# Postprandial cardiac hypertrophy in pythons

## This snake can synthesize fresh heart muscle to cope with extra metabolic demand.

Oxygen consumption by carnivorous reptiles increases enormously after they have eaten a large meal in order to meet metabolic demands, and this places an extra load on the cardiovascular system. Here we show that there is an extraordinarily rapid 40% increase in ventricular muscle mass in Burmese pythons (*Python molurus*) a mere 48 hours after feeding, which results from increased gene expression of muscle-contractile proteins. As this fully reversible hypertrophy occurs naturally, it could provide a useful model for investigating the mechanisms that lead to cardiac growth in other animals.

**Hearty meal: the python's cardiac ventricles grow after feeding.**

The heart is remarkable for its ability to remodel itself in response to altered functional demands. For example, chronic exercise training in mammals results in ventricular hypertrophy[1], which is beneficial because the resulting increase in stroke volume leads to a decrease in the resting and submaximal heart rates, and to an increase in filling time and in venous return[2].

Burmese pythons are considered to be an excellent model of extreme physiological upregulation[3]. While digesting, their metabolic rate may increase by up to 40-fold relative to the fasting rate and may be raised for as long as 14 days (ref. 3). This increase in oxygen consumption is accompanied by rapid remodelling of many physiological systems: within two days of feeding, there is a substantial increase in wet mass of the gastrointestinal system, kidneys, liver, pancreas, lungs, heart and stomach[4]. However, the cause of this remodelling, which could be increased protein synthesis or increased fluid content, is unclear[3,5].

To investigate the nature of cardiac hypertrophy in pythons following feeding, we obtained ventricles from three groups of Burmese pythons: fasting (fast of 28 days), digesting (two days after consuming rats equal to 25.0 ± 0.1% body mass) and post-digestion (28 days after the meal). (For methods, see supplementary information.) Oxygen consumption increased sevenfold and ventricular mass increased significantly ($P < 0.003$) by 40% during digestion (Table 1). This increase was fully reversible, as the ventricular mass returned to its fasting mass in post-digestion animals.

There was no change in the ventricular dry/wet mass ratio, indicating that the increased ventricular mass during digestion was not due to water shifts between extra- and intracellular compartments. Total protein, RNA and myofibrillar concentrations on a tissue-mass-specific basis did not change during digestion (Table 1). Mass-specific DNA concentration significantly decreased ($P < 0.01$), and this is consistent with the ventricular mass increase by cellular hypertrophy found in rats[6]. All of these measurements indicate a rapid new growth of ventricular tissue.

To investigate whether this growth was a result of increased transcription, we sequenced the isoforms of cardiac myosin heavy chains (GenBank accession numbers: AY773093 and AY773094). We found a significant increase in the expression of messenger RNA for heavy-chain cardiac myosin during digestion (Table 1), judging both by polymerase chain reaction with reverse transcription ($P < 0.0001$) and by band intensity on northern blots ($P < 0.0001$; Fig. 1).

We conclude that the newly synthesized protein results from increased transcription of the gene encoding cardiac myosin heavy chains and that cardiac hypertrophy follows from *de novo* addition of contractile elements. This cardiac hypertrophy is likely to have important consequences for oxygen transport and could explain why stroke volume in postprandial pythons is 50% greater than that measured in fasted animals doing maximal exercise[7].

This ventricular growth in postprandial pythons is very rapid compared with that in mammalian models, in which comparable increments in ventricular size take weeks to develop[8]. In addition, mammalian models may necessitate highly invasive procedures for variable aortic occlusion, such as hydraulic constrictors, inflatable cuffs or angioplasty balloons, which can induce acute congestive failure and aortic rupture

Table 1 **Cardiac hypertrophy following digestion in Burmese pythons**

| *Phase* | *Fasting* | *Digesting* | *Post-digestion* |
|---|---|---|---|
| Pre-feeding body mass (kg) | 0.702 ± 0.045 | 0.783 ± 0.126 | 0.721 ± 0.070 |
| Oxygen consumption (ml $O_2$ $min^{-1}$ $kg^{-1}$) | 0.67 ± 0.056 | 4.49 ± 0.385 * | 0.54 ± 0.070 |
| Ventricular mass (g wet mass) | 0.95 ± 0.070 | 1.34 ± 0.202 * | 1.07 ± 0.062 |
| Ventricular mass (% body mass) | 0.136 ± 0.006 | 0.168 ± 0.004 * | 0.137 ± 0.007 |
| Dry/wet mass of ventricles (%) | 22.4 ± 1.470 | 23.1 ± 1.91 | 20.3 ± 0.06 |
| Total protein (μg per mg ventricle) | 236 ± 17 | 208 ± 10 | 249 ± 24 |
| DNA (ng per mg ventricle) | 2.93 ± 0.11 | 2.54 ± 0.12 * | 3.09 ± 0.12 |
| RNA (μg per mg ventricle) | 0.96 ± 0.064 | 1.11 ± 0.080 | 1.08 ± 0.080 |
| Myofibrillar protein (μg per mg ventricle) | 32.9 ± 2.25 | 34.8 ± 3.78 | 30.7 ± 2.89 |
| Ventricular Mhc mRNA (Mhc/18S) | 0.97 ± 0.163 | 2.11 ± 0.221 * | 0.78 ± 0.139 |
| Ventricular Mhc mRNA (intensity) | 2,320 ± 526 | 31,650 ± 5,280 * | 5,140 ± 1,020* |

Mhc, cardiac myosin heavy chain; fasting, 28 d fasted; digesting, 48 h postprandial; post-digestion, 28 d postprandial. Ventricular Mhc mRNA expression was determined by semiquantitative reverse-transcription polymerase chain reaction (RT–PCR) and by northern blot (Fig. 1). In RT–PCR, Mhc mRNA is shown as a ratio (Mhc/18S) of 18S ribosomal subunit mRNA expression, which is considered to be constant. Quantitative mRNA expression measured by northern blot is given as intensity. $N = 6$ per group. Values are means ± 1 s.e.m. All experiments were carried out under University of California at Irvine Animal Research Committee Protocol Number 1999-2123.

*Significantly different ($P < 0.05$) from fasting value by one-tail *t*-test.

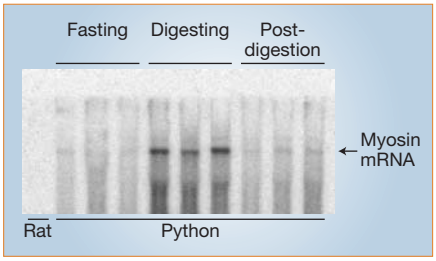


**Figure 1** Northern blot showing increase in myosin mRNA in the cardiac ventricles of postprandial Burmese pythons. Each lane represents RNA from an individual python ventricle, hybridized to a probe specific to the ventricular myosin heavy-chain isoform. The same blot hybridized with a probe for the python atrial isoform gave no detectable signal. The lane immediately to the left of the fasted samples contains rat ventricular RNA, showing that the probe does not crossreact. Fasting phase, 28 d fasted; digesting phase, 48 h postprandial; post-digestion phase, 28 d postprandial.

as well as hypertrophy. Because Burmese pythons naturally undergo a 40%, fully reversible increase in ventricular mass in the two days after a meal, they could provide an attractive model for investigating the fundamental mechanisms that lead to cardiac remodelling and ventricular growth[9]. The physiological stimuli underlying this hypertrophy are still unknown, but are likely to include neural and humoral factors.

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Synaesthesia

# When coloured sounds taste sweet

Synaesthesia is the involuntary physical experience of a cross-modal linkage — for example, hearing a tone (the inducing stimulus) evokes an additional sensation of seeing a colour (concurrent perception). Of the different types of synaesthesia, most have colour as the concurrent perception[1], with concurrent perceptions of smell or taste being rare[2,3]. Here we describe the case of a musician who experiences different tastes in response to hearing different musical tone intervals, and who makes use of her synaesthetic sensations in the complex task of tone-interval identification. To our knowledge, this combination of inducing stimulus and concurrent perception has not been described before.

Table 1 **Tastes triggered by tone intervals**

| *Tone interval* | *Taste experienced* |
|---|---|
| Minor second | Sour |
| Major second | Bitter |
| Minor third | Salty |
| Major third | Sweet |
| Fourth | (Mown grass) |
| Tritone | (Disgust) |
| Fifth | Pure water |
| Minor sixth | Cream |
| Major sixth | Low-fat cream |
| Minor seventh | Bitter |
| Major seventh | Sour |
| Octave | No taste |

Tastes experienced by synaesthete E.S. in response to different musical tone intervals are shown; in the case of the fourth and tritone intervals, however, complex visual and emotional perceptions, respectively, are induced. Note that dissonant tone intervals induce unpleasant tastes and consonant ones induce pleasant ones (for example, the minor second intervals induce sour tastes, and the major thirds induce sweet ones). There is also an apparent symmetry in some of the responses: the minor second and major seventh, which are mirror-image intervals in terms of octave equivalence, are both rated as sour, and the major second and minor seventh are both rated as bitter.

E.S. is a 27-year-old professional musician who is female, right-handed and of average intelligence[4] (IQ, 115). Whenever she hears a specific musical interval, she automatically experiences a taste on her tongue that is consistently linked to that particular interval (Table 1). Besides this exceptional interval-to-taste synaesthesia, she also reports the more common tone-to-colour synaesthesia, in which each particular tone is linked to a specific colour (for example, C and red; F sharp and violet).

Both synaesthetic perceptions have always been consistently reproducible. We repeatedly tested E.S. for over a year and have confirmed that her interval-to-taste synaesthesia is unidirectional: she does not hear tone intervals when exposed to taste. In addition, E.S. applies this synaesthesia in identifying tone intervals (which is evidence of a synaesthesia–cognition cascade).

To assess the influence of E.S.'s synaesthetic gustatory perception on her ability to identify tone intervals, we adapted the Stroop task[5] (for methods, see supplementary information). Four selected tone intervals (seconds and thirds) were presented while applying four differently tasting solutions (sour, bitter, salty and sweet) to E.S.'s tongue. Her task was to identify the tone intervals by pressing a particular button for each interval on a computer keyboard. Reaction times and errors were measured for trials in which the applied taste was either congruent or incongruent with the tone interval; tone intervals were also presented without taste stimulation.

We found that E.S.'s tone-interval identification was perfect and was significantly faster during the congruent condition compared with all the other conditions (Fig. 1). Five non-synaesthetic musicians were tested as controls using the same procedure: no significant between-condition differences were found. The reaction times of the controls were comparable to those of E.S. in the no-taste condition (Fig. 1).

To exclude conceptual priming effects as an explanation for these results (for example, the subject might imagine sourness when presented with 'sour' as either a taste or word), we also tested E.S. by showing her the word(s) describing each taste. We found no between-condition difference in this conceptual task (Fig. 1).

Together, these results indicate that E.S.'s performance in the gustatory Stroop task is most likely to be due to her extraordinary type of synaesthesia, in which a complex inducing stimulus leads to a systematic, concurrent gustatory sensation. This case differs from another gustatory synaesthete, S., who reported blended gustatory sensations (such as specific meals) in response to simple auditory stimuli (tones and sounds)[2]. E.S.'s application of her synaesthetic sensations in identifying tone intervals — a complex task that requires formal musical training — demonstrates that synaesthesias may be used to solve cognitive problems.

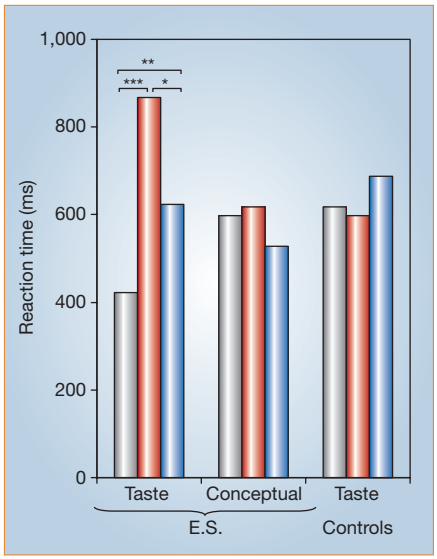


**Figure 1** Mean reaction times in a gustatory Stroop task linking perception of tone intervals with different tastes for congruent-taste (grey), incongruent-taste (red) and no-taste (blue) conditions for synaesthete E.S. and for five non-synaesthetic musicians (controls). In the 'Taste' condition, musical intervals were presented while solutions of different taste (citric acid, 20 g litre$^{-1}$; quinine, 60 mg litre$^{-1}$; salt, 10 g litre$^{-1}$; sucrose, 120 g litre$^{-1}$) were delivered to the subject's tongue. The 'Conceptual' condition followed the same procedure, except that words describing the tastes, instead of the tastes themselves, were visually presented 2 s before the tone interval. Non-parametric randomization tests were used for statistical comparison. For E.S., all statistical comparisons in the taste condition were associated with $P$ values of less than 0.01 ($^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$). For control subjects and for the conceptual condition, none of the comparisons revealed significant differences. The reaction time of E.S. in the no-taste condition is similar to those of the controls, but is faster in the congruent condition and slower in the incongruent condition.

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Supplementary methods.

Burmese pythons (*Python molurus*) (N=18) of undetermined sex with a body mass ranging from 0.503 - 1.286kg (mean mass = 0.729 ± 0.043 S. E. kg) were obtained from Bob Clark Captive Bred Reptiles, Oklahoma City, OK, USA. The snakes were kept in separate containers with access to water at 30°C with a 12h: 12h light: dark cycle in the University of California, Irvine animal housing facility. The animals fasted for 4 weeks, and then were divided into 3 groups: fasting, 2 days, and 28 days postprandial. Fasting animals (N=6) were sacrificed immediately following the four weeks of fasting; the remaining animals voluntarily ate rats equivalent to 25.0 ± 0.1 % of body mass. After two days, six snakes were sacrificed and the last group was sacrificed 28 days after the meal.

Pythons were decapitated and the ventricle was swiftly removed. After gentle pressure and blotting in saline, the remains of the major arteries were removed, and the wet mass of the ventricle was measured. The ventricles then were cut into 8 pieces, approximating transverse sections from apex to base. The sample used to compare wet and dry mass was weighed before freezing; all other pieces were freeze-clamped, wrapped in foil, and immersed in liquid nitrogen. All samples were frozen within 10 min following decapitation, and stored at – 80°C until analysis (up to 8 weeks).

*Oxygen consumption*

Oxygen consumption ($\dot{V}O_2$) was measured in the three experimental groups by flow-through respirometry at 30°C. Snakes were placed in the respiratory chambers and allowed to acclimate for at least 24 h before measurements were started. During measurements, the outflow from the chambers was dried (Drierite; Xenia, Ohio) and analyzed for % $O_2$ (S-3 A/I; Applied Electrochemistry; Sunnyvale, California). All gas exchange data are presented as standard temperature pressure dry (STPD).

*Dry/Wet mass*

The pre-weighed samples were dried in a lyophilizer for 2.5h at – 50°C and the dry mass/wet mass ratio was calculated.

*Myosin isoform sequencing*

Total RNA $[RNA]_{total}$ was isolated from a piece of frozen ventricle (~ 25mg) following homogenization in Tri-Reagent and extraction with BCP Reagent (Molecular Research Center, Cincinnati, OH). RNA was precipitated by addition of isopropanol, the samples were washed twice in 75% ethanol, and finally spun dry in a vacuum centrifuge. The RNA was solubilized in 10µl of RNA/DNase free water, and then heated to 50°C for 2 min. The $[RNA]_{total}$ was determined spectrophotometrically at 260nm.

*MHC sequence generation*

The 3' RACE (Rapid Amplification of cDNA Ends, Invitrogen, Carlsbad, CA) technique was applied to 3-5 µg of total RNA from the ventricles[1]. Total RNA was reverse-transcribed to cDNA and appended with an adapter primer at the 3' end, via SuperScript II (Invitrogen, Carlsbad, CA). Amplification of target DNA by PCR was performed using a 20 bp oligonucleotide of known identity in mysoin genes (5' agaaggagcaggacaccagc), lying 500bp upstream of the stop codon.

The PCR (Robocycler, Stratagene, La Jolla, CA) was carried out on 1 μl of cDNA in 25 μl of PCR reaction buffer[2]. The resulting 600-700bp fragments, containing MHC isoforms and other potentially unrelated sequences, were eluted from 1.5% agarose-TAE gels (Qiagen, Valencia, CA) into 35 μl of water. The eluted sequences were ligated (Rapid DNA Ligation Kit, Roche, Indianapolis, IN) into a pGEM-T vector (Easy Vector System, Promega, Madison, WI). Two μl of each cDNA sample were added to a cocktail with 1 μl of ligase, following the manufacturer provided protocol. Ligation proceeded for 30 min at room temperature, followed by termination at –20 °C. Competent DH5 cells (Invitrogen, Carlsbad, CA) then were transformed (2 μl of the resulting ligation reaction with 50 μl of cells) on ice for 30 min. The cells were briefly heat-shocked (37°C), then added to LB medium and incubated for 1 hour at 37 °C. Cells were collected by centrifugation and were plated for 14h on LB-medium agarose plates containing 100 g/ml ampicillin and X-gal. Colonies containing the DNA insert were incubated overnight at 37 °C, in 3ml LB-medium and ampicillin. Plasmid DNA was extracted by MiniPrep (Qiagen, Valencia, CA), and sequencing reactions (~200 total, bi-directional using SP6 and T7 primers) were performed using ABI Prism BigDye (Applied Biosystems, Foster City, CA) and HalfBD Enhancer reagent (Genetix, Charlestown, MA). Sequences were analyzed on an ABI Prism 3100 capillary sequencer (UCI-DNA Core Facility).

*MCH sequence analysis and semi-quantitative RT-PCR*

Sequences of ~600bp, representing the last ~500bp of each gene, and an additional 50-100bp of the 3' untranslated region, were compared by BLASTn analyses (NCBI) to other myosin sequences. Two sequences were identified as putative cardiac MHC isoforms. Identity as specifically α or β could not be resolved; however, each was uniquely obtained from either atrial or ventricular tissue. Primers for the ventricular isoform were identified by eye, utilizing the higher variability in the 3' untranslated region; these were modified in length to conform to a common annealing temperature of 55°C. A multiplex PCR reaction was performed on the individual ventricular samples; each reaction was comprised of the MHC common primer, the ventricular isoform-specific primer, and primers for the 18s ribosomal subunit together with a competing primer (10pmol/μl, 55°C annealing temperature)[1,2]. Care was taken to ensure that the PCR reaction was in the linear range by comparison with reactions of fewer or greater cycle numbers.

*Northern blots and hybridization.*

These measurements were accomplished as previously described[2]. Briefly, approximately 5 μg of total RNA were electrophoresed in 0.8% agarose gels in a buffer containing 8% formaldehyde, 20 mM 3(*N*-morpholino)-propanesulfonic acid, 5 mM sodium acetate, and 0.5 mM EDTA, pH 7.0. The RNA was transferred to a quiabrane (Quiagen) nylon membrane by the capillary method using 10× saline-sodium citrate and subsequently covalently cross-linked to the nylon membrane by UV light (UV crosslinker, Fisher Scientific). After drying at 80°C for 1 h to evaporate the formaldehyde, blots were stored at 4°C until used for hybridization. For hybridization, the 5' antisense oligonucleotides used above for the PCR reaction was used. Hybridization with the probe, washing, and exposure to the autoradiographic film was done simultaneously for 3 samples from each group. Intensity of the bands was determined through autoradiography, and then the probes were washed off the blots by boiling for 10-15 min in 1% SDS. The blots then were rehybridized with a general MHC probe to verify equal hybridization and gel loading. Autoradiograms were analyzed by laser-scanning densitometry (Molecular Dynamics).

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