Journal of Thermal Biology 30 (2005) 79–83

Journal of
THERMAL BIOLOGY

www.elsevier.com/locate/jtherbio

Lower selected body temperatures after food deprivation in the lizard *Anolis carolinensis*

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Received 12 May 2004; received in revised form 16 June 2004; accepted 9 July 2004

Abstract

Diet composition can affect body temperatures selected by lizards in a thermal gradient (SBT) but there is very little evidence to support a similar effect due to fasting. The latter could represent a means of energy conservation during periods of low food availability. This study investigated the impact of short-term food deprivation (5 days) on diurnal thermal preferences of male ($n = 10$) and non-reproductive female ($n = 22$) *Anolis carolinensis* using a repeated measures design. Mean SBT varied significantly over the 4 h measurement period, and was higher for males than females, overall. A small but significant change in SBT was detected between the two feeding treatments, with food deprivation leading to a mean decrease ranging from 0.43 ± 0.55 to 0.72 ± 0.46 °C (at different times of day) in females and from 0.61 ± 0.71 to 2.13 ± 0.76 °C in males. Previous studies may have failed to detect such subtle trends due to a lack of statistical power arising from small sample sizes. An explanation of these differences is they represent a feeding status dependent trade-off for maintenance of energy balance versus optimal food assimilation. However, (1) the estimated energy saving, and (2) the impact on food assimilation appear rather negligible even assuming that the observed decrease in SBT produces a similar lowering of field body temperature in the wild.

Keyword: Body temperature; Food-deprivation; Reptile; *Anolis carolinensis*

1. Introduction

Body temperatures selected by ectotherms (SBT) in laboratory thermal gradients reflect a complex interplay of physiological and behavioural mechanisms. The relative ease with which SBT can be measured in lizards has considerably enhanced our understanding of their behavioural thermoregulation. For example, the comparison of SBT with field body and operative temperatures provides the basis for evaluating how precisely a lizard thermoregulates in the wild (Hertz et al., 1993). Studies of the factors affecting SBT can also shed light on physiological mechanisms. For example, hypoxia leads to a decreased SBT in the lizard *Anolis sagrei* which can be removed by administration of an adenosine receptor antagonist, suggesting roles for arterial oxygen and adenosine in temperature regulation (Petersen et al., 2003).

To date there have been a small number of studies that have investigated the impact of feeding and diet on lizard SBT. A saturated fatty acid (SFA) diet leads to an increased SBT in *Amphibolurus nuchalis* (Geiser and Learmonth, 1994), while a high polyunsaturated fat (PUFA) intake leads to a commensurate decrease in *Tiliqua rugosa* (Geiser et al., 1992). Simandle et al. (2001) found no differences in daytime SBTs between

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0306-4565/$ - see front matter
doi:10.1016/j.jtherbio.2004.07.005

groups of lizards fed SFA or PUFA diets, but scotophase temperatures were higher in the latter group. In another study, Nussear et al. (1998) found no differences in SBT between *Sauromalus obesus* fed on diets differing in dietary fibre composition. Very little work seems to have been carried out on the effects of fasting in lizards although Sievert (1989) found a scotophase postprandial effect in *Crotaphytus collaris*. More studies have been carried out on snakes (see Touzeau and Sievert, 1993 for early review) and amphibia, with rather mixed results. For example, an increase in SBT after feeding has been observed in the toad *Bufo woodhousii* (O'Connor and Tracy, 1992; Witters and Sievert, 2001), and the snakes *Elaphe obseleta* (Blouin-Demers and Weatherhead, 2001) and *Nerodia sipedon* (Sievert and Andreadis, 1999) (at least during some parts of the day) but not in other species, such as *Bufo marinus* (Mullens and Hutchison, 1992). The majority of studies have recorded either no change in SBT or a postprandial increase, although the former may be a type II statistical error in some cases arising through small sample sizes and thus lack of statistical power.

The general finding of a reduced SBT in lizards after food deprivation could have implications for field energy budgets, particularly given that some taxa often spend substantial periods with little or no food in their gut (Huey et al., 2001). This work was aimed at testing whether fasting influenced SBT in the lizard *Anolis carolinensis*, a well-studied model organism in ecological research (e.g., Jenssen et al., 1996; Lovern et al., 2004). A slightly larger sample size was used than in many similar studies in order to increase the probability of detecting a small mean change in SBT.

2. Materials and methods

2.1. Study organism and laboratory housing

Anolis carolinensis is a small diurnal lizard with a maximum snout to vent length of 7.5 cm from South Eastern states of the USA. Ten adult males and 22 adult females were obtained from a commercial supplier; all animals were at least first generation captive bred. They were housed in a laboratory at Trotters World of Animals Zoological Gardens, UK, from mid-October 2003, under the following conditions. Ten $30 \times 45 \times 45$ cm cages containing numerous perches were used to house groups (1:1–2, M:F). Groups never consisted of more than 1 male ensuring no male dominance hierarchy, which can affect recovery from stress (Plavicki et al., 2004) and testosterone levels (which generally increase male activity in *A. carolinensis* and so could influence SBT [see Lovern et al., 2000; Yang and Wilczynski, 2001]). The summer reproductive period had finished when experimentation began so no females were gravid for at least 1 month before, during, or after the experimental period. A photoperiod of 12:12 LD (07:00–19:00 GMT) and a range of perch temperatures from 20 °C to approximately 35 °C were provided by a single 20 W bulb in each tank. Night temperatures within the holding tanks did not fall below 20 °C. All lizards were provided ad libitum with Anole Food (Zoo Med) daily, while brown crickets (*Gryllus* spp.), mealworms (*Tenebrio molitor*) and wax moth larvae (*Galleria mellonella*) were offered every two days. Fresh food was always provided at 10:00 hours. Live food was dusted weekly with vitamin powder. All 32 individuals were healthy and readily took food on a daily basis. Water was provided daily.

2.2. Selected body temperatures

A thermal gradient was constructed using a glass aquarium divided by a central wooden partition to create two compartments measuring $120 \times 20 \times 45$ cm. A 3 cm shelf was attached to both sides of the partition at a height of 10 cm from the substrate, running horizontally along the gradient. This provided a perch for lizards and was regularly used. The sides of the glass aquarium were covered so as to minimise disturbance. A mesh lid prevented escape but allowed light to enter the gradient uniformly from above (from overhead ceiling lights: exactly the same photoperiod was used as in the holding tanks).

One end of the thermal gradient was heated from below by two 150 W bulbs connected to a dimmer switch. The opposite end was cooled using ice packs, which were replaced as needed. This created a 12–50 °C temperature range at perch height. A 1 cm layer of coarse sand was used as a substrate.

In order to maximise the statistical power of the design, all individuals were tested under both "FED" and "FASTED" treatments described below, with the order of application differing between individuals (random assignment). We estimated that the statistical power to detect a true difference of 2 °C should be >0.99 for 32 individuals even in a simple paired observation design (based on standard deviations typically found in similar SBT studies). The FED treatment comprised a normal feeding regime while no food was offered for 5 days prior to the experiment under the FASTED treatment. The 5 day time period ensured the gut would be empty, while periods longer than this lead to severe depletion of liver glycogen and plasma glucose levels in *A. carolinensis* (Gist, 1972). After this period, lizards were individually placed in one of the gradients at 11:00 GMT and left undisturbed for 1 h to habituate to the new environment. No food was offered to the lizards during the experiment.

Cloacal temperatures were initially taken at 12:00, 14:00, 16:00 and 18:00; however, it was found that the first measurement (taken only 1 h after introduction to the gradient) was highly variable, contained many outliers and so was discarded (although note that even these noisy data showed the same trends as those described later). Individuals were captured using a small aquarium net so as to minimise disturbance and a thermocouple inserted approximately 5 mm into the cloaca. All readings were taken within 30 s of capturing the lizard, which was then immediately returned to the gradient. No gloves were used so we cannot rule out the possibility of minor heat transfer to the lizards. However we believe this is justified because: (1) it helped keep handling times to an absolute minimum, thus reducing stress (handling may increase SBT: Cabanac and Bernieri, 2000), (2) any putative effect would not introduce bias because any potential heat transfer would be similar for all individuals under both treatments, (3) the SBTs were actually lower than those recorded in a previous study on this species (Corn, 1971), which provides evidence against the effects of handling stress and/or heat transfer.

2.3. Statistical analysis

Data were analysed using a repeated measures analysis of variance (ANOVA) test, with sex as the between-individual factor and measurement time and feeding treatment as the within-subject factors.

3. Results

The mean deviation obtained by subtracting FASTED from FED SBTs for each individual was positive for all three measurement times for both sexes, suggesting that 5 day food deprivation decreases SBT in both males and females (see Table 1 and Fig. 1). The overall significance of the different feeding treatments was confirmed by the ANOVA ($F_{[1,30]} = 4.92$, $P = 0.034$).

Mean male SBT was higher than mean female SBT for all corresponding within-subject time/feeding groups

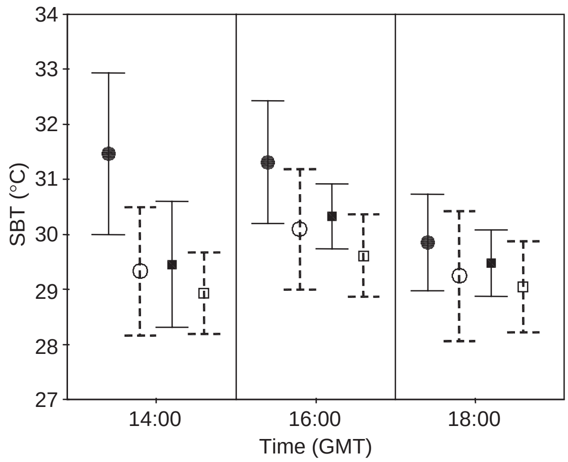

Fig. 1. Mean selected body temperatures (SBT) and 95% confidence intervals at the three measurement periods for FED males (●), fasted males (○), FED females (■), and FASTED females (□).

(Fig. 1). This between-sex difference was also confirmed by the ANOVA ($F_{[1,30]} = 4.70$, $P = 0.038$).

Finally, there was a significant change in SBT over the three measurement times ($F_{[2,47.49]} = 7.75$, $P = 0.003$). Note that measurement time showed evidence of deviation from the assumption of sphericity (approximate $\chi^2_{[2]}$ distributed test statistic = 8.863, $P = 0.12$), so Greenhouse–Geisser adjusted error degrees of freedom were used. None of the two or three-way interaction terms (i.e., Time*Sex, Time*Feeding, Feeding*Sex, or Time*Feeding*Sex) approached significance ($P > 0.17$ in all cases).

Table 1
Mean increases in SBT (°C) associated with feeding, based on within-individual changes for FED relative to FASTED treatments (±1 SE)

Time	Males	Females
14:00	2.13±0.765	0.53±0.711
16:00	1.22±0.833	0.72±0.461
18:00	0.61±0.713	0.43±0.549

4. Discussion

The comparison of SBT over a 4 h diurnal measurement period reveals a clear depression of SBT associated with food deprivation. This effect is quite subtle, however, 0.4–0.7 °C in females and 0.6–2.1 °C in males. Other studies on snakes have revealed much larger effects, with increased SBTs of around 2–5 °C (Slip and Shine, 1988; Blouin-Demers and Weatherhead, 2001; Dorcas et al., 1997) and increased field body temperatures of around 6 °C (Beck, 1996) after feeding. More substantial effects have been detected in amphibia, too, with a 2–3 °C increase being reported for a European newt (Gvoždík, 2003), although the effect on the toad *Bufo woodhousii* is more comparable to those here, with the difference ranging from approximately 0–2 °C, depending on the time of day (Witters and Sievert, 2001). Of course, the predominance of either large or no significant feeding effects could well be explained by lack of statistical power due to small sample sizes/high variances in previous work.

The feeding treatment differences also appears generally slightly smaller than those observed between SFA and PUFA diets, the latter leading to a decrease in SBT of around 2–3 °C (Geiser et al., 1992; Simandle et al., 2001). The possibility that the diet and food deprivation effects are mechanistically linked is worth considering. The latter effects are hypothesised to be related to the influence of plasma lipid type on membrane fluidity and fasting does produce changes in plasma lipoprotein lipids, in rats at least (Peinado-Onsurbe et al., 2001).

The shift in SBT could help to improve energy balance during periods of low food availability, as hypothesised for the toad *Bufo boreas* (Lillywhite et al., 1973) although the impact of such a subtle change might be negligible. Based on published data for this species (Jenssen et al., 1996), we estimated that a 0.5 °C decrease in body temperature would lead to a decrease in metabolic rate of around 3% at normal activity temperatures. While it is possible that the energy saving could be increased if greater differences were observed during the scotophase (not measured in this study), a change in SBT would only affect nocturnal field temperatures if it had a major impact on shelter site selection because the lizards are inactive at this time of day. Many other factors (in addition to SBT) will affect field body temperature (Huey and Slatkin, 1976) so it is questionable whether the observed pattern would lead to decreased energy expenditure at all.

The advantage of increased SBT during digestion could be increased metabolisable energy intake (MEI). Angilletta (2001) found that optimal MEI very closely corresponded to SBT of fed *Sceloporus* lizards, however, the temperature relationships of many components of MEI tend to peak at or just below 30 °C (e.g., Van Damme et al., 1991; Du et al., 2000; Xue-Feng et al., 2001). The latter could even lead to an increase in MEI with decreased temperatures in the 29–31 °C range. In sum, the idea that shifts in fasted versus fed SBTs are linked to a trade-off between energy balance and digestive efficiency is an attractive one. Unfortunately, supporting evidence that the small change observed here could have a substantial impact on these factors in the wild is lacking.

The increased SBT in males relative to females is somewhat surprising given that none of the latter were reproductive during the study. Many lizards show upward or downward shifts in SBT while gravid or pregnant (e.g., Daut and Andrews, 1993; Zari, 1998; Rock et al., 2002; Le Galliard et al., 2003), with a return to normal levels after oviposition or parturition. It is more unusual for differences between non-reproductive female and male lizards to be found, although lower female body temperatures have been described in some species (Patterson and Davies, 1978), and the exact opposite in others (Schall, 1977).

Finally, many analyses of SBT detect a significant time of day effect (e.g., Brown, 1996; Sievert and Paulissen, 1996). An interaction between time and feeding-state has been shown in a previous lizard study (Sievert, 1989) as well as studies on toads and snakes (Beck, 1996; Witters and Sievert, 2001), indicating that the observed differences may not remain constant over a 24 h period, in these snake and toad studies the greatest difference between fed and fasted individuals occurred during the main activity period of the species. Our results show come concordance with this inasmuch as the smallest differences between treatments within males and females occurred just prior to the scotophase. However, Sievert (1989) found that postprandial effects were large and significant during the scotophase, but not so during the day.

Future studies that determine the generality of the finding of decreased lizard SBT associated with food deprivation would be welcome, in addition to experimental studies that further-investigate potential benefits in terms of energy balance and assimilation efficiency. The basis for the difference could also be examined, with biochemical and physiological comparisons among alternative treatments that cause a change in SBT providing a starting point.

Acknowledgements

We thank Trotters World of Animals, Cumbria, UK, for logistic support during this project, and to two anonymous reviewers for their comments on an earlier draft of this report.

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