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Seasonal metabolic depression, substrate utilisation and changes in scaling patterns during the first year cycle of tegu lizards (*Tupinambis merianae*)

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Summary

The tegus increase in body mass after hatching until early autumn, when the energy intake becomes gradually reduced. Resting rates of oxygen consumption in winter drop to 20% of the values in the active season ($\dot{V}_{O_2}$=0.0636 ml g^{-1} h^{-1}) and are nearly temperature insensitive over the range of 17–25°C (Q_{10}=1.55). During dormancy, plasma glucose levels are 60% lower than those in active animals, while total protein, total lipids and β-hydroxybutyrate are elevated by 24%, 43% and 113%, respectively. In addition, a significant depletion of liver carbohydrate (50%) and of fat deposited in the visceral fat bodies (24%) and in the tail (25%) and a slight loss of skeletal muscle protein (14%) were measured halfway through the inactive period. Otherwise, glycogen content is increased 4-fold in the brain and 2.3-fold in the heart of dormant lizards, declining by the onset of arousal. During early arousal, the young tegus are still anorexic, although $\dot{V}_{O_2}$ is significantly greater than winter rates. The fat deposits analysed are further reduced (62% and 45%, respectively) and there is a large decrease in tail muscle protein (50%) together with a significant increase in glycogen (2–3-fold) and an increase in plasma glucose (40%), which suggests a role for gluconeogenesis as a supplementary energy source in arousing animals. No change is detectable in citrate synthase activity, but β-hydroxyacyl CoA dehydrogenase activities are strongly affected by season, reaching a 3-fold and 5-fold increase in the liver tissue of winter and arousing animals, respectively, and becoming reduced by half in skeletal muscle and heart of winter animals compared with late fall or spring active individuals. From hatching to late autumn, the increase of the fat body mass relatively to body mass is disproportionate (b=1.44), and the mass exponent changes significantly to close to 1.0 during the fasting period. The concomitant shift in the $\dot{V}_{O_2}$ mass exponent in early autumn (b=0.75) to values significantly greater than 1.0 in late autumn and during winter dormancy indicates an allometric effect on the degree of metabolic depression related to the size of the fat stores and suggests greater energy conservation in the smaller young.

Key words: dormancy, fasting, oxygen consumption, metabolic depression, scaling, lipid, glycogen, HOAD, CS, lizard, tegu, *Tupinambis merianae.*

Introduction

Over the past two decades, the mechanisms of torpor have been investigated most amply in small mammals that hibernate in cold environments and involve modulation of the control of appetite and fattening, thermoregulation, blood clotting and other functions associated with seasonal fluctuations in temperature and food supply in normoxic environments (Boyer and Barnes, 1999; Guppy and Withers, 1999). The ability to transform a euthermic pattern into dormancy and torpor apparently constitutes a widespread feature in the endotherms, as suggested by growing evidence on mammals and birds from tropical and subtropical climates, which exhibit a state of torpor during the cool-dry season or during the daytime when food intake is halted (Bicudo, 1996; Ortmann et al., 1996; Schmid and Speakman, 2000).

In addition to these classical model systems, many lower vertebrates are known to enter a state of reduced metabolism over winter or under the combined influence of air temperature and relative humidity (Pinder et al., 1992; Abe, 1995; Guppy and Withers, 1999; Storey, 2002). The metabolic correlates of seasonality in these animals probably share some fundamental attributes with mammalian species that undergo a fasting period during the annual cycle. For example, a substantial proportion of the energy supply during the hypometabolic state may derive from lipid oxidation, and the carbohydrate stores

apparently constitute a limited potential for sustaining energy expenditure at the whole body level, as opposed to the adjustments seen under anoxic conditions (Storey, 1996). Remarkably, unlike the situation in mammal and bird species, a large reduction in aerobic metabolism can be achieved in the lower vertebrates without the predominant effect on the energy requirements associated with endothermy and a high thermoregulatory capacity. The regulatory mechanisms involved in metabolic depression appear to be coupled to an endogenous, circannual rhythm superimposed on the circadian pattern of thermal behaviour and locomotor activity, as shown in terrestrial reptiles (Hismiller and Heldmaier, 1988; Foà et al., 1994). These aspects have been studied in detail in only a few species, and considerable research remains to be done in order to identify the general principles of regulation acting on these events.

Another largely unstudied aspect of seasonal fasting and hypometabolism concerns how these profound changes develop in newly born animals of small body mass and limited capacity to store substrates. Since much evidence suggests that metabolic depression is endogenous in nature, it is important to investigate how early this phenomenon manifests in the life cycle. Furthermore, the ontogenetic adjustment of the relationship between energy use and storage capacity as an essential part of the make up of seasonal dormancy also may be predicted. We have begun to address these questions by examining the occurrence of metabolic depression and associated changes in energy metabolism during the first year cycle of tegu lizards (*Tupinambis merianae*). The tegu is widely distributed throughout South America (Avila-Pires, 1995) and occurs in large numbers in southeastern Brazil. Winter at this latitude is synchronized with the dry season, when insects and other food sources become scarce. An innate rhythm is apparent soon after hatching and, in the adult stage, the reproductive activities are concentrated in the spring months, with foraging and energy intake becoming gradually reduced during summer until the lizards enter a state of continuous inactivity, spending 4–5 months underground at temperatures around 17°C in the autumn and winter months (Abe, 1995). Abe (1995) verified a marked reduction in oxygen consumption during winter to 20–30% of the resting values in lizards with a mean mass of 1270 kg, consumption rates becoming nearly temperature insensitive over the range of 17–25°C. Anorexia is also a marked feature of the annual cycle in tegu lizards and develops from late summer through early autumn irrespective of the high temperatures and wide food availability at this time of the year (H. R. Lopes and A. Abe, unpublished observation).

The present study is concerned with the questions of how fasting and the magnitude of metabolic depression in young tegus compare with these events in their adult counterparts and whether such changes are influenced by body mass in the growing lizards. We also focus on the absence of reproduction at this early stage of development, emphasising correlative shifts in the levels of metabolites, substrate stores and enzymes over the first year cycle. The results support the notion of an endogenous rhythm acting *via* appetite control and energy metabolism in the tegu and suggest a shift in the balance between energy expenditure and body mass during the hypometabolic state, related to fat storage capacity and increased energy conservation in the smaller progeny.

Materials and methods

Animal supply and maintenance

Young tegu lizards (*Tupinambis merianae* Duméril and Bribon) were obtained from a population reared outdoors in large pens in Rio Claro, southeastern Brazil. About two months after eclosion in the summer, the animals were moved to the laboratory and used in the experiments during their first annual cycle. The lizards were kept indoors in 120 litre cages equipped with incandescent lights set on an 8 h:16 h L:D photo- and thermal-period, in addition to the sunlight diffusing from outside. The lizards could freely alternate between warming and cooling their bodies by climbing onto a small platform or hiding in a wooden shelter among the sheets of paper covering the box floor. The animals were separated into small groups according to size to minimise fighting and competition for food and were fed every two days on raw meat, egg and fruits, enriched with minerals, having continuous access to drinking water.

A noticeable change in daily activity was seen in the early autumn, when the time spent on 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, being routinely inspected and weighed every 15 days with minimal disturbance. By early spring, they expelled a dried pellet of uric acid and began moving outside the shelter, promptly reacting when mechanically stimulated. These changes were taken as an indication of arousal, and the animals were then returned to the previous photo- and thermal-period, with free access to drinking water. During the first days of arousal they were still anorexic, and a gradual increase in the time spent on thermoregulation and food intake took place over the following weeks.

Minimum and maximum air temperatures were recorded daily using a thermometer placed in the shelter area. The mean ranges for each seasonal period were: early autumn, 21–26°C; late autumn, 18–23°C; winter, 15–20°C; early spring, 20–26°C; late spring, 23–30°C.

Oxygen consumption measurements

Resting oxygen consumption rates ($\dot{V}_{O_2}$) were measured during the annual cycle on groups of tegus as follows: ‘autumn activity’, subdivided into early and late autumn to include lizards exhibiting behaviour characteristic of the onset of dormancy; ‘winter dormancy’, including fasting and totally inactive lizards over 50–60 winter days; ‘arousal’ for lizards emerging from dormancy (90–100 days from the first winter day), subdivided into rehydrated, unfed animals (48–96 h after arousal) and fed animals (~1 week after arousal); ‘spring

activity', including fully active lizards 30–40 days after arousal.

The metabolic chamber consisted of a well-sealed acrylic box, having an inlet and an outlet fixed to the top of the lid. The lizards were transferred into individual metabolic chambers by mid-afternoon and kept in the shade for at least two hours prior to experiments, during which time room air could diffuse freely through an aperture. The chamber lids were then screwed shut, providing an effective gas-tight seal, and $\dot{V}_{O_2}$ was measured. The initial and final fractional O_2 concentrations were obtained by taking two samples, each of 10 ml, with a gas-tight syringe, the first after closing the chamber and the second after 60 min, in two consecutive series of measurements. Prior to sampling, the air inside the chamber was gently mixed. The sample was carefully injected into an oxygen analyzer (S-3; Applied Eletrochemistry, Pittsburgh, PA, USA), drawn through silica gel and ascarite and subsequently into the oxygen sensor *via* a pump at low speed. Reproducibility of the procedure was verified by the repeated injection of samples of gas mixtures containing known O_2 concentrations: the error was always less than 1%. Oxygen concentrations below 19% were avoided during the experiments. $\dot{V}_{O_2}$ was calculated according to the equation developed by Hill (1972) and expressed as ml O_2 h^{-1} g^{-1} at standard temperature and pressure.

Measurements were performed from 19.30 h to 23.30 h. Previous experiments revealed two $\dot{V}_{O_2}$ peaks during the daily cycle of the tegu, one in the late morning and the other in the early afternoon, followed by a progressive drop in $\dot{V}_{O_2}$ to the minimum values at 02.00–04.00 h. In one experimental series, resting rates were measured in six different groups of animals at 25±1°C, except during dormancy when 17±1°C was used. Both temperatures are representative of the mean variation encountered by the tegu within its underground shelter (Abe, 1995). The lizards were then killed to obtain blood and tissue samples for each specific seasonal period, as detailed below. In the other experimental series, resting $\dot{V}_{O_2}$ was measured on the same group of animals at 25°C and 17°C throughout the year to verify seasonal changes in the temperature effect, as calculated from the Q_{10} ratio. In all cases, body temperature was assumed to be in equilibrium with the air inside the chamber after the acclimation period.

Blood and tissue sampling

For blood and tissue analysis, one group of animals was killed in the morning after $\dot{V}_{O_2}$ was measured, and named according to the seasonal period as above. In these experiments, 'autumn activity' refers to late autumn, and 'arousal' to rehydrated, unfed animals. The animals were decapitated and blood samples were taken directly into pre-heparinised tubes (0.2 mg heparin per ml blood). A 0.1 ml sample was vigorously mixed with 0.2 ml of 0.6 mol l^{-1} perchloric acid (v/v), centrifuged at 6000 ***g*** and 4°C for 10 min, and stored at 10°C for lactate assay. The remaining volume was centrifuged to obtain plasma samples, then frozen in liquid nitrogen and stored at –80°C until analysis. The whole brain, liver, heart ventricle, white portion of the iliofibularis muscle, and a sample of the longitudinal tail muscle were quickly dissected and immediately frozen in liquid nitrogen and stored at –80°C for metabolite and enzyme assays. Finally, the two abdominal fat bodies were removed and weighed, and a sample of the whole tail was removed from the proximal third to assess cyclic changes in fat content. All tissue samples were stored at –80°C until analysis.

Analysis of blood and tissue metabolites

Blood osmolality was measured in 10 μl plasma samples using a vapour pressure Osmometer (5500; Wescor, Logan, UT, USA). Total protein was assayed in blood samples according to Lowry et al. (1951), using bovine serum albumin as a standard. Total lipids and β-hydroxybutyrate were measured in plasma samples using diagnostic kits purchased from LabTest (Belo Horizonte, MG, Brazil) and Sigma (St Louis, MO, USA), respectively. The first method is based on the sulpho-phospho-vaniline colorimetric reaction, and the second follows the enzymatic oxidation of β-hydroxybutyrate to acetoacetate. D-glucose and L-lactate concentrations were assayed in deproteinised samples according to standard enzymatic procedures (Bergmeyer, 1984); NAD^+ and $NADP^+$ reactions were monitored at 340 nm using a spectrophotometer (DU-70; Beckman, Fullerton, CA, USA) at 25°C. Values are expressed as mmol l^{-1}.

Total tissue water and protein were measured in duplicate samples of skeletal (tail) muscle. Water content was estimated by the accompanying mass loss in each tissue sample at 80°C until constant mass. For total protein analysis, tissue samples were homogenised in four volumes (v/w) of 0.6 mol l^{-1} PCA. The homogenate was centrifuged for 5 min at 10 000 ***g*** and the pellet redissolved in 0.6 mmol l^{-1} PCA, the procedure being repeated twice. The precipitate was solubilised in 2.5% KOH and protein content was measured (Lowry et al., 1951).

Glycogen content was assayed in liver, skeletal (tail) muscle, heart and brain. Frozen samples were homogenized in ice-cold 0.6 mol l^{-1} PCA, and two aliquot samples were taken. One was incubated with amyloglucosidase at 40°C for glycogen hydrolysis and D-glucose analysis, and the other was used to estimate background glucose. The assays were conducted following standard enzymatic procedures (Bergmeyer, 1984), and glycogen standards were used to control hydrolysis efficacy.

Total lipid content in liver, skeletal (tail) muscle and whole tail samples was measured in freshly thawed samples as described by Folk et al. (1957). Fresh masses of the abdominal fat bodies were taken as an estimate of the amount of fat in this deposit at different times during the annual cycle.

Enzyme assays

The activities of citrate synthase (CS), an indicator of tissue total aerobic capacity, and of β-hydroxyacyl CoA dehydrogenase (HOAD), an indicator of the capacity for fatty acid utilisation, were measured in skeletal muscle (iliofibularis, white portion), heart ventricle, liver and brain tissue. Freshly thawed samples were homogenised at approximately 4°C in

nine volumes of buffer (w/v) with a teflon–glass homogenizer, using the following composition: 20 mmol l^{-1} imidazol-HCl, pH 7.4, 2 mmol l^{-1} EDTA, 0.1% Triton X-100. Homogenates assigned to the CS assay were consecutively frozen at –80°C and thawed at 4°C three times for complete membrane disruption before centrifugation. All homogenates were centrifuged at 17 000 ***g*** and 4°C for 10 min, and the supernatant fractions kept ice-cold until assay.

Enzyme activities were measured spectrophotometrically (Beckman DU-70) at 25°C, by following the nicotinamide adenosine dinucleotide (NADH) and 5,5′-dithiobis 2-nitrobenzoic acid (DTNB) reactions at 340 nm and 412 nm, respectively, under saturating, non-inhibitory substrate conditions. Buffers and enzyme assays followed the standard approaches given in Bergmeyer (1984), and preliminary experiments were performed to check for control reaction rates (reactions omitting substrate) and to establish the optimal substrate and cofactor concentrations for the final procedure. Each tissue sample was assayed in duplicate, and enzyme activities were described as units per mg of tissue wet mass. Soluble protein concentration was measured in all tissue fractions using bovine serum albumin standards (Lowry et al., 1951), and 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. 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; HOAD: 50 mmol l^{-1} Tris-HCl (pH 7.0), 0.15 mmol l^{-1} NADH, 0.1 mmol l^{-1} acetoacetyl CoA.

Statistical analysis

A one-way analysis of variance (ANOVA) or the Kruskal–Wallis ANOVA on ranks procedure was used to test for differences between groups over the annual activity cycle. Means were then compared by the Student–Newman–Keuls or Dunn's tests for multiple comparisons, where appropriate. The correlations between $\dot{V}_{O_2}$, fat body mass or Q_{10} values and body mass were assessed using a least-squares linear regression method on log-transformed data, and the contribution of body mass in predicting the dependent variable was evaluated by the *F*-test. The analyses were based on Zar (1999) and performed using SigmaStat statistical software (Jandel Co.). The probability of error in the test results was generally assumed to be significant at $P \leq 0.05$.

Results

Changes in oxygen consumption rates ($\dot{V}_{O_2}$)

The resting, mass-specific $\dot{V}_{O_2}$ decreased by a mean of 50% from early to late autumn in newly hatched lizards. In winter, $\dot{V}_{O_2}$ stabilised at values 75–85% lower than those recorded in early autumn, with a concomitant decrease in body (experimental) temperature from 25°C to 17°C (Table 1). The neonates became continuously inactive and did not eat or drink over at least 90 days during winter. At the onset of arousal, they drank water abundantly but remained anorexic, while $\dot{V}_{O_2}$ and the overall activity increased to the levels seen in late autumn. Food intake began within ~1 week, leading to a further increase in $\dot{V}_{O_2}$ to values only 25% lower than those seen in early autumn, a non-significant difference. Thereafter, the lizards resumed intense activity, and $\dot{V}_{O_2}$ values measured 30–40 days after arousal were similar to those of early autumn, a state that continued during the first half of summer.

Body mass did not differ significantly among the experimental groups above (P=0.333). The scaling effect on seasonal fluctuations in $\dot{V}_{O_2}$ and on the degree of metabolic depression was then analysed in lizards whose body mass ranged from 37 g to 257 g. The relationship between body mass (M_b) and $\dot{V}_{O_2}$ changed with season during the first year cycle of the tegu (Table 1). In early autumn, $\dot{V}_{O_2}$ correlates with $M_b^{0.75}$, small animals having higher mass-specific metabolic rates than their larger counterparts. Mass exponents for the reduced metabolic rates were >1.0 during late autumn and winter dormancy and close to 1.0 during unfed arousal, implying the lack of increase in mass-specific metabolism with

Table 1. *Resting rates of mass-specific oxygen consumption ($\dot{V}_{O_2}$) and the scaling relationship with body mass in tegu lizards during the first annual cycle*

Seasonal activity state	$\dot{V}_{O_2}$ (ml O_2 h^{-1} g^{-1})	Body mass (g)	*a*	*b*	r^2
Autumn activity					
Early	0.0627±0.0057[b–e]	92.6±14.9	–0.75±0.37	0.75±0.19	0.60
Late	0.0309±0.0033[a,c–e,f]	111.1±18.7	–2.09±0.47	1.27±0.24	0.74
Winter dormancy	0.0119±0.0013[a,b,d–f]	104.4±12.1	–2.18±0.68	1.12±0.34	0.52
Arousal					
Unfed	0.0273±0.0023[a–c,e,f]	94.1±9.1	–1.50±0.72	0.94±0.37	0.40
Fed	0.0477±0.0042[a–d,f]	113.7±8.4	–2.81±0.60	1.72±0.29	0.77
Spring activity	0.0636±0.0045[b–e]	129.6±14.8	–1.13±0.429	0.96±0.20	0.70

Values are the mean ± 1 S.E.M. from 10–12 different animals measured at 25°C, except in winter dormancy when 17°C was used. Linear regressions were performed on log-transformed $\dot{V}_{O_2}$ (ml O_2 h^{-1}) and body mass (g) as described by $\log_{10} \dot{V}_{O_2} = a + b \log_{10} M_b$, where a is the intercept ± S.E.M., *b* is the slope ± S.E.M. and M_b is body mass; [a] indicates significant differences from early autumn, [b] from late autumn, [c] from winter dormancy, [d] from unfed arousal, [e] from fed arousal, [f] from spring activity (P<0.05).

decreasing mass during the hypometabolic state. According to the equations, a 3-fold increase in body mass leads to a 4-fold increase in $\dot{V}_{O_2}$ in late autumn and a 3.4-fold increase during winter dormancy. In both cases, the slopes are significantly different from that obtained for early autumn individuals (P<0.001), and the degree of metabolic depression in late autumn would be 30% *versus* 61% for lizards weighing 180 g and 60 g, respectively; later in winter, metabolic depression would reach 73% and 83% for the same body mass values. Thus, size-related differences may influence either the magnitude of $\dot{V}_{O_2}$ decrease and/or the time of entry into dormancy. At the onset of arousal, the $\dot{V}_{O_2}$ is somewhat enhanced in aphagic animals although the percentage of depression is still high, irrespective of body mass. The slope at this step is b=0.94 and is significantly different from that for winter dormancy (P<0.01). When feeding reinitiates, the exponent shifts remarkably to b=1.72 and, assuming a 3-fold increase in body mass, $\dot{V}_{O_2}$ would increase almost 7-fold. The calculated percentage of $\dot{V}_{O_2}$ depression would still be 53% in the smaller individuals as opposed to <10% in larger lizards, indicating that the larger the individual, apparently less time is necessary to accomplish the transition from dormancy to full activity. Later in spring, the slope for resting $\dot{V}_{O_2}$ is b=0.96 and within one month of arousal metabolic rates are similar, irrespective of body mass. Body mass accounted for more than 50% of $\dot{V}_{O_2}$ variability in most groups, with inter-individual variation being predominant in unfed, arousing animals. After correcting $\dot{V}_{O_2}$ for body mass, the slope of the relationship between $\dot{V}_{O_2}$ and body mass was significantly different from 0 only in fed individuals during arousal (b=0.722; P=0.035). In the other groups, variability was large, perhaps preventing a statistically significant correlation.

The $\dot{V}_{O_2}$ rates measured at 25°C and 17°C in the single group used for Q_{10} analysis showed a pattern similar to the above. Entry into dormancy occurred earlier that year and, although typical early autumn data were not available, aerobic metabolism at 25°C stabilised at values 77% lower during dormancy compared with rest in spring. A significant decrease was seen at 17°C, the magnitude being less along this lower temperature line (55%). There was no statistical difference between $\dot{V}_{O_2}$ values at these two temperatures during late autumn or winter dormancy, and the calculated Q_{10} is ~1.5 for these two groups (Table 2). During early arousal, there was a significant difference between the $\dot{V}_{O_2}$ values measured at 25°C and 17°C, causing an increase in Q_{10} values prior to food intake, which then further increased to almost 3.0 after feeding was reinitiated, increasing above this value in active spring individuals. Statistical analysis failed to show any significant correlation between body size and the Q_{10} effect in most groups in this experimental series, possibly due to the reduced sample size (N=6). An exception, however, was the spring activity group, in which a strong correlation was found (r^2=0.90; P<0.004). The extremely high mass exponent (b=9.35) suggests a much greater temperature sensitivity of $\dot{V}_{O_2}$ between 17°C and 25°C in larger individuals at this period of the first annual cycle.

Table 2. *Q_{10} values (mean ± S.E.M.) for the seasonal change in resting rates of oxygen consumption at 25°C and 17°C in young tegu lizards*

Seasonal activity state	Q_{10}	Body mass (g)
Autumn activity	1.50±0.066	99.27±16.6
Winter dormancy	1.55±0.053	90.86±18.1
Arousal		
Unfed	1.77±0.073	86.49±16.9
Fed	2.94±0.012	97.26±23.7
Spring activity	3.54±0.178	178.03±43.1

Measurements were made on six animals individually followed during their first annual cycle. Autumn activity corresponds to late autumn in Table 1.

Changes in body mass and composition

The tegus weighed ~15 g upon hatching and, in the laboratory, their mass increased by 5–7-fold during the 4–5 months up to mid-autumn. Thereafter, feeding and other activities became gradually reduced, and mass loss during dormancy reached 15% of the maximum mass in late autumn, as calculated on an individual basis for six animals (Table 2). More than half of this loss (62%) was quickly offset after water intake during the first days of arousal, suggesting that mass change is partially due to evaporative water loss, besides the use of other body stores. Body mass increased at progressively higher rates after feeding was reinitiated and increased more than 10-fold in the young lizards by the end of the first year cycle.

During dormancy, plasma protein concentration increased concomitantly with a peak in osmolality, which also increased slightly in the late autumn and arousal groups compared with active spring animals (Table 3). There was a significant drop in the water content of skeletal (tail) muscle during dormancy, suggesting some loss of fluid from tissue compartments. Other changes during dormancy included a pronounced drop in circulating glucose to 40% and an increase in the levels of total protein (24%), lipids (43%) and β-hydroxybutyrate (113%) in relation to spring activity values (Table 3). Particularly interesting is the almost completely restored glucose levels in arousing animals before food intake. Blood lactate did not change significantly, an indication that there is no substantial alteration in the rates of anaerobic glycolysis associated with metabolic depression in the tegu.

At the tissue level, there was no significant change in the soluble protein content of tissues examined. Total protein in tail muscle, however, was 14% less by mid-winter and the cumulative loss was almost 50% in the tail muscle of arousing animals compared with the values found in late autumn (Table 4). The uric acid pellet expelled on arousal is another strong indication of protein mobilisation during the prolonged fast, possibly intensified by the onset of arousal. Tail muscle glycogen content was reduced to half by mid-winter and increased almost 3-fold in unfed aroused individuals, suggesting the use of amino acids resulting from protein

Table 3. *Seasonal changes in blood plasma parameters during the first annual cycle of tegu lizards*

Seasonal activity state	Glucose (mmol l^{-1})	Lactate (mmol l^{-1})	Total lipids (mg ml^{-1})	β-Hydroxybutyrate (mmol l^{-1})	Protein (mg ml^{-1})	Urea (mg l^{-1})	Osmolality (Osm g^{-1} H_2O)
Autumn activity	–	–	5.22±0.63	–	47.4±2.0	20.4±4.2	307.1±5.3[b]
Winter dormancy	3.08±0.15[b]	1.13±0.16	5.67±0.36[b]	2.85±0.33[b]	53.3±2.2[b]	20.4±5.2	323.1±1.5[a,b]
Arousal	5.87±0.65	0.67±0.11	3.02±0.26[a]	–	40.2±2.0	21.1±4.8	315.4±6.2[a,b]
Spring activity	7.61±0.97	0.90±0.14	3.97±0.40	1.34±0.21	43.1±1.5	35.5±6.1	293.7±2.3

'Autumn activity' corresponds to late autumn, and 'arousal' corresponds to rehydrated, unfed animals in Table 1. Values are the mean ± S.E.M. from six animals; [a] indicates significant differences from autumn activity, [b] from spring activity (P<0.05).

catabolism as the carbon source for carbohydrate synthesis before feeding is reinitiated. Liver glycogen is higher in late autumn, reaching 474 μmoles (77 mg) of glycosyl units in a 100 g lizard, and reduces by 63% in mid-winter, while glucose levels remain constant (Table 4). The liver also regained its potential to accumulate glycogen in arousing animals, concomitant with a significant drop in its glucose content. A distinct trend was seen in the brain tissue, where glycogen is consistently higher in late fall, winter dormancy and arousal groups compared with spring activity, reaching a difference of almost 5-fold in the hypometabolic state. Similarly, heart glycogen is increased by 2-fold during winter dormancy compared with levels in late fall and spring activity, suggesting that these tissues may have the ability to preserve an endogenous source of glucose during the prolonged fasting. Together, these results support the idea that carbohydrate metabolism may be enhanced before food intake at the end of the prolonged fasting.

Before entry into dormancy, the young lizards deposited an average of 2.7% of their body mass as fat in the abdominal fat bodies. The size of this deposit gradually decreased during dormancy, by 24% after 50–60 days of winter and by 62% on arousal in early spring; the fat deposits were virtually depleted in active spring animals 30–40 days after arousal. The liver lipid content was fairly constant at 35 mg g^{-1} throughout the year, corresponding to 53–70 mg in a 100 g lizard during the different seasons. In skeletal (tail) muscle, lipids averaged 24 mg g^{-1} muscle in late autumn animals, showing a slight tendency to drop in dormant and arousing animals. The fat content of the whole tail sample was 2–4-fold higher (79.2 mg g^{-1} tail) than in the skeletal muscle alone. This fat was reduced 25% by mid-winter and 45% in unfed arousing animals, compared with active spring individuals, suggesting that fat from a subcutaneous deposit may constitute another important energy source in fasting animals.

The correlation between body mass and abdominal fat body size was examined to test for a scaling effect on the deposition and mobilization pattern of this energy store. In late autumn animals, the fat body mass correlates with body mass with b=1.44 (P<0.000; Fig. 1), implying that a 3-fold increase in body mass leads to a 5-fold increase in fat deposits. This correlation was also significant after transforming fat content into an index of body mass (b=0.44; P=0.020), confirming that, on entry into dormancy, larger animals possess substantially more fat per unit mass available in this deposit. Halfway through dormancy, the relationship becomes less disproportionate, although larger animals still have a fat surplus of 18% in the fat bodies, as calculated for the body size

Table 4. *Seasonal changes in tissue composition during the first annual cycle of tegu lizards*

	Seasonal activity state	Tail muscle	Liver	Heart	Brain
Protein (mg g^{-1})	Autumn activity	70.5±2.57 (212.5±18.6)	121.6±4.79	64.9±2.20	62.6±2.24
	Winter dormancy	65.7±1.85 (183.3±10.5)	129.6±6.01	62.1±2.24	60.1±2.41
	Arousal	63.9±4.10 (119.8±21.4)[a]	120.8±6.34	59.9±2.00	66.1±0.76
	Spring activity	60.2±1.87 (146.1±14.3)[a]	132.6±4.75	61.6±2.22	70.3±3.00
Glycogen (mg g^{-1})	Autumn activity	0.83±0.24	36.2±6.51[b–d]	1.04±0.18	1.22±0.28[d]
	Winter dormancy	0.36±0.07[c]	13.2±7.33	2.56±0.48[a,c,d]	2.59±0.19[a,d]
	Arousal	1.29±0.28	21.5±3.76	1.64±0.24	1.50±0.13[d]
	Spring activity	0.78±0.18	13.4±2.83	1.10±0.17	0.49±0.03
Lipid (mg g^{-1})	Autumn activity	23.6±6.07[b,c]	34.5±0.79	–	–
	Winter dormancy	11.3±0.90[c]	33.0±4.06	–	–
	Arousal	15.0±1.08	38.5±1.96	–	–
	Spring activity	20.3±3.46	29.9±1.14	–	–

Protein values correspond to the soluble fraction after centrifugation; total protein content in tail muscle is indicated in parentheses. 'Autumn activity' corresponds to late autumn, and 'arousal' to rehydrated, unfed animals in Table 1. Values are the mean ± S.E.M. from six animals; [a] indicates significant differences from autumn; [b] from winter dormancy; [c] from arousal; [d] from spring activity (P<0.05).

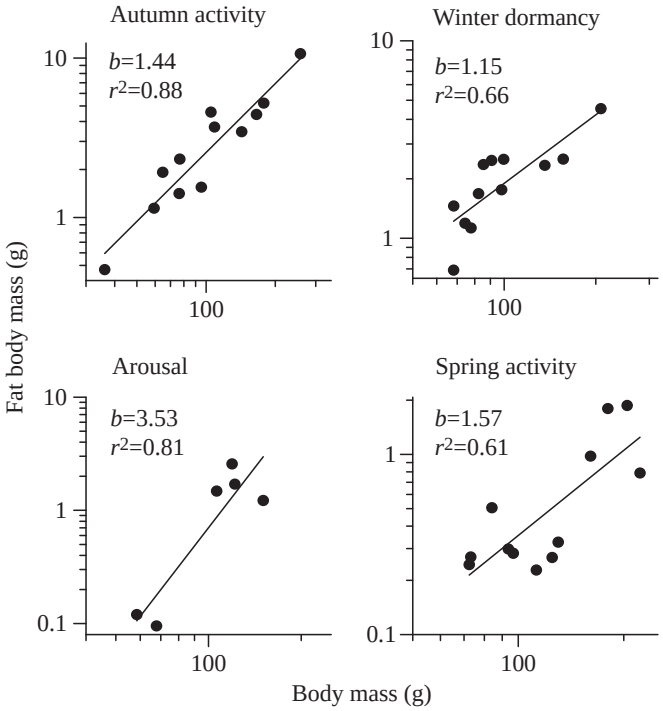

Fig. 1. The allometric relationship between fat body mass (M_{fb}) and body mass (M_b) of young tegu lizards (*Tupinambis merianae*) at distinct activity states during the first annual cycle. Autumn activity corresponds to late autumn, and arousal to unfed, rehydrated animals in Table 1. Linear regressions were performed on log-transformed M_{fb} (g) and M_b (g) as described by $\log_{10} M_{fb}=a+b \log_{10} M_b$.

range considered. The amount of fat mobilised during dormancy up to mid-winter would be approximately 16% for a small animal *versus* 35% for a large animal, and, therefore, small lizards apparently utilize fat from this deposit at lower rates than do larger ones. At the onset of arousal, however, the slope is remarkably high (b=3.53) and the correlation is statistically significant despite a smaller sample size in this group (P=0.014, N=6). This shift in scaling pattern implies ample differences in fat body size according to body mass in early arousal, and the correlation predicts that this deposit is virtually exhausted in the smallest individuals. The variability was very high in this group and the correlation was not significant after values were corrected for body mass (P>0.2). In late spring, fat bodies are largely reduced although still vary with body mass according to b>1, suggesting that the size-related differences observed in late autumn would continue in the active, growing animal.

Enzyme activities

Maximum CS and HOAD activities in the tissues of young tegus are given in Table 5. CS activity was higher in the heart and brain, both highly oxidative tissues, and typically lower in skeletal (iliofibularis) muscle. The maximum activity was constant in brain, liver and skeletal muscle sampled during the different seasons, with a tendency to decrease in the heart during dormancy (P=0.059). HOAD activity was strongly affected by season in most tissues except the brain, where it was nearly constant in all groups. In heart and skeletal muscle, HOAD activity was reduced during winter dormancy and arousal compared with late autumn and spring values. In the liver, the changes were remarkable, with decreased activity in late autumn and almost 3-fold higher rates in dormant and arousing lizards, reaching nearly 5-fold in active spring animals. The pattern of change of enzyme activities calculated per tissue wet mass and per mass of soluble tissue protein was very similar, indicating that the influence of seasonal fluctuations in tissue water content and/or unspecific effects on the soluble protein content are negligible.

Table 5. *Enzyme activities in selected tissues of young tegu lizards during the first annual cycle*

	Seasonal activity state	CS	HOAD
IL muscle	Autumn activity	1.51±0.13	1.36±0.10
	Winter dormancy	1.29±0.12	0.67±0.04[a,d]
	Arousal	1.54±0.17	1.01±0.12[a,d]
	Spring activity	1.57±0.13	1.31±0.09
Heart	Autumn activity	29.65±4.60	12.11±0.26
	Winter dormancy	23.04±1.11	7.19±0.43[a,d]
	Arousal	28.90±1.17	8.73±0.52[a,d]
	Spring activity	22.80±4.76	12.57±0.63
Liver	Autumn activity	6.53±0.67	6.67±0.47
	Winter dormancy	6.87±1.07	18.95±3.10[a,d]
	Arousal	5.36±0.36	17.27±1.58[a,d]
	Spring activity	7.82±0.78	31.17±1.80[a–c]
Brain	Autumn activity	16.44±1.24	2.38±0.11
	Winter dormancy	17.40±1.37	2.80±0.19
	Arousal	17.80±0.58	2.44±0.07
	Spring activity	16.93±1.16	2.71±0.19

'Autumn activity' corresponds to late autumn, and 'arousal' to unfed, rehydrated animals in Table 1. Values are the mean ± 1 S.E.M. from six animals in U g^{-1} wet mass; [a] indicates significant differences from autumn activity; [b] from winter dormancy; [c] from arousal; [d] from spring activity (P<0.05). IL, iliofibularis, white portion.

Discussion

The present study provides evidence for an intrinsic rhythm underlying the annual activity cycle in young tegu lizards. Consistent changes were seen in newly hatched animals at different levels of structural organisation and entail a hypometabolic condition of duration and magnitude equivalent to that seen in hibernating and aestivating species (reviewed by Guppy and Withers, 1999). Furthermore, a scaling effect on the processes of energy storage and utilisation was found to be associated with winter dormancy, which may play an important role in the ensuing stages of return to activity and continued growth during the first year cycle of the tegu.

The mechanisms that trigger dormancy are still poorly

understood (Guppy et al., 1994; Storey, 2002). In young tegus, they result in a stepwise depression of aerobic metabolism in the whole animal, initially causing resting $\dot{V}_{O_2}$ to drop by 50% from early to late autumn at 25°C, associated with anorexia and a clear departure from the normal routine. This intermediate condition extends for a few weeks until the animals remain in their artificial refuges in the mornings, spending 3–4 months inactive during the first annual cycle. During this time, metabolic rates are stable at ~20% of the resting value without detectable variation within the daily cycle (S. C. R. de Souza and J. E. de Carvalho, unpublished observations). The reverse change on arousal is also gradual, with a partial increase in aerobic metabolism (22–25%) occurring in aphagic animals measured 48 h after water intake, increasing further after food intake recommences a few days later.

In mammals and birds, the interpretation of changes in $\dot{V}_{O_2}$ with torpor is complex mainly because of their inherent capability to generate heat and thermoregulate in normothermic conditions. The underlying mechanisms may be switched off and an ensuing large Q_{10} effect provides the animal with an alternative route for substantial reduction in energy expenditure that may be supplemented by lesser savings derived from metabolic depression (Guppy and Withers, 1999). In the tegu, the situation is clearly different, with the hypometabolic condition relying heavily on a temperature-independent mechanism for energy conservation on a long-term basis. During late autumn and winter dormancy, $\dot{V}_{O_2}$ decreases markedly in newly hatched lizards compared with resting $\dot{V}_{O_2}$ in early autumn, the rates becoming nearly constant over the temperature range of 17–25°C. Accordingly, the calculated Q_{10} is low, ~1.5 during the hypometabolic condition, in marked contrast to the large Q_{10} effect (3.5) during spring activity, suggesting that the temperature sensitivity of the metabolic reactions is somehow reduced during dormancy. Thus, a torpid tegu in its refuge at 17°C would acquire total metabolic savings equivalent to a much larger drop in body temperature, to approximately 8°C. The percent depression in winter compared with resting conditions in spring decreases when calculated along the lower temperature line, reaching 55% at 17°C, in contrast to 77% at 25°C. Overall, the reduced rates of oxidative metabolism during winter dormancy apparently meet the limit for long-term survival at a very low energy cost.

The young tegus undergo a period of intense growth from hatching in early summer until autumn, body mass increasing by 5–7-fold under laboratory conditions. Thereafter, they become anorexic and their weight losses result in a progressively negative balance during winter dormancy, leading to a net weight loss of 15% until arousal in spring. Although the size range among siblings is narrow, analysis of the individual data revealed an allometric effect on the magnitude of metabolic depression during the first year cycle, with a clear trend towards higher energy conservation in the smaller lizards. Initially, the exponent relating $\dot{V}_{O_2}$ and body mass shifts from b=0.75 in early autumn individuals to b>1.0 during the hypometabolic condition, an indication that the extent of depression is largely unproportional. The absolute rates of metabolism increase by 35%, with a 3-fold increment in body size in the early stages of depression, a difference of 18% remaining during winter dormancy when metabolic costs are reduced to their lowest and small differences become meaningful in terms of substrate savings for the duration of the hypometabolic condition. During early arousal, $\dot{V}_{O_2}$ rates are somewhat enhanced in the aphagic individuals, and at this step the energy expenditure per mass unity would be similar irrespective of body mass (b=0.94). However, a few days after feeding is reinitiated, the exponent rises remarkably to b=1.72, suggesting that the larger the individual the less time is necessary to accomplish the full transition from dormancy to activity. In late spring, the slope for resting $\dot{V}_{O_2}$ returns to b=0.96, and this close proportion between body mass and energy expenditure may be associated with the intense growth period that follows arousal in young lizards.

Interspecific comparisons of $\dot{V}_{O_2}$ data from adult, heterothermic birds and mammals suggest a body mass influence on the extent of metabolic depression in smaller species, which undergo deep hibernation (body temperature <10°C), the negative slope for the mass-specific rates not being observed during torpor (Geiser, 1988). This shift in the scaling pattern has been ascribed to the limited capacity of smaller species to store energy as fat. A similar effect is not seen when the different categories of heterotherms are analysed as a single group, the mass exponents being indistinguishable and <1.0 (Guppy and Withers, 1999). By contrast, the $\dot{V}_{O_2}$ changes in young tegus are the product of a complex interaction with a seasonal rhythm superimposed on the developmental process. Within the ontogenetic context of mammals and other vertebrates, the allometric exponent for whole animal metabolism varies with the stage of development, and different phases are recognised within this relationship over the life cycle (Wieser, 1984). Briefly, the exponent is close to 1.0 during the early developmental stages, the period of most rapid growth, and from then on the basal metabolic rates tend to follow the surface rule, a pattern that prevails during most of the life cycle; a crossover point of these two lines is reached after a given degree of adult body mass is attained. This suggests that the slope of b=0.75 for autumn activity in the first year cycle of the tegu may not constitute a definite pattern for the relationship, being restricted to the transition from a period of intense growth during entry into dormancy when food intake declines and growth processes are halted to ensure that a suitable amount of energy is deposited mostly as fat in the body stores. In this case, larger animals with their larger fat deposits may reduce energy expenditure earlier on entry into dormancy. Roughly, starting from a mean body mass of 15 g on hatching (N=24), the young lizards grow at the fastest rate of 1.0 g day^{-1} during summer, growth decreasing subsequently to 0.4 g day^{-1} and to 0.2 g day^{-1} during the early and late autumn months, until reaching a negative balance. After a prolonged pause in the anabolic processes during winter dormancy, the young enter another period of positive energy intake, their growth

rates becoming increasingly higher in spring when metabolic rates show a closer relationship with body mass. Supportive data suggesting the downregulation of endocrine mechanisms that promote somatic growth during metabolic depression were recently obtained with ground squirrels (*Spermophilus lateralis*), an effect presumably associated with the change in nutritional status during the hibernating period (Schmidt and Kelley, 2001). Thus, while the exact significance of the mass exponent for aerobic metabolism in early autumn activity remains unresolved, the significant shifts in scaling pattern, both on entry and on arousal from the hypometabolic condition, strongly suggest a body mass influence on the mechanisms of metabolic depression in young tegus.

A seasonal pattern of lipid cycling coexists with the shifts in aerobic metabolism during the first annual cycle of tegu lizards, as revealed by the consistent changes in the mass of the visceral fat bodies and in the amount of fat in the subcutaneous tail deposits. Seasonal variation was also found in the potential for fatty acid oxidation in various tissues, as shown by the remarkable changes in HOAD activity, which increases several fold from late autumn to spring activity in liver tissue, becoming reduced in skeletal and heart muscle in dormant lizards. Apparently, these effects were independent of changes in tissue water content and strongly suggest the tissue-specific regulation of HOAD expression during the annual cycle, related to the processes of fat deposition and mobilization as well as to energy spare and overall metabolic depression during dormancy. The progressive increase of liver capacity for fatty acid oxidation would limit the use of modest glycogen reserves, and this pattern, together with the constancy of CS in most tissues examined, emphasises the aerobic nature of seasonal dormancy in the tegu.

Seasonal cycles of fat deposition and mobilisation correlate with food availability in many reptiles, most lipids being stored subcutaneously and/or in visceral fat bodies (Derickson, 1976). At most, fat body lipids make up 50% of the total storage in the species examined, a variable fraction being allocated for gametogenesis and other processes in preparation for reproduction during winter and in early spring. The tegus reach reproductive maturity by their third year cycle; prior to this point, changes in lipid stores would be closely related to energy expenditure for whole body maintenance during the fasting period and for the metabolic increase seen upon arousal. The allometric patterns for the changes in $\dot{V}_{O_2}$ and fat body mass are coherent in this context. In late autumn, the slope for the correlation between fat body mass and body mass is b=1.44, revealing that for a body size difference of 3–4-fold, a disproportionately larger amount of fat will accumulate in larger young individuals. By mid-winter, the shift in allometric pattern to a close correlation with body mass suggests that smaller animals drain fat from the fat body at lower rates than do larger lizards. This is in good agreement with the greater depression of aerobic metabolism seen in smaller young individuals. At the time of arousal, these fat body deposits are virtually exhausted in the smaller individuals, while substantial fat is still available in the larger tegus, causing the mass exponent to shift to b>1 again. Given the importance of this fat deposit, this may imply a limited capability of the smaller animals to sustain the higher rates of metabolism required to actively hunt for food and to avoid death by inanition upon return to activity. The events preparatory to dormancy in the subsequent annual cycles are probably anticipated, given that the arrest of routine activities generally occurs earlier, and usually extends further, in individuals at later stages of development (H. R. Lopes and A. S. Abe, unpublished observations).

The physiological events relating the size of the fat stores to energy intake and expenditure are becoming clearer as a consequence of studies on obesity in humans and laboratory mammals (for a review, see Ahima and Flier, 2000) and its correlates in hibernating mammals (Boyer and Barnes, 1999). The adipocyte is now established as the source of numerous peptides secreted in the plasma, such as leptin, whose levels correlate positively with total adipose mass. Leptin secretion may act as a self-regulating system to sustain both a given degree of lipid reserve and animal body mass. The action of leptin on energy expenditure is probably exerted *via* the hypothalamus through an effect on the production of thyroid hormones and by a direct effect on cellular respiration in peripheral tissues (Reidy and Weber, 2000). In hibernating mammals, the mechanisms of body mass control may involve more complex interactions of leptin with other molecules that effect a seasonal modulation of leptin sensitivity and an apparent dissociation of its anorectic and metabolic effects (Boyer et al., 1997; Klingenspor et al., 2000). A leptin-like molecule has been detected in the plasma and tissues of fish and reptiles (Johnson et al., 2000; Niewiarowski et al., 2000); while its physiological function in lower vertebrates has yet to be elucidated, these findings provide a promising scenario for the investigation of potential mechanisms linking the control of body mass and degree of adiposity to the energy expenditure during the annual cycle of young tegus.

Other sources of energy for the dormant tegu are carbohydrates in the liver and skeletal muscle. Liver glycogen in the late autumn averaged 223 μmol g^{-1}, accounting for 3.6% of the mass of the liver and corresponding to less than half the liver glycogen reserve in overwintering amphibians and other lower vertebrates that display varying capacities of hypoxia tolerance (Boutilier et al., 1997; Scapin and Giuseppe, 1994; Lutz and Nilsson, 1997). A rough calculation predicts that during dormancy a 100 g lizard could survive for 2–3 days exclusively on liver carbohydrates for oxidative processes (assuming that 0.84 litres of O_2 are required per 1 g of carbohydrate oxidized); the same calculation for fat deposited in the fat bodies provides 170 days (assuming that 2.0 litres of O_2 are required per 1 g of fat oxidized). This carbohydrate store was reduced to a limit of ~60% in animals sampled halfway through the inactive period. Plasma lactate remained nearly constant in relation to active animals, further suggesting that glycolytic ATP production does not play an important role in the long-term maintenance of dormant tegus, unlike the case in vertebrates in which metabolic depression is associated with oxygen deprivation. However, the small carbohydrate store in

the liver may be essential during the initial period of fasting, providing energy at reduced rates for glucose-dependent tissues such as the brain, renal medulla and retina (Guppy et al., 1987) until the availability of alternative substrates like ketone bodies increases in the blood. The high ratio of HOAD to CS enzyme activities in the liver tissue of dormant tegus and arousing animals suggests that the liver may be a site of ketogenesis from the incomplete oxidation of fatty acids (Stuart and Ballantyne, 1997), accounting for the increase in β-hydroxybutyrate in the circulating plasma in dormant tegus. Given the reduced glucose supply, this metabolite may become a supplementary energy source for specific tissues in the tegu; in agreement, our recent findings have revealed increased enzyme activity related to the oxidation of ketone bodies in the brain of dormant tegus (J. E. de Carvalho, M. S. C. Bianconcini and S. C. R. de Souza, unpublished results).

The energy supply for such tissues, which typically show a preference for carbohydrate as an energy substrate, may be even more challenged in young tegus during emergence from the hypometabolic condition, when they must rely on reduced body stores to increase metabolic rate and succeed in the search for food. Despite the low levels of glucose in the circulating plasma, glycogen content is increased several fold in the brain and in the heart of dormant lizards compared with spring active individuals and may constitute a readily available source of glucose for these high-priority tissues at the onset of arousal. Notably, brain glycogen levels reached 2.6 mg g^{-1} (16 μmol glycosyl units g^{-1}) in dormant lizards, a high content typically found in the brain of anoxia-tolerant species as crucian carp, goldfish and freshwater turtles (Lutz and Nilsson, 1994). The delivery rate of glucose to the brain is presumably lower during metabolic depression, and glycogen deposition in this condition may be facilitated by a sustained potential of synthesis and reduced rates of carbohydrate usage. At the onset of arousal (2–4 days), brain glycogen decreased by 42% concomitant with a significant increase in blood glucose and skeletal muscle glycogen, suggesting that another source of glucose is made available for the tissues before feeding is reinitiated.

The substantial reduction in tail muscle total protein in arousing tegus (50%) is consistent with the general idea that amino acids from protein breakdown play an important role as precursors for glucose synthesis at the end of a prolonged fasting period, in addition to glycerol from fatty acid oxidation (Moon, 1988). Protein is also an important source of energy for both large and small hibernating mammals (Cherel et al., 1995; Tinker et al., 1998), and an adequate balance of fat and protein use is apparently part of a common repertory in several spontaneous fasters, the control of which is poorly known (Robin et al., 1998; Mellish and Iverson, 2001). The degree of protein catabolism correlates with the size of the initial fat reserves in the species examined; the young tegus, with their small size and limited capacity to store fat, may derive more energy from protein during the fasting period, and particularly on arousal, than at later stages of development. The high cumulative protein loss from skeletal white muscle in the young may nevertheless compromise locomotion and hunting capabilities upon return to activity. In hibernating bears, net protein loss varies from 4% to 10% in two muscle types heavily used for locomotion, with no significant muscle atrophy and only modest changes in fibre type composition (Tinker et al., 1998); similarly, the seasonal fast in hedgehogs entails a loss in total body protein of ~10%, irrespective of the duration of hypothermia (Cherel et al., 1995). Thus, the comparatively high loss of protein from the tail muscle of arousing tegus strengthens the importance of this substrate as an energy source during the fasting period of the first year cycle and implies a differential rate of proteolysis in distinct muscle types, preventing the impairment of functions like locomotion and lung ventilation.

Our results suggest a downregulatory mechanism that acts on the energy metabolism of tegu lizards, in which body size apparently sets a limit to substrate storage capacity in the small young and thus to their ability to survive during the prolonged fast soon after hatching. In mammals, the standard rates of metabolism are due mostly to mitochondrial respiration, of which ~80% is coupled to ATP synthesis and ~20% is used to compensate a proton leak across the inner membrane that bypasses ATP production (Rolfe and Brown, 1997). A similar composition apparently occurs in ectotherms (Hulbert and Else, 1981; Brand et al., 1991). A regulatory effect on the rate of ATP synthesis has been shown during the hypometabolic condition by altering substrate supply to metabolic pathways (Storey, 1997) and by influencing other molecular mechanisms that define the rates of ATP production by the mitochondrial inner membrane (Martin et al., 1999; St-Pierre et al., 2000). The ATP demand for protein synthesis, ion pumping and other energy-consuming processes is reduced in several hibernators and facultative anaerobes, as discussed in the reviews by Guppy et al. (1994) and by Boyer and Barnes (1999).

Attempts have been made to quantify the degree of metabolic change in individual tissues of a few species, although no general trend is apparent (Flanigan et al., 1991; Land et al., 1993; Fuery et al., 1998). In the tegu, the ventilatory pattern becomes episodic during dormancy, with intervals lasting up to 26 min at 17°C; even so, the relative cost of the work of breathing would account for an estimated 50% of total metabolic rate (Andrade and Abe, 2000). This high energy cost implies that the downregulation of cellular mechanisms is largely unproportional in different tissues and even among different muscle types in the tegu, involving tissue-specific modulation of substrate flux through the metabolic pathways. Given that the skeletal muscle mass constitutes a high percentage of the vertebrate body, a substantial reduction of the total energy cost may result from the pronounced decrease of enzyme activities related to substrate flux in the glycolytic pathway in this tissue, as recently observed in dormant tegus (J. E. de Carvalho, M. S. C. Bianconcini and S. C. R. de Souza, unpublished results). In hypoxic hibernating frogs, there is a reduction of 50% in proton leak in skeletal muscle as a consequence of a reduced electron flux through the mitochondrial membrane (St-Pierre et al.,

2000); no change, however, is detectable in normoxic frogs. While a role played by uncoupling proteins on the regulatory change of proton leak rate remains controversial, much interest has emerged regarding the paradoxical findings of an increased expression of these proteins during induced starvation and its association with fat metabolism (for a review, see Duloo and Samec, 2000; Boss et al., 2000). The proteins may be involved in the regulation of lipid use as an energy fuel and in the control of body mass, with a more pronounced effect in the predominantly fast glycolytic white muscles, associated with their greater capacity to alternate between glucose and lipids as substrates. Thus, the size of the adipose tissue mass may affect both the degree of energy expenditure and substrate preference of the large skeletal muscle mass in animals that spontaneously undergo fasting periods during their annual cycle.

To our knowledge, a scaling influence on the magnitude of metabolic depression has not yet been the subject of investigation at the cellular level, and therefore the regulatory mechanisms involved are even less clear. In a phylogenetic context, many cellular processes are known to be allometrically related to body mass in the same way as is standard metabolism (Else and Hulbert, 1985; Porter et al., 1996). Hulbert and Else (2000) propose that a possible unifying factor in this arrangement may be the amount and lipid composition of the cell membranes. Besides the body mass effect, these authors consider that such factors are also under the influence of ontogenetic changes, dietary manipulation and stress conditions, with the ensuing effects being exerted on the activities of membrane-bound proteins, such as sodium pumps and mitochondrial uncoupling proteins. Thus, the potential for a regulatory effect on a variety of energy-demanding processes would endow the cell membranes with the capacity to influence energy expenditure during metabolic depression as well, as shown in a study with aestivating snails (Stuart et al., 1998). According to this idea, a change in cell membrane structure and function would modify the cost of living while maintaining the same factorial proportion to body size. Given the results obtained with the tegu, however, it is tempting to speculate that the disproportionate mass of adipose tissue in young individuals may transmit a signal of distinct amplitude to the tissues in which energy is consumed, thus promoting a deviation in allometric pattern concomitant with the depressing effect.

In conclusion, the present study contributes to our understanding of the mechanisms underlying metabolic depression associated with spontaneous fasting in terrestrial reptiles and provides perspective on the means by which seasonal events are conciliated with growth and developmental changes during the early stages of the tegu lizard's life cycle. The results obtained should aid in elucidating potential mechanisms by which the balance between body mass and energy expenditure may be modulated, favouring a close relationship with the size of available substrate stores in a state that allows no energy intake and demands reduced expenditure.

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