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RESEARCH ARTICLE

The consequences of seasonal fasting during the dormancy of tegu lizards (*Salvator merianae*) on their postprandial metabolic response

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ABSTRACT

Tegu lizards (*Salvator merianae*) aestivate for up to 5 months during Brazil's winter, when they retreat to burrows and halt most activities. Dormant tegus reduce their gastrointestinal (GI) mass, which allows a substantial energy economy. This strategy, however, implies that the first post-dormancy digestion would be more costly than subsequent feeding episodes as a result of GI atrophy. To address this, we determined the postprandial metabolic response (SDA) of the first (M1), second (M2) and several (RM) feeding episodes after tegus' dormancy. Another group of tegus (PF) was subjected to an extra 50 day fasting period after arousal. Glucose, triglycerides and uric acid levels were checked before and after feeding. M1 digestion lasted twice as long and cost twofold more when compared with M2 or RM, in agreement with the idea that GI atrophy inflates digestion cost at the first post-dormancy meal. The SDA response was similar in M2 and RM, suggesting that the GI tract was fully reorganized after the first feeding. The SDA cost was equal in PF and RM, implying that the change in state per se (dormant to arousal) triggers the regrowth of GI, independently of feeding. Fasting tegus at M1 presented higher triglyceride and lower uric acid levels than fed tegus, indicating that fasting is mainly sustained by fat storage. Our results show that seasonal fasting imposes an extra digestion cost to tegus following their next feeding, which is fully paid during their first digestion. This surplus cost, however, is negligible compared with the overall energetic savings from GI tract atrophy during the dormancy period.

KEY WORDS: Metabolism, Dormancy effects, Gastrointestinal tract, Atrophy, Specific dynamic action, Blood metabolites

INTRODUCTION

All animals require energy from food ingestion to perform their activities, such as routine metabolism, physical movement, growth and reproduction. When food resources are scarce, several animals are able to minimize their energy expenditure by physiological and morphological changes (Secor and Carey, 2016). Indeed, the lack of luminal nutrients decreases the intestinal mass and structure, and the change of state from gut atrophy to increased tissue growth upon refeeding is well documented among vertebrate groups (Carey and Cooke, 1991; Karasov and McWilliams, 2005; Starck, 1999, 2005). These reversible changes in animals' traits due to the variability in the environmental conditions, referred to as phenotypic flexibility (Piersma and Drent, 2003), are very often considered as crucial to the adaptation and survival of organisms in the face of environmental challenges (Barnes et al., 2010). In this regard, the digestive system seems to be one of the most responsive to environmental changes (Starck, 2003) and, therefore, it has been proposed that the gut flexibility could be treated as an attribute with fundamental relevance for overall animal performance (Naya et al., 2008), particularly for those that experience natural variation in food/fasting (e.g. Carey, 1990, 1995, 2005; Carey et al., 2003; Chernetsov, 2012; McWilliams and Karasov, 2014).

Many ectothermic vertebrates become seasonally inactive when fluctuations in environmental conditions temporally restrict their activities (Gregory, 1982; Pinder et al., 1992; Hailey and Loveridge, 1997; see Wang et al., 2006; McCue, 2010, and references therein). During these dormancy periods, animals usually retreat into refuges and experience a suite of behavioral, morphological and physiological adjustments. For example, dormant individuals often cease feeding and relax thermoregulation (Bicego et al., 2007; Withers and Cooper, 2010), which may lead to substantial decreases in body temperature, metabolism, ventilation and cardiovascular function (Secor et al., 1994; Piersma and Lindström, 1997; Holmberg et al., 2003; Wang et al., 2003; Andersen et al., 2005; Secor, 2005a; Bicego et al., 2007; McCue, 2007; da Silveira et al., 2013). Also, as feeding is halted, the maintenance of a fully functional gastrointestinal (GI) tract, known to have a high energetic cost (Cant et al., 1996; Secor, 2003, 2005b; Starck, 2005), would become energetically detrimental to dormant animals. Accordingly, metabolic depression in dormant fasting animals optimizes the use of accumulated energetic reserves until activity is resumed (Hume et al., 2002; Wang et al., 2006; Secor, 2005a,b; do Nascimento et al., 2016).

The Argentine black and white tegu lizard, *Salvator* (formerly *Tupinambis*) *merianae* (Duméril and Bibron 1839), is a large-bodied teiid widely distributed in South America (Vanzolini et al., 1980; Ávila-Pires, 1995). Tegus exhibit a marked seasonal cycle of activity, being active during spring and summer (Abe, 1995). By late autumn and winter, they retreat into burrows dug in the ground and aestivate for 3–5 months in a fasting dormant state (Abe, 1983, 1995; Andrade and Abe, 1999; Andrade et al., 2004; Klein et al., 2006). By early spring, tegus arouse from dormancy and resume feeding, which, in this species, occurs regularly throughout the active season (see Andrade et al., 2004). Dormant tegus exhibit a

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number of seasonal physiological adjustments, including decreases in resting metabolic rate and body temperature sensitivity (Abe, 1983, 1995; de Souza et al., 2004; Milsom et al., 2008), changes in thermoregulatory behavior (Milsom et al., 2008), and adjustments in the respiratory gas transport cascade (Andrade and Abe, 1999; Andrade et al., 2004) and in the use of metabolic substrates (de Souza et al., 2004). In addition, dormant tegus undergo morphological and structural changes in cardiac muscles (da Silveira et al., 2013) and decrease the GI tract mass (do Nascimento et al., 2016). Similar to other ectothermic animals, this decreased mass of the GI tract in tegu lizards may contribute to their seasonal metabolic depression; however, upon arousal, the digestion of their first post-dormancy meal may impose an increased energetic cost as a result of the required regrowth of GI tissues (see also Zaidan and Beaupre, 2003; Zeng et al., 2014). In the present study, we examined the effect of seasonal fasting during the dormancy of tegu lizards, *S. merianae*, on their postprandial metabolic response (specific dynamic action, SDA) to their first and second meals ingested after arousal from dormancy compared with a regular feeding episode during the active season. We anticipated that the first meal post-dormancy will elicit a greater SDA response than the second one, while the response of regularly fed active lizards will be of smaller magnitude. As the end of dormancy seems to trigger GI regrowth in tegus independently of food ingestion (do Nascimento et al., 2016), we also determined the SDA response of a group of lizards that was fasted for an extra period of 50 days after arousal from dormancy. If the transition from dormancy to activity indeed triggers the regrowth of the GI tract independently of food ingestion, we anticipated that the SDA response of this prolonged fasting group would be smaller than that of animals fed immediately after arousal from dormancy. Finally, we quantified changes in plasma content of triglycerides, glucose and uric acid before and after feeding to gain insight into possible shifts in the mobilization of metabolic substrates among experimental treatments.

MATERIALS AND METHODS

Animal supply and maintenance

We used 46 juvenile tegu lizards obtained from a scientific breeding facility (IBAMA 673766) at the University of São Paulo State (UNESP), in the city of Rio Claro, São Paulo state, Brazil. During the active season (September to May), animals were maintained in plastic boxes (32×21×10 cm), covered with wood shavings with free access to a plywood shelter and to a heating lamp under a 12 h:12 h light:dark photoperiod. Animals were fed three times a week with minced meat supplemented with vitamins and minerals. At the beginning of the dormancy period (around May), animals reduced the intake of food and spent less time basking. Thereafter, voluntary feeding progressively reduced until late June, when the tegus become completely inactive. At this point, food was no longer offered, the heat lamp was turned off, and the wood shavings were replaced with moist straw grass. By early September, tegus arouse from dormancy, photoperiod was re-established, and feeding was resumed according to the desired experimental protocol. Only animals judged to be healthy and that were not shedding their skin were used in the experiments.

Experimental protocol

SDA was determined by quantifying the rates of oxygen uptake ($\dot{V}_{O_2}$) before and after meal ingestion. Measurements were taken for the first (M1) and second (M2) meals post-dormancy, the first meal post-dormancy after an extra fasting period of 50 days (PF), and for a regular meal (RM) during the active season when animals were under a frequent feeding regime. Thus, the approximate fasting duration previous to the determination of the postprandial metabolic response was 3 months for M1, 15 days for M2 and RM, and 5 months for PF. Different individual lizards were measured under each experimental treatment.

Metabolic measurements started with fasting tegus being weighed and transferred into a hermetically sealed respirometric chamber (PVC, ∼500 ml) kept inside a climatic chamber (model 122FC, Eletrolab, São Paulo, SP, Brazil) set at 30°C (±1°C) under constant dark. Fasting $\dot{V}_{O_2}$ rates were measured for 2 days, with the first day aimed at acclimation and not used for later analysis. Then, measurements were paused and animals returned to their maintenance boxes overnight. The next morning, they were force-fed with ground lean beef (eye of the round, less than 5% fat) up to 10% of their own body mass and measurements resumed until $\dot{V}_{O_2}$ returned to fasting levels.

Blood samples for the determination of plasma metabolite content were harvested from terminally sampled fasting and fed tegus. Although the general maintenance and feeding protocol were identical to that described above, these animals were distinct from those used for metabolic measurements and were killed for the purpose of a different study. For all experimental groups, except RM, fasting animals were sampled after being kept at the experimental temperature of 30°C (±1°C) for 24 h. Fed animals were also kept at 30°C and sampled at 30 h post-feeding. Fasting and fed tegus in the RM group, which had free access to a heating lamp, were sampled during the daytime when their body temperature typically varied from 30 to 37°C. All animals were killed by decapitation following procedures approved by the Ethics Committee on Animal Use (CEUA) of the University of São Paulo State (UNESP), municipality of Rio Claro, São Paulo State, Brazil (protocol numbers: 0108, 17 January 2011; and 5143, 16 July 2012).

Respirometry

$\dot{V}_{O_2}$ was measured via an intermittently closed respirometry system. In this system, an air pump connected to a mass flow meter (SS4 Subsampler, Sable Systems International, Las Vegas, NV, USA) generated a constant airflow (150 ml min^{-1}) directed to a multiple flow controller (Multiplexer v2.0, Sable Systems) set for ventilating the respirometric chambers with external air for 60 min (open phase), while measuring the seventh chamber (closed phase) for 10 min. During the open phase, ambient air was pumped through the system at a flow rate of 150 ml min^{-1} in order to renew the air of the respirometric chambers. During the closed phase, the air from the animal chamber was circulated, in a closed loop, through an oxygen analyzer (model PA-1, Sable Systems International) at the same flow rate. Changes in oxygen concentration were monitored, via an A/D interface (UI-2 Data Acquisition Interface, Sable Systems International), at a rate of 1 Hz and were used to calculate $\dot{V}_{O_2}$ (see Klein et al., 2006). Because our system allowed for alternation between the open and closed phases among the seven respirometric chambers, we were able to attain a metabolic measurement for each individual animal every 70 min. The whole apparatus was controlled by data acquisition software (Datacan V, Sable Systems International), which was also used for data analysis.

Blood metabolites

Immediately after decapitation, the extravasated blood from the carotids was collected with an insulin syringe and immediately centrifuged at 13,000 rpm for 5 min. Afterwards, blood plasma was harvested from the supernatant fraction and stored at −20°C until

analysis. Blood metabolite concentrations were determined for plasma samples by colorimetric assays using commercially available analytical kits for triglycerides (TG Kit: cat. no. 06800, Laborlab, Guarulhos, SP, Brazil), glucose (GLUC Kit: cat. no. 02200, Laborlab) and uric acid (URIC Kit, Anvisa no. 100.970.10141, Laborclin Products for Laboratory, Pinhais, PR, Brazil). Triglycerides, glucose and uric acid were measured at 505, 505 and 520 nm, respectively, using spectrophotometry (model 600 Plus, Femto, São Paulo, SP, Brazil). Procedures and the appropriate use of standards followed the instructions provided with the determination kits.

Treatment and data analysis

We used SigmaPlot 12.5 (Systat Software, San Jose, CA, USA) for statistical comparison and to generate box-and-whisker plots. From the oxygen uptake measurements, we quantified the following variables: resting metabolic rate (RMR), as the average $\dot{V}_{O_2}$ for the 24 h immediately before meal ingestion; maximum $\dot{V}_{O_2}$ attained during digestion ($\dot{V}_{O_2,peak}$); time to reach $\dot{V}_{O_2,peak}$ (T_{peak}); SDA factorial scope, as the ratio between $\dot{V}_{O_2,peak}$ and RMR; and SDA duration, as the time elapsed from meal ingestion until the postprandial $\dot{V}_{O_2}$ returned to RMR levels. All these variables were extracted after a best-fit curve procedure was adjusted to our data using sub-routines of the software TableCurve 2D (Systat Software). Typically, these regressions provided r^2 values greater than 0.9.

SDA energetic cost was estimated as the sum of the oxygen consumed above the RMR level for the duration of the postprandial metabolic response, assuming that 1 ml of oxygen used in aerobic metabolism resulted in the expenditure of 0.0198 kJ (Gessman and Nagy, 1988). The energy content of the meal (ME) was estimated from Boback et al. (2007) and the SDA coefficient (%SDA) was calculated as the amount of energy spent for meal digestion as a percentage of ME.

Parameters associated with the SDA response were compared among groups by a one-way ANOVA, followed, whenever necessary, by a pairwise multiple comparison procedure (Holm–Sidak method). Whenever the data failed to attend the premise of normality and/or homoscedasticity of variances, they were logarithmized and the test was repeated. If the data still did not meet the necessary assumptions, the non-parametric test ANOVA on ranks was used, followed, if necessary, by the Student–Newman–Keuls test.

Blood metabolite parameters were compared by a two-way ANOVA, with treatment (M1, M2 and PF) and state (fasting versus fed) as factors. Whenever differences among groups were detected, we applied a *post hoc* Tukey's test to access the origin of the variation. All data are presented as means±s.e.m. Significance was attributed at the level of $P<0.05$.

RESULTS

Postprandial metabolic response

Tegus body mass did not differ among M1, M2 and PF groups (mean 31.27 g; Table 1; $P>0.1$; $F_{3,20}=26.624$). However, for the RM group, which received food regularly for approximately four consecutive months, body mass was statistically greater than that for all other groups (77.97 g; Table 1; $P<0.001$). Nevertheless, analyses of covariance indicated that RMR was not affected by body mass among groups ($P>0.01$, $F_{3,20}=0.704$). The meal energy content (ME) was the same among all groups (Table 1).

RMR rates did not differ among groups (Table 1; $P>0.1$, $F_{3,20}=1.701$). Maximum rates of oxygen uptake ($\dot{V}_{O_2,peak}$) during digestion occurred at approximately 23 h post-feeding in all groups (Fig. 1, Table 1; $P>0.1$, $F_{3,20}=1.904$). $\dot{V}_{O_2,peak}$ was higher in the M2 than in the PF group ($P<0.05$), but not in comparison to the other groups ($P>0.1$, $F_{3,20}=3.121$). The SDA factorial scope reached values near 2.5-fold above RMR in all treatments (Table 1; $P>0.05$, $F_{3,20}=1.559$). Digestion duration was significantly longer for the M1 group ($P<0.001$) compared with that for all other groups. Also, the M2 group digested their meal faster than the PF group (Table 1; $P<0.05$, $F_{3,20}=11.471$). The extra energy spent during digestion (i.e. SDA) for the M1 group was higher than that for all other groups (Table 1; $P<0.001$, $F_{3,20}=15.311$) as was the respective SDA coefficient (Table 1; $P<0.001$, $F_{3,20}=15.311$).

Blood metabolites

Tegus sampled before and after feeding for blood metabolites did not differ in body mass (overall mean 26.92±1.5 g; Table 2; $P>0.1$, $F_{5,16}=1.146$). For the M1 group only, feeding lowered the plasma concentration of triglycerides ($P<0.001$, $F_{2,16}=13.232$) and elevated that of uric acid (Fig. 2, Table 2; $P<0.05$, $F_{2,16}=0.896$). For all the other groups, triglyceride and uric acid concentration before and after the meal did not differ ($P>0.1$ in all cases).

Triglyceride plasma concentration in the M1 group was higher than that in the M2 and PF groups ($P<0.001$ in both cases) during fasting, but this difference disappeared after feeding (Fig. 2A, Table 2; $P>0.1$ in all cases). Uric acid plasma concentration during fasting was higher in the PF group than in the M1 group ($P<0.05$). After feeding, there was no difference in uric acid plasma concentration among groups (Fig. 2C, Table 2; $P>0.1$ in all

Table 1. Metabolic and energetic parameters associated with the SDA of *Salvator merianae* fed on meals of 10% of their body mass at 30°C

	M1	M2	PF	RM
Body mass (g)	28.93±3.24^{a}	28.75±3.07^{a}	36.16±4.77^{a}	77.97±6.41^{b}
RMR (ml O_2 kg^{-1} h^{-1})	109.97±11.6^{a}	119.27±6.2^{a}	94.05±8.4^{a}	112.25±4.4^{a}
$\dot{V}_{O_2,peak}$ (ml O_2 kg^{-1} h^{-1})	295.58±24.8^{a,b}	280.34±7.27^{a}	237.74±8.30^{b}	260.62±7.91^{a,b}
T_{peak} (h)	23.06±4.53^{a}	17.29±3.85^{a}	30.01±3.97^{a}	21.69±2.69^{a}
Scope ($\dot{V}_{O_2,peak}$/RMR)	2.72±0.08^{a}	2.38±0.15^{a}	2.62±0.24^{a}	2.32±0.04^{a}
SDA duration (h)	138.64±8.44^{a}	69.32±3.73^{b}	102.97±13.69^{c}	83.22±6.57^{b,c}
SDA (kJ kg^{-1})	290.87±33.68^{a}	126.73±9.62^{b}	162.61±11.99^{b}	148.78±9.88^{b}
ME (kJ kg^{-1})	659.00	659.00	659.00	659.00
SDA coefficient (%)	44.14±5.11^{a}	19.23±1.46^{b}	24.68±1.82^{b}	22.58±1.50^{b}

M1, first meal after arousal; M2, second meal after arousal; PF, first meal after a 50 day fasting period following arousal; RM, regular meal; RMR, resting metabolic rate; $\dot{V}_{O_2,peak}$, maximum oxygen uptake during digestion; T_{peak}, time taken to reach $\dot{V}_{O_2,peak}$; Scope, the factorial scope of $\dot{V}_{O_2,peak}$ in relation to the RMR; SDA, SDA energetic cost; SDA duration, the period from meal ingestion to when postprandial $\dot{V}_{O_2}$ returns to RMR levels; ME, meal energy content; SDA coefficient, percentage of the energy expended for SDA in relation to the energy intake (ME). Values are means±s.e.m. $N=6$ for all groups.
P-values significant at the 0.05 level are indicated by different letters.

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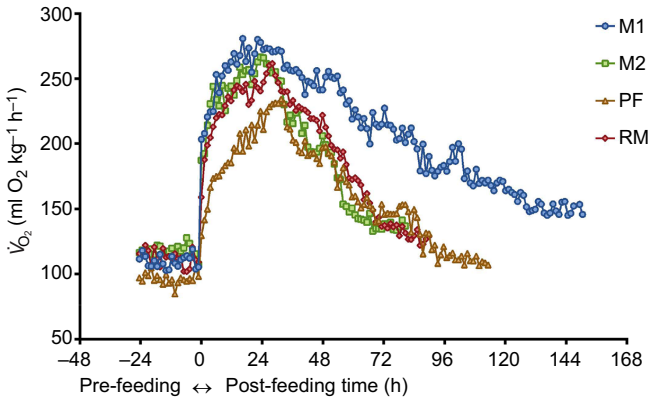

Fig. 1. Time course variation of postprandial metabolic response ($\dot{V}_{O_2}$) of *Salvator merianae* groups. Groups were fed a meal equal to 10% of body mass at 30°C. M1, first meal after arousal (blue circles); M2, second meal after arousal (green squares); PF, first meal after a 50 day fasting period following arousal (orange triangles); and RM, regular meal (red diamonds). Time zero indicates the moment of meal ingestion. The interval between two consecutive points is 70 min, and each point represents the mean $\dot{V}_{O_2}$ for N=6 tegus for all groups. Standard error bars were omitted for clarity of data.

cases). Glucose levels showed no differences among groups, either during fasting or after feeding (Fig. 2B, Table 2; P>0.1 in all cases, $F_{2,16}$=1.941).

DISCUSSION

The effectiveness of physiological mechanisms adopted by animals during fasting in cold seasons is pivotal to their survival and lifetime fitness (Boutilier et al., 1999). Animal aestivation is usually followed (or even preceded) by phenotypic adjustments at different organizational levels, which may be fundamental to sustaining life-maintaining performance. In this sense, the digestive system is well known to be highly responsive to environmental changes (Starck, 2003). For example, several vertebrate species, tegu lizards included, decrease their digestive performance during overwintering as an adaptive strategy to reduce energy expenditure throughout this period. In contrast, when animals become active in the next season, it has been proposed that the first bout of feeding is likely more expensive in terms of digestion costs because of the reorganization of intestinal organs. In fact, little is known about the effects of refeeding and how the animals recover from long fasting events (McCue et al., 2017). Here, we found that the first feeding episode after fasting dormancy in *S. merianae* is accompanied by additional costs of digestion.

Dormancy effects on postprandial metabolism

The postprandial metabolic response of *S. merianae*, as is usual for lizards (Secor, 2001), was characterized by a steep increase in $\dot{V}_{O_2}$ soon after meal ingestion that reached a peak between the first and the second day after feeding. Afterwards, there was a gradual decrease in metabolism until its return to the RMR levels within 3–6 days. This general pattern of response was not affected by experimental treatment and was very similar to that observed for adult tegus (Klein et al., 2006). However, juvenile lizards during the active season (present study) exhibited a smaller factorial increment in postprandial $\dot{V}_{O_2}$ and a shorter digestion duration than adults at the same period (see Klein et al., 2006). As a result, juvenile tegus seem to spend less energy on meal digestion (SDA coefficient ~20%; present study) compared with adults (SDA coefficient ~36%; Klein et al., 2006). Such a possibility, however, is confounded by differences in meal type and size.

Seasonally dormant fasting animals often reduce the mass of GI organs, decreasing the energy that, otherwise, would be spent in the maintenance of a high-cost system during a period of inactivity (Cant et al., 1996; Ferraris and Carey, 2000; Secor, 2001, 2005a,b; Secor et al., 2012; Zeng et al., 2014). This strategy is generally accepted as important in assisting the global metabolic depression observed in seasonally inactive animals (McCue, 2010; Secor and Carey, 2016), but imposes recovery costs as animals resume their activities. Indeed, in the case of tegu lizards, our results showed that they spent approximately twice as much energy digesting their first meal post-dormancy compared with the second and subsequent feeding events. As dormant tegus also experience a reduced GI mass during dormancy (do Nascimento et al., 2016), this difference can be safely assumed to reflect the extra costs of recovering the GI tract from a quiescent state. Considered on a longer temporal scale, this additional cost is overly compensated by the economy in energy expenditure yielded by GI tract atrophy during inactivity. Dormant tegus at their normal dormancy temperature (i.e. ~17°C; Abe, 1995) depress metabolic rate by 80% in relation to that of early autumn active animals at 25°C (de Souza et al., 2004). On this basis, we conservatively estimate that a 100 g tegu would conserve approximately 425.8 kJ over a dormancy period extending for 100 days, which is quite typical for this species (see Andrade et al., 2004). Therefore, the extra energy spent by our tegus on the first meal post-dormancy (compared with subsequent feeding events) represents less than 4% (16.4 kJ) of the total energy preserved during dormancy.

Even though our estimate does not account for the contribution of the atrophy of other organ systems (besides the GI tract) to the seasonal organismal depression in energy metabolism, it seems clear that reduced GI tract mass during seasonal fasting advantageously compensates for the extra costs of its regrowth upon the ingestion of the first meal post-dormancy. Similar energetic considerations have already been proposed for other vertebrates, such as amphibians (Cramp and Franklin, 2003; Naya et al., 2009b; Bizjak-Mali et al., 2013; Tamaoki et al., 2016), mammals (Piersma and Lindström, 1997; Starck, 1999; Carey, 2005) and other reptiles (Gist, 1972; Holmberg et al., 2003; Christel et al., 2007; Naya et al., 2008, 2009a);

Table 2. Plasma metabolite content of tegu lizards, before and after meal ingestion, for the first (M1) and the second (M2) meals post-dormancy, and after a prolonged fasting (PF) after arousal from dormancy

	M1		M2		PF	
	Fasting	Fed	Fasting	Fed	Fasting	Fed
Body mass (g)	28.38±3.97^{a}	25.24±2.39^{a}	27.22±4.45^{a}	32.81±3.92^{a}	21.66±2.31^{a}	24.21±2.02^{a}
Triglycerides (mmol l^{-1})	27.76±3.29^{a,*}	11.99±0.57^{b}	3.13±1.38^{c}	7.22±2.50^{b}	6.41±1.58^{c}	6.34±1.52^{b}
Glucose (mmol l^{-1})	10.89±0.90^{a,b}	7.46±0.60^{b}	11.23±1.23^{a}	10.64±1.46^{b}	9.41±2.13^{a}	12.41±3.19^{b}
Uric acid (mmol l^{-1})	0.14±0.02^{a,*}	0.32±0.03^{b}	0.26±0.04^{a,c}	0.30±0.01^{b}	0.36±0.13^{c}	0.45±0.06^{b}

Values are shown as means±s.e.m. N=4 for fasting and fed animals from groups M1 and M2; N=3 for PF fasting and fed groups. P-values significant at the 0.05 level are indicated by different letters (among groups) and by an asterisk (between fasting and fed animals from the same group).

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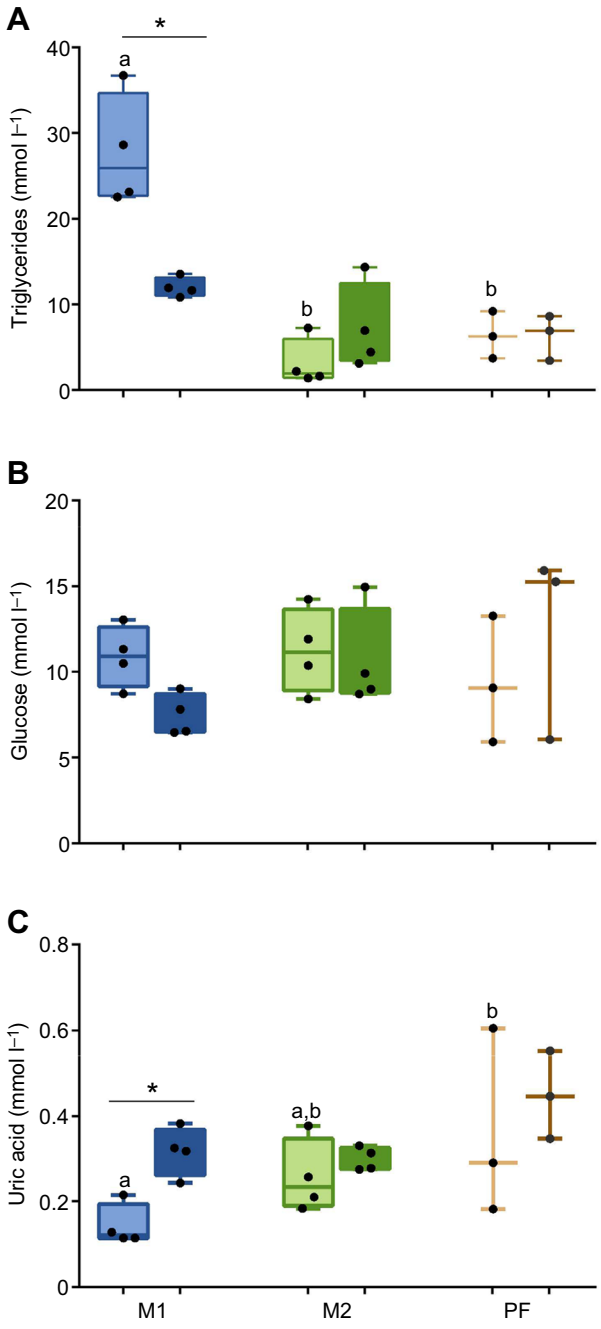

Fig. 2. Concentration of blood metabolites in plasma of fasting and fed *S. merianae* for M1, M2 and PF groups. (A) Triglycerides, (B) glucose and (C) uric acid. Light (on the left) and dark (on the right) colors indicate fasting and fed tegus, respectively. Data are shown as box plots. The lower and the upper extremities of the box indicate the 25% and 75% percentiles; the central line is the median; and whiskers indicate the full data range. Black dots indicate individual data points. *N*=4 for fasting and fed animals from the M1 and M2 groups; *N*=3 for fasting and fed animals from the PF group. *P*-values significant at the 0.05 level are indicated by different letters (among groups) and by an asterisk (between fasting and fed animals from the same group).

therefore, it may well represent a common pattern of response for animals engaging in seasonal fasting (Starck, 2003; Wang et al., 2006; McCue, 2010; Secor and Carey, 2016). Finally, the same rationale for energy trade-off between increase and reduction of the GI tract mass in response to feeding and fasting, respectively, is present in some snake species that feed infrequently (see Secor and Diamond, 2000; Starck and Beese, 2001, 2002; Zaidan and Beaupre, 2003; Andrade et al., 2005; Wang et al., 2006; McCue et al., 2012).

The greater cost of the first meal post-dormancy in tegu lizards compared with subsequent feeding events can be entirely ascribed to the increased duration of meal digestion. Indeed, while postprandial $\dot{V}_{O_2}$ rates (including $\dot{V}_{O_2,peak}$) were similar among treatments, digestion duration of the first meal post-dormancy was 2-fold longer than that of the second meal. These results agree with studies in fishes, in which fasting prolongation increased digestion duration but not the magnitude of the metabolic increment (Fu et al., 2011; Zeng et al., 2014). As fasting is prolonged, different physiological systems, besides the GI tract, are likely to be depressed, including those involved with sustaining metabolic processes. Thus, when feeding is resumed, the greater metabolic response expected to be required by the processes of GI tract regrowth might be constrained by a metabolic ceiling. In this scenario, we anticipate that these processes would persist in time, prolonging SDA duration (see Zaidan and Beaupre, 2003). Our findings of a significant effect of fasting on the SDA coefficient run contrary to observations made by Overgaard et al. (2002) in fasting Burmese pythons. However, as warned by Zaidan and Beaupre (2003), their maximum fast length was only 60 days, in comparison with our 90–150 day fast. Additionally, our tegus fasted at relatively low temperatures in association with winter inactivity, which may involve the atrophy of other organ systems in addition to the GI tract compared with shorter fast lengths during active summer periods (Zaidan and Beaupre, 2003; e.g. da Silveira et al., 2013). In this regard, for some snakes, and probably for all Squamata, the energetic expense of morphological regrowth of the gut may be low for short fasting periods (Starck and Beese, 2001; Overgaard et al., 2002). In contrast, prolonged fast lengths, such as those experienced seasonally, are likely to entail a higher degree of GI tract regrowth in response to the subsequent feeding event (Zaidan and Beaupre, 2003).

Our results clearly indicate that GI regrowth from the depressed state induced by seasonal fasting can be entirely accomplished upon the ingestion of the first post-dormancy meal, although refeeding is not required for remodeling of the gut (see below). Accordingly, postprandial metabolic rate, SDA duration and SDA coefficient did not differ between the second and subsequent feeding events. This agrees with the feeding biology of *S. merianae*, which, soon after the end of the dormancy period, resume their activities and start to forage actively for food on a daily basis (Abe, 1983, 1995). Meal ingestion in tegu lizards is believed to occur frequently (Winck et al., 2011), with captive animals accepting food every other day (Lopes and Abe, 1999; Manes et al., 2007). In this context, once the GI tract has recovered from its quiescent seasonal fasting-induced state upon the first feeding event, the frequent delivery of food would justify its fully functional maintenance for the duration of the activity period.

The SDA response of lizards subjected to the extra fasting period after the end of the seasonal dormancy was intermediate between the responses exhibited by lizards fed their first meal soon after dormancy and those fed a second time or regularly. This pattern holds true for postprandial metabolic rate, SDA duration and SDA coefficient and indicates that, at least partly, GI tract regrowth may occur independently of food intake, probably triggered by the transition from a dormant to an active state. A similar response has been documented in the striped burrowing frog, *Ranoidea alboguttata*, in which the regrowth of the GI tract happens not only by re-feeding but also by arousal from aestivation (Cramp and

Franklin, 2005). Possibly, while some processes associated with digestive function are regulated endogenously, others are triggered by exogenous factors (see Naya et al., 2011). Finally, juvenile tegu lizards gradually increase their metabolism immediately after arousal from dormancy even if held under fasting conditions (de Souza et al., 2004), and this response, we suspect, may at least partially reflect the costs of GI tract regrowth.

Substrate utilization

Many reptiles experiencing extended fasting or subjected to seasonal dormancy rely primarily on lipid metabolism from fat stores (de Souza et al., 2004; El-Deib, 2005; Haddad, 2007; McCue, 2007; 2008; Oliveira et al., 2013; see also Price, 2017). Indeed, lipid stores are significantly depleted during the dormancy of tegu lizards, and this response is accompanied by increases in enzymatic activity involved with β-oxidation metabolism in the liver, by the elevation of circulating levels of ketone bodies and by the reduction of non-esterified fatty acid levels (de Souza et al., 2004; Haddad, 2007). In agreement with these observations, we found that the levels of triglycerides were elevated in fasting tegus arousing from seasonal dormancy in comparison to that of animals fed the first meal after arousal and to both fasting lizards that have already fed once and lizards under a prolonged fasting (i.e. unfed tegus from groups M2 and PF, respectively). For tegu lizards subjected to the extra fasting period after arousal from dormancy, we found low levels of triglycerides and high concentrations of uric acid under fasting, although these conclusions should be interpreted with caution as estimates were based on only three individuals in the PF group. As metabolic rate is known to gradually increase after the end of dormancy even under fasting (de Souza et al., 2004), the prolonged post-dormancy fasting we imposed on this particular experimental group may have led to the mobilization of constitutive proteins, resulting in an elevation of plasma uric acid concentration and a decrease in triglyceride levels. Independent of treatment, feeding tended to be accompanied by an increase in circulating uric acid levels, probably related to a peak of protein oxidation occurring 24 h after meal ingestion, as seen in pythons (McCue et al., 2015). Differences in uric acid levels reached statistical significance only for newly aroused animals. These results were probably due to the fact that fasting M2 tegus still had moderate levels of uric acid from the first feeding event and fasting animals from the PF group, as discussed above, were probably using energy from muscle stores. Glucose homeostasis was kept relatively constant among different treatments and regardless of feeding. This result is congruent with the findings of de Souza et al. (2004), in which circulating glucose was almost completely restored in arousing tegus, even after a 60% drop in glucose levels during dormancy.

Animals that engage in seasonal dormancy commonly exhibit an orchestrated chain of behavioral, morphological and functional adjustments often considered to be pivotal for minimizing metabolic expenditure and defending endogenous energy stores. Less appreciated is the fact that some of these restructuring processes will impose costs upon their re-activation as the animals resume their activities. At least in terms of the physiological and energetics parameters for feeding, our results shows that: (1) these costs can be significant enough to be detectable; (2) they can be entirely covered within a short time period after dormancy ends; (3) some restructuring processes may be partially triggered not by a specific stimulant (e.g. meal ingestion) but by a change in state; and (4) any GI tract regrowth costs are likely to be compensated for by the energy saved by the atrophy processes during the dormancy period. With regard to this last point, seasonal metabolic depression is suggested as an ecologically advantageous strategy for many animal species (see also Secor and Carey, 2016).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: R.S.B.G., M.R.S., D.V.A.; Methodology: R.S.B.G., M.R.S., M.N.G.-F., B.F.G.; Validation: B.F.G., D.V.A.; Formal analysis: R.S.B.G.; Investigation: R.S.B.G., M.R.S., M.N.G.-F., B.F.G.; Data curation: M.N.G.-F., B.F.G.; Writing - original draft: R.S.B.G., M.R.S.; Writing - review & editing: R.S.B.G., D.V.A.; Visualization: R.S.B.G., M.R.S., A.S.A., D.V.A.; Supervision: D.V.A.; Project administration: R.S.B.G., D.V.A.; Funding acquisition: A.S.A., D.V.A.

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References

Abe, A. S. (1983). Observations on dormancy in tegu lizard, *Tupinambis teguixin* (Reptilia, Teiidae). *Naturalia* **8**, 135-139.

Abe, A. S. (1995). Estivation in South American amphibians and reptiles. *Braz. J. Med. Biol. Res.* **28**, 1241-1247.

Andersen, J. B., Rourke, B. C., Caiozzo, V. J., Bennett, A. F. and Hicks, J. W. (2005). Postprandial cardiac hypertrophy in pythons. *Nature* **434**, 37-38.

Andrade, D. V. and Abe, A. S. (1999). Gas exchange and ventilation during dormancy in the tegu lizard *Tupinambis merianae*. *J. Exp. Biol.* **202**, 3677-3685.

Andrade, D. V., Sanders, C., Milsom, W. K. and Abe, A. S. (2004). Overwintering in Tegu Lizards. In *Life in the Cold: Evolution, Mechanisms, Adaptation, and Application* (ed. B. M. Barnes and H. V. Carey), pp. 339-348. Fairbanks: University of Alaska.

Andrade, D. V., Cruz-Neto, A. P., Abe, A. S. and Wang, T. (2005). Specific dynamic action in ectothermic vertebrates: a review of determinants of postprandial metabolic response in fishes, amphibians and reptiles. In *Physiological and Ecological Adaptations to Feeding in Vertebrates* (ed. J. M. Starck and T. Wang), pp. 306-324. Enfield, NH: Science Publishers.

Ávila-Pires, T. C. (1995). *Lizards of Brazilian Amazonia (Reptilia: Squamata)*. Leiden: Zoologische Verhandelingen.

Barnes, B., Gordon, M., Sato, K. and Hoppeler, H. (2010). Survival in a changing world. *J. Exp. Biol.* **213**, 853.

Bicego, K. C., Barros, R. C. H. and Branco, L. G. S. (2007). Physiology of temperature regulation: comparative aspects. *Comp. Biochem. Physiol.* **147A**, 616-639.

Bizjak-Mali, L., Sepčić, K. and Bulog, B. (2013). Long-term starvation in cave salamander effects on liver ultrastructure and energy reserve mobilization. *J. Morphol.* **274**, 887-900.

Boback, S. M., Cox, C. L., Ott, B. D., Carmody, R., Wrangham, R. W. and Secor, S. M. (2007). Cooking and grinding reduces the cost of meat digestion. *Comp. Biochem. Physiol.* **148A**, 651-656.

Boutilier, R. G., Tattersall, G. J. and Donohoe, P. H. (1999). Metabolic consequences of behavioural hypothermia and oxygen detection in submerged overwintering frogs. *Zoology* **102**, 111-119.

Cant, J. P., McBride, B. W. and Croom, W. J., Jr. (1996). The regulation of intestinal metabolism and its impact on whole animal energetics. *J. Anim. Sci.* **74**, 2541-2553.

Carey, H. V. (1990). Seasonal changes in mucosal structure and function in ground squirrel intestine. *Am. J. Physiol.* **259**, R385-R392.

Carey, H. V. (1995). Gut feelings about hibernation. *News Physiol. Sci.* **10**, 55-61.

Carey, H. V. (2005). Gastrointestinal responses to fasting in mammals: lessons from hibernators. In *Physiological and Ecological Adaptations to Feeding in Vertebrates* (ed. J. M. Starck and T. Wang), pp. 229-254. Enfield, NH: Science Publishers.

Carey, H. V. and Cooke, H. J. (1991). Effect of hibernation and jejunal bypass on mucosal structure and function. *Am. J. Physiol.* **261**, G37-G44.

Carey, H. V., Andrews, M. T. and Martin, S. L. (2003). Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. *Physiol. Rev.* **83**, 1153-1181.
Chernetsov, N. (2012). Fuel deposition rate and energy efficiency of stopovers. In *Passerine Migration* (ed. N. Chernetsov), pp. 37-57. Berlin: Springer.
Christel, C. M., DeNardo, D. F. and Secor, S. M. (2007). Metabolic and digestive response to food ingestion in a binge-feeding lizard, the Gila monster (*Heloderma suspectum*). *J. Exp. Biol.* **210**, 3430-3439.
Cramp, R. L. and Franklin, C. E. (2003). Is re-feeding efficiency compromised by prolonged starvation during aestivation in the green striped burrowing frog, *Cyclorana alboguttata*. *J. Exp. Zool.* **300A**, 126-132.
Cramp, R. L. and Franklin, C. E. (2005). Arousal and re-feeding rapidly restores digestive tract morphology following aestivation in green-striped burrowing frogs. *Comp. Biochem. Physiol.* **142A**, 451-460.
da Silveira, L. C., do Nascimento, L. F. R., Colquhoun, A., Abe, A. S. and de Souza, S. C. R. (2013). Cardiac hypertrophy and structural and metabolic remodeling related to seasonal dormancy in the first annual cycle in tegu lizards. *Comp. Biochem. Physiol.* **165A**, 371-380.
de Souza, S. C. R., Carvalho, J. E., Abe, A. S., Bicudo, J. E. P. W. and Bianconcini, M. S. C. (2004). Seasonal metabolic depression, substrate utilization and changes in scaling patterns during the first year cycle of tegu lizards (*Tupinambis merianae*). *J. Exp. Biol.* **207**, 307-318.
do Nascimento, L. F. R., da Silveira, L. C., Nisembaum, L. G., Colquhoun, A., Abe, A. S., Mandarim-de-Lacerda, C. A. and de Souza, S. C. R. (2016). 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*). *Comp. Biochem. Physiol.* **195A**, 55-64.
El-Deib, S. (2005). Lipid changes in blood serum and tissues of the Egyptian cobra "*Naja haje haje*" during the hibernation cycle. *J. Therm. Biol.* **30**, 51-59.
Ferraris, R. P. and Carey, H. V. (2000). Intestinal transport during fasting and malnutrition. *Annu. Rev. Nutr.* **20**, 195-219.
Fu, S. J., Pang, X., Cao, Z. D., Peng, J. L. and Yan, G. J. (2011). The effects of fasting on the metabolic interaction between digestion and locomotion in juvenile southern catfish (*Silurus meridionalis*). *Comp. Biochem. Physiol.* **158A**, 498-505.
Gessman, J. A. and Nagy, K. A. (1988). Energy metabolism: errors in gas-exchange conversion factors. *Physiol. Zool.* **6**, 507-513.
Gist, D. H. (1972). The effects of starvation and refeeding on carbohydrate and lipid reserves of *Anolis carolinensis*. *Comp. Biochem. Physiol.* **43**, 771-780.
Gregory, P. T. (1982). Reptilian hibernation. In *Biology of the Reptilia* (ed. C. Gans and F. H. Pough), pp. 53-154. Physiology D. Physiological Ecology, Vol. 13. London: Academic Press.
Haddad, L. S. (2007). O papel dos lipídios na reorganização metabólica associada à dormência sazonal nos lagartos teiú, *Tupinambis merianae* (Sauria, Teiidae). *PhD Dissertation: Instituto de Biociências*, Universidade de São Paulo, São Paulo, Brazil.
Hailey, A. and Loveridge, J. P. (1997). Metabolic depression during dormancy in the African tortoise *Kinixys spekii*. *Can. J. Zool.* **75**, 1328-1335.
Holmberg, A., Kaim, J., Persson, A., Jensen, J., Wang, T. and Holmgren, S. (2003). Effects of digestive status on the reptilian gut. *Comp. Biochem. Physiol.* **133A**, 499-518.
Hume, D., Beiglböck, C., Ruf, T., Frey-Roos, F., Bruns, U. and Arnold, W. (2002). Seasonal changes in morphology and function of the gastrointestinal tract of free-living alpine marmots (*Marmota marmota*). *J. Comp. Physiol.* **172B**, 197-207.
Karasov, W. H. and McWilliams, S. R. (2005). Digestive constraints in mammalian and avian ecology. In *Physiological and Ecological Adaptations to Feeding in Vertebrates* (ed. J. M. Starck and T. Wang), pp. 87-112. Enfield, NH: Science Publishers.
Klein, W., Perry, S. F., Abe, A. S. and Andrade, D. V. (2006). Metabolic response to feeding in *Tupinambis merianae*: circadian rhythm and a possible respiratory constraint. *Physiol. Biochem. Zool.* **79**, 593-601.
Lopes, H. R. and Abe, A. S. (1999). Biologia reprodutiva e comportamento do teiú, *Tupinambis merianae*, em cativero (Reptilia, Teiidae). In *Manejo y Conservación de Fauna Silvestre en América Latina* (ed. T. G. Fang, O. L. Montenegro and R. E. Bodmer), pp. 259-272. La Paz, Bolivia: Editorial Instituto e Ecología.
Manes, M. E., Parry, H. V. and Manlla, A. (2007). Growth and feeding conversion responses to different protein and energy supplies in *Tupinambis merianae* (Squamata: Teiidae). *App. Herpetol.* **4**, 279-285.
McCue, M. D. (2007). Snakes survive starvation by employing supply- and demand-side economic strategies. *Zooloogy* **110**, 318-327.
McCue, M. D. (2008). Fatty acid analyses may provide insight into the progression of starvation among squamate reptiles. *Comp. Biochem. Physiol.* **151A**, 239-246.
McCue, M. D. (2010). Starvation physiology: reviewing the different strategies animals use to survive a common challenge. *Comp. Biochem. Physiol.* **156A**, 1-18.
McCue, M. D., Lillywhite, H. B. and Beaupre, S. J. (2012). Physiological responses to starvation in snakes: low energy specialists. In *Comparative Physiology of Fasting, Starvation, and Food Limitation* (ed. M. D. McCue), pp. 103-131. Berlin, Germany: Springer Verlag.
McCue, M. D., Passement, C. A. and Guzman, R. M. (2015). Digesting pythons quickly oxidize the proteins in their meals and save the lipids for later. *J. Exp. Biol.* **218**, 2089-2096.
McCue, M. D., Terblanche, J. S. and Benoit, J. B. (2017). Learning to starve: impacts of food limitation beyond the stress period. *J. Exp. Biol.* **220**, 4330-4338.
McWilliams, S. R. and Karasov, W. H. (2014). Spare capacity and phenotypic flexibility in the digestive system of a migratory bird: defining the limits of animal design. *Proc. R. Soc. B* **281**, 20140308.
Milsom, W. K., Andrade, D. V., Brito, S. P., Toledo, L. F., Wang, T. and Abe, A. S. (2008). Seasonal changes in daily metabolic patterns of tegu lizards (*Tupinambis merianae*) placed in the cold (17°C) and dark. *Physiol. Biochem. Zool.* **81**, 165-175.
Naya, D. E., Veloso, C. and Bozinovic, F. (2008). Physiological flexibility in the Andean lizard *Liolaemus bellii*: seasonal changes in energy acquisition, storage and expenditure. *J. Comp. Physiol.* **178B**, 1007-1015.
Naya, D. E., Veloso, C., Sabat, P. and Bozinovic, F. (2009a). Seasonal flexibility of organ mass and intestinal function for the Andean lizard *Liolaemus nigroviridis*. *J. Exp. Zool.* **311A**, 270-277.
Naya, D. E., Veloso, C., Sabat, P. and Bozinovic, F. (2009b). The effect of short- and long-term fasting on digestive and metabolic flexibility in the Andean toad, *Bufo spinulosus*. *J. Exp. Biol.* **212**, 2167-2175.
Naya, D. E., Veloso, C., Sabat, P. and Bozinovic, F. (2011). Physiological flexibility and climate change: the case of digestive function regulation in lizards. *Comp. Biochem. Physiol.* **159A**, 100-104.
Oliveira, A. S., Candioto, C. G., Santos, D. M. S., Pereira, J. G., Sousa, A. L. and Machado, C. R. (2013). Effects of fasting and refeeding on the metabolic functions of the turtle *Kinosternon scorpioides* (Linnaeus, 1766) raised in captivity. *Pesqui. Vet. Bras.* **33**, 1041-1044.
Overgaard, J., Andersen, J. B. and Wang, T. (2002). The effects of fasting duration on the metabolic response to feeding in *Python molurus*: an evaluation of the energetic costs associated with gastrointestinal growth and upregulation. *Physiol. Biochem. Zool.* **75**, 360-368.
Piersma, T. and Drent, J. (2003). Phenotypic flexibility and the evolution of organismal design. *Trends Ecol. Evol.* **18**, 228-233.
Piersma, T. and Lindström, A. (1997). Rapid reversible changes in organ size as a component of adaptive behaviour. *Trends Ecol. Evol.* **12**, 134-138.
Pinder, A. W., Storey, K. B. and Ultsch, G. R. (1992). Estivation and Hibernation. In *Environmental Physiology of the Amphibians* (ed. M. E. Feder and W. W. Burggren), pp. 250-276. Chicago: University of Chicago Press.
Price, E. R. (2017). The physiology of lipid storage and use in reptiles. *Biol. Rev.* **92**, 1406-1426.
Secor, S. M. (2001). Regulation of digestive performance: a proposed adaptive response. *Comp. Biochem. Physiol.* **128A**, 565-577.
Secor, S. M. (2003). Gastric function and its contribution to the postprandial metabolic response of the Burmese python, *Python molurus*. *J. Exp. Biol.* **206**, 1621-1630.
Secor, S. M. (2005a). Evolutionary and cellular mechanisms regulating intestinal performance of amphibians and reptiles. *Integr. Comp. Biol.* **45**, 282-294.
Secor, S. M. (2005b). Physiological responses to feeding, fasting and estivation for anurans. *J. Exp. Biol.* **208**, 2595-2609.
Secor, S. M. and Carey, H. V. (2016). Integrative physiology of fasting. *Compr. Physiol.* **6**, 773-825.
Secor, S. M. and Diamond, J. M. (2000). Evolution of regulatory responses to feeding in snakes. *Physiol. Biochem. Zool.* **73**, 123-141.
Secor, S. M., Stein, E. D. and Diamond, J. (1994). Rapid upregulation of snake intestine in response to feeding: a new model of intestinal adaptation. *Am. J. Physiol.* **266**, G695-G705.
Secor, S. M., Taylor, J. R. and Grosell, M. (2012). Selected regulation of gastrointestinal acid-base secretion and tissue metabolism for the diamondback water snake and Burmese python. *J. Exp. Biol.* **215**, 185-196.
Starck, J. M. (1999). Structural flexibility of the gastro-intestinal tract of vertebrates – implications for evolutionary morphology. *Zool. Anz.* **238**, 87-101.
Starck, J. M. (2003). Shaping up: how vertebrates adjust their digestive system to changing environmental conditions. *Anim. Biol.* **53**, 245-257.
Starck, J. M. (2005). Structural flexibility of the digestive system of tetrapods – patterns and process at the cellular and tissue level. In *Physiological and Ecological Adaptations to Feeding in Vertebrates* (ed. J. M. Starck and T. Wang), pp. 175-200. Enfield, NH: Science Publishers.
Starck, J. M. and Beese, K. (2001). Structural flexibility of the intestine of Burmese python in response to feeding. *J. Exp. Biol.* **204**, 325-335.
Starck, J. M. and Beese, K. (2002). Structural flexibility of the small intestine and liver of garter snakes in response to feeding and fasting. *J. Exp. Biol.* **205**, 1377-1388.
Tamaoki, K., Okada, R., Ishihara, A., Shiojiri, N., Mochizuki, K. and Goda, T. (2016). Morphological, biochemical, transcriptional and epigenetic responses to fasting and refeeding in intestine of *Xenopus laevis*. *Cell. Biosci.* **6**, 2.
Vanzolini, P. E., Ramos-Costa, A. M. M. and Vitt, L. J. (1980). *Répteis das caatingas*, 161 p. Rio de Janeiro, RJ: Academia Brasileira de Ciências.

Wang, T., Zaar, M., Arvedsen, S., Vedel-Smith, C. and Overgaard, J. (2003). Effects of temperature on the metabolic response to feeding in *Python molurus*. *Comp. Biochem. Physiol.* **133A**, 519-527.

Winck, G. R., Blanco, C. C. and Cechin, S. Z. (2011). Population ecology of *Tupinambis merianae* (Squamata, Teiidae): home-range, activity and space use. *Anim. Biol.* **61**, 493-510.

Withers, P. C. and Cooper, C. E. (2010). Metabolic depression: a historical perspective. In *Aestivation: Molecular and Physiological Aspects. Prog. Mol. Subcell. Biol.* (ed. C. A. Navas and J. E. Carvalho), pp. 1-23. Berlin: Springer.

Zaidan, F., III and Beaupre, S. J. (2003). Effects of body mass, meal size, fast length, and temperature on specific dynamic action in the timber rattlesnake (*Crotalus horridus*). *Physiol. Biochem. Zool.* **76**, 447-458.

Zeng, L.-Q., Fu, S.-J., Li, X.-M., Li, F.-J., Li, B., Cao, Z.-D. and Zhang, Y.-G. (2014). Physiological and morphological responses to the first bout of refeeding in southern catfish (*Silurus meridionalis*). *J. Comp. Physiol.* **184B**, 329-346.