Effect of fur removal on the thermal conductance and energy budget in lactating Swiss mice

Zhi-Jun Zhao* and Jing Cao

School of Agricultural Science, Liaocheng University, Liaocheng, Shandong 252059, China

*Author for correspondence (e-mail: zhaozj73@yahoo.com.cn)

SUMMARY

The limits to sustained energy intake (SusEI) are important because they determine the ceiling restricting all the competing physiological processes. A recent hypothesis is that SusEI is constrained by the capacity to dissipate heat. However some previous data for Swiss mice are inconsistent with this hypothesis. To examine the role of limits to heat dissipation on SusEI, the body temperature, thermal conductance and lactation performance were measured in dorsally shaved Swiss mice. Shaving reduces external insulation and the heat dissipation limitation hypothesis predicts such animals should be capable of eating more food and raising heavier litters. Shaved mice had a significantly higher thermal conductance and a faster reduction in body temperature following noradrenaline injection. At peak lactation, shaved mice spent more time in feeding behaviour, and increased food intake above that observed in non-shaved controls, indicating that limits on SusEI might be imposed by the capacity to dissipate heat. However, shaved females did not spend more time suckling their pups, and did not raise heavier litters, which is inconsistent with the expectations of the heat dissipation limitation hypothesis. The strong correlations between resting, feeding and suckling behaviour at peak lactation suggested that there might be a trade-off in the time distribution between the behavioural patterns. These data suggest that limits on performance may be set at different levels in different strains or species. In MF1 mice studied previously the limit on milk production imposed by maximal mammary secretion capability may lie above that for heat dissipation, hence when the latter was increased the mice produced more milk and raised heavier litters. In Swiss mice the opposite might be the case. Hence when the heat dissipation capacity was increased this did not translate into heavier litters, i.e. supporting the peripheral limitation hypothesis. Further work in a range of additional species or strains will be necessary to establish whether the more normal condition is for SusEI in animals during late lactation to be set by combined peripheral demands or by the heat dissipation capacity.

Key words: behaviour, energy budget, fur removal, heat dissipation limitation, lactation, sustained energy intake, thermal conductance.

INTRODUCTION

Sustained energy intake (SusEI) is the maximal rate of energy intake that animals can sustain over sufficiently long periods so that energy demands are fuelled by food intake rather than by transient depletion of energy reserves (Speakman and Krol, 2005). In many situations, the SusEI may be constrained. The factors causing these constraints on SusEI are important because they establish the upper energetic limits on the ability of animals to distribute, survive and reproduce (Karasov, 1986; Peterson et al., 1990; Hammond and Diamond, 1997; Speakman, 2000; Krol and Speakman, 2003a; Krol and Speakman, 2003b; Speakman and Krol, 2005). Earlier studies suggested that the SusEI might be imposed by food availability; called the extrinsic limitation hypothesis (Hammond and Diamond, 1997; Speakman and Krol, 2005). However, the extrinsic limitation hypothesis is inconsistent with the fact that most animals do not increase food intake when given free access to unlimited food (Akbar and Gorman, 1993; Akbar and Gorman, 1996; Hammond and Diamond, 1997; Speakman and Krol, 2005). To explain such situations two further hypotheses emerged – the central and peripheral limitation hypotheses (Weiner, 1992; Peterson et al., 1990; Hammond and Diamond, 1997; Speakman and Krol, 2005; Speakman, 2007). The central limitation hypothesis suggested the constraint on SusEI might be imposed by the capacity of the gastrointestinal tract to acquire, process and absorb energy. The peripheral limitation hypothesis in contrast suggested that the limits

on SusEI were imposed by the expenditure capacities of the energy-consuming organs, such as skeletal muscle during physical exercise, brown adipose tissue and muscles during cold exposure and the mammary glands during lactation (Hammond and Diamond, 1997; Speakman and Krol, 2005; Speakman, 2007; Speakman, 2008).

Lactation is the most energetically demanding period encountered by small mammals (Hammond and Diamond, 1997; Johnson et al., 2001a; Johnson et al., 2001b; Thompson and Nicol, 2002; Speakman and Krol, 2005; Speakman, 2007). Hammond and Diamond (Hammond and Diamond, 1997) calculated the sustained metabolic scope (SusMS, the ratio SusEI/resting metabolic rate, RMR) of laboratory mice and found that maximal SusMS was 3.6 for physical exercise, 4.8 for heat production during cold exposure, and 6.5 for lactation. This suggests that lactation is an excellent model for the study of factors limiting SusEI (Hammond and Diamond, 1997). When litter sizes were manipulated at birth, both Swiss Webster mice and MF1 mice raising additional offspring were not able to further increase food intake to match the greater number of pups, showing that SusEI was limited, but it was not possible to determine whether the limitation was acting centrally or peripherally (Hammond and Diamond, 1992; Johnson et al., 2001a). Other studies showed that both house mice (*Mus domesticus*) and deer mice (*Peromyscus maniculatus*) were not capable of increasing food intake to meet the combined energy demands of lactation and wheel-running activity, suggesting that limits to SusEI might be set

centrally (Perrigo, 1987). However, inconsistent with the central limitation hypothesis, food intake in MF1 mice fed a low-energy diet during the lactation period exceeded that observed in lactating mice fed normal diet (Speakman et al., 2001). Moreover, when exposed to cold many lactating rodents, including Swiss Webster mice (Hammond et al., 1994), cotton rats (*Sigmodon hispidus*) (Rogowitz, 1998), deer mice (Hammond and Kristan, 2000), MF1 mice (Johnson and Speakman, 2001) and Brandt's voles (Zhang and Wang, 2007), elevate their food intake above the asymptotic food intake of lactating subjects at 21°C, indicating that central factors were not limiting.

In the light of the above studies, it would be expected that limits to SusEI might be imposed peripherally by the capacity of mammary glands to produce milk. Rogowitz (Rogowitz, 1998) found that milk production did not differ significantly between lactating cotton rats at 8 and 21°C, providing support for the peripheral limitation hypothesis. However, Johnson and Speakman (Johnson and Speakman, 2001) and Krol and Speakman (Krol and Speakman, 2003b) working on MF1 mice found that there were significant differences in milk production across different ambient temperatures, with more milk being produced at lower temperatures (Johnson and Speakman, 2001; Krol and Speakman, 2003a; Krol and Speakman, 2003b; Krol et al., 2003). This result was inconsistent with the peripheral limitation hypothesis.

To explain these data it was suggested that the capacity of lactating animals to dissipate heat might impose a limit on SusEI (Krol and Speakman, 2003a; Krol and Speakman, 2003b; Speakman and Krol, 2005). This hypothesis could explain why animals at 21°C were not able to increase food intake to meet the increased energy demands imposed by raising larger litter sizes, to meet the combined energy demands of lactation and concurrent pregnancy, or a combination of locomotive activity and lactation (Perrigo, 1987; Hammond and Diamond, 1992; Koiter et al., 1999; Johnson et al., 2001a; Johnson et al., 2001b; Johnson et al., 2001c; Krol and Speakman, 2003a; Krol and Speakman, 2003b; Zhang et al., 2008). The heat dissipation limitation hypothesis also explained why lactating mice exposed to cold were able to increase food intake and milk production as in these cold conditions the limits on heat dissipation capacity were relaxed (Hammond et al., 1994; Rogowitz, 1998; Hammond and Kristan, 2000; Speakman et al., 2001; Zhang and Wang, 2007). When lactating MF1 mice were maintained at thermoneutrality (30°C), they ate less food and exported less energy as milk, and consequently raised lighter litters than mice of the same strain maintained at 21 or 8°C (Johnson et al., 2001a; Johnson and Speakman, 2001; Krol and Speakman, 2003a; Krol and Speakman, 2003b). These studies were all consistent with the heat dissipation limitation hypothesis. Moreover, Krol et al. (Krol et al., 2007) found that shaving off the dorsal fur of lactating MF1 mice elevated food intake beyond the asymptotic food intake of controls, and increased energy output as milk, leading to heavier litters. This manipulation provides very strong support for the heat dissipation limitation hypothesis (Krol et al., 2007).

Despite the large amount of data explained by the heat dissipation limitation hypothesis some data remain unexplained. In particular, Hammond and colleagues (Hammond et al., 1996) manipulated Swiss mice by surgically removing some mammary tissue. They found that such mice were unable to upregulate the milk production of the remaining tissue which would be predicted from the heat dissipation limitation hypothesis. These data are consequently more consistent with the peripheral limitation idea that SusEI is defined by the summed metabolic demands of contributing metabolic processes. The reasons for this difference between MF1 and Swiss

mouse strains remain uncertain. In the present study we aimed to repeat the shaving experiment performed by Krol and colleagues (Krol et al., 2007), but instead used Swiss mice to establish whether this strain is also limited by heat dissipation capacity.

Because the thermal conductivity of the insulating surface (subcutaneous fat, skin and fur) is one of most important factors affecting heat flow between an animal and its environment, fur removal is an effective means for examining the heat dissipation limitation hypothesis (Conley and Porter, 1980; Krol et al., 2007). The key benefit of this approach compared with simply changing the ambient temperature is that ambient temperature affects both mother and pups; hence any effect on the mother could be mediated by changes in pup demands at the altered ambient temperature. In contrast, shaving affects the heat dissipation ability of the mother independent of her pups and thus provides a more robust test of the hypothesis. According to the heat dissipation hypothesis, the high metabolic demands of digestion, milk production and suckling may lead to hyperthermia. Thus, the females would be forced to disrupt suckling, which might lead to a reduction in suckling stimulus, and ultimately a lower milk production (Speakman and Krol, 2005). It has been found that rats with their ventral fur removed spend more time in the nest than unshaved individuals (Croskerry et al., 1978). We explored these ideas by also monitoring the behaviour over time of shaved and unshaved Swiss mice during late lactation.

The absence of an effect of shaving might arise from a lack of impact on the capacity of the animals to dissipate heat, e.g. if insufficient fur was removed or if the animal compensated by redistributing subcutaneous fat. To eliminate these possibilities we measured the impact of dorsal fur removal directly on thermal conductivity and noradrenaline (NA)-induced body temperature changes. During lactation we examined the variation in food intake, maternal body mass, litter size and litter mass of shaved mice and non-shaved controls. Finally, behaviours including general activity, grooming, feeding, resting and suckling were recorded in both shaved and non-shaved mice at peak lactation. It was predicted that fur removal would lead to a significant increase in thermal conductivity. If the heat dissipation limitation hypothesis was correct for these mice this manipulation would lead to increases in food intake matched by increases in litter masses of the shaved mice compared with unshaved controls. Compared with control mice, shaved mice were also expected to spend more time in feeding behaviour and suckling behaviour, and less time in general physical activity.

MATERIALS AND METHODS
Animals and experimental protocol

Virgin female Swiss mice, 8–9 weeks old, were obtained from a laboratory colony from the Experimental Animal Centre of Shandong University, and were housed in groups of 10 on a 12 h L:12 h D photoperiod cycle with lights on at 07:00 h and an environmental temperature of 23±1°C. Standard rodent chow (produced by the Animal Centre of Shandong Province) and water were available *ad libitum*. After a 1 week adaptation, the mice were housed individually in plastic cages (29 cm×18 cm×16 cm) with fresh sawdust bedding.

Experiment 1

To examine the time course of body temperature change in shaved mice after NA-induced maximal thermogenesis, 12 females were assigned randomly into either a control group (N=6) or a shaved group (N=6) in which the mice were shaved dorsally (mass of shaved fur, 0.406±0.064 g). One week later, both controls and

shaved mice were injected dorsally subcutaneously with NA. The dose of NA was mass dependent and calculated according to Heldmaier et al. (Heldmaier et al., 1982). Prior to NA injection and at 10 min intervals following the injection, rectal body temperature was measured using a digital mouse thermometer (produced by the Beijing Normal University). The probe of the thermometer was inserted 3 cm into the rectum and a reading was taken after 1 min.

Experiment 2

To confirm the effect of fur removal on thermal conductance, eight female Swiss mice were shaved dorsally (mass of shaved fur, 0.408±0.021 g). RMR was measured prior to fur removal (as a baseline) and on day 7 after fur shaving, using a closed-circuit respirometer as described previously (Gorecki, 1975; Wang et al., 2000; Zhao and Wang, 2005; Zhao and Wang, 2007). Briefly, single mice were placed in a 3.6-l metabolic chamber after 4 h of food deprivation. The chamber temperature was controlled within ±0.5°C by a water bath. Water and carbon dioxide in the chamber were absorbed using silica gel and KOH, respectively. After 1 h stabilization, oxygen consumption of the animals was determined at a thermoneutral temperature of 30±0.5°C (Speakman and Rossi, 1999) for a further 1 h at 5 min intervals. Two continuous stable minimum recordings were taken to calculate minimal oxygen consumption which was defined as RMR. Before and after each measurement, body mass and rectal temperature (hereafter referred to as body temperature) were determined. RMR was finally corrected to standard temperature and pressure (STP) conditions. All measurements were carried out between 09:00 h and 12:00 h. Thermal conductance was calculated according to the following equation (McNab, 1980; Zhan and Wang, 2004):

$$C = RMR / (T_b - T_a) , \qquad (1)$$

where C (ml O_2 h^{-1} °C^{-1}) is thermal conductance including the heat dissipation caused by water evaporation; RMR (ml O_2 h^{-1}) is the resting metabolic rate at 30±0.5°C; T_b (°C) is the body temperature; and T_a (°C) is the ambient temperature.

Experiment 3

Sixty-five individually housed female Swiss mice aged 10–11 weeks were paired with males for 11 days. Pregnancy was detected by an increase in body mass and food intake over the following 7 days. After parturition (day 0 of lactation), litter size and mass were measured on a daily basis, except for days 1–2 of lactation. Based on litter size on day 6, 50 lactating mice were separated at random into either a non-shaved lactating group (NL, N=25) or shaved lactating group (SL, N=25). In the SL group the mice were shaved dorsally on day 7 of lactation. The removed fur was weighed. All pups were weaned on day 17 of lactation.

Body mass and food intake

The females were weighed during pregnancy and on days 3–17 of lactation. Food intake was also measured in both pregnancy and throughout lactation. Previous studies have suggested that spillage of diet mixed with the bedding was less than 2% and thus was considered negligible (Johnson et al., 2001a); food intake was therefore calculated as the mass of food missing from the hopper every day (Johnson et al., 2001a; Johnson et al., 2001b). There was no significant difference in food intake on days 11–15 of lactation by repeated measurements, so the asymptotic food intake during the peak lactation was calculated as the mean daily food intake over this period.

Behavioural observation

On days 14–16 of lactation, observations were made on 40 lactating females (NL, N=20; SL, N=20) with their litters by two observers over a total period of 8 h at two points in the day: 05:00–09:00 h and 17:00–21:00 h. A low-power (30 W) red light was used in the dark observation. The observation of each mouse was made for a period of 30 s in sequence, and the dominant behaviour over the 30 s interval was classed into one of four categories: general activity, grooming, feeding and resting behaviour, as described previously (Speakman and Rossi, 1999; Speakman et al., 2001). The general activity involved rearing up, climbing on the cage bars and walking around the cage. Grooming behaviour included self-grooming and grooming their pups. Both eating food and drinking water were referred to as feeding behaviour. Resting behaviour included being inactive wherever they were. In addition, during the period of grooming and resting, if the mouse suckled pups the dominant behaviour was also generally defined as suckling behaviour. A series of 20 cages were observed over the 8 h for 10 min periods. Thus, in total, each mouse was observed 48 times and the percentage of time spent in each behaviour was defined as a percentage of each behaviour across the 8 h (% each behaviour).

Statistical analysis

All values are expressed as means ± s.e.m. and analysed using SPSS 13.0 statistical software. The level of significance was set at P<0.05. Experiment 1, the variations in T_b over the 80 min measurements, was analysed using repeated measures ANOVA. Significant differences between days were detected using multiple *post hoc* comparisons by the least significant difference (LSD) method. Differences between control and shaved groups on any day points were then investigated using independent-sample *t*-tests. Experiment 2, body mass, T_b, RMR and thermal conductance between shaved and unshaved mice, was analysed using paired-samples *t*-tests. Experiment 3, the percentage of time spent in each behaviour, and body mass, food intake, litter mass and size on any day over the period of lactation, was analysed using independent-sample *t*-tests. For percentage data, arcsine square-root transformation was performed prior to analysis to normalize the data. The fluctuations of body mass, food intake, litter mass and size during lactation were analysed using repeated measures ANOVA; where appropriate, multiple *post hoc* comparisons were performed using the LSD method. The asymptotic food intake at peak lactation was defined as the period (days 11–15) during which no significant difference in food intake between days was detected using repeated measures (Speakman et al., 2001). Pearson correlation analysis was used to detect possible correlation of litter mass with litter size or maternal body mass, asymptotic food intake with litter mass, litter size or maternal body mass, percentage of feeding behaviour with activity, grooming, suckling or resting behaviour, and percentage of suckling behaviour with activity, resting behaviour or litter mass. The regression lines were compared using analysis of covariance (ANCOVA) (Krol and Speakman, 2003a).

RESULTS
Experiment 1

Prior to NA injection, T_b averaged 36.7±0.2°C and 36.3±0.1°C in control and shaved mice, respectively (t_{10}=1.972, P=0.08) (Fig. 1). After NA injection, T_b of control mice increased during the first 30 min period, reaching a maximum of 37.4±0.1°C at 30 min, and thereafter declined (repeated measures ANOVA, $F_{8,40}$=10.044, P=0.00). The T_b of controls was 36.5±0.1°C at 80 min which was similar to that before NA injection. The shaved mice exhibited a similar time course
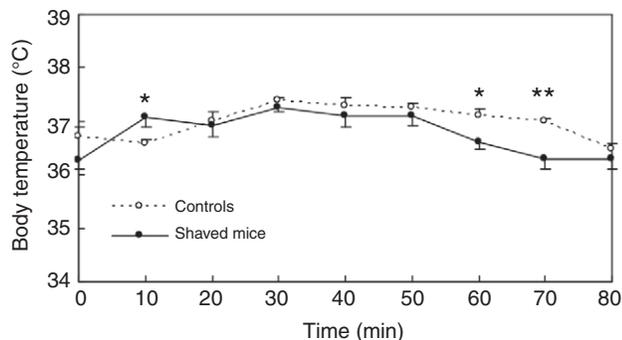
Fig. 1. Change of body temperature of controls and dorsally shaved Swiss mice after subcutaneous injection of noradrenaline (NA). Compared with controls, the body temperature of shaved mice was significantly higher at 10 min during the increasing phase and lower at 60 and 70 min over the decreasing phase (10 min, t_{10}=2.433, P=0.04; 60 min, t_{10}=2.611, P=0.03; 70 min, t_{10}=3.433, P=0.006). Values are means ± s.e.m. *P<0.05; **P<0.01.

of fluctuation in T_b following NA injection (repeated measures ANOVA, $F_{8,40}$=16.750, P=0.00), in which the highest value averaged 37.3±0.1°C at 30 min and the lowest averaged 36.3±0.2°C 80 min after NA injection. For both controls and shaved mice, the change of T_b showed an increasing phase from 0 to 30 min and a decreasing phase from 30 min to the end of the experiment. During the increasing phase, T_b was significantly higher in the shaved group than in controls at 10 min (t_{10}=2.433, P=0.04). There was no group difference at 30 min (t_{10}=1.034, P=0.33). However, shaved mice showed a lower T_b than controls at 60 and 70 min (60 min, controls 37.1±0.1°C, shaved mice 36.6±0.1°C, t_{10}=2.611, P=0.03; 70 min, controls 37.0±0.1°C, shaved mice 36.3±0.2°C, t_{10}=3.433, P=0.006). Finally, no difference was found at the end of experiment (80 min, t_{10}=0.714, P=0.49) (Fig. 1). Overall these data show that shaved mice cooled down more rapidly following NA injection, consistent with an elevated heat dissipation capacity due to shaving.

Experiment 2
T_b before the RMR measurement was lower in shaved mice than the baseline (Table 1), whereas no group difference was found in T_b after RMR measurement. The mean T_b was also lower in shaved mice compared with the baseline. RMR increased significantly by 18.6% 1 week after fur shaving than the baseline. Thermal conductance was 16.4% higher in shaved mice (Table 1).

Experiment 3
Body mass
Both the NL and SL groups showed significant increases in maternal body mass during late pregnancy (days –5 to –1, repeated measures ANOVA; NL, $F_{4,96}$=410.739, P<0.001; SL, $F_{4,96}$=409.588, P<0.001) (Fig. 2A). However, neither NL nor SL mice showed

significant fluctuations in body mass throughout lactation (days 3 to 17, repeated measures ANOVA; NL, $F_{14,336}$=0.730, P=0.74; SL, $F_{14,336}$=1.510, P=0.11). Maternal body mass did not differ significantly between the NL and SL group on any day during late pregnancy and throughout lactation (day –1, NL 68.85±1.28 g, SL 67.29±1.52 g, t_{48}=0.787, P=0.44; day 6, NL 50.39±0.82 g, SL 49.64±1.01 g, t_{48}=0.580, P=0.57; day 17, NL 49.49±0.78 g, SL 48.94±1.03 g, t_{48}=0.418, P=0.68) (Fig. 2A).

Food intake
There was no difference in food intake between the NL and SL group during pregnancy (day –5, NL 8.81±0.15 g day^{-1}, SL 8.87±0.25 g day^{-1}, t_{48}=0.186, P=0.84) (Fig. 2B). Food intake increased significantly by 155% in the NL group from 8.43±0.48 g day^{-1} on day 0 to 21.52±0.55 g day^{-1} on day 17 (repeated measures ANOVA, $F_{17,408}$=370.052, P<0.001). The SL group also showed an increase in food intake over the lactation period, in which the maximum intake averaged 23.64±0.70 g day^{-1} on day 11 of lactation, 162% higher than that on day 0 (9.01±0.37 g day^{-1}; days 0–17, repeated measures ANOVA, $F_{17,408}$=74.342, P<0.001). No significant difference was found in food intake between the NL and SL group prior to shaving (day 6, NL 20.31±0.51 g day^{-1}, SL 20.81±0.45 g day^{-1}, t_{48}=0.738, P=0.46). After shaving, food intake was 9%, 11%, 12% and 9% higher in the SL group than in the NL group on days 11, 13, 15 and 17 of lactation (day 11, t_{48}=2.410, P=0.02; day 13, t_{48}=2.400, P=0.02; day 15, t_{48}=2.361, P=0.02; day 17, t_{48}=2.182, P=0.03). On days 11–15 of lactation, both NL and SL mice showed relatively stable food intake (day 11–15, repeated measures ANOVA, NL, $F_{4,96}$=1.63, P=0.17; SL, $F_{4,96}$=1.14, P=0.35). So, the asymptotic food intake was calculated based on daily food intake across these days, which averaged 21.51±0.44 g day^{-1} in the NL group and 23.00±0.51 g day^{-1} in the SL group. SL mice had a 7% higher asymptotic food intake than NL mice (NL, t_{48}=2.22, P=0.03).

Litter mass and size
On day 3 of lactation, litter mass averaged 29.74±1.26 g and 29.66±1.21 g in the NL and SL group, respectively (t_{48}=0.046, P=0.90) (Fig. 3A). Before fur shaving, there was no difference in litter mass between the NL and SL group (day 6, NL 44.04±1.70 g, SL 45.76±1.75 g, t_{48}=0.704, P=0.46). Both NL and SL mice increased their litter mass significantly throughout lactation (repeated measures ANOVA; NL, $F_{14,336}$=479.684, P<0.001; SL, $F_{14,336}$=310.497, P<0.001), but no significant difference was found between the two groups on any day (e.g. day 17, NL, 84.34±2.60 g; SL, 90.24±2.64 g, t_{48}=1.592, P=0.68) (Fig. 3A). Similarly, throughout the lactation mean pup mass of both the NL and SL group increased significantly from 2.79±0.07 g and 2.81±0.07 g on day 3 to 8.69±0.26 g and 9.09±0.33 g on day 17 of lactation, respectively (repeated measures ANOVA, NL, $F_{14,336}$=439.271, P<0.001; SL, $F_{14,336}$=259.090, P<0.001) (Fig. 3B). There was also

Table 1. Effect of fur removal on RMR and thermal conductance in female Swiss mice

	Baseline (N=8)	Fur removed (N=8)	t	P
Body mass (g)	36.3±1.5	36.8±1.4	1.774	0.12
T_b before RMR measurement (°C)	36.8±0.2	36.3±0.1	3.401	0.02
T_b after RMR measurement (°C)	38.5±0.1	38.3±0.1	1.317	0.21
Mean T_b (°C)	37.7±0.1	37.3±0.1	2.667	0.03
RMR (ml O_2 h^{-1})	84.7±4.3	100.5±4.5	5.382	0.00
Thermal conductance (ml O_2 h^{-1} °C^{-1})	10.8±0.6	12.5±0.5	4.400	0.00

T_b, body temperature; RMR, resting metabolic rate; data presented as means ± s.e.m.
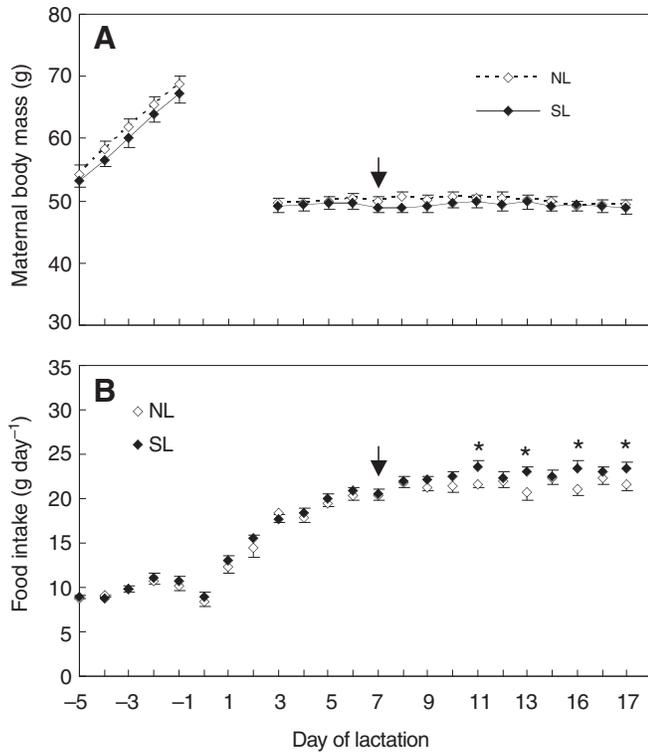
Fig. 2. Effect of fur removal on maternal body mass (A) and food intake (B) in lactating Swiss mice. Food intake increased significantly in both the non-shaved lactating (NL) group and shaved lactating (SL) group throughout lactation (days 0 to 17, repeated measures ANOVA; NL, $F_{17,408}=370.052$, $P<0.001$; SL, $F_{17,408}=74.342$, $P<0.001$), in which SL mice had higher food intake compared with NL mice on days 11, 13, 15 and 17 of lactation (day 11, $t_{48}=2.410$, $P=0.02$; day 13, $t_{48}=2.400$, $P=0.02$; day 15, $t_{48}=2.361$, $P=0.02$; day 17, $t_{48}=2.182$, $P=0.03$). Arrow, SL females were dorsally shaved on day 7 of lactation. Values are means ± s.e.m. *$P<0.05$.
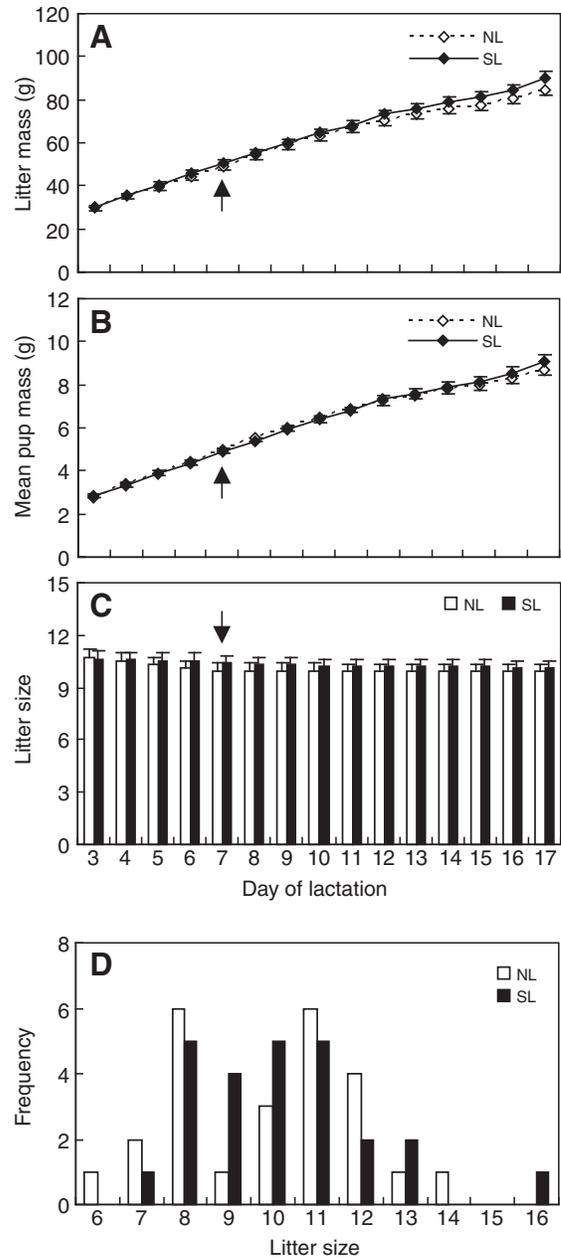

Fig. 3. Effect of fur removal on litter mass (A), mean pup mass (B) and litter size (C) in lactating Swiss mice, and histogram of the litter sizes at weaning of NL and SL mice (D). No significant difference was found in litter mass, litter size and mean pup mass between the NL and SL group throughout lactation. Arrow, SL females were dorsally shaved on day 7 of lactation. Values are means ± s.e.m.

no significant difference in mean pup mass between the NL and SL group at any day points over the lactation period (day 3, $t_{48}=0.140$, $P=0.89$; day 17, $t_{48}=0.947$, $P=0.35$) (Fig. 3B).

On day 3 of lactation, litter size averaged 10.7±0.4 (range 6–14) in the NL group and 10.6±0.4 (range 7–16) in the SL group ($t_{48}=0.133$, $P=0.90$). The SL mice showed the same litter size as the NL mice prior to fur removal (day 6, NL 10.1±0.4, SL 10.6±0.4, $t_{48}=0.753$, $P=0.46$) (Fig. 3C). On day 17 of lactation, litter size averaged 9.9±0.4 (range 6–14) (Fig. 3D) in NL mice, which did not differ from the litter size of 10.2±0.4 (range 7–16) (Fig. 3D) in SL mice ($t_{48}=0.410$, $P=0.68$) (Fig. 3C). In addition, neither the NL nor SL group showed notable changes in litter size distribution over the lactation period. For NL females, the minimum litter size was 6 pups and the maximum was 14 pups at weaning, and mothers with litter sizes of 8–11 pups accounted for 64% of a total of 25 NL females (16/25). SL females had a similar litter size distribution to NL females, with the smallest litter size being 7 and the largest 16, and mothers raising 8–11 pups accounting for 76% of the 25 SL females (19/25) (Fig. 3D).

On day 17 of lactation, there was a positive correlation between maternal body mass and litter mass in the SL mice (SL, $y=1.33x+24.93$, $R^2=0.272$, $P<0.01$) (Fig. 4A), but not in the NL mice (NL, $R^2=0.075$, $P=0.18$). There was a significant difference between slopes (ANCOVA, $F_{2,47}=6.419$, $P=0.003$) but not between intercepts (ANCOVA, $F_{1,48}=2.534$, $P=0.12$). Litter size was positively correlated with litter mass in both NL and SL groups (day 17, NL,

$y=4.63x+38.39$, $R^2=0.562$, $P<0.001$; SL, $y=3.24x+57.34$, $R^2=0.249$, $P<0.01$) (Fig. 4B). A steeper slope of the regression line was found for NL mice than SL mice (ANCOVA, $F_{2,47}=16.489$, $P<0.001$). The asymptotic food intake was correlated with maternal body mass in both NL and SL groups (NL, $y=0.25x+9.14$, $R^2=0.199$, $P=0.03$; SL, $y=0.20x+13.06$, $R^2=0.172$, $P=0.04$) (Fig. 5A), in which there were significant differences between slopes (ANCOVA, $F_{2,47}=8.039$, $P=0.001$) and intercepts (ANCOVA, $F_{1,48}=4.940$, $P=0.03$). There was a significant correlation between asymptotic food intake and litter mass on day 17 of lactation in both NL and SL mice (NL, $y=0.11x+12.13$, $R^2=0.437$, $P<0.001$; SL, $y=0.14x+10.02$, $R^2=0.565$,
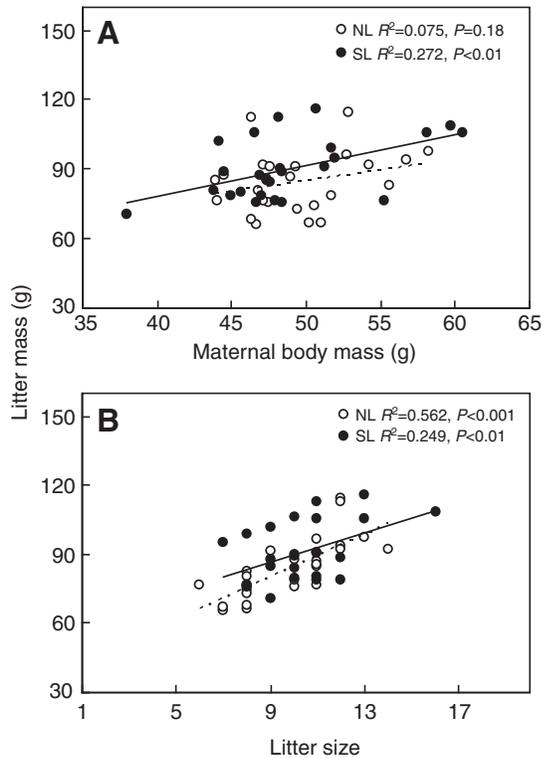
Fig. 4. Relationship between litter mass and maternal body mass (A) and litter size (B) in lactating Swiss mice. On day 17 of lactation, there was a positive correlation between maternal body mass and litter mass in SL mice (y=1.33x+24.93), but not in NL mice. Litter size was positively related to litter mass in both the NL and SL group (NL, y=4.63x+38.39; SL, y=3.24x+57.34). Values are means ± s.e.m.
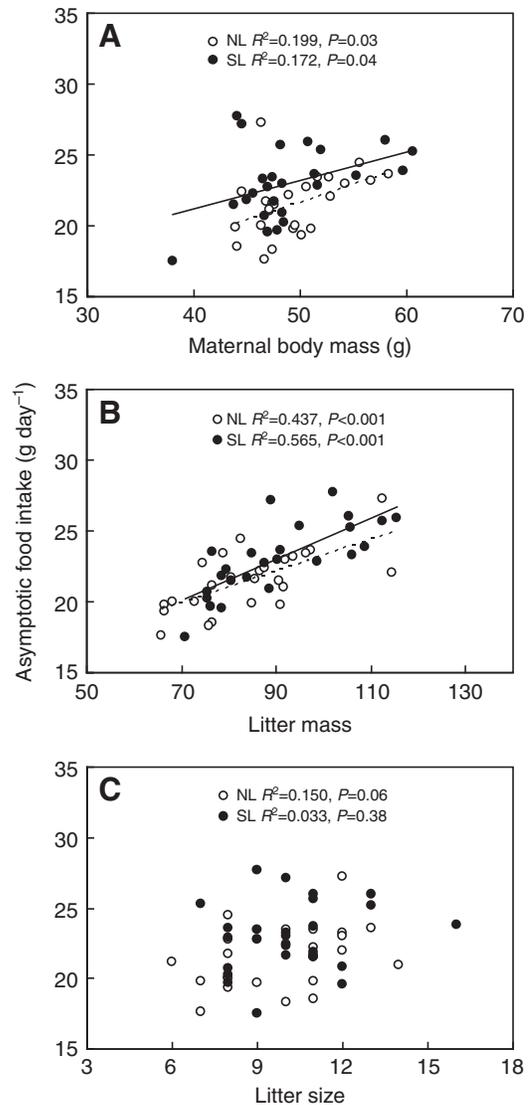

Fig. 5. Relationship between asymptotic food intake (mean food intake on days 11–15 of lactation) and maternal body mass (A), litter mass (B) and litter size (C) in lactating Swiss mice. The asymptotic food intake was positively correlated with maternal body mass on day 17 of lactation in both the NL and SL group (NL y=0.25x+9.14, SL y=0.20x+13.06, A). A correlation was also found between asymptotic food intake and litter mass (NL y=0.11x+12.13, SL y=0.14x+10.02, B). Values are means ± s.e.m.

$P<0.001$; no group effect, ANCONA, $F_{1,47}$=1.872, $P>0.05$) (Fig. 5B), in which SL mice showed a significantly steeper slope than NL mice (ANCOVA, $F_{2,47}$=28.943, $P<0.001$). However, the relationship between asymptotic food intake and litter size did not reach a significant level in either the NL or SL group (NL, R^2=0.150, P=0.06; SL, R^2=0.033, P=0.38) (Fig. 5C).

Behavioural observation

Over the 8h observation period, the percentage of time spent in general activity averaged 9.5±1.5% and 7.9±1.2% in NL and SL groups, respectively (t_{38}=0.826, P=0.41) (Fig. 6). The percentage of grooming behaviour also did not show any significant difference between the NL and SL group (NL 11.3±1.7%, SL 8.8±1.2%, t_{38}=1.200, P=0.24). Neither the percentage of suckling nor the percentage of resting behaviour differed significantly between NL and SL mice (suckling, NL 40.3±2.9%, SL 44.9±2.7%, t_{38}=1.157, P=0.25; resting, NL 50.0±3.2%, SL 46.3±2.3%, t_{38}=0.958, P=0.34). The percentage of feeding behaviour, however, was significantly higher in the SL group than in the NL group (NL 29.2±2.5%, SL 37.1±2.6%, t_{38}=2.149, P=0.04) (Fig. 6).

The percentage of time spent in general activity was not correlated with the time spent feeding (Fig. 7A). There was a negative correlation between the time spent in grooming and feeding behaviour in the SL group (y=−0.25x+17.84, R^2=0.282, P=0.02), but not in the NL group (R^2=0.041, P=0.33) (Fig. 7B). Moreover, the percentage of time spent feeding was significantly correlated with the time spent in resting behaviour in both the NL

(y=−0.78x+72.77, R^2=0.387, $P<0.001$) and SL groups (y=−0.64x+70.04, R^2=0.531, $P<0.001$) (Fig. 7C). There were significant differences between slopes (ANCOVA, $F_{2,47}$=18.959, $P<0.001$) but not between intercepts (ANCOVA, $F_{1,48}$=0.919, P=0.34). Significant correlation was also found between feeding and suckling behaviour in the SL group (y=−0.49x+62.92, R^2=0.221, P=0.02), but not in the NL group (R^2=0.141, P=0.06) (Fig. 7D). SL mice showed a significantly steeper slope than NL mice (ANCOVA, $F_{2,47}$=5.314, P=0.008) whereas no significant difference was found in the intercept for the regression on feeding and suckling behaviour (ANCOVA, $F_{1,48}$=1.339, P=0.25).

The percentage of time spent in suckling behaviour was negatively correlated with the time spent in general activity in the NL group (y=−0.87x+48.63, R^2=0.198, P=0.03) (Fig. 8A), but not in the SL group (R^2=0.120, P=0.09). A significantly positive correlation
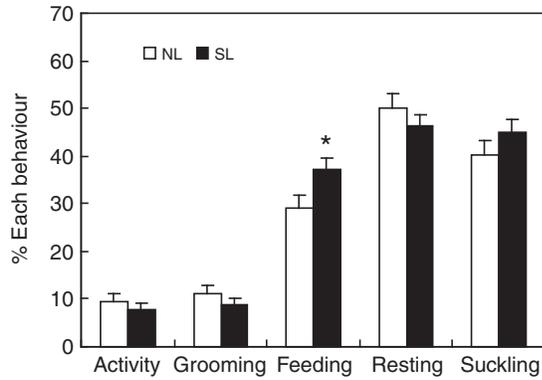
Fig. 6. The percentage of time spent in general activity and grooming, feeding, suckling and resting behaviour in lactating Swiss mice over the 8 h observation on days 1416 of lactation. The percentage of time spent in feeding behaviour was significantly higher in the SL group compared with the NL group (t_{38}=2.149, P=0.04). Values are means ± s.e.m. *P<0.05.

between suckling and resting behaviour was found in both the NL and SL groups (NL, y=0.64x+8.20, R^2=0.483, P<0.001; SL, y=0.82x+7.17, R^2=0.483, P<0.001) (Fig. 8B), with SL mice showing a significantly steeper slope than NL mice (ANCOVA, $F_{2,47}$=23.241, P<0.001). However, neither NL nor SL mice showed a significant correlation between the percentage of time spent in suckling behaviour and litter mass (NL, R^2=0.060, P=0.24; SL, R^2=0.063, P=0.23) (Fig. 8C).

DISCUSSION

The capacity to dissipate heat mainly depends on the thermal conductivity of the insulating surface (e.g. subcutaneous fat, skin and fur) and the temperature difference between an animal and its environment (Conley and Porter, 1980; Krol et al., 2007). As expected, the present study shows that the NA-induced rise in body temperature decreased faster in shaved mice than in non-shaved controls, because of the higher thermal conductance caused by dorsal fur removal. The fact that thermal conductance was 13.6% higher in shaved mice than in non-shaved individuals and the fact that they cooled faster following NA administration demonstrates that the experimental fur removal was sufficient to increase the ability of the mice to dissipate heat.

In the present study, there was no significant effect of fur removal on maternal body mass over the lactation period in Swiss mice, consistent with the data reported previously in MF1 mice (Krol et al., 2007). The asymptotic food intake was 7% higher in shaved lactating mice, which was similar to an approximately 18% increase in RMR and 16% increase in thermal conductance for shaved mice. In addition, shaved MF1 mice assimilated more energy in late lactation than non-shaved individuals (Krol et al., 2007). Similar results have also been reported in non-reproductive animals, such as harvest mice (*Reithrodontomys megalotis*) (Pearson, 1960) and Siberian hamsters (*Phodopus sungorus*) (Kauffman et al., 2001; Kauffman et al., 2003). This increase in food intake is inconsistent with the central limitation hypothesis that limits on SusEI are imposed centrally by the capacity of the gastrointestinal tract to process food. The data are, however, consistent with either the peripheral limitation hypothesis or the idea that limits on SusEI might be imposed by the ability to dissipate heat.

The energy demands of animals during lactation vary with litter size, as litter mass is related to litter size in many rodent species,
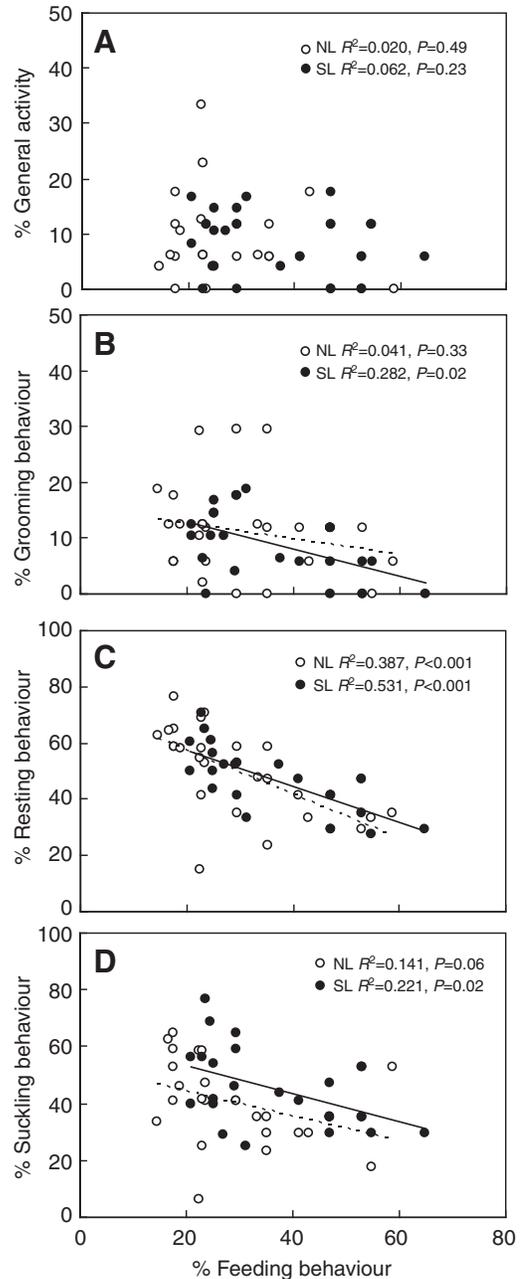

Fig. 7. Relationship between the percentage of time spent feeding and the percentage of time spent in general activity (A) and grooming (B), resting (C) and suckling (D) behaviour over the 8 h observation on days 14–16 of lactation in Swiss mice. There was a negative correlation between the percentage of time spent in grooming behaviour and the percentage of time spent in feeding behaviour in the SL group (y=–0.25x+17.84) but not in NL group. The percentage of time spent in feeding behaviour was negatively correlated with resting behaviour in both NL and SL mice (NL y=–0.78x+72.77, SL y=–0.64x+70.04). Moreover, a correlation was found between the percentage of time spent in feeding and suckling behaviour in the SL group (y=–0.4853x+62.92), but not in the NL group. Values are means ± s.e.m.

e.g. MF1 females decrease their asymptotic daily food intake during late lactation in relation to the extent of the reduction in litter size but cannot increase their food intake to match their new litter size (Johnson et al., 2001a; Speakman, 2007; Speakman, 2008; Hammond and Diamond, 1992; Zhang et al., 2008; Krol and
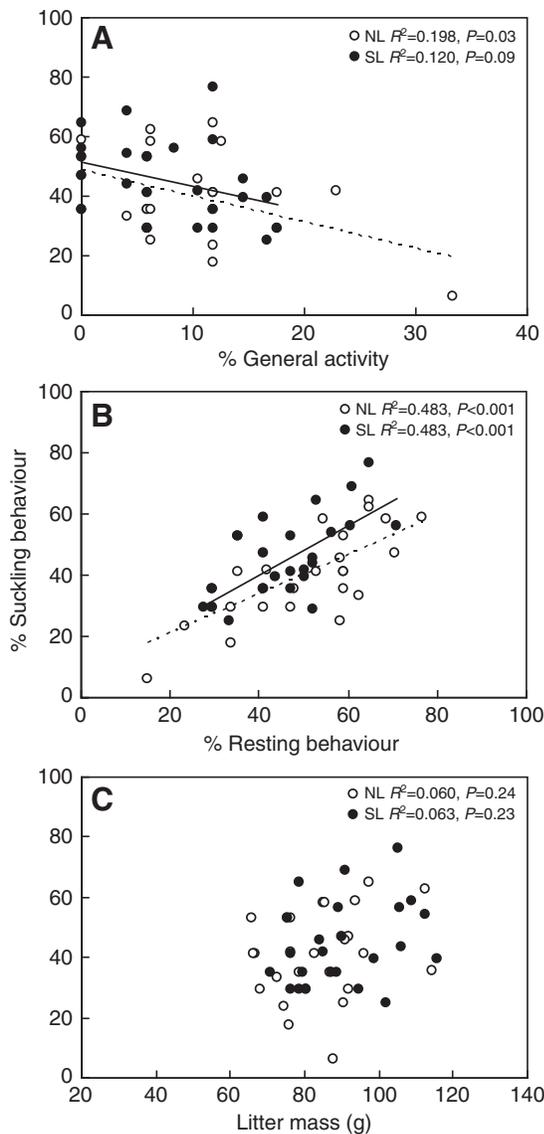
Fig. 8. Relationship between the percentage of time spent in suckling behaviour and general activity (A), resting behaviour (B) and litter mass (C) in lactating Swiss mice. The percentage of time spent in suckling behaviour was negatively correlated with general activity in the NL group ($y=-0.87x+48.63$), but not in the SL group. Positive correlations between the percentage of suckling behaviour and resting behaviour were found in both the NL and SL group (NL $y=0.64x+8.20$, SL $y=0.82x+7.17$). Values are means ± s.e.m.

Speakman, 2003a). Such a relationship between litter size and mass was also found in Swiss mice in this study. However, the present data did not show significant differences in litter size and mass between shaved mice and control individuals. In contrast, shaved female MF1 mice exported on average 15.2% more energy as milk at peak lactation than non-shaved controls (Krol et al., 2007). The litters produced by shaved MF1 mice were consequently 15.4% heavier than the litters weaned by non-shaved mothers (Krol et al., 2007). Swiss mice and MF1 mice therefore appear to respond differently to the shaving manipulation. If different amounts of fur were removed for the two studies, this might explain the contrasting results. When MF1 mice were dorsally shaved, approximately 0.18–0.30 g of fur was removed on day 6 of lactation. These mice

were re-shaved on days 10 and 14 of lactation (see Krol et al., 2007). For Swiss mice, the fur removed averaged 0.34 g (ranging from 0.26 to 0.39 g) on day 7 of lactation. Consequently there was no notable difference in fur removal between the two mouse strains.

A potential mechanism linking heat dissipation capacity to lactation performance would be suckling behaviour. Maternal mice might be forced to disrupt suckling to prevent development of hyperthermia in the nest. A decreased suckling stimulus caused by continual disruption of suckling would lead to lower oxytocin, and ultimately a lower level of milk production (Croskerry et al., 1978; Speakman and Krol, 2005). Consistent with the heat dissipation limitation hypothesis, over the lactation period, maternal rats with fur removed spent more time with their pups in the nest compared with non-shaved individuals (Croskerry et al., 1978; Leon et al., 1985). For Swiss mice, the behaviour at peak lactation was observed and classified into one of five categories: general activity, grooming, feeding, suckling and resting behaviour. Both shaved mice and non-shaved controls showed a large reduction in general activity at peak lactation, which was similar to the behavioural patterns observed in MF1 mice (Speakman et al., 2001) and rats (Slonaker, 1924). The percentage of time spent suckling, however, did not differ significantly between shaved and non-shaved mice, which suggests that there was no effect of fur removal on suckling behaviour at late lactation.

Trade-offs in time distribution might be employed by animals to cope with the high energy demands for lactation. In the present study, for NL mice no significant correlation was found between the percentage of time spent suckling and feeding, whereas SL mice spent more time feeding at the cost of reducing the time spent suckling. Also, for SL mothers feeding was negatively related to resting and grooming, but not to activity. These results might suggest that the greater the energy demand that SL mice experience to meet the energy output associated with raising pups and increases in thermal conductance following shaving, the more time they spend feeding, and the less time they spend suckling, resting and grooming. Moreover, the strong positive correlation between the percentage of time spent suckling and resting indicates that suckling mainly occurred during resting. A negative correlation between suckling behaviour and general activity in non-shaved mice suggests that the mothers spent more time suckling pups at the expense of general activity. Similarly, Speakman and colleagues (Speakman et al., 2001) found that MF1 mice fed a low-energy diet spent more time feeding (approximately 45% of the dark phase and 35% of the light phase), and less in general activity (Speakman et al., 2001). Consistent with these data for MF1 mice, Swiss mice also increased the percentage of time spent feeding when they ate more food in late lactation. Overall the behavioural responses of the two strains during lactation did not appear substantially different.

The contrasting responses of the MF1 mice, which increased milk production in response to shaving (Krol et al., 2007), and the Swiss mice, which did not (this study), may suggest that the relative limits on the capacity of the mammary glands to synthesize milk and the capacity to dissipate heat may be different in the two strains. We suggest that in the MF1 mouse the restriction on mammary secretion may be greater than the limit on heat dissipation. Hence, after these mice were shaved, they increased their milk production and raised heavier litters because they then had the capacity to increase milk production. In contrast, in Swiss mice the maximal capacity of the mammary glands to secrete milk may lie below the heat dissipation limit. Consequently when this strain was shaved they demonstrated a greater heat dissipation capacity and ate more food to compensate, but they could not translate this increased intake into milk production

because their mammary glands were already working at capacity. As anticipated in this situation there was no increase in suckling time and no increase in litter mass. These data therefore support the peripheral limitation hypothesis rather than the heat dissipation hypothesis. This explanation is consistent with the observation that Swiss mice were unable to respond to surgical removal of some of their mammary tissue by elevation of milk production in the remaining tissue (Hammond et al., 1996) which is also consistent with the peripheral limitation hypothesis. Overall these data suggest that the limitations on lactation performance and SusEI may not be the same in all species, or even between different strains of mice. Further work will be necessary to establish whether the heat dissipation limitation hypothesis (as found in MF1 mice) or the peripheral limitation hypothesis (as found in Swiss mice) is the more usual constraint on SusEI.

In summary, in the present study the dorsally shaved Swiss mice had a significantly higher thermal conductance and a faster reduction in body temperature following NA injection. Dorsal fur removal was therefore an effective manipulation to increase heat dissipation capacity. Shaved Swiss mice increased food intake during lactation beyond that observed in non-shaved individuals, indicating that limits to SusEI might be imposed by the capacity to dissipate heat. However, shaved females did not raise heavier litters and did not spend more time suckling than non-shaved controls, which is inconsistent with the expectations of the heat dissipation limitation hypothesis, but consistent with the peripheral limitation hypothesis. There appeared to be a trade-off in the time distribution for general activity, grooming, feeding, resting and suckling behaviour. Shaved mice spent more time feeding and ate more food, to meet the extra demands caused by the experimentally elevated thermal conductance. The contrasting responses of MF1 mice (Krol et al., 2007) and Swiss mice (this study) suggest limits on SusEI imposed by different factors differ between different species or strains, leading to a diversity of findings across studies.

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