betadine and a subcutaneous injection of lidocain provided local anaesthesia before the vertebral artery was accessed through a 5 cm ventrolateral incision for occlusive cannulation with a polyethylene catheter (PE50) containing heparinised saline (50 IU ml^{-1}). The incision was closed with monofilament suture (3-0) and the catheter fastened to the skin. All snakes recovered for 24 h at 30°C in a climate chamber to allow f_H, MAP and plasma stress hormones to return to resting values (Olesen et al., 2008).

Blood pressures were measured by connecting the catheters to pressure transducers (model PX600; Baxter 117 Edwards, Irvine, CA, USA), calibrated daily against a water column, and connected to a preamplifier built in-house to allow pressure traces to be recorded using a Biopac MP100 data acquisition system (Biopac Systems, Goleta, CA, USA) at 200 Hz. f_H was derived from the pulsatile pressure signal. pH, P_{CO_2}, P_{O_2}, Na^+, K^+, Ca^{2+}

of the heparinised arterial blood samples were measured using the automated GEM premier 3500 (Instrumentation Laboratory, Bedford, MA, USA). This *in vitro* blood gas analyser relies on potentiometry to measure pH, P_{CO_2} and Na^+, Ca^{2+} and K^+, whilst P_{O_2} is measured amperometrically. We have recently verified this method against classic radiometer electrodes on python blood, and used the temperature correction determined by Malte et al. (Malte et al., 2014). Hct was measured after centrifugation at 10,000 ***g*** for 3 min from the proportion of cell volume to total volume.

Experimental protocols

To ensure resting measurements of MAP and f_H, the recovered snakes were kept in their cylinders in the climate chamber to reduce disturbance during measurements. Snakes were left undisturbed for >1 h after connecting the catheters to the pressure transducers after which resting MAP and f_H had been determined. An arterial blood sample was then taken and analysed for blood gases and Hct, whereupon temperature was increased in steps of 2°C every second hour as described above. After the measurements at 40°C, the temperature was returned to 30°C and similar to the measurements of $\dot{V}_{O_2}$, the snakes were fed 20% of their body mass, after which the protocol was repeated once the snakes were 24 h into the postprandial period. When all measurements had been completed the snakes were euthanised by intravascular infusion of pentobarbital (100 mg kg^{-1}).

Statistics

The effect of temperature on $\dot{V}_{O_2}$ was tested using one-way analysis of variance (ANOVA) for fasting and digesting snakes, respectively. The effects of temperature and digestive state on blood parameters were analysed using a two-way repeated-measures ANOVA. Data was log-transformed in cases where normality and homoscedasticity did not fulfil the requirements for ANOVA and if this was insufficient, one-way ANOVA on ranks (Kruskal–Wallis test) were used separately for fasting and digesting snakes (testing separately for the effect of temperature). All data are presented as means ± s.e.m.

Acknowledgements

Special thanks to Heidi Meldgaard Jensen for animal care and husbandry and Rasmus Buchanan for technical assistance.

Competing interests

The authors declare no competing financial interests.

Author contributions

T.W., D.F. and J.O. designed the experiments; D.F. performed the experiments; T.W., D.F. and J.O. analysed data and wrote the MS.

Funding

This study was funded by the Danish Research Council.

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