J Exp Biol Advance Online Articles. First posted online on 5 December 2013 as doi:10.1242/jeb.092841
Access the most recent version at http://jeb.biologists.org/lookup/doi/10.1242/jeb.092841

Reduction of blood oxygen levels enhances postprandial cardiac hypertrophy in Burmese python (*Python molurus*)

Christopher E. Slay[1,2], Sanne Enok[2,3], James W. Hicks[1], Tobias Wang[2*]

[1]Department of Ecology and Evolutionary Biology, University of California Irvine, Irvine, CA, USA

[2]Zoophysiology, Institute for Biological Sciences, Aarhus University, Aarhus, Denmark.

[3]Inderdisciplinary Nanoscience Center, Aarhus University, Arhus, Denmark

running header: Postprandial cardiac growth

Keywords: Cardiac hypertrophy, cardiovascular regulation, Burmese python, reptile, heart, digestion, postprandial, SDA, anemia

*Corresponding Author:
Professor Tobias Wang
Zoophysiology, Department of Biosciences
Aarhus University
Building 1131, Universitetsparken
8000 Aarhus C
DENMARK
tel (+45) 5137 7737
email: tobias.wang@biology.au.dk

Summary

Physiological cardiac hypertrophy is characterized by reversible enlargement of cardiomyocytes and changes in chamber architecture, which increase stroke volume and $\dot{V}O_2max$ *via* augmented convective oxygen transport. Cardiac hypertrophy is known to occur in response to repeated elevations of O_2 demand and/or reduced O_2 supply in several species of vertebrate ectotherms, including postprandial Burmese pythons (*Python molurus*). Recent data suggest postprandial cardiac hypertrophy in *P. molurus* is a facultative rather than obligatory response to digestion, though the triggers of this response are unknown. Here we hypothesize that an O_2 supply-demand mismatch stimulates postprandial cardiac enlargement in Burmese pythons. To test this hypothesis, we rendered animals anemic prior to feeding, essentially halving blood oxygen content during the postprandial period. Fed anemic animals had heart rates 126% higher than fasted controls, which, coupled with a 71% increase in mean arterial pressure suggests fed anemic animals were experiencing significantly elevated cardiac work. We found significant cardiac hypertrophy in fed anemic animals, which exhibited ventricles 39% larger than fasted controls and 28% larger than fed controls. These findings support our hypothesis that those animals with a greater magnitude of O_2 supply-demand mismatch exhibit the largest hearts. The "low O_2 signal" stimulating postprandial cardiac hypertrophy is likely mediated by elevated ventricular wall stress associated with postprandial hemodynamics.

1. Introduction

Burmese pythons, like many large snakes, utilize an intermittent "sit-and-wait" feeding strategy, where prolonged fasts are punctuated by brief and voracious feeding bouts when prey is available (Pope, 1961). Digestion of these large meals (up to 25-100% of their body mass) is associated with pronounced upregulation of a suite of digestive functions (Secor and Diamond, 1997) and a large postprandial increase in oxygen uptake ($\dot{V}O_2$), termed specific dynamic action (SDA), where $\dot{V}O_2$ may exceed that during aerobic activity and last for several days (Secor and Diamond, 1997; Secor and Diamond, 1998; Secor, 2008a; McCue, 2008; Secor, 2008b; Cox and Secor, 2008; Secor and White, 2010; Wang *et al.*, 2001). To support the high $\dot{V}O_2$ during digestion, cardiac output increases drastically above resting values through a combination of increased stroke volume and heart rate (Secor *et al.*, 2000; Secor and White, 2010). This hemodynamic response is mitigated largely by a reduction in cholinergic tone and positive chronotropic effects of non-adrenergic-non-cholinergic (NANC) factors, including an increased histaminergic tone (Wang *et al.*, 2001; Skovgaard *et al.*, 2008; Enok *et al.*, 2012, 2013).

The rise in stroke volume has been linked with a 40% increase in ventricular mass within 48h of eating (Andersen *et al.*, 2005) that Riquelme *et al.* (2011) described as "physiologic" in nature and triggered by humoral factors, including increased levels of circulating free fatty acids. The universality and stimulus of the postprandial cardiac hypertrophy, however, remain unclear since Jensen *et al.* (2010) found no postprandial cardiac hypertrophy in Burmese or Ball pythons (*Python regius*) under a similar experimental protocol. Jensen *et al.* (2010) argued, therefore, that postprandial cardiac hypertrophy should be considered a "facultative" rather than "obligatory" response to feeding and the lack of postprandial cardiac hypertrophy was recently reported in two additional studies (Hansen *et al.*, 2013; Enok *et al.*, 2013).

The correlation between the magnitudes of SDA and postprandial cardiac hypertrophy is not well understood. Thus, while $\dot{V}O_2$ consistently increases following feeding, the postprandial cardiac hypertrophy is inconsistent. We hypothesize that postprandial cardiac hypertrophy is triggered when systemic metabolic demand outpaces systemic oxygen delivery. To investigate this hypothesis, we induced an oxygen supply-demand mismatch in postprandial pythons by rendering specimens anemic prior to feeding with the prediction that anemic pythons would exhibit greater postprandial cardiac hypertrophy than fasted pythons with normal blood oxygen levels.

2. Materials and Methods

2.1. Animal acquisition and husbandry

Burmese pythons (*Python molurus*; Kuhl 1820; n=31) of both sexes were acquired from commercial vendors and housed for several months prior to experimentation at the vivarium facilities of Aarhus University or the University of California, Irvine. Animals ranged from 0.24 kg to 11.5 kg with a mean body mass of 1.83 kg ± 0.52 kg. Snakes were kept in individual vivaria at 27-30ºC, and had access to heated surfaces that reached 32ºC. A 12h light:12h dark photoperiod was maintained. All animals always had access to water, vigorously consumed rodent meals every 1-2 weeks, and gained mass during captivity. All snakes fasted for a minimum of 28 days prior to experimentation. Animals were housed and treated according to Danish Federal Regulations and UCI IACUC protocol 2009-2821.

2.2. Surgical procedures

Snakes (27 of the 31) were instrumented with arterial catheters for measurement of blood pressure (MAP) and heart rate (f_H), as well as for withdrawal of arterial blood samples to determine blood oxygen concentration (C_{O_2}) and blood pH. To induce anesthesia, individual snakes were placed in a sealed container containing gauze soaked in isoflurane (Baxter, Allerød, Denmark) until they lost muscle tone and could be intubated for artificial ventilation with 2% isoflurane at 5 breaths min^{-1} and 50 ml kg^{-1}tidal volume, using a vaporizer (EZ-155, EZ Systems, Bethleham, PA, USA) and an HI 665 Harvard Apparatus respirator (Holliston, Massachusetts, USA). A 5 cm incision close to the cloaca enabled the dorsal aorta to be accessed by blunt dissection, so a catheter (PE-50) containing heparinized saline (50 IU/ml), could be inserted and externalized via a small cutaneous puncture and secured to the skin with 2-0 braided silk suture. Approximately 0.15ml of whole blood was then withdrawn from the catheter to determine hematocrit (Hct) by spinning the blood in glass capillaries for 3 min at 12,000 rpm.

Subsets of 14 randomly selected snakes were rendered “anemic” (see discussion of experimental groups, below) by withdrawing blood while the snakes were still anaesthetized. Aliquots of 10% of the estimated blood volume (6-7% of body mass; Lillywhite and Smits, 1984) were placed in sterile 1.5 ml Eppendorf tubes and centrifuged at 6,000 rpm for 5 min. The supernatant plasma was returned via the arterial catheter. Hct was re-measured 15 min after

reinjection of plasma and the process was repeated until Hct was reduced to approximately 10% (mean 10.1 ± 0.3%).

The snakes were ventilated with room air until they regained muscle tone and resumed spontaneous ventilation. They were then returned to their enclosures, given access to water, and placed in a 30ºC temperature-controlled chamber. Animals were allowed to recover from surgery undisturbed in their enclosures for 24 h to ensure low plasma catecholamine levels (Olesen *et al*., 2008).

2.3. Experimental and feeding protocols

Following the 24 h recovery period, we measured MAP, and f_H from each snake while they remained minimally disturbed in the climactic chamber. The catheters were connected to pressure transducers (PX600, Baxter Edwards, Irvine, CA) calibrated with a vertical water column and connected to an in-house built amplifier sampling at 200 Hz (MP100 BioPac Systems, Inc., Goleta, CA). MAP and f_H were analysed over 5-10 min intervals.

Each animal was randomly assigned to one of 4 treatments: fasted-control (N=8), fasted-anemia (N=6), fed-control (N=9), or fed-anemia (N=8). Following the measurements of MAP and f_H, the "fasted" animals remained undisturbed at 30º, whereas "fed" animals consumed rodent meals equivalent to 25 ± 0% body mass. Contingent upon catheter patency, 48 h after recovery (72 h after surgery), MAP and f_H were obtained and the rate-pressure product (RPP) was calculated (f_H x MAP) from each animal. Cardiac output and thus work was not measured, but we used the RPP as a proxy for myocardial work. From each animal, an arterial blood sample of approximately 0.5 ml was withdrawn to determine C_{O_2} (Tucker, 1967), blood pH (glass electrode maintained at 30ºC and connected to a PHM 73; Radiometer, Copenhagen, Denmark), and Hct. Immediately after blood sampling, adrenergic and cholinergic tones were assessed by sequential infusion of atropine and propranolol (see Enok *et al*., 2012) and calculated from the standard equations, modified for use of f_H rather than R-R interval (*e.g*. Altimiras *et al*., 1997):

$$\text{Chol}\ (\%) = \frac{\frac{1}{f_{H_{cont}}} - \frac{1}{f_{H_{atr}}}}{\frac{1}{f_{H_{dbl}}}} * 100 \qquad \text{(Eq. 1)}$$

and

$$\text{Adr}\ (\%) = \frac{\frac{1}{f_{H_{dbl}}} - \frac{1}{f_{H_{atr}}}}{\frac{1}{f_{H_{dbl}}}} * 100 \qquad \text{(Eq. 2)}$$

where $f_{H_{cont}}$ is the control heart rate, $f_{H_{atr}}$ is the heart rate following administration of atropine, and $f_{H_{dbl}}$ is the double blocked heart rate (i.e. following administration of atropine and propranolol).

2.4. Sacrifice and Tissue Harvest

Immediately following assessment of autonomic tone, animals were euthanized via intraperitoneal injection of sodium pentobarbital (>100 mg kg^{-1}) whereupon a long ventral incision allowed for the heart, liver, stomach, small intestine, large intestine, and kidneys to be removed. All organs were rinsed with isotonic saline and blotted dry with gauze to remove blood and chyme before determining wet mass. A small representative sample was removed from each organ and weighed before and after it had been dried in an oven at 60ºC for 72 h to determine the dry mass:wet mass (M_D:M_W) ratio.

2.5 Statistical Analyses

Mass-specific organ mass (gram of tissue per kg body mass), C_{O_2}, f_H, and MAP data were compared using two-way analysis of variance (ANOVA) and post-hoc Tukey's HSD in JMP statistical software (Version 7, SAS Institute, Inc., Cary, NC) following assurance of homogeneity of variance and normal distribution of data. Post-hoc tests were performed only when the ANOVA yielded significance ($p \leq 0.05$), and were considered significant when $p \leq 0.05$. Hematocrit, adrenergic tone, cholinergic tone, and M_D:M_W were arcsin square root transformed and compared using a two-way ANOVA in JMP. "Effects," where reported, are the results of the effect tests conducted as part of the ANOVA model and are distinguished by the single degree of freedom. Regression plots were generated using GraphPad Prism (Version 6, GraphPad Software, La Jolla, CA, USA) and slopes were analyzed using the software's linear regression analysis. Slopes of the regression lines were considered significantly different from 0 at the level of $p \leq 0.05$. All values are reported as mean ± s.e.m.

3. Results

3.1 Hematological parameters and blood gases

Our experimental procedure for rendering animals anemic resulted in significantly reduced blood oxygen carrying capacity (Table 1). At the time of sacrifice, anemic animals (both fed and fasted) exhibited 61% lower Hct than controls ($F_{1,28} = 103.0$, $p < 0.0001$) and 53% lower arterial C_{O_2} than control animals ($F_{1,19} = 27.8$, $p < 0.0001$). Among fed animals, C_{O_2} was significantly reduced in anemic animals as compared to control animals ($F_{3,19} = 9.4$, $p < 0.0001$), which was critical for testing the hypothesis. Arterial pH did not differ between anemic and control snakes and was not affected by digestion ($F_{3,21} = 1.15$, *ns*).

3.2 Cardiovascular parameters

While manipulation of Hct alone did not significantly elevate f_H of fasting snakes, feeding elicited significant increases in f_H among both anemic (50% increase) and control (78% increase) snakes. Coupling anemia with feeding, however, resulted in a 126% difference between fasted controls and fed anemic snakes (Figure 1A; $F_{3,20} = 9.2$, $p < 0.001$)

MAP was 85% higher in fed controls than in fasted controls and 71% higher in fed anemic animals than in fasted controls (Figure 1B; $F_{3,20} = 5.9$, $p < 0.05$). As a consequence of the markedly elevated f_H and MAP, particularly in fed anemic snakes, RPP was 2.9-fold higher in fed anemic snakes than in fasted controls (Figure 1C; $F_{3,20} = 6.2$, $p < 0.05$).

The changes in f_H were attended by changes in autonomic tone on the heart (Figure 2). Feeding alone elicited a 42% reduction in adrenergic tone among control animals, but the response was blunted in anemic animals, resulting in more modest 27% reduction. The greatest reduction in adrenergic tone was the 47% difference between fasted anemic snakes and fed control snakes (Figure 2A; $F_{3,16} = 10.3$, $p = 0.001$).

There were significant effects of digestive status ($F_{1,16} = 10.4$, $p < 0.05$) and Hct ($F_{1,16} = 5.0$, $p < 0.05$) on cholinergic tone (Figure 2B), with a modest difference existing between fasted controls and fed controls (47%), and a more impressive difference between fasted controls and fed anemic animals (73%)(Figure 2B; $F_{3,16} = 8.1$, $p < 0.005$).

The effect of feeding alone was significant in determining double-blocked f_H ($F_{1,17}$ = 16.9, $p < 0.005$) whereas Hct did not have a significant effect ($F_{1,17}$ = 2.0, *ns*), but fed anemic animals had higher f_H than either group of fasted animals ($F_{3,17}$ = 6.9, $p < 0.005$) (Figure 2C).

3.3 Cardiac hypertrophy

Heart mass of snakes with normal Hct did not increase during digestion, and anemia did not elicit cardiac growth in fasting snakes (Figure 3A). However, the anemic snakes euthanized 48h into digestion had a ventricular mass of 1.8 g kg^{-1}, which is 39% larger than the ventricle of fasting snakes with normal Hct. Thus, there was a significant difference ($F_{3,30}$ = 3.0, $p < 0.05$) in ventricular wet mass between treatments. The effects of Hct on ventricular mass ($F_{1,30}$ = 5.3, $p < 0.05$) were greater than the effects of digestion ($F_{1,30}$ = 2.6, *ns*). There was no significant difference in the dry:wet mass ratio between treatments (Figure 3B; $F_{3,30}$ = 0.7, *ns*)

Total wet heart mass, *i.e.* combined ventricular and atrial wet masses, also differed between treatments (Figure 3C) ($F_{3,30}$ = 3.9, $p < 0.05$), with fed anemic animals again having the largest hearts (38% larger than hearts of fasted controls) animals, but not significantly larger than hearts of fasted anemic or fed control snakes. Hct exerted a greater effect on ventricular mass ($F_{1,29}$ = 7.3, $p < 0.05$) than digestion ($F_{1,29}$ = 2.9, *ns*). Atrial wet mass was also 36% greater in fed anemic animals than in fasted controls ($F_{3,30}$ = 4.3, $p < 0.05$), again, with a significant effect of Hct ($F_{1,29}$ = 8.8, $p < 0.05$) but not feeding status ($F_{1,29}$ = 3.5, *ns*). There was no significant difference in atrial dry:wet mass ratio between groups of animals ($F_{1,29}$ = 0.3, *ns*).

We correlated ventricular mass with f_H and the rate-pressure product, where this value estimates myocardial oxygen consumption and thus provides a proxy for cardiac work (Figure 4). Ventricular mass was positively and linearly correlated with both heart rate (Figure 4A; $p < 0.05$, R^2 = 0.31) and the rate-pressure product (Figure 4C; $p < 0.05$, R^2 = 0.22), however the relationship between f_H and ventricular mass was significant among fed animals ($p < 0.001$, R^2 = 0.69; Figure 4A), but not fasted animals (*ns*, R^2 = 0.00; Fig 4B).

3.4 Plasticity of the digestive organs

Stomach wet mass was significantly greater in fed control than fasted control animals (Table 2; $F_{3,30}$ = 5.2, $p < 0.05$), but there was no difference in stomach dry mass between groups ($F_{3,30}$ = 2.4, *ns*). Wet mass of the small intestine was also significantly larger in digesting snakes ($F_{3,30}$ =

10.0, $p < 0.0001$), with similar trends for dry mass (albeit with no statistical difference between fasted anemic and fed control intestines; $F_{3,30} = 16.0$, $p < 0.005$). There were no significant differences in large intestine mass (wet - $F_{3,30} = 1.5$, *ns*; dry - $F_{3,29} = 2.3$, *ns*) or liver wet mass ($F_{3,30} = 2.4$, *ns*), whereas liver dry mass differed significantly between groups ($F_{3,30} = 16.5$, $p < 0.005$). Fed anemic animals exhibited higher kidney wet mass than fasted controls (84% enlargement; $F_{3,30} = 5.3$, $p < 0.05$), but there were no significant changes in kidney dry mass ($F_{3,27} = 1.7$, *ns*). While growth of the small intestine was due only to digestion ($F_{1,30} = 27.1$, $p < 0.0001$) and not Hct ($F_{1,30} = 1.7$, *ns*), both digestion ($F_{1,30} = 7.8$, $p < 0.05$) and Hct ($F_{1,30} = 7.1$, $p < 0.05$) had significant effects on kidney wet mass.

4. Discussion

Our study confirms that feeding alone does not elicit postprandial cardiac hypertrophy. Animals confronted with the simultaneous challenges of increased O_2 demand (digestion) and reduced O_2 supply (anemia) do, however, exhibit postprandial cardiac hypertrophy when compared to fasted, un-manipulated controls. This suggests that cardiac hypertrophy is triggered when oxygen supply/delivery cannot meet the elevated metabolic demands of digestion. Interestingly, cardiac mass of several other ectothermic vertebrates also responds to oxygen supply and demand mismatches, such alligators reared in hypoxia (Warburton et al., 1995; Crossley et al., 2005; Owerkowicz *et al.*, 2009) or fish rendered anemic (e.g. Sun et al., 2009; Simonot and Farrell, 2005).

Our findings conflict with the previous reports of an obligatory postprandial cardiac hypertrophy (*e.g.* Andersen *et al*., 2005; Riquelme *et al*., 2011), and supports the proposal that postprandial cardiac hypertrophy is a facultative response in pythons (Jensen *et al*., 2011; Hansen *et al*., 2013; Enok *et al*., 2013). In contrast, postprandial enlargement of the small intestine, liver, and kidneys seems consistent amongst studies (Secor and Diamond, 1995; Secor and Diamond 1998; Starck and Beese, 2001; Ott and Secor, 2006; Cox and Secor, 2008; Jensen *et al*., 2011; Hansen *et al*., 2012; Enok *et al*., 2013). Consistent with the idea that expansion of the intestine is stimulated by the presence of chyme in the intestine (Secor *et al*., 2000), there was no effect of reducing Hct on the rise in intestinal mass during digestion, though it is impressive that significant intestinal hypertrophy occurs in animals with severe oxygen limitation. Enlargement of the stomach seems to be another facultative response to digestion, as

it is noted in some studies (Secor and Diamond, 1995, Jensen et al., 2011), but not others (Cox and Secor, 2008; Ott and Secor, 2006). As in other studies (Secor and Diamond, 1995; Jensen et al. 2011), kidney wet mass increased with digestion, but we also note that snakes with reduced Hct had enlarged kidneys, which may result from a stimulation of erythropoietic functions, but dry kidney mass did not differ between groups.

As shown in earlier studies (Wang *et al.*, 2001; Skovgaard *et al.*, 2008; Enok *et al.*, 2012, 2013), the postprandial tachycardia is largely governed by a reduction of cholinergic tone on the heart, whereas the adrenergic tone actually decreases during digestion. In the double-blocked heart, there was also a rise in the postprandial f_H resulting from a circulating NANC factors (Skovgaard *et al.*, 2008), although the specific nature of the stimulus remain to be identified (Enok *et al.*, 2012). Given that the NANC factor is likely to be released in direct response to digestion, possibly as a peptide from the digestive organs, it is not surprising that anemia did not affect the double-blocked f_H. The rise in f_H of the anemic snakes was likely a barostatic response to vasodilation and an attending lowering of total peripheral resistance in response to lowered blood C_{O_2}, but could also result from stimulation of chemoreceptors (Wang *et al.*, 1994; 1997; Andersen *et al.*, 2003). In contrast to previous studies on digesting snakes, the postprandial tachycardia in our study was associated with a significant rise in MAP. However, because MAP did not increase proportionally to the rise in f_H, and because stroke volume is likely to have been elevated, digestion was probably attended by a reduced total peripheral resistance as blood flows to the digestive organs increase during digestion (Secor *et al.*, 2000; Starck and Wimmer, 2005; Secor and White, 2010). In addition, lowering of Hct is likely to have reduced blood viscosity and hence could have alleviated the workload on the heart. However, anemia did not influence MAP, and the anemic snakes therefore did have a higher rate pressure product than animals with normal Hct.

The observation that the postprandial cardiac hypertrophy of pythons is facultative rather than obligatory indicate that other factors than circulating signal molecules are involved, and our results suggest that increased cardiac work or myocardial oxygen consumption stimulate the postrandial cardiac growth in pythons. Compared to resting animals, postprandial cardiac growth was elicited in anemic snakes with significantly higher rate pressure product, suggesting increased workload and greater mechanical stress on the ventricles. In mammals, the molecular pathways stimulating physiologic cardiac hypertrophy are stimulated by increased mechanical

stress, such that increased workload stimulates myocytes to synthesize and release growth factors, including insulin-like growth factor I (IGF-I; Serneri *et al*., 1999; Hill and Olson, 2008). These growth factors are then involved in paracrine and/or autocrine activation of the phosphatidylinositol 3'-kinase (PI3K)-Akt-mTOR pathway, which ultimately leads to synthesis of contractile elements (Dorn and Force, 2005; Shiojima and Walsh, 2006; Dorn, 2007; Hill and Olson, 2008). AMPK, Akt, GSK3β, and mTOR, all signalling molecules in mammalian physiologic hypertrophy pathways mediated by mechanical stress, are known to be active in the python model (Riquelme *et al.*, 2011). This suggests that the cardiac hypertrophy in pythons occur in response to elevated mechanical stress on ventricular myocytes. This obviously does not rule out that circulating factors, such as free fatty acids (Riquelme *et al*., 2011), may contribute to the postprandial hypertrophy. Nevertheless, such humoral regulation does not appear adequate without a sufficient elevation of cardiac work and mechanical stress.

4.5 General conclusions

Despite the universal presence of gastrointestinal hypertrophies in fed pythons, our study supports the concept that postprandial cardiac hypertrophy is not an obligatory response to elevated oxygen demands associated with digestion in the python. We describe postprandial cardiac hypertrophy in fed anemic animals, whose hearts are operating at significantly elevated f_H (as mediated by reduced C_{O_2}, subsequently reduced cholinergic tone, and the presence of a significant NANC tone), and elevated cardiac work (as indicated by the rate pressure product). We posit that regardless of the potential for other humoral signals (Riquelme *et al*., 2011), significantly elevated cardiac work is required to "trigger" the postprandial hypertrophy *via* common physiologic hypertrophy signaling pathways. However, the precise level of cardiac work needed to induce cardiac hypertrophy is difficult to assess from the current analysis, as the experimental paradigm depends on a group analysis. Experiments measuring systemic flow, f_H, MAP, $\dot{V}O_2$, and heart size/mass need to be correlated during fasting and digestion, within individual animals. Advanced imaging techniques, which are becoming increasingly accessible to comparative physiologists (e.g. Hansen et al., 2013), in combination with classical physiological measurements would provide the information to determine the trigger level needed to induce postprandial cardiac hypertrophy in the Burmese python.

5. List of abbreviations

ANOVA – analysis of variance

C_{O_2} – blood oxygen concentration

f_H – heart rate

Hct - hematocrit

NANC – non-adrenergic, non-cholinergic

MAP – mean arterial blood pressure

SDA – specific dynamic action

SMR – standard metabolic rate

RPP – rate pressure product

Acknowledgements

We owe many thanks to Rasmus Buchanan and Heidi Jensen for technical assistance and animal husbandry. Funding of experimental work was provided by the Danish Research Council to TW; National Science Foundation [grant IOS-0922756 to JWH; Graduate Research Fellowship to CES]; and Society for Integrative and Comparative Biology [Fellowship for Graduate Student Travel to CES].

References

Altimiras, J., Aissaoui, A., Tort, L. and Axelsson, M. (1997). Cholinergic and adrenergic tones in the control of heart rate in teleosts. How should they be calculated? *Comp. Biochem. Physiol.* **118A**, 131-139.

Andersen, J.B., Hedrick, M.S. and Wang, T. (2003) Cardiovascular responses to hypoxia and anemia in the toad *Bufo marinus*. *J. Exp. Biol.* **206**, 857-865.

Andersen, J., Rourke, B.C., Caiozzo, V.J., Bennett, A.F. and Hicks, J.W. (2005) Postprandial cardiac hypertrophy in pythons. *Nature* **434**, 37-28.

Badeer, H.S. (1964) Biological significance of cardiac hypertrophy. *Am. J. Cardiol.* **14**, 133-138.

Busk, M., Overgaard, J., Hicks, J.W., Bennett, A.F. and Wang, T. (2000) Effects of feeding on arterial blood gases in the American alligator *Alligator mississippiensis*. *J. Exp. Biol.* **203**, 3117-3124.

Cox, C.L. and Secor, S.M. (2008) Matched regulation of gastrointestinal performance in the Burmese python, *Python molurus*. *J. Exp. Biol.* **211**, 1131-1140.

Crossley, DA and Altimiras, J.J. (2005) Cardiovascular development in embryos of the American alligator *Alligator mississippiensis*: effects of chronic and acute hypoxia. *J. Exp. Biol.* **208**, 31-39.

Dorn II, G.W. (2007) The Fuzzy Logic of Physiological Cardiac Hypertrophy. *Hypertension* **49**, 962-970

Dorn II, G.W. and Force, T. (2005) Protein kinase cascades in the regulation of cardiac hypertrophy. *J. Clin. Invest.* **115**, 527-537.

Dorn II, G.W., Robbins, J. and Sugden, P.H. (2003) Phenotyping hypertrophy: eschew obfuscation. *Circ. Res.* **92**, 1171-1175.

Drabkin, D.L., and Austin, J.H. (1935) Spectro- photometric studies. II. Preparations from washed blood cells; nitric oxide hemoglobin and sulfhemoglobin. *J. Biol. Chem.*, **112,** 51.

Eme, J. Owerkowicz, T., Gwalthney, J., Blank, J.M., Rorke, B.C. and Hicks, J.W. (2009) Exhaustive exercise training enhances aerobic capacity in American alligator (*Alligator mississippiensis*). *J. Comp. Physiol. B.* **179**, 921-931.

Enok, S., Simonsen, L.S., Pedersen, S.V., Wang, T. and Skovgaard, N. (2012) Humoral regulation of heart rate during digestion in pythons (*Python molurus* and *Python regius*). *Am. J. Physiol.* **302**, R1176-R1183.

Enok S., Simonsen L. S. and Wang T. (2013) The contribution of gastric digestion and ingestion of amino acids on the postprandial rise in oxygen consumption, heart rate and growth of visceral organs in pythons. *Comp. Biochem. Physiol.* **165A**, 46-53.

Hansen, K., Pedersen, P.B.M., Pedersen, M. and Wang, T. (2013) Magnetic Resonance Imaging volumetry for non-invasive measures of phenotypic flexibility during digestion in Burmese pythons. *Physiol. Zool.* **86**, 149-158.

Hicks, J.W. and Wood, S.C. (1985) Temperature regulation in lizards: effects of hypoxia. *Am. J. Physiol*. **248**, R595-R600.

Hill, J.A. and Olson, E.N. (2008) Mechanisms of disease: Cardiac plasticity. *New Engl. J. Med.* **13**, 1370-1380.

Hunter, J.J. and Chien, K.R. (1999) Mechanisms of disease: Signaling pathways for cardiac hypertrophy and failure. *New Engl. J. Med*. **341**, 1276-1283.

Jensen, B., Larsen, C.K., Nielsen, J.M, Simonsen, L.S. and Wang, T. (2011) Change of cardiac function, but not form, in postprandial pythons. *Comp. Biochem. Physiol*. **160**A, 35-42.

Lillywhite, H.B. and Smits, A.W. (1984) Lability of blood volume in snakes and its relation to activity and hypertension. *J. Exp. Biol.* **110**, 267-274.

McCue, M.D. (2006) Specific dynamic action: A century of investigation. *Comp. Biochem. Physiol.* **144A**, 381–394

Olesen, M.G, Bertelsen, M.F., Perry, S.F. and Wang, T. (2008) Effects of preoperative administration of butorphanol or meloxicam on physiologic responses to surgery in ball pythons. *J. Am. Vet. Med. Assoc.* **233**, 1883-1888.

Ott, B.D. and Secor, S.M. (2006) Adaptive regulation of digestive performance in the genus *Python*. *J. Exp. Biol.* **210**, 340-356.

Overgaard, J and Wang, T. (2002) Increased blood oxygen affinity during digestion in the snake *Python molurus*. *J. Exp. Biol.* **205**, 3327-3334.

Overgaard, J., Busk, M., Hicks, J.W., Jensen, F.B. and Wang, T. (1999) Respiratory consequences of feeding in the snake *Python molurus*. *Comp. Biochem. Physiol.* 124A, 359-365.

Owerkowicz, T., Elsey, R.M. and Hicks, J.W. (2009) Atmospheric oxygen level affects growth trajectory, cardiopulmonary allometry, and metabolic rate in American alligator (*Alligator mississippiensis*). *J. Exp. Biol.* **212**, 1237-1247.

Pope, C.H. (1961) The Giant Snakes. Knopf, New York, NY.

Riquelme, C.A., Magida, J.A., Harrison, B.C., Wall, C.E., Marr, T.G., Secor, S.M. and Leinwand, L.A. (2011) Fatty Acids Identified in the Burmese Python Promote Beneficial Cardiac Growth. *Science* **334**, 528-531.

Shiojima, I. and Walsh, K. (2006) Regulation of cardiac growth and coronary angiogenesisby the Akt/PKB signaling pathway. *Genes Dev*. **20**, 3347-3365.

Skovgaard, N., Møller, K., Gesser, H. and Wang, T. (2008) Histamine induces postprandial tachycardia through a direct effect on cardiac H_2-receptors in pythons. *Am. J. Physiol.* **296**, R774-R785.

Secor, S.M. (2008a) Digestive physiology of the Burmese python: broad regulation of integrated performance. *J. Exp. Biol.* **211**, 3757-3774

Secor, S.M. (2008b) Specific dynamic action: a review of the postprandial metabolic response. *J. Comp. Physiol. B.* **159**, 1-56.

Secor, S.M. and Diamond, J. (1995) Adaptive responses to feeding in Burmese pythons: pay before pumping. *J. Exp. Biol.* **198**, 1313-1325.

Secor, S. M. and Diamond, J. (1997) Determinants of post-feeding metabolic response in Burmese pythons (*Python molurus*). *Physiol. Zool.* **70**, 202–212;

Secor, S.M. and Diamond, J. (1998) A vertebrate model of extreme physiological regulation. *Nature* **395**, 659-662

Secor, S.M. and White, S.E. (2010) Prioritizing blood flow: cardiovascular performance in response to the competing demands of locomotion and digestion for the Burmese python, Python molurus. *J. Exp. Biol.* **213**, 77-88.

Secor S.M., Hicks J.W. and Bennett AF. (2000) Ventilatory and cardiovascular responses of a python (*Python molurus*) to exercise and digestion. *J. Exp. Biol.* **203**, 2447–2454.

Secor, S.M., Whang, E.E., Lane, J.S., Ashley, S.W. and Diamond, J. (2000). Luminal and systemic signals trigger intestinal adaptation in the Burmese python (*Python molurus*). *Am. J. Physiol.* **279**, G1177-G1187.

Secor, S.M., Nagy, T.R., Johnston, K.E. and Tamura, T. (2001) Effect of feeding on circulating micronutrient concentrations in the Burmese python (*Python molurus*). *Comp. Biochem. Physiol.* **129A**, 673-679.

Serneri, G.G.N, Modesti, P.A., Boddi, M., Cecioni, I., Paniccia, R., Coppo, M., Galanti, G., Simonetti, I., Vanni, S., Papa, L. et al. (1999) Cardiac growth factors in human hypertrophy: relations with myocardial contractility and wall stress. *Circ. Res.* **85**, 57-67

Simonot, D.L. and Farrell, A.P. (2007) Cardiac remodeling in rainbow trout *Oncorhynchus mykiss* Walbaum in response to phenylhydrazine-induced anemia. *J. Exp. Biol.* **210**, 1574-1584.

Starck, J. M. and Beese K. (2001). Structural flexibility of the intestine of Burmese python in response to feeding. *J. Exp. Biol.* **204**, 325–335.

Starck J. M., and Wimmer C. (2005). Patterns of blood flow during the postprandial response in ball pythons, *Python regius. J. Exp. Biol.* **208**, 881-889.

Sun, X., Hoage, T., Bai, P., Ding, Y., Chen, Z., Zhang, R., Huang, W., Jahangir, A., Paw, B., Li, Y.G. et al. (2009). Cardiac hypertrophy involves both myocyte hypertrophy and hyperplasia in anemic zebrafish. *PLoS ONE.* **4**, e6596.

Tucker, V.A. (1967) Method for oxygen content and dissociation curves on microliter blood samples. *J. Appl. Physiol.* **23**, 410–414.

Warburton, S.J., Hastings, D. and Wang, T. (1995) Responses to chronic hypoxia in embryonic alligators. *J. Exp. Biol.* **73**, 44-50.

Wang, T., Burggren, W. and Nobrega, E. (1995) Metabolic, ventilator, and acid-base responses associated with Specific Dynamic Action in the toad *Bufo marinus*. *Physiol. Zool.* **68**, 192-205.

Wang, T., E. Krosniunas, and J.W. Hicks (1997). The role of cardiac shunts in the regulation of arterial blood gases. *Am. Zool*. **37**, 12-22.

Wang, T., L.G.S. Branco and M.L. Glass (1994). Ventilatory responses to hypoxia in the toad *Bufo paracnemis* before and after a decrease in haemoglobin oxygen-carrying capacity. *J. Exp. Biol*.**186**, 1-8.

Wang, T, Taylor, E.W., Andrade, D. and Abe, A.S. (2001) Autonomic control of heart rate during forced activity and rest in the snake *Boa constrictor*. *J. Exp. Biol.* **204**, 3553-3560.

Wang, T., Busk, M. and Overgaard, J. (2001) The respiratory consequences of feeding in amphibians and reptiles. *Comp. Biochem. Physiol.* **128A**, 535-549.

Wagner, P.D. (1996) Determinants of maximal oxygen transport and utilization. *Ann. Rev. Physiol.* **58**, 21-50.

Table 1. Blood parameters in fasting and fed pythons. Values which share Latin character superscripts are not statistically different from one another.

	Fasted		Fed	
	Control	Anemic	Control	Anemic
C_{O_2} (mM)	3.24±0.84[a,b]	1.76±0.47[b]	3.94±0.24[a]	1.69±0.26[b]
Hct (%)	24.0±1.7[a]	7.4±0.5[b]	21.1±1.7[a]	9.9±1.0[b]
pH	7.49±0.08[a]	7.65±0.05[a]	7.60±0.09[a]	7.66±0.04[a]

439

440

441

Table 2. Visceral organ masses. Values which share Latin character superscripts (lower case for wet mass and upper case for dry mass) are not statistically different from one another.

	Wet mass (g kg^{-1})				Dry mass (g kg^{-1})			
	Fasted		Fed		Fasted		Fed	
	Control	Anemic	Control	Anemic	Control	Anemic	Control	Anemic
Stomach	12.9 ± 0.8^{a}	15.2 ± 1.1^{ab}	17.7 ± 2.2^{b}	17.0 ± 1.2^{ab}	2.7 ± 0.2^{A}	2.7 ± 0.1^{A}	3.6 ± 0.4^{A}	3.6 ± 0.3^{A}
Small Intestine	14.5 ± 1.4^{a}	17.1 ± 0.8^{a}	25.4 ± 3.2^{b}	29.6 ± 1.7^{b}	2.8 ± 0.3^{A}	3.0 ± 0.1^{AB}	4.6 ± 0.9^{BC}	5.9 ± 0.4^{C}
Large Intestine	8.7 ± 0.7^{a}	10.7 ± 1.0^{a}	10.2 ± 0.8^{a}	13.2 ± 2.6^{a}	2.2 ± 0.5^{A}	1.45 ± 0.1^{A}	1.4 ± 0.2^{A}	1.7 ± 0.2^{A}
Liver	17.5 ± 1.4^{a}	18.7 ± 1.9^{a}	22.1 ± 2.6^{a}	25.5 ± 2.8^{a}	5.1 ± 0.4^{A}	4.7 ± 0.5^{A}	6.1 ± 0.9^{AB}	8.2 ± 0.8^{B}
Kidney	4.5 ± 0.4^{a}	5.9 ± 0.4^{ab}	6.0 ± 0.6^{ab}	7.7 ± 0.8^{b}	0.9 ± 0.1^{A}	1.1 ± 0.1^{A}	1.4 ± 0.3^{A}	1.4 ± 0.2^{A}

442

443

Figure legends

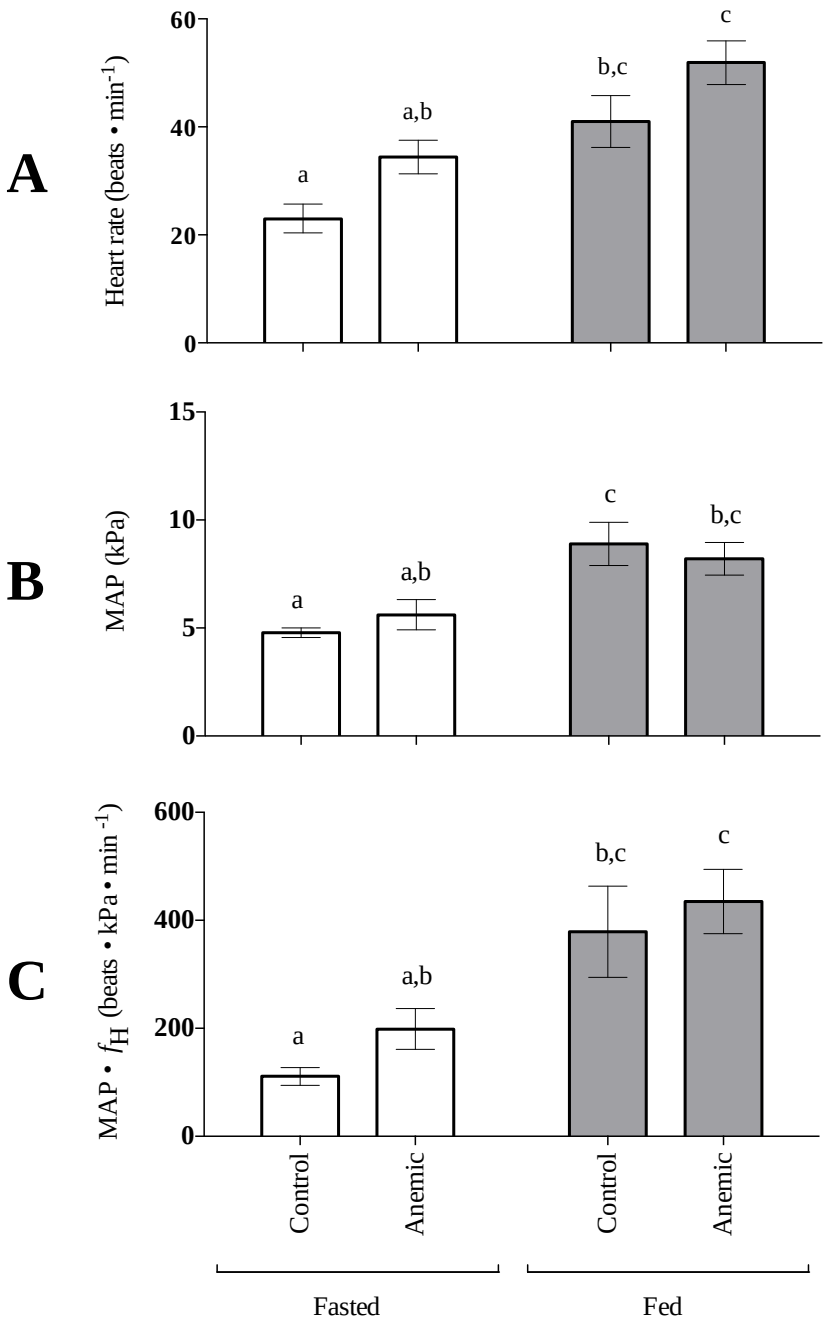
Figure 1. (A) Heart rates (f_H) in fasting and postprandial (48 h into digestion) Burmese pythons (*Python molurus*). Heart rate was significantly higher in the digesting snakes compared to fasting. (B) Mean arterial blood pressure was significantly higher in digesting snakes compared to fasting control snakes, while anemia did not influence MAP. (C) The rate-pressure product (proxy for cardiac work) was significantly higher in digesting snakes compared to fasted controls. Groups with the same Latin character do not differ significantly. All data is presented as mean ± s.e.m. Fasted controls, N=4; fasted anemic, N=5; fed controls, N=6; fed anemic, N=7.

Figure 2. Adrenergic and cholinergic cardiac tones in fasting and postprandial (48 h into digestion) Burmese pythons (*Python molurus*). Both cholinergic and adrenergic tones were lower in digesting snakes, and the cholinergic tone was reduced during anemia in both fasting and digesting snakes. Groups with the same Latin character do not differ significantly. All data is presented as mean ± s.e.m. Fasted controls, N=3; Fed controls, N=4; Fasted anemic, N=4; Fed anemic, N=7.

Figure 3. Ventricular wet mass, dry to wet mass ratio (M_D:M_W) and total heart mass fasting and postprandial (48 h into digestion) Burmese pythons (*Python molurus*). Ventricular wet mass (A), was significantly higher in the fed anemic snakes compared to fasting control snakes, while there were differences in M_D:M_W. Error bars represent s.e.m. Groups with the same Latin character do not differ significantly. All data is presented as mean ± s.e.m. Fasted control, N=8; Fasted anemic, N=6; Fed control, N=9; Fed anemic, N=8.

Figure 4. Correlation between ventricular wet mass and the rate-pressure product (RPP) in fasting and fed controls and anemic Burmese pythons. The RPP is equal to the product of the mean arterial blood pressure (MAP) and heart rate (HR) and is a proxy for cardiac work.

476 Figure 1



477
478
479
480
481
482

Figure 2

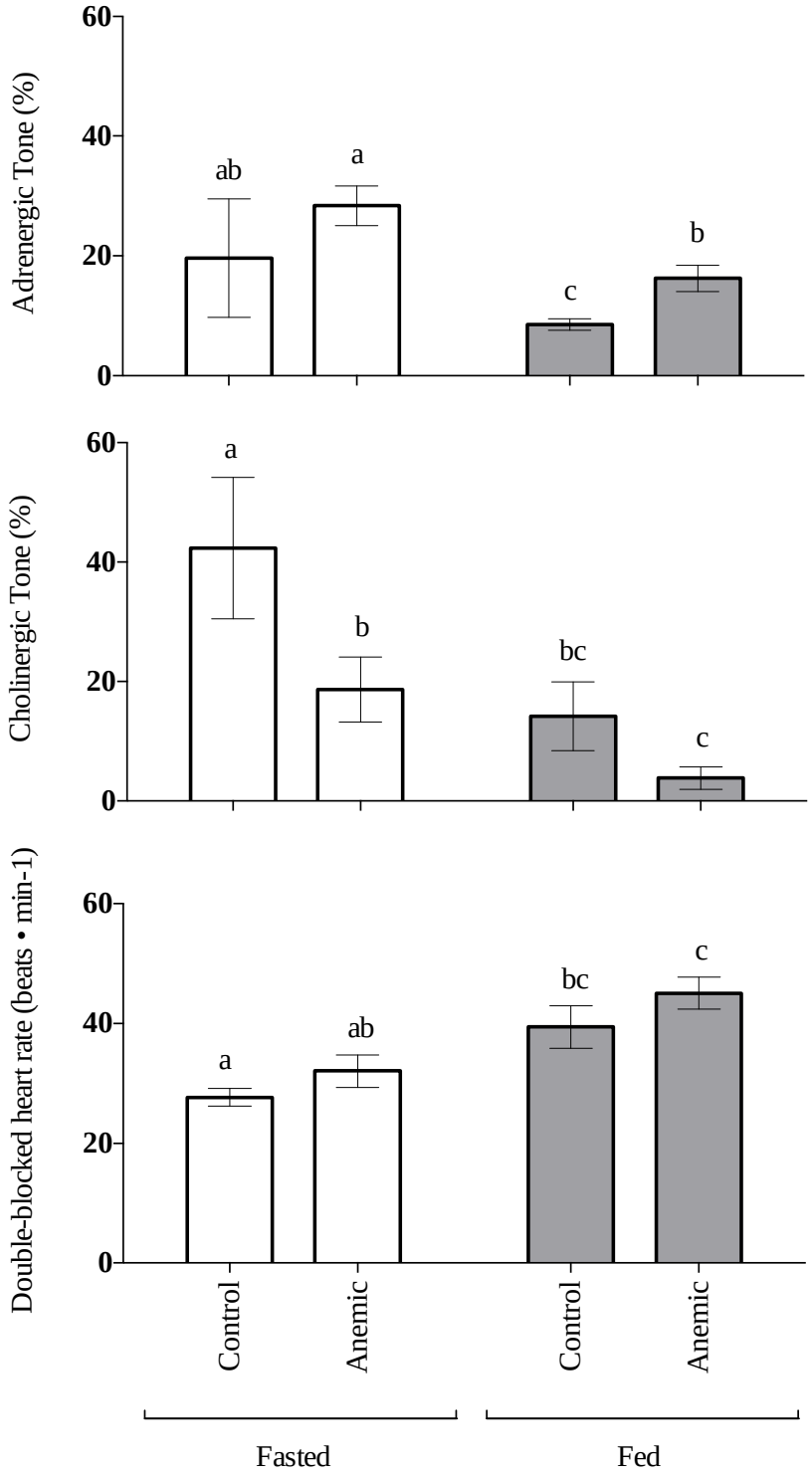


Figure 3

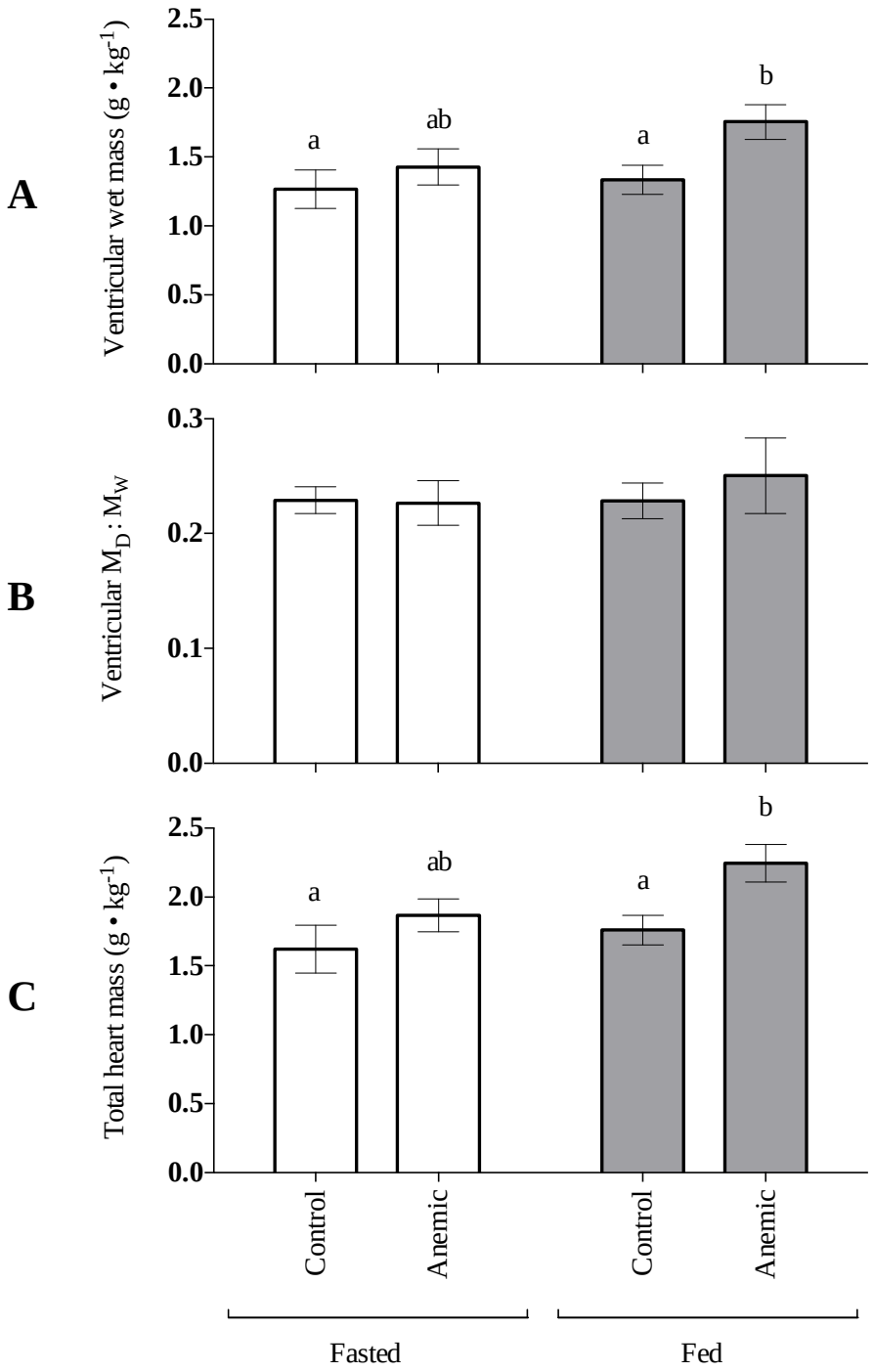
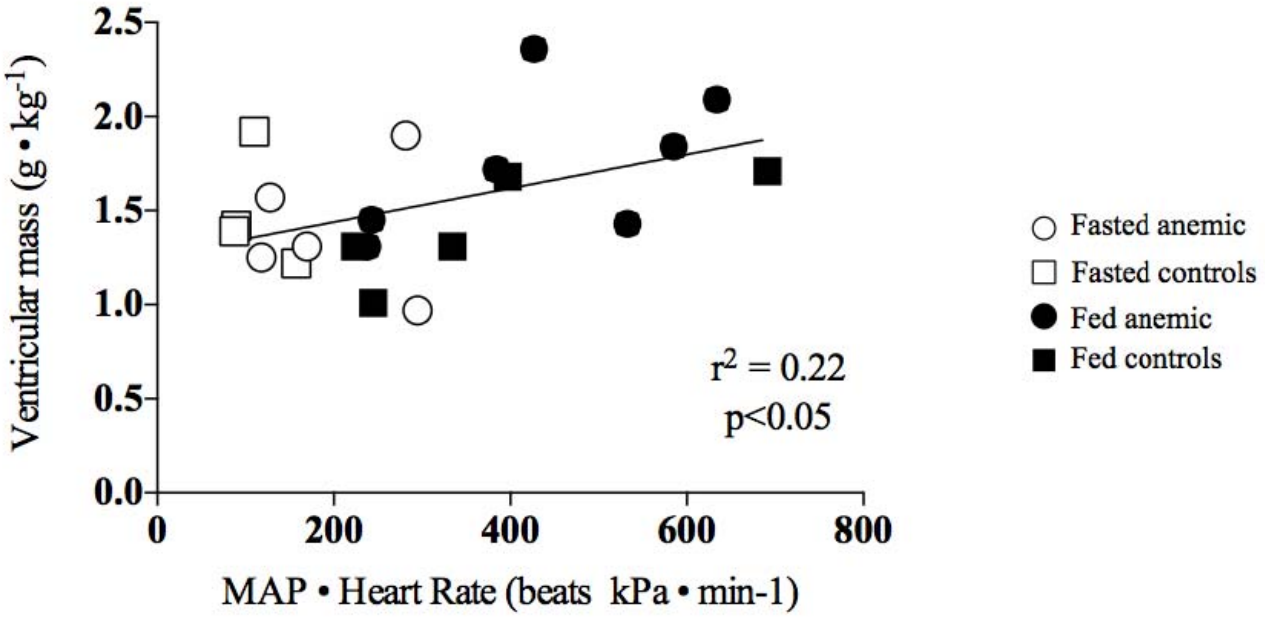
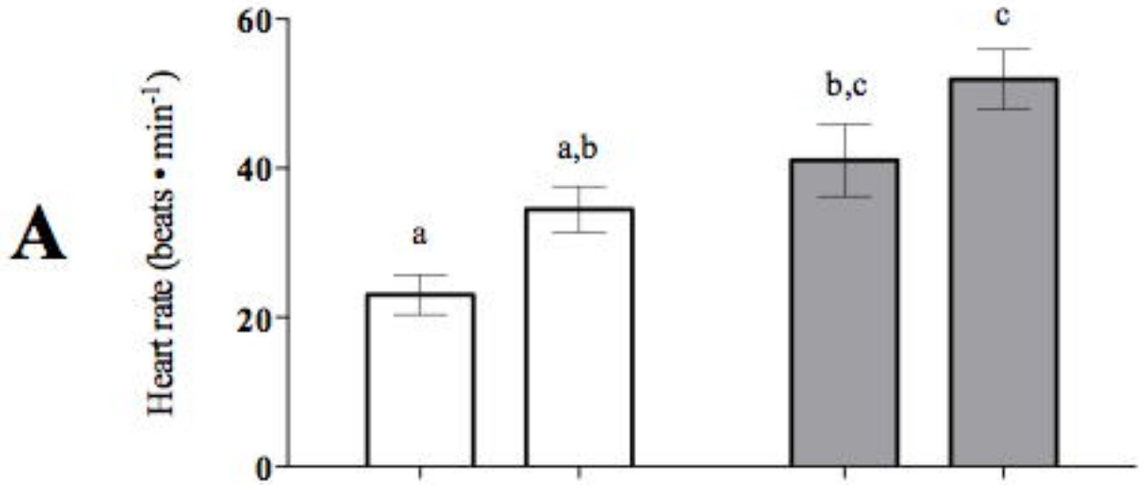
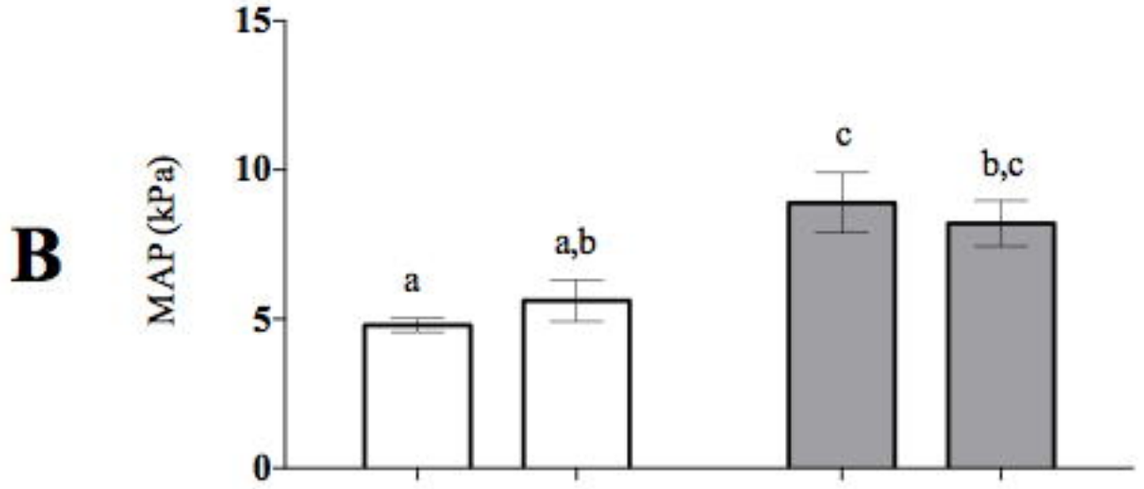
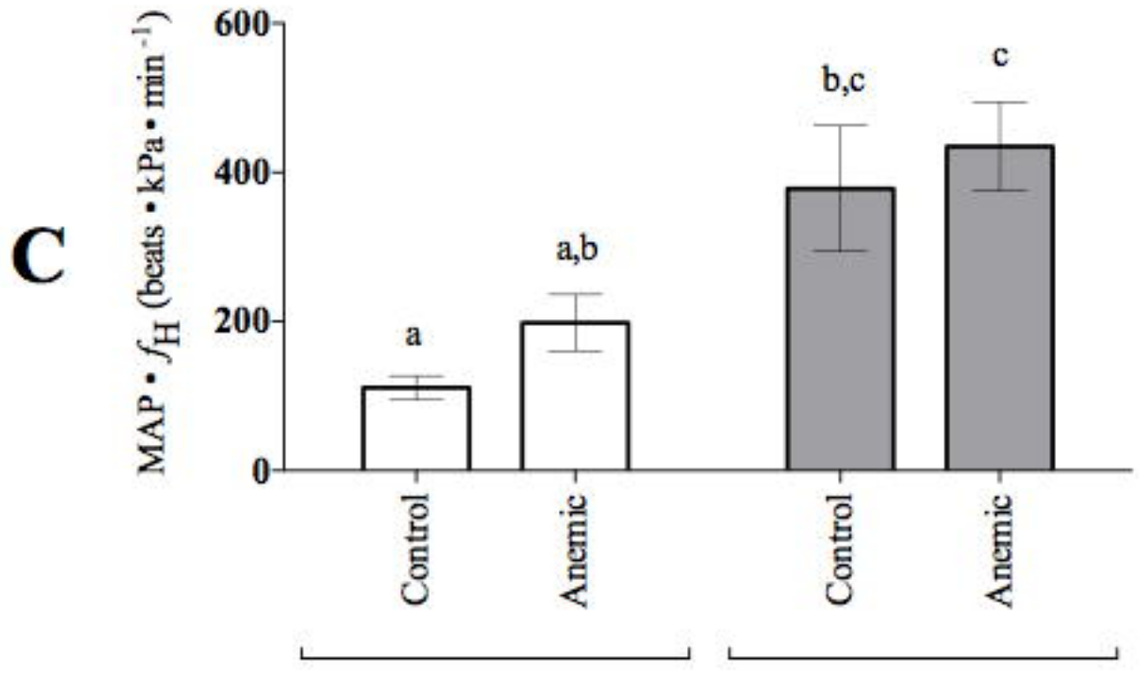


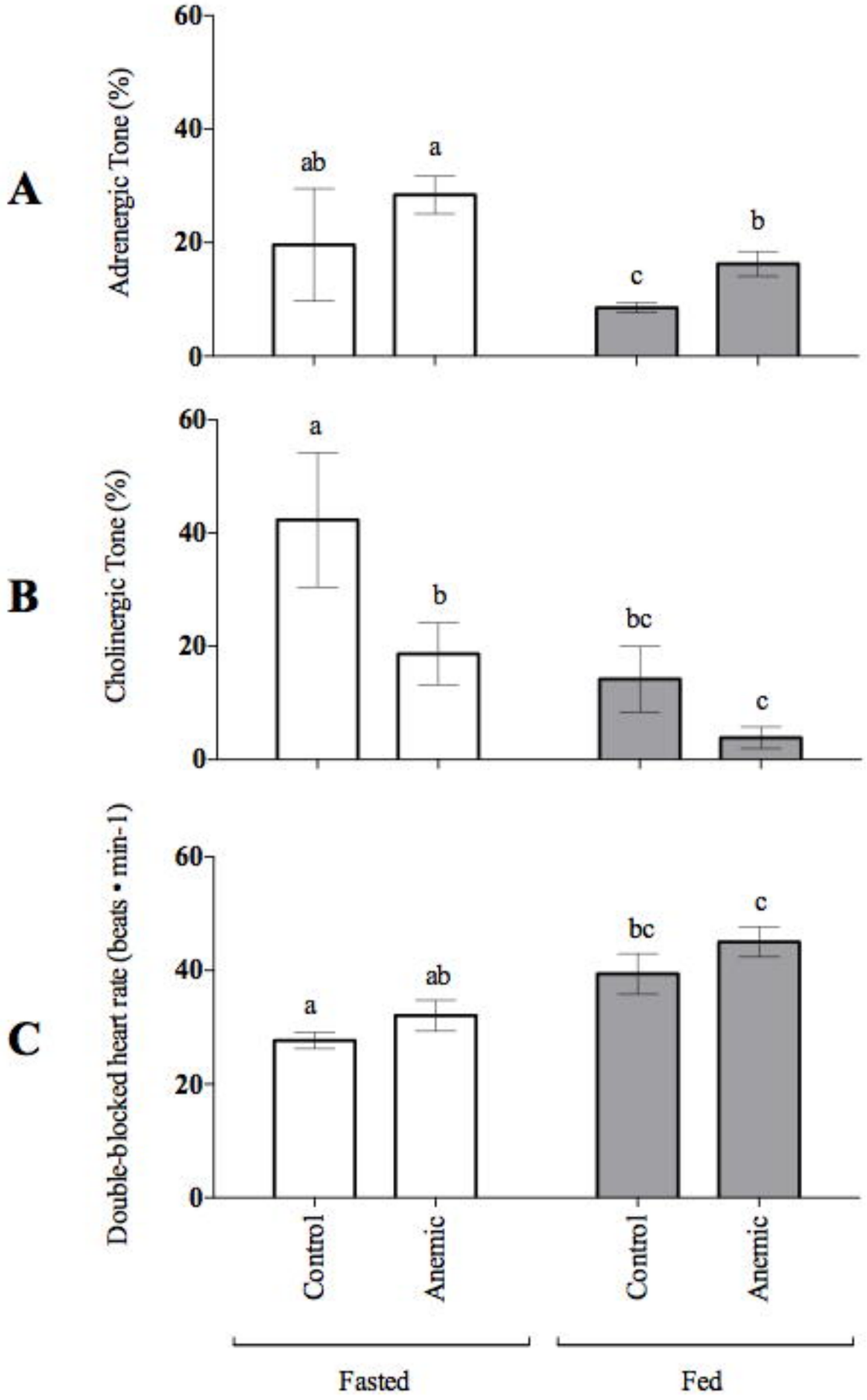
Figure 4

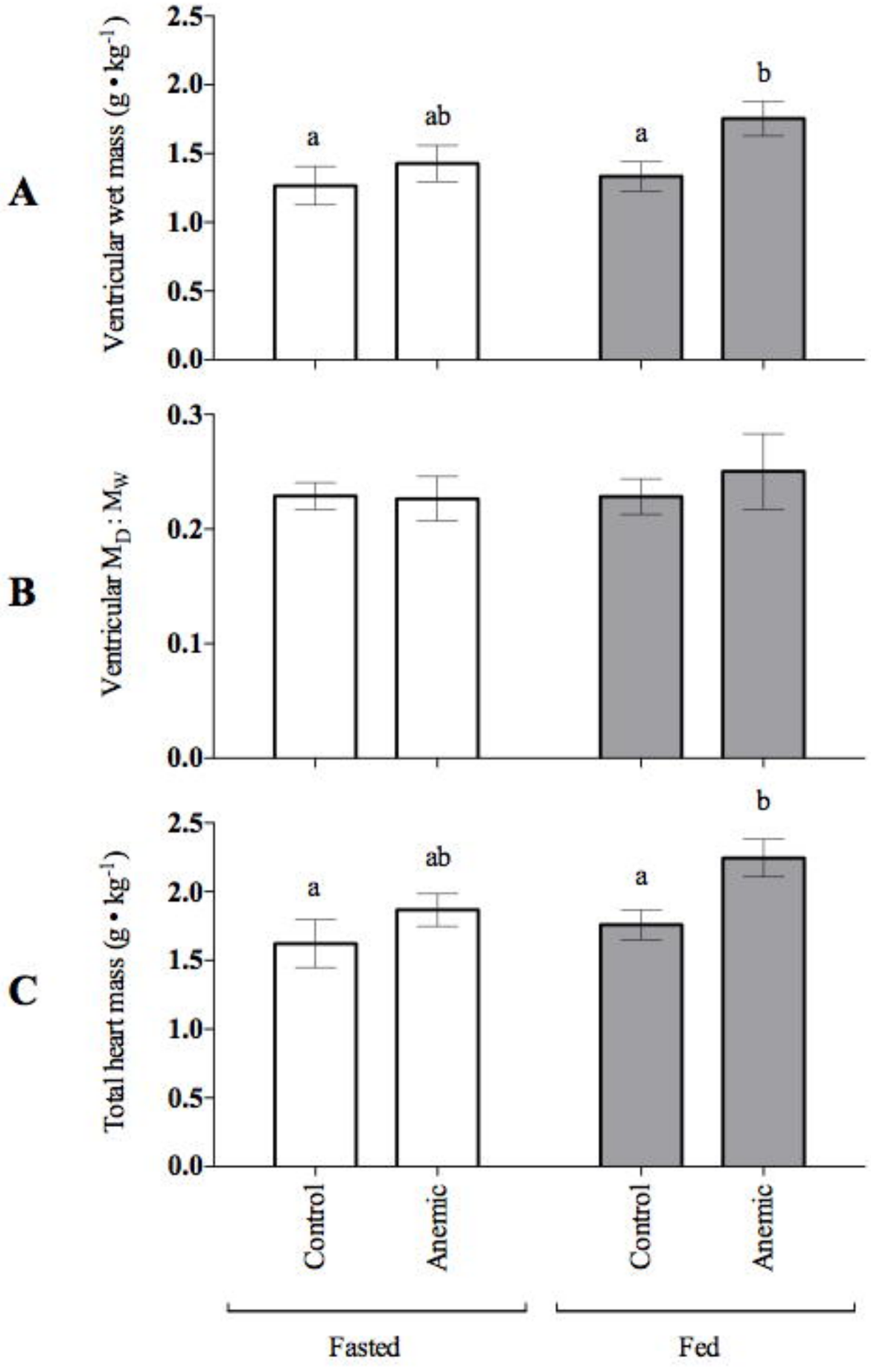


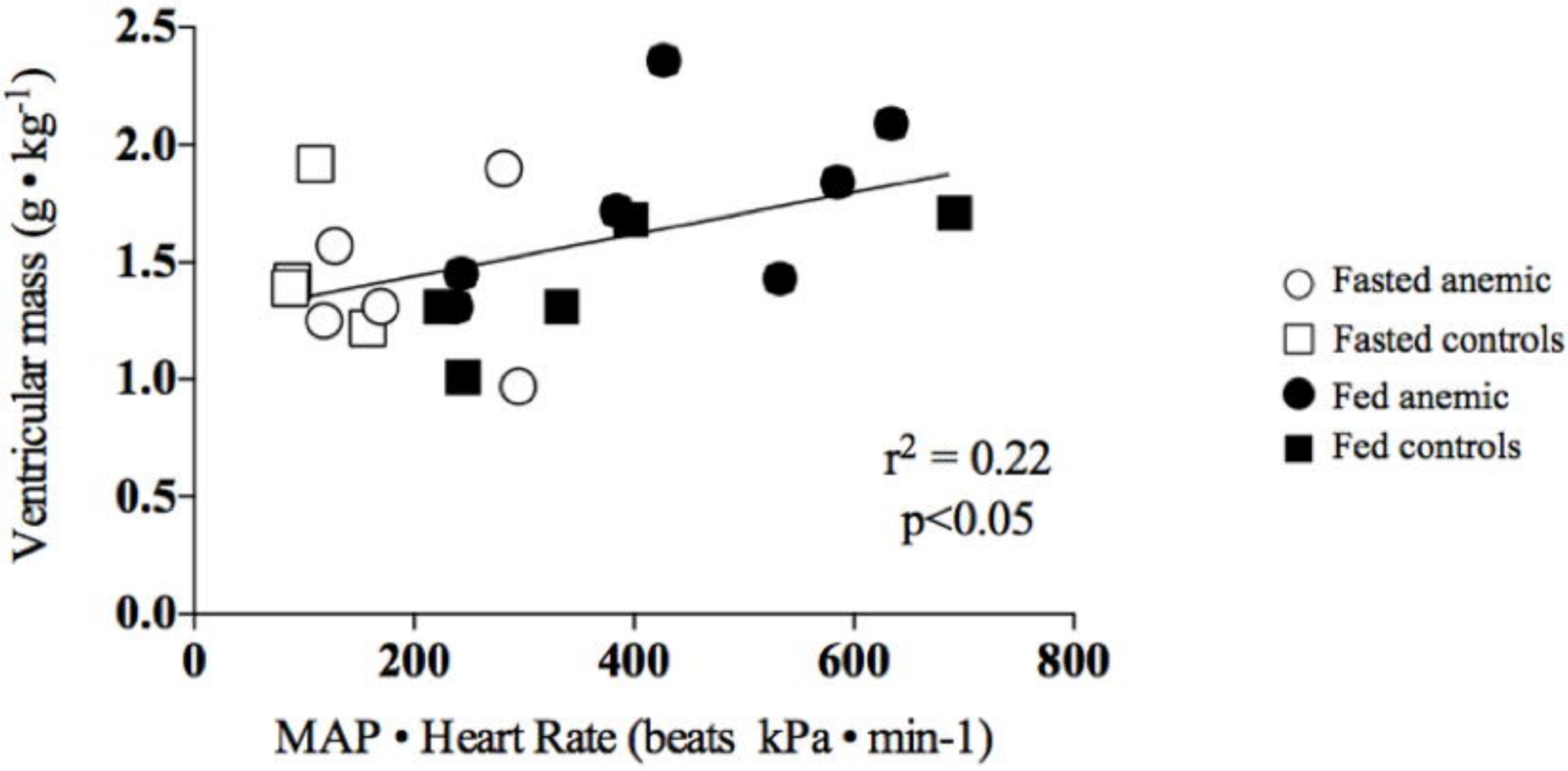

A
Heart rate (beats • min-1)
60
40
20
0
a
a,b
b,c
c


B
MAP (kPa)
15
10
5
0
a
a,b
c
b,c


C
MAP • fH (beats • kPa • min-1)
600
400
200
0
a
a,b
b,c
c
Control
Anemic
Control
Anemic
Fasted
Fed


A
Adrenergic Tone (%)
60
40
20
0
ab
a
c
b
B
Cholinergic Tone (%)
60
40
20
0
a
b
bc
c
C
Double-blocked heart rate (beats • min-1)
60
40
20
0
a
ab
bc
c
Control
Anemic
Control
Anemic
Fasted
Fed


A
Ventricular wet mass (g • kg-1)
2.5
2.0
1.5
1.0
0.5
0.0
a
ab
a
b
B
Ventricular MD : MW
0.3
0.2
0.1
0.0
C
Total heart mass (g • kg-1)
2.5
2.0
1.5
1.0
0.5
0.0
a
ab
a
b
Control
Anemic
Control
Anemic
Fasted
Fed


Ventricular mass (g • kg-1)
2.5
2.0
1.5
1.0
0.5
0.0
0
200
400
600
800
MAP • Heart Rate (beats kPa • min-1)
r2 = 0.22
p<0.05
Fasted anemic
Fasted controls
Fed anemic
Fed controls