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RESEARCH ARTICLE

Ambient temperature shapes reproductive output during pregnancy and lactation in the common vole (*Microtus arvalis*): a test of the heat dissipation limit theory

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SUMMARY

The heat dissipation limit theory suggests that heat generated during metabolism limits energy intake and, thus, reproductive output. Experiments in laboratory strains of mice and rats, and also domestic livestock generally support this theory. Selection for many generations in the laboratory and in livestock has increased litter size or productivity in these animals. To test the wider validity of the heat dissipation limit theory, we studied common voles (*Microtus arvalis*), which have small litter sizes by comparison with mice and rats, and regular addition of wild-caught individuals of this species to our laboratory colony ensures a natural genetic background. A crossover design of ambient temperatures (21 and 30°C) during pregnancy and lactation was used. High ambient temperature during lactation decreased milk production, slowing pup growth. The effect on pup growth was amplified when ambient temperature was also high during pregnancy. Shaving fur off dams at 30°C resulted in faster growth of pups; however, no significant increase in food intake and or milk production was detected. With increasing litter size (natural and enlarged), asymptotic food intake during lactation levelled off in the largest litters at both 21 and 30°C. Interestingly, the effects of lactation temperature on pup growth were also observed at smaller litter sizes. This suggests that vole dams trade-off costs associated with hyperthermia during lactation with the yield from investment in pup growth. Moreover, pup survival was higher at 30°C, despite lower growth, probably owing to thermoregulatory benefits. It remains to be seen how the balance is established between the negative effect of high ambient temperature on maternal milk production and pup growth (and/or future reproduction of the dam) and the positive effect of high temperatures on pup survival. This balance ultimately determines the effect of different ambient temperatures on reproductive success.

Key words: heat dissipation, litter size enlargement, sustained energy intake, fur removal, ambient temperature, lactation, pup mortality.

INTRODUCTION

Reproduction comprises a period of high energy demand (McNab, 2002; Speakman, 2008). Because success and failure during reproduction directly translate into reproductive success, reproduction is an appropriate period in which to study limits in energy intake and processes in life history. During the search for the mechanistic nature of limitations on sustained energy intake, studies originally focused on two hypotheses: a peripheral or central limitation on sustained energy intake (Speakman and Krol, 2005b). A central limitation would be imposed by the capacity of the intestine and associated organs to process energy. This ‘central’ limit is therefore equivalent to a limit on the ability to extract resources from the environment. The utilisation of these resources could also be limited, and the inability to utilise further energy might then feed back into the uptake process and, thereby, limit energy intake (the peripheral limitation hypothesis).

Studies in laboratory mice (*Mus musculus*) suggest that neither of these two hypotheses may be valid. Compared with 21–23°C, lactating mice in the cold (5–8°C) (Hammond et al., 1994; Johnson and Speakman, 2001) increased their energy intake (refuting the central limitation hypothesis) and also increased their milk production (Johnson and Speakman, 2001) (refuting the peripheral limitation hypothesis). Mice that went through gestation and

lactation in a hot (thermoneutral) environment (30°C) produced less milk compared with mice held at 21°C (Krol and Speakman, 2003b). Pups also grew more slowly in this hot environment compared with a cold (8°C) or warm (21°C) environment (Krol and Speakman, 2003a). These latter findings led to the postulation of the heat dissipation limit theory (Speakman and Krol, 2011). It states that mice have to dissipate heat that is produced when energy is metabolised or else they risk hyperthermia. The risk of hyperthermia associated with high levels of intake thus limits energy intake, leading to reduced milk production and impaired pup growth.

There is considerable evidence to support this theory and also several lines of evidence that do not support it (Rogowitz, 1998; Zhao and Cao, 2009; Zhao et al., 2010; Valencak et al., 2010). For example, in support of the theory, increased ambient temperature decreased milk production and piglet growth in sows (*Sus scrofa*) (Black et al., 1993; Renaudeau and Noblet, 2001) and milk production also declined with increasing temperature in dairy cattle (*Bos taurus*) (Igono et al., 1992). In both species, relieving heat loss by either cooling the floor (sows) (Silva et al., 2006) or spraying with water (dairy cattle) (Igono et al., 1985) decreased body temperature and increased milk production. Exposure to high temperatures (close to 30°C) during lactation has also been shown to decrease pup growth in lab strains of rats (*Rattus norvegicus*)

(Jansen and Binard, 1991; Leon and Woodside, 1983; Morag et al., 1969). By contrast, data that do not support the theory include the observation that cold exposure (10°C) did not increase milk production and retarded pup growth in cotton rats (*Sigmodon hispidus*) (Rogowitz, 1998), and shaving did not elevate milk production or pup growth in Swiss mice (Zhao and Cao, 2009; Zhao et al., 2010). Valencak et al. suggested that temperature manipulations of European brown hares (*Lepus europaeus*) also did not support the theory (Valencak et al., 2010) – but this interpretation of the raw data has been questioned (Speakman and Krol, 2011). Two studies in common voles (*Microtus arvalis*) have investigated the effect of ambient temperature on reproductive output (Daksetse and Martinet, 1977; Martinet and Daksetse, 1976). The interpretation of these data is difficult because different diets and photoperiod regimes were applied simultaneously. In general, however, higher temperatures tended to reduce pup growth, while increasing pup survival. In a recent study, Brandt's voles (*Lasiopodomys brandtii*) raising litters that were larger than average produced less milk and their pups grew slower, but caught up during the last five days of lactation when they are also self-feeding, to reach a similar weaning mass at 30°C compared with 21°C (Wu et al., 2009).

In mice, exposure to very high (36°C) ambient temperatures during gestation has negative effects on survival and growth of pups. The contribution of pups and dams to this effect was investigated by cross-fostering pups born at 36°C to dams at 21°C and by cross-fostering pups born at 21°C to dams at 36°C. In both experiments, the negative effects of the high ambient temperature treatment were reduced (Pennycuik, 1966). One study in mice showed that exposure to cold had a small negative effect on pup growth, but no effect on milk production (Barnett and Dickson, 1984). However, exposure to the cold for 10 generations increased pup growth and milk production in the same study.

Most studies reviewed above were conducted using laboratory or husbandry animals. Numerous generations of selection under these domestic conditions might have shifted the genetic makeup of these animals away from those in the field (e.g. resulting in enlarged litter sizes). It will be interesting to determine, therefore, whether these studies can be extrapolated to the natural situation, and hence whether the heat dissipation limitation theory has wider validity. In the field, an energetic ceiling in great tits (*Parus major*) that is reached during parental effort is reduced with increasing temperatures [measured using doubly labelled water (Tinbergen and Dietz, 1994; Tinbergen and Verhulst, 2000)]. Wild lactating squirrels (*Sciurus hudsonicus*) built less-insulated nests with increasing ambient temperature and with increasing heat stress induced by larger litters (Guillemette et al., 2008), and short-tailed field vole (*Microtus agrestis*) females build poorer insulated nests than males (Redman et al., 1999). These findings suggest that animals may also routinely face heat dissipation problems in the wild. Increases in ambient temperature can also have profound effects on food abundance (e.g. increasing seedfall, which increases population growth of deer mice) (Falls et al., 2007) or timing of the peak in food supply [e.g. global warming causes mistimed reproduction in great tits, *Parus major* (Visser et al., 1998), and blue tits, *Parus caeruleus* (Thomas et al., 2001)].

The effects of ambient temperature on food abundance and timing confound conclusions that can be drawn for the mechanism of the effect of ambient temperature on reproductive output in the field. For instance, Tinbergen and Verhulst concluded that the energetic ceiling reached in great tits was not due to increased heat stress due to hard work, because feeding rate was not affected by temperature in the same study (Tinbergen and Verhulst, 2000). Changes in food

abundance, possibly caused by ambient temperature itself, could result in similar feeding rates with different foraging costs. Generalisations of effects of ambient temperature on reproductive output (e.g. fledging mass, weaning weight or survival) should also be treated with care. Food abundance and other direct effects of temperature on the offspring's environment together shape reproductive output [e.g. in rabbits, cold soil temperatures increase nest mortality (Rödel et al., 2008)].

The confounding effects of temperature on food supply therefore confuse any conclusion from observational field data alone that heat dissipation might limit reproductive output. In the present study, common voles (*Microtus arvalis* Pallas 1779) were used in a controlled laboratory setting to test the heat dissipation limit theory, thereby investigating its wider validity. These voles were bred in a colony that is regularly supplied with wild-caught individuals. Therefore, genetic changes in response to selection induced by direct artificial selection and/or the artificial laboratory environment are expected to be far less significant than in laboratory or husbandry animals. Common voles also produce small litters, whereas many laboratory strains or domesticated livestock have been selected for large litter size (Sikes and Ylönen, 1998). Compared with wild-derived mice, a control group that consisted of a cross of four different lab strains produced litters that were twice as large [averaging 4.4 and 9.8 pups, respectively (Miller et al., 2000)]. With increasing litter size, asymptotes in food intake are reached in lab strains of mice. After a certain litter size, maximal food intake during lactation does not increase further, resulting in reduced pup growth and survival (Hammond and Diamond, 1992; Johnson et al., 2001; Krol and Speakman, 2003a). In the present study, these limits were investigated in common voles nursing natural and experimentally enlarged litter sizes. Furthermore, the significance of heat dissipation as a limiting factor was evaluated using warm (21°C) and hot [30°C, this is close to the thermoneutral zone of common voles, which is approximately 31°C (Cretegnny and Genoud, 2006; Trojan and Wojciechowska, 1967)] ambient temperatures during lactation, and by experimentally increasing heat dissipation capacity of dams by shaving their fur, as was done previously in MF1 mice (Krol et al., 2007) and Swiss mice (Zhao and Cao, 2009). During summer, burrow temperatures of common voles can reach the high temperature range used in this laboratory study. Burrow temperatures have been reported to lie between 9 and 19°C in Finland and were reported to closely follow soil temperature (Hackman, 1963). In southern Germany (Seewiesen, Bavaria), soil temperatures at burrow depth (10cm below the surface) can reach 30°C in summer and coincided with observations of increased surface activity (M.P.G., unpublished).

In the studies mentioned above, animals were moved to a different (hot or cold) temperature and were then compared with a control group that stayed at the temperature from which they came. Strictly, such a design cannot discriminate between the confounding factor of switching ambient temperature and the ambient temperature itself. Furthermore, in most previous studies, the temperature treatment was also given throughout pregnancy and lactation, making it impossible to discriminate between effects of ambient temperature that occurred during pregnancy or lactation. To circumvent these problems, a cross-design of two different temperature treatments at pregnancy and lactation was used in this study.

MATERIALS AND METHODS

Animals

Voies were bred in our indoor breeding colony in which one or two females are paired with one male. This breeding colony (Gerkema

et al., 1993) was established with wild-caught individuals from a population at the Lauwersmeer area in The Netherlands. Inbreeding was avoided. Temperature in the colony was not controlled and was subject to outside temperature variation (18–28°C). The light:dark (LD) cycle was maintained at 14h:10h. Pups were weaned at approximately day 18 of lactation and were housed together in same-sex groups. Only virgin females were used in the described experiments. Animals were moved to a temperature-controlled room at 20.8±0.2°C with a LD cycle of 12h:12h at least one week before mating commenced. During the experiment, animals were housed in 15×32.5×13 cm (width×length×height) plastic cages containing sawdust bedding (Lignocel hygienic animal bedding, Rettenmaier, Rosenberg, Germany) and without any additional nesting material. The cages were mounted with wire tops. Standard laboratory rodent food (Arie Blok Woerden AM-II diet, rodent chow 10 mm, 17.3 kJ g⁻¹; Arie Blok, Woerden, The Netherlands) and tap water was provided *ad libitum*. Care and use of the animals followed local animal welfare laws and the experiments were performed under licence 5210 from the Animal Experimentation Committee of the University of Groningen.

Experiment 1: ambient temperature

Four- to seven-week-old female voles were individually paired with a single male for a 4–11 day mating period at 21°C (20.8±0.2°C). After 11 days, the female voles were allocated to two temperatures during pregnancy: T_{P21} (20.8±0.2°C) and T_{P30} (29.5±0.1°C). These two groups were balanced for body mass, which was measured when the male was removed. One day after parturition (day 1 of lactation), half of these groups were again allocated to a different 'lactation' temperature: T_{L21} (20.8±0.2°C) and T_{L30} (29.5±0.1°C). This created a four-group design ($T_{P21;L21}$, $N=16$; $T_{P30;L21}$, $N=18$; $T_{P30;L30}$, $N=24$; $T_{P21;L30}$, $N=16$), which allowed us to test the effect of ambient temperature on reproductive output during pregnancy and lactation and its interaction. Animals that did not get pregnant or lost their pups at an early stage of lactation were used as non-reproductive controls ($T_{P21;L21}$, $N=12$; $T_{P30;L21}$, $N=14$; $T_{P30;L30}$, $N=13$; $T_{P21;L30}$, $N=12$) and were allocated to the different temperatures in the same way. Mean litter size at birth in this study was 3.57 (±1.28). This is comparable to litter sizes of 3.5 on average that are produced in first-generation offspring of wild-caught voles (Heise and Rozenfeld, 1999).

Dams and pups were weighed daily (using a Kern PCB balance, precision of 0.01 g) throughout lactation. Food intake and water intake were also measured daily. Only small amounts of food fell from the hopper into the bedding. These pieces were collected and weighed. Moisture loss of the food was measured and food intake was corrected accordingly where appropriate (in the cases in which a substantial amount of fresh food was added). Water loss from water bottles owing to evaporation was measured and was found to be negligible and equal between the ambient temperatures. The timing of weighing was kept constant throughout the experiment and was completed within 2.5 h. Approximately half of the non-reproductive controls were measured twice ($N=27$), 3 days apart; the others ($N=24$) were measured each day for a similar period as the reproductive animals.

Experiment 2: litter size manipulation

Four- to seven-week-old female voles were individually paired with a single male for a 4 day mating period at 21°C. In this experiment, the natural litter a dam produced was doubled using age-matched pups from dams that had produced litters before ($N=25$). As a control, a group of dams ($N=27$) had half of their nest replaced with

donor pups, maintaining their natural litter size. Donor dams were also allocated to the two pregnancy temperatures and pups were only donated to dams that were in the same pregnancy temperature group. Daily weighing, pairing and attribution to pregnancy temperature were as described for experiment 1. Pups were added at the end of day 1 of lactation and were allocated directly or early the next day to their lactation temperature.

Experiment 3: fur shaving

Seven- to nine-week-old female voles were individually paired with a single male for an 11 day mating period at 21°C. Mating occurred at 21°C for 11 days at 7–9 weeks of age. After this, the dams were kept at 30°C during pregnancy and lactation. Half of the dams had a large part of their dorsal fur shaved off at day 2 of lactation. The shaved ($N=27$) and non-shaved ($N=25$) groups were balanced for body mass of the dam and litter size. Fur shaving was performed while the animal was immobilised by holding the tail and head with two hands. A second experimenter performed the shaving using a Babylliss E840XE electrical razor with a 2-cm shaving blade. This resulted in 0.19±0.04 g of fur removal. Animals were not re-shaved because there was little re-growth of fur during the lactation period. Non-shaved controls were handled in a similar way as the shaved animals while the razor with an attached top-piece was moved across their backs. In this way, the animals felt and heard the razor but no hair was removed. Both experimental groups were handled for the same time (shaved, 6.0±1.4 min; control, 5.7±0.6 min).

Structural analysis

At day 18 of lactation, all dams were killed by the use of CO₂ gas. The lengths of their ceacum and small and large intestine were measured to the nearest 0.5 cm. The tail was removed, and tail mass (±0.01 g) and length (using digital callipers, ±0.01 mm) were measured.

Milk energy output

We measured the milk energy output (MEO) on a subset of 31 animals in the main temperature manipulation experiment (14 at 30°C and 17 at 21°C) and also on 36 individuals in the shaving experiment. MEO was quantified as the difference between apparent digestible energy intake (DEI) and the daily energy expenditure (DEE) (Krol and Speakman, 2003b), which has been shown to be the least variable approach to estimating milk production. To estimate DEI, we used the dry food intake on days 12 and 13 multiplied by an assimilation efficiency for voles of 80% (Krol et al., 2005). DEE (kJ day⁻¹) was measured using the doubly labelled water (DLW) technique (Butler et al., 2004; Lifson and McClintock, 1966). This method has been previously validated by comparison to indirect calorimetry in a range of small mammals (e.g. Speakman and Krol, 2005a). On day 12 of lactation, the animals were weighed (Kern PCB balance, precision of 0.01 g) and a known mass of approximately 0.3 ml DLW (665,460 ppm ¹⁸O; 328,410 ppm ²H) was administered by intraperitoneal injection. Syringes were weighed before and after administration (Sartorius balance, precision of 0.0001 g) to calculate the exact mass of DLW injected. Blood samples (approximately 30–50 µl) were taken by tail-tipping after 1 h of isotope equilibration to estimate initial isotope enrichments (Krol and Speakman, 1999). Animals were sampled again on day 14 of lactation, and final blood samples (30–50 µl) were taken as close as feasible to whole 24 h periods (Speakman and Racey, 1988) to estimate isotope elimination rates. Taking samples over multiple 24 h periods minimises the substantial day-to-day variability in DEE (Berteaux et al., 1996; Speakman et al., 1994). During the shaving

experiment, approximately half the animals were measured over days 11–13 rather than days 12–14. Background isotope enrichments were evaluated from individuals that were not injected (Speakman and Racey, 1987). Blood samples were immediately heat sealed into $2 \times 100 \mu\text{l}$ glass capillaries, which were stored at room temperature. Capillaries that contained the blood samples were then vacuum distilled (Nagy, 1983), and water from the resulting distillate was used to produce CO_2 (Speakman et al., 1990) and H_2 (Speakman and Krol, 2005a). The isotope ratios $^{18}\text{O}:^{16}\text{O}$ and $^2\text{H}:^1\text{H}$ were analysed using gas source isotope ratio mass spectrometry (Optima, Micromass IRMS and Isochrom μG , Manchester, UK). Samples were run alongside three lab standards for each isotope (calibrated to international standards) to convert delta values to ppm. Isotope enrichments were converted to values of DEE using a single pool model as recommended for this size of animal (Speakman, 1993). There are several alternative approaches for the treatment of evaporative water loss in the calculation (Visser and Schekkerman, 1999). We chose the assumption of a fixed evaporation of 25% of the water flux [eqn 7.17 (Speakman, 1997)], which has been established to minimise error in a range of conditions (Van Trigter et al., 2002; Visser and Schekkerman, 1999). We also measured DEE using DLW in 22 non-reproductive voles.

Statistical analysis

All statistical analyses were performed using restricted maximum likelihood (REML) fitted mixed models, using JMP 7.0 software. These models included nest as a random effect, in this way correcting for repeated measures of each nest. Linear fits were drawn over time in the cases in which the relationship appeared to be linear, as it was for pup growth. For others, time was fitted as a categorical variable. To investigate significant group interaction effects, each combination of two groups was run in the model to determine which groups differed significantly. If time was fitted as a categorical variable, *post hoc t*-tests ($\alpha=0.05$) were used. Pup survival, including failed nests, was analysed with contingency tables using χ^2 tests. Limits with increasing litter size were investigated with segmented regression (Muggeo, 2003) using segmented, a package for R. This analysis iteratively finds the breakpoint of two linear fits. The significance of this breakpoint was evaluated using Akaike's information criterion (AIC). When the segmented regression model did not converge, we regarded the breakpoint as non-significant. Error bars in graphs depict s.e.m. to aid visual assessment of the significance of the differences presented. Data are presented in the text as means \pm s.d.

Each of the separate sets of treatments was performed within a batch of animals that came from our breeding colony. This approach was chosen because it excludes the possibility that confounding effects of time and batch are mistaken for a treatment effect. For this reason, we refrained from making direct comparisons between different sets of treatments (i.e. the shaving treatment and the ambient temperature treatment). For the ambient temperature experiment, we chose to pool data from this batch with data from dams that raised natural litter sizes in the litter size experiment, in which the same four-group ambient temperature design was applied, to increase statistical power. Histograms of mean pup mass of the four ambient temperature groups were inspected for both batches, and no reason to reject pooling was found.

RESULTS

Equality of treatment groups

The temperature groups could not be statistically distinguished in terms of dam mass at day 1 of lactation ($F_{3,116}=0.66$, $P=0.58$),

number of pups at day 2 ($F_{3,114}=0.97$, $P=0.41$) and mean pup mass at day 2 ($F_{3,114}=0.55$, $P=0.65$). The dam mass of the animals that had their litters enlarged was significantly higher at day 1 of lactation compared with the dams with natural litters (24.2 ± 3.6 vs 26.0 ± 4.15 ; $F_{1,118}=5.07$, $P=0.03$). Mean pup mass at day 2 did not differ significantly between these groups ($F_{1,116}=3.33$, $P=0.07$). The shaved and non-shaved control voles could not be statistically distinguished in terms of dam mass at day 1 of lactation ($F_{1,55}=0.01$, $P=0.90$), number of pups at day 2 ($F_{1,55}=0.02$, $P=0.89$) and mean pup mass at day 2 ($F_{1,55}=0.35$, $P=0.55$).

Experiment 1: ambient temperature

Dam body mass

All four groups showed different patterns of change in dam body mass during lactation (Fig. 1). A small drop during the first days of lactation was followed by increasing body mass or an attenuation of the decline. These patterns differed over time. In the model, day of lactation was fitted as a categorical variable and was highly significant ($F_{17,1189}=13.47$, $P<0.0001$). The interaction term between lactation day and temperature group was also highly significant ($F_{51,1189}=5.88$, $P<0.0001$). Including litter size at birth or mean litter size over the lactation period did not change any of the conclusions (estimate=1.42–1.43, $F_{1,69}=27.0$ – 28.0 , $P<0.0001$). *Post hoc t*-tests on least square means from the model revealed that, from day 7 of lactation onwards, the $T_{P30; L21}$ group differed from the $T_{P30; L30}$ group. From day 10 of lactation onwards, the $T_{P21; L30}$ group differed significantly from the $T_{P30; L21}$ group. At the end of lactation (day 17–18), the dams from the $T_{P30; L30}$ group weighed significantly less than the other three groups. The differences between $T_{P30; L21}$ and $T_{P21; L21}$, between $T_{P21; L21}$ and $T_{P21; L30}$ and between $T_{P21; L30}$ and $T_{P30; L21}$ did not reach statistical significance.

Food intake

Food intake before day 3 of lactation was excluded from the analysis. The data were analysed for the two lactation temperatures separately, because of strong reductions in food intake in response to increasing lactation temperature (Fig. 2; $F_{1,72}=84.0$, $P<0.0001$). Food intake increased significantly over the lactation period at both lactation

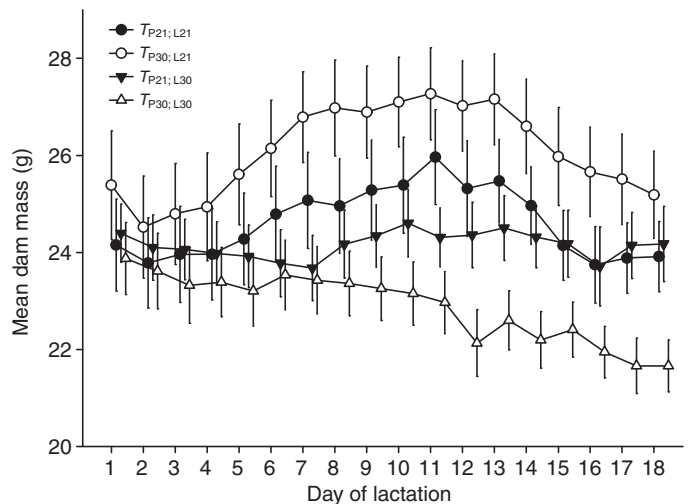


Fig. 1. Dam mass over the lactation period for each ambient temperature group. Note the difference in patterns over time in each group. The groups differ in the degree of increase in mass during mid-lactation. The different groups are slightly offset on the x-axis to improve graph reading. Values are means \pm s.e.m.

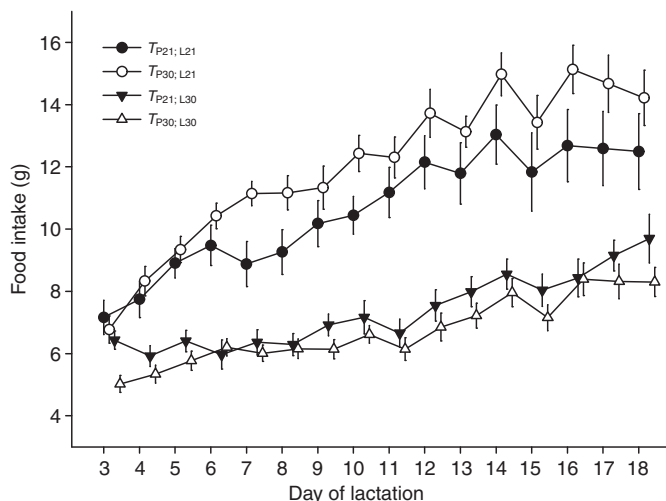


Fig. 2. Daily food intake over the lactation period for each ambient temperature group. T_{L30} causes a strong reduction in food intake. All groups increase food intake during the course of lactation and reach an asymptote. The different groups are slightly offset on the x-axis to improve graph reading. Values are means \pm s.e.m.

temperatures ($F_{15,494-583}=41.1-32.82$, $P<0.0001$). *Post hoc* matched-pair *t*-tests revealed that, at both lactation temperatures, food intake increased until day 14 of lactation, after which an asymptote was reached. Pregnancy temperature had no significant effect on food intake for either lactation temperature (21°C, $F_{1,32}=2.72$, $P=0.11$; 30°C, $F_{1,38}=1.74$, $P=0.20$) and no significant interaction with lactation day was found (21°C, $F_{15,479}=1.24$, $P=0.24$; 30°C, $F_{15,568}=1.24$, $P=0.24$). When dam mass at the beginning of lactation was added as a covariate to the model ($F_{1,31-37}=26.73-8.64$, $P<0.01$), this did not change any of the conclusions.

Water intake

Backward step-wise selection (smallest *P*-value of parameter removed was 0.08) of a full factorial model including lactation day, pregnancy temperature and lactation temperature resulted in a model that included lactation temperature ($F_{1,72}=0.03$, $P=0.85$), lactation day ($F_{16,1138}=68.3$, $P<0.0001$) and its interaction ($F_{16,1138}=2.84$, $P=0.0001$). *Post hoc t*-tests revealed that the steep increase seen at a lactation temperature of 30°C at days 17 and 18 of lactation is responsible for the significance of this interaction term (only these two days proved significant; Fig. 3). When dam mass at the beginning of lactation was added as a covariate to the model ($F_{1,70}=11.6$, $P=0.001$), this did not change any of the conclusions.

MEO and DEE

Ambient temperature groups differed in their MEO (Fig. 4; $F_{3,26}=10.5$, $P=0.0009$). The model included litter mass as a significant predictor (estimate=1.77, $F_{1,26}=26.2$; $P<0.0001$). A *post hoc t*-test on least square means from the model revealed that only lactation temperature significantly affected milk production. DEE increased significantly with dam mass (estimate=2.80, $F_{1,27}=8.25$, $P=0.008$) and litter size (estimate=3.78, $F_{1,27}=4.50$, $P=0.043$), and was significantly higher in the T_{L21} group than the T_{L30} group (91.8 ± 16.3 kJ vs 59.3 ± 13.5 kJ; $F_{1,27}=9.09$, $P=0.006$). Reproductive voles had DEE values similar to those of non-reproductive controls ($F_{1,49}=0.01$, $P=0.91$) when controlling for body mass (estimate=3.30, $F_{1,49}=52.7$, $P<0.0001$) and lactation temperature

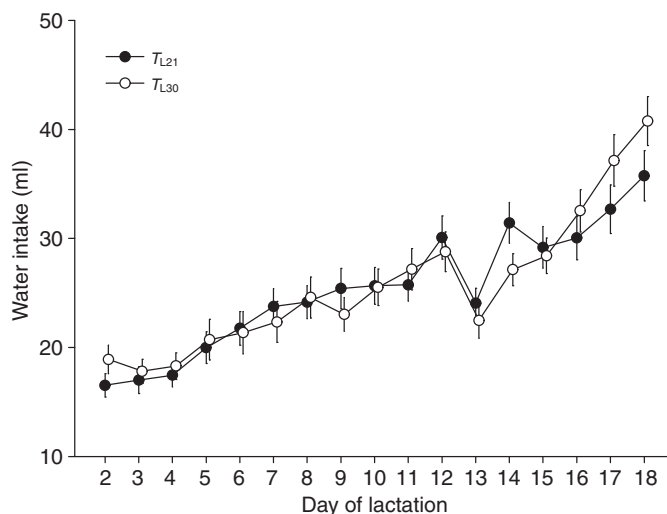


Fig. 3. Water intake for the two lactation temperature groups. The intake is remarkably similar for both groups, meaning that T_{L30} causes a strong increase in water intake relative to food intake. Towards the end of lactation, water intake increases, even more so for T_{L30} . The different groups are slightly offset on the x-axis to improve graph reading. Values are means \pm s.e.m.

($F_{1,49}=38.4$, $P<0.0001$) by adding them as covariates to the statistical model.

Pup growth

Growth of pups differed significantly between groups, as revealed by the significant interaction between lactation day and temperature group (Fig. 5; $F_{3,1253}=57.7$, $P<0.0001$). This interaction was highly significant ($P<0.001$) for all combinations of groups, except for $T_{P21;L21}$ and $T_{P30;L21}$ ($P=0.61$). When dam mass at the beginning of lactation and the number of pups born were included as covariates in the model, this did not change any of the conclusions. Dam mass was positively related to pup mass (estimate=0.11, $F_{1,68,0}=59.14$, $P=0.004$). Litter size was negatively associated with pup mass (estimate=-0.40, $F_{1,68,0}=12.8$, $P=0.0006$).

Non-reproductive voles

Food and water intake, and DEE

Backward step-wise selection (smallest *P*-value of parameter removed was 0.68) of a model that included pregnancy and lactation temperature and mean dam mass at days 15 and 18 as a covariate resulted in a model that included lactation temperature ($F_{1,48}=4.73$, $P<0.0001$) and mean dam mass ($F_{1,48}=8.80$, $P<0.0001$). The same analysis was done with water intake as the dependent variable and resulted in a model that included pregnancy temperature ($F_{1,46}=5.32$, $P=0.026$) and mean dam mass ($F_{1,46}=38.6$, $P<0.0001$). Non-reproductive voles from T_{P30} drank less than animals from T_{P21} (10.8 ± 5.6 and 12.3 ± 4.3 ml, respectively). DEE was lower at 30°C than at 21°C (77.1 ± 14.9 kJ vs 55.8 ± 17.0 kJ; $F_{1,19}=40.0$, $P<0.0001$; including body mass as covariate, estimate=3.54, $F_{1,19}=55.7$, $P<0.0001$); no effect of pregnancy temperature on DEE was found ($F_{1,18}=0.05$, $P=0.82$).

Experiment 2: litter size manipulation

Litter size

The experimental manipulation of litter size itself did not induce differences in pup growth when litter size was added as a covariate. This was tested using the same model as for natural litter sizes. Data

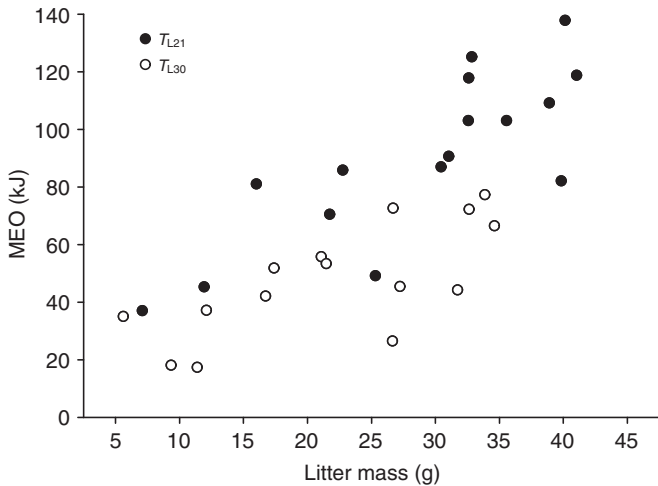


Fig. 4. Milk energy output (MEO) versus litter mass. T_{L30} reduced milk transfer to pups.

from the experimentally enlarged litters were added to this data set. Litter size at day 2 was included as a covariate ($F_{1,92,0}=16.60$, $P<0.0001$) and enlarged was included as a fixed predictor ($F_{1,92,0}=0.36$, $P=0.56$).

To investigate possible limitations of food intake with increasing litter size at both lactation temperatures, asymptotic food intake (day 13, 14 of lactation, the same period as the DEE measures in experiment 1) was regressed against mean litter size (Fig. 6). Independent food intake by pups during this period is small (this study) (see also Migula, 1969). Pups also start thermo-regulating from day 10 of lactation onwards, and reach full adult thermoregulatory capacity gradually between days 13 and 17 of lactation (Bashenina, 1960). A significant breakpoint was detected at 5.9 pups [95% confidence interval (CI)=4.3–7.4], with a slope before the breakpoint of 1.37 (95% CI=1.0–1.7) and after the breakpoint of 0.21 (95% CI=–0.48–0.90).

The limit in asymptotic food intake at the largest litter sizes was expected to result in reduced pup growth. Although mean litter size

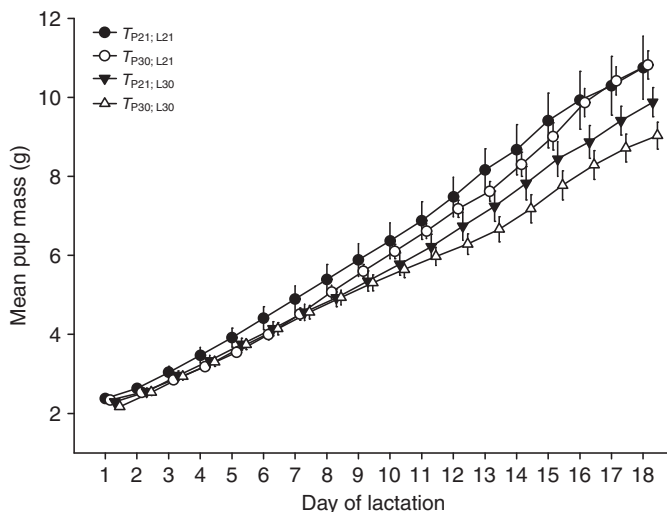


Fig. 5. Growth of pups (mean pup mass) over the lactation period for each ambient temperature group. The different groups are slightly offset on the x-axis to improve graph reading. Values are means \pm s.e.m.

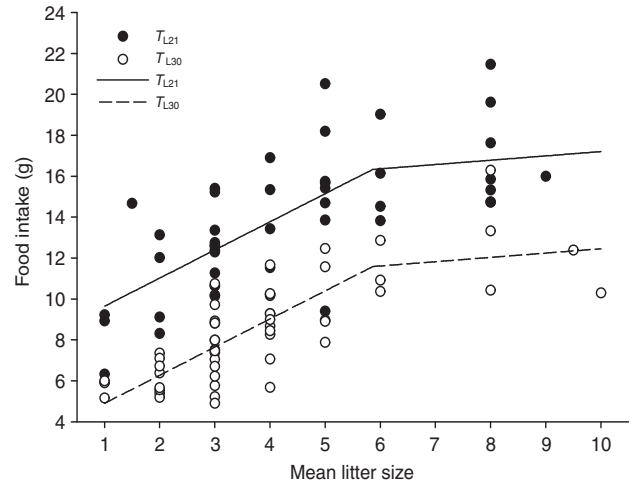


Fig. 6. Asymptotic food intake (days 13, 14) versus mean litter size. Below a litter size of six there was a significant increase in food intake. In larger litters, a limit on sustainable food intake was reached.

during lactation indeed showed a negative relationship with mean pup mass at the end of lactation ($F_{1,95}=8.29$, $P=0.0049$), controlling for lactation temperature ($F_{1,95}=6.87$, $P=0.010$), no significant breakpoint was detected (Fig. 7). The interaction between lactation temperature and litter size was also not significant ($F_{1,94}=0.97$, $P=0.33$).

Comparison between reproductive and non-reproductive voles

Food intake

The food intake of non-reproductive voles was compared with the average asymptotic food intake (lactation days 13 and 14) of the reproductive voles, and a model was fitted that included reproductive status, lactation temperature and their interaction, and dam mass as a covariate ($F_{1,142}=54.0$, $P<0.0001$). The interaction in this model proved to be highly significant ($F_{1,142}=23.7$, $P<0.0001$), indicating a relatively larger reduction in food intake in response to increased ambient temperature (from T_{L21} to T_{L30}) of reproductive compared with non-reproductive voles (Fig. 8).

Water intake

The same models that were fitted for food intake were also fitted for asymptotic water intake (lactation days 13 and 14) of reproductive and non-reproductive voles. The interactions between reproductive state and lactation temperature proved non-significant ($F_{1,142}=0.42$, $P=0.51$). Left in the model was the main effect of reproduction (non-reproductive, 11.7 ± 4.93 ml; reproductive, 28.8 ± 11.5 ml; $F_{1,144}=107.7$, $P<0.0001$) and body mass ($F_{1,144}=28.4$, $P<0.0001$). To test whether water intake of reproductive voles increased irrespective of their increased food intake, two additional factors were included in the model: food intake and lactation temperature. In this model, reproductive state was still significant ($F_{1,142}=5.08$, $P=0.026$), indicating that lactation induced increased water intake irrespective of the increased food intake it induced.

Experiment 3: fur shaving

Food and water intake

Shaving did not lead to significant differences in food or water intake over the lactation period (main effect: food, $F_{1,50}=1.16$, $P=0.29$; water, $F_{1,50}=0.37$, $P=0.55$; interaction with lactation day: food,

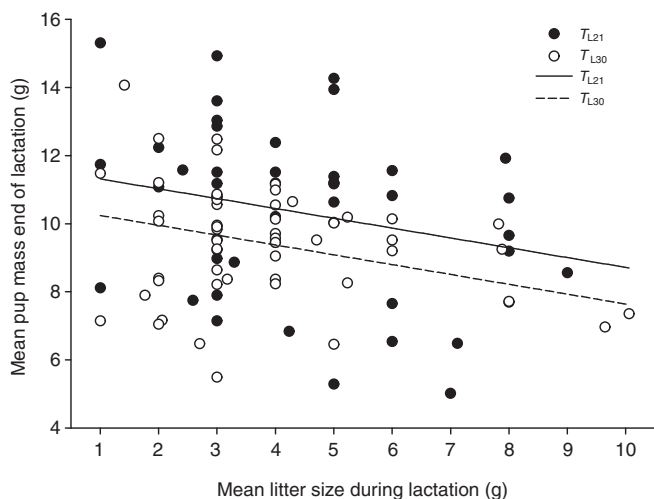


Fig. 7. Mean pup mass at the end of lactation declined significantly with litter size for both lactation temperatures. No significant breakpoint was detected.

$F_{16,796}=0.28$, $P=1.00$, water, $F_{16,796}=0.55$, $P=0.91$). However, the trend in food intake was in the expected direction (Fig. 9). When dam mass at the beginning of lactation was added as a covariate to the model, this did not change any of the conclusions. No differences were found within the non-reproductive animals (lowest $P=0.44$).

Dam body mass

There was no main effect of shaving on body mass of the dam ($F_{1,50}=0.21$, $P=0.65$), neither was there a difference in the pattern over time (non-significant interaction; $F_{17,849}=0.41$, $P=0.98$). Body mass decreased significantly over time ($F_{17,866}=9.51$, $P<0.0001$) from 30.8 ± 6.7 at day 1 to 29.0 ± 6.3 at day 18.

Milk output

Shaved dams did not increase milk production ($F_{1,29}=0.90$, $P=0.35$; Fig. 10). The model included nest mass ($F_{1,29}=90.5$, $P<0.0001$) as a significant covariate.

Pup growth

Pups from shaved dams grew significantly faster, as was revealed by the interaction between lactation day and treatment group in the mixed model (Fig. 11; $F_{1,881}=22.68$, $P<0.0001$). When dam mass at the beginning of lactation and the number of pups born were included as covariates in the model, this did not change any of the conclusions.

Comparative structural analysis

Ambient temperature

The effect of the ambient temperature group on intestine length was analysed for non-reproductive controls and dams with natural and enlarged litters combined (Fig. 12). These different groups were coded in one fixed factor (experimental group). The model also included body mass at the time the animals were sacrificed as a covariate (for two animals these data were missing; for these, mass measured earlier that day was used). Lactation temperature ($F_{1,145}=4.99$, $P=0.027$) and experimental group ($F_{2,145}=10.4$, $P<0.0001$) had a significant effect on intestine length, but pregnancy temperature did not ($F_{1,144}=0.44$, $P=0.51$). The interaction between lactation temperature and experimental group proved non-significant ($F_{2,143}=0.50$; $P=0.61$). *Post hoc t*-tests on the residuals of total

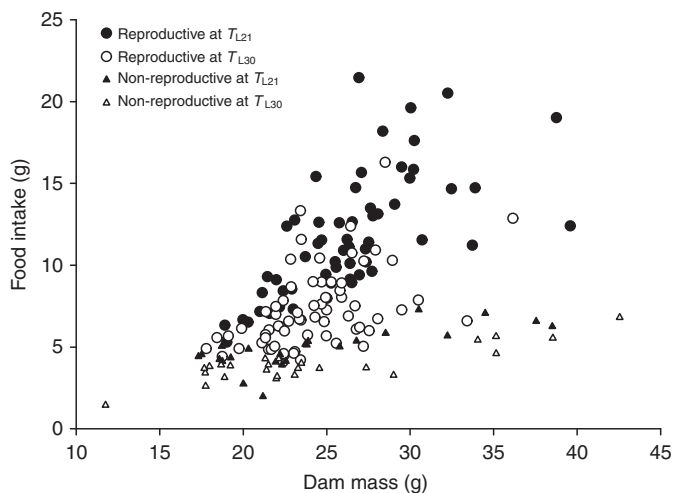


Fig. 8. Food intake (mean of days 13, 14) increased with dam mass. Reproductive animals had a higher daily food intake and T_{L30} induced a stronger reduction in food intake in reproductive voles than in non-reproductive voles.

intestine length corrected for body mass revealed that the non-reproductive controls differed from the reproductive animals. Within the reproductive animals, natural and experimentally increased litter sizes did not differ from each other. Residual total intestine length (corrected for body mass) did not predict mean pup mass (corrected for litter size and lactation temperature by including these factors in the model) at day 18 of lactation ($F_{1,92}=0.28$, $P=0.60$).

To estimate which part of the intestine was responsible for these differences in total intestine length, separate models were fitted for caecum, small intestine and large intestine length. Lactation temperature was only a significant factor for small intestine length ($F_{1,145}=5.66$, $P=0.019$). Caecum and large intestine length were also shorter at T_{L30} , but not significantly so ($F_{1,145}=0.44-1.93$, $P=0.51-0.17$). Experimental group was significant in all three models ($P<0.002$). Using the same models described above but with tail mass or length as the dependent variable did not yield any

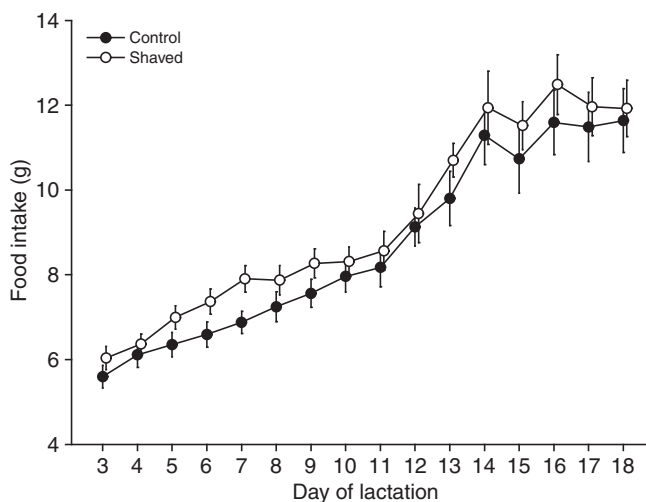


Fig. 9. Daily food intake over the lactation period for the shaving treatment groups. The differences in food intake did not reach significance, but were in the expected direction. The groups are slightly offset on the x-axis to improve graph reading. Values are means \pm s.e.m.

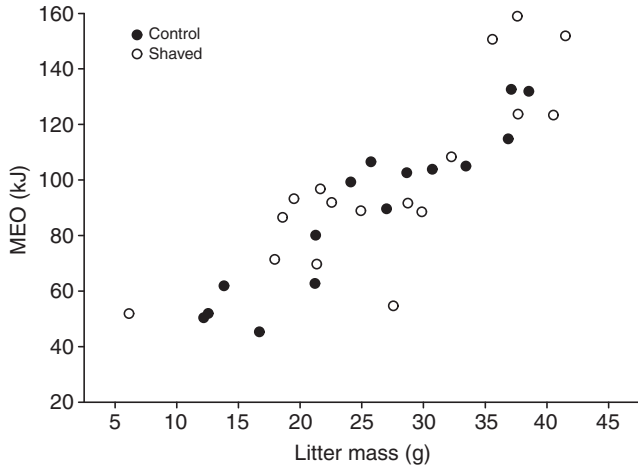


Fig. 10. Milk energy output (MEO) in the shaving experiment is plotted against litter mass. No effect of treatment was detected statistically.

significant effects (lowest P -value=0.27). The tail is of interest because it is an important thermoregulatory organ in many rodents (Raman et al., 1983).

Shaving

Using models that included the same parameters as described for ambient temperature revealed no significant effects of shaving on either intestine length or tail mass ($P>0.56$). As found in the analysis of ambient temperature, the non-reproductive controls in the shaving experiments also had shorter intestines compared with the reproductive animals ($F_{1,59}=6.0, P<0.001$).

Comparative survival analysis

Ambient temperature and litter size

The effects of ambient temperature on pup survival were analysed for natural and experimentally enlarged litter sizes separately,

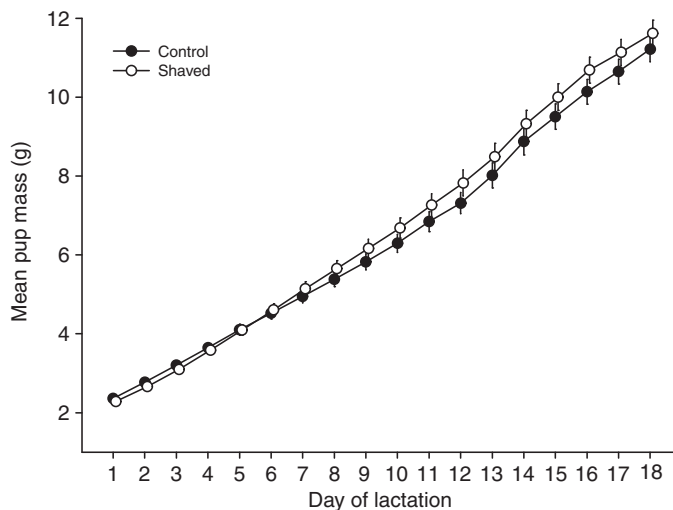


Fig. 11. Growth of pups (mean pup mass) over the lactation period for shaved dams and non-shaved controls. The different groups are slightly offset on the x-axis to improve graph reading. Values are means \pm s.e.m.

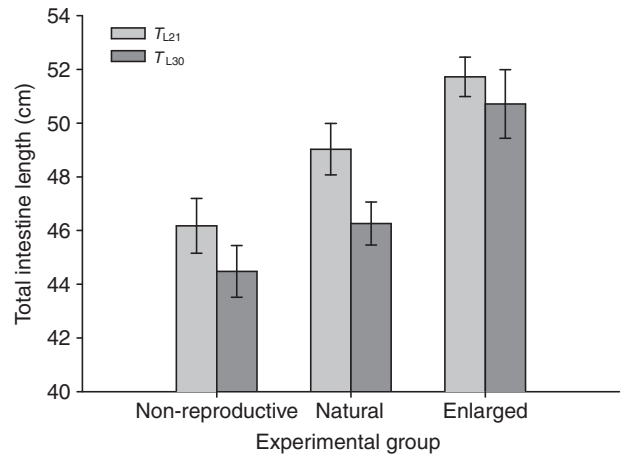


Fig. 12. Total intestine length of non-reproductive voles and dams that had a natural or enlarged litter for the two lactation temperatures. Increased temperature induced shorter intestine length and lactation induced longer intestines. Values are means \pm s.e.m.

because within enlarged litters more pups died ($\chi^2=4.62, P=0.03$; 19.3% pups died in natural litters compared with 28.5% in the enlarged litters).

Ambient temperature group affected pup mortality in both natural and enlarged litter sizes ($\chi^2=12.6, P=0.006, N=322$ pups; $\chi^2=22.2, P=0.0001, N=200$ pups, respectively) (Fig. 13). To investigate effects of pregnancy temperature within each lactation temperature, separate contingency tables were calculated. Only within natural litter sizes and at T_{L30} did T_{P30} significantly reduce pup mortality ($\chi^2=5.82, P=0.016, N=182$ pups).

Shaving

The same pattern that was observed for ambient temperature in pup mortality was also observed in the shaving experiment. Shaving was accompanied by increased pup mortality (non-shaved, 2.1%; shaved, 12.7%; $\chi^2=7.31, P=0.007, N=212$ pups).

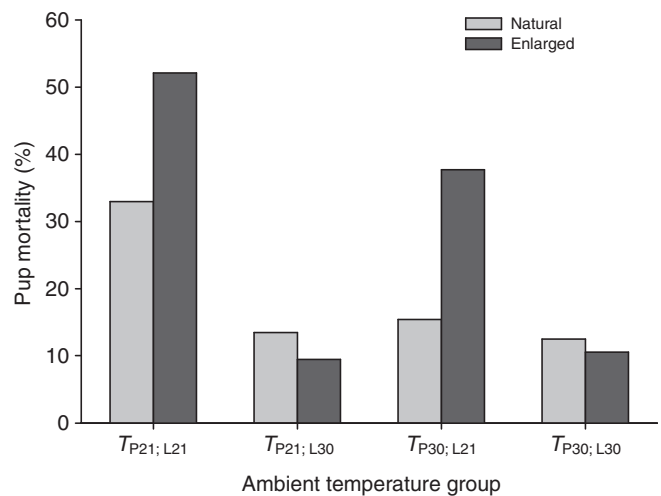


Fig. 13. Pup mortality (the percentage of pups born that died during lactation) for each ambient temperature group for natural and enlarged litters. Litter size enlargement induced pup mortality at T_{L21} . Within natural litter sizes, more pups died at $T_{P21; L21}$ compared with the other groups.

DISCUSSION

The reduced food intake of the mothers when transferred to higher temperature might be interpreted as a simple response of an endothermic mammal to the reduced energy demands for thermoregulation. However, if this were the case we would not anticipate any reduction in the investment of the females in their offspring at the higher temperature. Indeed, because energy demands on thermoregulation were lower at higher temperatures, one might anticipate that investment in offspring might increase at these high temperatures if the total energy intake was limited by some factor unrelated to temperature. By contrast, the heat dissipation limitation theory predicted that milk production would decline at high temperature and hence pup growth would be reduced. The reduced milk production and retarded pup growth (Fig. 5) at high temperature was therefore consistent with the heat dissipation limit theory. An alternative explanation for the poor growth of the pups at the higher temperature was not that their growth was restricted by the milk supply from mothers working under a heat dissipation constraint, but rather that the pups were themselves constrained by their own heat dissipation capacity. This might happen because of the relatively large volumes of milk that the pups ingest, and the fact the milk delivered to them is at the body temperature of the lactating dam. The pups might therefore be at risk of hyperthermia and the easiest way to control this would be to restrict their intake of milk. Therefore, mothers might downregulate their milk production at 30°C because of reduced demand from the pups, which would also retard their growth. The temperature manipulation data are unable to distinguish between this hypothesis and the heat dissipation limitation hypothesis. However, when the females were shaved the growth rate of the pups was significantly increased. Although food intake and milk production were also increased, these latter effects did not reach statistical significance. Nevertheless, the positive trends in these traits, combined with the significant increase in pup growth when the mothers were shaved, are inconsistent with the idea that hyperthermia in the pups was responsible for the reduced milk production and retarded growth in the pups raised at 30°C. This is because shaving the mothers would not affect the heat dissipation capacity of the pups. Overall, the data are most consistent with the heat dissipation limitation theory – although other hypothetical explanations are possible (see below).

These findings in the common vole are consistent with previous studies in other species such as laboratory mice, rats and hamsters, domesticated livestock and Brandt's voles. Therefore, this study increases the wider validity of the heat dissipation limit theory. Long-term selection in the laboratory, and in domestic livestock, for higher litter sizes and/or elevated milk production seems unlikely to be the only factor causing a heat dissipation limit.

The effect of shaving on pup growth in this experiment replicates the shaving experiment by Krol et al. (Krol et al., 2007). Although there was slightly higher mortality in the shaved group, the resultant slightly reduced litter size did not explain the difference in growth because the growth effect remained when litter size was included as a covariate in the analysis. Although the pups from shaved dams grew faster, we did not find any significant effect on either food intake or MEO, although in both cases the direction of the effect of shaving was consistent with the elevated growth. Further research that manipulates heat dissipation without changing ambient temperature is required to substantiate the heat dissipation limit theory. Our data contrast the recent study which reported no effect of dam shaving at 23°C on pup growth in Swiss mice (Zhao and Cao, 2009), despite elevated food intake of the dam. However, milk production was not quantified in that study, and so it is not clear

whether the shaved females increased their milk production (as predicted by the heat dissipation limit theory) but for some reason this did not translate to greater growth, or whether the females did not translate the elevated intake into milk (which is inconsistent with the heat dissipation limit theory). More recently, Zhao et al. quantified milk production in shaved and unshaved Swiss mice and suggested that there was no significant effect of shaving on milk production (Zhao et al., 2010), although the method employed to establish this effect [after Krol and Speakman (Krol and Speakman, 2003b)] was less precise than the isotope methods used by Krol et al. (Krol et al., 2007) and in the present study. The reasons for the differences between studies remain obscure. More studies of shaving (or other direct manipulations of heat dissipation of the dam) at peak lactation, linked to simultaneous measures of pup growth, food intake and milk production, are clearly required. Even more so because such experiments can conclusively show whether the heat dissipation capacity of the dam is responsible for the differences in pup growth, food intake and milk production at different ambient temperatures or whether these effects stem from effects mediated by the offspring.

Pregnancy temperature

It is intriguing that not only ambient temperature during lactation, but also ambient temperature during pregnancy influenced pup growth in lactation (Fig. 5). Pregnancy at 30°C decreased pup growth when lactation temperature was also 30°C, but pup growth was not affected by pregnancy temperature when lactation temperature was 21°C. If similar interactions between pregnancy and lactation temperature also exist in other species, this means that studies in which dams went through gestation and lactation at the same temperature may have overestimated the effects of high ambient temperatures during lactation on pup growth.

The factor that was most closely linked to the decline in pup growth in the $T_{P30; L30}$ group was the decline in body mass of the dam by approximately 10% over the lactation period (Fig. 1). This was probably not due to a limit in food intake, because their food intake did not differ from that of the $T_{P21; L30}$ group (Fig. 2). With the same amount of food intake, dams at $T_{P21; L30}$ lost less mass and their pups grew faster. This suggests that either the dams or the pups from the $T_{P30; L30}$ group required more energy for specific processes, possibly because of detrimental, but yet unknown, effects of high temperatures during pregnancy or because of differences in assimilation efficiency. The absence of effects of $T_{P30; L30}$ on MEO has to be seen in the light of the limited sample size for this measure.

Water intake

Lactation induced higher water intake, also when controlling for the increased food intake it induced. Moreover, at 30°C, pups and dams drank more water relative to the amount of food they consumed compared with pups and dams at 21°C (Fig. 3). At both lactation temperatures, water intake was found to be similar except for a steep increase at 30°C at the end of lactation. This difference in water intake cannot be attributed to increased food intake, because at 30°C food intake was substantially lower. This relative increase in water intake could mean that dams are not able to meet the water demands of pups. An alternative interpretation to the heat dissipation limitation hypothesis, therefore, is that water intake limited pup growth at 30°C. The steep increase at the end of lactation (at this time pups start to drink from water bottles on their own; this study) might reflect this increased need for water of pups. This need could arise from the decreased water transfer through milk to pups during lactation at 30°C by dams. Whether this effect will also be present

with more naturalistic food like grass roots, which contains substantial amounts of moisture in comparison to lab chow, remains to be investigated.

Structural analysis

Reproduction increased intestine length and T_{L30} resulted in shorter intestine lengths compared with T_{L21} (Fig. 12). In mice and other small mammals (including voles), similar results for both reproductive state and temperature have been reported previously (Derting and Austin, 1998; Krol et al., 2003; Speakman and McQueenie, 1996; Wu et al., 2009; Zhang and Wang, 2007). A shorter intestine length at T_{L30} suggests that the total capacity of the gut of the dam is not responsible for the reduced energy flow to pups reducing their growth at this temperature, because they can maintain a larger gut at T_{L21} . The intestine length probably shortened (or lengthened less) because of decreased food intake induced by heat dissipation problems of the dam and/or by decreased pup demand at higher ambient temperatures.

Limits

An asymptote in food intake was reached at the end of lactation between days 14 and 18 (Fig. 2), as has been previously reported in mice (Johnson et al., 2001). This asymptotic intake has been inferred to reflect a limit on sustained energy intake (Speakman and Krol, 2005b) because a direct consequence of the plateau in intake is that offspring from larger litters end up smaller than offspring from smaller litters. There is substantial evidence to suggest that such smaller individuals are at a disadvantage at weaning relative to the larger offspring that are derived from smaller litters. It is argued, therefore, that if females were able to ingest more food, allowing the offspring from larger litters to wean at the equivalent body weight to those in smaller litters, they would do so. The same asymptotic intake was also observed in voles, and while this was not formally demonstrated to be due to a limit, the same arguments potentially apply to voles that have been applied to other small rodents. The fact that this asymptotic intake was higher at 21°C compared with 30°C is also consistent with previous work on mice and Brandt's voles (Johnson and Speakman, 2001; Krol and Speakman, 2003a; Speakman and Krol, 2005b; Wu et al., 2009) and also follows the predictions of the heat dissipation limit theory (Speakman and Krol, 2010). As a result, MEO was also lower at 30°C.

Although on the face of it these data suggest that there might be a limitation on sustained food intake that is imposed by heat dissipation, our litter size manipulation study suggests that things are a little more complex. In line with earlier studies, asymptotic food intake reached a plateau at the largest litter sizes (Johnson et al., 2001; Krol and Speakman, 2003a), but this was not reflected in pup mass at the end of lactation. Increased mortality in the largest litters might be responsible for this effect. This suggests that the asymptote in food intake that was reached at the end of lactation is flexible up to a litter size of six in common voles (Fig. 7), which is at the top of their natural litter size range (Boyce and Boyce, 1988). A levelling off of food intake could therefore be the result of a physiological limitation (such as a heat constraint), pup demand or a behavioural decision about restricted investment. Further separation between these hypotheses is not currently possible.

Strikingly, the lactation temperature effects we found were not limited to these large litters (Figs 1, 2, 4 and 5). This shows that heat dissipation in common voles does not only potentially modulate a limit in sustainable food intake in large litters as in mice, but may also affect the investment of dams in smaller litters in which no

limit in sustained food intake is reached. This suggests that either pup demand is decreased at 30°C or that dams that nurse small litter sizes trade-off benefits of larger offspring with costs of increasing food intake, which induces heat production. In a wider perspective, this could mean that heat dissipation could also be important in shaping reproductive investment in other species that do not reach a physiological limit in energy intake during reproduction, as it is in common voles. Costs associated with increased heat production (e.g. sub-lethal effects of hyperthermia or costs associated with the need to increase heat loss, for example by decreasing fur density) could be traded off with benefits from increased reproductive output, rather than hyperthermia-induced mortality imposing a strict physiological limitation (Speakman and Krol, 2010).

Pup mortality

A positive effect of T_{L30} was found on pup survival (Fig. 13). The most likely benefit of ambient temperature to pups is body temperature maintenance between nest visits of the dam. The fact that pup mortality is higher at T_{L21} might mean that the overall fitness yields from the reproductive effort at T_{L21} is comparable to or even lower than fitness yield when lactating at T_{L30} . These effects could be amplified by effects of ambient temperature on juvenile vole survival, as found in an earlier study (Martinet and Daketse, 1976). Survival after weaning was lower at colder temperatures. The body mass pups needed to survive was also lower at 33°C compared with the colder temperatures (Martinet and Daketse, 1976). Higher pup mortality was also found in nests from shaved dams compared with non-shaved controls. The cause of this effect is unknown at present.

Different survival rates between temperatures and shaving treatments warrant caution in the interpretation of the results on pup growth. It is possible that these results can be attributed to selective mortality of low quality pups, litters and/or dams in the different experimental groups. The increased growth at $T_{P30; L21}$ with comparable survival to T_{L30} argues against this, suggesting that selection through pup mortality can only be partly responsible for the differences in growth found under the different ambient temperature treatments.

Reproductive success

Offspring survival was higher at high ambient temperature, but growth was reduced. In terms of reproductive success, the advantage of increased offspring survival is reduced by the disadvantage of decreased weaning mass. Large body size is associated with increased survival (Boonstra and Krebs, 1979), recruitment (Wauters et al., 1993) and reproduction [e.g. attractiveness, pup growth (Solomon, 1993) (present study)] in small mammals. A reliable estimate of these positive effects of mass is not possible given that it is not known how these factors will add up in terms of reproductive success in *M. arvalis*. From the point of view of the dam there is also a possible loss of reproductive value. Dams in the $T_{P30; L30}$ group lost mass during lactation and this could reduce their own survival and growth of their future litters. Future experiments could measure the reproductive success of adults raised to weaning in T_{L21} or T_{L30} and the future reproductive success of the dams that raised them in a semi-natural situation. A similar experiment using shaved and unshaved individuals and their pups would answer the same questions in a semi-natural context. Future experiments in a more natural, ecologically relevant situation could test the ecological validity of the heat dissipation theory. The present lab study shows that temperature affects lactation in a microtine rodent species with natural genetic makeup and with small litter sizes. Whether the effects reported here are also present in a field situation remains to

be investigated. A recent non-breeding study that focused on survival of shaved *versus* non-shaved *Microtus californicus* in the field found no effects (Kenagy and Pearson, 2000).

CONCLUSIONS

The results of the ambient temperature and shaving experiments were consistent with the suggestion that common voles may face a heat limitation problem when they are lactating, potentially supporting the heat dissipation limit theory (Speakman and Krol, 2010). However, not all data were consistent with this theory. In particular, shaving of adult females did not produce the expected significant increases in food intake and milk production, although a significant positive effect on pup growth was observed. Further tests of the heat dissipation limit theory with additional shaving experiments (or other direct manipulations of heat dissipation of the dam) are clearly required. The new insights into the involvement of pregnancy temperature in shaping pup growth are intriguing, although we do not yet understand the mechanism behind this. Furthermore, voles with natural litter sizes did not reach a limit in sustained food intake, but effects of temperature on food intake and pup growth were shown. This suggests that heat dissipation problems may also play a role in shaping reproductive investment when sustained food intake is not limited. We also suggest that, in addition to a potential negative effect of heat on dams, there is a benefit of heat to pups, leading to increased pup survival. The possible involvement of water intake in heat dissipation adds increasing complexity to the known effects of ambient temperature on reproductive output. It remains to be seen how the balance between the negative effect of high ambient temperature on pup growth and/or future reproduction of the dam and the positive effect on pup survival affects reproductive success in the field. This balance ultimately determines the effect of different ambient temperatures on reproductive success.

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