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Cardiac hypertrophy and structural and metabolic remodeling related to seasonal dormancy in the first annual cycle in tegu lizards

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ABSTRACT

Morpho-functional adjustments in the heart of juvenile tegu lizards (*Tupinambis merianae*) were analyzed at distinct seasonal periods to investigate how the demands of growth and of energy saving are reconciled during the first annual cycle. The relative ventricular mass (Mv) was 31% and 69% larger in late autumn and winter dormancy, respectively, compared to early autumn. This effect did not persist during unfed arousal, suggesting that protein accumulates in the heart during hypometabolism and is degraded on arousal. Both the hypertrophy and the atrophy were disproportionate in the largest individuals. In contrast, Mv was smaller in lizards that were starved during spring activity compared to fed lizards, this effect being larger in smaller individuals. In late autumn and winter dormancy the spongy myocardium had 8% of the section area covered by lacunary spaces, which expanded after food intake during arousal and reached 29% in spring activity together with higher density of cardiomyocytes. Total and soluble proteins per mass unity were unchanged, and maximum activities of selected enzymes suggest sustained glycolytic and aerobic capacities during hypometabolism. Results indicate that important structural adjustments occur in the heart in anticipation of dormancy, and that the protein balance in the tissue is maintained at winter temperatures ~17 °C.

1. Introduction

Many vertebrate species exhibit seasonal dormancy during the annual cycle, and this is characterized by fasting and by a marked depression of energy metabolism that is associated with the inhibition, or even cessation, of several physiological functions. These animals are able to predict the phase of harsh climate conditions and/or food scarcity and prepare themselves through a series of metabolic and behavioral adjustments (Guppy and Withers, 1999; Heldmaier et al., 2004). These adjustments allow, among other activities, the storage of energy substrates, and thus are an extraordinary example of phenotypic flexibility. One remarkable aspect of seasonal dormancy is the maintenance of a reduced level of cardiac function. Despite the low heart rate (HR) and the low blood flow in the lumen of the organ, the capacity of the heart to rapidly increase its rate of activity seems to be conserved throughout dormancy. The ability of these animals to withstand large fluctuations in energy supply without compromising the functional capacity of the heart raises questions concerning the nature of the adaptive changes that occur in the heart tissue in association with the seasonal cycle and how early in the life cycle the corresponding traits would be activated. Furthermore, the changes in the heart should occur in anticipation of the long period of fasting during dormancy; therefore, another question of interest is whether a sudden curtailing of food resources in the months of activity would cause similar changes in the hearts of these animals.

The present study examined these questions during the first annual cycle of juvenile tegu lizards of the species *Tupinambis merianae*, which is widely distributed throughout South America (Avila-Pires, 1995). After hatching in the first half of summer, tegus grow at a high rate until the autumn. At this point, aerobic metabolism starts to be gradually depressed in the whole animal, which causes the resting VO_2 value to decline before stabilizing at ~20% of the resting value during winter dormancy (Souza et al., 2004). During arousal, a partial increase (25%) in aerobic metabolism occurs in aphagic animals, and its rates increase further after food intake recommences a few days later. In contrast with small mammals that go into deep torpor at body temperatures near 0 °C (Geiser, 1988), a similar degree of metabolic depression occurs in tegus at mild winter temperatures of ~17 °C, which implies that temperature-independent mechanisms regulate energy metabolism in these lizards. In dormant tegus, HR stabilizes at approximately 12 bpm and is independent of changes in body temperature, while HR is positively correlated with body temperature in active animals and can reach 100 bpm (Abe, 1983; Galli et al., 2006). Additionally, the spongy layer of the myocardium of the majority of reptiles is supplied with blood in the lacunary interfibrillar spaces, and this blood is continuous

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with that flowing in the lumen of the organ (Tota, 1983). Therefore, the maintenance of structural integrity and functional capacity of the heart during winter months should involve adjustments that compensate for the altered rate of perfusion of the myocardium.

Marked changes in morphology and energy metabolism in the mixed myocardium of ectothermic species have been reported to occur during the ontogenetic development (Tota, 1983; Cerra et al., 2004). To our knowledge, no previous study has integrated development and seasonal dormancy at early stages of life. In newly hatched tegus, morphological and physiological changes would be the products of a complex interaction in which a seasonal rhythm is superimposed on the developmental process. A previous study of the tegus has shown significant shifts in both the mass exponent for aerobic metabolism and the mass of fat bodies during the first annual cycle, both on entry into winter dormancy and on arousal, which suggests that body mass size would set a limit to the capacity to survive the first annual cycle (Souza et al., 2004). Here, we focus on the high energy demand associated with growth and on the small capacity for substrate storage in newly hatched individuals to test the hypothesis that dormancy in tegus at winter temperatures of ~17 °C is achieved by sustaining the rates of anabolic processes in the heart and thereby preserving its protein composition and tissue integrity. In small mammals, protein synthesis ceases when the body temperature reaches 18 °C (van Breukelen and Martin, 2002), however, recent evidence has shown that the protein balance in select tissues is maintained (Lee et al., 2012). Because significant amounts of lean mass are catabolized in the course of fasting during dormancy in juvenile tegus (Souza et al., 2004), we also hypothesized that some protein from the heart stores may be catabolized, thereby compromising both the aerobic capacity of the smallest lizards and their fast return to activity. We examined the morphology of the heart tissue and analyzed protein fractions from the heart, and we integrated the data with our knowledge about the activities of the enzymes involved in supplying energy to the heart muscle to improve our understanding of how the demands of growth and of energy saving are reconciled in the first annual cycle.

2. Materials and methods

2.1. Animal supply and maintenance

Newly hatched tegu lizards (*Tupinambis merianae* Duméril and Bribon) were obtained from a population reared outdoors in large pens in Rio Claro, southeastern Brazil. After hatching in the summer, the animals were maintained indoors in 120-liter cages equipped with incandescent lights and were placed under a 9 · h:15 · h light: dark photo- and thermal-period in addition to the sunlight diffusing from outside. The lizards could freely alternate between warming and cooling their bodies by climbing onto a small platform or by hiding either in a wooden shelter or among the sheets of paper covering the floor of the box. The animals were fed every two days on a diet of raw meat, eggs and fruits that was enriched with minerals, and they had continuous access to drinking water.

A noticeable change in daily activity was observed in the early autumn, with a progressive shortening of the time spent on thermoregulation and a gradual decrease of food intake until the lizards became continuously inactive inside the shelter. The animals were kept in the shade throughout the winter months, and by early spring they had expelled a dried pellet of uric acid and begun to move outside the shelter. These changes were taken as an indication of arousal, and the animals were returned to the previous photo- and thermal-period, with free access to drinking water. A gradual increase in the time spent on thermoregulation and food intake took place over the subsequent weeks. The minimum and maximum air temperatures were recorded daily using a thermometer placed in the shelter area. The mean ranges for each seasonal period were as follows: early autumn, 21–26 °C; late autumn, 18–23 °C; winter, 15–20 °C; early spring, 20–26 °C; late spring, 23–30 °C.

2.2. Tissue sampling

For tissue analysis, groups of lizards were weighed before being killed by decapitation during the annual cycle and named according to the seasonal period as follows: '**autumn activity**', which, for some assays, was divided into two sub-groups named '**early**' (mean body mass ± SE = 175 ± 36 g) and '**late**' (192 ± 30 g), which were killed in the first and second half of autumn, respectively; '**winter dormancy**' (184 ± 23 g), which included totally inactive, fasting lizards after 60–70 winter days; '**arousal**', which, for some assays, was divided into two sub-groups named '**unfed**' (164 ± 17 g) and '**fed**' (177 ± 33 g), which comprised rehydrated lizards killed at 48–72 h after emerging from dormancy (i.e., 90–100 days from the first winter day) and lizards that were killed after their first meal at 6–8 days after arousal, respectively; '**spring activity**', which included fully active, '**fed**' lizards (195 ± 23 g), which were sacrificed at 30–40 days after arousal, and '**unfed**' lizards (305 ± 59 g), which were starved for 20 days during spring activity. This last sub-group was used in some assays to investigate the effects of unanticipated starvation during activity, thereby allowing a comparison of these effects with those of anticipated fasting during winter dormancy. This period of starvation during spring activity roughly corresponds to 50–60 days of fasting during winter dormancy, as estimated from the oxygen consumption rates of newly hatched tegus in the corresponding seasons (Souza et al., 2004).

The lizards were killed between 10:00 and 12:00 h. After 72 h of starvation, each animal was weighed and decapitated, and the heart was quickly removed and transferred to ice-cold saline in a Petri dish for further dissection. The ventricle was separated from the atria and weighed, and a lateral fragment of the ventricular wall was cut and transferred to fixative solution (4% formaldehyde in phosphate buffer, pH 7.4). The remaining tissue was cut into small pieces, quickly frozen in liquid nitrogen and stored at −80 °C until needed.

2.3. Histology and morphometry

The tissue fragments were taken from the fixative solution and the ends were removed to avoid the apex of the heart, which could contain an area of convergent fibers. The fragments were cut twice by incisions with random orientations and the resulting parts were dehydrated through a graded series of ethanol solutions, with a final dehydration step in 100% ethanol. The dried tissue was cleared in xylol and embedded in Paraplast Plus (Sigma Aldrich) with the tissue orientation randomized relative to the reference plane (the surface of the bench). Serial sections of 10 μm thickness were cut and stained with hematoxylin and eosin for histological analysis.

Four animals were analyzed per group using a light microscope (Nikon Eclipse E1000, NY, USA), with eight fields per animal randomly selected for analysis. Images were captured by a video camera (Low Light Integrating i308) connected to a computer. The area density of lacunary spaces (A_A) was determined by point-counting planimetry as $A_A = \sum PP/\sum PT$, where PP is the number of points hitting the lacunary spaces and PT is the total number of test points on the section. The numerical density of the myocytes (Nv) was determined in the spongy myocardium by the optical disector method (West and Gundersen, 1990; Mandarim-de-Lacerda, 2003). The sampling procedure was designed to achieve a final count of at least 200 nucleoli per animal. Counting was performed using a 100X oil-immersion objective and the density was determined as Nv = QA/disector, where QA is the number of nucleolus counted in the test frame plane and disector is the test volume determined by the product of the test area and the thickness of the disector (3 μm). All variables were measured using Stereo Investigator 2000 software (MicroBrightField 2000, Inc., Colchester, VT, USA).

2.4. Analysis of tissue metabolites

Total tissue water, proteins, and lipids were measured in samples of the heart ventricle. Water content was estimated from the mass lost when each tissue sample was incubated at 60 °C until it reached a constant mass. For total protein analysis, the tissue samples were homogenized in four volumes (v/w) of 0.6 M perchloric acid (PCA). The homogenate was centrifuged for 5 min at 10000 **g**, and the pellet was redissolved in 0.6 mM perchloric acid (PCA). This procedure was repeated twice. At the final round, the precipitate was solubilized in 2.5% KOH and its protein content was measured (Lowry et al., 1951). The sarcoplasmic and myofibrillar protein fractions were separated according to the method of Bates and Millward (1983) as modified by Somero and Childress (1990). The tissue was homogenized in buffer solution (20 mM imidazole-HCl pH 7.4, 2 mM EDTA, 0.1% Triton X-100) and centrifuged at 3000 **g** for 20 min at 4 °C. The supernatant was saved as part of the sarcoplasmic fraction. The pellet was resuspended in low-salt buffer (50 mM K_3PO_4 pH 7.0, 0.1% Triton X-100) and centrifugation was performed as in the preceding step. This procedure was repeated twice, and the three supernatant fractions were combined as the sarcoplasmic fraction, while the washed pellet constituted the insoluble myofibrillar fraction. The pellet was resuspended in a 10 mM solution of urea in glacial acetic acid and incubated overnight under constant agitation for complete solubilization. The protein concentrations were measured (Lowry et al., 1951) with bovine albumin as the standard. Total lipid content was measured in freshly thawed samples as described by Folch et al. (1957) and was determined by the colorimetric method described by Frings et al. (1972).

2.5. Enzyme assays

In heart ventricle samples, maximum activity (*Vmax*) values were measured for hexokinase (HK; EC 2.7.1.1, an indicator of the capacity of a tissue for the aerobic use of glucose), pyruvate kinase (PK; EC 2.7.1.40; an indicator of the capacity for carbohydrate utilization), lactate dehydrogenase (LDH; EC 1.1.1.27; an indicator of the capacity for anaerobic carbohydrate utilization and lactate oxidation), β-hydroxyacyl CoA dehydrogenase (HOAD; EC 1.1.1.35; an indicator of the capacity for fatty acid utilization), citrate synthase (CS; EC 2.3.3.1; an indicator of total aerobic capacity), and three enzymes that are indicators of amino acid oxidation, namely, alanine aminotransferase (ALT; EC 2.6.1.2), aspartate aminotransferase (AST; EC 2.6.1.1), and glutamate dehydrogenase (GLDH; EC 1.4.1.2). Freshly thawed samples were homogenized at approximately 4 °C in nine volumes (v/w) of buffer (20 mM imidazole-HCl pH 7.4, 2 mM EDTA, 0.1% Triton X-100) with an Ultra Turrax homogenizer (Janke & Kunkel, IKA, Labortechnick, Staufen, Germany). The homogenates were sonicated on ice (Janke & Kunkel) using three bursts of 30 s duration and 30% amplitude at 30 s intervals to achieve complete membrane disruption. The samples were then centrifuged at 17000 **g** and 4 °C for 10 min, and the supernatant fractions were kept ice-cold until the assays were performed.

Enzyme activities were measured spectrophotometrically (Beckman DU-70) at 25 °C by following the nicotinamide adenosine dinucleotide (NADH) and 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) reactions at 340 nm and 412 nm, respectively, under saturating, non-inhibitory substrate conditions. The buffers and enzyme assays used were in accordance with the standard approaches given in Bergmeyer (1984), and preliminary experiments were performed to check for control reaction rates (i.e., in reactions omitting the substrate) and to establish the optimal substrate and cofactor concentrations for the final procedure. Each tissue sample was assayed in duplicate, and enzyme activities were described as units per mg of tissue wet mass. Soluble protein concentration was measured in all tissue fractions using bovine serum albumin standards (Lowry et al., 1951), and the enzyme activities were calculated per soluble protein mass to account for any bias that might occur due to a change in tissue water content and/or unspecific effects on the soluble protein content. The assay conditions were as follows: **HK**, 50 mM imidazole-HCl (pH 7.0), 30 mM glucose, 1 mM ATP, 5 mM $MgCl_2$, 100 mM KCl, 5 mM DTT, 0.5 mM NADP, 1 U/mL glucose-6-phosphate dehydrogenase; **PK**, 50 mM imidazole-HCl (pH 7.0), 5 mM ADP, 10 mM $MgCl_2$, 100 mM KCl, 0.15 mM NADH, 0.02 mM fructose 1,6-bisphosphate, 4.2 U/mL lactate dehydrogenase, 5 mM phospho*enol*-pyruvate; **LDH**, 50 mM imidazole-HCl (pH 7.0), 5 mM DTT, 0.15 mM NADH, 1 mM pyruvate; **HOAD**, 50 mM imidazole-HCl (pH 7.0), 0.15 mM NADH, 0.1 mM acetoacetyl CoA; **AST**, 50 mM imidazole-HCl (pH 7.0), 10 mM α-ketoglutarate, 40 mM aspartate, 0.15 mM NADH, 5 mM DTT, 0.025 mM pyridoxal phosphate, 2.2 U/mL malate dehydrogenase; **ALT**, 50 mM imidazole-HCl (pH 7.0), 5 mM xx, 0.025 mM pyridoxal phosphate, 0.15 mM NADH, 4.0 U/mL LDH, 10 mM α-ketoglutarate, 200 mM L-alanine; **GLDH**, 50 mM imidazole-HCl (pH 7.0), 100 mM ammonium acetate, 1 mM ADP, 5 mM DTT, 0.15 mM NADH, 10 mM α-ketoglutarate; **CS**, 50 mM Tris–HCl (pH 8.0), 0.3 mM acetyl CoA, 0.1 mM DTNB, 0.5 mM oxaloacetate.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) or the Kruskal–Wallis ANOVA on ranks procedure was used to test for differences between the groups over the annual cycle. The means were then compared by the Student–Newman–Keuls or Dunn's tests for multiple comparisons, as appropriate. The differences between groups of 'fed' and 'unfed' individuals in the spring activity group were analyzed separately from the effects of the season using Student's *t* or Mann-Whitney's test. The correlation between ventricular mass and body mass was assessed using the least-squares linear regression method on log-transformed data, and the contribution of body mass in predicting the dependent variable was evaluated by the *F*-test. The analyses were based on Zar (1999) and were performed using SigmaStat statistical software (Systat Scientific Software.). The test results and the probability of error are given in the text or in the tabulated results. Differences were considered to be significant at $P \leq 0.05$ and marginally significant at $0.1 \geq P \geq 0.05$.

3. Results

3.1. Effects of body mass and season on the heart ventricle mass

The body mass (Mb) of newly hatched tegu lizards did not differ significantly among the experimental groups analyzed during the annual cycle ($F = 0.343$; $P = 0.848$), while a marginally significant difference ($t = -2.042$; $P = 0.055$) was detected between the sub-groups of fed and unfed individuals in the spring activity group. The lack of a difference in body mass among the groups was most likely a consequence of our sampling scheme, in which the lizards were selected from cages where they were maintained separated according to body size. In contrast, the heart ventricle mass (Mv) showed a tendency to vary according to season ($H = 8.547$; $P = 0.073$), and differences among groups were accentuated after the ventricle mass value was converted into a percentage of body mass value (Fig. 1). In early autumn, the heart ventricle in the neonates was an average of 0.16% of body mass, and this value increased significantly to 0.27% after 60 days of winter dormancy and to 0.21% during unfed arousal compared with early autumn and winter dormancy, respectively ($H = 19.344$; $P < 0.001$). Later in the spring, the heart ventricle was 0.24% of body mass in active, fed tegus and was an average of 0.19% of body mass in the group of animals which were starved for 20 days. While this difference is only marginally significant ($t = 1.967$; $P = 0.063$), it suggests that the effects of the unanticipated starvation during spring activity were different from those of fasting during winter dormancy.

The correlation between body mass and heart ventricle mass was examined to test for a scaling effect on the changes described above. During early autumn, Mv correlates with Mb according to the exponent $b = 0.70$ in the neonates, and smaller animals therefore reach

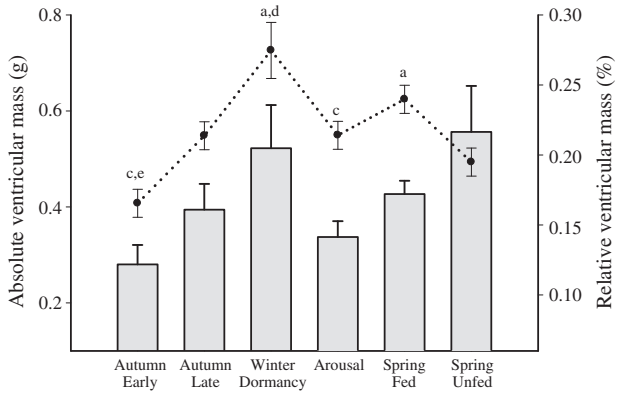

Fig. 1. The heart ventricle mass of tegu lizards (*T. merianae*) during the first annual cycle. Bars represent the ventricle mass in grams, and filled circles represent the ventricle mass converted into a percentage of body mass. Values are mean ± S.E.M from *N* different animals, with *N* as given in Table 1. Significant differences among groups ($P < 0.05$) were detected only for the relative ventricular mass as follows. [a] indicates significant differences from early autumn, [c] from winter dormancy, [d] from arousal, [e] from spring activity.

this season with a larger heart ventricle relative to their body mass than their larger counterparts (Table 1; Fig. 2). This pattern persisted throughout autumn, with an exponent of $b = 0.81$ found for the late autumn group of animals, so that by entry into dormancy, a 3-fold increase in Mb led to a 2.4-fold increase in Mv in juvenile tegus. After 60 days of dormancy, there is a shift in this scaling pattern that results in an exponent of $b = 1.14$, which implies that the ventricle increases in mass disproportionately in the largest individuals during winter dormancy. On this basis, we would expect an Mv increase of 7% for a 100 g lizard versus 53% for a 3-fold larger individual between late autumn and the end of the winter dormancy period. At the onset of arousal, however, there is an opposite shift in the exponent to $b = 0.78$, which suggests a marked reduction of ventricle mass in larger lizards and predicts mass percentages for small and large individuals who are very similar to those of late autumn. In the spring activity group, the exponent changed to $b = 0.51$, which was significantly different from the values found in the arousal group ($t = 33.78$, $P < 0.001$) and the early and late autumn activity groups ($t = 20.421$, $P < 0.001$ and $t = 34.924$, $P < 0.001$, respectively). This difference suggests that a disproportionate increase in the size of the ventricle occurred in the smallest lizards after feeding was reinitiated. Assuming a 3-fold increase in Mb, Mv would increase 1.8-fold, which equates to a smaller scaling effect than was found in the late autumn and winter dormancy groups. Overall, Mv in a 100 g tegu would be 25% larger during spring activity than in late autumn, while it would be virtually unchanged in a 3-fold larger individual. After 20 days of starvation in spring, the exponent changed to $b = 0.75$ and was significantly different from that of the group of fed individuals ($t = 32.65$; $P < 0.001$).

3.2. Heart ventricle morphology

The architecture of the ventricular muscle in juvenile tegus is similar to that previously described for other reptiles (Poupa and Lindström, 1983; Tota, 1983). Taking the spring activity group as a reference (Fig. 3-E), we observed that the external layer, the epicardium (Ep), consists of a thin layer of serous tissue associated with many blood vessels, while the underlying mixed myocardium is composed of a compact outer layer (Co) and a spongy inner layer (Es). The muscle fibers in the compact layer are densely packed and organized, and the arrangement of these fibers suggests a subdivision of the compact layer into an outer sublayer, with fibers oriented longitudinally, and an inner sublayer, with fibers oriented in a circular direction. The spongy layer is formed from a loose network of interwoven fibers (trabeculae) separated by lacunae (La) that communicates with the ventricular cavity. The innermost layer, the endocardium, lines the surface of the trabeculae and is formed by flattened endothelial cells with oval nuclei, which stained deeply with hematoxylin in our histological analysis. This arrangement was found to change dramatically during the annual cycle (Fig. 3, A–E), and estimates of the area density of lacunary spaces A_A indicated significant differences among the different seasons ($F = 19.83$, $P < 0.001$; Table 2). In the autumn activity group, the spongy myocardium appeared firmly packed, with thick trabeculae and reduced A_A, which only covered approximately 8% of the total section area. This arrangement was maintained in the winter dormancy group and in rehydrated, arousing individuals. Soon after food intake, a clear tendency to increase A_A was observed, with the lacunae covering 14% of the total section area, and this was accompanied by a thinning of the trabeculae. Later, during spring activity, the spongy myocardium contains ample lacunae, which correspond to 29% of the total section area. This area density was significantly different from that found in all previous phases ($P < 0.001$).

Based on these two contrasting arrangements, we tested the hypothesis that changes occur in Nv between the periods of dormancy and activity by examining the trabeculae of the spongy myocardium in winter dormancy and spring activity individuals. The Nv is 37% lower during winter dormancy compared with spring activity ($t = 11.0$, $P = 0.032$) (Fig. 4; Table 2), and substantial variation persists after correcting the Nv value to take account of the percentage of tissue occupied by lacunary spaces (25%; $t = -1.99$, $P = 0.086$). Assuming a constant and small volume for the interstitial components of heart muscle (endotheliocytes, fibroblasts, smooth muscle, collagen fibers), the cardiomyocyte volume was derived, and this was an average of 2,098 μm^3 in winter dormancy versus 1,376 μm^3 in spring activity, which equates to a difference of 52% ($t = 3.893$, $P = 0.008$). The total number of cardiomyocytes estimated for a 100 g tegu, based on the Nv value and the relative ventricle mass in the respective seasons, is two-fold lower in winter dormancy than in spring activity ($t = -2.97$, $P = 0.021$).

The coefficient of error, which is an estimate of the stereological noise, was in the range of 0.13–0.19 for A_A, and of 0.06–0.07 for Nv.

Table 1
Heart ventricle mass and the scaling relationship with body mass in tegu lizards (*T. merianae*) during the first annual cycle.

Seasonal activity state	*N*	*a*	*b*	r^2	*F*	*P*
Autumn activity						
Early	4	−2.116 ± 0.085	0.699 ± 0.0381 [b,c,d,e]	0.994	337.156	0.003
Late	10	−2.254 ± 0.188	0.811 ± 0.084 [a,c,d,e]	0.921	93.408	<0.001
Winter dormancy	14	−2.883 ± 0.311	1.140 ± 0.140 [a,b,d,e]	0.848	66.735	<0.001
Arousal	17	−2.205 ± 0.156	0.784 ± 0.071 [a,b,c,e]	0.889	119.935	<0.001
Spring activity						
Fed	15	−1.538 ± 0.118	0.514 ± 0.052 [a,b,c,d]	0.881	95.933	<0.001
Unfed	7	−2.116 ± 0.155	0.752 ± 0.064*	0.966	139.953	<0.001

Linear regressions were performed on log-transformed ventricle mass (g) and body mass (g) as described by $\log_{10} Mv = a + b \log_{10} Mb$, where *a* is the intercept ± S.E.M., *b* is the slope ± S.E.M., Mv is ventricle mass and Mb is body mass. [a] indicates significant differences from early autumn, [b] from late autumn, [c] from winter dormancy, [d] from arousal, [e] from spring activity, * between fed and unfed animals during spring activity ($P < 0.05$).

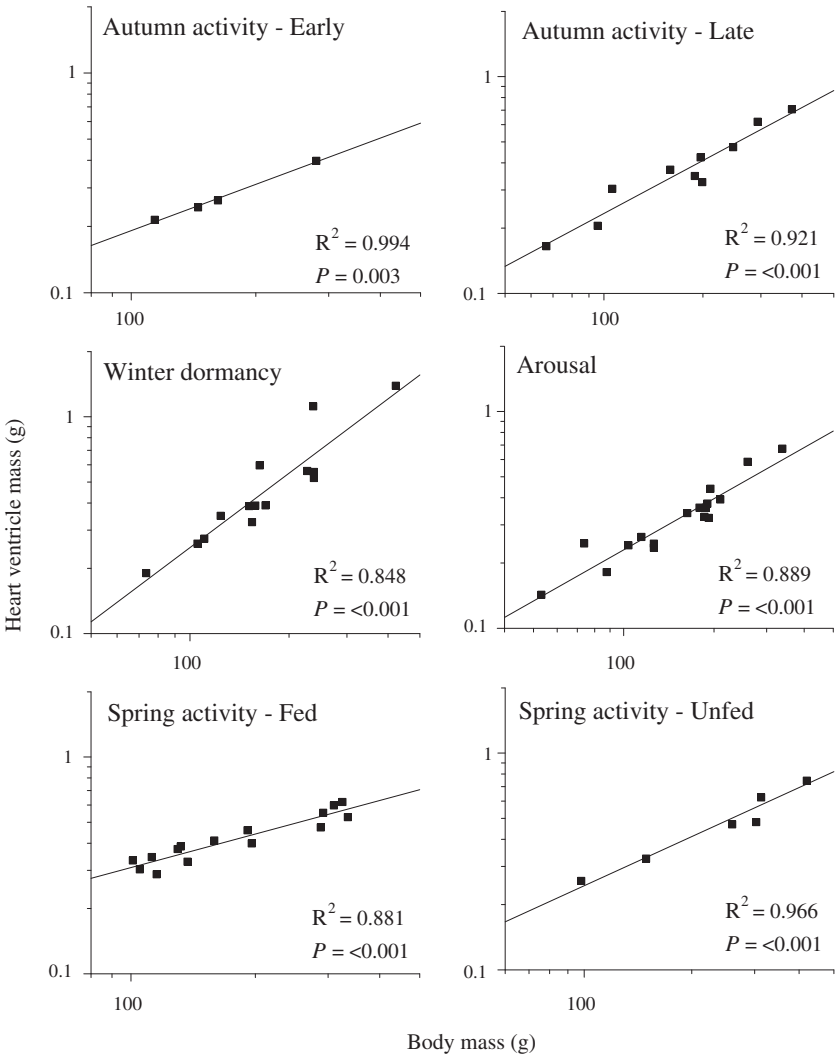

Fig. 2. The allometric relationship between heart ventricle mass (Mv) and body mass (Mb) of tegu lizards (*T. merianae*) at distinct activity states during the first annual cycle. Linear regressions were performed on log-transformed Mv (g) and Mb (g) as described by $\log_{10}\mathrm{Mv} = a + b\ \log_{10}\mathrm{Mb}$.

3.3. Macromolecular composition of the heart ventricle

There was little variability in the water content of tissue samples as a function of season ($F = 2.484$, $P = 0.09$), and this was mostly associated with an increase of 1.7% in the value for winter dormancy compared with that for spring activity (Table 3). During spring activity, there was no detectable difference in the water content of the heart ventricle in fed and unfed individuals. The total protein content calculated per tissue mass (wet and dry) did not vary throughout the annual cycle ($F = 0.380$, $P = 0.769$ and $F = 0.662$, $P = 0.584$, respectively), nor did it vary between the fed and unfed groups of animals in spring activity ($t = -0.714$, $P = 0.494$). However, there is evidence of selective changes in the muscle protein fractions during the annual cycle. The content of myofibrillar protein showed a clear tendency to vary as a function of season ($H = 7.38$, $P = 0.061$), with values during autumn activity and winter dormancy being 40% lower than during arousal and spring activity. The variability in the myofibrillar protein content in the group of re-hydrated, arousing animals was high, and this is possibly why our statistical test indicated only a marginal level of significance (Table 3). The sarcoplasmic protein content also showed a tendency to vary with season ($F = 2.77$, $P = 0.078$); however, the pattern of change differed from that of the myofibrillar fraction, with mean values that were 20% lower in the winter dormancy and arousal groups compared with the autumn and spring activity groups. There were significant changes in lipid content, with a 21–25% decrease found during winter dormancy and arousal compared with autumn and spring activity ($F = 9.14$, $P < 0.001$). A similar change was observed in the cardiac ventricle of animals starved during spring activity compared with fed individuals, indicating that it is primarily related to fasting ($t = 2.573$, $P = 0.033$).

3.4. Enzymes activities

The *Vmax* values measured at 25 °C in samples of the ventricular muscle of juvenile tegus are presented in Table 4. PK, LDH, AST and CS activities were remarkably constant among the different seasons ($F = 0.716$, 1.275, 1.48, and 1.90, respectively) while the activity of HK ($F = 4.11$, $P = 0.02$), GLDH ($F = 4.89$, $P = 0.02$), ALT ($F = 8.34$, $P = 0.001$) and HOAD ($F = 2.87$, $P = 0.064$) differed as a function of season. HK activity was lower in the autumn activity (by 26%) and winter dormancy (by 29%) groups compared to the spring activity group. GLDH activity decreased progressively during dormancy, and reached values that were significantly lower at arousal (by 24%) relative to those found during spring activity. ALT activity was 64% lower in winter dormancy relative to both spring activity values and the values recovered at the moment of arousal before food intake. HOAD activity showed a similar tendency and was decreased by 25% in the winter dormancy group. Between the fed and unfed sub-groups in the spring activity group, PK and CS activities decreased by 11% ($U = 6.0$, $P = 0.065$) and 22% ($t = 3.4$, $P = 0.007$), respectively,

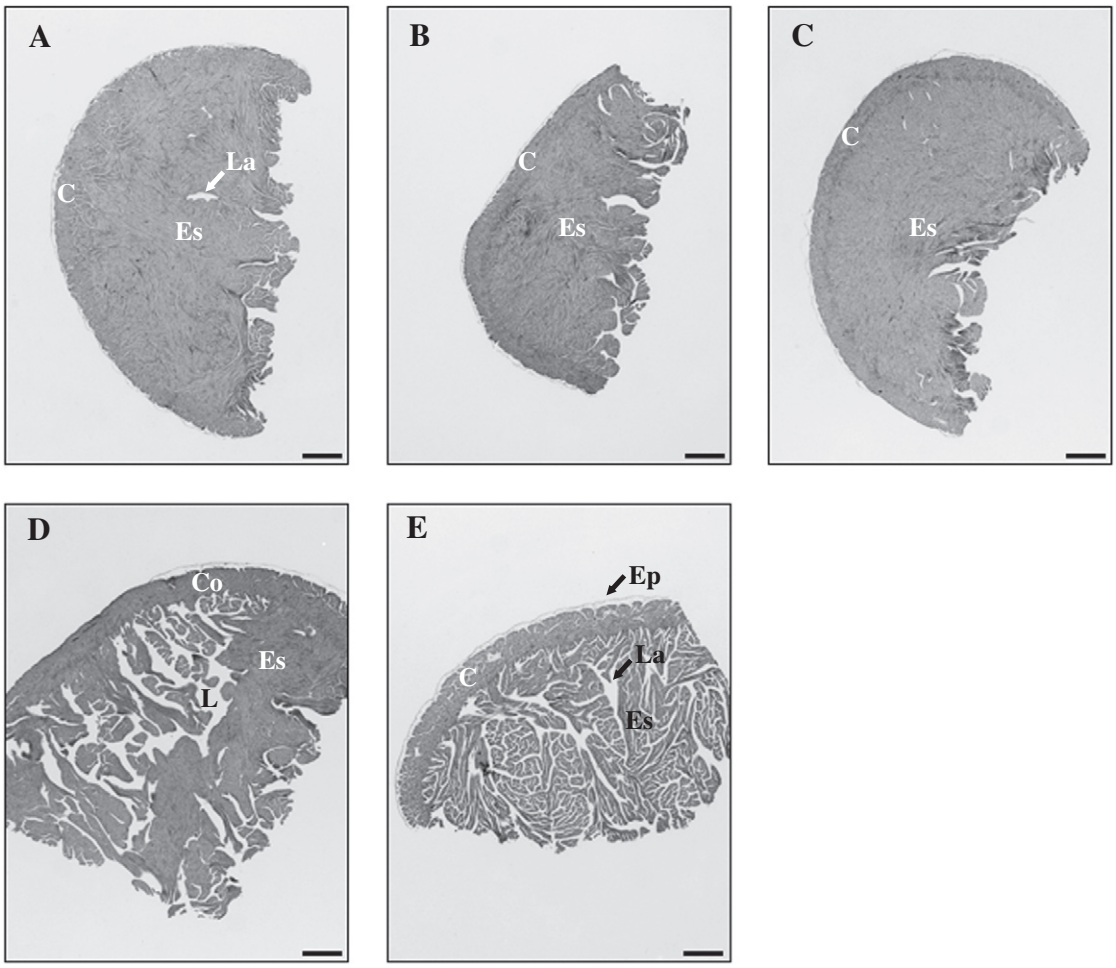

Fig. 3. Light micrographs of the ventricular wall from hearts of tegu lizards (*T. merianae*) showing the epicardium (Ep), the compact myocardial layer (Co), the spongy myocardial layer (Es) and lacunary spaces (La), at distinct activity states during the first annual cycle. A- autumn activity; B- winter dormancy; C- arousal unfed; D- arousal fed; E- spring activity. HE. Scale bar, 400 μm.

but there were no detectable differences between these sub-groups with regard to LDH and HOAD activities (Table 4).

There was no difference in the water-soluble protein concentration of extracts from the groups analyzed, and the pattern of change in the *Vmax* values was similar when calculated per mass of soluble protein and per wet tissue mass.

4. Discussion

The present study provides evidence of remarkable changes in the mass of the ventricle and in the architecture of the heart during the first annual cycle in tegu lizards. There is cardiac hypertrophy during late autumn and winter dormancy, and this progresses in parallel with HR reduction and VO_2 depression at the whole body level, which makes it a very intriguing phenomenon. The hypertrophy involves proportional adjustment of the protein content, and the scaling patterns suggest that smaller protein stores in the heart may restrict the capacity of the organ to rapidly return to activity in the smallest individuals. There is also considerable remodeling of the spongy myocardium, which reverses after arousal and food intake. According to the enzyme activity data, the capacity of the myocardium to oxidize a variety of substrates is preserved throughout the year, with down-regulation at sites of substrate entry into the oxidative pathways. Overall, adaptive changes in the heart of the tegu appear to occur in anticipation of winter dormancy, thereby preserving the potential for increase in HR that accompanies the return to activity.

The relative ventricle mass was 28% higher by mid-winter compared with late autumn in tegus, and this change was not related to differences in body mass or substrate stores between the experimental groups. The glycogen and lipid content represents at most 0.5% of the wet mass of the ventricle in juvenile tegus, and our data show that the total lipid level is lower in the heart during winter dormancy, while an earlier study showed that the glycogen level is increased (Souza et al., 2004). In this earlier study, a net weight loss of 15% was measured in individual tegus after 90–100 days of winter dormancy, and this was mostly due to evaporative water loss. A similar effect may have influenced the Mv percentages measured halfway

Table 2
Area density of lacunar spaces (A_A) and cardiomyocyte numerical density (Nv) in the spongy myocardial layer of the heart ventricle from tegu lizards (*T. merianae*) at distinct activity states during the first annual cycle.

Seasonal activity state	A_A (cm^0)	Nv (mm^3)
Autumn activity	0.08 ± 0.02	-
Winter dormancy	0.08 ± 0.00	479878.6 ± 23286.1*
Arousal		-
Unfed	0.08 ± 0.01	-
Fed	0.14 ± 0.03	
Spring activity	0.29 ± 0.02[a,b,c,d]	759326.9 ± 74809.7

Values are the mean ± S.E.M from 4–5 different animals. [a] indicates significant differences from early autumn, [b] from late autumn, [c] from winter dormancy, [d] from arousal, * between winter dormancy and spring activity ($P < 0.05$).

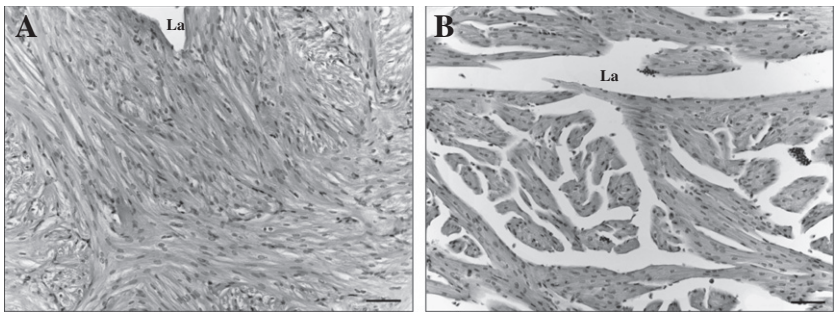

Fig. 4. Light micrographs of the spongy myocardial layer from hearts of tegu lizards (*T. merianae*) showing lacunary spaces (La), at distinct activity states during the first annual cycle. A- winter dormancy; B- spring activity. HE. Scale bar, 400 μm.

through dormancy in the present study. Despite this, the hypertrophy we observed was greatly disproportionate among siblings, being less than 10% for a 100 g tegu and higher than 50% for a 3-fold larger individual. This pattern is contrary to the influence of the different rates of evaporative water loss through the body surfaces of small and large lizards. Together, these data provide evidence of a tissue-specific regulation of the cardiac mass in juvenile tegus that is related to seasonality.

Cardiac hypertrophy during winter dormancy has previously been observed in lizard and squirrel species (Wickler et al., 1991; Naya et al., 2009), although the mechanisms underlying this process are largely unknown. In fish exposed to low temperatures, cardiac hypertrophy was attributed to the extension of the ventricle filling time and the greater stretching of the myocardium that are associated with a decrease in HR rather than to a direct effect of acclimation to low temperatures (Aho and Vornanen, 2001). In newly hatched tegus, hypertrophy may be triggered by a similar mechanism, with smaller tegus having some limitation in their response to the stimulus. The shifts in the scaling exponent that occur during entry into dormancy and in early arousal indicate that the changes in Mv are disproportionate in the largest individuals. The resting VO_2 rate per mass unit increases by 25% in arousing animals irrespective of body size (Souza et al., 2004), implying an increase in heart function that is compatible with the demands of an increased metabolic rate at the whole body level, which is larger in the largest individuals. Following the first meal after arousal, however, the percent VO_2 changes indicate that the smaller the individual, the greater the time necessary to accomplish the transition from dormancy to full activity. This apparent disadvantage would be overcome later in spring, when our data suggest that smaller individuals undergo a markedly greater increase in the relative Mv, which becomes 25% larger in a 100 g tegu and is virtually unchanged in a 3-fold larger individual in relation to late autumn activity.

The increased Mv could be due to cardiomyocyte hypertrophy, hyperplasia, or both and would compensate for the potential increase in peripheral resistance caused by vasoconstriction and redistribution of blood during winter dormancy. The lower numerical density of cardiomyocytes (Nv) and the larger cardiomyocyte volume in winter dormancy suggest that the larger relative Mv and thicker trabeculae result, at least in part, from cardiomyocyte hypertrophy. Evidence of cellular hypertrophy associated with an increase in cardiac mass has been found in estivating lungfish, in which it occurs without any change in the general structure of the myocardium (Icardo et al., 2008). Interestingly, the morphological changes in the ventricle of newly hatched tegus appeared early in the annual cycle, during autumn activity, raising the possibility that it could be part of ontogenetic development in the newly hatched individuals rather than a process related to seasonality. In some vertebrates possessing a mixed myocardium, heart growth and development appear to involve a reduction of the lacunary spaces and an increase in the thickness of the trabeculae (Poupa and Lindström, 1983). In newly hatched tegus, however, the morphological arrangement observed during autumn activity and winter dormancy reverses completely one month after arousal, as suggested by the increased A_A and Nv that are concomitant with an increase in the rate of somatic growth during spring activity. Thus, it seems highly unlikely that this arrangement is associated with ontogenetic development. Data suggesting formation of thicker trabeculae during winter dormancy were also obtained for a frog species (Barni et al., 1994). The results from our analysis of tegus strongly suggest that structural changes in the ventricle are initiated early in autumn in anticipation of winter dormancy. The HR presumably decreases together with the VO_2 decrease, while the Mv becomes increasingly larger during the autumn and winter months. During arousal, reversal of the morphological changes are gradual, with no alteration detected at 48 h after water intake and a complete rearrangement observed after food intake a few days later, which is consistent with the gradual increase in resting VO_2 under similar conditions (Souza et al., 2004). In contrast, the Mv was smaller before feeding was reinitiated in early arousal, with one possible explanation for this significant mass loss being that tissue components are mobilized and used as energy substrates to sustain the increased HR in arousing individuals.

The dense aspect of the spongy myocardium in the tegu, observed in the autumn activity and winter dormancy groups, could be the cause of the gradual increase in the ventricular mass or the consequence of the thickening of the muscle trabeculae that offsets the increased heart size. In their study of icefish, O'Brien et al., (2000) emphasized that larger hearts need thicker walls to support the tension required

Table 3
Seasonal changes in heart muscle composition during the first annual cycle of newly hatched tegu lizards.

Seasonal activity state	Water (%)	Total Protein (mg. g^{-1})	Sarcoplasmic protein (mg.g^{-1})	Miofibrilar protein (mg.g^{-1})	Total lipids (mg.g^{-1})
Autumn activity	80.31 ± 0.31	133.99 ± 8.41	101.07 ± 12.57	14.08 ± 0.89	18.50 ± 0.59[b,c]
Winter dormancy	81.11 ± 0.17	143.33 ± 10.15	87.12 ± 6,49	15.37 ± 0.48	14.53 ± 0.22[a,d]
Arousal	80. 63 ± 0.38	146.66 ± 11.04	84.65 ± 4.75	24.59 ± 5.51	14.39 ± 0.26[a,d]
Spring activity					
Fed	79.77 ± 0.49	136.77 ± 7.86	114.61 ± 10.74	27.46 ± 3.96	19.24 ± 1.38[b,c]
Unfed	80.64 ± 0.29	145.13 ± 8.70	-	-	14.72 ± 0.44*

Values are the mean ± 1 S.E.M from 4–7 different animals. [a] indicates significant differences from autumn activity, [b] from winter dormancy, [c] from arousal, [d] from spring activity, * between fed and unfed spring activity animals ($P < 0.05$).

Table 4
Maximal activities of enzymes in cardiac muscle of newly hatched tegu lizards during the first annual cycle.

Seasonal activity state	HK	PK	LDH	GLDH	ALT	AST	HOAD	CS
Autumn activity	2.33 ± 0.21[d]	103.3 ± 11.9	793.3 ± 55.6	7.05 ± 0.17[c]	1.85 ± 0.21[b]	169.5 ± 8.3	8.82 ± 0.57	29.6 ± 2.2
Winter dormancy	2.22 ± 0.21[d]	120.1 ± 7.4	680.5 ± 30.9	6.39 ± 0.53	0,55 ± 0.08[a,c,d]	148.4 ± 11.0	7.07 ± 0.80	37.6 ± 2.6
Arousal	2.61 ± 0.22	113.1 ± 5.4	738.9 ± 36.2	5.46 ± 0.08[a,d]	1.41 ± 0.16[b]	151.1 ± 9.8	7.97 ± 0.48	32.7 ± 3.5
Spring activity								
Fed	3.14 ± 0.17[a,b]	114.8 ± 4.7	763.1 ± 50.0	7.17 ± 0.68[c]	1.53 ± 0.15[b]	168.0 ± 6.6	9.42 ± 0.52	36.4 ± 2.1
Unfed	–	102.0 ± 3.7	833.9 ± 56.0	–	–	–	9.52 ± 1.48	28.4 ± 1.1*

Values are the mean ±1 S.E.M from 6–7 animals in $U.g^{-1}$ wet mass. [a] indicates significant differences from autumn activity, [b] from winter dormancy, [c] from arousal, [d] from spring activity,* between fed and unfed spring activity animals ($P < 0.05$).

to generate internal pressure equivalent to that of a smaller heart. Maintenance of the perfusion of the spongy layer could be achieved by the conversion of the contracted lacunae into small canals that could sustain the communication between the luminal and coronary blood, as occurs during the ontogeny of mammals (Tota, 1983). Alternatively, the thicker trabeculae, along with the reduction in the HR, may imply decreased perfusion of the spongy myocardium during winter dormancy. This effect would be accompanied by metabolic adjustments at the cellular level that would allow a new equilibrium to be reached while avoiding tissue damage and preserving capacity for the moment of arousal. In hibernating mammals, blood flow in the heart is maintained during torpor, and this most likely serves to avoid cardiac arrest during hypothermia (Burlington and Milson, 1993; Kudej and Vatner, 2003). Later, during spring activity, the configuration of the spongy myocardium that we observed in tegus would ensure that high intra-ventricular pressures develop with relatively low tension of the muscle trabeculae, because the thousands of small compartments of the spongy myocardium can act as auxiliary pumps to the larger pump of the outer compact layer (Tota, 1983; O'Brien et al., 2000). Moreover, the reduction in the thickness of the trabeculae results in an increase in the surface area of the endothelial endocardium, thereby ensuring effective oxygen diffusion to the spongy myocardium. Together, these changes would support the increased demand imposed on the myocardium by the increased activity and growth rates occurring in spring.

In the spring activity group, the Nv was 58% higher and the calculated cell volume was 52% smaller compared with the winter dormancy group, which suggests that in this phase the heart ventricle increases in size through hyperplasia that accompanies increased somatic growth in juvenile tegus. The disproportionate increase in Mv in smaller individuals further emphasizes the importance of this mechanism for cardiac growth after arousal, adjusting heart function to support continuing growth and development. Given the concomitant increase in the A_A, however, it is possible that Nv is somewhat overestimated due to 'draining' and 'shrinking' of the cardiomyocytes, with the cells becoming closer to each other. After correcting Nv for the percentage of tissue area occupied by lacunary spaces, Nv is 34% higher during spring activity than during winter dormancy, and the total number of ventricular myocytes estimated for a 0.3 g heart is 2-fold higher during spring activity than during winter dormancy (270 million versus 137 million, respectively). These values are consistent with those reported for trout (300 million cardiomyocytes in a 0.66 g heart ventricle) and considerably larger than those reported for rats (48 million in a 0.67 g heart ventricle) (Anversa et al., 1978; Clark and Rodnick, 1998). The volume of one cardiomyocyte in the tegu, as calculated from the Nv values, averages 2098 μm^3 during winter dormancy and 1376 μm^3 during spring activity, and this is consistent with estimates from linear measurements made for other ectotherms (2300 μm^3 for varanid lizard and turtle; 2900 μm^3 for frog, 2500 μm^3 for rainbow trout) and about ten times smaller than estimates made for mammals (34400 μm^3 for rat, 30000 μm^3 for rabbit) (Satoh et al., 1996; Clark and Rodnick, 1998; Vornanen et al., 2002; Galli et al., 2006; Shiels and White, 2008; Galli et al., 2009). The smaller volume of the cardiomyocyte in ectotherms implies a high surface to volume ratio for calcium uptake from the extracellular medium because the cells lack transverse tubules and a well-developed sarcoplasmic reticulum (Helle, 1983; Poupa and Lindström, 1983; Galli et al., 2006). The reduction of cell volume during spring activity that we observed in juvenile tegus would be advantageous by enhancing the exchange of substances between the intracellular and extracellular compartments and by optimizing the ionic currents and cardiac performance. Thus, increases of Mv apparently occur by a combination of hypertrophy and hyperplasia, with the emphasis on each of the mechanisms varying as part of the repertoire of adjustments that reconcile growth and maturation with the endogenous seasonal rhythm.

Cardiac hypertrophy in the tegu during autumn and winter months, together with the reduction of mass during arousal, are not associated with changes in the total protein content per mass unit. This result contrasts with previous data obtained from studies of skeletal muscle in tegus, which showed protein content values 14-18% decreased by mid-winter and values 50–67% decreased during early arousal (Souza et al., 2004; S. C. R. Souza, unpublished), and suggests high rates of proteolysis during the prolonged period of fasting and inactivity. Cardiac hypertrophy together with the unchanged total protein level may result from continued protein synthesis through the autumn and winter months, as the body temperature of tegus during winter dormancy is stable approximately 17 °C, combined with decreased rates of protein degradation. In many hibernating mammals, rates of protein synthesis are severely reduced at the very low body temperatures that are typical of torpor (~4 °C) (van Breukelen and Martin, 2002). Recently, Lee et al., (2012) found a significant increase in liver, heart, small intestine, and BAT nitrogen isotope ratios associated with lean mass loss, while they found no change in skeletal muscle, corroborating the idea that the protein balance would be restored in selected tissues during the episodes of arousal and re-warming that occur in the hibernation period. Interestingly, this anabolic model of hibernation could apply to winter dormancy in the tegu. Moreover, the protein content per mass unit in the ventricle of the tegu remained constant after a period of starvation during spring activity, which emphasizes the existence of a hierarchy of protein mobilization from endogenous sources during fasting, as observed in hibernating mammals (Steffen et al., 1991; Wickler et al., 1991).

These effects observed at the total protein level may not reveal adjustments occurring in the sub-cellular fractions. The myofibrillar proteins are reduced in the ventricle during the autumn and winter months in a manner concomitant with larger cardiac mass, and the relationship between these variables reverses at the onset of arousal. This pattern differs from that of the sarcoplasmic fraction, the protein content of which mirrors the changes in the HR and does not recover until feeding recommences. There may be independent regulation of the synthesis of contractile and non-contractile proteins in the heart muscle; for instance, in the rat heart, the myofibrillar fraction is much more sensitive to fasting than is the soluble fraction (Preedy and Sugden, 1989). While the time taken for the new myofibrillar elements to be synthesized in the heart of the arousing tegu is unknown, the smaller heart mass and higher whole body oxygen consumption at arousal strongly suggest that proteins had been degraded and the resulting amino acids used as oxidative substrates and/or as precursors of new proteins. The degree of heart

atrophy would be 8% for a 100 g lizard versus 38% for a 3-fold larger animal during arousal in relation to winter dormancy, and this would offset the disproportionate gain of mass during entry into dormancy. Likewise, larger tegus accumulate more fat in their fat body deposits before entry into dormancy, and substantial fat is still available at the onset of arousal in these animals, while fat deposits are virtually exhausted in smaller individuals (Souza et al., 2004). Therefore, the energy required for the sudden increase in HR and metabolism in arousing animals may come from stored fatty acids in addition to amino acids produced by the breakdown of proteins. The more than 3-fold higher activity of ALT in the ventricle of arousing tegus, together with the high constant AST activity, may also indicate an increased contribution of amino acids to augment the Krebs cycle and oxidative phosphorylation to pace that of β-oxidation during fat catabolism. Aspartate is mobilized through the coupled activities of AST and ALT, which thereby form oxaloacetate for the Krebs cycle, and this is the main means for augmenting the pool of Krebs cycle intermediates in tissues such as the cardiac muscle (Hochachka and Somero, 2002). Overall, the smaller juvenile tegus, with their smaller ventricle and reduced substrate stores, would have a limited capability to sustain the higher HR upon return to activity.

The stable concentration of soluble proteins in the ventricular tissue of the tegu throughout the annual cycle is paralleled by the stable activities of selected enzymes of intermediary metabolism. PK, LDH and CS activities were high and constant in all experimental groups, which suggest that the capacity for carbohydrate and lactate oxidation would be preserved during seasonal dormancy. This pattern of regulation may rely on a decreased rate of degradation and on an increase in the half-life of soluble cardiac proteins, as has been observed in hibernating mammals (Epperson et al., 2010). This would contribute to the maintenance of protein balance and to energy savings in the organ during the hypometabolic condition. Dormancy in the tegu is a continuous process in which conservation of the metabolic capacities of the heart are important for the moment of arousal at the end of the winter season. The regulation of selected steps in the metabolic pathways, however, was indicated by the lower HK activity observed during autumn and winter dormancy. This enzyme catalyzes the first step in the metabolism of glucose. Down-regulation of HK activity together with maintenance of PK activity was observed in estivating animals (Brooks and Storey, 1992; Cowan et al., 2000; Frick et al., 2008a). This adjustment may reflect the importance of reserving blood glucose for the tissues that typically show a preference for carbohydrates, such as the central nervous system, as opposed to the myocardium, which has the capacity to oxidize a variety of substrates. The high, sustained levels of PK activity would preserve the potential to generate energy from the glycogen readily available from tissue stores, thereby allowing rapid increases in HR. LDH activity is highest in the ventricle in tegus, where it is found to be higher than in the myocardium of many other vertebrates (Driedzic and Gesser, 1994). Because the LDH isozyme present in the cardiac muscle predominantly operates as a pyruvate reductase (Hochachka and Somero, 2002), the constant LDH activity in the ventricle of juvenile tegus suggests that the potential for oxidizing the lactate formed in other tissues is also preserved during the hypometabolic condition. The rate of uptake of glucose or lactate in the circulating blood is proportional to its plasma concentration (Kodde et al., 2007), and unlike glucose, lactate is constantly present at slightly higher levels (25%) in the plasma of dormant tegus compared with the levels observed during spring activity (Souza et al., 2004). In the cardiac muscle of mammals, approximately equal amounts of the pyruvate used for the formation of acetyl-CoA are derived from glycolysis and from the oxidation of lactate (Stanley et al., 2005). In contrast, estimates of the ratio of PK activity to LDH activity in the ventricle of the tegu are lower than 0.2 in all experimental groups. Thus, it appears that lactate could be used continuously by the ventricular muscle for aerobic energy production during winter dormancy.

HOAD activity was significantly lower in the hearts of dormant and arousing tegus, which confirmed previous observations of the cardiac muscle of juvenile tegus (Souza et al., 2004) and was similar to the patterns of change in the hearts of estivating lungfish (Frick et al., 2008b). As indicated by RQ values of nearly 0.7, fatty acids are the preferred energy substrate during aerobic metabolic depression (Carey et al., 2003), and HOAD inhibition in the heart of the tegu most likely contributes to matching the fatty acid flux to the low rate of mitochondrial oxidation and to energy saving. The heart is remarkable for its metabolic flexibility, and its adjustments seem to rely upon stoichiometric efficiency (Hochachka and Somero, 2002). In normoxia, approximately 10–40% of the acetyl-CoA produced in the myocardium of vertebrates comes from the oxidation of glucose, while 60–90% arises from the β–oxidation of fatty acids (Driedzic and Gesser, 1994; Kodde et al., 2007). Despite the lower HOAD activity, the relative contribution of fatty acids to the lower energy expenditure in dormant tegus may be even higher than during activity, thereby favoring the highest yield of ATP per mole of substrate oxidized. The lower ratio of HOAD activity to CS activity observed during winter dormancy (0.17) further suggests that the potential of CS to condense acetyl-CoA is considerably greater than the capacity for formation of acetyl-CoA by HOAD, which highlights the importance of mechanisms that conserve the capacity of the tissue for a sudden increase in metabolism. Despite a severe depression of resting VO_2 and HR during winter dormancy (Abe, 1983; Souza et al. 2004), no change in CS activity was detected throughout the year. The regulation of the aerobic capacity at the tissue level could be achieved through other mechanisms, such as modification of the volume density of the mitochondria, alteration in the area of the inner mitochondrial membrane and the concentration of enzymes (St-Pierre and Boutilier, 2001). The unchanged CS activity level in the tegu suggests that the cardiac hypertrophy that occurs in the autumn and winter months is most likely accompanied by proportional adjustments in one or more of these traits, with the resulting outcome being an increased aerobic capacity of the whole organ per body mass unit.

The consequences of seasonality on energy metabolism in the tegu heart are in marked contrast with the effects of starvation during spring activity. In particular, CS activity decreases significantly together with atrophy of the organ, implying a severe reduction in the aerobic capacity of juvenile individuals both at the organ and the whole body level. This unbalanced protein loss differs from the changes observed in arousing, unfed tegus, in which CS activity was constant despite the decrease in cardiac mass. HOAD activity was unchanged and the ratio of HOAD activity to CS activity increased slightly in the hearts of starved tegus, which is likely to lead to increased formation of ketone bodies in the tissue. An increased capacity for the oxidation of lactate over glucose was indicated by constant LDH activity and an increased ratio of LDH activity to CS activity. Thus, adjustments promoting lactate oxidation would be a conservative aspect of the metabolic response to fasting in the tegu heart whatever the circumstance.

In conclusion, this study contributes to our understanding of the cardiac changes related to the challenges of seasonal dormancy during growth and development at early stages of life. Remarkable changes in the mass and architecture of the organ occur in anticipation of dormancy and arousal, while the conservative nature of the energy metabolism would preserve the potential for fast increases in the HR. The scaling patterns suggest an allometric effect on the magnitude of cardiac hypertrophy and atrophy, which may allow smaller individuals, with smaller hearts and substrate stores, to meet the limit for survival during the first annual cycle.

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