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Morphological and metabolic adjustments in the small intestine to energy demands of growth, storage, and fasting in the first annual cycle of a hibernating lizard (*Tupinambis merianae*)

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ABSTRACT

Seasonal plasticity in the small intestine of neonatal tegu lizards was investigated using morphometry and analysis of enzymes involved in supplying energy to the intestinal tissue. In the autumn, the intestinal mass (Mi) was 1.0% of body mass and the scaling exponent $b = 0.92$ indicated that Mi was larger in smaller neonates. During arousal from dormancy Mi was 23% smaller; later in spring, Mi increased 60% in relation to the autumn and the exponent $b = 0.14$ indicated that the recovery was disproportionate in smaller tegus. During the autumn, the intestinal villi were greatly elongated; by midwinter, the Hv, SvEp, and VvEp were smaller than during the autumn (59%, 54%, 29%) and were restored to autumn levels during spring. In the active tegus, the maximum activity (*Vmax*) of enzymes indicated that the enterocytes can obtain energy from different sources, and possess gluconeogenic capacity. During winter, the *Vmax* of CS, HOAD, GDH, PEPCK was 40–50% lower in relation to the autumn and spring, while the *Vmax* of HK, PK, LDH, AST was unchanged. The hypoglycemia and the mucosal atrophy/ischemia during winter would prevent the enterocytes from using glucose, whereas they could slowly oxidize fatty acids released from body stores and amino acids from the tissue proteolysis to satisfy their needs of energy. Contrastingly, starvation during spring caused severe mass loss (50%); the tissue protein and the VvEp and VvLP did not change while the thickness of the muscular layer increased 51%, which suggested different effects along the length of the organ. In addition, the *Vmax* of the glycolytic enzymes was lower, indicating that a regulatory mechanism would spare blood glucose for vital organs during unanticipated food restriction.

1. Introduction

Many vertebrates have the ability to undergo a state of dormancy during the annual cycle. This state manifests as a decrease in the rates of energy intake and expenditure throughout the whole organism as a result of a complex suite of morphological, physiological and biochemical adjustments that are coupled to a circa-annual rhythm that is superimposed on the circadian pattern of activity (Williams et al., 2012; Storey and Storey, 2007; Carey et al., 2003). In addition to hypometabolism, another feature of seasonal dormancy is long-term fasting. All vertebrates exhibit adaptive responses to a lack of food, although they differ in the amount of time it can be tolerated, and the absence of nutrients in the intestinal lumen results in structural and physiological changes in the gastrointestinal tract (McCue, 2010). The gastrointestinal tract is a very metabolically active tissue and is costly to maintain; it accounts for 20–30% of the basal metabolic rate, the major components of which include Na^+, K^+ ATPase-ion linked transport systems and protein turnover (Rolfe and Brown, 1997). Accordingly, this tissue undergoes marked atrophy in response to a decrease in demand, which assists in energy conservation (Wang et al., 2006; Secor, 2005; Hume et al., 2002). In marked contrast to sporadic feeding habits and to unpredictable shortfalls in the food supply, fasting during winter dormancy is preceded by anticipatory modulation of the control of appetite and fattening, which prepares the animal for the depletion of energy stores and restricted nutrient intake (Florant and Healy, 2012; Xing et al., 2012).

Abbreviations: AST, aspartate aminotransferase; CS, citrate synthase; DTNB, 5,5′-dithiobis 2-nitrobenzoic acid; Ep, epithelium; GDH, glutamate dehydrogenase; HK, hexokinase; HOAD, β-hydroxyacyl CoA dehydrogenase; Hv, villus height; LDH, lactate dehydrogenase; LP, lamina propria; Mb, body mass; Mi, intestinal mass; ML, muscular layer; NADH, nicotinamide adenosine dinucleotide; PEPCK, phosphoenolpyruvate carboxykinase; PK, pyruvate kinase; Sv, surface density; *Vmax*, maximum enzyme activity; Vv, volume density.

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The regulatory mechanisms that mediate adjustments in nutrient intake over seasonal time scales in hibernators have not yet been uncovered (Perry and Wang, 2012). One of the fundamental questions is whether increases in the functional capacity of the intestinal tract serve as a way to regulate levels of body adiposity prior to winter dormancy. The idea underlying this question is that the physiological demand that is placed on the visceral organs for energy storage would involve higher rates of nutrient intake and absorption within the intestinal tissue. Later on during the preparatory phase, anorexia progressively develops irrespective of food availability, indicating a reversal in the modulation of food intake and satiety. This shift in control of the appetite coexists with downregulation of intestinal protein turnover, which promotes a gradual atrophy of the organ over the winter, concomitant with tissue remodeling (Carey et al., 2003; Hume et al., 2002). In this context, another question of interest concerns the fuel that supports the metabolism of the intestinal cells and satisfies their own energy needs in response to the large shifts in nutritional status and energy demand that occur during the annual cycle. In the fed state, the intestinal mucosa is presented with a complex mixture of arterial and luminal fuels that can provide nutrition for the cells that comprise the tissue (Burrin, 2002). However, during long-term fasting, the sources of nutrients become limited, a condition that is associated with reduced blood perfusion in the intestinal mucosa (Wang et al., 2006).

The present study addresses these questions by examining adjustments in energy demands in the small intestine of juvenile tegu lizards (*Tupinambis merianae*) in response to seasonal variation. This species is widely distributed throughout South America (Avila-Pires, 1995). Newly hatched animals feed exclusively on abundant insect populations in the natural environment, which become reduced during the autumn and winter dry seasons (Dessem, 1985; A. S. Abe, personal communication). After they hatch during the summer, the small tegus grow at high rates until the autumn; at the same time, fat is deposited in tissue stores at increasingly high rates to fuel metabolism during the winter (Souza et al., 2004). Aerobic metabolism is gradually depressed during late autumn, causing resting VO_2 at 17 °C to stabilize at ~20% of the resting values throughout winter dormancy (Souza et al., 2004). This period of metabolic suppression during the annual cycle in the tegu has been considered a form of active winter sleep, i.e., hibernation (Milsom et al., 2012, 2008). A partial increase in aerobic metabolism occurs in arousing, aphagic animals, and it increases further after food intake recommences a few days later.

This study integrates morphometric analysis of structural properties of the intestinal tissue and analysis of enzymes involved in supplying energy to the intestinal tissue in juvenile tegus because these factors are important determinants of the functional capacity of the organ. The small intestine is the first tissue exposed to the diet, and it has a key regulatory role in the digestion, absorption and availability of dietary protein and amino acids to enable continued growth and development in neonatal individuals. It was hypothesized that: (1) the relationship between body mass and intestinal mass would be disproportionate in juvenile tegus, and together with tissue structural and metabolic properties, would adjust the organ capacity to the increased demands of growth and storage during the autumn; (2) given the small body mass and limited storage capacity of neonatal tegus, considerable atrophy and restructuring of the small intestine through winter would allow reduced maintenance costs; and (3) the oxidative capacity of the intestinal cells would be retained in the remaining tissue by reorganization of energy metabolism, preserving the potential of the tissue to regenerate and to quickly restore the absorptive function at the time of arousal. Additionally, the consequences of unanticipated starvation on the small intestine of juvenile tegus were analyzed to contrast with those of fasting during winter dormancy, in an attempt to highlight adaptive differences related to seasonal plasticity.

2. Materials and methods

2.1. Animal supply and maintenance

Tegu lizards (*T. merianae* Duméril and Bribon) were obtained from a population that was reared outdoors in large pens in Rio Claro, southern Brazil. Two months after the animals hatched during the summer, they were moved to the laboratory and used in the experiments during their first annual cycle. The lizards were kept indoors in 120-liter cages and were exposed to thermal and photo periods. These periods were determined by incandescent lights that were set on 8 h:16 h L:D period, in addition to the sunlight that diffused in from outside. The lizards could freely alternate between warming and cooling their bodies by climbing onto a small platform or hiding in a wooden shelter that was located among the sheets of paper covering the box floor. The animals were separated into groups of 5–6 individuals according to size to minimize fighting and competition for food. The lizards were fed everyday on raw meat, egg and fruits that were enriched with minerals and had continuous access to drinking water.

In mid-autumn, the time devoted to thermoregulation became progressively shorter and food intake gradually reduced until the lizards became continuously inactive inside the shelter. The animals were kept in the shade throughout the winter months and were returned to the previous photo- and thermal periods after arousal in early spring. Aroused lizards had free access to drinking water, and a gradual increase in the time devoted to thermoregulation and food intake took place over the following weeks.

2.2. Tissue sampling

For tissue analysis, groups of lizards were weighed and killed by decapitation during the annual cycle and were classified according to the seasonal period in which they were sacrificed: 'autumn activity', which included lizards exhibiting behavior characteristic of the onset of dormancy; 'winter dormancy', which included fasting lizards and those that were totally inactive over 50–60 winter days; 'arousal', which included rehydrated, unfed lizards 48–96 h after they emerge from dormancy (90–100 days from the first winter day); and 'spring activity', which including fed and fully active lizards 30–40 days after arousal. Additionally, one group of lizards was deprived of food for 20 days during spring activity to analyze the effects of unanticipated starvation in active individuals. This period of starvation would require roughly the same amount of energy to support whole body metabolism as that required during seasonal fasting, as estimated from the oxygen consumption rates of dormant and active tegus at the respective temperatures (Souza et al., 2004).

The lizards were killed between 10:00 and 12:00, and the digestive tract was dissected. The middle portion of the alimentary canal, corresponding to the small intestine, was excised. The lumen of the organ was exposed by a longitudinal incision and was carefully rinsed with physiological saline to remove food residues. The tissue was blotted dry on filter paper and weighed. One sample was then taken from the proximal third, cut into small fragments and transferred to fixative solution (4% formaldehyde in phosphate buffer; pH 7.4) for histology. The remaining tissue was cut into small pieces, and aliquots were quickly frozen in N_2 and stored at −80 °C for assays.

2.3. Histology and morphometry

Tissue fragments of approximately 0.5 cm^2 were removed from the fixative solution and dehydrated through a graded series of ethanol, concluding with 100%. The dried tissue was cleared in xylol and embedded with Paraplast Plus (Sigma Aldrich) in an orientation that was randomized relative to a reference plane. Serial vertical sections of 5 μm thickness were cut transverse to the muscular walls and to the

mucosa, and sections were mounted on slides and stained with hematoxylin and eosin.

Five animals per group were randomly selected, and three sections per animal were analyzed using a light microscope (Nikon Eclipse E1000, Japan). Images were captured by a camera (Low Light Integrating i308) connected to a computer. Linear measurements of the thickness of the muscular layer (ML) and of the maximum height of the villi (Hv) were performed by randomly taking three measurements of the extent between the serous layer and the mucosa and five measurements of the extent between the muscularis and the tip of the largest villi using a Stereo Investigator (MicroBrightField 2000, Inc. Colchester, VT, USA). The epithelial surface density (Sv) and the volume density (Vv) of the mucosa were estimated according to classical stereological methods reviewed in Mandarim-De-Lacerda (2003) and using the test system proposed by Gundersen et al. (1988). The test was performed within three randomly selected fields of the image, which was projected on the computer screen. The vertical orientation of the sections was followed, and the number of times that the cycloid arcs intercepted the internal and external boundaries of the epithelium was counted. The Sv was calculated from the average of internal and external measurements according to the formula $Sv = 2\,I / LT$, where I = the average number of arc intersections with the epithelial surface and LT = the total length of the test lines. The Vv was estimated by counting the number of test points that fell within the structures analyzed, i.e., the absorptive epithelium and the lamina propria, which were used in the formula $Vv = PP / PT$, where PP = the number of test points within the structures and PT = the total number of test system points.

Photomicrographs were taken with an inverted microscope (Nikon Eclipse TE 300, Japan) equipped with a digital camera (Nikon D40, Japan). Image editing and processing were performed using Adobe Photoshop (CS3 Extended version 10.0).

2.4. Water and total protein content

Total tissue water was measured in freshly thawed tissue samples and was based on the mass loss that occurred in each sample at 60 °C until a constant mass was achieved; this measure was expressed as $g \cdot g^{-1}$ tissue. For total protein analysis, frozen samples were homogenized in four volumes (*v*/*w*) of 0.6 mol l^{-1} perchloric acid in an UltraTurrax homogenizer (Janke & Kunkel, IKA, Labortechnick, Staufen, Germany). The homogenate was centrifuged for 5 min at 10,000*g*, and the pellet was redissolved in four volumes of 0.6 mol l^{-1} PCA; this procedure was repeated twice. The precipitate was solubilized in 2.5% KOH, and protein content was measured using bovine serum albumin standards (Lowry et al., 1951). Each tissue sample was assayed in duplicate, and total protein was expressed in $mg \cdot g^{-1}$ wet and dry tissues.

2.5. Enzyme assays

The maximum activity (*Vmax*) of hexokinase (HK), pyruvate kinase (PK), lactate dehydrogenase (LDH), and phosphoenolpyruvate carboxykinase (PEPCK), which are enzymes of carbohydrate metabolism; of β-hydroxyacyl-CoA dehydrogenase (HOAD), which is an indicator of the capacity for fatty acid utilization; of glutamate dehydrogenase (GDH) and aspartate aminotransferase (AST), which are indicators of amino acid oxidation; and of citrate synthase (CS), which is an index of tissue total aerobic capacity, was measured in samples of the small intestine. Freshly thawed samples were homogenized at approximately 4 °C in nine volumes (*w*/*v*) of buffer (20 $mmol \cdot l^{-1}$ imidazole-HCl, pH 7.4, 2 $mmol \cdot l^{-1}$ 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, IKA, Labortechnik, Staufen, Germany) using three 30%-amplitude bursts of 30 s duration and at 30 s intervals for complete membrane disruption. The homogenates were then centrifuged at 17,000*g* at 4 °C for 10 min, and the supernatant fractions were kept ice-cold until assays were conducted. 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 samples using bovine serum albumin standards (Lowry et al., 1951), and enzyme activities were calculated per soluble protein mass to verify any biased tendency due to a change in tissue water content and/or to unspecific effects on the soluble protein content. Assay conditions were as follows: HK, 50 mmol l^{-1} imidazole-HCl (pH 7.0), 1 mmol l^{-1} ATP, 5 mmol l^{-1} $MgCl_2$, 100 mmol l^{-1} KCl, 5 mmol l^{-1} DTT, 0.5 mmol l^{-1} NADP, 1 U/ml glucose-6-phosphate dehydrogenase, 30 mmol l^{-1} glucose; PK, 50 mmol l^{-1} imidazole-HCl (pH 7.0), 5 mmol l^{-1} ADP, 10 mmol l^{-1} $MgCl_2$, 100 mmol l^{-1} KCl, 0.15 mmol l^{-1} NADH, 0.02 mmol l^{-1} fructose 1,6-bisphosphate, and 4.2 U/ml lactate dehydrogenase, 5 mmol l^{-1} phosphoenol-pyruvate; LDH, 50 mmol l^{-1} imidazole-HCl (pH 7.0), 5 mmol l^{-1} DTT, 0.15 mmol l^{-1} NADH, 1 mmol l^{-1} pyruvate; PEPCK, 50 mmol l^{-1} imidazole-HCl (pH 7.0), 0.2 mmol l^{-1} dGDP, 1 mmol l—1$MnSO_4$, 20 mmol l^{-1} $NaHCO_3$, 0.15 mmol l^{-1} NADH, 1 U/ml malate dehydrogenase, 0.5 mmol l^{-1} phosphoenolpyruvate; HOAD, 50 mmol l^{-1} imidazole-HCl (pH 7.0), NADH 0.15 mmol l^{-1}, 0.1 mmol l^{-1} acetocaetil CoA; GDH, 50 mmol l^{-1} imidazole-HCl (pH 7.0), ADP 1 mmol l^{-1} DTT and 5 mmol l^{-1}, NADH at 0.15 mM ammonium acetate 100 mmol l^{-1}, 10 mmol l^{-1} α-ketoglutarate; AST, 50 mmol l^{-1} imidazole-HCl (pH 7.0), 40 mmol l^{-1} α-ketoglutarate, 0.15 mmol l^{-1} NADH, 5 mmol l^{-1} DTT, 0.025 mmol l^{-1} pyridoxal phosphate, 2.2 U/ml malate dehydrogenase, 10 mmol l^{-1} L-aspartate; CS, 50 mmol l^{-1} Tris–HCl (pH 8.0), 0.3 mmol l^{-1} acetyl CoA, 0.1 mmol l^{-1} DTNB, 0.5 mmol l^{-1} oxaloacetate.

2.6. Statistical analysis

The correlation between the intestinal mass and the body mass was assessed using a least squares linear regression method applied to the logarithm of the data. The contribution of body mass in predicting the dependent variable, indicated by the R^2 regression coefficient, was evaluated by analysis of variance (ANOVA), and differences between mass exponents '*b*' were evaluated by Student *t*-test. A one-way ANOVA or the Kruskal–Wallis ANOVA on ranks procedure was used to test for differences between groups over the annual cycle. Means were then compared by the Student–Newman–Keuls or Dunn's tests for multiple comparisons, where appropriate. The differences between groups of 'fed' and 'unfed' (starved) individuals in spring activity were analyzed separate from the effects of season using the Student *t*-test. The analyses were based on Zar (1999) and performed using SigmaStat statistical software (Systat Scientific Software). The test results and probability of error are given in the text or in the tabulated results, differences being considered significant at $P \leq 0.05$ and marginally significant at $0.1 \geq P \leq 0.05$.

3. Results

3.1. Effects of body mass and season on the mass of the small intestine

The body mass (Mb) of the tegu lizards did not differ among the experimental groups that were analyzed during the annual cycle ($F = 0.355$; $P = 0.786$), although a marginally significant difference was detected between the sub-groups of fed and unfed (starved) individuals in the spring activity group ($t = -1.870$; $P = 0.076$) (Table 1). Otherwise, the intestinal mass (Mi), both in grams and as a percentage of Mb, significantly differed as a function of season ($F = 11.00$ and $H = 27.69$, respectively; $P < 0.001$). In the autumn activity group, Mi represented an average of 1.0% of the Mb in juvenile tegus, and this percentage gradually decreased to 0.88% after 60 days of winter and to 0.77% at the onset of arousal in early spring. Later in spring, Mi was more than two-fold larger in the group of active, fed tegus. In addition, the mass of the organ was 50% smaller in the sub-group of lizards that

Table 1
Small intestinal mass and the scaling relationship with body mass in the first annual cycle of tegu lizards.

Seasonal activity state	Intestinal Mass (g) (%)		Body Mass (g)	a	*b*	R^2	*N*	*P*
Autumn activity	2.04 ± 0.26[c,d]	1.04 ± 0,05[c]	197.4 ± 261	−1.80 ± 0.31	0.92 ± 0.14	0.85	10	0.0002
Winter dormancy	1.63 ± 0.22[d]	0.88 ± 0,04[d]	190.6 ± 26.9	−1.82 ± 0.26	0.89 ± 0.12[c]	0.83	14	<0.0001
Arousal	1.29 ± 0.10[a,d]	0.77 ± 0.03[a,d]	168.1 ± 15.4	−2.08 ± 0.23	0.98 ± 0.11[b]	0.84	17	<0.0001
Spring activity — fed	2.65 ± 0.21[a,b,c]	1.65 ± 0.21[b,d]	188.7 ± 22.5	0.09 ± 0.32	0.14 ± 0.14	0.076	14	0.3410
Spring activity — unfed	2.19 ± 0.30	0.85 ± 0.08[*]	287.7 ± 57.4	−1.11 ± 0.18	0.60 ± 0.07	0.92	8	0.0002

Values are the mean ± S.E.M. Linear regressions were performed with the log-transformed values and described by the equation $\log_{10} Mi = a + b \log_{10} Mb$, where a is the intercept ± SEM, *b* is the slope ± SEM, Mi is the intestinal mass and Mb body mass.
[a] Indicates significant differences from autumn activity.
[b] Indicates significant differences from winter dormancy.
[c] Indicates significant differences from arousal.
[d] Indicates significant differences from spring activity.
[*] Indicates significant differences between fed and unfed spring activity animals ($P < 0.05$).

were starved during this season compared with normally fed individuals ($t = 2.77$; $P = 0.012$).

The correlation between Mi and Mb was examined for scaling effects associated with the seasonal changes in Mi. In the autumn activity group, Mi correlated with Mb according to the exponent $b = 0.92$, suggesting that smaller tegus reach this season with a slightly larger Mi than their larger counterparts (Table 1). The mass exponent for the correlation after 60 days of winter dormancy was similar, however, it increased significantly to $b = 0.98$ in the group of unfed, arousing lizards. This result suggests that Mi disproportionately decreases in smaller individuals at the end of winter before feeding recommences; according to the equations, the mass loss would be 30% in a 100 g lizard versus 25% in a 3-fold larger individual. Later in the spring, the exponent was almost zero ($b = 0.14$) in the group of active lizards, indicating a remarkable increase of Mi in the smallest lizards; in a 100 g lizard, this increase was calculated to be 3.9-fold in relation to the onset of arousal. Overall, during spring activity, Mi became almost 3-fold larger in a 100 g tegu and virtually unchanged in a 300 g tegu in relation to the values in similarly sized individuals during autumn activity. After food deprivation in spring, the mass exponent was $b = 0.60$ and was significantly larger than the value for the group of fed individuals ($P < 0.001$). This shift in the scaling pattern suggests considerable differences in the effect of food deprivation related to body mass; accordingly, the correlation predicts that the Mi would decrease by 60% in a 100 g tegu versus 16% in a 3-fold larger individual.

3.2. Histology and morphometry

During autumn activity, the proximal mucosa in the small intestine of juvenile tegus resembles the tissue that covers the inner wall of the duodenum of mammals. The villi are multitudinous, thread-like projections that are lined by an epithelium (Ep) that is mainly composed of absorptive cells, with interspersed Paneth cells and goblet cells, and that lies on a basal lamina (Figs. 1 and 2). The crypts are not developed, and the lamina propria forms a complex connective tissue that is composed of loose connective tissue with blood and lymph vessels, nerve fibers and smooth muscle fibers in addition to immune cells—mainly lymphocytes and macrophages. The muscularis mucosae consists of a very thin layer of tissue that separates the lamina propria (LP) of the submucosa, which is composed of dense connective tissue filled with large-caliber arterioles. The muscular layer (ML), which is composed of one circular and one longitudinal layer, is well developed in juvenile tegus.

The Ep configuration that was observed during the autumn changed remarkably in the following seasons (Figs. 1 and 2). After 60 days of winter dormancy, the tissue switched from predominantly simple columnar to pseudo-stratified, with a large number of superimposed cells near the basal lamina; this arrangement persisted in arousing, unfed lizards. Later in the spring, smaller numbers of cells were present, and the Ep contained both simple columnar and pseudo-stratified regions. Additionally, the villus height (Hv) and the density of the epithelial surface (SvEp) were significantly different among the experimental groups as a function of season ($H = 217.18$ and $F = 11.74$, respectively; $P < 0.001$) (Table 2; Figs. 1 and 2). During the autumn activity, the Hv was 19% larger and significantly different from the Hv in the spring activity group. After 60 days of winter dormancy, the Hv was 50% smaller and the SvEp was 20% smaller in relation to the corresponding values measured during the autumn, and there was no further change after the end of winter in the group of rehydrated, arousing lizards. One month later in the spring, the Hv and SvEp were 59 and 54% larger, respectively, compared to the values during early arousal and had recovered to the autumn activity levels. Likewise, the volume density of the epithelium (VvEp) and of the lamina propria (VvLP) differed among the experimental groups ($F = 9.96$, $P < 0.001$ and $H = 11.14$, $P = 0.011$, respectively). Multiple comparisons tests indicated that VvEp and VvLP were similar in the autumn and spring activity groups. During winter dormancy, VvEp was 29% smaller and significantly different from that in the autumn activity group, and no further change was detected in arousing tegus. Otherwise, VvLP was unchanged in the group of winter dormancy and was 31% smaller in the arousal group relative to the values during the autumn. The density volume of the entire mucosa, which combines VvEp and VvLP, also differed significantly among the experimental groups ($F = 27.811$; $P < 0.001$), mostly due to values in arousing tegus that were 31% smaller than those in the autumn group. Later, during spring activity, VvEp and VvLP were ≈50% larger than during arousal and were restored to autumn levels. The thickness of the muscular layer (ML) also differed significantly among the experimental groups ($H = 19.901$; $P < 0.001$), mostly due to values in the winter dormancy group that were ≈20% smaller than those in the autumn and spring activity groups.

The morphological changes in the group that was starved during spring activity were less pronounced than those in the winter dormancy group (Table 2; Figs. 1 and 2). The Hv and SvEp were ≈20% smaller ($U = 1237.0$, $P < 0.001$, and $t = 3.187$, $P = 0.011$, respectively); there was no detectable difference in VvEp and VvLP, although their sum, which corresponds to the density volume of the entire mucosa, was 11% smaller and significantly different in relation to that in normally fed individuals. The thickness of the ML was 51% larger in the group of starved individuals compared with the group of fed individuals ($t = -11.635$; $P < 0.001$), indicating a change in a direction opposite to the change that occurred during the winter fasting.

3.3. Water and total protein content

There was little, although significant, variability in the water content of tissue samples during the annual cycle ($F = 6.206$; $P = 0.002$). The multiple comparisons test indicated that it was associated with the content in rehydrated, arousing tegus, which was 3–4% larger than that of autumn and spring activity groups of tegus; all other measures were similar (Table 3). The total protein per mass unit also differed among

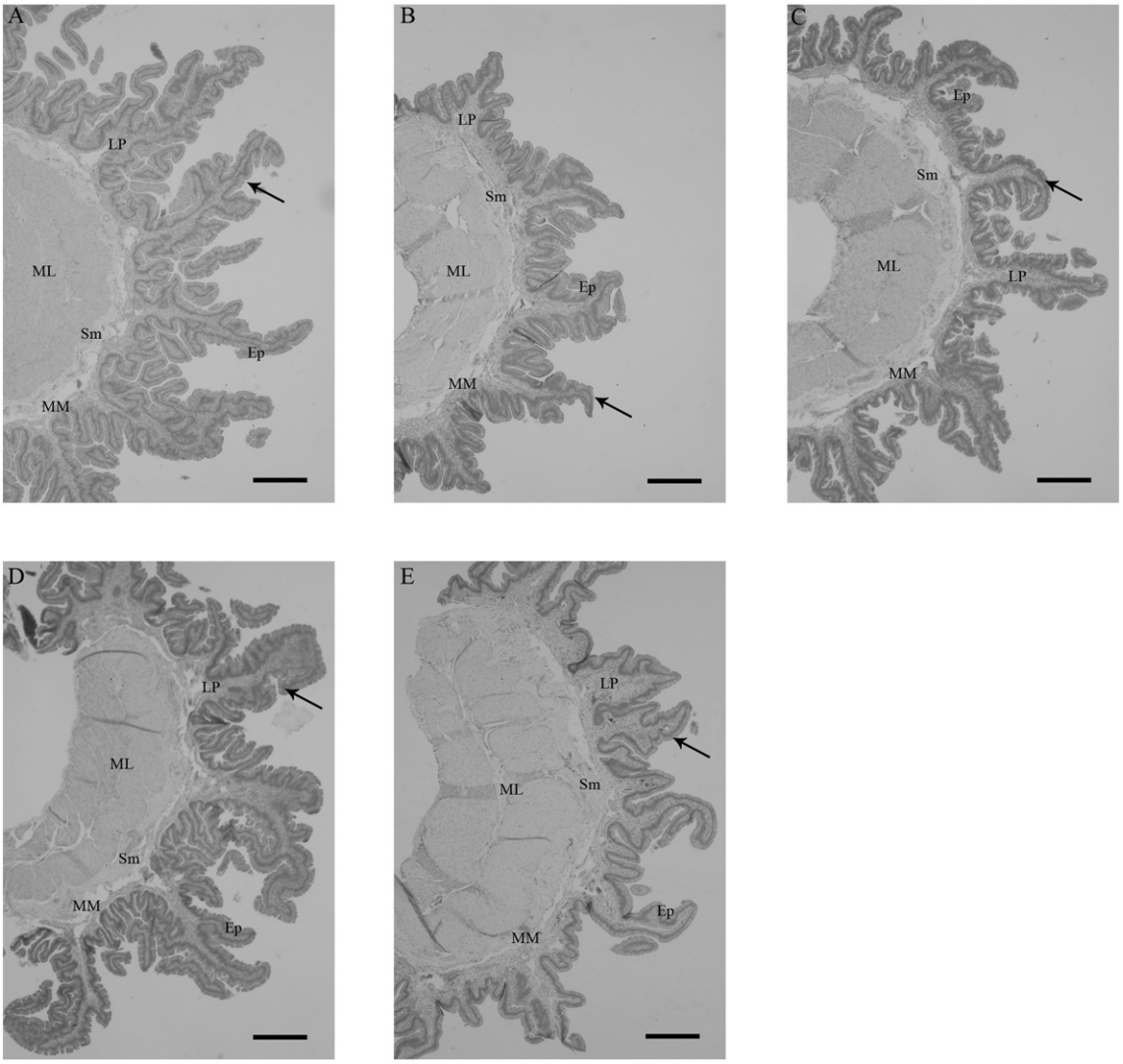

Fig. 1. Light micrograph sections of the proximal small intestine of juvenile tegu lizards. A) autumn activity; B) winter dormancy; C) arousal unfed and rehydrated; D) spring activity, normally fed; and E) spring activity, starved for 20 days. ML, muscular layer; MM, muscular mucosae; Sm, submucosa; LP, lamina propria; Ep, epithelium. The arrows point to villus. HE. Scale bar, 200 μm.

the experimental groups, both in the wet and in the dry tissue samples ($F = 7.02$, $P < 0.001$ and $F = 2.95$, $P = 0.047$, respectively), and the variability was associated with the content in the winter dormancy group, which was 23% smaller than that in the autumn and spring activity groups. In contrast, there were no significant differences in the water and total protein content between the groups of starved and fed lizards during spring activity.

3.4. Enzyme activities

The maximum activity (*Vmax*) of metabolic enzymes in samples of the small intestine is presented in Fig. 3. The activity rates of HK, PK and LDH were remarkably constant in the experimental groups ($H = 4.50$, $P = 0.212$; $F = 1.302$, $P = 0.292$; and $F = 1.50$, $P = 0.234$, respectively), suggesting that the capacity for carbohydrate oxidation per mass unit of the intestinal tissue is maintained during the annual cycle. In contrast, the *Vmax* of HOAD and CS was strongly affected by season ($F = 11.08$, $P = 0.011$; and $F = 8.98$, $P < 0.001$, respectively). According to the multiple comparisons test, the activity rates of both enzymes were highest in the autumn and spring activity groups and were ≈50% lower during winter dormancy and arousal. Additionally, the *Vmax* of PEPCK differed among the experimental groups ($F = 3.99$, $P = 0.016$); this difference was mostly due to activity rates that were 44% lower during winter dormancy and arousal compared with those during spring activity. The *Vmax* of GDH also changed as a function of season ($F = 4.28$; $P = 0.013$), and the multiple comparisons test indicated that this measure was 27% lower during midwinter and 40% lower at the onset of arousal compared with that during autumn activity. The *Vmax* of AST was high and constant throughout the year.

In contrast to the results for the tegus that were fasting during winter, the enzymes HK, PK and LDH were significantly less active in the tegus that were starved during the spring than in the fed individuals ($t = 2.38$, $P = 0.031$; $t = 2.84$, $P = 0.012$; and $t = 3.06$, $P = 0.008$, respectively). Other enzyme activities were also reduced during starvation; PEPCK values were 28% smaller ($t = 1.96$, $P = 0.069$), HOAD and CS values were 46% smaller ($U = 63.000$, $P = 0.011$; and $U = 67.000$, $P = 0.003$, respectively), and GDH and AST values were slightly, although not significantly, smaller than those of normally fed individuals.

There was significant variability in the content of soluble proteins in tissue extracts as a function of season ($F = 13.12$; $P < 0.001$), and it was mostly due to the values that were 35–40% smaller in tissue extracts obtained from arousing tegus relative to those in the tegus in the autumn activity and spring activity groups. Similarly, the soluble protein

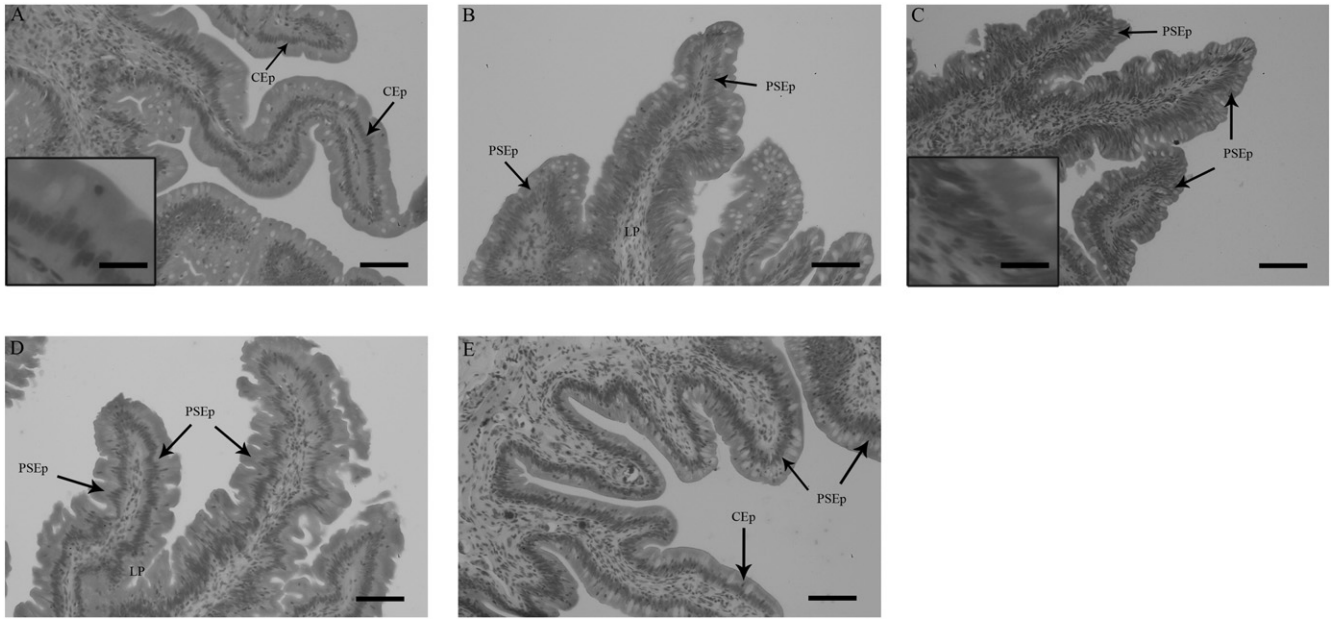

Fig. 2. Light micrograph of the proximal small intestine of juvenile tegu lizards, highlighting the epithelial lining cells. A) autumn activity; B) winter dormancy; C) arousal unfed and rehydrated; D) spring activity, normally fed; and E) spring activity, starved for 20 days. LP, lamina propria; CEp, simple columnar epithelium; PSEp, pseudostratified epithelium. HE. Scale bars, 50 μm, and 25 μm (insets).

content was 29% smaller in extracts obtained from individuals that were starved during spring activity ($t = 3.65$; $P < 0.001$). These changes in the content of soluble proteins conform with the consistent suppression of several metabolic enzymes in the tissue during the winter, as described above.

4. Discussion

4.1. Morphological changes and correlations with growth and seasonality

Tegu lizards undergo a period of intense growth after they hatch in early summer, and the mass of the small intestine (Mi) was found to be an average of 1.0% of body mass (Mb) around mid-autumn. Interestingly, in the smaller lizards, the mass of this organ was slightly larger than that in bigger lizards. The reason for this pattern is unclear. One possible explanation may arise from previous evidence that the decrease in the metabolic rate that occurs during the transition from activity to dormancy takes place later in smaller neonates (Souza et al., 2004). The slower progression to dormancy would be associated with continuously high nutrient intake and biosynthesis rates in the intestinal epithelium, preserving the mass of the organ. Otherwise, the largest neonates showed a faster progression to dormancy, which would be presumably accompanied by some degree of mucosal atrophy and a decrease in the mass of the organ.

The Mi in tegus that were arousing from winter dormancy was 26% smaller than that in tegus in the autumn group. This difference can be taken as an approximation of the organ atrophy that took place over 90 days of fasting in winter and indicates that the degree of atrophy in juvenile tegus was small compared to that in other reptiles and amphibians that have been investigated, which were mostly adult animals. Among the species examined, intestinal atrophy averages 34% and may reach 50 to 65%; apparently, there is no correlation between intestinal mass loss and body size or phylogeny, however there is correlation with feeding habits, and species that commonly fast for long periods have larger mass losses (Zaldúa and Naya, 2014; Secor, 2005). In juvenile tegus, the degree of atrophy was slightly greater in smaller lizards: 30% in a 100 g lizard versus 25% in a 300 g lizard. This small difference may become meaningful in terms of substrate savings over the duration of the hypometabolic condition. In addition to differences in the size of energy stores in the autumn, previous studies with juvenile tegus have indicated that the degree of reactivation of the metabolic rate upon arousal is considerably smaller in the smallest individuals (da Silveira et al., 2013; Souza et al., 2004). One month later in spring, the Mi was more than two-fold larger than that in similarly sized individuals during the autumn. In adult mammals, a large increase in the Mi was recorded three months after emergence from hibernation which would be necessary to facilitate attainment of the energy and nutrient demands required for reproductive events (Hume et al., 2002; Carey, 1990). In

Table 2
Structural changes in the proximal small intestine of tegu lizards.

Seasonal activity state	ML (mm)	Hv (mm)	V_VLP (%)	V_VEp (%)	S_VEp (mm)
Autumn activity	0.485 ± 0.034	1.091 ± 0.083[b,c,d]	34.72 ± 0.90[c]	38.75 ± 2.40[b,c]	15.53 ± 0,68[b,c,d]
Winter dormancy	0.390 ± 0.046	0.579 ± 0.029[a]	29.72 ± 1.80	26.25 ± 2.70[a,d]	12.56 ± 1,70[a,d]
Arousal	0.435 ± 0.044	0.576 ± 0.054[a,d]	23.26 ± 1.21[a,d]	27.66 ± 1.60[a,d]	12.32 ± 0,63[a,d]
Spring activity — fed	0.412 ± 0.031	0.918 ± 0.040[b,c,d]	35.56 ± 4.20[c]	40.00 ± 2.33[b,c]	18.97 ± 0,67[b,c,d]
Spring activity — unfed	0.622 ± 0.040[*]	0.721 ± 0.042[*]	32.75 ± 1.20	34.14 ± 1.68	14.80 ± 1,22[*]

Values are the mean ± S.E.M. from 5 to 6 animals of the following variables: muscular layer thickness (ML), villus maximum height (Hv), lamina propria volume density (V_VLP), epithelial volume density (V_VEp), and epithelial surface density (S_VEp).
[a] Indicates significant differences from autumn activity.
[b] Indicates significant differences from winter dormancy.
[c] Indicates significant differences from arousal.
[d] Indicates significant differences from spring activity.
[*] Indicates significant differences between fed and unfed spring activity animals ($P < 0.05$).

Table 3
Seasonal changes in small intestine tissue composition in juvenile tegu lizards.

Seasonal activity state	Water (%)	Total protein ($mg \cdot g^{-1}$)		Soluble protein ($mg \cdot g^{-1}$)
		Wet tissue	Dry tissue	
Autumn activity	81.81 ± 0.68[c]	45.92 ± 1.45[b]	259.56 ± 12.89[b]	40.06 ± 2.44[b,c]
Winter dormancy	83.07 ± 0.58	35.37 ± 1.06[a,d]	210.33 ± 8.02[a]	31.87 ± 1.60[a,d]
Arousal	84.55 ± 0.50[a,d]	40.21 ± 2.32	237.60 ± 16.21	26.15 ± 1.57[a,d]
Spring activity — fed	80.97 ± 0.62[c]	45.21 ± 2.15[b]	237.17 ± 7.55	43.33 ± 2.80[b,c]
Spring activity — unfed	82.88 ± 0.52	44.22 ± 1.72	259.86 ± 10.26	30.72 ± 1.95[*]

Values are the mean ± S.E.M. from 7 to 10 animals.
[a] Indicates significant differences from autumn activity.
[b] Indicates significant differences from winter dormancy.
[c] Indicates significant differences from arousal.
[d] Indicates significant differences from spring activity.
[*] Indicates significant differences from fed and unfed spring activity animals ($P < 0.05$).

juvenile tegus, the relatively faster increase in the Mi following arousal resulted in remarkable differences in the mass of the organ — 2.4% of body mass in a 100 g lizard versus 1.0% in a 300 g individual. Thus, after emergence from dormancy, a large fraction of the energy intake would be invested to increase the capacity to process food, particularly in the smallest tegus, in order to subsequently benefit from the energy intake to increase the rate of growth and to continue development.

Starvation during spring activity caused a decrease of Mi that was larger than the decrease that occurred during spontaneous fasting during winter in juvenile tegus, although both conditions require roughly similar amounts of energy to support the whole body metabolism. Additionally, much larger differences in the degree of intestinal atrophy were observed, amounting to 50% in a 100 g lizard versus 17% in a 300 g one. The large amount of protein drawn from the intestine would become available as a supplementary energy source to the tissue until food could be found. Nevertheless, food restriction during the spring would lead to considerable loss of the intestinal capacity to absorb nutrients in small neonates, which would ultimately hinder the processes of growth and development after emergence from dormancy.

In addition to changes in the mass of the organ, tissue remodeling in the small intestine may help reconcile the demands of growth with those of seasonality in the tegus. During the autumn, the growth rates of newly hatched individuals become progressively reduced while those of lipid deposition become increasingly higher until the animals enter into dormancy (Souza et al., 2004). In the present study, the small intestine of the tegus displayed a large surface density of epithelium (SvEp) and very tall villi during the autumn. This configuration would be part of the events that prepare for winter dormancy by providing the organ with a greater capacity for nutrient uptake in juvenile tegus. Halfway through winter dormancy, substantial changes in this arrangement were already apparent: the maximum height of the intestinal villi (Hv) was reduced by half, and the SvEp, the volume density of epithelium (VvEp), and the thickness of the muscular layer (ML) were all reduced in relation to the corresponding values in the autumn; the lamina propria, however, was virtually unaltered. These changes may account for some of the mass loss in the organ, which reduces the energy costs of maintenance during the hypometabolic condition. Likewise, intestinal atrophy during hibernation in small mammals is associated with decreases in villus height and epithelial surface area; notably, some absorptive cells are replaced during the short periods of arousal, when the body temperature increases and allows increases in the rates of protein synthesis (Carey, 1990, 1995; Van Breukelen and Martin, 2002). The mitotic activity is usually very high in the small intestine compared to other sections of the gastrointestinal tract, and low rates of cell proliferation can be maintained for many days without food intake after emergence from hibernation (Hume et al., 2002). In the present study, clusters of cells were observed in the villi at the tip of the lamina propria halfway through dormancy, suggesting that some level of proliferative activity remains during winter dormancy at the moderately high body temperature of the tegu.

The structural adjustments in the small intestine of the tegus became more extensive by the end of the winter, when the volume density of the lamina propria (VvLP) was smaller, intensifying the degree of atrophy of the whole mucosa. Interestingly, there were no additional changes in Hv, SvEp, and VvEp beyond those that occurred during the first half of the winter. Thus, the changes in selected components of the small intestine developed over different time scales during the seasonal fasting, suggesting that regulatory mechanisms modulate this specificity of action. One month after feeding was reinitiated in the spring, a full recovery of the organ was accomplished by an increase in Hv together with an enlargement of SvEp, VvEp, and the lamina propria core; besides a more folded surface, the greater than two-fold increase in the Mi could possibly involve an increase in tube length and/or diameter. Additionally, the layer of cells that lines the epithelial surface changed from pseudo-stratified to simple columnar; the cells also appeared to become more voluminous. In snakes, food ingestion following a prolonged period of fasting induces an increase in the size of enterocytes, which has been attributed to the incorporation of fat droplets and fluid inside the cells (Secor and Diamond, 1998; Stark and Beese, 2002; Lignot et al., 2005). In juvenile tegus, an alternative hypothesis would be the occurrence of true hyperplasia of the epithelial cells via increased amounts of structural proteins and cells. Together, the adjustments would increase the capacity of the organ to absorb nutrients, allowing the processes of growth and development to recommence after the animals emerge from dormancy.

Starvation during the spring caused markedly different effects on the intestinal morphology relative to those that occurred during spontaneous fasting during the winter, indicating that the regulatory mechanisms are not the same. The reduction in the mass of the organ was almost two-fold larger during starvation, and this reduction was associated with smaller-magnitude changes in Hv and SvEp and with the absence of changes in VvLP. This pattern differs from that seen in the small intestine of toads, in which the adjustments during hibernation and short-term fasting were similar (Naya et al., 2009). Intriguingly, the thickness of the ML was much greater (by ≈51%) in the starved tegus. A large increase in ML thickness in slices from the proximal part of the organ concomitant with a large decrease in the mass of the whole organ suggests that mass loss during starvation was more severe in distal parts of the organ. Consequently, preservation of the intestinal mucosa in the proximal part of the organ would allow some of the tissue's capability to absorb nutrients to be retained and used any time that food became available. While the primary cause for the increase in the ML thickness in the starved tegus cannot be ascertained with the present data, it is noteworthy that this adjustment was not observed in winter dormant lizards. Starvation is known to alter the composition of intestinal microbiota in mammals, leading to changes in the concentrations of short chain fatty acids which are potential mediators of the effects of gut microbiota on intestinal immune function (Sonoyama et al., 2009). Under many circumstances, such as nutritional imbalance, a change in the fatty acids composition activates an

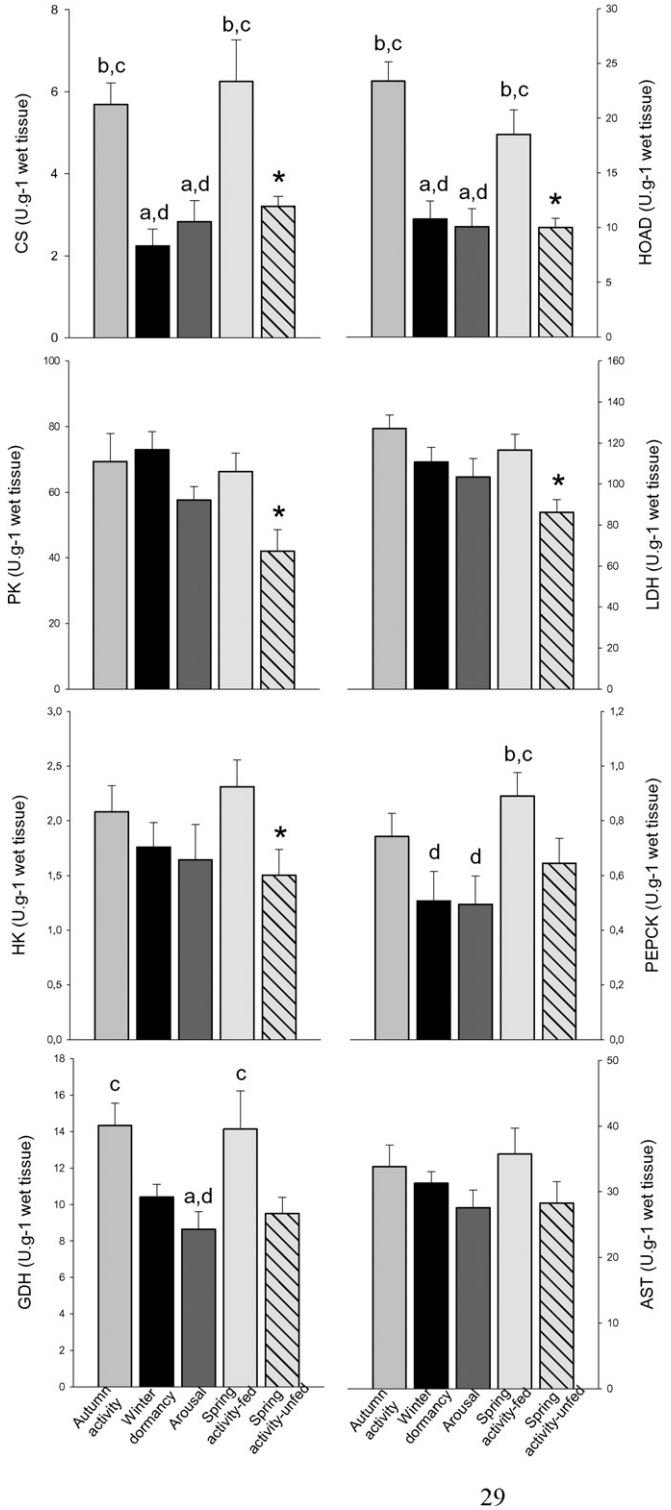

Fig. 3. Maximum activity of enzymes in small intestine tissue samples of juvenile tegu lizards. Values are the mean ± S.E.M from 7 to 10 animals in $U \cdot g^{-1}$ wet mass. a indicates significant differences from autumn activity, b from winter dormancy, c from arousal, d from spring activity,* significant difference between fed and unfed spring activity animals ($P < 0.05$).

inflammatory response in the intestinal mucosa that may lead to hypertrophy of smooth muscle cells (Serikov et al., 2010). During hibernation, there is evidence showing that a change in the microbiota is accompanied by increased levels of mucosal anti-inflammatory cytokines, which promote immune tolerance (Carey et al., 2013; Kurtz and Carey, 2007).

4.2. Compositional changes and metabolic reorganization

The adjustments in the mass and structure of the small intestine that occurred in the juvenile tegus were unrelated to the changes in the tissue water content. During winter dormancy, the water content was unaltered, suggesting that the total loss of fluid from tissue compartments was negligible. Because the tegus do not drink water or urinate throughout the winter, behavioral and physiological mechanisms might help minimize water loss through the body surfaces and maintain water balance. For instance, the ventilation pattern of adult tegus becomes episodic during dormancy, and apnea intervals can last up to 26 min (Andrade and Abe, 1999). Additionally, the cloacal space in the tegus that were killed during dormancy was filled with an aqueous fluid (L. F. R. do Nascimento, unpublished observation), which could be directed back to the intestinal lumen, where water can be reabsorbed through the capillary network of the mucosal layer (Walker and Liem, 1994). At the onset of arousal, a small increase in the tissue water content results from the intake of large amounts of water and possibly from some fluid retention in the interstitial space of the gastrointestinal tract. This, however, was insufficient to override the smaller measurements for the morphological variables.

Otherwise, protein turnover in the small intestine of the juvenile tegus was strongly affected by season. In addition to the smaller mass of the organ, the protein content per gram of tissue was smaller during winter dormancy and arousal than during the autumn, and this effect was associated to a great extent with reduced amounts of soluble proteins. At the onset of arousal, the levels of soluble proteins remained low while the total content was slightly higher, suggesting differential regulation of the synthesis rates of structural proteins in the rehydrated lizards. The small increase in the tissue water content in the aroused tegus could lead to activation of pathways that promote protein synthesis in enterocytes, which would prepare the tissue for the return to functionality (Haussinger et al., 1994). Amino acids from endogenous sources may be used as a substrate for protein deposition in the small intestine, particularly from protein catabolism in the heart and skeletal muscle (da Silveira et al., 2013; Souza et al., 2004). Additionally, proteins from intestinal cells that are discharged into the lumen of the organ could be hydrolyzed and the amino acids could be reabsorbed by the tissue, as suggested for hibernating mammals (Carey, 1995). This recycling mechanism could supply the intestinal tissue of the tegu with amino acids, which could then be used for energy production or incorporated into new proteins.

In marked contrast to fasting during winter dormancy, starvation during spring activity caused no changes in the total protein content of the small intestine. The mismatch between unchanged total protein and severely reduced organ mass and soluble proteins suggests that structural proteins may be well conserved in the tissue during starvation. Additionally, these results are congruent with the idea of regional differences in the adjustments to starvation in juvenile tegus that were presented above. In mammals, the rate of protein synthesis progressively diminishes along the length of the small intestine, and there are proximal to distal differences in protein balance during starvation (Stoll et al., 2000). In active tegus, similar differences could lead to a high degree of atrophy in the distal portion while preserving the composition and structural configuration in the proximal portion of the organ in the face of an unpredictable food shortage.

In actively feeding tegus, the soluble proteins represented ≈90% of the total protein content in the intestinal tissue and consisted mostly of metabolic enzymes. This fraction was 35% smaller during the emergence from winter dormancy compared to during the autumn and was 29% smaller in the animals that were starved during the spring compared to normally fed individuals, indicating the downregulation of the enzymes in response to the interruption of food intake. Accordingly, the activity of the enzymes HOAD and CS in the small intestine of the tegus was consistently reduced during winter dormancy and starvation and was restored only after feeding was reinitiated. The activity

of HOAD was high in the intestine compared to other tegu tissues that were already analyzed (Souza et al., 2004). The ratio of HOAD activity to CS activity was also high (3–4), suggesting considerable potential for fatty acid oxidation and that lipid may be an important source of energy for the tissue. During winter dormancy and during starvation in the spring, despite the marked reduction of HOAD and CS activity, the ratio of HOAD activity to CS activity remained very high (≈ 5), suggesting that the tissue may obtain energy by slowly oxidizing fatty acids released from fat stores. Additionally, the intestinal tissue might become another site of ketogenesis in addition to the liver, providing heart and brain tissues with an alternative source of energy when food intake is interrupted and carbohydrate stores become depleted.

In the fed state, the intestinal mucosa is presented with a complex mixture of arterial and luminal fuels that provide nutrition for the enterocytes (Burrin, 2002). In mammals, only a small proportion of the glucose that is taken up from the lumen of the intestine is metabolized, indicating that flux through this enzyme is normally under inhibitory control within these cells (Newsholme and Carrié, 1994). In juvenile tegus, the activities of the glycolytic enzymes HK and PK in the small intestine were similar to the values previously found in heart and brain tissues (da Silveira et al., 2013; Souza et al., 2004). These tissues typically show high rates of glucose uptake and oxidation, and thus the small intestine has the potential to actively use glucose in aerobic pathways of energy production. Unlike the activity of HOAD and CS, the activities of the enzymes HK, PK and LDH were unchanged throughout the annual cycle, and the ratio of PK activity to LDH activity, which is an indicator of anaerobic capacity for carbohydrate oxidation (Hochachka et al., 1992), was nearly constant around 0.5. The proportion of glucose that is completely oxidized to CO_2 in the small intestine of mammals is substantially less than that of either glutamate or glutamine, and most of the pyruvate that is derived from glucose is converted into lactate and alanine and exported to the portal circulation (Stoll et al., 1999). In fully active tegus, any LDH activity in excess of the capacity to form pyruvate might similarly favor the production of lactate in the intestine, which could be exported, used in amino acid transamination, or a combination of these alternatives, in accordance with short-term food availability. During winter dormancy, however, the main carbohydrate stores are depleted in juvenile tegus, and they become hypoglycemic (Souza et al., 2004); this condition, combined with the mucosal atrophy and the ensuing ischemia in the tissue, probably results in very low glucose uptake and its use as substrate by enterocytes. Otherwise, the activity rates of all glycolytic enzymes were lower in the tegus that were starved during the spring, indicating that under conditions of long-term interruption of food intake, a regulatory mechanism in the small intestine acts to spare blood glucose for the glucose-dependent tissues, whose energy demands are high during spring activity.

In addition to its capacity for glucose oxidation, strong evidence supports a role for the small intestine as a site of endogenous glucose production in mammals, in addition to the liver and the kidney. The mechanisms of glucose homeostasis and the role of glucose in reptile metabolism are poorly understood, as reviewed by Polakof et al. (2011). The present data show that the activity of the rate-limiting gluconeogenic enzyme PEPCK in the small intestine of the tegu is comparable to that in the intestine of the rat, suggesting that the tissue of neonatal tegus possesses gluconeogenic capacity. Souza et al. (2004) found plasma glucose levels of 7.6 mM in juvenile, active tegus; this level is higher than the average reported for reptiles, and decreased 60% in winter dormant individuals. Remarkably, at the onset of arousal, blood glucose was restored concomitant with an increase of glycogen in the skeletal muscle, suggesting that another source of glucose is made available to the body tissues before feeding is reinitiated. In small mammals, intestinal glucose production can contribute 20–25% of total endogenous glucose production during long-term fasting (Croset et al., 2001); the expression of PEPCK was strongly upregulated in the intestinal cells of mice, while that of LDH decreased by almost 3-fold, suggesting that the enterocytes acquire the capacity to produce glucose rather than lactate using amino acids from protein breakdown (Penhoat et al., 2014; Sokolović et al., 2007). The adjustments that occur in the tegu would be another, as indicated by the significantly lower PEPCK activity and the high and constant LDH activity observed during winter dormancy and during starvation in the spring. The downregulation of PEPCK matches the smaller capacity of the tissue to synthesize ATP for enzymatic reactions in the empty intestine, while some gluconeogenic activity might continue at very low rates using glycerol, which is derived from fatty acid catabolism, or with amino acids, which are derived from protein breakdown, as precursors.

Amino acids are a major source of mucosal energy generation in mammals, and in absolute molar terms, the uptake of some amino acids via the arterial and luminal routes is nearly equivalent in the small intestine (Burrin, 2002). There is abundant evidence that in addition to glutamine, glutamate and aspartate are equally important intestinal fuels (Burrin and Stoll, 2009). Once in enterocytes, a large fraction of the dietary amino acid intake is metabolized via conversion to glutamate, which is readily converted to α-ketoglutarate by means of various aminotransferases and a dehydrogenase in reversible reactions. In the small intestine of the tegu, the activity of AST was high and constant throughout the annual cycle as well as during starvation in the spring, and the activity rates of this enzyme were more than twice the rates of GDH, therefore suggesting that glutamate would be preferably metabolized by an aminotransferase. In mammals, glutamate and aspartate-containing peptides enter mucosal cells by co-transport; once inside the cells, they are hydrolyzed to their amino acid constituents (Blachier et al., 2009). According to these authors, the glutamate concentration in enterocytes and the intestinal mucosa is very high, and this amino acid is extensively metabolized through various pathways, including those involved in protein synthesis. Thus, the proteolysis that occurs in the intestinal tissue during winter dormancy and during food restriction in juvenile tegus could provide the enterocytes with amino acids that could satisfy, at least in part, their own energy needs for continuing function. Additionally, during arousal from dormancy, amino acids from the mucosa and from other endogenous sources can be incorporated into new proteins to quickly restore some functional capacity of the organ before feeding recommences.

5. Conclusions

The present study contributes to the understanding of the mechanisms underlying hibernation in a tropical lizard and provides insight into how this seasonal phenotype is expressed in the small intestine and accommodates the energy demands of growth and development in newly hatched individuals. During the autumn, the disproportionate relationship between body mass and intestinal mass may sustain a high capacity for energy intake in the smallest tegus; this component, together with greatly elongated villosities at the mucosal surface, may take part in the events to prepare for winter dormancy. During the long-term winter fasting, the intestinal mass loss was relatively modest; following arousal, however, there was a very large increase in the organ's relative mass in the smaller individuals, indicating that a large fraction of the energy intake is invested to increase their capacity to process food before the reinitiation of growth and development. Overall, these data highlight the benefits of winter dormancy for offspring survival under the energetically unfavorable conditions of the winter (dry) season. Additionally, the results indicated marked contrasts in the adjustments in response to fasting during the winter and to starvation during the spring. During the winter, the mass loss in the whole organ gradually increased; this effect was accompanied by losses in the total and soluble proteins and by rearrangement of the intestinal mucosa. In contrast, starvation during the spring caused a severe reduction in the organ mass that was also associated with uneven changes in protein content and structure, suggesting regional differences in the adjustments along the length of the organ. The enzymatic activities indicated that the energy requirements of the small intestine would

be fulfilled through the capacity of the tissue to obtain energy from different sources. Furthermore, the relative contribution of the different substrates may be dynamically regulated as a function of nutritional changes during the annual cycle, allowing the tissue to accomplish important physiological functions that are necessary for the growth and development of the juveniles.

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