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Limits to sustained energy intake. X. Effects of fur removal on reproductive performance in laboratory mice

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Summary

The maximum rate of sustained energy intake (SusEI) may limit reproductive effort and other aspects of animal performance. We have previously suggested that lactating mice are not limited centrally by the alimentary tract or peripherally by the mammary glands, but that the limits to SusEI are imposed by the capacity of the animal to dissipate body heat generated as a by-product of processing food and producing milk. To explore the nature of the limits to SusEI, we bred MF1 laboratory mice at 21°C and then dorsally shaved lactating females to reduce their external insulation and thereby elevate their capacity to dissipate body heat. These mice increased their food intake by 12.0% and assimilated on average 30.9 kJ day^{-1} more energy than unshaved animals. With nearly identical mean litter sizes (11.4 pups for shaved and 11.3 pups for unshaved mice), shaved mothers exported 15.2% (22.0 kJ day^{-1}) more energy as milk than control individuals. The elevated milk production of shaved mice enabled them to wean litters that were 15.4% (12.2 g) heavier than offspring produced by unshaved mice. Our results argue against central, peripheral or extrinsic limits to SusEI at peak lactation and provide strong support for the heat dissipation limit hypothesis. More generally, we see many situations where heat dissipation may be a previously unrecognised factor constraining the evolution of endothermic animals – for example, the latitudinal and altitudinal trends in clutch and litter sizes and the migration patterns of birds.

Key words: heat dissipation limit, lactation, digestive efficiency, metabolizable energy intake, daily energy expenditure, milk production, pup energy content.

Introduction

The sustained maximum rate of energy intake (SusEI) is an important trait that imposes an upper constraint on animal performance (e.g. Drent and Daan, 1980; Kirkwood, 1983; Peterson et al., 1990; Weiner, 1992; Hammond and Diamond, 1997; Speakman, 2000; Speakman and Król, 2005a; Anderson and Jetz, 2005). It is therefore a key component of our understanding of limits on reproductive output, which is essential for modelling the impact of global climate change on animal distributions (Thomas et al., 2001; Humphries et al., 2002). In addition, limits on SusEI have important ramifications for our understanding of human endurance performance (Hammond and Diamond, 1997) and productivity of domesticated livestock.

Recent attempts to elucidate the nature of the limits to SusEI have focused on lactation, which is energetically the most demanding period for female mammals (e.g. Perrigo, 1987; Weiner, 1987; Kenagy et al., 1989; Hammond and Diamond, 1992; Hammond and Diamond, 1994; Hammond et al., 1994; Rogowitz and McClure, 1995; Hammond et al., 1996; Koteja, 1996; Speakman and McQueenie, 1996; Rogowitz, 1998; Hammond and Kristan, 2000; Johnson and Speakman, 2001; Johnson et al., 2001a; Johnson et al., 2001b; Johnson et al., 2001c). Early studies of food intake at peak lactation suggested that the limits on SusEI were imposed centrally by the capacity of the alimentary tract and associated organs, such as the liver, to process the ingested food (e.g. Kirkwood, 1983; Perrigo, 1987; Hammond and Diamond, 1992; Hammond and Diamond, 1994; Koteja, 1996). This idea was supported by experiments on lactating mice and guinea pigs *Cavia porcellus* that were energetically challenged by adding extra pups (Hammond and Diamond, 1992; Künkele, 2000; Johnson et al., 2001a) or by extending lactation (Hammond and Diamond, 1994), and did not breach the upper limit of food intake established in unmanipulated mothers. This 'central limitation hypothesis' was not supported, however, when animals were challenged with different modes of energy expenditure. Specifically, mice and hispid cotton rats *Sigmodon hispidus* forced to lactate at low ambient temperatures were able to increase their food intake well beyond a previously supposed centrally mediated limit (Hammond et al., 1994; Rogowitz, 1998; Hammond and Kristan, 2000; Johnson and Speakman, 2001). Consequently, it was suggested that lactating animals were limited not centrally, but peripherally, most likely by the capacity of mammary glands to produce milk (Hammond et al., 1994). According to this idea, females with artificially enlarged litters or prolonged lactation

could not respond to the increased demands, because their mammary glands were already working at maximal capacity, and any further increase in food intake could not be transformed into greater milk production. However, animals lactating at low temperatures were able to elevate their food intake because the increased demands for thermoregulation were additional to and independent from milk production.

A fundamental prediction of the 'peripheral limitation hypothesis' is that mammary glands at peak lactation should work at maximal capacity regardless of ambient temperature. To evaluate this assumption, we performed a series of measures of food intake and milk production in mice exposed to 30°C, 21°C and 8°C (Johnson et al., 2001a; Johnson and Speakman, 2001; Król and Speakman, 2003a; Król and Speakman, 2003b). Consistent with the peripheral limitation model, food intake was the lowest at 30°C and the highest at 8°C. Conflicting with the peripheral limitation idea, however, milk energy output (MEO) was not constant across the different temperatures, but mirrored the pattern of food intake. Greater MEO at lower ambient temperatures (87.7 kJ day^{-1} at 30°C, 166.7 kJ day^{-1} at 21°C and 288.0 kJ day^{-1} at 8°C) could potentially be explained by increasing energy demands of the pups. However, pups weaned at lower temperatures were also heavier (6.1 g at 30°C, 7.0 g at 21°C and 7.3 g at 8°C). Hence, the colder it got, the more food lactating mice ate, the more milk they produced, and the heavier pups they raised. Our results were inconsistent with a limitation imposed either centrally by the capacity of the alimentary tract or peripherally by the capacity of the mammary glands. Other attempts to test these limits were also inconclusive (for a review, see Speakman and Król, 2005a). The lack of clear separation between the central (alimentary tract) and peripheral (mammary gland) effects suggests that the limits on SusEI may act elsewhere.

We have recently proposed that the limits to SusEI at peak lactation might be imposed by the capacity of the animal to dissipate body heat generated as a by-product of processing food and producing milk (Król and Speakman, 2003a; Król and Speakman, 2003b; Król et al., 2003). The heat flow between an animal and its environment strongly depends on the temperature gradient (the difference between body and ambient temperatures) and the thermal conductivity of the insulating surface (subcutaneous fat, skin and fur) (e.g. Scholander et al., 1950; Hammel, 1955; Conley and Porter, 1980). Accordingly, when lactating females were challenged with manipulations that did not involve alterations of ambient temperature and/or thermal conductivity to enhance heat flow, they could not increase their food intake because this would make them dangerously hyperthermic. Examples of such manipulations are experiments performed at room temperature (approximately 21°C) that aimed to increase litter size (Hammond and Diamond, 1992; Künkele, 2000; Johnson et al., 2001a), prolong lactation (Hammond and Diamond, 1994), increase demands of precocial pups by withholding solid food early in lactation (Laurien-Kehnen and Trillmich, 2003), force lactating animals to run for food (Perrigo, 1987) or make them simultaneously pregnant during lactation (Biggerstaff and Mann, 1992; Johnson et al., 2001a; Koiter et al., 1999). However, when the capacity of animals to dissipate heat was increased, by lowering ambient temperature (8°C), mice were able to elevate their food intake to produce more milk and heavier pups (Johnson and Speakman, 2001). By contrast, when we reduced the temperature gradient between the animal and the environment by exposing mice to 30°C, they responded by decreasing food intake, milk production and the size of their offspring (Król and Speakman, 2003a; Król and Speakman, 2003b).

Importantly, raising ambient temperature affects not only the amount of body heat that can be dissipated by the mother, but also by her pups. If the limits to SusEI are, for example, set by the capacity of homeothermic pups to dissipate heat associated with their intense growth rate, then the expected response of the pups exposed to higher ambient temperatures would be to slow down their growth. Under this scenario, pups would have decreased demands for milk which, *via* reduced stimulus from suckling, would lead to downregulation of milk production and an associated decrease in maternal food intake. Hence, the reduced SusEI of females lactating at higher ambient temperatures (Król and Speakman, 2003a) does not indicate whether the limits to SusEI are intrinsic (heat dissipation capacity of the mother) or extrinsic (heat dissipation capacity of the pups), or perhaps unrelated to the dissipation of heat [for examples of other potential limiting factors see (Speakman and Król, 2005a)]. These effects can be separated by manipulation of the heat flow between the female and the environment without affecting the thermal balance of the pups. This can be achieved by changing the thermal conductivity of the female by shaving off some of the fur.

The insulation provided by mammalian fur significantly reduces heat loss to the environment (e.g. Scholander et al., 1950; Barnett, 1959; Knight, 1987; Reynolds, 1993). Seasonal increases in the density, thickness and length of fur have been shown to decrease thermal conductance in winter (e.g. Hart, 1956; Morrison and Tietz, 1957; Conley and Porter, 1980; Jacobsen, 1980). Furthermore, the presence of fur substantially contributes to energy savings during torpor or hibernation by decreasing body heat loss and reducing energy expenditure during periodic arousals from torpor and subsequent intervals of normothermia (Snapp and Heller, 1981; Kauffman et al., 2001a; Kauffman et al., 2004). Conversely, partial or complete fur removal in non-reproductive mice, hamsters and voles is associated with increased food intake and energy expenditure, reflecting enhanced costs of thermoregulation (Pearson, 1960a; Kenagy and Pearson, 2000; Kauffman et al., 2001b; Kauffman et al., 2003). The association between high thermal conductance due to lack of fur and elevated costs of thermoregulation has also been demonstrated in genetically hairless mice (Mount, 1971; Heldmaier, 1974). The effects of fur removal on the energy budget during reproduction, however, have not previously been investigated.

To explore the nature of the limits to SusEI, we bred MF1 laboratory mice (*Mus musculus* L.) at 21°C and then dorsally shaved lactating females to reduce their external insulation and thereby elevate their capacity to dissipate body heat. The heat dissipation limit hypothesis predicts that under such conditions mice should have elevated food intake and milk production that would result in enhanced reproductive performance. Any increases in food intake could also be explained by higher costs of thermoregulation due to fur removal, and would not be inconsistent with the peripheral or extrinsic limitation

hypotheses. However, data on milk production and reproductive performance can unambiguously separate the heat dissipation hypothesis from alternative ideas. Reduction in milk production and reproductive performance by shaved mice would be consistent with the central limitation hypothesis. The peripheral and extrinsic limit hypotheses would be consistent with unchanged milk production and reproductive performance. To test the heat dissipation hypothesis, we measured food intake, daily energy expenditure, milk production and reproductive performance (evaluated by litter size, litter mass, pup body mass, and pup energy content) of shaved mice and compared these traits with the same parameters measured in unshaved mice. The effects of shaving were also evaluated in non-reproductive mice.

Materials and methods

Animals

We used 82 virgin female mice (*Mus musculus* L., outbred MF1) individually housed in shoebox cages (44 cm× 12 cm×13 cm) containing sawdust, and exposed to a 12 h:12 h light:dark cycle (lights on 07:00 h) at an ambient temperature of 21°C (range 20–22°C) and relative humidity of 59% (range 54–64%). Food (CRM, Pelleted Rat and Mouse Breeder and Grower Diet, Special Diets Services, BP Nutrition, Witham, Essex, UK) and water were available *ad libitum*. Animals were acclimated to the experimental conditions for 1 week when they were between 9–11 weeks old. After the acclimation period, 66 randomly selected females were paired with males for 11 days. The remaining 16 females went through the protocol as non-reproductive individuals. Pregnant mice (N=63) were checked twice a day to determine the day of parturition (day 0 of lactation). Females that gave birth to less than 10 pups (N=11) or whose litter size decreased during lactation (N=12) were excluded from the experiment. On day 18 of lactation, all animals that contributed to the final sample size (40 lactating mice with offspring and 16 non-reproductive mice) were sacrificed.

Body mass, food intake and reproductive performance

The body mass, food intake, litter size and litter mass of females raising 10 or more pups (N=40) were recorded (±0.01 g) every other day, from day 4 to the end of lactation (day 18). Food intake was calculated from the difference between the amount of food provided and that left in the hopper. The amount of food consumed over each 2-day period was averaged and presented as the daily food intake for the second day of the period. Simultaneous measurements of body mass and food intake were also performed on non-reproductive females.

To evaluate metabolizable energy intake, sawdust was collected from the cages between days 12–14 of lactation. Sorting through the sawdust revealed that all mice removed some food from the hopper that was then fragmented and left uneaten in the cage as orts. The amount of fragmented food did not vary significantly between shaved and unshaved mice (data and statistical analyses not shown). Since sorting through the sawdust was performed for only one of the seven food-intake trials, the consistency of food wasting across trials repeated on the same individuals was unknown. Therefore, food-intake data reported for days 4–18 of lactation were not corrected for the amount of orts generated by mice on days 12–14 of lactation, but detailed calculations of energy balance and milk production at peak lactation were made using the food intake corrected for orts production (details below).

Shaving protocol

Once the measurements on day 6 of lactation were completed, we assigned the mice to two groups (shaved and unshaved) that were matched for litter size (lactating mice) or body mass (non-reproductive mice). All mice were then anaesthetized with gaseous isoflurane for approximately 10 min. During this time, 20 lactating and 8 non-reproductive mice were shaved dorsally (using a Wella Contura Hair Clipper, Basingstoke, Hants, UK) to remove approximately 0.18–0.30 g of fur (Fig. 1); the remaining mice were not shaved (lactating and non-reproductive unshaved control groups). Based on the data available for 6 mice that went through the shaving protocol and were then completely shaved *post mortem*, the amount of fur shaved off during lactation corresponded to 72.1±2.2% (range 69.4–74.4%) of total fur mass. Hair regrowth was prevented by repeating the shaving protocol on days 10 and 14 of lactation. The mean litter sizes for shaved and unshaved mice were 11.4±1.1 and 11.3±1.0 pups, respectively (N=20 for both groups).

Metabolizable energy intake

Measurements of metabolizable energy intake (MEI) were made on days 12–14 of lactation. Females and their litters were placed in cages with fresh sawdust and a weighed portion of food was added to the hopper on day 12 of lactation. Samples of the food were taken to determine dry mass content (90.7±0.1%, N=10), and the food remaining in the hopper was

Fig. 1. Lactating MF1 mouse with dorsal fur shaved off to increase heat loss capacity.

reweighed on day 14 of lactation. Any uneaten, fragmented food and faeces were removed from the cage, dried to a constant mass at 60°C and weighed. Simultaneous measurements of MEI were also performed on non-reproductive females. The gross energy content of food (17.97±0.15 kJ g^{-1} dry mass, N=3) and faeces were measured by bomb calorimetry (Gallenkamp Autobomb Adiabatic Bomb Calorimeter, Rowett Research Institute Analytical Services, Aberdeen, UK). We calculated the dry food consumption of each female (g day^{-1}) from food removed from hopper (g day^{-1})×food dry mass content (%)–dry uneaten food from the cage floor (g day^{-1}). Daily energy intake (kJ day^{-1}) was calculated from the dry food consumption (g day^{-1})×food energy content (kJ g^{-1} dry mass). The energy lost through defecation (kJ day^{-1}) was calculated from dry faecal production (g day^{-1})×faecal energy content (kJ g^{-1} dry mass). MEI was estimated as the difference between energy consumed and defecated, assuming that urinary energy loss was 3% of the digestible energy intake (Drożdż, 1975). The apparent digestive efficiency was calculated as the percentage of gross energy intake that was digested.

Daily energy expenditure

We measured daily energy expenditure (DEE) using the doubly labelled water (DLW) technique (Lifson and McClintock, 1966; Speakman, 1998) on days 15–17 of lactation. This method has been previously validated by comparison to indirect calorimetry in a range of small mammals (e.g. Speakman and Racey, 1988a) and provides an accurate measure of DEE over periods of several days (Speakman and Król, 2005b). Day-to-day variability in estimated energy metabolism suggests measurements spanning multiple 24 h periods may give a superior representation of energy metabolism (Speakman et al., 1994; Berteaux et al., 1996). Studies of lactating mammals suggest that recycling of isotopes between a mother and her offspring is negligible (Scantlebury et al., 2000).

Animals were weighed (±0.01 g) and injected intraperitoneally with approximately 0.25 g (lactating mice) or 0.15 g (non-reproductive mice) of water containing enriched ^{18}O (27.8 atom%) and ^{2}H (15.9 atom%). Syringes were weighed before and after administration (±0.0001 g) to calculate the mass of DLW injected. Blood samples were taken after 1 h of isotope equilibration to estimate initial isotope enrichments (Król and Speakman, 1999; Visser et al., 2000a) and were also collected from unlabelled animals to estimate the background isotope enrichments (Speakman and Racey, 1987) (method D). Blood samples were immediately heat sealed into 2×50 μl glass capillaries and stored at room temperature. A final blood sample was taken approximately 48 h later (Speakman and Racey, 1988b) to estimate isotope elimination rates. 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 Król, 2005b). The isotope ratios ^{18}O:^{16}O and ^{2}H:^{1}H were analysed using gas source isotope ratio mass spectrometry (ISOCHROMμGAS system and IsoPrime IRMS, Micromass, Manchester, UK). We ran three high-enrichment standards each day alongside the samples and corrected all the raw data to these standards to minimise the problems of inlet cross contamination (Meijer et al., 2000).

Initial isotope dilution spaces (mol) were calculated by the intercept method (Coward and Prentice, 1985), then converted to g assuming a molecular mass of body water of 18.020 and expressed as a percentage of body mass before injection. We used the intercept method because the actual body water pool estimated by desiccation (using mice excluded from the main part of this experiment due to small or unstable litter sizes), was more accurately predicted by the intercept approach than by the plateau approach. The actual body water pool for the mice that were desiccated averaged 69.1±5.3% (day 17 of lactation), whereas their mean oxygen dilution space (N_o) was calculated as 69.4±3.0% by the intercept method and 72.3±3.7% by the plateau method (N=14 for all means). Similarly, the deuterium dilution space (N_d) averaged 74.9±2.4% for the intercept method and 77.3±3.0% for the plateau method (N=14 for both means). Final dilution spaces were inferred from the final body mass, assuming the same percentage of body water as measured for the initial dilution spaces. The isotope elimination rate (k) was calculated following published methods (Lifson et al., 1955). We used the single-pool model equation 7.17 (Speakman, 1997) to calculate the rate of CO_2 production, as recommended for animals weighing under 1 kg (Visser and Schekkerman, 1999; Visser et al., 2000b; Speakman and Król, 2005b). Energy equivalents of the rate of CO_2 production were calculated using a conversion factor of 24.026 J ml^{-1} CO_2, derived from the Weir equation (Weir, 1949) for a respiratory quotient of 0.85 (Speakman, 1997).

Milk energy output (MEO)

We used the DLW data to evaluate MEO, calculated from the difference between MEI and DEE (Król and Speakman, 2003b). MEI and DEE were not measured simultaneously to avoid possible changes in animal behaviour or feeding patterns as a result of DLW injections/bleeding (Król and Speakman, 2003a; Speakman and Król, 2005b). Animals were in energy balance when MEO was evaluated, as indicated by their stable body mass (Fig. 2A).

Pup energy content

Eight litters raised by shaved and nine litters raised by unshaved females were sacrificed on day 18 of lactation. These were desiccated to a constant mass and then ground up and used to evaluate the gross energy content of the pups (Gallenkamp Autobomb Adiabatic Bomb Calorimeter, Rowett Research Institute Analytical Services, Aberdeen, UK). Mean values of pup body mass, pup dry mass and gross energy content of pups (dry and live mass) were calculated for each litter.

Statistics

Data are reported as mean ± s.d. (N=sample size). Measurements repeated on the same individuals (body mass, food intake, litter mass and pup body mass) were analysed using two-way repeated measures ANOVA, with group (shaved *versus* unshaved mice) and day of lactation as factors. When the effect of group or the interaction 'group×day' was significant, the Holm–Sidak multiple comparison procedure was applied to determine differences between the groups within each day. For single measurements, we used either t-test or ANCOVA with an appropriate covariate that was selected by screening the data

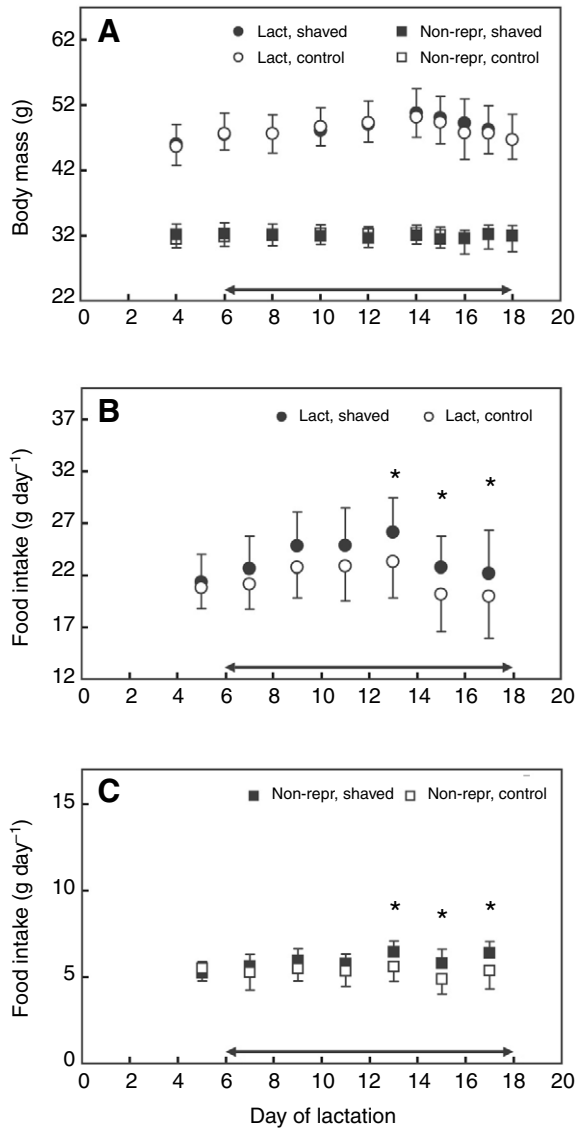

Fig. 2. Body mass (A) and food intake (B) of shaved (*N*=20) and unshaved control (*N*=20) mice during lactation. Double-headed arrows indicate period when dorsal fur was removed to increase heat loss capacity (days 6–18 of lactation). Body mass (A) and food intake (C) of non-reproductive shaved (*N*=8) and unshaved control (*N*=8) mice are also shown. *Days with significant differences between shaved and control mice of the same reproductive status. Values are means ± 1 s.d. Filled symbols, shaved mice; open symbols, unshaved control mice; circles, lactating mice; squares, non-reproductive mice.

for potential relationships between measured variables. These relationships were described using Pearson correlation coefficients and *P*-values. Arcsine-square-root transformations were performed prior to analysis for percentage data (apparent digestive efficiency and pup dry mass content). For non-reproductive mice, we calculated the percentage error in DEE relative to MEI as 100×(DEE-MEI)/MEI. All statistical analyses were conducted using SigmaStat for Windows, version 3.5 (Systat Software Inc.). Statistical significance was determined at $P<0.05$. All tests were two-tailed unless stated otherwise.

Results

Body mass and food intake

The body mass of lactating females allocated to shaved and unshaved groups averaged 46.0±3.0 g and 45.7±3.0 g, respectively, prior to shaving (day 4 of lactation) and 46.7±3.9 g and 46.7±3.0 g, respectively, at the end of the experiment (day 18 of lactation) (*N*=20 for all means, Fig. 2A). Body mass did not vary between the shaved and unshaved groups, but varied across days of lactation (two-way repeated measures ANOVA; group, $F_{1,342}=0.1$, $P=0.78$; day, $F_{9,342}=32.1$, $P<0.001$; interaction group×day, $F_{9,342}=1.5$, $P=0.15$). The mean food intake of lactating shaved and control mice was 21.3±2.6 g day^{-1} and 20.8±2.0 g day^{-1}, respectively, prior to shaving (day 4 of lactation) and 22.2±4.1 g day^{-1} and 20.0±4.1 g day^{-1}, respectively, at the end of the experiment (day 18 of lactation) (*N*=20 for all means, Fig. 2B). Shaved lactating mice ate more food than controls and food intake varied with day of lactation (two-way repeated measures ANOVA; group, $F_{1,228}=5.3$, $P<0.027$; day, $F_{6,228}=23.2$, $P<0.001$; interaction group×day, $F_{6,228}=1.5$, $P=0.19$). Multiple comparisons for the groups within each day revealed that the difference in food intake between shaved and unshaved mice was significant for days 12–13 ($P=0.007$), 14–15 ($P=0.015$) and 16–17 ($P=0.037$). On these days, shaved mice ate on average 2.9, 2.6 and 2.2 g day^{-1} more food than unshaved mice, respectively. Over the whole period of manipulation (days 6–18), lactating shaved mice ate on average 26.2 g more food than lactating unshaved individuals.

The body mass of non-reproductive females allocated to shaved and unshaved groups averaged 32.2±1.6 g and 31.5±1.4 g, respectively, prior to shaving and 32.0±1.5 g and 31.9±2.5 g, respectively, at the end of the experiment (*N*=8 for all means, Fig. 2A). Body mass was not affected by shaving or the day of experiment (two-way repeated measures ANOVA; group, $F_{1,126}=0.01$, $P=0.92$; day, $F_{9,126}=1.5$, $P=0.14$; interaction group×day, $F_{9,126}=1.8$, $P=0.08$). The mean food intake of non-reproductive shaved and control mice was 5.2±0.6 g day^{-1} and 5.5±0.8 g day^{-1}, respectively, prior to shaving and 6.4±0.7 g day^{-1} and 5.4±1.1 g day^{-1}, respectively, at the end of the experiment (*N*=8 for all means, Fig. 2C). The response of mice to the treatment strongly depended on the day of the experiment (two-way repeated measures ANOVA; group, $F_{1,84}=3.0$, $P=0.10$; day, $F_{6,84}=4.5$, $P<0.001$; interaction group×day, $F_{6,84}=3.4$, $P=0.005$). For days 5–11, the difference in food intake between shaved and control mice was not significant ($P>0.05$). However, on days 12–13, 14–15 and 16–17, shaved mice had a significantly higher food intake than unshaved individuals; the differences between the groups averaged 0.8 g day^{-1} ($P=0.007$), 0.9 g day^{-1} ($P=0.015$) and 1.0 g day^{-1} ($P=0.037$), respectively. Over the whole period of manipulation (days 6–18), non-reproductive shaved mice ate on average 8.1 g more food than non-reproductive unshaved individuals.

Metabolizable energy intake and digestive efficiency

The faecal production of lactating mice was highly correlated with food consumption ($r=0.84$, $P<0.001$, $N=40$; data not shown). When food consumption was included as a covariate, faecal production did not vary significantly between shaved and unshaved mice (ANCOVA, food, $F_{1,36}=69.6$, $P<0.001$; group,

$F_{1,36}$=0.5, P=0.47; interaction food×group, $F_{1,36}$=0.5, P=0.49). The gross energy content of faeces did not vary significantly between shaved (17.02±0.26 kJ g^{-1} dry mass) and control mice (17.00±0.18 kJ g^{-1} dry mass) (t-test, t_{34}=0.3, P=0.80, N=20 for both groups). On days 12–14 of lactation, the mean MEI of shaved and unshaved mice was 297.2±33.2 kJ day^{-1} and 266.3±35.2 kJ day^{-1}, respectively (N=20 for both groups). The difference between the groups (30.9 kJ day^{-1}) was significant (t-test, t_{37}=2.9, P=0.007). Apparent digestive efficiency did not vary significantly between lactating shaved (79.9±1.8%) and unshaved (79.8±1.2%) mice (t-test, t_{33}=0.2, P=0.84, N=20 for both groups).

In non-reproductive mice, faecal production and food consumption were also highly correlated (r=0.90, P<0.001, N=16; data not shown). After correcting for differences in food consumption, faecal production did not vary significantly between shaved and control mice (ANCOVA, food, $F_{1,12}$=34.1, P<0.001; group, $F_{1,12}$=0.03, P=0.86; interaction food×group, $F_{1,12}$=0.04, P=0.84). The gross energy content of faeces averaged 16.71±0.24 kJ g^{-1} dry mass for shaved mice and 16.86±0.17 kJ g^{-1} dry mass for unshaved individuals (N=8 for both groups). These values were not significantly different (t-test, t_{12}=1.4, P=0.20). On days 12–14 of the experiment, non-reproductive shaved and control mice assimilated on average 75.7±8.2 kJ day^{-1} and 66.2±8.2 kJ day^{-1}, respectively (N=8 for both groups). The difference between the groups (9.5 kJ day^{-1}) was significant (t-test, t_{13}=2.3, P=0.039). Apparent digestive efficiency did not vary significantly between non-reproductive shaved (78.9±1.4%) and unshaved (78.8±1.5%) mice (t-test, t_{13}=0.1, P=0.90, N=8 for both groups).

Daily energy expenditure

Results of the DLW measurements are presented in Table 1. Between days 15 and 17 of lactation, DEE of shaved and unshaved mice averaged 130.7±13.5 kJ day^{-1} and 121.8±14.8 kJ day^{-1}, respectively (N=20 for both groups). The difference between the groups (8.9 kJ day^{-1}), as anticipated with higher expenditure in the shaved mice, was significant (one-tailed t-test, t_{37}=2.0, P=0.027).

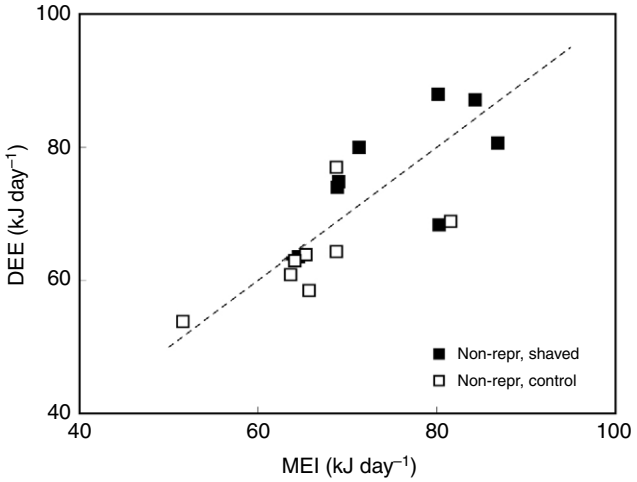

Fig. 3. Relationship between daily energy expenditure (DEE) and metabolizable energy intake (MEI) in non-reproductive shaved (filled squares; N=8) and unshaved control (open squares; N=8) mice. The line of equality is shown.

In non-reproductive mice, the DEE of shaved and unshaved individuals averaged 77.0±8.6 kJ day^{-1} and 63.7±7.0 kJ day^{-1}, respectively (N=8 for both groups). The difference between the groups (13.3 kJ day^{-1}) was significant (t-test, t_{13}=3.4, P=0.005), and DEE and MEI were highly correlated (r=0.76, P=0.001, N=16) (Fig. 3). On average, estimated DEE (70.4± 10.2 kJ day^{-1}) differed from MEI (70.9±9.3 kJ day^{-1}) by –0.5±9.1% (N=16 for all means).

Milk energy output and reproductive performance

On average, shaved mice exported 22.0 kJ day^{-1} more energy as milk at peak lactation (166.4±31.1 kJ day^{-1}) than control individuals (144.5±30.8 kJ day^{-1}) (t-test, t_{37}=2.2, P=0.031, N=20 for both groups). Milk production was not correlated with maternal body mass (Fig. 4A, r=–0.02, P=0.91, N=40) or litter size (Fig. 4B, r=0.14, P=0.38, N=40). There was, however, a

Table 1. *Results of doubly labelled water measurements of daily energy expenditure performed on lactating shaved or unshaved mice and on non-reproductive shaved or unshaved female mice*

	Lactating mice		Non-reproductive mice	
Trait	Shaved	Unshaved	Shaved	Unshaved
Body mass (g)[a]	50.0±3.3	49.4±3.3	31.5±1.8	32.1±2.0
k_d (h^{-1})[b]	0.038±0.005	0.038±0.007	0.013±0.002	0.012±0.002
k_o (h^{-1})[c]	0.050±0.006	0.050±0.008	0.024±0.002	0.020±0.003
k_o/k_d	1.312±0.045	1.303±0.049	1.867±0.094	1.782±0.146
N_d (% of body mass)[d]	75.1±3.2	73.9±3.9	73.3±10.8	73.6±10.7
N_o (% of body mass)[d]	68.8±2.2	67.4±3.7	66.5±9.5	67.4±9.9
N_d/N_o	1.091±0.028	1.096±0.011	1.102±0.026	1.092±0.033
DEE (kJ day^{-1})[e]	130.7±13.5	121.8±14.8	77.0±8.6	63.7±7.0

DEE, daily energy expenditure (between days 15 and 17 of lactation in lactating mice; see text).

Values are means ±s.d.; N=20 for shaved and unshaved lactating mice; N=8 for non-reproductive shaved and unshaved mice.

[a]Body mass before injection; [b]elimination rate of ^2H; [c]elimination rate of ^{18}O; [d]deuterium (N_d) and oxygen (N_o) dilution spaces expressed as % of body mass before injection; [e]daily energy expenditure was measured between days 15 and 17 of lactation (non-reproductive mice were measured on the same days as lactating mice).

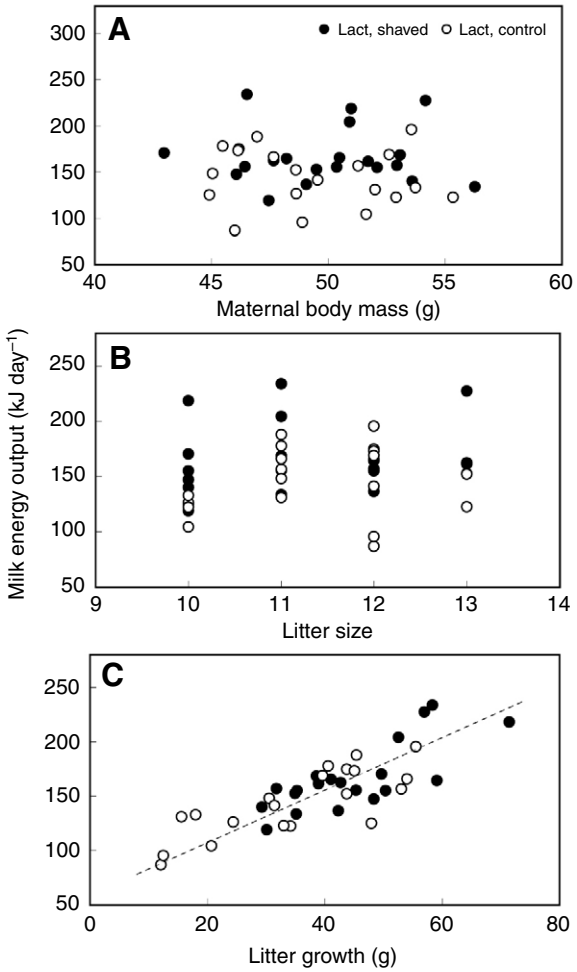

Fig. 4. Milk energy output (MEO) of shaved (filled circles; N=20) and unshaved control (open circles; N=20) mice plotted against maternal body mass (A), litter size (B) and litter growth (C). Maternal body mass refers to day 15 of lactation. Litter growth is defined as the difference between litter mass on day 18 and day 6 of lactation. Only the correlation between MEO and litter growth is significant (for statistical details, see Results). The fitted line represents reduced major axis regression for the pooled data (N=40), described by y=58.9+2.4x.

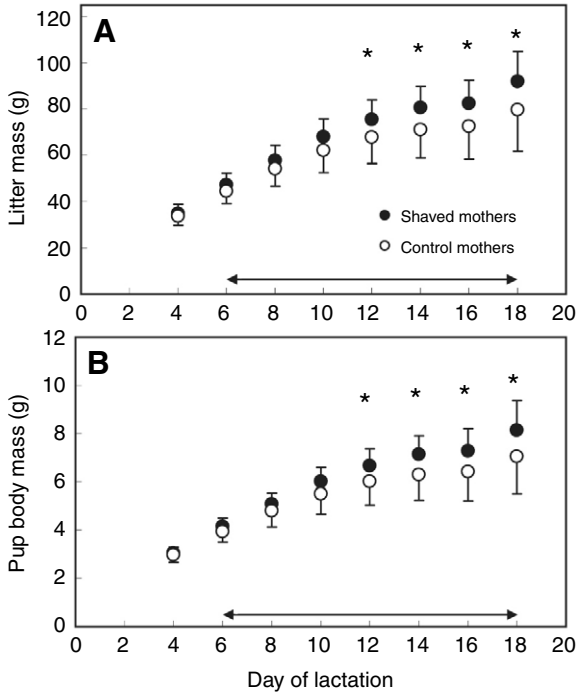

Fig. 5. Litter mass (A) and pup body mass (B) of shaved (filled circles; N=20) and unshaved control (open circles; N=20) mothers. Double-headed arrows indicate period when mothers had dorsal fur shaved off to increase heat loss capacity (days 6–18). *Days with significant differences between offspring raised by shaved and control mice. Values are means ± 1 s.d.

significant positive correlation between MEO and litter growth rate (Fig. 4C, r=0.80, P<0.001, N=40).

The effect of shaving on litter mass varied during the lactation period (two-way repeated measures ANOVA; group, $F_{1,266}$=5.7, P=0.022; day, $F_{7,266}$=473.7, P<0.001; interaction group×day, $F_{7,266}$=6.0, P<0.001) (Fig. 5A). For days 4–10 of lactation, there was no significant difference between the litter mass of shaved and unshaved mice (P>0.05). However on days 12, 14, 16 and 18, litters raised by shaved mice were heavier than litters of unshaved females by an average of 7.7 g (P=0.016), 9.6 g (P=0.003), 9.8 g (P=0.003) and 12.2 g (P<0.001), respectively (note that no measurements were made on odd numbered days). At the end of lactation, the litter masses of shaved and control mice averaged 91.8±13.0 and 79.6±17.9 g, respectively (N=20 for both groups). The analyses performed on mean pup body mass (litter mass divided by litter size) yielded similar results (two-way repeated measures ANOVA; group, $F_{1,266}$=5.9, P=0.020; day, $F_{7,266}$=433.8, P<0.001; interaction group×day, $F_{7,266}$=5.7, P<0.001) (Fig. 5B). For days 4–10, the body mass of the pups of shaved and control mothers did not vary significantly (P>0.05). On days 12, 14, 16 and 18, pups raised by shaved mothers were heavier than pups of unshaved females by an average of 0.7 g (P=0.016), 0.8 g (P=0.003), 0.9 g (P=0.002) and 1.1 g (P<0.001), respectively. At the end of lactation, mean pup mass was 8.1±1.2 g for shaved mice and 7.1±1.5 g for control individuals (N=20 for both groups).

Greater pup body mass was associated with higher dry mass content (Fig. 6A, r=0.95, P<0.001, N=17) and higher gross energy content of pup dry mass (Fig. 6B, r=0.89, P<0.001, N=17). Consequently, there was also a positive correlation between pup body mass and gross energy content of pup live mass (Fig. 6C, r=0.94, P<0.001, N=17). Pups raised by shaved and unshaved mice did not differ in dry mass content (ANCOVA, pup mass, $F_{1,13}$=76.0, P<0.001; group, $F_{1,13}$=0.6, P=0.42; interaction pup mass×group, $F_{1,13}$=0.7, P=0.41), gross energy content of dry mass (ANCOVA, pup mass, $F_{1,13}$=41.4, P<0.001; group, $F_{1,13}$=1.7, P=0.21; interaction pup mass×group, $F_{1,13}$=1.7, P=0.22) or gross energy content of live mass (ANCOVA, pup mass, $F_{1,13}$=80.5, P<0.001; group, $F_{1,13}$=1.0, P=0.34; interaction pup mass×group, $F_{1,13}$=1.1, P=0.33).

Discussion

We have previously suggested that lactating mice are not limited centrally by the alimentary tract or peripherally by the

mammary glands, but that the limits to SusEI may be imposed by the capacity of the female to dissipate body heat generated as a by-product of processing food and producing milk (Król and Speakman, 2003a; Król and Speakman, 2003b; Król et al., 2003). If this idea is correct, then any manipulation that increases the heat dissipation capacity should result in elevated food intake and milk production that would translate into enhanced reproductive performance. Indeed, when lactating mice were exposed to cold conditions, their responses were consistent with the heat dissipation limit hypothesis (Hammond et al., 1994; Johnson and Speakman, 2001). However, manipulations that involve changes in ambient temperature do not separate maternal effects from the extrinsic effects associated with the thermoregulatory responses of the pups.

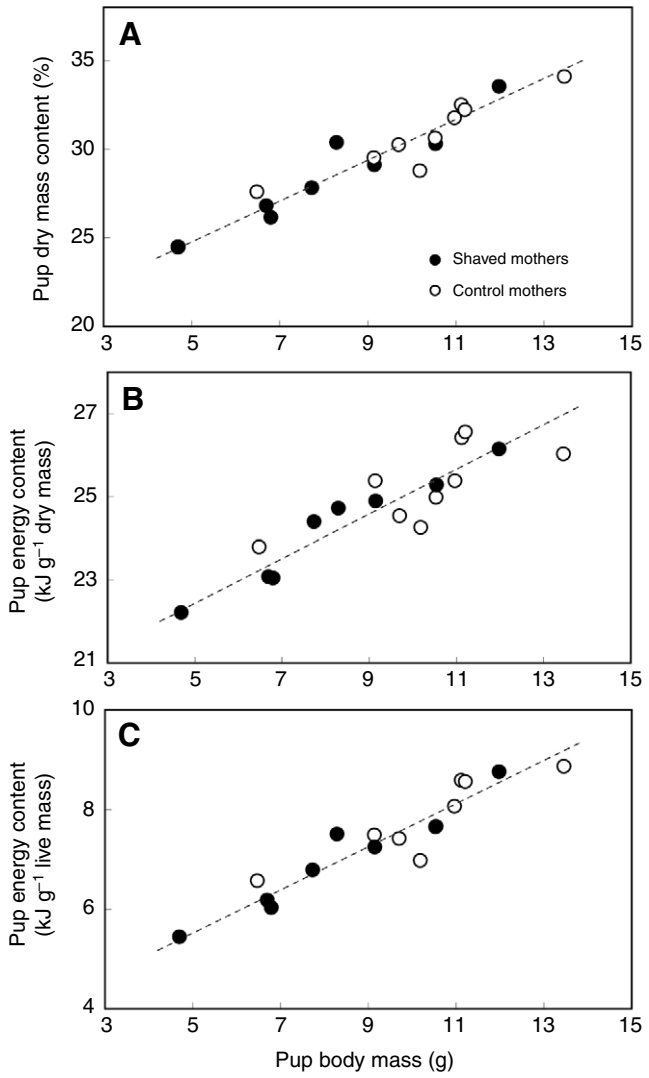

Fig. 6. Pup dry mass content (A), gross energy content of pup dry mass (B) and gross energy content of pup live mass (C) plotted against pup body mass. Values are means for litters raised by shaved (filled circles; N=8) and unshaved control (open circles; N=9) mothers. All correlations are significant (for statistical details, see Results). The fitted lines represent reduced major axis regressions for the pooled data (N=17), described by y=19.0+1.2x (A), y=19.8+0.5x (B) and y=3.3+0.4x (C).

The approach we took in the current experiment was to increase the heat dissipation capacity of females lactating at 21°C by shaving off their dorsal fur and comparing their reproductive performance with unshaved controls. Importantly, shaving did not induce compensatory changes in body mass (Fig. 2A). Furthermore, compensatory changes in body fat content were also unlikely since shaved and unshaved mice had similar isotope-derived body water content (Table 1). At peak lactation, the shaved mice ate on average 12.0% (2.5 g day^{-1}/21.1 g day^{-1}) more food than unshaved controls (Fig. 2B). This result is incompatible with the central limitation hypothesis but consistent with the peripheral and heat dissipation limit hypotheses. When we took into account individual variation in food wasting and individual estimates of apparent digestive efficiency, shaved mice at peak lactation assimilated on average 30.9 kJ day^{-1} more energy than unshaved individuals. As predicted exclusively by the heat dissipation limit hypothesis, this extra energy was used to generate more milk. Milk production was calculated from the difference between MEI and DEE (Król and Speakman, 2003b) and we validated our application of the DLW technique in non-reproductive mice, in which estimates of MEI and DEE closely matched each other (Fig. 3). With nearly identical litter sizes (11.4 pups for shaved and 11.3 pups for unshaved mice), shaved mothers exported at peak lactation on average 15.2% (22.0 kJ day^{-1}/144.5 kJ day^{-1}) more energy as milk than control individuals (Fig. 4). If the increase in MEO induced by shaving (22.0 kJ day^{-1}) was fuelled by the extra energy that was assimilated (30.9 kJ day^{-1}), the efficiency of conversion of MEI to MEO would be 71.1% (22.0 kJ day^{-1}/30.9 kJ day^{-1}). This value is in good agreement with the net milk production efficiencies reported for other mammals (e.g. Romero et al., 1976; Baldwin et al., 1980; Freetly et al., 2006). The litters weaned by shaved mice were on average 15.4% (12.2 g/79.6 g) heavier than the litters produced by control mothers (Fig. 5A). Similarly, the individual pups raised by shaved mice were on average 1.1 g heavier than the control pups (Fig. 5B). The differences in litter and pup body masses were not associated with changes in body composition of produced offspring, since pups raised by shaved and unshaved mice had similar dry mass and energy contents (Fig. 6). Taken together, when we increased the capacity of lactating mice to dissipate body heat by shaving off their dorsal fur, these mice were able to eat more food, generate more milk and wean heavier offspring than mice with intact fur. Our results argue against central, peripheral or extrinsic limits to SusEI at peak lactation and provide strong support for the heat dissipation limit hypothesis.

Experimental alterations of maternal heat load by factors other than ambient temperature have also been performed in Wistar rats (Leon et al., 1978). The first manipulation involved shaving off the ventral fur of females on the day of parturition. These rats spent more time with their pups than unshaved controls, supporting the idea that chronic hyperthermia of lactating females may constrain maternal behaviour if contact with the litter further increases their body temperature, forcing the termination of nest bouts to dissipate the heat load (e.g. Croskerry et al., 1978; Leon et al., 1985; Scribner and Wynne-Edwards, 1994). The second manipulation (Leon et al., 1978) aimed to decrease ability of female rats to dissipate body heat

by removing their tails – a major avenue for heat flow when heat production exceeds heat loss to the environment. Accordingly, rats without tails spent less time with their offspring than sham-operated intact mothers, whereas females with half their tail removed had an intermediate amount of contact time. If we assume that the time spent suckling is positively correlated with the daily rate of milk transfer from mother to offspring, then the behavioural observations of rats that were shaved or had their tails removed would be consistent with the heat dissipation limit hypothesis.

Further support for the heat dissipation hypothesis comes from the correlated responses of mice that have been divergently selected for many generations for high and low heat loss (Nielsen et al., 1997a; Nielsen et al., 1997b). As indicated by a weigh-suckle-weigh method, mice selected for high heat loss produced on average 20.6% (1.70 g/1.41 g) more milk over a 2 h collection period than the low heat loss mice (McDonald and Nielsen, 2006). Furthermore, the litters weaned by mice selected for high heat loss were on average 10.1 g heavier than the litters produced by the low heat loss line. These results are in good agreement with the effects of fur removal presented in the current study, but lack direct measures of daily MEO to be a complete proof of the heat dissipation limit hypothesis.

When we removed dorsal fur in non-reproductive mice, they responded to the elevated heat dissipation capacity in a similar way to lactating mice – by increasing their food intake (Fig. 2C). On average, shaving increased food intake of non-reproductive mice by 17.6% (0.9 g day^{-1}/5.3 g day^{-1}). A similar result was found for non-reproductive Siberian hamsters (*Phodopus sungorus*) that were housed under a long day photoperiod at an ambient temperature of 23°C – these animals increased their food intake by approximately 20% following complete fur removal (Kauffman et al., 2001b). In the current study, non-reproductive mice with the reduced external insulation assimilated on average 9.5 kJ day^{-1} more energy than control mice. Moreover, shaving increased energy expenditure of non-reproductive mice by 13.3 kJ day^{-1}, which corresponds to 20.8% increase above the level measured in unshaved controls (13.3 kJ day^{-1}/63.7 kJ day^{-1}) (Table 1). Surprisingly, as a consequence of complete fur removal, DEE of non-reproductive California voles (*Microtus californicus*) living in natural winter conditions increased by only 10% (Kenagy and Pearson, 2000). However, the degree to which fur conserved energy in that study was probably underestimated because of behavioural adjustments of the voles, which in winter tend to form social aggregations by sharing nests with four or five other individuals (Pearson, 1960b; Hayes et al., 1992). Much higher (35%) increases in oxygen consumption following fur removal were reported in non-reproductive harvest mice (*Reithrodontomys megalotis*) held in a short-day photoperiod at 18°C and 24°C, although these conclusions should be interpreted with caution as only one animal was tested (Pearson, 1960a). Coupled with the observation that fur removal does not induce changes in locomotor activity (Kauffman et al., 2003), simultaneous occurrence of elevated assimilation (9.5 kJ day^{-1}) along with the elevated energy expenditure (13.3 kJ day^{-1}) in non-reproductive mice that were shaved (present study), clearly suggests that these increases may reflect a compensatory response to increased costs of thermoregulation. This conclusion is supported by 38% increase in the mass and activity of brown adipose tissue (BAT) reported in genetically hairless mice (Heldmaier, 1974).

By contrast to the non-reproductive mice, the majority of the additional energy that was assimilated by lactating mice as a result of fur removal (30.9 kJ day^{-1}) was not metabolised, and therefore did not reflect possible costs of thermoregulation. Instead, the extra energy assimilated was exported as milk and therefore was not included as carbon dioxide production in the direct evaluation of total DEE. As indicated by the DLW data (Table 1), the increases in energy expenditure of lactating mice induced by shaving averaged 8.9 kJ day^{-1} (7.3% increase above the level measured in unshaved controls). This increase probably represents metabolism associated with converting dietary energy into milk (e.g. Romero et al., 1976; Baldwin et al., 1980; Freetly et al., 2006) and/or metabolism associated with postabsorptive processing (heat increment of feeding), because these two processes are difficult to discriminate (e.g. Parry, 1983; Blaxter, 1989; Wieser, 1994).

Shaving did not appear to induce thermogenic heat production in lactating mice as it did in non-reproductive animals, suggesting that the amount of heat generated as a by-product of lactogenesis was great enough to substitute for the heat that would otherwise be needed to maintain normal body temperature. Our estimates of lactogenic heat production are 48.0 kJ day^{-1} (166.4 kJ day^{-1}×28.9%) for shaved mice and 41.7 kJ day^{-1} (144.5 kJ day^{-1}×28.9%) for unshaved controls, whereas the estimated cost of thermoregulation caused by fur removal in non-reproductive mice was 13.3 kJ day^{-1}. If the performance of lactating mice is limited by the total amount of heat they can dissipate, then the increasing heat load due to milk production is expected not only to substitute for thermogenic heat, but also to effectively reduce non-reproductive components of the energy budget. Otherwise, completely additive lactogenic and thermogenic costs would exacerbate heat dissipation problems and increase a risk of developing maternal hyperthermia. The ability to utilise lactogenic heat to substitute for heat generated solely for thermogenesis is supported by downregulation of the thermogenic function of BAT during lactation, as shown by tissue hypothrophy, a decrease in mitochondrial biogenesis, reduced expression of the gene encoding uncoupling protein 1 (UCP1), lower protein levels of UCP1 and a reduction in the noradrenaline-induced non-shivering thermogenesis (e.g. Agius and Williamson, 1980; Trayhurn et al., 1982; Villarroya et al., 1986; Trayhurn, 1989; Nizielski et al., 1993; Johnson et al., 2001b; Xiao et al., 2004; Zhang and Wang, 2007).

In summary, we have shown that lactating mice with their dorsal fur shaved off ate more food, produced more milk and weaned heavier offspring than unshaved mice. Our verification of the heat dissipation limit hypothesis is important because it indicates that ambient temperature changes will have direct effects on reproductive performance, as well as indirect effects *via* impacts on food supply. This means that the consequences of climate change may be felt more immediately and directly by endotherms than has hitherto been suspected. Understanding the links between constraints on heat dissipation, life-history traits and biogeography may improve our ability to model the ecosystem impacts of global climate change. More generally, we see many situations where heat dissipation limits could be a

previously unrecognised factor constraining the evolution of endothermic animals. For example, the ability to dissipate body heat may provide valuable insights into latitudinal and altitudinal trends in clutch and litter sizes (e.g. Bohning-Gaese et al., 2000; Cooper et al., 2005; Virgos et al., 2006). Furthermore, thermal constraints during flapping flight could explain why birds tend to migrate at high altitude and/or during cloudless nights (e.g. Klaassen, 1996; Léger and Larochelle, 2006).

List of abbreviations

BAT	brown adipose tissue
DEE	daily energy expenditure
DLW	doubly labelled water
MEI	metabolizable energy intake
MEO	milk energy output
SusEI	sustained energy intake
UCP1	uncoupling protein 1

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