Central Limits to Sustainable Metabolic Rate Have No Role in Cold Acclimation of the Short-tailed Field Vole (Microtus agrestis)

Regina M. McDevitt
John R. Speakman
Department of Zoology, University of Aberdeen, Aberdeen AB9 2TN, Scotland, United Kingdom
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Abstract
We investigated the extent to which the changes in basal metabolic rate (BMR), gut morphology, and food intake (FI) that typically occur during cold acclimation in small mammals can be explained by the concept of alimentary-mediated limits to sustainable metabolic energy expenditure. Adult short-tailed field voles (Microtus agrestis) were cold stressed by continuous exposure to 5°C. Exposure for 10, 20, 50, and 100 d (n = 6 in all cases) produced significant changes in oxygen consumption (V̇O₂), mass, FI, and the dry weight of a variety of morphological parameters when compared with voles that were not cold exposed (n = 8). At 10°, 20°, and 25°C, V̇O₂ (mL·min⁻¹) increased significantly with the duration of cold exposure. After 100 d cold exposure, V̇O₂ had increased by more than 50% at each test temperature. Food intake (g·d⁻¹) increased significantly by 106% after 10 d cold exposure but did not increase further with increased exposure time. The ratio of FI (g·h⁻¹) to BMR (J·h⁻¹) was 1.2 in controls and increased to 2.7 after 10 d cold exposure. Thereafter the ratio decreased, and after 100 d cold exposure FI:BMR was not significantly different from control levels. The mass of cold-exposed voles increased significantly with duration of exposure. Masses of the following morphological parameters increased with increasing duration of cold exposure: whole body mass, carcass, skeleton, pelage, subcutaneous fat, liver, kidney, lung, and interscapular brown fat (BAT). There was no significant relationship between duration of cold exposure and the masses of muscle, large intestine, heart, and brain. Using stepwise multiple regression analysis we showed that variation in BMR was linked mostly to changes in skeletal mass. However, when skeletal mass was removed as an independent variable from the analysis, BAT, muscle, and gut mass entered as significant predictors, together explaining 55.5% of the variation in BMR. Although FI increased during cold exposure, the increase (106%) was apparently insufficient to precipitate a hypertrophic response in the gut. Nevertheless BMR did increase as duration of cold exposure increased, probably linked to an increase in BAT mass and thus thermogenic capacity. We cannot support the hypothesis that the changes that typ-
Introduction

All aspects of animal behavior and morphology have to some extent implications for energy expenditure. Energy expenditure therefore potentially represents a common currency by which one may be able to evaluate the costs that are associated with different behavioral and morphological evolutionary strategies. Implicit in the "energetics" approach is the assumption that the rate at which animals are able to expend energy is limited, possibly by limits in their ability to acquire energy. Although it is frequently asserted that such limits exist, for example, "obtaining adequate energy may be the most important challenge facing wild animals" (Nagy 1987, p. 111) and "rate of energy expenditure may be limited by the rate of acquisition" (McNab 1980, p. 106), the actual nature of the limits on energetic performance have proved elusive.

Over the very short term (seconds and minutes), endotherms are capable of expending energy at very high levels (Bartholemew 1982). However, these maximal levels of energy expenditure can only be maintained for short durations (McGillivary 1971). The sustainable metabolic energy expenditure of an animal, which is less than the short-term maximal rate, can be defined as the energy expenditure that can be sustained over a more protracted period (hours or days). Measurements of free-living energy expenditures over such protracted periods are commonly made using the doubly labeled water technique (Lifson and McClintock 1966; Mullen 1973; Nagy 1980; Speakman and Racey 1988) and are frequently referred to as field metabolic rate (FMR). Interspecific comparisons of FMR (Framstad et al. 1981; Nagy 1987) have revealed that the major factor influencing it is body mass, with the scaling exponent somewhere between 0.7 and 0.8. Different groups (divided by ecology), however, have significantly different intercepts, and thus at any given body mass there is a large range in the FMR that any particular species can sustain. These differences in FMR are exemplified by a comparison of two animals of similar mass. The long-eared bat (Plecotus auritus) weighs 10 g and has a maximum FMR of about 25–30 kJ·d⁻¹ (Speakman and Racey 1987), while the common shrew (Sorex araneus) has the same body mass but a maximal FMR of about 120–140 kJ·d⁻¹ (Poppitt 1988). These differences call into question the assumption...
that there exists a common limit on the level at which animals can expend energy.

The pattern of variation in FMR is mirrored by the pattern of variation in basal metabolic rate (BMR), which also scales with an exponent of about 0.75 on body mass (Kleiber 1961; Framstad et al. 1981; McNab 1983; Bozinovic 1992). Comparisons of BMR in different groups divided by ecology (McNab 1980) or phylogeny (Bennett and Harvey 1987; Elgar and Harvey 1987) also reveal intergrade differences in energy expenditure. Although the nature of these intergrade differences, and whether they rest in ecology (McNab 1992) or phylogeny (Harvey and Pagel 1991), has been the focus of some recent debate, the similarity of the pattern of variation in BMR with respect to variation in FMR is striking.

Drent and Daan (1980) were probably the first to indicate that levels of FMR might be causally linked to variations in BMR. They noted that, independent of the absolute levels of energy expenditure, the FMR was commonly at most around four times the BMR for the same species. This led to the notion that there might be a ceiling to the sustainable energy expenditure that is not absolute but a fixed multiple of BMR. More recently the level of this supposed ceiling at four times the BMR has been questioned. Bryant and Tatner (1991) examined all the published data of FMR for birds \( n = 663 \) measurements on 32 species) and found that at least 30% of measurements exceeded the supposed four-times-BMR limit, although none exceeded seven times BMR. Similarly Peterson, Nagy, and Diamond (1990) from a more restricted comparison also came to the conclusion that seven times BMR represented the level of the ceiling on sustainable energy expenditure. In contrast, Masman et al. (1989) reviewed about 30 studies of birds feeding nestlings, which is generally considered the most energetically stressful period of reproduction, and confirmed the three- to four-times limit.

There are two potential mechanisms that might underlie the proposed ceiling on sustainable metabolic rate. The first is that the ceiling represents a common limit imposed by the ability of the animal to absorb energy via the gastrointestinal tract. Kirkwood (1983) found that metabolizable energy intake also scales closely with BMR such that the maximum metabolizable energy intake is three to six times the BMR. The linkage may arise because animals that process large quantities of food have enlarged gastrointestinal tracts and livers, and both of these organs are inferred to contribute the greatest component of the BMR (Webster 1988). The limit to metabolizable energy intake matches closely the supposed maximum limits of FMR. The second hypothesis is that the metabolic limits are more peripherally imposed, reflecting the constraints, for example, in the cardiovascular or re-
spiratory system (Pasquis, Lacaisse, and Dejours 1970; Lechner 1978), or availability of substrates for thermogenesis (Wang 1978).

Hammond and Diamond (1992) explicitly tested the ceiling hypothesis by manipulating litter size in mice. They found that at peak lactation the mice would not take in more energy than 7.2 times their BMR, independent of how many offspring they were fostering. Hammond and Diamond (1992) claimed that this experiment supported first the notion that there is a ceiling to sustained energy expenditure, second that it demonstrated that the level of the ceiling is around 7.2 times BMR, and third (from results concerned with substrate transport across the gut) that it indicated that the ceiling is mediated centrally by limits in the alimentary canal.

During the process of thermal acclimation (when animals are exposed to continuous temperatures below their lower critical temperature), previous studies have indicated that several changes consistently take place. The subject animals, by definition, experience an increase in thermogenic energy demands. They respond by an immediate increase in their food intake (FI). Later they show hypertrophy of the gut and liver and also have elevations in their BMR (Gross, Wang, and Wunder 1985; Haim, Van Arde, and Skinner 1990). One interpretation of these changes is that they represent changes in the animal due to the alimentary-mediated ceiling on metabolic performance. That is, when initially exposed in the cold, the animals have to expend energy beyond their imposed ceiling for energy intake. The animals immediately increase their FI as much as possible and meet the deficit in expenditure from stored reserves. To increase FI further, a longer-term response is hypertrophy of the gut and liver. However, a consequence of this hypertrophy of the food processing machinery is an increase in BMR.

In this article we explicitly explore the possibility that the process of thermal acclimation can be explained in the context of the centrally mediated ceiling to sustainable energy expenditure, using as a model animal the short-tailed field vole (*Microtus agrestis*).

**Material and Methods**

**Study Animals**

Short-tailed field voles, *Microtus agrestis* Linnaeus, were live-trapped in the northeast of Scotland (57°N) in October 1991, when population density is highest (Corbet and Harris 1991). A grid of 6 × 10 live traps (Longworth, Penlon, U.K.) was established in a young Sitka spruce plantation (approximately 4 yr old), which provided sufficient ground cover for field voles. Traps were baited with chopped carrot, packed with dry hay for insulation,
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and laid out along established vole runs. The trapping program ran for 3

wk, with traps set continuously and checked between 0800 and 1000 hours

and 1600 and 1800 hours daily. In total, 89 M. agrestis were captured during

504 trap hours. Of these, 56 were fully mature adults and 33 were subadults.

Voles were separated into age classes on the basis of mass and the appearance

of external genitalia. Adult male voles were easily sexed as they had obvious

testes, and adult females had nipple patches and/or perforated vaginas,

which indicates that they had been reproducitively active. Corbet and Harris

(1991) reported that juvenile M. agrestis from western Scotland had a mean

mass of 20.8 g and adults were between 30 and 40 g. However, they do not

report either the range or standard deviations of their data. In the present

study the mean mass of subadults was 16.7 ± 1.6 g (range 14.1–18.6 g) and

that of the adults was 25.1 ± 4.9 (range 19.8–34.1 g). When there was any

ambiguity about the reproductive status and thus age of a vole, it was classed

as a subadult and excluded from the experiment.

When brought into the laboratory, the voles were kept in a constant-
temperature room at 25° ± 1.0°C, the lower critical temperature of M. agrestis

(R. M. McDevitt, unpublished data), and under a 14L:10D photoperiod cycle.

The normal range of ambient temperatures experienced by these voles in

October 1991 was a maximum of 9.8° ± 2.8°C and a minimum of 3.8° ± 2.9°C

(meteorological weather station, Cultery field station, Aberdeen).

Voles were kept singly in rodent boxes (30 cm X 15 cm X 15 cm) provided

with sterilized wood chips (5 cm in depth) as substrate and with wood wool

for nesting material (weighing approximately 10 g). Rodent chow (Special

Diet Service, BP Nutrition, U.K.) and water were provided ad lib. and were

supplemented on a weekly basis with fresh vegetables.

All captured voles were kept under these maintenance conditions for 3

wk, the primary aim of which was to standardize the thermal history of the

voles, as this was not known for each individual vole. The second aim was

to familiarize the voles with laboratory conditions and thus minimize stress

levels. Thirty-two voles were chosen at random from the pool of adult an­

imals and randomly divided into five groups (n = 6 in each of the cold-
exposed groups and n = 8 in the control voles). The length of time these

groups were cold exposed varied from 0 (control voles that were not cold

exposed at all) to 10, 20, 50, and 100 d at 5°C. The number of male and

female voles in each of the groups was as follows: control (four males, four

females), 10 d (six males), 20 d (one male, five females), 50 d (two males,

four females), and 100 d (four males, two females). During cold exposure,

voles were moved to a constant-temperature-controlled room (5.0° ± 2.0°C)

with the same photoperiod (14L:10D) as maintenance conditions. The voles

were kept in rodent boxes of the same dimensions as under maintenance
conditions and were provided with the same amount of substrate and bedding as the control voles. Food intake (rodent chow only) and body mass (g) were monitored on a weekly basis for the duration of the study. A control group of voles maintained at 25°C (i.e., maintenance conditions) for 100 d was not included in the experimental protocol because such a control group would be warm acclimated relative to the temperatures that acclimatized voles would normally experience in October. Thus any comparison of a 100-d control at 25°C and the 100-d experimental animals at 5°C would detect the difference between cold and warm acclimation and not the effects of cold acclimation alone.

**Respirometry Protocol**

Oxygen consumption ($\dot{V}O_2$) was measured with an open circuit respirometry system. Animals were placed in a sealed Perspex metabolic chamber (25 × 10 × 10 cm) with openings at opposite ends for incurrent and excurrent airflows. The metabolic chamber was kept inside a constant temperature incubator (INL-401N-010, Gallenkamp), which maintained the experimental temperature in the incubator at ±0.5°C. The chamber was divided by perforated Perspex sides into three sections. The vole was kept in the middle section downstream of silica gel that was kept in the portion closest to the incurrent airstream. A wire gauze platform covered the floor of the animal's section to elevate it above any excreta and urine voided during experiments. Ambient air was pumped through the system and the flow was maintained at $500 \pm 20.0$ mL· min$^{-1}$ (metered continuously with an Alexander Wright DM3A flowmeter). Before and after passing through the flowmeter, air was dried by passing over the silica gel. After entering the incubator and still upstream of the metabolic chamber, ambient air was first passed through a length of copper tubing (1 m) to ensure that it equilibrated with the incubator temperature.

Excurrent air from the chamber was subsampled, and 400 mL· min$^{-1}$ was drawn through one channel of a two-channel oxygen sensor (N-37m, S-3A, Applied Electrochemistry) by using a flow control (R-2, Applied Electrochemistry). Reference air was also pumped through the incubator by a second pump and drawn through the second channel of the oxygen analyzer in the same way. The lengths of tubing carrying both air samples were kept the same to minimize pressure differences. Before entering the oxygen sensor both reference and expired air were dried over silica gel and passed through Carbasorb (BDH) to remove CO$_2$. The difference between these two oxygen concentrations ($\Delta$O$_2$) was continuously monitored at 40-ms intervals with an A to D converter (uPD7002), and the mean was recorded
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A control group (100 d old) was studied. The control group was acclimatized to 5°C and not the effects of acclimatization on metabolism were compared. A control chamber (25°C; condenser, oxygen analyzer, and excurrent air) was divided by a Perspex sheet to the experimental side. The Perspex sheet was divided by a Perspex sheet to the middle of the animal's body mass.

VO_2 was maintained at the middle of the cage. Air was passed through a flowmeter and still monitored at 40-ms intervals. The mean was recorded every 5 s with an on-line microcomputer (Acorn). The minimum VO_2 (mL·min^{-1}) was calculated over 10-min intervals for each hour.

Prior to VO_2 determinations, each animal was weighed to the nearest 0.1 g (Sartorius, pan balance) and placed inside the metabolic chamber without food or water. The vole was not food deprived prior to being placed in the chamber but were left for an hour before the start of measurements (Hayes, Speakman, and Racey 1992). Atmospheric pressure (mmHg) and the temperature of air passing through the flowmeter were measured, and VO_2 was adjusted to standard temperature and pressure. All measurements took place between 0900 and 1700 hours, the naturally quiescent part of the circadian rhythm of M. agrestis (Hansson and Grodzinski 1970) but were not controlled further with respect to time of day. Each VO_2 measurement lasted for 3 h since the minimum measurement is dependent on duration but reaches a lower asymptote at about 2 h (Hayes et al. 1992). Oxygen consumption was measured in the control voles after 3 wk in captivity, during which time they were maintained at 25°C and 14L:10D. The thermoneutral zone (TNZ) in autumn-acclimatized M. agrestis had previously been determined to be 25°-30°C (R. M. McDevitt, unpublished data). Oxygen consumption was measured in the voles at two temperatures within the TNZ (25°C and 30°C) and at three temperatures below this (10°C, 15°C, and 20°C). The temperature that VO_2 was measured at was chosen randomly from the test temperatures, and the VO_2 of each vole at that temperature from the group was measured within the same 48-h period. After each 3-h VO_2 measurement, the control voles were returned to maintenance conditions. The cold-exposed voles were moved to the cold after first spending the same time as the control voles (3 wk) at maintenance conditions. The VO_2 of each cold-exposed group of voles was measured following the same procedure used for the control voles, except measurements were made at 10°C, 20°C, and 25°C, and afterward voles were returned to the cold room (5°C).

Morphological Protocol

After each vole had spent the required amount of time exposed to the cold and VO_2 had been measured at each experimental temperature, it was killed (by CO_2 overdose) and dissected. Brown adipose tissue (BAT) depots from the interscapular region were removed from the freshly killed voles, and wet weights of this tissue were recorded. Internal organs including the large and small intestine, liver, kidney, lungs, heart, and brain were removed, weighed wet, and then dried to a constant mass (3 d at 60°C) and reweighed (Oertling R20; accuracy: 0.01 g). The animals were skinned and all visible...
subcutaneous fat was removed and weighed and dry weights were obtained as for the internal organs. The small intestine was removed and its relaxed length was measured, to the nearest millimeter (Kannon callipers). Both portions of the intestinal tract were washed out with saline using a 10-mL syringe before being weighed and then dried as other tissues. The body length (nose to vent) and skull length (condylobasal) were also measured (Kannon callipers). The remaining carcass was also weighed wet and after drying. Dried carcasses were ashed in a furnace at 350°C for 48 h before being reweighed. Ashed carcass weight (i.e., skeletal mass) was subtracted from dried carcass weight to yield the muscle mass component of the carcasses.

Statistical Analysis

Data were analyzed by ANOVA, multiple least squares regression, and stepwise multiple regression with the Minitab software package (Ryan, Joiner, and Ryan 1985). We evaluated differences after ANOVA using the minimum significant difference (MSD) (Sokal and Rohlf 1981). Unless otherwise stated data quoted directly are means ± 1 SD. The data were analyzed for the effect of gender on all the parameters, and no significant relationship was found. However, the reduced sample sizes were too small to draw any meaningful conclusions from this nonsignificant result.

Results

Metabolic Rate in Cold-acclimated Voles

The lowest VO₂ over a 10-min interval during the 3-h measurement period in short-tailed field voles was influenced significantly by both the temperature of measurement (T₂), with greater energy demands at lower temperatures, and by the duration (in days) that the voles had spent exposed to the cold (D) (fig. 1). There was also a significant effect on VO₂ of the interaction between these two variables (F = 2.95, P = 0.026). The least squares fit multiple regression (\( VO₂ = 4.22 - 0.102 T₂ + 0.0185 D - 0.000845(T₂ \times D) \)) explained 62.6% of the variation in VO₂ (F = 36.36, P ≤ 0.001). When compared to that of control voles, the VO₂ in voles cold exposed for 100 d (n = 4) increased by more than 50% at all three measurement temperatures: 55.1% (25°C), 58.9% (20°C), and 52.2% (10°C).

During the experiments, some voles in the 100-d group died before they had spent 100 d in the cold and their VO₂ was measured. A bias therefore may have been introduced into the data set, as the surviving voles were the
were obtained wri the rats and its relaxed (opened) collars. Both rats were housed in using a 10-mL syringe. The body temperature was also measured 1 h before and after the rats were allowed for 48 h before the experiment was started and continued with the experiment of the car-

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Fig. 1. Oxygen consumption (\(\dot{V}O_2\), mL·min^{-1}) in Microtus agrestis during continuous exposure to constant cold (5°C) for different time periods: control, 10 d, 20 d, 50 d, and 100 d. Data points are means ± 1 SD in the control group (n = 8) and are means only in the cold-exposed groups (n = 6).

largest in the group. To account for this potential bias, the data were analyzed both with and without the 100-d animals included. When the data for the voles exposed for 100 d were excluded the \(\dot{V}O_2\) was still highly significantly related to the \(T_s\) (\(F= 114.11, P < 0.001\)), duration of cold exposure (\(F= 8.95, P < 0.001\)), and the interaction of these two factors (\(F= 3.38, P < 0.024\)). The variation in \(\dot{V}O_2\) with duration of cold exposure was consistent across all three measurement temperatures (fig. 2). Oxygen consumption increased significantly from that of control voles when compared to those exposed to 5°C for 20 d at test temperatures of 10°C and 20°C but not at 25°C (MSD ≥ 1.067, P ≤ 0.05). After 100 d exposure, \(\dot{V}O_2\) was significantly higher at each temperature than that of control voles (MSD ≥ 1.193, P ≤ 0.05). However, from 20 d onward there was no significant increase in \(\dot{V}O_2\) at any temperature when either the 50-d (MSD < 1.067, P > 0.05) or 100-d (MSD < 1.093, P > 0.05) voles were compared to the 20-d animals.

Food Intake and Mass in Cold-acclimated Voles

Food intake in cold-exposed Microtus agrestis increased significantly by 106% from 2.93 ± 0.61 g·d^{-1} in the control voles (n = 8) to 6.06 ± 3.25
Fig. 2. Oxygen consumption ($\bar{V}O_2$, mL·min$^{-1}$) of cold-exposed (5°C) Microtus agrestis at 10°C, 20°C, and 25°C at different time intervals of exposure. Data are means ± 1 SD.

$\dot{V}O_2$ = 22, $t = 4.1$, $P = 0.0005$) in voles that had spent 1 wk at 5°C ($n = 24$). The FI of the voles exposed for 100 d (8.25 ± 0.01 g·d$^{-1}$, $n = 2$) was also significantly higher than that of the controls by 185% (df = 6, $t = 23.6$, $P \leq 0.001$). However, FI was not significantly related to duration of cold exposure ($F = 0.47$, $P = 0.495$) from day 7 to day 100, that is, after the initial cold exposure (fig. 3). Clearly these data were not well balanced because voles were removed from the cold room for measurement of $\dot{V}O_2$ and killed for morphological analysis. If we restricted the analysis to the four voles for which repeated measures were available for 14 wk of exposure, the same nonsignificant trend was apparent ($F = 1.23$, $P = 0.289$).

During cold exposure, mass increased significantly with increasing length of cold exposure ($F = 27.01$, $P \leq 0.001$). Duration of exposure explained 21.1% of the variation in mass of cold-exposed voles (fig. 4). There was no significant difference between mass of the control voles (20.35 ± 1.35 g, $n = 8$) and that of voles after exposure to 5°C for 1 wk (19.95 ± 2.42 g, $n = 24$). However, after 100 d at 5°C, body mass of cold exposed voles (30.14 ± 0.50 g, $n = 4$) was significantly greater than that of the control animals (df = 5, $t = 14.9$, $P \leq 0.001$). Again, these data may be biased. However, the relationship between mass and duration of cold exposure remained significant ($F = 10.29$, $P = 0.002$) if we included only the four voles that survived for 14 wk, although less (10.1%) of the variation in mass was explained by this relationship. Hence the original relationship (fig. 4) was not due to differential mortality/removal of voles favoring larger individuals, but a progressive deposition of tissue throughout the period of exposure (see also below).
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1 wk at 5°C (n = 24, g · d⁻¹, n = 2) consisted of 85% (df = 6, t = 2.3, P = 0.07) of the duration of exposure that is, after the well balanced measurement of VO₂ throughout the week of exposure, food intake was not related to the duration of cold exposure; however, after 1 wk FI in control voles was significantly lower than in the cold-exposed voles. Data are means ± 1 SD; value labels are sample sizes. Note that the error bar for the data at week 14 is smaller than the dimensions of the data bar and is not visible at this scale.

The ratio of FI to BMR, that is, VO₂ measured at 25°C, the lower critical temperature, with both quantities expressed in energy expenditure per unit time, throughout the duration of cold exposure initially increased by a factor of 2.2, from 1.29 ± 0.26 in controls to 2.85 ± 1.23 after 10 d cold exposure (fig. 5). This increase marginally failed to reach significance (df = 5, t = 2.3, P = 0.07) because of the large variation in the FI:BMR ratios of the 10-d voles. Between 10 and 100 d, the FI:BMR ratio declined to 1.35 ± 0.31, which was also not significantly greater than in the controls.

Changes in Gross Morphology during Cold Acclimation

Regression analysis of morphological parameters revealed that the different organs varied in their response to cold exposure (fig. 6A–C and table 1). Whole body mass in the killed voles was significantly related to duration of cold exposure (F = 17.87, P ≤ 0.001; see also fig. 4 for trend in live voles) as was carcass (F = 27.47, P ≤ 0.001), skeletal (F = 9.91, P = 0.005), and pelage mass...
Fig. 4. Mass (g) of *Microtus agrestis* during continuous exposure to 5°C, where C is the mass of the control voles and the 0 time point is the mass of the cold-exposed voles before they are placed in the cold. The mass of cold-exposed voles increased significantly with increasing exposure time. This relationship remained significant if only the 100-d voles are used in the analysis. Data are means ± 1 SD; value labels are sample sizes.

(\(F = 8.80, P = 0.007\)) (fig. 6A). Of the viscera, the masses of the liver (\(F = 15.43, P \leq 0.001\)), kidneys (\(F = 19.56, P \leq 0.001\)), and lungs (\(F = 12.77, P = 0.002\)) were significantly related to duration of cold exposure (fig. 6B). The dry weights of the heart and brain were not significantly affected by duration of exposure. Brain weight remained remarkably constant with 0.0% variance explained by changes in cold-exposure duration (\(F = 1.00, P = 0.327\)) (fig. 6C). Neither the length of the small intestine (\(F = 0.55, P = 0.464\)) (table 2) nor the mass of the large intestine (\(F = 3.79, P = 0.064\)) increased significantly with duration of cold exposure (fig. 6B). The muscle mass component of carcass mass did not increase significantly with cold exposure (\(F = 0.28, P = 0.604\)); however, the mass of subcutaneous fat did (\(F = 10.73, P = 0.003\)). Of all the morphological parameters considered, only subcutaneous fat (\(F = 7.64, P = 0.014\)), carcass dry mass (\(F = 6.46, P = 0.023\)), and BAT wet mass (\(F = 50.33, P \leq 0.001\)) were significantly correlated with total animal mass. Mass of the interscapular BAT increased significantly (\(F = 20.18, P \leq 0.001\)) with duration of cold exposure, and a significantly large increase (\(df = 4, t = 6.0, P = 0.005\)) occurred between days 50 and 100 (fig. 6B). Other organs also followed this
of the liver ($F = 12.77, P = 0.014$). A significant positive relationship existed in both body length ($F = 4.34, P = 0.046$) and skull length ($F = 12.40, P = 0.002$) and duration of cold exposure (table 2). A significant increase in body length occurred between 50 and 100 d ($df = 4, t = 5.0, P = 0.008$) but not in skull length.

When the morphological data for the voles exposed for 100 d were excluded, only the lungs ($F = 4.40, P = 0.048$), carcass ($F = 4.77, P = 0.040$), and skeletal mass ($F = 10.54, P = 0.004$) had a significant positive relationship with duration of cold exposure, although the relationship to BAT mass approached significance ($F = 3.32, P = 0.08$). The effect of exposure on body length was lost, but the increase in skull length remained significant ($F = 16.45, P \leq 0.001$).

The Relationship between BMR and Morphological Parameters during Cold Acclimation

A stepwise multiple regression between BMR and 15 morphological parameters (masses of the live vole, carcass, skeleton, pelage, large intestine,
Fig. 6. Morphological changes in Microtus agrestis during exposure to 5°C. Data are means ± 1 SD, and a significant relationship between mass and duration of cold exposure is indicated by a single asterisk (P < 0.01) and two asterisks (P < 0.001). A significant difference between dry mass at 50 d compared to 100-d voles is indicated by a dollar sign (P ≤ 0.05). A, Dry mass (g) of vole carcass, skeletal mass, pelage, and subcutaneous fat; B, dry mass of liver, kidneys, and large intestine (BAT mass is wet weight); C, dry mass of lungs, heart, and brain.
liver, kidneys, lungs, heart, brain, subcutaneous fat, and BAT [g] and the length of the body, small intestine, and skull [cm], *F* to enter = 4) resulted in only one variable, skeletal mass, entering as significant (*t* = 5.27), which explained 54.7% of the variation in \( \dot{V}O_2 \) at 25°C (=BMR). The least squares fit regression equation (*F* = 27.77, *P* < 0.001) was BMR = 1.05 + 0.453 skeletal mass. In a stepwise multiple regression with forward inclusion but excluding skeletal mass as a predictor variable, with *F* to enter still equal to 4, three variables entered as significant: gut mass (*t* = 2.13), BAT mass (*t* = 3.60) and muscle mass (*t* = −2.67). The best fit equation was BMR = 0.959 + 2.14 gut + 2.17 BAT − 0.300 muscle, and it explained 55.5% of variation in BMR. Changes in gut mass with duration of exposure were not significant, yet changes in the mass of BAT were, so the change in BMR with duration of exposure was more closely related to the change in BAT mass than changes in gut mass.

**Discussion**

Previous studies on small rodents have similarly demonstrated an increase in metabolic rate, both BMR (Adolph 1950; Haim et al. 1990) and average daily metabolic rate, after prolonged cold exposure (Hansson and Grodzinski 1970). Adolph and Lawrow (1951), for example, found an increase of 28%
Table 1
Morphological changes in Microtus agrestis during long-term exposure to 5°C

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<tr>
<td>Vole</td>
<td>20.3 (1.2)</td>
<td>23.7 (3.5)</td>
<td>22.3 (2.3)</td>
<td>24.0 (2.3)</td>
<td>30.2 (.4)</td>
</tr>
<tr>
<td>Carcass</td>
<td>3.1 (.3)</td>
<td>3.4 (.6)</td>
<td>3.3 (.5)</td>
<td>3.8 (.4)</td>
<td>5.0 (.3)</td>
</tr>
<tr>
<td>Pelage</td>
<td>2.7 (.4)</td>
<td>3.3 (1.0)</td>
<td>3.2 (.5)</td>
<td>3.5 (.5)</td>
<td>4.5 (.1)</td>
</tr>
<tr>
<td>Skeleton</td>
<td>2.4 (.5)</td>
<td>1.1 (.5)</td>
<td>1.5 (.4)</td>
<td>1.4 (.6)</td>
<td>2.0 (.1)</td>
</tr>
<tr>
<td>WAT</td>
<td>1.3 (.3)</td>
<td>1.6 (.5)</td>
<td>1.8 (.7)</td>
<td>1.7 (.3)</td>
<td>2.4 (.4)</td>
</tr>
<tr>
<td>BAT</td>
<td>.19 (.06)</td>
<td>.24 (.09)</td>
<td>.27 (.05)</td>
<td>.29 (.07)</td>
<td>.52 (1 × 10⁻¹)</td>
</tr>
<tr>
<td>Intestine</td>
<td>.36 (.05)</td>
<td>.39 (.04)</td>
<td>.38 (.03)</td>
<td>.44 (.10)</td>
<td>.48 (.01)</td>
</tr>
<tr>
<td>Liver</td>
<td>.34 (.03)</td>
<td>.34 (.10)</td>
<td>.37 (.06)</td>
<td>.36 (.05)</td>
<td>.60 (.08)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>.06 (3 × 10⁻³)</td>
<td>.08 (.01)</td>
<td>.08 (6 × 10⁻³)</td>
<td>.08 (6 × 10⁻³)</td>
<td>.11 (.01)</td>
</tr>
<tr>
<td>Lungs</td>
<td>.04 (6 × 10⁻³)</td>
<td>.04 (.01)</td>
<td>.05 (8 × 10⁻³)</td>
<td>.05 (5 × 10⁻³)</td>
<td>.06 (4 × 10⁻³)</td>
</tr>
<tr>
<td>Heart</td>
<td>.04 (6 × 10⁻³)</td>
<td>.05 (.01)</td>
<td>.05 (9 × 10⁻³)</td>
<td>.05 (6 × 10⁻³)</td>
<td>.06 (9 × 10⁻³)</td>
</tr>
<tr>
<td>Brain</td>
<td>.11 (.14)</td>
<td>.11 (6 × 10⁻³)</td>
<td>.11 (.01)</td>
<td>.11 (3 × 10⁻³)</td>
<td>.12 (4 × 10⁻³)</td>
</tr>
</tbody>
</table>

Note. Data are mean mass in grams with SD in parenthesis; n = 8 for 0-d exposures; n = 6 for 10-, 20-, 50-, and 100-d exposures; WAT is white adipose tissue from subcutaneous depots only and BAT is interscapular brown adipose tissue. All masses are dry weight except for whole vole and BAT masses, which are wet weights.

* Significant relationship between mass and duration of cold exposure at P ≤ 0.01.

** Significant relationship between mass and duration of cold exposure at P ≤ 0.001.
Alimentary Limits to Sustained Metabolic Rate

TABLE 2

Morphological changes in Microtus agrestis during long-term cold exposure (5°C)

<table>
<thead>
<tr>
<th>Duration of Cold Exposure (d)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine</td>
<td>24.7 ± 2.3</td>
<td>25.5 ± 1.5</td>
<td>25.0 ± 1.8</td>
<td>25.0 ± 1.5</td>
<td>26.3 ± 1.8</td>
</tr>
<tr>
<td>Body*</td>
<td>9.6 ± .5</td>
<td>9.7 ± .3</td>
<td>9.3 ± .3</td>
<td>9.6 ± .2</td>
<td>10.4 ± .1</td>
</tr>
<tr>
<td>Skull**</td>
<td>2.2 ± .1</td>
<td>2.3 ± .1</td>
<td>2.4 ± .2</td>
<td>2.5 ± .6</td>
<td>2.5 ± .7</td>
</tr>
</tbody>
</table>

Note. Data are mean length (cm) ± 1 SD; n = 6 for 0-, 10-, 20-, 50-, and 100-d exposures; n = 4 for 100-d exposures.

* Significant at P < 0.05.

** Significant at P < 0.01.

The increase in BMR of 50% reported here is greater than in these previous studies. This probably reflects the relatively long duration of exposure, because the changes were progressive up to about 20 d, while many previous studies of responses to cold exposure have lasted for less than 20 d. A similar pattern of progressive increase in metabolism up to around 35 d exposure in rats was reported by Cottle and Carlson (1954).

Food intake (dry matter and digestible energy) has been shown previously to increase during cold exposure (e.g., Gross et al. 1985; Haim et al. 1990). Both energy consumption and assimilation efficiency increase with decreasing acclimation temperature (Weiner 1987). Few studies have continuously monitored FI in cold-exposed mammals. Our results indicate that, as long as there was an increased energy stress, energy consumption remained high compared to control levels. Similar results have been found in rats (Cottle and Carlson 1954). The process of acclimation therefore principally reflected
the animals altering their capacity and mechanism of heat production rather than increasing insulation to reduce the heat losses and hence also ultimately FI requirements. Although we reported an increase in food consumption, which is consistent with the bulk of previous studies of cold acclimation in small mammals, we also found a significant increase in pelage mass, which is not consistent with previous findings (Hart 1965; Heldmaier 1989). However, the increase in pelage in the present study was not sufficient to obviate the need for some increased thermogenesis, and therefore an increase in food consumption was necessary to meet this demand.

The gastrointestinal tract and associated organs have been identified as the tissues most responsive to changes in energy demands, either due to thermoregulatory costs (Gross et al. 1985) or reproductive expenditure (Fell, Smith, and Cambell 1963; Barnett 1973; Hammond and Diamond 1992). In the present study, we found no relationship between gut morphology (mass or length) and the duration of cold exposure in *Microtus agrestis*. The increase in FI recorded here was relatively small (106%) when compared with that reported in previous studies (Fell et al. 1963; Barnett 1973; Hammond and Diamond 1992). Hence, the increase in FI when voles were exposed to the cold may have been insufficient to necessitate an increase in gut mass or length because the gut could handle the increased intake without a reduction in digestive efficiency. This interpretation does, however, suggest these voles had a substantial safety margin for increased processing capacity (Hammond and Diamond 1992), which we cannot explain.

Acclimation to the cold involves an increase in cold tolerance (Hart 1953). This is primarily achieved by an increase in the capacity for nonshivering thermogenesis (NST) (Feist and Rosenmann 1976). An increase in NST, or an increased sensitivity to noradrenaline, has been previously demonstrated in cold-acclimated rodents (Feist and Rosenmann 1976; Klaus, Heldmaier, and Ricquier 1988). The principle site for NST is BAT (Foster and Frydman 1979; Himms-Hagen 1986). Several previous studies have also demonstrated that BAT increases in mass during the process of cold acclimation (Tarkkonen 1971; Bukowiecki et al. 1982; Bukowiecki and Collet 1983), as we found in *M. agrestis*.

In cold-acclimated rats, liver, heart, and kidney mass increased but subcutaneous fat, muscle, and pelage mass decreased (Heroux and Campbell 1959). In that study it was suggested that the animals were drawing on their bodily reserves to satisfy thermogenic requirements instead of increasing energy intake. Because most of the recorded morphological parameters considered in *M. agrestis* increased in mass in response to prolonged cold exposure, the increased cost of thermoregulation must have been fueled entirely by the observed increase in energy intake.
Previous studies in rats have also indicated a progressive increase in whole body mass during cold acclimation (Cottle and Carlson 1954). We know of no previous studies in which the responses of skeletal parameters like skeletal mass and condylobasal length to cold acclimation have been reported. We cannot eliminate the possibility that the increases reported in this study did not represent changes occurring within individuals but rather were the result of an unfortuitous chance allocation of individuals to the groups. The progressive pattern of change, however, make this unlikely. Also, because the voles used in this study were all adults, on the basis of mass and external appearance of reproductive organs, these progressive changes do not reflect developmental growth from juvenile to adult. Skeletal changes during cold acclimatization have been previously established in many small mammals (Pucek 1970; Pasanen 1971; Lynch 1973; Merritt 1986), but these normally involve regression in the cold (winter) and not expansion. There has been much speculation about the advantages and disadvantages of differences in body size for energetics and thus the likely direction of selection on body size for animals exposed to periods of cold. A general conclusion has been that increased body size is advantageous in cold climates (e.g., Lindstedt and Boyce 1985; Millar and Hickling 1990). Our data on phenotypic skeletal changes of voles exposed to the cold are in general agreement with this hypothesis. However, the validity of the predictions from energetics is questionable (Millar and Hickling 1992; Speakman 1992, 1993; Hickling and Millar 1993).

Metabolizable energy intake in a wide selection of mammals and birds, measured during energetically demanding conditions, has been quantified as between three and six times BMR (Kirkwood 1983). We found that the FI:BMR ratio between the controls and cold-exposed animals increased to a peak 10 d after exposure. This increase indicates that energy intake increased at a faster rate than any concomitant increase in basal metabolism. At the peak, however, the ratio of FI to BMR was only 2.7. This is well below the previously inferred limits to sustainable metabolic rate of approximately four to seven times BMR (Drent and Daan 1980; Masman et al. 1989; Peterson et al. 1990; Bryant and Tatner 1991). This level of increased FI was apparently insufficient to stimulate an increase in gut mass or length. Nevertheless, there was still a progressive increase in the BMR as duration of cold exposure increased.

The increase in BMR appears to have been linked most closely to changes in skeletal mass, in contrast to other studies, which have linked BMR to either the gut (Webster 1988) or the cardiovascular system (e.g., Lechner 1978). However, both gut and BAT mass did assume importance as BMR predictors, in addition to muscle mass, when skeletal mass was removed as
a predictor. Gut changes with duration of cold exposure were not significant, yet changes in the mass of BAT were, so the continuous increase in BMR probably most closely reflects the progressive increase in mass of BAT and the increased thermogenic capacity.

We have shown that the pattern of increased BMR in response to cold exposure in *M. agrestis* occurs even when the level of increased FI is insufficient to precipitate an increase in gut mass or length. This increase in BMR probably reflects mostly the increased mass of BAT, which grows during cold exposure to allow increased NST. These data therefore do not support the hypothesis that the pattern of changes observed during cold acclimation reflect central limits to sustainable metabolic rate.

**Acknowledgments**

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not significant, increase in BMR not associated with mass of BAT and do not support cold acclimation


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