SHAVING INCREASES DAILY ENERGY EXPENDITURES IN FREE LIVING ROOT 

VOLES

Paulina A. Szafranśka¹*, Karol Zub¹, Monika Wieczorek¹, Aneta Książek², John R. Speakman³, Marek Konarzewski²

¹ Mammal Research Institute Polish Academy of Sciences, 17-230 Białowieża, Poland
² University of Białystok, 15-950 Białystok, Poland
³ Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, Scotland, and State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China.

*Corresponding author: e-mail: pszafran@ibs.bialowieza.pl

Running title: Shaving increases DEE in wild voles

Keywords: body mass, doubly labelled water, fur insulation, mammal, metabolic rate, thermoregulation,
ABSTRACT

Experimental manipulation of energy expenditure has long been recognized as an effective means for identifying causative effects and avoiding confounded interpretations arising from spurious correlations. This approach has been successfully applied mainly in studies on birds, particularly on reproducing adults, while manipulations in mammals have proved more problematic. Here we tested the hypothesis that shaving off 50% of the dorsal pelage should effectively increase energy expenditure in the wild root voles (*Microtus oeconomus*) in their natural environment. We measured daily energy expenditures (DEE), using doubly labelled water (DLW), in shaved and unshaved voles at the beginning of winter. The difference in DEE (corrected for body mass and year effects) between experimental and control group fluctuated from 11.5% to 17.3%. Probability of recapture over the 3-days DEE assay was strongly dependent on body mass but did not differ between shaved and unshaved animals, however the prevalence of larger (heavier) shaved individuals was observed. Shaved animals lost more weight between the release and recapture. Shaving therefore appears an effective method of increasing costs of total daily energy expenditures in wild endotherms in their natural environment.
INTRODUCTION
The idea to overload animals’ energy expenditures to increase their total energy budget came from classical studies on parental effort (Drent and Daan 1980). Experimental manipulations in animal energetics have mainly been successfully applied in studies on reproducing birds. This is because of a relative ease of implementing such manipulations through changing clutch size (Knowles et al. 2010) or the costs of flight (by feather clipping: Carrascal and Polo 2006; Barron et al. 2013). Experimental manipulations of energy expenditures outside breeding season, particularly in mammals, are far more difficult and are mostly limited to laboratory conditions, which allows for manipulation via control of the ambient temperature (Selman et al. 2008; Chappell et al. 2012), food quality or quantity (Cao et al. 2009; Gutowski et al. 2011), litter size (Simons et al. 2011; Zhao et al. 2013) or the composition of respired air (Rosenmann and Morrison 1974; Cheviron et al. 2013).

The lack of effective means of manipulation of energy expenditures in free ranging, non-reproducing mammals is reflected in the scarcity of such studies, which mainly rely on natural, uncontrolled variation of environmental factors (Nagyet et al. 1999; Nagy 2005), and therefore, are inherently correlative in nature. The aim of this study was to test under natural conditions the hypothesis that shaving, as a method of increasing costs of thermoregulation, increases total daily energy expenditures (DEE). Measurements using pelt-covered, internally heated metal casts of animals, indicated that naked skin increases thermal conductance (Jofré and Caviedes-Vidal 2003) and opposite, long and thick fur decreases rate with which heat dissipates (Chappell 1980; Reynolds 1993). Laboratory experiments on living animals indicated that 60% removal of the total dorsal pelage in Siberian hamsters increased energy intake up to 44% (Kaufman et al. 2001). Shaving the backs of elephant shrews and rock mice increased their thermal conductance measured by increase in resting metabolic rate by approximately 25% and 10%, respectively (Boyles et al. 2012). There were also several studies which demonstrated that shaving increased food consumption in females, energetically overload by lactation (in mice by Król et al. 2007; Zhao and Cao 2009; Zhao et al. 2010; in voles by Simons et al. 2011 and in hamsters by Paul et al. 2012).

Field studies, however, were more equivocal. Meadow voles had only a small and statistically insignificant increase of DEE in shaved compared with unshaved individuals (Kenagy and Pearson 2000). These studies, however, were based on a very small sample sizes of few individuals. Here we report the results of much larger study, in which we tested the
efficacy of shaving in the root vole (*Microtus oeconomus*) - a small rodent characterized by a wide range of adult body mass (BM; 20-60 g).

RESULTS AND DISCUSSION

Following injection with DLW to estimate DEE, a total of 119 unshaved and 121 shaved (Fig. 1) voles were released back to their natural environment at the beginning of climatic winter. Initial body mass of unshaved (control) and shaved (experimental) individuals averaged 27.1±6.7 g and 28.9±8.55 g (±SD), respectively. Nested ANOVA showed no difference in body mass between these groups (F\(_{2,236}=3.59, p=0.06, \text{fig. 1A}\)), but a significant effect of year nested within shaving treatment (F\(_{2,236}=4.97, p=0.008\)).

Within 3 days after release we managed to recapture 25.6% of shaved and 25.2% of unshaved individuals. The probability of recapture shaved and unshaved individuals did not differ after one, two or three days (Lifetest procedure, Wilcoxon test with \(d.f.=2, p>0.3\)). However, the probability of recapture of shaved voles strongly depended on the initial body mass, as reflected by a higher proportion of heavier individuals (Lifetest procedure, Wilcoxon test with \(d.f.=1, p>0.002, \text{Fig. 2A and 2B}\)). This resulted in a significant difference in body mass distribution between the shaved (mean BM=31.9 g) and unshaved (mean BM=27.3 g) recaptured voles (Fig. 2, Fisher exact test, \(p=0.007\)), which may suggest different energetic constrains affecting voles larger than 30 g (Fig. 3). Higher trapability of larger shaved individuals could be attributed to their elevated activity (Kenagy 1973) and longer foraging time, which compensated for the excessive heat loss. Increased mobility of starving animals was observed in some species (Cao et al. 2009). Shaving caused an increase in food intake in laboratory setting (Kaufman et al. 2001; 2004), which in natural conditions is related to activity time – a potential component of DEE (Fyhn et al. 2001; Jodice et al. 2003).

Shaving also decreased individual body mass: between first and second trapping shaved voles lost more weight (mean=2.1 g) than unshaved ones (mean=0.4 g; ANCOVA, effect of shaving: F\(_{1,67}=5.45, p=0.02, \text{Fig. 2B}\)). The effect of time period (1–3 days) between release and recapture was not significant (F\(_{1,67}=1.13, p=0.3\)). Independently of shaving treatment larger individuals lost more weight (F\(_{1,67}=19.16, p<0.0001\), interaction body mass x treatment was not significant and therefore not included in the final model). The decrease in body mass was most likely caused by increased energy expenditures, which was also observed in shaved golden-mantled squirrels in laboratory conditions (Kauffman et al. 2004) and in shaved meadow voles in the field (Kenagy and Pearson 2000).
In the first study year we successfully measured DEE in 20 shaved and 12 unshaved animals and in the second year in 11 shaved and 18 unshaved voles. Raw DEE of shaved and unshaved animals averaged 100.6±27.1 kJ/day and 80.8±20.0 kJ/day, while their body masses at release averaged 34.2±10.1 g and 28.6±5.5 g, respectively. DEE (corrected for body mass and year) was higher for shaved than unshaved animals (Fig. 4, Table 1). Back transformed DEE values (corrected for the effect of body mass and year) derived from ANCOVA model (Table 1) were significantly higher in shaved than unshaved animals (97.2 kJ/day and 78.6 kJ/day, respectively).

DEE differed significantly between years (Table 1). During first year DEE averaged 109.2 kJ/day and 91.9 kJ/day, whereas during second year only 82.7 kJ/day and 73.2 kJ/day (for shaved and unshaved, respectively). Thus, DEE of unshaved animals (corrected for the effect of body mass) was 17.3% and 11.5% lower in first and second year, respectively, than of shaved individuals. To our knowledge this is the first successful demonstration of the effectiveness of such manipulation of mammalian DEE. In free-ranging meadow voles (Kenagy and Pearson 2000), elephant shrews and rock mice (Boyles et al. 2012) there was no significant influence of shaving treatment on DEE or on heterothermy index (Boyles et al. 2012). However, those studies relied on small samples sizes (7, 12 and 13 individuals, respectively) and may therefore have been insufficiently powered to detect a significant effect.

Our experiment was carried out at the beginning of winter at low ambient temperatures and lack of snow cover, which exposed voles to the cold. The higher DEE under milder conditions in 2008 than under colder conditions in next year, suggest not only effect of increased thermoregulation costs, but also changes in behavior. Similar effect of weather on energy expenditures was also observed in red squirrels (Fletcher et al. 2012).

We demonstrated that under such conditions shaving is an efficient method of increasing daily energy expenditures in wild animals in their natural environment by increasing costs of thermoregulation and probably also by modification of activity.

MATERIALS AND METHODS

Study area and experimental design

The study was conducted in the Biebrza River valley, (ca. 53°N, 23°E) NE Poland. The study area was located in the marshes dominated by the tussock-forming sedges, where the most common small mammal was the root vole. The experiment was carried out during two years, at the beginning of climatic winter in this region: November 1-16, 2008, and November 20 -
December 6, 2009. Mean daily temperatures during our study were 4.8°C in 2008 and 2.5°C in 2009. There was no snow cover during study periods and the mean daily precipitations were 0.41 mm and 0.53 mm, respectively (www.weatheronline.pl).

The natural population of the root voles was enclosed in the 1-ha sized area by a 70 cm high fence, covered with plastic coat and dug down to a depth of 30 cm. The fence prevented animals from climbing or digging tunnels.

In both years, voles were captured in live box traps and transported to the laboratory, located near the study area, where they were weighted, sexed and marked individually by microchips (Microchip DS.CO. S. C., Poland). Then, they were randomly assigned to either the experimental (shaved) or control (unshaved) group. Animals handled under the Local Research Ethics Committee in Białystok, permits no. 9/2008, 1/2009.

Extra thermoregulatory costs

We shaved off dorsal pelage of voles from the tail up to neck and on both sides up to the line elbow–knee (Fig. 1). We estimated the shaved patch as 50% of the total back surface. During shaving procedure one of the experimenters handled an animal while the second one shaved fur off using a human beard trimmer (Remington PG-200C) with no tip attached, which allowed us to shave voles to bare skin. Animals were not anaesthetized prior to procedure due to their high sensitivity to any sedative in this species. Voles from the control group went through the same procedure and were immobilized in the same way as voles from the experimental group. Instead of hair cutting, however, we were only touching their backs with the working trimmer for two minutes, which was the time needed for shaving the experimental animals. The experiment was conducted on 124 voles in November 2008 (experimental group: 35 males and 28 females, control: 35 males and 26 females) and 116 voles in November 2009 (experimental: 33 males and 25 females; control: 35 males and 23 females).

Daily energy expenditures

Daily energy expenditures (DEE) were estimated by the doubly labelled water method (DLW) (Butler et al. 2004). This technique is based on isotopes of oxygen (^{18}O) and hydrogen (^{2}H), known quantities of which are injected into the animals and then the initial isotope enrichment is estimated in an blood sample taken 1 h after injection (Król and Speakman 1999). The CO_{2} production was calculated based on the differential washout of ^{18}O and ^{2}H over a period of 24-72 hours, when the final blood sample was taken (Speakman et al. 1994). To calculate the rate of
CO$_2$ production we used the single-pool model equation 7.17 (Speakman 1997) as recommended for animals below 1 kg (Speakman and Król 2005). As the final step, energy equivalents of the rate of CO$_2$ were converted to DEE (kJ/day) assuming a respiratory quotient of 0.85 (Speakman 1997). The initial and the final (50 μl each) blood samples were taken from the retro-orbital sinus which is commonly used method in rodents study (e.g., Klein et al. 1997). Voles are very sensitive to any anaesthesia, hence we bleed conscious animal. In frame of three days, as the DLW method required, we were able to recapture 25.8% in 2008 and 31.9% in 2009 out of all injected animals.

Data analyses

We used nested ANOVA to test the effect of shaving treatment and study year (nested within shaving treatment) on the initial body mass of all DLW injected individuals (N=240). A nested design was used because categories of the nested factor (year) within each level of the main factor (shaving treatment) were different (Quinn and Keough 2002). We estimated the effect of shaving on the probability of recapture in the course of DLW assay by means of the procedure Lifetest (SAS 9.2, SAS Institute Inc., Cary, NC, USA), which allowed us to model the right-censored characteristics of our data set, reflecting the fact that individual voles were recaptured between 1-3 days following their release. The data set was divided into two groups representing the effect of shaving, the effect of study year was controlled as the strata, and body mass as a covariate.

To analyse changes in body mass of recaptured individuals over the duration of the DLW assay we used ANCOVA with the difference in BM between first (initial body mass) and second capture as a dependent variable, shaving treatment and year nested within shaving treatment as factors, duration of DLW assay (in hours) and initial body mass as covariates. To test the effect of shaving on DEE we used ANCOVA with DEE (kJ/day) as a dependent variable, shaving treatment and year nested within shaving treatment as factors and body mass as a covariate. Based on this model we calculated predicted values of DEE for each individual, and the means for each treatment groups. In all models described above the respective interactions were also included and retained in the final models only when statistically significant. While analyzing daily energy expenditures both BM and DEE were log$_e$ transformed to take into account an allometric relation between those two traits (McNab 2002). The above analyzes were carried out in STATISTICA and the R package (R Development Core Team 2012).
ACKNOWLEDGEMENTS

We would like to thank numerous students, especially Britta Adam and Izabela Podbielska, for their help in field work and technical assistance. We also thank Tomasz Samojlik for drawing Fig. 1. Catherine Hambly and Peter Thompson provided technical support for the isotope analyzes. We are grateful to the Institute of Biology in Białystok for allowing us to use their Field Station in Gugny. We are also thankful to two anonymous reviewers for their valuable comments.

COMPETING INTERESTS

No competing interests declared.

AUTHOR CONTRIBUTIONS

All authors were involved in the conception and design of the study and revising the article. P.A.S., K.Z., M.W. and A.K. executed data collection from study animals in the field, J.R.S. was responsible for the DLW isotope analyses.

FUNDING

This study was supported by the Polish Ministry of Science and Higher Education grant NN304349335 to P.A.S.. J.R.S. was supported by a 1000 talents professorship of the Chinese government.

LITERATURE CITED

Carrascal L.M., and V. Polo. 2006. Effects of wing area reduction on winter body mass and foraging behaviour in coal tits: field and aviary experiments. Anim Behav 72:663–672.


Figures’ legends:

Figure 1. Schematic illustration of fur removed from the vole’s back.

Figure 2. (A) Body mass of all individuals released with DLW in 2008 and 2009 (range of body mass marked by dotted line); (B) body mass of fraction recaptured within 3 days, body mass when releasing and when recaptured connected by dotted line (○ – shaved, ● – unshaved, mean ± SE, sample size in brackets).

Figure 3. Body mass distribution of shaved and unshaved voles recaptured within 3 days of DLW assay.

Figure 4. Relationship between body mass and daily energy expenditures (DEE, estimated by doubly labelled water) corrected for the year of study.
Figure 1.

Figure 2.
Figure 3.

Figure 4.
Table 1. Factors influencing Daily Energy Expenditures (measured by doubly labelled water) in voles under natural conditions. Only significant interactions are presented, data log\(_e\) transformed.

<table>
<thead>
<tr>
<th>Source of variation in DEE</th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass</td>
<td>0.33</td>
<td>1</td>
<td>0.33</td>
<td>94.26</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Shaving</td>
<td>0.02</td>
<td>1</td>
<td>0.02</td>
<td>4.69</td>
<td>0.03</td>
</tr>
<tr>
<td>Year (Shaving)</td>
<td>0.09</td>
<td>2</td>
<td>0.05</td>
<td>13.15</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Shaving x body mass</td>
<td>0.02</td>
<td>1</td>
<td>0.02</td>
<td>4.32</td>
<td>0.04</td>
</tr>
</tbody>
</table>