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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