

SHORT COMMUNICATION FOR *THE JOURNAL OF EXPERIMENTAL BIOLOGY*

# Intraspecific scaling of arterial blood pressure in the Burmese python

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## SUMMARY

30 Interspecific allometric analyses indicate that mean arterial blood pressure (MAP) increases  
31 with body mass of snakes and mammals. In snakes, MAP increases in proportion to the  
32 increased distance between the heart and the head, when the heart-head vertical distance is  
33 expressed as  $gH$ , and the rise in MAP is associated with larger heart to normalize wall stress  
34 in the ventricular wall. Based on measurements of MAP in Burmese python ranging from  
35 0.9-3.7 m in length (0.20-27 kg), we demonstrate that although MAP increases with body  
36 mass, the rise in MAP is merely half of that predicted by heart-head distance. Scaling  
37 relationships within individual species, therefore, may not be accurately predicted by existing  
38 interspecific analyses.

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## INTRODUCTION

The mean arterial blood pressure (MAP) generated by the heart of animals provides the driving force that ensures adequate perfusion of the various vascular beds to match metabolic requirements. MAP is made up of two principal components: the pressure required to overcome total systemic vascular resistance (TPR) as well as the pressure required to overcome the vertical distance between the heart and upper extremities of the body, *i.e.* the gravitational (hydrostatic) pressure (Gauer and Thorn, 1965; White and Seymour, 2014). The gravitational pressure correlates directly to the vertical distance above the heart ( $h$ ) and can be quantified as  $\rho gh$ , where  $\rho$  equals the density of blood and  $g$  is acceleration due to gravity (Gauer and Thorn, 1965). Because the vertical distance between the heart and the upper extremities typically increases as animals get larger, the greater gravitational forces predict that MAP increases with body size (Seymour, 1987; White and Seymour, 2014). Consistent with this view, the exceptionally high MAP in giraffes of around 250mmHg, well above other similar-sized mammals, is typically viewed as an adaptation to overcome the large vertical distance between the heart and head, and hence provide a normal perfusion pressure of the cerebral circulation (*e.g.* Patterson et al., 1965; Brøndum et al., 2009). In birds, MAP is higher than in mammals, but because MAP does not scale with body mass nor the distance between heart and head, it has been suggested that factors other than gravity, such as metabolic rate, have major impacts on MAP (Seymour and Blaylock, 2000).

The cardiovascular system of long-bodied animals, such as snakes, is particularly affected by gravity and terrestrial and arboreal species are endowed with effective physiological mechanisms and structural adaptations that prevent pooling of blood in the lower body parts and maintains cardiac filling when body position is altered (Lillywhite, 1987, 2005; Lillywhite and Donald, 1994; Lillywhite and Gallagher, 1985; Seymour and Arndt, 2004; Lillywhite et al., 2012). An interspecific comparison of 16 individuals belonging to nine different species of terrestrial snakes revealed a significant rise in MAP with body length, such that MAP increased proportionally to the distance between the heart and head when expressed as the rise in gravitational pressure ( $\rho gh$ ; Seymour, 1987).

Interspecific allometric analyses often provide fundamental relationships that transcend taxonomic differences (Savage et al., 2008), however intraspecific and interspecific scaling relationships can differ significantly (Heusner, 1982; Thompson and Withers, 1997; Chappell and Ellis, 1987). To further understand how body size and head to heart distances affect MAP, we provide an intraspecific allometric analysis of heart rate ( $f_H$ ), ventricular mass and MAP in the Burmese python

75 (*Python molurus*). As Seymour (1987), we measured resting undisturbed snakes in a horizontal  
 76 position.

## 77 RESULTS AND DISCUSSION

78 The scaling relationship for ventricular mass in the python (Fig. 1; Table 1) was almost identical to the  
 79 relationship reported for terrestrial snakes (Seymour, 1987). In contrast the effects of body mass on  
 80 MAP in the python (Fig. 1; Table 1) was considerably less than measured in terrestrial snakes  
 81 (Seymour, 1987). Regardless, in accordance with the previous allometric analysis (Seymour, 1987),  
 82 MAP and ventricular mass of the Burmese pythons increased positively with body mass (Fig. 1; Table  
 83 1), while  $f_H$  decreased (Table 1). A regression through all data points predicts that  $f_H$  decreases from  
 84 22.7 to 9.5  $\text{min}^{-1}$  when a Burmese python grow from 200g to 30 kg. We did not measure stroke  
 85 volume, but given that ventricular mass decreases from 1.8 to 1.2  $\text{g kg}^{-1}$  over the same body mass  
 86 interval, it seems very likely that cardiac output decreases similarly to reduction in  $f_H$ . This implies that  
 87 most of the rise in MAP with increased body mass must be due to a rise in TPR. Interestingly, the rise  
 88 in MAP when expressed relative to the heart-head distance was also considerably lower than reported  
 89 in the previous interspecific analysis for snakes (Seymour, 1987; Fig. 2) where the relationship  
 90 between MAP, measured in resting, horizontal snakes, and the heart-head distance, converted into a  
 91 gravitational pressure ( $\text{pgh}$ ), had a slope of 1.18. If head-heart distance perfectly predicts MAP, this  
 92 slope should be 1.0. In the 15 pythons where we have heart-head distances and MAP, the slope of the  
 93 relationship was less than half that reported for terrestrial snakes (Seymour, 1987) (slope = 0.49 vs  
 94 1.18), and significantly less than 1.0 ( $p=0.0193$ ). Thus, at least in Burmese pythons, MAP is not  
 95 simply related to the heart-head distance. This result is not necessarily surprising. In a resting,  
 96 horizontal snake, or any terrestrial vertebrate in a horizontal position, the heart and head are  
 97 essentially at the same gravitational potential. Thus, the additional gravitational pressure component  
 98 of MAP is eliminated and the hemodynamic challenges of perfusing the head are minimized.

99 A variety of factors may account for the different MAP scaling relationship found in *P. molurus*  
 100 compared to existing interspecific analyses. First, pythons, unlike any other group of snakes, have  
 101 functional intraventricular separation of the pulmonary and systemic circulations, and hence sustain  
 102 relatively high MAP, while keeping pressures in the pulmonary circulation low (Wang et al., 2003;  
 103 Jensen et al., 2010). Consequently, MAP of pythons is already sufficiently high to overcome  
 104 gravitational stress when the head is elevated. In addition, although the rise in MAP is well below that

predicted by the longer heart-head distance, the reflexive neurogenic regulation of blood pressure may be sufficiently effective to overcome any vertical challenges whenever the head is raised above heart level (e.g. Lillywhite and Donald, 1994). Finally, the steeper interspecific scaling slope of MAP and head-heart distance reported by Seymour (1987) may have been biased by inclusion of many snake species adapted to an arboreal life style (scansorial).

Allometric analyses are generally insightful; however, interspecific and intraspecific analyses may depict dissimilar relationships. For example, the scaling of body mass and metabolism have yielded significantly different mass exponents when analysed intraspecifically *versus* interspecifically (e.g. Heusner, 1987). Therefore, it is not surprising that within the Burmese python, the allometric relationship of MAP and heart-head distance differs significantly from a phylogenetically-diverse, interspecific relationship.

## MATERIALS AND METHODS

Sixty-one Burmese pythons (*Python molurus*, Linnaeus, 1758) with a body mass between 0.2 and 27 kg were purchased from a commercial supplier and kept at Aarhus University in vivaria equipped with a heating system that provided temperatures between 25 and 32°C. In addition, three of the largest individuals were studied at UNESP in Brazil. The snakes had free access to water and were fed once a week. Food was withheld at least two weeks prior to experimentation. The animals grew during captivity and appeared healthy.

Anesthesia was induced by inhalation of 5% isoflurane (Baxter, Denmark) to allow intubation for mechanical ventilation (1-2% isoflurane at 5-10 breaths min<sup>-1</sup> and 50 mL kg<sup>-1</sup>) using a Harvard apparatus mechanical ventilator. Snakes heavier than 2 kg were manually ventilated with an Ambu bag. Subsequently, a 5 cm ventrolateral incision was made anterior to the heart or posterior to the kidney, for the occlusive insertion of a polyethylene catheter (PE50 or PE90 containing heparinized saline, 50IU ml<sup>-1</sup>) in the vertebral artery or dorsal aorta respectively. Snakes were allowed to recover for 1-2 days at 30°C.

To measure MAP, the arterial catheter was connected to a disposable pressure transducer (Model PX600; Baxter Edwards, Irvine, CA) calibrated against a static water column and recorded with a Biopac MP100 data acquisition system (Biopac Systems, Inc., Goleta, CA) at 100hz.  $f_H$  was derived from the pulsating pressure signal.

All measurements were conducted on fully recovered, fasting and undisturbed snakes kept at 30°C for a minimum of 12h in climatic chambers that also served to reduce visual and auditory disturbance during measurements. Resting MAP and  $f_H$  were measured one hour after connecting the catheters to alleviate the influence handling stress on MAP and  $f_H$ . All snakes studied in Aarhus were sacrificed after measurements (200 mg kg<sup>-1</sup> pentobarbital, i.p), whereupon length was measured and hearts were harvested. The data reported in the present study were collected in connection with other experiments to determine regulation of the cardiovascular system and the experiments were conducted in accordance with Danish Federal Regulations.

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## AUTHOR CONTRIBUTIONS

All authors contributed to conception, design and execution of the study, interpretation of the findings.

S.E., J.W.H. and T.W. wrote the manuscript, which was subsequently edited by C.S and A.S.A.

## COMPETING INTERESTS

No competing interests declared.

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## List of abbreviations

$f_H$	heart rate
g	is acceleration due to gravity
H	vertical distance above the heart
MAP	mean arterial blood pressure
TPR	total systemic vascular resistance
$\rho$	density of blood

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## FIGURE LEGENDS

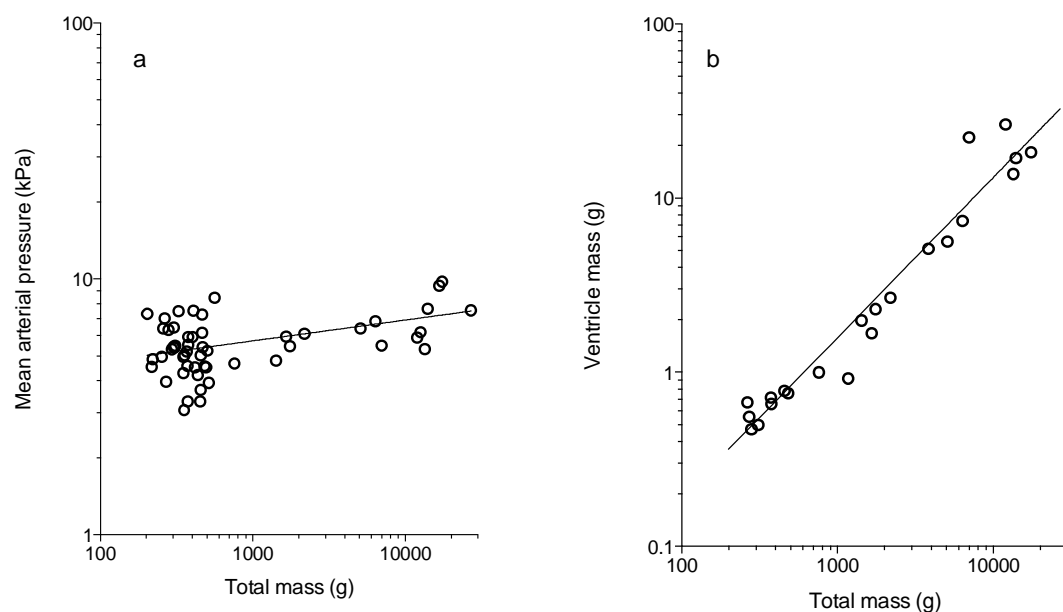
Figure 1. a: Mean arterial blood pressure as a function of body mass for *Python molurus* (N = 53). b: Ventricle mass as a function of total body mass for *Python molurus* (N = 22). Values are from fasted resting snakes at 30°C. Axes are logarithmic and the regression equation and statistics are presented in Table 1.

Figure 2. Mean arterial blood pressure as a function of head-heart pressure (pgh) from 16 *Python molurus*. A straight line with the equation  $Y = 0.4942X + 4.017$  is fitted to the data (black,  $r^2 = 0.3229$ ). A regression from Seymour et al. 1987 is shown in red ( $Y = 1.18X + 2.26$ ). Y=mean arterial pressure (kPa) and X=heart to head distanced converted to kPa. Blood was assumed to have a density of 1.05 g/ml.

Table 1: Allometric equations for ventricular mass (Mv), mean arterial blood pressure (MAP) Head-heart distance heart rate ( $f_H$ ) and body mass (Mb). All values were measured on fasting and undisturbed Burmese pythons (*Python molurus*) at 30°C.

Y	X	Regression	a	b	$r^2$	n
Mv (g)	Mb (g)	$Y = aX^b$	0.00277	0.9187	0.958	22
MAP (kPa)	Mb (g)	$Y = aX^b$	3.248	0.0786	0.197	53
$f_H$ (min <sup>-1</sup> )	Mb (g)	$Y = aX^b$	56.87	-0.1735	0.680	54
Head-heart (cm)	Total length (cm)	$Y = aX + b$	0.1905	2.814	0.971	23

Figure 1



260 Figure 2:

