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DAILY ENERGY EXPENDITURE IN THE POUCHED MOUSE
(SACCOSTOMUS CAMPESTRIS PETERS 1896)

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ABSTRACT

Previous studies of the pouched mouse (Saccostomus campestris) have revealed that during reproduction there is no increase in food consumption, although resting energy demands, measured by respirometry, increase substantially. One explanation for this anomalous situation is that these mice may routinely use torpor to compensate their energy budgets.

We measured the daily energy expenditure of eleven individual pouched mice over three consecutive days using the doubly-labelled water technique. The mice were housed in cages where they had free access to food and water, at an average temperature of 26.5 °C, and exposed to a natural photoperiod (February Pretoria).

There was a large day-to-day variation in energy expenditure within each individual. The coefficient of variation in daily energy demand averaged 24.5%. By comparing the correlation of estimates for consecutive and non-consecutive days we established that this variation was not a consequence of errors in the isotopic technique.

The scaling exponent of the measures of energy expenditure to mass was 1.196 (sd = 0.37 : not significantly different to 1.0). We corrected all the estimates to a mean mass of 61.3 g using a scaling exponent of 1.0. We then compared the daily energy demands with expected energy requirements for endothermic animals at rest in respirometers. All the estimates of daily energy expenditure exceeded those anticipated from the resting costs, and averaged about 2.1x greater. We found no compelling evidence therefore that Saccostomus campestris routinely utilizes long (>8 hours) bouts of torpor to compensate its energy budget.

INTRODUCTION

The pouched mouse (Saccostomus campestris) is a small fossorial cricetomyinid rodent which is found throughout southern Africa (Kingdon, 1974), ranging northwards to the equator in central Kenya (Neal, 1984). It is sexually dimorphic, with males being slightly heavier than females (Skinner and Smithers, 1990). Although the range in mass for each

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sex is large. *S. campestris* is absent from arid areas (rainfall less than 250 mm annually) and is most abundant in habitats where there are areas of open sand in which it can burrow easily (Kingdon, 1974). In South Africa pouched mice breed between October and February (Swanepoel, 1972), but further north the breeding season is later (Smithers and Wilson, 1979).

Perrin and Clarke (1987) investigated the bioenergetics of pregnancy and lactation in a single *S. campestris* that was housed in the laboratory. In contrast to studies of most other small rodents (Studier, 1979; Innes and Millar, 1981; Glazier, 1985) they suggested that throughout pregnancy and lactation the intake of food by the mouse did not increase above that of nonreproducing individuals. Indeed, during pregnancy the intake per mouse decreased. This anomaly was compounded by the fact that measures of respiratory energy expenditure indicated that during pregnancy, energy expenditure (per gram) was increased by 55%, and during lactation by 110%, the latter being significantly greater than in nonreproducing individuals. In contrast, most other reproducing small rodents show no increase in resting energy expenditure between pregnancy and lactation (Nicoll and Thompson, 1987).

Animals may cope with the energy demands of pregnancy and lactation in two fundamentally different ways. Firstly, increase in energy requirements may be met by increasing the food intake, or withdrawing stored energy from fat reserves. Most small mammals appear to use this method (reviewed in Racey and Speakman, 1987). Perrin and Clarke (1987) suggested that the absence of an increase in energy ingestion in *Saccosomus* might be a consequence of withdrawing stored fat and protein.

The second method is to reduce expenditure on some other component of the budget. We have previously shown in the brown long-eared bat (*Plecotus auritus*) that the pattern of energy expenditure throughout the reproductive period reflects a complex oscillation between increasing food intake and compensatory mechanisms (Speakman and Racey, 1987; Racey and Speakman, 1987). The most potent method for achieving such energy savings is to reduce body temperature by becoming torpid. The utilization of torpor by *S. campestris* might therefore explain the lack of increase in its energy demands throughout reproduction.

Ellison and Skinner (1990) have shown that some individual *S. campestris* (30 – 40%) will fall into torpor in respirometers when exposed to low temperatures. However, at the high temperatures which characterize the breeding season, the mice do not normally use torpor during the relatively short measurements that are made in respirometers. It is interesting, however, that measurements of the nonshivering thermogenic capacity of summer-acclimated (February) *S. campestris* reveal a higher level than expected (Haim et al., 1991). Small insectivorous bats also have functionally active brown adipose tissue throughout their reproductive period (Trayhurn et al., 1990), presumably to support the high levels of thermogenesis necessary to rewarm from periodic torpor. The high thermogenic capacity of immediately post-reproductive *S. campestris* may thus indicate that outside of respirometers the utilization of torpor is more frequent. This would be consistent with anecdotal accounts of individuals found in the wild that are sluggish and easily caught by hand (Skinner and Smithers, 1990; Shortridge, 1971; Kingdon, 1974).
In this paper we investigated the energy expenditure of summer-acclimated (February) *S. campestris* held in cages, using the doubly-labelled water technique (Lifson and McClintock, 1966; Nagy, 1980; Speakman and Racey, 1988a). We then compared the measured levels of energy expenditure with those expected from measurements of nontorpid individuals in respirometers. By making this comparison we hoped to establish the extent to which the animals use torpor when they are not in respirometers.

**METHODS**

We used 11 individual pouched mice (5 male and 6 female), all of which were not breeding but were adult. The mice were all captured from the Vaalkop Dam Nature reserve, South Africa (25°S), except one which was captured at Bithulie (Orange Free State) (20°S). All the individuals were measured on the same three days during the austral summer (February), approximately three weeks after capture.

**EXPERIMENTAL PROTOCOL**

The doubly-labelled water (DLW) technique is a method for the measurement of animal energy expenditure. It depends on the differential turnover of oxygen and hydrogen isotopes introduced into the animal's body water (Lifson and McClintock, 1966; Nagy, 1980; Speakman and Racey, 1988a). We have previously shown that any metabolic consequences of the procedures of the technique are undetectable (Speakman et al., 1991).

Individual mice were removed from their cages, weighed and then injected intraperitoneally with about 0.4 ml of a mixture of heavy oxygen (18O : 20 APE) and heavy hydrogen (2H : 10 APE). The mice were returned to their cages for a period of 90 minutes and a small (80 µl) sample of blood was collected for isotopic analysis by sectioning the end of the tail. The mouse was then returned to its cage. All the initial blood samples from the 11 individuals were removed over a period of three hours on the first day of measurement. Hence the measurements were at worst 88% simultaneous. At exactly 24 hours, 48 hours, and 72 hours, further blood samples were collected from each individual for isotopic analysis. The importance of timing samples exactly at 24-hour intervals has been previously highlighted (Speakman and Racey, 1988b). We also collected background blood from two individuals to assess the background abundance of the isotopes (method B: Speakman and Racey, 1987).

Blood samples were analyzed in at least duplicate for their levels of 18O, using CO2 prepared by the guanidine chemical conversion process (Wong and Klein, 1987) and using H2 prepared by the uranium reduction process (Wong and Klein, 1987) for 2H. All gases were analyzed by isotope ratio mass spectrometry.

Carbon dioxide production was calculated using eq 36 of Lifson and McClintock (1966). Estimates of CO2 production were made for each separate day, and a total of three-day measurements was also made using the initial and very final sample. In this way we generated data which were suitable for both inter-individual comparisons (three-day data) and intra-individual comparisons (consecutive one-day estimates). For two mice we were
unable to collect sufficient blood for isotopic analysis at 48 h and for these two individuals only two estimates were available: one spanning one day and one spanning two days. The estimated CO₂ production was converted to energy expenditure assuming an RQ of 0.8 and an energy equivalence of 20.9 J.ml O⁻¹.

RESULTS

The oxygen isotope was eliminated from *S. campestris* about twice as fast as the hydrogen isotope (Fig. 1). The oxygen isotope curve starts higher than the hydrogen curve simply because more oxygen isotope was injected into the body. Across all individuals the ratio of the isotope elimination rates for the two labels was 0.55 (sd = 0.05, n = 11). It is evident from Fig. 1 that the differences in the gradients between the two isotopes was different from day to day. Consequently the energy expenditure for each individual varied on a day-to-day basis. We calculated the daily energy expenditure for each animal, for each day that
initial and final isotope determinations were available \((n = 29)\).

The day-to-day variability in energy expenditure between individuals was very large. Among those individuals for which three estimates of daily energy expenditure were available, the coefficient of variation in daily energy demand averaged 24.5\% \((sd = 7.4\%, \ n = 9)\). There are two potential sources of this high intra-individual variability. On one hand, it may reflect analytical errors in the isotopic techniques or, alternatively, it may reflect true biological variability.

One potential source of error is erroneous isotopic analyses. If some of the analyses were erroneous, there would be a strong negative correlation between the energy expenditure measured on one day and that measured on the next day. The reason why isotopic errors should lead to such a negative correlation is clarified in Fig. 2. When we consider the situation across individuals, this negative correlation might be masked by the effects of other variables on energy expenditure. It is necessary therefore to consider the correlations not between energy expenditures but between the deviation in energy expenditure on given days from the average for that individual over the whole three-day period. However, this leads to further complications since the values contributing to an average are to some extent bound to be negatively correlated, particularly when the average is calculated from relatively few data.

We assessed the extent of negative correlation over consecutive days by plotting the deviation of energy expenditure measured one day from the three-day mean against the deviation of energy expenditure measured the next day from the three-day mean. We did this for all the individuals for the comparison of day one to day two and for day two to day three \((n = 18)\). To assess the extent of negative correlation over non-consecutive days we compared the deviation of energy expenditure on day one to that on day three \((n = 9, \text{Fig. 3b})\). In both cases there was a weak negative correlation; the \(r^2\) in the consecutive sample was 27.6\% and in the nonconsecutive sample 25.7\%. It was clear therefore that erroneous isotopic analyses were not a major contributory factor to the large variability among the individuals.

To investigate the potential contribution of torpor to the variability within individuals, we compared the observed levels of daily energy demand to the resting levels measured by respirometry (Haim et al., 1991). In this previous paper we removed the effects of mass on energy expenditure by using a direct proportional substitution. Because daily energy expenditure may scale differently than resting energy expenditure, to remove the effect of mass we plotted all the log converted data as a function of log body mass (Fig. 4). The least squares fit scaling exponent for these data was 1.19 \((95\% \text{ C.I.} = 0.92–1.22)\), which did not differ significantly from 1.0. We corrected the estimates to a mean mass of 61.3\,g (after Packard and Boardman, 1987). The distribution of these mass-corrected estimates across all individuals is shown in Fig. 5. The daily energy expenditures were mostly well in excess of the resting energy expenditures measured by respirometry. On average the daily energy expenditures were 138\,mL\,O\_2/hr \,(range 63 to 249, sd = 44.8, \ n = 31), whereas the measured resting energy expenditure at 26.5 °C was estimated to be equivalent to 66 mls O\_2/hr for a 61.3 gram animal.
Fig. 2. (a) A hypothetical time course for isotopic enrichment decline when there is no day-to-day variation in energy expenditure. The curves for both oxygen and hydrogen are linear (when enrichment is log converted). (b) When there is a deviant isotopic analysis (arrowed), the discontinuity in the decline means that on the day previous to the erroneous analysis there will be a lower than expected energy expenditure but this will be offset by a greater than expected energy expenditure the next day. A strong negative relationship between the deviation of energy expenditure from the overall mean measured on one day and the deviation on the next day will thus result from erroneous isotopic analyses.
Fig. 3. (a) The correlation between deviation of energy expenditure from the three day mean on one day and the deviation from the mean on the consecutive day. There is a negative relationship ($r^2 = 27.6\%$). (b) The correlation between deviation of energy expenditure from the mean measured one day and that measured two days later. There is still a weakly negative relationship ($r^2 = 25.7\%$).
Fig. 4. Daily energy expenditure of *Saccostomus campestris* measured by the doubly-labelled water technique as a function of body mass. Data from eleven individuals, measured on either two or three days each, are pooled. The best fit regression for the log transformed data had an exponent of 1.196 ($sd = 0.37$).

Fig. 5. Distribution of energy expenditures of *Saccostomus campestris* corrected for mass differences to a standard mass of 61.3 g (mean). The range for resting values measured by respirometry at endothermic body temperatures and the same ambient temperature (Mean = 26.5 °C) is also shown. On average the daily energy demands were 2.1× the resting energy expenditure.
DISCUSSION

Day-to-day individual variability in energy expenditure measured in the present study was high (Coefficient of variation = 24.5%), considering the animals were maintained under constant environmental conditions and in close captivity. Apart from biological causes, one potential source of such variability is inaccuracy in the technique. Many validations of the doubly-labelled water technique have been performed (see review of results in Speakman and Racey, 1988b). These indicate that on average in mammals the technique has an accuracy of +3.4%. However, the precision error is much larger than this (c. +/- 12%), and in any case this average across several validation studies masks some large differences between species. The source of such variability remains obscure, and since there has yet been no validation study performed on S. campestris it is difficult to assess directly the likely contribution that error in the technique makes to the total variability.

One possible source of error in the technique is the precision and accuracy of isotopic analyses. We previously assessed the guanidine preparation technique for measuring abundance of $^{18}\text{O}$ in water samples and this indicates that once the sample is encapsulated the precision and accuracy are very good (Speakman et al., 1990). Nevertheless, some samples may be fractionated during collection, such as for example if there is variation in the time for sealing the pipettes. This could result in erroneous isotopic analyses, contributing to the observed variation. The absence of a strong negative correlation between measured energy expenditure on one day and the next however, indicates that erroneous isotopic analyses did not contribute to the high day-to-day variability.

Perrin and Clarke (1987) measured the resting energy expenditure, at 20 °C, of a single S. campestris as it progressed from nonpregnancy, through pregnancy and lactation. The average levels of mass corrected energy expenditure were 95.6 mls O$_2$/h, 133.8 mls O$_2$/h and 200.3 mls O$_2$/h respectively. The levels of daily energy expenditure measured in the current study for nonreproductive animals, averaging 138 mls O$_2$/g/h, were thus in the mid-range of these previous estimates. The high levels of daily energy expenditure, when compared to the resting levels of reproductive animals, were surprising because the present animals were housed at an average of 26 °C, and consequently had much lower thermoregulatory energy requirements (Haim et al., 1991). Indeed the daily energy requirements of our mice averaged 2.1× the resting energy requirements of nonreproductive animals measured by respirometry at the same temperatures (Haim et al., 1991). The high levels of daily energy expenditure when compared to resting animals presumably reflects the costs associated with activity and with feeding. Since none of the measured energy requirements were lower than the costs anticipated from resting animals (after Haim et al., 1991) it could be inferred that these animals were probably not using torpor as a compensatory mechanism in their energy budgets.

It might be argued that the reason these animals were not using torpor was that their energy demands were lower than those of reproducing animals. Therefore, they did not need to compensate costs to bring their energy requirements down because they were already relatively low (albeit significantly greater than anticipated from the resting
expenditures). This seems unlikely for two reasons. Firstly, the average level of daily energy requirement was approximately 3 to 4× the measured basal metabolism (Haim et al., 1991). It has been suggested that this level is around the sustainable limit for long-term energy expenditure (Drent and Daan, 1980; Peterson et al., 1990). Secondly, the levels of digested daily food intake reported during pregnancy and nonpregnancy by Perrin and Clarke (1987) (lactation data not quoted in paper but text says levels were the same) are equivalent to approximately 70 ml O₂/animal/hr, which is well below the daily energy demands reported here. This would confirm that the energy requirements we recorded were neither particularly low, nor at the same level to which the reproductive energy expenditures had already been compensated. We cannot rule out in using these data the possibility that the mice were using infrequent short duration bouts of torpor, which in an animal of this size would be energetically advantageous. However, routine prolonged bouts of torpor are unlikely since their presence would imply unrealistically long durations of high-cost activity for animals restricted in cages. For example, 8 hours in torpor would imply at least 8 hours of activity at above 6× BMR. Direct measurement of energy expenditure of active animals in the daytime (Haim et al., 1988) suggests that such levels of energy expenditure are unlikely. There is no strong evidence therefore from the present data that long bouts of torpor are routinely used by these mice at these temperatures at this time of the year.

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REFERENCES


