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Shaving increases daily energy expenditure in free-living root voles

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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 to studies on birds, particularly on reproducing adults, whereas 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 wild root voles (*Microtus oeconomus*) in their natural environment. We measured daily energy expenditure (DEE), using doubly labelled water 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 day DEE assay was strongly dependent on body mass, but did not differ between shaved and unshaved animals; however, a prevalence of larger (heavier) shaved individuals was observed. Shaved animals lost more weight between the first and second trapping. Shaving therefore appears to be an effective method of increasing the cost of total DEE in wild endotherms in their natural environment.

KEY WORDS: Body mass, Doubly labelled water, Fur insulation, Mammal, Metabolic rate, Thermoregulation

INTRODUCTION

The idea to overload an animal's energy expenditure to increase its total energy budget came from classical studies on parental effort (Drent and Daan, 1980). Experimental manipulations of animal energetics have mainly been successfully applied in studies on reproducing birds. This is because of a relative ease of implementing such manipulations by changing clutch size (Knowles et al., 2010) or the costs of flight (by feather clipping) (Carrascal and Polo, 2006; Barron et al., 2013). Experimental manipulation of energy expenditure outside the breeding season, particularly in mammals, is far more difficult and is mostly limited to laboratory conditions, which allows for manipulation via control of the ambient temperature (Selman et al., 2008; Chappell et al., 2007), 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).

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The lack of effective means of manipulation of energy expenditure in free ranging, non-reproducing mammals is reflected in the scarcity of such studies, which mainly rely on natural, uncontrolled variation of environmental factors (Nagy 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 expenditure (DEE). Measurements using pelt-covered, internally heated metal casts of animals indicated that naked skin increases thermal conductance (Jofré and Caviedes-Vidal, 2003) and by contrast, long and thick fur decreases the rate by which heat dissipates (Chappell, 1980; Reynolds, 1993). Laboratory experiments on living animals indicate that 60% removal of the total dorsal pelage in Siberian hamsters increased energy intake up to 44% (Kauffman et al., 2001). Shaving the backs of elephant shrews and rock mice increases their thermal conductance by increasing the resting metabolic rate by ~25% and 10%, respectively (Boyles et al., 2012). There are also several studies which demonstrate that shaving increases food consumption in females that are already energetically overloaded by lactation in mice (Król et al., 2007; Zhao and Cao, 2009; Zhao et al., 2010), in voles (Simons et al., 2011) and in hamsters (Paul et al., 2010).

Field studies, however, are 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 were based on a very small sample size. Here, we report the results of a much larger study, in which we tested the efficacy of shaving in the root vole *Microtus oeconomus* Pallas 1776, which is a small rodent characterized by a wide range of adult body mass (20–60 g).

RESULTS AND DISCUSSION

Following injection with doubly labelled water (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 (BM) of unshaved (control) and shaved (experimental) individuals averaged 27.1±6.7 g and 28.9±8.55 g (±s.d.), respectively. Nested ANOVA showed no difference in body mass between these groups ($F_{2,236}$=3.59, P=0.06, Fig. 1A), but a significant effect of year nested within shaving treatment ($F_{2,236}$=4.97, P=0.008).

Within 3 days of release we managed to recapture 25.6% of shaved and 25.2% of unshaved individuals. The probability of recapture of shaved and unshaved individuals did not differ after 1, 2 or 3 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, Fig. 2A,B). This resulted in a significant difference in body mass distribution between the

Fig. 1. Schematic illustration of extent of fur shaved from the back of the root vole *Microtus oeconomus.*

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 weighing more 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 has been observed in some species (Cao et al., 2009). Shaving caused an increase in food intake in the laboratory setting (Kauffman et al., 2001; Kauffman et al., 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 the 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, 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). Independent of shaving treatment, larger individuals lost more weight ($F_{1,67}$=19.16, P<0.0001, interaction body mass × treatment was not significant and therefore not included in the final model). The decrease in body mass was most likely caused by increased energy expenditure, 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^{-1} and 80.8±20.0 kJ day^{-1}, 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^{-1} and 78.6 kJ day^{-1}, respectively).

DEE differed significantly between years (Table 1). During the first year DEE averaged 109.2 kJ day^{-1} and 91.9 kJ day^{-1}, whereas during second year only 82.7 kJ day^{-1} and 73.2 kJ day^{-1} (for shaved and unshaved, respectively). Thus, the DEE of unshaved animals (corrected for the effect of body mass) was 17.3% and 11.5% lower in the 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 the 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 the following year, suggests not only an effect of increased thermoregulation costs, but also changes in behaviour. A similar effect of weather on energy expenditure was also observed in red squirrels (Fletcher et al., 2012). We demonstrate that under cold conditions shaving is an efficient method of increasing daily energy expenditure in wild animals in their natural environment by increasing the cost 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 over 2 years, at the beginning of climatic winter in this region: 1–16 November 2008 and 20 November to 6 December 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 (http://www.weatheronline.pl).
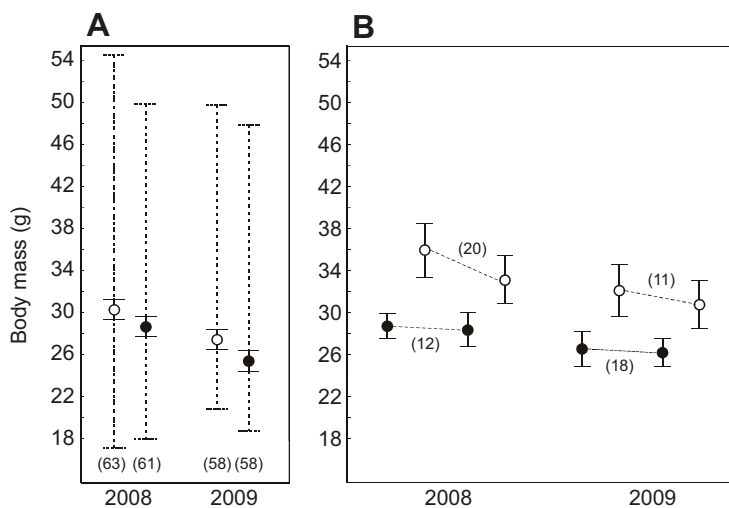

Fig. 2. Body mass of shaved and unshaved root voles. (A) Mean (±s.e.) body mass of all individuals released in 2008 and 2009 (range of body mass indicated by dotted line). (B) Mean (±s.e.) body mass of fraction recaptured within 3 days; body mass when released and when recaptured is connected by a dotted line. Open circles, shaved animals; filled circles, unshaved animals. Number of individuals in shown in parentheses.
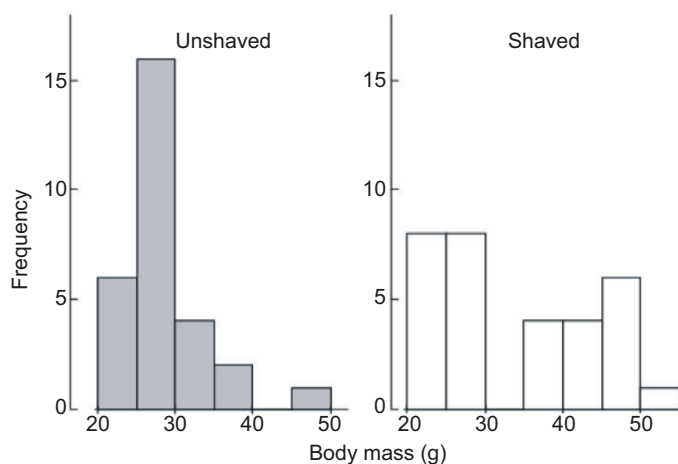
Fig. 3. Body mass frequency distribution of unshaved and shaved root voles recaptured within 3 days.

The natural population of the root voles was enclosed in the 1 ha (10,000 m^2) area by a 70-cm-high fence, covered with a plastic coating 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 weighed, sexed and microchipped (Microchip Ds.co. s.c., Głogów, Poland). Then, they were randomly assigned to either the experimental (shaved) or control (unshaved) group. Animals were handled under the Local Research Ethics Committee in Białystok, permits no. 9/2008, 1/2009.

Extra thermoregulatory costs
We shaved off the dorsal pelage of voles in the direction from the tail up to the neck and up to the midline between the elbow and the knee on both sides (Fig. 1). We estimated the shaved patch to be 50% of the total back surface. During the shaving procedure, one of the experimenters handled an animal while a second shaved the fur using a human beard trimmer (Remington PG-200C) with no tip attached, which allowed us to shave voles to the bare skin. Animals were not anaesthetized prior to the procedure because of a 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 2 min, 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
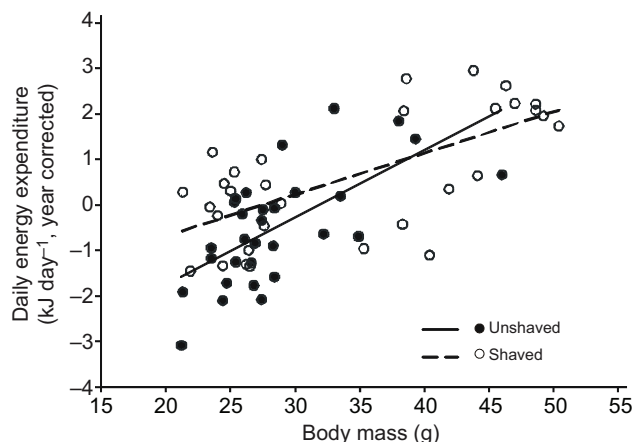

Fig. 4. Relationship between body mass and daily energy expenditure. DEE was estimated using doubly labelled water in unshaved (filled circles and solid line) and shaved (open circles and dashed line) root voles and was corrected for the year of study.

Table 1. Factors influencing daily energy expenditure in root voles under natural conditions

Source of variation in DEE	s.s.	d.f.	m.s.	F	P
Body mass	0.33	1	0.33	94.26	<0.0001
Shaving	0.02	1	0.02	4.69	0.03
Year (shaving)	0.09	2	0.05	13.15	<0.0001
Shaving x body mass	0.02	1	0.02	4.32	0.04

Only significant interactions are presented; data are log$_e$ transformed.

November 2009 (experimental: 33 males and 25 females; control: 35 males and 23 females).

Daily energy expenditure
Daily energy expenditure (DEE) was estimated using the doubly labelled water (DLW) method (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 a 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^{-1}) assuming a respiratory quotient of 0.85 (Speakman, 1997). The initial and final blood samples (50 μl each) were taken from the retro-orbital sinus which is commonly used with rodents (e.g. Klein et al., 1997). Voles are very sensitive to any anaesthesia, hence we bled conscious animals. Over the 3 days that the DLW method required, we were able to recapture 25.8% of all injected animals in 2008 and 31.9% in 2009.

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 and 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^{-1}) 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. For the analysis of DEE, both BM and DEE were log$_e$ transformed to take into account an allometric relationship between these two traits (McNab, 2002). The above analyses were carried out using STATISTICA and the R package (R Development Core Team, 2012).

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