Associations between energetics and over-winter survival in the short-tailed field vole *Microtus agrestis*

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Summary

1. Over-winter mortality in small mammals is influenced strongly by low ambient temperatures. Individuals with greater thermogenic capacities might then be expected to survive better than those with lower thermogenic capacities.

2. To test this hypothesis, short-tailed field voles *Microtus agrestis* (Linnaeus) were captured during the winters of 1995/96 and 1996/97 at two field sites near Aberdeen (57°N). The captured animals were tagged and taken back to the laboratory, where their resting metabolism (RMR), thermogenic capacity (NA induced metabolism excluding RMR) and body mass were measured.

3. Body mass, RMR and thermogenic capacity did not differ significantly between the start and end of winter in voles that were captured at both times.

4. Body mass varied significantly over the winter months, being lowest in January and highest in March. Thermogenic capacity also varied over the winter and the variation was linked significantly with changes in ambient temperature, suggesting that either voles with greater thermogenic capacity were more likely to be active on cold nights, or voles were flexible in their thermogenic capacities. RMR did not vary significantly over the winter.

5. Animals that survived the winter had a significantly higher residual RMR than those that died (or permanently emigrated) but the survivors did not have significantly greater body masses, RMRs, thermogenic capacities or residual thermogenic capacities.

Key-words: *Microtus agrestis*, non-shivering thermogenesis (NST), resting metabolic rate (RMR), survival.

Introduction

During winter, in the arctic and temperate zones, many small mammals face an energy crisis. At the same time that ambient temperatures decline, the primary productivity of the environment also declines. Because animals face a potential imbalance in supply and demand there has been strong selective pressure for the evolution of physiological, anatomical and behavioural adaptations that enhance their probability of survival over the winter. For animals that do not migrate or hibernate, behavioural strategies include huddling in communal nests to reduce heat loss (Contreras 1984; Bazin & MacArthur 1992; Hayes, Speakman & Racey 1992) and a reduction in winter activity levels to avoid exposure to low temperatures (Erkinaro 1961; McDevitt & Andrews 1997; Bright et al. 1996). Anatomical adaptations include increased fur thickness to increase insulation (Walsberg 1991) and reduction in body mass (Iverson & Turner 1974; Agrell et al. 1991). These latter modifications of pelage insulation have been observed most widely in larger mammals because of their advantageous surface-to-volume ratios, which readily permit the expansion of fur depth without hindering activity. Consequently physiological strategies tend to be of more importance in smaller mammals (Scholander et al. 1950). The physiological strategies employed by small mammals include an increased capacity for endogenous heat production, particularly non-shivering thermogenesis. Non-shivering thermogenesis occurs in a number of organs, but the main site is in the brown adipose tissue (BAT) and is mediated primarily by noradrenaline (NA) (Mory et al. 1984). Non-shivering thermogenic capacity can be quantified by measuring the peak metabolic response (= oxygen consumption) following
relationship does not point to a simple threshold in Y et despite the evident links between over winter capacity, and all (or almost all) the animals would die. Variables between winters – because in some mild winters one might anticipate that mortality would be highly absolute and residual RMR also had higher absolute and mass effects had been removed. Mice with higher absolute and residual RMR (because in Speakman 1996) pointed to a potential cost associated with maintaining a NST capacity. Animals which maintain a high NST capacity. This observation is important because it helps to explain the diversity of resting metabolic rates that are observed routinely across individual small mammals. Resting metabolic rates show large variation between individuals and in most populations it is true that for animals of any given body mass there is a two-fold range in RMR (because in Speakman 1996). Since animals operating high RMR must ingest more food (necessitating increased foraging times and perhaps increased risk of predation when foraging) to fuel this metabolism there must be some selective advantage favouring these individuals, which generally balances the negative aspects of their greater expenditure. Several studies have shown that their NST capacity was exceeded. If the winter was influenced by mean temperature in February, which was the coldest month during the winter (Berry et al. 1969).

If winter mortality of small mammals is often dependent on ambient temperature, and by implication their capabilities to generate heat by NST, a question arises as to why these animals do not expand their thermoregulatory capacities routinely to greater levels, thus allowing them to survive until the following summer. One possible explanation is that there is a physiological limit on the extent of NST capacity. This seems an unlikely explanation, however, for two reasons. First, our previous measures indicate that even for animals of the same body mass there is a substantial variation in NST capacity (McDevitt & Speakman 1996; Haim et al. 1997). Moreover, if most animals did have high capacity one might speculate that mortality would be highly variable between winters – because in some mild winters all the animals would have sufficient capacity to survive, yet in other severe winters none would have sufficient capacity, and all (or almost all) the animals would die. Yet despite the evident links between over winter mortality and ambient temperature the nature of this relationship does not point to a simple threshold in NST being responsible.

Speakman (1996) observed in wood mice Apodemus sylvaticus (Linnaeus) that NST capacity (by NA injection) was linked to variations in resting metabolic rate – even after the effects of shared variation due to body mass effects had been removed. Mice with higher absolute and residual RMR also had higher absolute and residual NST. This observation is important because it points to a potential cost associated with maintaining a high NST capacity. Animals which maintain a high NST capacity would also face elevated total energy demands, since RMR comprises a major component of the total daily energy budget (30–50% of total energy costs) (Bryant & Tatner 1991; Nagy et al. 1999; Speakman 2000).

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This hypothesis makes several predictions and assumptions, which we aimed to explore in more depth in the present study. First, it assumes a link between RMR and NST which is robust to the manner in which NST is quantified. Speakman (1996) used only a single method for quantifying NST (peak including RMR), yet we have shown previously (Jackson et al. submitted) that different expressions of NST are possible. Secondly, the hypothesis assumes that animals have relatively poor capacities to alter their RMR and NST as a function of changing weather patterns throughout a given winter – they are assumed to enter winter as high or low strategists and be unable to respond to short-term weather changes by altering strategies.

In this study we aimed to test these assumptions by exploring the links between NST and RMR in a large population of individuals drawn from the wild at the start of winter. Secondly, we aimed to investigate the extent to which RMR and NST were being modulated on a day-to-day basis as winter progresses. Finally, we aimed to investigate whether over-winter survival of individual voles was correlated with their thermoregulatory attributes at the start of the winter.
Survival in voles

Over-winter

633–640
Ecology
Journal of Animal

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Met Office Dyce, for both winters. The Met office was
ambient temperatures were provided by Aberdeen
Met office (Erkinaro 1961). Trapping commenced each year in
November and continued monthly until March. Over the
two winters we trapped on a total of 79 nights, with
November and December (39 nights) and at the end of
winter, March (20 nights). To reduce emigration from
the trapping grids, field sites were chosen to include
natural barriers at their boundaries such as less favour-
able habitats such as agricultural farmland, which might
reduce the likelihood of emigration from the field site.

In addition, in March of each year additional traps
were placed around each of the grids (10–50 m), to catch
any voles that may have emigrated from the grid. Voles
were placed in the chamber for 2 h to allow resting
metabolic rate RMR to be measured. This time period
was chosen since previous studies have shown
peaks of activity at dusk and dawn for this species
(Erkinaro 1961). Trapping commenced each year in
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In addition, in March of each year additional traps
were placed around each of the grids (10–50 m), to catch
any voles that may have emigrated from the grid. Voles
were considered to have not survived the winter if they
were caught in November/December and not captured
again at the end of the winter period, i.e. in March,
although this may include individuals that had perman-
ently emigrated. Voles returned to the laboratory
were kept in an outdoor enclosure at the same approximate
ambient temperatures and photoperiods prevailing
at the study sites. The voles were kept at the laboratory
no longer than 2 days then taken back and released as
close as possible to the trapping station where they
were caught. A total of 564 voles were caught over the
two winters, of which 282 were recaptures. A total of
173 voles had their RMR measured and of these, 97
also had their thermogenic capacity measured.

**STATISTICAL ANALYSIS**

Analysis of variance (ANOVA) and analysis of covariance
(ANCOVA), binary logistic regression, t-tests and linear
regression were used to determine body mass and activity
using the Minitab statistical package (Ryan et al. 1985). Mass, RMR, thermogenic
capacity and peak NA induced metabolism were all log-
transformed to normalize the data. Subsequent statisti-
cal analyses were performed using the log-transformed
data. All the data presented are from individual voles,
i.e. no recaptures were included in the analyses.

**Results**

**EFFECT OF BODY MASS**

There was a significant positive relationship between
RMR and body mass of the voles (Fig. 1a, linear regres-
sion, \( F = 86.43, r^2 = 0.333, P < 0.001 \)), and a significant
positive relationship between thermogenic capacity and body mass (Fig. 1b, linear regres-
sion, \( F = 23.92, r^2 = 0.195, P < 0.001 \)). A similar relationship was found
between peak NA induced metabolism and body mass
(linear regression, \( F = 42.57, r^2 = 0.301, P < 0.001 \)).
Voles captured at site 2 in the winter of 1996/97 were heavier (averaged over all months) than voles from site 1 in the winter of 1995/96 (Table 1, difference by ANOVA, $F = 36.11, P < 0.001$). The RMR of voles at the two sites, their thermogenic capacities and body masses are shown in Table 1. RMR was significantly higher in voles from site 1 in the winter 95/96 (ANOVA, $F = 19.09, P < 0.001$). Thermogenic capacity was significantly higher in voles from site 2 winter 1996/97 (ANOVA, $F = 18.47, P < 0.001$); however, if the variation due to body mass was removed, then RMR and thermogenic capacity were not significantly different between years (ANCOVA, $F = 3.66, P = 0.057$; ANCOVA, $F = 2.27, P = 0.136$). For subsequent analysis the data from both years were pooled.

**GENDER DIFFERENCES**

Male voles (mean mass $= 19.83\, g$, SD $= 3.82$, $n = 164$) were significantly heavier than female voles (mean mass $= 18.39\, g$, SD $= 2.71$, $n = 109$, difference by ANOVA, $F = 11.54, P = 0.001$). RMR and thermogenic capacity were not significantly different between male and female voles, before or after the effect of body mass was removed (ANOVA, $F = 3.01, P = 0.085$; ANOVA, $F = 2.44, P = 0.122$, corrected for body mass; ANCOVA, $F = 0.21, P = 0.645$; ANCOVA, $F = 0.10, P = 0.747$).

**RELATIONSHIP BETWEEN RMR AND THERMOCAPACITY**

There was a significant positive relationship between the RMR and the peak NA induced metabolism (Fig. 2a: linear regression, $F = 21.31, r^2 = 0.179, P < 0.001$). This relationship remained significant but was much weaker, when thermogenic capacity was used as the dependent variable (Fig. 2b, linear regression, $F = 3.94, P = 0.049$). However, all three traits (RMR, thermogenic capacity and peak NA induced metabolism) were related to body mass (Fig. 1a,b). If the shared variation due to mass was removed then the relationships between RMR and these two variables disappeared (linear regression, $F = 0.84, P = 0.361$ for thermogenic capacity and linear regression, $F = 2.69, P = 0.104$, for peak NA induced metabolism). Thus, larger voles had greater RMRs, thermogenic capacities and greater peak NA induced metabolism but for any given mass RMR was not related to thermogenic capacity or peak NA induced metabolism.

**MONTHLY CHANGES**

Body mass of the voles varied over the months (not including recaptures, Fig. 3, ANOVA, $F = 8.96, P < 0.001$). Body mass was highest in March and lowest in January. Resting metabolic rate did not vary significantly between the months (ANOVA, $F = 1.7, P = 0.15$); however, peak NA induced metabolism did vary significantly over the months.
The variation in peak %NA induced metabolism showed the opposite pattern to average monthly changes in air temperature (Fig. 3b). Daily average air temperature was negatively related to peak %NA induced metabolic rate (Fig. 4, Linear regression, $F = 4.34, r^2 = 0.042, P = 0.04$). There was no significant change in body mass, RMR, residual RMR, thermogenic capacity or residual thermogenic capacity in the same voles at the start and the end of the winter period (Table 2).

**Survival**

Body mass, RMR, residual RMR, thermogenic capacity and residual thermogenic capacity were compared at the start of the winter (November/December - body mass, $N = 190$; RMR, $N = 119$; thermogenic capacity, $N = 65$) for voles that subsequently survived to the end of the winter (March - mass, $N = 27$, RMR, $N = 22$, thermogenic capacity, $N = 9$) and voles that subsequently did not survive (or had permanently emigrated). Voles that survived the winter did not have a significantly
different RMR to voles that did not (or had emigrated) (Table 3, \(t\)-test, \(t = 1.21, P = 0.19\)). However, voles that survived the winter had a significantly higher residual RMR at the start of the winter (November/December) than voles that did not survive (or had emigrated; Table 3, \(t\)-test, \(t = 2.08, P = 0.045\)). Voles that had survived the winter did not have a significantly different body mass, thermogenic capacity or residual thermogenic capacity from voles that had died or emigrated (Table 3). An alternative way to examine these data is to consider survival as the dependent trait and explore the effects of the individual aspects of morphology and physiology on this parameter using binary logistic regression. We entered year, body mass, RMR, residual RMR, thermogenic capacity and residual thermogenic capacity as predictors in this analysis. Confirming our previous analysis the only predictor that emerged as significantly correlated with survival (or residency) was residual RMR (Fig. 5: \(\chi^2 = 3.87, P < 0.05\)).

**Discussion**

**Relationship between NST and RMR**

The question of whether RMR is related to an animal’s capacity for NST has important ecological, evolutionary and thermoregulatory implications. Speakman (1996) suggested that a possible reason for the large interindividual variation in RMR observed in a number of species could be attributed to variation in thermogenic capacity. Significant interindividual variation in RMR has been observed in a number of species (Haim 1987; Speakman 1996; McLean & Speakman 2000) and was also evident in the present study. There is little agreement in the literature as to whether RMR is related to NST. A number of interspecific studies have shown conflicting results. Haim & Izhaki (1993) found a negative relationship between RMR and NST measured over 21 species. However, Sparti (1992) investigated the relationship between RMR and NST in 12 species of shrew and found no relationship between RMR and NST as well as no relationship between RMR and \(V_O\)\(_2\) max. However, these previous studies concerned interspecific links between RMR and NST, and therefore

**Table 2.** Results from paired \(t\)-tests comparing the mass, RMR, residual RMR, thermogenic capacity and residual thermogenic capacity of the same voles at the start of the winter (Nov/Dec) and at the end of the winter (March)

<table>
<thead>
<tr>
<th>Start of the winter</th>
<th>End of the winter</th>
<th>(N)</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>(t)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (g)</td>
<td>27</td>
<td>18.004</td>
<td>2.107</td>
<td>19.195</td>
<td>4.02</td>
<td>1.85</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>RMR (Watts)</td>
<td>16</td>
<td>0.3903</td>
<td>0.0946</td>
<td>0.4259</td>
<td>0.0364</td>
<td>1.39</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Residual RMR (Watts)</td>
<td>16</td>
<td>0.0059</td>
<td>0.0361</td>
<td>0.0279</td>
<td>0.0505</td>
<td>0.47</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Thermogenic capacity (Watts)</td>
<td>7</td>
<td>0.3988</td>
<td>0.1585</td>
<td>0.3282</td>
<td>0.1905</td>
<td>0.66</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Residual thermogenic capacity (Watts)</td>
<td>7</td>
<td>0.0305</td>
<td>0.0472</td>
<td>0.0211</td>
<td>0.0506</td>
<td>0.33</td>
<td>0.75</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.** Results of \(t\)-tests comparing voles mass, RMR, residual RMR, thermogenic capacity and residual thermogenic capacity following NA injection at the start of the winter between voles that had survived and voles that had not survived the winter (or permanently emigrated)

<table>
<thead>
<tr>
<th></th>
<th>Non-survivors</th>
<th>Survivors</th>
<th>(t)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Mean</td>
<td>SD</td>
<td>(N)</td>
<td>Mean</td>
</tr>
<tr>
<td>Mass (g)</td>
<td>18.71</td>
<td>3.13</td>
<td>190</td>
<td>18.16</td>
</tr>
<tr>
<td>RMR (Watts)</td>
<td>0.409</td>
<td>0.0174</td>
<td>119</td>
<td>0.4146</td>
</tr>
<tr>
<td>Residual RMR (Watts)</td>
<td>0.0001</td>
<td>0.0329</td>
<td>119</td>
<td>0.0147</td>
</tr>
<tr>
<td>Thermogenic capacity (Watts)</td>
<td>0.3137</td>
<td>0.065</td>
<td>65</td>
<td>0.3158</td>
</tr>
<tr>
<td>Residual thermogenic capacity (Watts)</td>
<td>-0.002</td>
<td>0.119</td>
<td>65</td>
<td>-0.003</td>
</tr>
</tbody>
</table>

**Fig. 5.** Survival (or permanent emigration) of short-tailed field voles in relation to their residual log resting metabolic rate (RMR) (Watts). The raw data are shown as open circles. Calculated survival (or permanent emigration) for the data split into 10 centiles of the RMR distribution are shown as open squares and the fitted logistic regression curve shown as a solid line with solid circles at the corresponding 10 centiles of the RMR distribution.
cannot be easily compared with the present intraspecific study. The results of the present study suggested that there is no relationship between RMR and NST (expressed as peak NA induced metabolism or thermogenic capacity) in individual Microtus agrestis when the shared variation due to body mass was removed. Speakman (1996) found that RMR was a positively related to NST in individual Wood mice A. sylvaticus and this relation remained even when the shared variation due to mass was eliminated. The difference between studies was unlikely to reflect the manner in which NST was quantified. NST can be expressed in a number of ways, which have different biological and physiological relevance. Peak NA induced metabolism which includes RMR may be biologically more important because it reflects the animal’s total capacity for generating heat (Wunder & Gettinger 1996), but NST excluding RMR (=thermogenic capacity) may be physiologically more relevant because it concerns only the heat produced as a result of NA stimulation. Speakman (1996) estimated NST as metabolic response to NA injection including RMR (peak NA induced metabolism). We found, however, that expressing the NST in this manner did not result in a significant relationship after the shared variation due to mass was removed, calling into question the generality of the previous result.

ENERGETIC STRATEGY AND SURVIVAL

We found that the RMR and NST of voles entering winter was not significantly different from the RMR and NST of the same individuals coming out of the winter. This might indicate that voles have a fixed energetic strategy over the winter months with respect to these traits. If this was the case one might also predict that variation in the average levels of these traits over the winter would also not be significant. This was indeed the case for RMR, however, NST did vary significantly over time and the pattern of variation in NST was related to the variations in ambient temperature. Similar results were also reported by Merritt & Zegers (1991) who investigated seasonal changes in RMR and NST in Southern red-backed voles C. gapperi (Vigors). The discrepancy between the lack of differences in NST in the same individuals over the entire winter compared with the significant variation across individuals in relation to ambient temperatures, could reflect one of two things. First, the significant variation in NST capacity with temperature might reflect variations in activity of voles with high and low NST in relation to variations in ambient temperature. Animals with higher NST may have had a greater likelihood of being active on cold days and thus a greater probability of being trapped. Alternatively, RMR may be fixed before entering the winter but NST may not and be capable of responding to short-term changes in temperature. At present we are unable to separate these explanations.

Voles with a higher residual RMR survived the winter. However, we have shown that voles that have a high residual RMR do not necessarily also have a high residual NST capacity, and this was consistent with the observation that voles which survived the winter did not have a higher capacity for NST. These results agreed with the findings of Berry et al. (1969), who found that house mice with a higher BMR were more likely to survive the winter, but there was no significant link with ‘metabolic performance’ (metabolic response to 5 °C averaged over 100 min) of the mice. If there is no thermoregulatory advantage to a higher RMR, the question arises as to why voles with a higher residual RMR entering winter survive better (or are less likely to emigrate)? The evolution of endothermy in vertebrates has been hypothesized to be linked to selection for increased maximal aerobic capacity, therefore leading to increased RMR levels as a response (Bennett & Ruben 1979). Previous studies have demonstrated a significant interspecific correlation between RMR and maximal rates of oxygen consumption (VO₂max) both induced by cold exposure and locomotion (MacMillen & Hinds 1992). Similar trends have been shown intraspecifically (Hayes & Garland 1995). A possible explanation for the enhanced survival of voles with higher residual RMR found here could be that voles with higher residual RMR also had higher VO₂max. Maximum oxygen consumption has been shown previously to be correlated with voluntary and maximum running speed in untrained mice (Friedman et al. 1992). Voles in the present study with higher residual RMRs may have had higher running speeds, which may have afforded them an advantage when escaping from predators.

Acknowledgements

This work was supported by NERC grant (GR3/00910) and a BBSRC studentship to DMJ. We are grateful to Sam Snart for assistance with the trapping, Graham Horgan and John Dunbar for statistical advice and to the landowners who allowed us to work on their land.

References


