

CORRECTION

Correction: Both thyroid hormone levels and resting metabolic rate decrease in African striped mice when food availability decreases (doi: 10.1242/jeb.151449)

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There was an error published in *J. Exp. Biol.* (2017) **220**, 837-843 (doi: 10.1242/jeb.151449).

A mistake was made by the authors in the calculation of RMR values. The corrected Results section, tables and figures follow and we indicate where the significance of relationships changed. There are no changes to the conclusions of the paper.

Mass-adjusted RMR ($\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$) was higher in the moist season than in the dry season ($t_{29,17}=-3.40$, $P=0.001$). Whole-animal RMR also varied seasonally (ANCOVA: $F=39.82$, $P<0.0001$). Whole-animal RMR did not correlate with individual body mass ($r_{38}=0.29$, $t=1.90$, $P=0.06$, $N=40$; Fig. S1), while in the previously published version it was significant at $P=0.04$.

Association of T₃ levels and RMR

T₃ levels ($\chi^2=5.43$, d.f.=7, $P=0.01$; Table 2, Fig. 2) and season ($\chi^2=26.32$, d.f.=7, $P<0.001$; Table 2, Fig. 2) influenced log-transformed whole-animal RMR, while sex and body mass had no significant effect (sex: $\chi^2=0.21$, d.f.=7, $P=0.63$; body mass: $\chi^2=2.06$, d.f.=7, $P=0.15$; Table 2). In the moist season, log-transformed T₃ levels and log-transformed whole-animal RMR were negatively correlated ($r_{16}=-0.54$, $t=-2.62$, $P=0.018$, $N=18$; Fig. 2), while they were not correlated in the dry season ($r_{20}=-0.08$, $t=-0.38$, $P=0.70$, $N=22$; Fig. 2). T₃ levels ($\chi^2=14.43$, d.f.=6, $P=0.001$; Table S1, Fig. S2), season ($\chi^2=23.59$, d.f.=6, $P<0.001$; Table S1, Fig. S2) and their interaction ($\chi^2=32.86$, d.f.=4, $P<0.001$; Table S1, Fig. S2) influenced log-transformed mass-adjusted RMR, whereas sex had no influence ($\chi^2=1.32$, d.f.=6, $P=0.24$; Table S1).

Table 2. Relationship between RMR and T₃ levels

| | Estimate | s.e. | <i>t</i> | <i>P</i> |
|----------------|----------|-------|----------|-------------------|
| Intercept | 2.918 | 0.090 | 32.20 | <0.0001 |
| T ₃ | -0.111 | 0.030 | -3.63 | 0.0010 |
| Sex | -0.012 | 0.084 | -0.15 | 0.8815 |
| Season | 0.452 | 0.078 | 5.75 | <0.0001 |
| Body mass | 0.102 | 0.044 | 2.32 | 0.0310 |

RMR, resting metabolic rate. Results of the linear mixed model examining the relationship between whole-animal RMR (kJ day^{-1} , log-transformed) and T₃ levels (pmol l^{-1}). The model controlled for random intercepts of individual ID and group ID. R^2 conditional for the model: $R^2=0.96$. Significant contrasts are in bold. Previously, body mass was not a significant predictor of RMR.

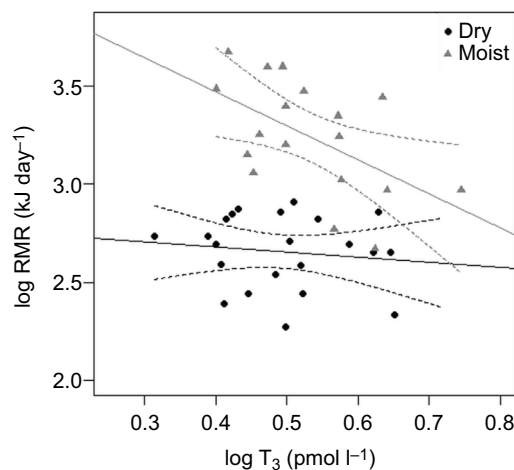


Fig. 2. Relationship between log-transformed T₃ levels and log-transformed whole-animal RMR. Data are presented for the dry season ($N=22$) and moist season ($N=18$). Linear regression lines (dry season: $y=2.96x-0.08$; $R^2=-0.04$, $P=0.70$; moist season: $y=4.22x-0.69$; $R^2=0.25$, $P=0.01$) are shown as continuous lines, and dashed lines indicate the 95% CI.

Table S1. Relationship between RMR and T_3 levels

| | Estimate | s.e. | <i>t</i> | <i>P</i> |
|---------------|----------|-------|----------|-------------------|
| Intercept | 0.134 | 0.081 | 1.65 | 0.1064 |
| T_3 | -0.066 | 0.046 | -1.41 | 0.1664 |
| Sex | -0.069 | 0.080 | -0.86 | 0.3913 |
| Season | 0.332 | 0.065 | 5.08 | <0.0001 |
| T_3 *season | -0.108 | 0.050 | -2.14 | 0.0377 |

Linear mixed model examining the relationship between mass-adjusted RMR ($\text{ml O}_2 \text{g}^{-1} \text{h}^{-1}$, log-transformed) and T_3 levels (pmol l^{-1}). The model controlled for random intercepts of individual ID and group ID. R^2 conditional for the model: $R^2=0.99$. Significant contrasts are given in bold. Previously, the interaction between T_3 and season was not significant.

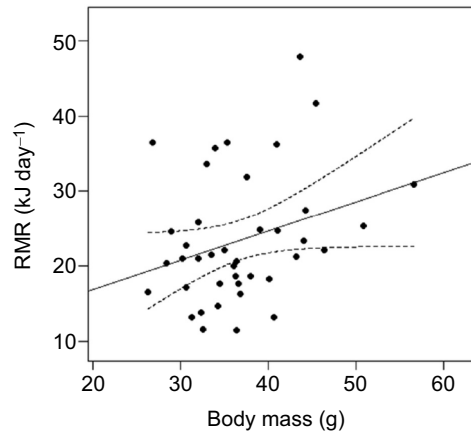


Fig. S1. Relationship between body mass and whole-animal RMR. The linear regression line ($y=9.21x+0.38$; $R^2=0.06$, $P=0.06$) is shown and dashed lines indicate the 95% CI.

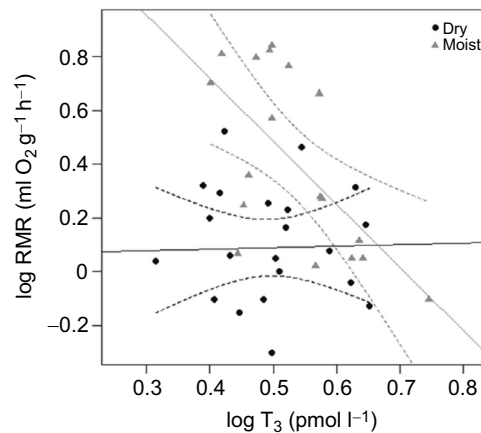


Fig. S2. Relationship between log-transformed T_3 levels and log-transformed mass-adjusted RMR in the dry season (filled circles) and moist season (grey triangles). Linear regression lines (dry season: $y=0.06x+0.02$; $R^2=-0.04$, $P=0.92$; moist season: $y=1.65x-1.01$; $R^2=0.36$, $P=0.004$) are shown as continuous lines, and dashed lines indicate the 95% CI.

There are no changes to the conclusions of the paper. The authors apologise for any inconvenience this may have caused.

RESEARCH ARTICLE

Both thyroid hormone levels and resting metabolic rate decrease in African striped mice when food availability decreases

Rebecca Rimbach^{1,*}, Neville Pillay¹ and Carsten Schradin^{1,2}

ABSTRACT

In response to variation in food availability and ambient temperature (T_a), many animals show seasonal adaptations in their physiology. Laboratory studies showed that thyroid hormones are involved in the regulation of metabolism, and their regulatory function is especially important when the energy balance of an individual is compromised. However, little is known about the relationship between thyroid hormones and metabolism in free-living animals and animals inhabiting seasonal environments. Here, we studied seasonal changes in triiodothyronine (T_3) levels, resting metabolic rate (RMR) and two physiological markers of energy balance (blood glucose and ketone bodies) in 61 free-living African striped mice (*Rhabdomys pumilio*) that live in a semi-arid environment with food shortage during the dry season. We predicted a positive relationship between T_3 levels and RMR. Further, we predicted higher T_3 levels, blood glucose levels and RMR, but lower ketone body concentrations, during the moist season when food availability is high compared with summer when food availability is low. RMR and T_3 levels were negatively related in the moist season but not in the dry season. Both RMR and T_3 levels were higher in the moist than in the dry season, and T_3 levels increased with increasing food availability. In the dry season, blood glucose levels were lower but ketone body concentrations were higher, indicating a change in substrate use. Seasonal adjustments in RMR and T_3 levels permit a reduction of energy expenditure when food is scarce, and reflect an adaptive response to reduced food availability in the dry season.

KEY WORDS: Triiodothyronine, Metabolism, Energetics, Fasting, Starvation, Drought

INTRODUCTION

Thyroid hormones are important regulatory hormones that affect a multitude of physiological processes such as the metabolism of protein, fat and carbohydrates, growth and non-shivering thermogenesis (Freaker and Oppenheimer, 1995; Kim, 2008; Silva, 2005, 2011). Moreover, thyroid hormones regulate seasonal adjustments of body mass, food intake and exercise (Barrett et al., 2007; Ciloglu et al., 2005; Ebling and Barrett, 2008), and are also involved in the timing and cessation of reproduction in seasonal breeders (Billings et al., 2002; Evans et al., 1966; Thrun et al., 1996). Thus, thyroid hormones could enable adaptive physiological responses to environmental and seasonal change. Here, we refer to

any physiological adjustment of an organism in response to an environmental stimulus resulting in the improved ability of that organism to cope with its changing environment as physiological adaptation (Boratynski et al., 2017; Brinkmann et al., 2014; Rymer et al., 2016). However, physiological adaptation is also often called acclimatization (Noakes et al., 2017; Petit et al., 2013).

Thyroid hormones, especially triiodothyronine (T_3), the major biologically active form of thyroid hormones (Freaker and Oppenheimer, 1995; Yen, 2001), stimulate tissue oxygen consumption, which results in corresponding effects on metabolic rate (Hulbert, 2000; Kim, 2008; López et al., 2013). Accordingly, a positive relationship between thyroid hormone levels and metabolic rate has been found in captive reptiles (Joos and John-Alder, 1990), birds (Vézina et al., 2009) and mammals (Banta and Holcombe, 2002; Brinkmann et al., 2016; Li et al., 2010), including humans (reviewed in Hulbert, 2000; Kim, 2008). In contrast, other studies report no relationship between metabolic rate and thyroid hormones in captive mammals (Nilssen et al., 1984; Ostrowski et al., 2006), including humans (Bernstein et al., 1983; Johnstone et al., 2005). However, little information is available concerning the relationship between thyroid hormones and metabolic rate for animals living under natural conditions (exceptions are studies on birds: Chastel et al., 2003; Elliott et al., 2013; Welcker et al., 2013; Zheng et al., 2014; and a study of free-ranging Arctic ground squirrels *Urocitellus parryii*; Wilsterman et al., 2015), and the reported results are ambivalent. Some studies report a positive relationship between T_3 levels and metabolic rate (Chastel et al., 2003; Welcker et al., 2013) while others found no relationship (Burger and Denver, 2002). This inconsistency in results highlights the need for additional studies examining the relationship between thyroid hormone levels and metabolic rate of free-living animals, especially mammals. Additionally, there is a paucity of data concerning seasonal changes in the relationship between thyroid hormone levels and resting metabolic rate (RMR) or basal metabolic rate (BMR) in free-living animals because most studies examined both factors only during one season (i.e. the breeding season: Chastel et al., 2003; Elliott et al., 2013; Welcker et al., 2013). To our knowledge, seasonal variation in the relationship between thyroid hormone levels and metabolic rate has not been studied in free-living mammals.

Many animals have to adjust their physiology to maintain a state of energy balance throughout seasonal changes in food availability and/or ambient temperature (T_a). Thyroid hormones are believed to play a key role in the regulation of seasonal and acute changes in metabolism, and the regulating function of thyroid hormones should be especially important during periods of reduced food availability, when animals should lower energy expenditure (Brinkmann et al., 2016; Flier et al., 2000; Fuglei and Øritsland, 1999; Zhan et al., 2009). Accordingly, laboratory studies on humans (Carlson et al., 1977; Gardner et al., 1979), other mammals and birds found that thyroid hormone levels decrease when individuals are food deprived (Brinkmann et al., 2016;

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Diano et al., 1998; Mustonen et al., 2008; Nieminen et al., 2001) and increase during periods of high food intake (Fuglei et al., 2000; Stokkan et al., 1985). However, few field studies tested whether free-living animals show seasonal changes in thyroid hormone levels as an adaptation to changes in food availability.

Adaptations to periods of low food availability and starvation also involve changes in the mobilization of fuel substrates. Food shortage leads to decreased blood glucose levels (Larsen et al., 1985; Pambu-Gollah et al., 2000; Schradin et al., 2015). Instead of glucose, animals start metabolizing their fat reserves, producing ketone bodies as fuel for many different tissues (Delaere et al., 2010; McCue, 2010; Robinson and Williamson, 1980). Consequently, a combination of blood glucose and ketone body levels can be used as physiological markers for energy balance and state of fasting.

Maintaining energy balance is particularly challenging for animals living in seasonal environments where they experience reduced food availability. However, few studies have simultaneously considered the relationship between environmental factors (e.g. T_a and food availability) and seasonal changes in thyroid hormones and metabolic rate of free-living animals. The African striped mouse, *Rhabdomys pumilio* (Sparman 1784) is a good model species for such studies because it inhabits the semi-arid Succulent Karoo in south-western Africa, an environment with pronounced seasonal changes in food availability (Schradin and Pillay, 2006) and T_a (Scantlebury et al., 2006; Schradin and Pillay, 2004). Striped mice can lose 10% or more of their body mass during the hot dry season (Schradin and Pillay, 2005). The physiology and energy balance of multiple individuals can be studied (Schradin et al., 2015) because they are small rodents that are trap-happy. We predicted that: (i) T_3 levels, blood glucose levels and RMR will be higher during the moist season in spring, when food availability is high and T_a is low; (ii) ketone body concentrations will be higher during the dry season as a response to low food availability; there is a positive relationship between (iii) food availability and T_3 levels and (iv) T_3 levels and RMR; and (v) there is a negative relationship between T_a and T_3 levels.

MATERIALS AND METHODS

Study site and animals

We collected data in the dry seasons (January 2013, December 2013–February 2014, November 2014–April 2015) and moist seasons (August–November 2013, June 2014, June 2015–August 2015) of three consecutive years in the Goegap Nature Reserve, South Africa (29°41.56'S, 18°1.60'E). Fieldwork for this study was carried out under the necessary licences and was in accordance with the relevant South African animal welfare regulations. Animals were captured and handled following protocols approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC 2007/10/01, AESC 2007/39/04 and AESC 2014/40/B). The study area is characterized by moist winters (mean annual rainfall of 160 mm at our field site), followed by high food availability in spring and hot dry summers with low food availability (Schradin and Pillay, 2006). At the field site, striped mice are facultative group living, and groups typically consist of one breeding male, one to four breeding females and their philopatric adult offspring of both sexes (Schradin and Pillay, 2004). For individual recognition, we marked all striped mice permanently with numbered ear-tags (National Band and Tag, Newport, KY, USA) and temporarily with hair dye (Inecto, Pinetown, South Africa).

Blood sampling and hormone assays

We collected blood samples of a total from 61 adult, non-breeding philopatric striped mice. Ten individuals were sampled in both a dry

season and a moist season, thus resulting in a total of 71 blood samples for analysis of T_3 , glucose and ketone levels (dry season: 25 females and 21 males; moist season: 11 females and 14 males). We took blood samples early in the morning shortly after mice emerged from their nest, to control for possible circadian rhythms in hormone excretion (Burger and Denver, 2002). We captured individuals using Sherman-style live-traps (26×9×9 cm), which were placed close to a group's nest. We watched traps from a distance of 10 m and as soon as an individual entered a trap we removed it and anaesthetized it with diethyl ether. We took a blood sample (250–500 μ l) from the sub-lingual vein (Heimann et al., 2009; Schradin et al., 2009). The first blood drops were used to measure blood glucose levels using a One Touch Ultra Glucometer (LifeScan, Inc., Milpitas, CA, USA) and ketone body concentrations using a Freestyle Optium Blood β -Ketone meter (Abbott Diabetes Care Ltd, Witney, Oxon, UK), and then blood was collected for hormone analyses. Blood collection was terminated after 3 min to avoid an influence of elevated glucocorticoid concentrations on hormone levels (Romero and Reed, 2005). Blood sampling was terminated when all individuals of a group were sampled or, at the latest, 1 h after sunrise. After blood sampling, we weighed individuals (± 0.1 g) using an electronic balance (Yamada HC-3, Yuet Hing Company, Kowloon, Hong Kong) and released mice after 15–20 min at their nest. We left the blood samples at room temperature for 45 min, after which we centrifuged them twice for 10 min at 1000 rpm, and subsequently froze the remaining serum in aliquots of 60 μ l at -20°C until further processing at the endocrinology laboratory of the IPHC-DEPE at CNRS, Strasbourg, France.

We measured free serum T_3 using commercially available enzyme-linked immunosorbent assay (ELISA) kits from Cusabio (College Park, MD, USA). Sensitivity of the assay was 0.38 pmol l^{-1} . We assayed the samples in duplicate and followed the assay procedures for serum that accompanied these kits, but we made minor modifications concerning the standards used. Preliminary data showed that striped mice have low thyroid hormone concentrations (similar to desert rodents: Yousef and Johnson, 1975), and thus we created an additional low-concentration standard by using half the normal volume of the lowest existing standard. We validated the kit using striped mice serum prior to use by both the standard addition method and using tests of parallelism. Serial dilution curves closely followed the standard curve. Intra- and inter-assay variability were estimated using a pool created from striped mice serum. Intra-assay variation was 9.12% (9 measurements), while inter-assay variation was 16.5% (5 different measurements). Recovery of samples added to the standard curve was 101.07% for a pool created from male striped mice serum and 92.31% for a pool created from female striped mice serum.

RMR measurements

Within 3 days of blood sampling, we measured oxygen consumption ($\text{ml O}_2 \text{ h}^{-1}$) of the same individuals in the respirometry laboratory at the field site using an open circuit respirometry system (FoxBox, Sable Systems, NJ, USA). Traps were set in the mornings near the nests and checked after 1 h. Trapped mice were brought to the laboratory immediately, where they were weighed and then placed in one of three respirometry chambers (1000 cm^2 each). We initiated O_2 measurements and video recordings of the mice in the metabolic chambers using a webcam (Microsoft HD webcam). The metabolic chambers were immersed in a propylene container and the temperature was controlled using a temperature controller (Pelt5, Sable Systems).

The RMR of striped mice was measured at $30 \pm 1^\circ\text{C}$, which lies within the species' thermoneutral zone (Scantlebury et al., 2006). Readings were taken every 3 s for 4×10 min per individual within the 3 h of measurements. The flow of air ($\sim 700 \text{ ml min}^{-1}$) into the chambers was controlled by a flow regulator (FB8, Sable Systems) placed upstream, and measurements of oxygen consumption were taken using an oxygen analyser (FoxBox, Sable Systems). The analyser was calibrated to an upper and lower value in dry air weekly. After the measurements, mice were weighed again, rewarded with a small amount of food and subsequently released at their nest. For analyses, we used the mean of the lowest 89 readings (equal to 4.45 min) of oxygen consumption for each individual, when it was seen to be at rest in the video. Of the 71 individuals tested, 43% (31 mice: 16 females and 15 males) remained restless in the respirometry trials and were thus excluded; hence, the relationship between RMR and T_3 levels was examined in 40 individuals in total (dry season: 11 females and 11 males; moist season: 9 females and 9 males).

Food availability

Biweekly we conducted plant surveys (on the 1st and the 15th of each month) in eight randomly located monitoring plots (each 4 m^2), using the Braun–Blanquet method (Werger, 1974). We recorded the number of edible plant species per plot and used the average number of plant species recorded in all eight plots for further analysis (Schradin and Pillay, 2006; Schradin et al., 2015).

T_a

For every individual, we recorded average T_a in the week prior to the T_3 measurement using an automated weather station (Orion Weather Science, South Africa), which recorded outside temperature every 10 min.

Statistical analysis

T_3 levels, RMR, glucose levels and ketone body concentrations differed significantly from normality (Shapiro–Wilk W -tests: $P > 0.05$), and we log-transformed RMR and T_3 levels to reach normality. Body mass data were normally distributed, and we used t -tests to examine seasonal differences in mass-adjusted RMR, T_3 levels and body mass. We used an ANCOVA, with body mass as a co-variate, to examine seasonal differences in whole-animal RMR; Wilcoxon rank sum tests to determine seasonal differences in glucose levels and ketone body concentrations; and Pearson's product-moment correlations to examine whether T_3 levels (log-transformed) correlated with whole-animal RMR (log-transformed) in the dry and the moist season, and to assess whether body mass correlated with whole-animal (not corrected for body mass) RMR.

To assess the relationship between T_3 levels and food availability (model 1, response variable: log-transformed T_3 levels), T_3 levels and RMR (model 2, response variable: log-transformed whole-animal RMR), and T_3 levels and T_a (model 3, response variable: log-transformed T_3 levels), we fitted LMMs with the 'glmer' function from the lme4 package (<https://CRAN.R-project.org/package=lme4>). We used individual ID and group ID as random factors in all models. In model 1, we used food availability and sex as explanatory variables. In model 2, we used T_3 levels, sex and season as explanatory variables and body mass as a co-variate. We included body mass as a co-variate because of the strong positive correlation between metabolic rate and body mass (Gillooly et al., 2001; Kleiber, 1947). In model 3, we used mean ambient temperature and sex as explanatory variables. We tested for interactions between the variables T_3 and sex, T_3 and season, and season and body mass

(model 2), and dropped all interaction terms as a result of non-significance. To determine the significance of full models compared with corresponding null models, we used likelihood ratio tests (R function 'anova' with argument test set to 'Chisq'). Only if this likelihood ratio test revealed a significant difference did we consider the significance of the individual predictors. We standardized (z -transformed) all numeric predictors for more accurate model fitting and to facilitate comparisons of model estimates (Schielzeth, 2010). For model validation, we visually inspected $Q-Q$ plots and scatterplots of residuals plotted against fitted values, and we checked for the assumptions of homogeneous and normally distributed residuals. To assess model stability, we ran diagnostics (dfbetas) that did not suggest the existence of influential cases, and variance inflation factors (all < 2) indicated that there was no collinearity between variables (Zuur et al., 2010). To derive variance inflation factors, we used the function 'vif' of the R package car (Fox and Weisberg, 2011). We determined $R^2_{(c)}$ (where 'c' stands for conditional) for the minimal adequate models with the function 'r.squaredGLMM' from the MuMIn package (v.1.9.13, <https://CRAN.R-project.org/package=MumIn>). $R^2_{(c)}$ indicates the variance explained by both fixed and random factors. To obtain more reliable P -values, we used the function 'pvals.fnc' from the package languageR (Baayen, 2010). In this function, P -values are based on Markov Chain Monte Carlo (MCMC) sampling (<https://CRAN.R-project.org/package=languageR>; Baayen, 2010). All analyses were conducted in R (v.3.2.4, <https://www.r-project.org/>). All statistical tests were two tailed, and the statistical threshold was set at $P \leq 0.05$.

RESULTS

Seasonal variation in T_3 levels, physiological markers, RMR and body mass

T_3 levels were higher in the moist season than in the dry season ($t_{54.83} = -2.36$, $P = 0.021$). Blood glucose levels were higher (Wilcoxon rank sum test: $W = 394.5$, $P = 0.04$) whereas ketone body concentrations were lower ($W = 831.5$, $P < 0.001$) in the moist season than in the dry season. Mass-adjusted RMR ($\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$) was higher in the moist season than in the dry season ($t_{26.67} = -4.09$, $P < 0.001$). Whole-animal RMR also varied seasonally (ANCOVA: $F = 51.26$, $P < 0.0001$). Both females and males were heavier in the moist season than in the dry season (mean \pm s.d.: females: 35.5 ± 6.4 versus 31.8 ± 4.0 g, $t_{16.75} = -2.09$, $P = 0.048$; males: 45.4 ± 8.5 versus 35.3 ± 4.6 g, $t_{23.15} = -5.68$, $P < 0.001$). Whole-animal RMR positively correlated with individual body mass ($r_{38} = 0.30$, $t = 1.97$, $P = 0.04$, $N = 40$; Fig. S1).

Relationship between T_3 levels and food availability

Food availability significantly influenced log-transformed T_3 levels ($\chi^2 = 6.15$, d.f. = 5, $P = 0.013$; Table 1), and there was a positive relationship between food availability and T_3 levels (Fig. 1). Sex did not influence log-transformed T_3 levels ($\chi^2 = 1.21$, d.f. = 5, $P = 0.26$; Table 1).

Table 1. Relationship between T_3 levels and food availability

| | Estimate | s.e. | t | P_{MCMC} |
|-------------|----------|-------|--------|-------------------|
| Intercept | 1.153 | 0.040 | 28.661 | 0.0001 |
| Food plants | 0.061 | 0.024 | 2.492 | 0.0196 |
| Sex | 0.041 | 0.049 | 0.837 | 0.4452 |

T_3 , triiodothyronine. Results of the linear mixed model testing for an association between T_3 levels (pmol l^{-1} , log-transformed) and food availability. The model controlled for random intercepts of individual ID and group ID. R^2 conditional for the model: $R^2 = 0.27$. Significant contrasts are in bold. P_{MCMC} , P -values based on Markov Chain Monte Carlo sampling.

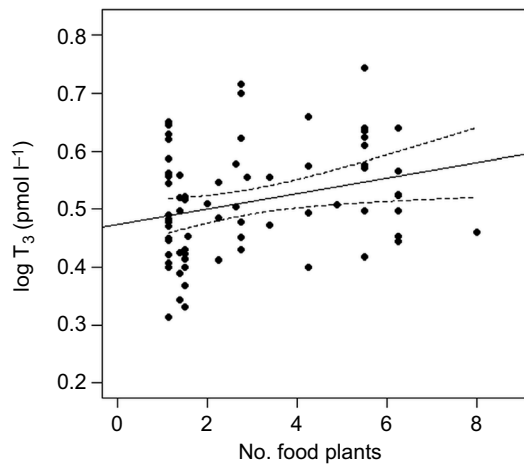


Fig. 1. Relationship between food availability (number of food plants) and log-transformed T_3 levels. $N=71$ samples. The linear regression line ($y=1.08x+0.03$; $R^2=0.07$, $P=0.018$) is shown as a continuous line and dashed lines indicate the 95% confidence interval (CI).

Association of T_3 levels and RMR

T_3 levels ($\chi^2=6.28$, d.f.=7, $P=0.01$; Table 2, Fig. 2) and season ($\chi^2=33.64$, d.f.=7, $P<0.001$; Table 2, Fig. 2) influenced log-transformed whole-animal RMR, while sex and body mass had no significant effect (sex: $\chi^2=0.08$, d.f.=7, $P=0.77$; body mass: $\chi^2=0.41$, d.f.=7, $P=0.51$; Table 2). In the moist season, log-transformed T_3 levels and log-transformed whole-animal RMR were negatively correlated ($r_{16}=-0.54$, $t=-2.57$, $P=0.020$, $N=25$; Fig. 2), while they were not correlated in the dry season ($r_{20}=-0.12$, $t=-0.56$, $P=0.57$, $N=46$; Fig. 2). T_3 levels ($\chi^2=18.65$, d.f.=6, $P<0.001$; Table S1, Fig. S2) and season ($\chi^2=34.08$, d.f.=6, $P<0.001$; Table S1, Fig. S2) influenced log-transformed whole-animal RMR, whereas sex had no influence ($\chi^2=2.46$, d.f.=6, $P=0.11$; Table S1).

Influence of T_a on T_3 levels

Mean T_a ($\chi^2=4.53$, d.f.=5, $P=0.03$; Table 3) influenced log-transformed T_3 levels, with log-transformed T_3 levels increasing with decreasing T_a (Fig. 3). Sex did not influence log-transformed T_3 levels ($\chi^2=1.05$, d.f.=5, $P=0.30$; Table 3).

DISCUSSION

To summarize the results, we found seasonal adjustments in physiological markers of energy balance, T_3 levels and RMR in striped mice, which reflected changes in food availability. As predicted, blood glucose levels were lower but ketone body concentrations were higher in the dry season when food availability was low compared with the food-rich moist season, indicating a change in fuel use. T_3 levels increased with increasing

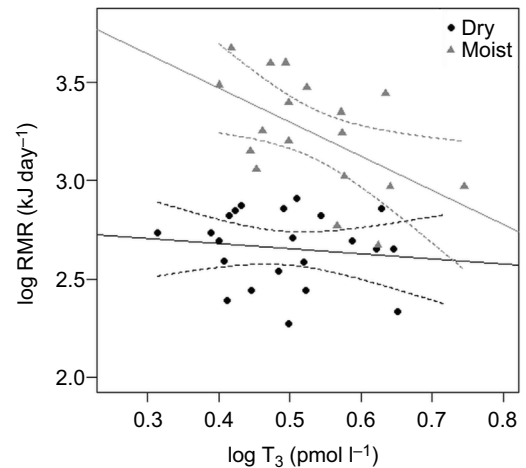


Fig. 2. Relationship between log-transformed T_3 levels and log-transformed whole-animal RMR. Data are for the dry season ($N=46$) and moist season ($N=25$). Linear regression lines (dry season: $y=2.78x-0.11$; $R^2=-0.03$, $P=0.57$; moist season: $y=4.15x-0.74$; $R^2=0.29$, $P=0.02$) are shown as continuous lines, and dashed lines indicate the 95% CI.

food availability and decreased with increasing T_a . The seasonal variation in RMR was associated with T_3 levels. Unexpectedly, however, we found a negative instead of the predicted positive relationship between T_3 levels and RMR in the moist season, and no relationship between them in the dry season. Reduced RMR and low T_3 levels in the dry season are likely to reflect an adaptive mechanism to reduce energy expenditure when food resources are scarce.

Both physiological markers of energy balance – glucose levels and ketone body concentrations – showed predicted seasonal variation. Glucose is the end product of carbohydrate metabolism and is the primary energy source for the body. In concurrence, glucose levels were higher in the moist season, when food availability was high and striped mice spent large portions of the day foraging (Rimbach et al., 2016; Schradin, 2006). A similar result was reported in a long-term study on striped mice at our field site, where blood glucose levels increased with increasing food availability (Schradin et al., 2015). Likewise, glucose levels of common frogs (*Rana temporaria*) and Norwegian reindeer (*Rangifer tarandus tarandus*) were lower during periods of low food availability (Larsen et al., 1985; Smith, 1949). In contrast to glucose levels, ketone body concentrations were higher in the dry season, when striped mice experienced reduced food availability. Similarly, ketone body concentrations increased in response to low availability of food or starvation in Bornean orangutans (*Pongo pygmaeus*), Arctic foxes (*Alopex lagopus*) and black bears (*Ursus americanus*) (Fuglei et al., 2000; Knott, 1998; LeBlanc et al., 2001). Likewise, a recent study found that the health of striped mice deteriorated (i.e. elevated blood parameters indicative of liver injury

Table 2. Relationship between RMR and T_3 levels

| | Estimate | s.e. | t | P_{MCMC} |
|-----------|----------|-------|--------|-------------------|
| Intercept | 2.668 | 0.084 | 31.670 | 0.0001 |
| T_3 | -0.101 | 0.037 | -2.720 | 0.0484 |
| Sex | 0.018 | 0.080 | 0.230 | 0.6142 |
| Season | 0.567 | 0.081 | 6.970 | 0.0001 |
| Body mass | 0.044 | 0.057 | 0.770 | 0.9212 |

RMR, resting metabolic rate. Results of the linear mixed model examining the relationship between whole-animal RMR (kJ day^{-1} , log-transformed) and T_3 levels (pmol l^{-1}). The model controlled for random intercepts of individual ID and group ID. R^2 conditional for the model: $R^2=0.75$. Significant contrasts are in bold.

Table 3. Relationship between T_3 levels and T_a

| | Estimate | s.e. | t | P_{MCMC} |
|------------|----------|-------|--------|-------------------|
| Intercept | 1.341 | 0.099 | 13.472 | 0.0001 |
| Mean T_a | -0.009 | 0.004 | -2.172 | 0.0501 |
| Sex | 0.052 | 0.049 | 1.057 | 0.3330 |

Results of the linear mixed model examining the relationship between T_3 levels (pmol l^{-1} , log-transformed) and ambient temperature (T_a). The model controlled for random intercepts of individual ID and group ID. R^2 conditional for the model: $R^2=0.31$. Significant contrasts are in bold.

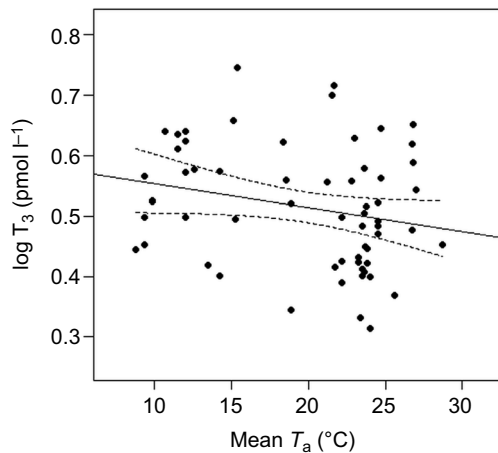


Fig. 3. Relationship between mean T_a and log-transformed T_3 levels. $N=71$ samples. The linear regression line ($y=1.35x-0.008$; $R^2=0.03$, $P=0.07$) is shown as a continuous line and dashed lines indicate the 95% CI.

and of toxicity) during the long and hot dry season, and that ketone body concentrations decreased from the dry season to the subsequent moist season when food availability increased again (Schoepf et al., 2016). Our results indicate that striped mice depleted their glycogen stores and mobilized energy from adipose tissue during the dry season. Elevated concentrations of ketone bodies signal that individuals were fasting or starving (McCue, 2010), and suggest that individuals were using more energy than they could gain from ingested food sources. These results indicate that striped mice were food restricted during the dry season and had to find a way to cope with reduced energy availability.

As predicted, both RMR and T_3 levels were lower in the hot dry season when food was restricted compared with the moist season with high food availability. Similar seasonal changes in RMR have been reported in several other mammals (Merkt and Taylor, 1994; Ostrowski et al., 2006; Song and Wang, 2006; Tomasi and Mitchell, 1996). Striped mice were heavier in the moist season, which is one likely reason for the elevated RMR in this season because RMR increases with increasing body mass (Gillooly et al., 2001; Kleiber, 1947). Low T_a in the moist season is also likely to have contributed to the elevated RMR of striped mice. Similarly, cold-acclimated house mice (*Mus musculus*) had higher BMR and larger metabolically active organs than warm-acclimated mice (Konarzewski and Diamond, 1995). In sum, the seasonal changes in RMR indicate an adaptive response to reduced food (i.e. energy) availability in summer and increased thermoregulatory needs in spring.

T_a also influenced T_3 levels, which increased with decreasing T_a and with increasing food availability. At our study site, high levels of food availability coincide with low T_a during the moist season (Schradin and Pillay, 2006). Our findings are consistent with previous studies that report upregulated thyroid activity in response to low T_a (El-Nouty et al., 1978; Tomasi, 1991; Tomasi and Mitchell, 1996), an adaptive response because thyroid hormones stimulate metabolic heat production (non-shivering thermogenesis) during cold exposure (Kim, 2008). Moreover, thyroid hormones also stimulate food intake and regulate the level of exercise (Ciloglu et al., 2005; Kong et al., 2004; Stokkan et al., 1985). Therefore, elevated T_3 levels of striped mice during the moist season are probably adaptive because they should result in an elevation in metabolic heat production when individuals experience low T_a and promote increased foraging when food availability is high.

Both RMR and T_3 levels varied with seasonal changes in food availability and T_a , and thus T_3 could have a direct influence on RMR, as indicated by several laboratory studies (Banta and Holcombe, 2002; Joos and John-Alder, 1990; Li et al., 2010). To our knowledge, this study is the first to examine seasonal changes in the relationship between thyroid hormone levels and RMR in a free-living mammal. Interestingly, we found different relationships between T_3 levels and RMR in the two seasons. During the hot dry season, we found no relationship between RMR and T_3 levels. Similarly, several studies on humans failed to find an association between circulating concentrations of T_3 and either BMR or RMR (Bernstein et al., 1983; Johnstone et al., 2005). Likewise, there was no correlation between T_3 levels and RMR in Arabian oryx (*Oryx leucoryx*) during the summer, when food and water were restricted (Ostrowski et al., 2006). The lack of a correlation in the oryx was interpreted to result from the involvement of thyroid hormones in the release of non-esterified fatty acids from adipocytes when the animals reached a negative energy balance (Heimberg et al., 1985), which may also be the case in the striped mice. The reasons, however, for the negative relationship between striped mice RMR and T_3 levels in the moist season are currently unclear, as normally T_3 is expected to increase metabolic rate. In the Succulent Karoo, food availability is high in the moist season and striped mice gain body mass in preparation for the subsequent breeding season and to survive the next dry season (Schradin et al., 2014). Many seasonal breeding bird and mammal species utilize thyroid hormones for timing the onset and cessation of reproductive activity (Evans et al., 1966; Hulbert, 2000; Thrun et al., 1996). The negative relationship between RMR and T_3 levels may result from interactions between T_3 and steroid hormones such as testosterone, which are also involved in the regulation of reproduction. For example, an inverse phase relationship between seasonal changes in thyroid hormone levels and testosterone levels has been suggested for red foxes (*Vulpes vulpes*) (Maurel and Boissin, 1981). Alternatively, the relationship between T_3 levels and RMR may be influenced by seasonal changes in leptin levels. Falling leptin levels decrease energy expenditure and suppress the thyroid axis (Flier et al., 2000), and in striped mice, leptin levels decrease when food availability increases (Schradin et al., 2014). Extensive blood sampling (for measurements of T_3 , testosterone and leptin) combined with RMR measurements throughout the moist and subsequent breeding season may resolve whether interactions between T_3 levels and testosterone and T_3 levels and leptin can explain the negative relationship found in this study.

Conclusions

Striped mice show seasonal changes in their RMR, T_3 levels and physiological markers of energy balance. These changes are likely to reflect adaptive responses to differences in food availability and T_a , which permit a reduction of energy expenditure when food is scarce. While we found no relationship between RMR and T_3 levels in the dry season, we found a negative relationship in the moist season, which deviates from previous, mainly laboratory, studies. Further studies will be necessary to assess the causes of this unexpected result.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

R.R., N.P. and C.S. contributed to conception and design; R.R. collected blood samples and conducted metabolic rate measurements; C.S. contributed to the establishment of the RMR measurements and ran ELISA kits; R.R. analysed the data and all authors contributed to interpretation of the results and writing of the article.

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Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.151449.supplemental>

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Table S1. Relationship between RMR and T₃ levels. Linear Mixed Model examining the relationship between mass-adjusted RMR (ml O₂ g⁻¹h⁻¹, log-transformed) and T₃ levels (pmol L⁻¹). The model controlled for random intercepts of individual ID and group ID. R² conditional is given for the model. Significant contrasts are given in bold.

| R² = 0.98 | Estimate | SE | t | P _{MCMC} |
|-----------------------------|----------|-------|--------|-------------------|
| Intercept | 0.729 | 0.114 | 6.379 | 0.0188 |
| T ₃ | -0.237 | 0.026 | -9.021 | 0.0084 |
| Season | 0.435 | 0.062 | 6.989 | 0.0001 |
| Sex | -0.116 | 0.076 | -1.532 | 0.1096 |

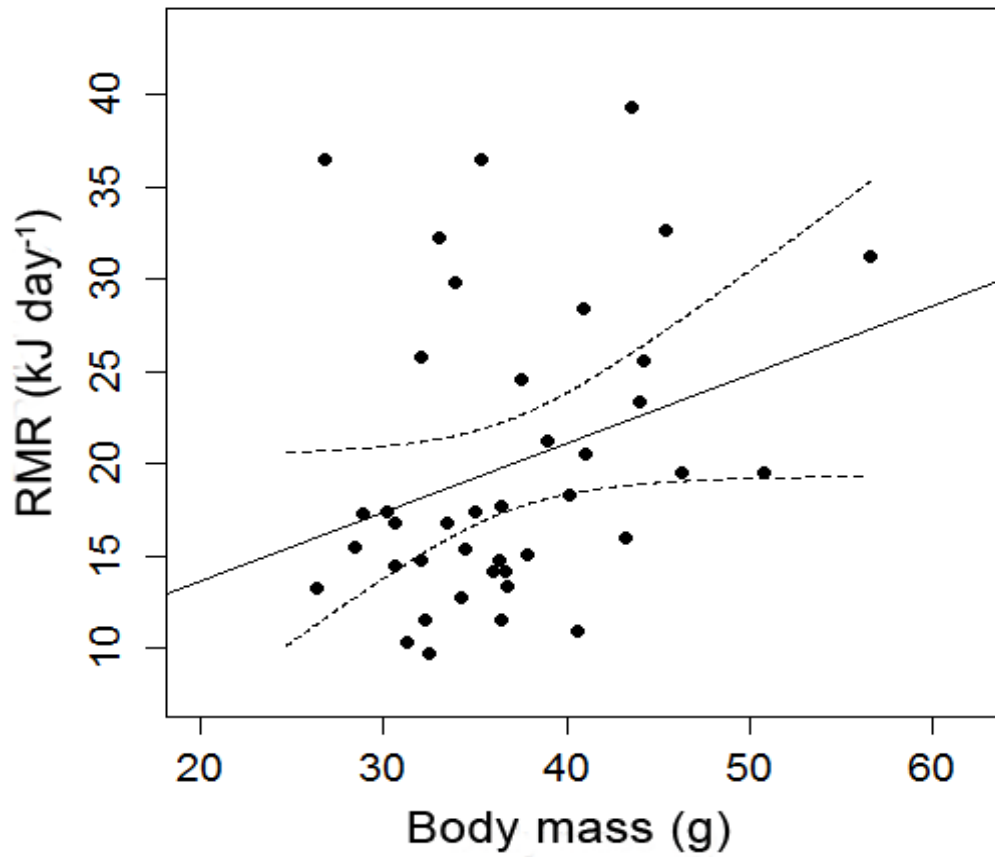


Fig. S1. Relationship between body mass and whole-animal RMR. The linear regression line ($y = 6.17x + 0.37$; $R^2 = 0.06$, $P = 0.048$) is shown and dashed lines indicate the 95% c.i.

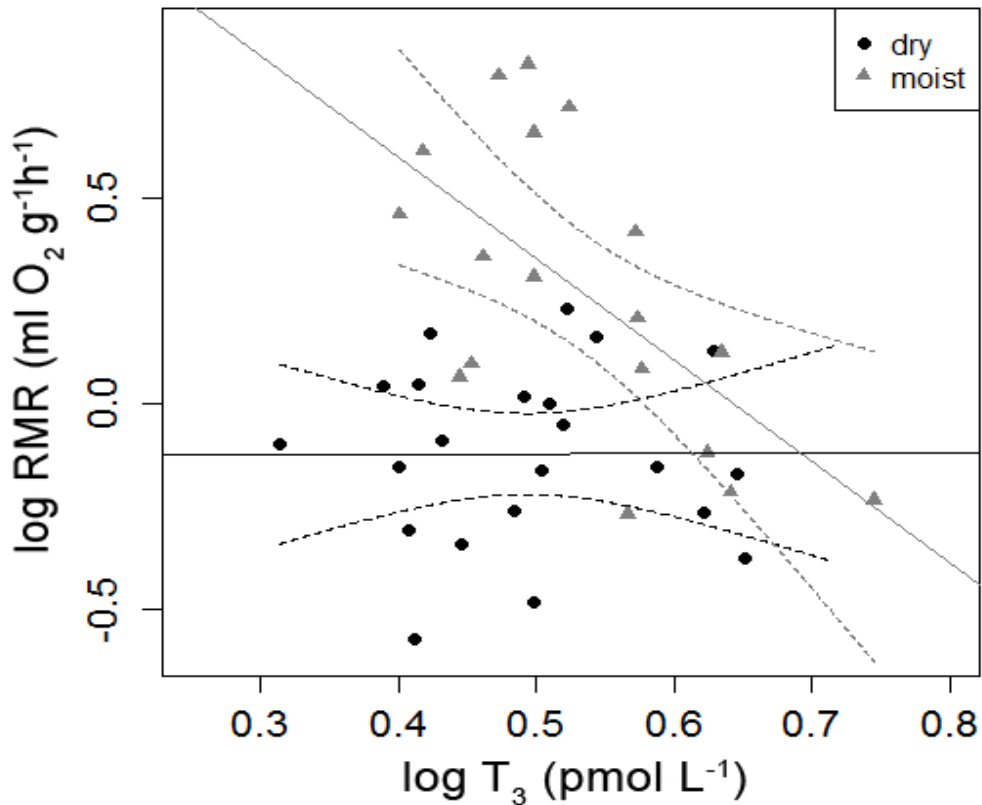


Fig. S2. Relationship between log-transformed T₃ levels and log-transformed mass-adjusted RMR in the dry season (filled circles) and moist season (grey triangles). Linear regression lines ($y = -0.12x + 0.001$; $R^2 = -0.05$, $P = 0.99$; moist season: $y = -1.58x - 1.06$; $R^2 = 0.38$, $P = 0.005$) are shown as continuous lines, and dashed lines indicate the 95% c.i.