RESEARCH ARTICLE

Limits to sustained energy intake. XX. Body temperatures and physical activity of female mice during lactation

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SUMMARY

Lactating animals consume greater amounts of food than non-reproductive animals, but energy intake appears to be limited in late lactation. The heat dissipation limit theory suggests that the food intake of lactating mice is limited by the capacity of the mother to dissipate heat. Lactating mice should therefore have high body temperatures (T_b), and changes in energy intake during lactation should be reflected by variation in T_b. To investigate these predictions, 26 mice (*Mus musculus*) were monitored daily throughout lactation for food intake, body mass, litter size and litter mass. After weaning, 21 days postpartum, maternal food intake and body mass were monitored for another 10 days. Maternal activity and T_b were recorded every minute for 23 h a day using implanted transmitters (vital view). Energy intake increased to a plateau in late lactation (days 13–17). Daily gain in pup mass declined during this same period, suggesting a limit on maternal energy intake. Litter size and litter mass were positively related to maternal energy intake and body mass. Activity levels were constantly low, and mice with the largest increase in energy intake at peak lactation had the lowest activity. T_b rose sharply after parturition and the circadian rhythm became compressed within a small range. T_b during the light period increased considerably (1.1°C higher than in baseline), and lactating mice faced chronic hyperthermia, despite their activity levels in lactation being approximately halved. Average T_b increased in relation to energy intake as lactation progressed, but there was no relationship between litter size or litter mass and the mean T_b at peak lactation. These data are consistent with the heat dissipation limit theory, which suggests performance in late lactation is constrained by the ability to dissipate body heat.

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INTRODUCTION

Lactation is one of the most energy demanding periods in the life cycle of a female mammal (Butte and King, 2005; Speakman, 2008; Wade and Schneider, 1992). Typical energy intake during lactation may be two to four times greater than that of non-reproductive animals (Speakman and McQueenie, 1996; Studier, 1979). There is a large body of work, mainly conducted in small rodents, that suggests that the intake of energy at peak lactation is limited. Energy intake at peak lactation reaches an asymptote (Johnson et al., 2001a; Johnson et al., 2011b; Król et al., 2003; Król et al., 2007; Zhao and Cao, 2009), and several studies have shown that when female mice are presented with artificially enlarged litters they do not elevate their intake to compensate for the increased demands of the offspring (Hammond and Diamond, 1992; Johnson et al., 2001b; Laurien-Kehnen and Trillmich, 2003). This failure to modulate intake in relation to litter size results in offspring that wean at a much reduced body mass compared with those from small litters (natural or artificially manipulated) (Duah et al., 2013; Hammond and Diamond, 1992; Johnson et al., 2001b; Zhao et al., 2013b). Indeed, if litters are made very large, the female may be unable to deliver sufficient resources to ensure their survival (Hammond and Diamond, 1992). Other experimental manipulations have also suggested that the intake of the female at peak lactation is limited.

If females become pregnant while already lactating (Johnson et al., 2001b), are forced to run for their food (Perrigo, 1987; Schubert et al., 2009; Zhao et al., 2013a) or are infected with parasites (Kristan and Hammond, 2000), they do not elevate their total intake to cope with these additional demands, leading to trade-offs in the amount of milk they deliver to their offspring. Mice did elevate the mass of food ingested if they were presented with a low quality food, which had been diluted with cellulose (Speakman et al., 2001), but in this case the net energy intake remained at around the same level as that observed when feeding on more digestible food.

Several hypotheses have been advanced to explain the limitations in food intake at peak lactation. These have included the 'central limitation hypothesis', which suggests that energy budgets are limited by the capacity of the alimentary tract to absorb and process food; the 'metabolic theory of ecology', which suggests that metabolic rates are limited by the geometry of the fractal supply network distributing absorbed resources to their sites of use; the 'peripheral limitation hypothesis', suggesting that total demand is a sum of the demands of individual tissues in the periphery, each working under unique physiological constraints; and the 'heat dissipation limitation (HDL) theory', which suggests that the constraining factor is the capacity to dissipate body heat and risk of hyperthermia (reviewed in Speakman and Król, 2005; Speakman

and Król, 2011; Piersma and van Gils, 2010). Varying the ambient temperature experienced by animals during lactation has repeatedly shown that female animals modulate their intake at peak lactation in relation to the prevailing ambient temperature (Hammond et al., 1994; Jansen and Binard, 1991; Johnson and Speakman, 2001; Hammond and Kristan, 2000; Leon and Woodside, 1983; Morag et al., 1969; Rogowitz, 1998; Wu et al., 2009; Zhang and Wang, 2007). Such data are incompatible with, and hence disprove, the central limitation hypothesis and the metabolic theory of ecology, but are consistent with the two other ideas – the peripheral limitation hypothesis and the HDL theory.

Support for the peripheral limitation hypothesis and the HDL theory has been mixed. Hammond et al. (Hammond et al., 1996) surgically removed some of the mammary tissue of Swiss Webster mice. The rationale for this experiment was that if the mammary tissue sets the limits on intake and it is normally working at capacity, it would not be possible for the remaining tissue to compensate for the tissue that had been surgically removed. In line with the peripheral limitation hypothesis, the tissue remaining after surgery did not elevate its milk production capacity. The peripheral limitation hypothesis also predicts that under different temperature conditions mice would generate constant milk supplies for their offspring because the tissues would always be working at capacity. Supporting this prediction, in lactating cotton rats (*Sigmodon hispidus*), food intake was elevated in the cold, but milk production was not (Rogowitz, 1998). However, in MF1 mice, milk production varied in relation to ambient temperature, being higher in the cold and lower when it was hot, suggesting that the mammary gland did not limit the energy budget (Johnson and Speakman, 2001; Król et al., 2003). This result was therefore more consistent with the HDL theory. This effect was also later demonstrated in Brandt's voles (*Lasiopodomys brandtii*) (Wu et al., 2009) and common voles (*Microtus arvalis*) (Simons et al., 2011). Furthermore, when lactating MF1 mice were shaved to increase their capacity to dissipate heat, they increased food intake and milk production and they weaned larger pups than unshaved mice (Król et al., 2007). In contrast, shaving Swiss mice (Zhao and Cao, 2009; Zhao et al., 2010) and hamsters (*Phodopus sungorus*) (Paul et al., 2010) increased food intake, demonstrating an increase in heat loss, but had no significant effect on milk production or pup growth. In common voles, shaving resulted in significantly increased pup growth (Simons et al., 2011), and the effects on food intake and milk production were in the anticipated direction from the HDL theory, but the latter effects were not statistically significant.

Although support for the HDL theory is not universal, this theory is consistent with several other observations of females at peak lactation. For example, there are sporadic reports that body temperature (T_b) was elevated during lactation in various species, including Norway rats (*Rattus norvegicus*) (Leon et al., 1978), Mongolian gerbils (*Meriones unguiculatus*) (Weinandy and Gattermann, 1995), Sprague-Dawley rats (*Rattus norvegicus*) (Eliason and Fewell, 1997) and dwarf hamsters (*Phodopus campbelli* and *P. sungorus*) (Scribner and Wynne-Edwards, 1994). In addition, lactating rodents reduce wheel-running activity and spontaneous locomotor activity (Scribner and Wynne-Edwards, 1994; Speakman et al., 2001; Weinandy and Gattermann, 1995; Zhao et al., 2013a), and thermogenesis in brown adipose tissue (BAT) is also suppressed (Król et al., 2011; Trayhurn, 1983; Trayhurn and Jennings, 1987). During physical activity, heat production is elevated (Refinetti, 2003; Weinert and Waterhouse, 1998). Reductions in physical activity during lactation may therefore serve to reduce this competitive heat load. Moreover, BAT contributes to basal heat production and hence

suppressing it may achieve similar reductions in heat production that would otherwise compete with the heat produced during milk synthesis, within the overall capacity to dissipate body heat. The fact that the currently available data do not unequivocally support one hypothesis over the other, combined with the fact that females may vary their intake in different situations, has reopened the question of whether intake is actually limited at all at peak lactation (Speakman and Król, 2011; Valencak et al., 2010). The suggestion has been made that the limits or constraints on intake may only reflect differential pup demand and the investment 'strategy' of the lactating female (Valencak et al., 2010: Zhao et al., 2013b; see also discussion in Speakman and Król, 2005).

In the current paper we aimed to monitor the T_b and physical activity levels of female mice prior to reproduction (baseline), during lactation and over a 10 day post-lactation period. Data for the period of pregnancy for the same individuals have already been published (Gamo et al., 2013). We simultaneously monitored food intake, body mass and pup growth. We predicted that if food intake at peak lactation is limited, rather than being dependent on pup demand, there would be a suppression in pup growth rates over the period of asymptotic food intake in late lactation, compared with earlier in lactation and during the subsequent period when the offspring start to feed themselves. If the HDL hypothesis is correct we expected that mice in lactation would be hyperthermic relative to their own body temperatures prior to and following breeding. We predicted the extent of this hyperthermia across individuals would be related directly to the level of energy intake, and would therefore increase throughout lactation as levels of energy intake increased. At peak lactation, hyperthermia would be expected to be related to the level of food intake and the litter mass being supported, because litter mass is closely related to milk production rates (Zhao, 2011). As observed in other studies, we expected that during physical activity the T_b would be elevated above that at rest. However, if the reduction in physical activity during lactation serves to reduce heat production, we predicted that there would be a reduction in the extent of elevation of T_b during physical activity relative to resting conditions when animals were lactating. Moreover, we anticipated that across individuals the reduction in physical activity would be related to the level of peak energy intake. In previous studies, observations were often terminated at peak lactation or when the offspring wean. In the current study we followed females for an additional 10 days to monitor how rapidly the female returns to the baseline state (if at all) when lactation is over.

MATERIALS AND METHODS
Animals and housing
We monitored T_b and physical activity of 26 female mice mated at age 12 weeks (*Mus musculus* Linnaeus 1758, outbred MF1 strain; supplied by Harlan UK, Bicester, UK). Monitoring was continuous, with records taken every minute, for 23 h per day, for the whole of lactation. For 18 of these individuals we also monitored them over a 10 day post-lactation period. Data were collected using passive implanted transmitters, VitalView telemetry and a data acquisition system (Mini Mitter, Bend, OR, USA). The total data set comprised ~1 million measurements each of physical activity and temperature. The raw data set will be made freely available to anyone wishing to use it on a collaborative basis. Please contact the corresponding author if you require access. Because of the restricted availability of monitoring equipment, the study was conducted over 3 years: 2005 (*N*=8), 2006 (*N*=8) and 2007 (*N*=10). The same 26 animals were also monitored throughout pregnancy, in the context of energy compensation mechanisms during gestation, details of which are

presented in Gamo et al. (Gamo et al., 2013). Mice were kept at a temperature and humidity of 21±1°C and 50–60%, respectively, under a 12 h:12 h light:dark photoperiod. We monitored food intake, body mass, litter size and pup growth on a daily basis. Eight females matched for age were monitored as non-reproductive controls.

Experimental design in lactation and post-lactation
Daily food mass in the hopper as well as body mass were measured daily between 13:00 and 14:00 h in 2005, and between 09:00 and 10:00 h in 2006 and 2007 (for details, see Gamo et al., 2013). Food and energy intake were calculated using the same methods described previously (Król et al., 2007; Gamo et al., 2013). The day when pups were first observed was defined as the day of parturition, which was referred to as day of lactation 0 (DOL 0) following the convention established in Johnson et al. (Johnson et al., 2001a). No measurements of food and body mass were made on DOL 0 to avoid disturbance. Food mass measurement was restarted from the day after parturition (DOL 1); however, monitoring the mass of mothers was restarted from DOL 2. Between DOL 2 and DOL 20, litter size and litter mass were also recorded. The pups were removed on DOL 21. Subsequently, some of the mothers (N=18 from year 2006 and 2007) remained in the same cages to be monitored over the following 10 days as a post-lactation period from lactation. T_b and activity were recorded using the same protocols as detailed in Gamo et al. (Gamo et al., 2013).

A transponder failed in one animal on DOL 15. The sample size for T_b and activity was therefore 26 until DOL 14, and 25 thereafter. In addition, the sample sizes for these parameters were reduced to 24 on DOL 19 and 22 on DOL 20, because of corrupted files after download. The sample size for T_b and activity was 17 mice during post-lactation. We have previously shown that T_b is elevated during the period during and immediately following physical activity (Gamo et al., 2013). We therefore defined the temperature for active animals 'active T_b' as comprising the periods when physical activity was recorded by the transmitter plus the 15 min following cessation of activity. 'Inactive T_b' was the T_b when the mice were inactive (as recorded by the transmitter) and not within 15 min of activity having occurred.

Data analysis
All data are expressed as means ± s.d. (N=sample size). Energy intake and body mass of reproductive mice were compared with values in non-reproductive mice by repeated-measures ANOVA. Changes in energy intake and body mass of the mothers, and growth of offspring with days of lactation or post-lactation, were also assessed using repeated-measures ANOVA. Tukey *post hoc* tests were conducted to locate significant differences.

T_b and activity counts were analysed using repeated-measures ANOVA, including time of a day and reproductive status (baseline, lactation and post-lactation) as factors. When significant differences were found, *post hoc* Tukey tests were conducted to locate significant differences. Furthermore, mean T_b and activity counts were grouped into sequential 5-day periods of lactation – DOL 1–5, DOL 6–10, DOL 11–15 and DOL 16–20 – and the effect of time of day and reproductive status were determined using ANOVA and Tukey *post hoc* tests. The relationships between energy intake, body mass, litter size, litter mass, T_b and activity counts were assessed by linear regression analysis. Further, mean T_b was analysed by generalised linear model (GLM) to establish the effects of activity counts, energy intake and maternal body mass on T_b. All statistical analyses were carried out using R (R Development Core Team, 2007).
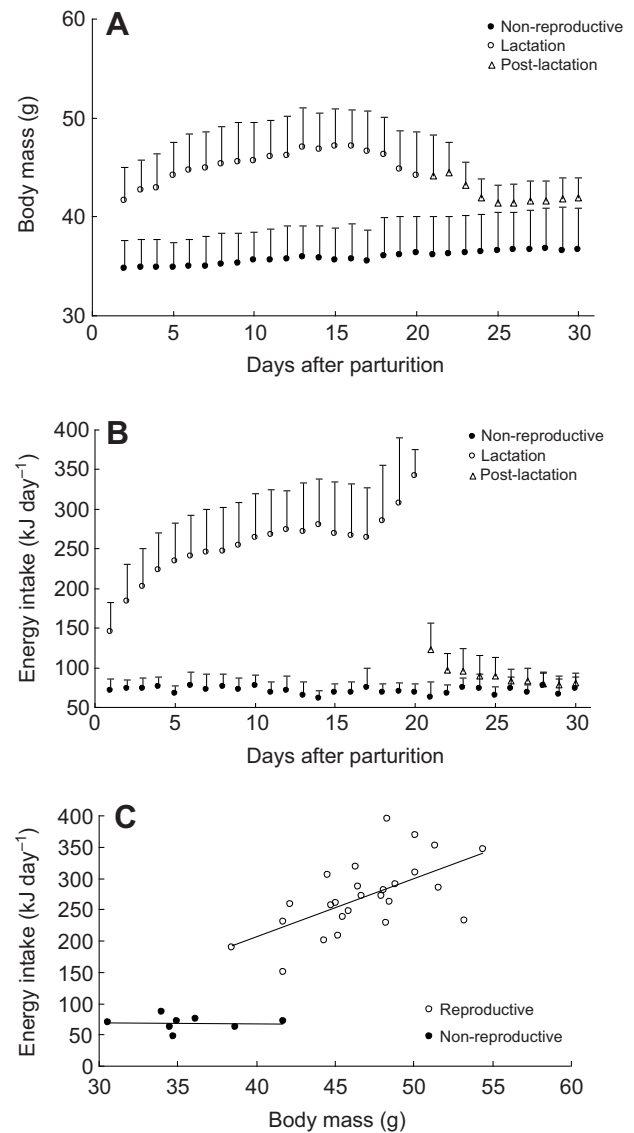

Fig. 1. (A) Body mass and (B) energy intake in reproductive and non-reproductive mice. All data are shown as means ± s.d. Closed circles represent energy intake in non-reproductive mice (N=8). Open circles and triangles indicate energy intake during lactation (N=26) and post-lactation (N=18), respectively. (C) The relationship between asymptotic energy intake and body mass. Plots represent mean intake between DOL 13 and 17 (N=26) and for non-reproductive individuals across the same days (N=8). Regressions are expressed by y=–0.09x+70.93 (R^2=0.001, P=0.953) for non-reproductive mice and y=9.27x–164.60 for reproductive mice (R^2=0.35, P=0.001).

RESULTS
Energy intake and body mass
Lactating mice weighed significantly more than non-reproductive mice (ANOVA, day: $F_{18,604}$=4.18, P<0.001, reproductive status: $F_{1,604}$=846.56, P<0.001, interaction of day and status: $F_{18,604}$=0.85, P=0.65; Fig.1A). In lactation, body mass increased significantly from DOL 2 to DOL 13 (one-way ANOVA, $F_{11,300}$=4.91, P<0.001). During the post-lactation period, reproductive mice were significantly heavier than non-reproductive mice, but did not change their body mass significantly over time (ANOVA, day: $F_{9,236}$=1.80, P=0.07, reproductive status: $F_{1,236}$=226.54, P<0.001, interaction of status and day: $F_{9,236}$=1.06, P=0.39).

Energy intake during lactation and post-lactation was compared with that of non-reproductive mice (Fig. 1B). Lactating mice consumed significantly more food than non-reproductive mice and increasingly so as lactation progressed (ANOVA, day: $F_{19,630}=13.48$, $P<0.001$, reproductive status: $F_{1,630}=1589.01$, $P<0.001$, interaction of day and status: $F_{19,630}=4.51$, $P<0.001$). Mean energy intake of the non-reproductive mice was 71.1 kJ day^{-1} ($N=8$) over the 19 days that coincided with lactation (DOL 1–20). Lactating mice progressively increased daily energy intake until DOL 12 (one-way ANOVA, $F_{11,490}=14.93$, $P<0.001$; Fig. 1A) and stabilised between DOL 13 and 17 at 270.3 kJ day^{-1}, the mean asymptotic daily energy intake. After DOL 17, offspring were able to eat food from the hoppers by themselves, which led to the increase in apparent energy consumption between DOL 18 and 20 (Fig. 1A). After weaning (DOL 21), energy intake of the mothers immediately dropped to an average of 122.7 kJ day^{-1}, which was still significantly higher than the energy intake of non-reproductive mice over the same time period (ANOVA, day: $F_{9,231}=4.57$, $P<0.001$, reproductive status: $F_{1,231}=56.63$, $P<0.001$, interaction of status and day: $F_{9,231}=3.70$, $P<0.001$). Energy intake returned to that observed in baseline on DOL 22. Fig. 1C shows the relationships between the asymptotic daily energy intake and mean body mass between DOL 13 and 17 for non-reproductive and reproductive mice. In non-reproductive mice, there was no significant relationship between daily energy intake and body mass ($P=0.953$). In contrast, energy intake was significantly positively related to body mass in reproductive mice ($y=9.268x-164.6$, $R^2=0.35$, $F_{1,24}=14.01$, $P=0.001$). Heavier mothers consumed more energy at peak lactation than lighter ones.

Litter size and litter mass

Litter size varied from 3 to 15 pups across the individual mothers on DOL 2. Mean litter mass increased from 21.03±5.33 g on DOL 2 to 97.39±22.27 g on DOL 20 (Fig. 2A). Mean pup mass and daily body mass gain were calculated from the number of pups and total litter mass (Fig. 2B). Although the mean mass of the pups increased as lactation advanced (ANOVA, $F_{18,471}=154.66$, $P<0.001$), their daily mass gain was significantly reduced in late lactation; i.e. growth rate dropped from 0.63±0.15 g day^{-1} in early lactation (DOL 6) to 0.29±0.12 g day^{-1} in late lactation (DOL 15) (Tukey pairwise comparisons, $P<0.01$). Growth rate increased again to match that in early lactation after DOL 17 when the young started eating solid food.

Maternal energy intake, maternal mass, litter size and litter mass were averaged for each mother during peak lactation, i.e. DOL 13–17. Individual means of energy intake were related with litter size and litter mass (Fig. 3). There was a positive non-linear relationship between maternal energy intake and litter size ($y=75.26\log x+107.6$, $R^2=0.18$, $F_{1,24}=5.43$, $P=0.029$; Fig. 3A). Removing the smallest litter (three pups), however, resulted in the relationship between the two variables becoming non-significant ($P=0.313$). Maternal energy intake was highly correlated with litter mass both including ($y=2.63x+73.00$, $R^2=0.50$, $F_{1,24}=23.73$, $P<0.001$; Fig. 3B) or excluding the smallest litter ($y=2.62x+73.82$, $R^2=0.38$, $F_{1,23}=14.25$, $P<0.001$). Maternal body mass was significantly related to litter size ($y=0.67x+40.88$, $R^2=0.20$, $F_{1,24}=6.10$, $P=0.021$; Fig. 4A) and litter mass ($y=0.12x+37.77$, $R^2=0.28$, $F_{1,24}=7.98$, $P=0.009$; Fig. 4B). When the smallest litter was eliminated from the analysis, the significance in the relationship between maternal body mass and litter size became only marginal ($P=0.068$). However, maternal body mass was still related to litter mass when the smallest litter was excluded ($y=0.13x+37.43$, $R^2=0.18$, $F_{1,23}=5.17$, $P=0.033$).
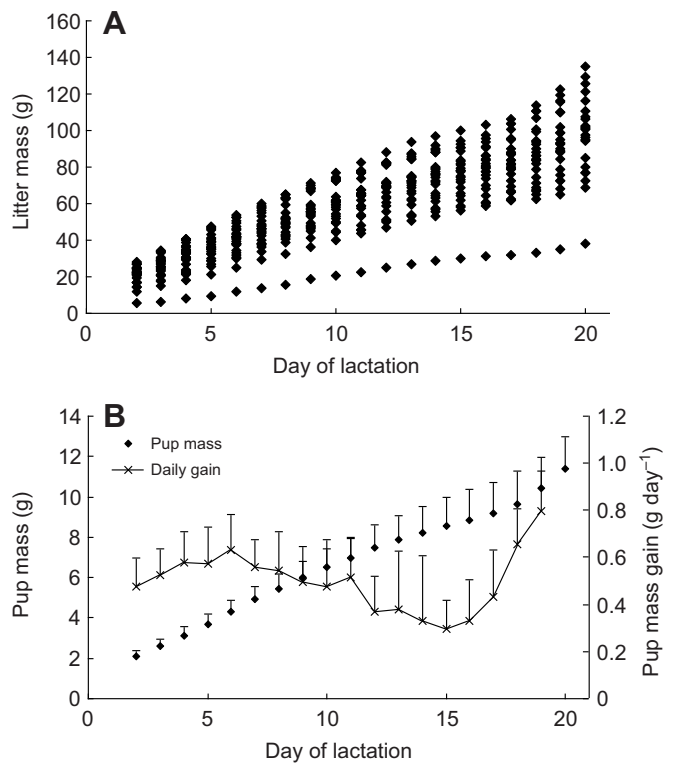
Fig. 2. (A) Mass growth of individual litters through lactation (DOL 2–20). (B) Mean pup mass and daily gain in mean pup mass. All data are shown as means + s.d. ($N=26$). Closed diamonds express pup mass on average across 26 litters. Crosses plus line express daily gain of averaged pup mass across 26 litters.

Activity levels

Changes in mean daily activity levels during baseline, lactation and post-lactation are shown in Fig. 5. Mean activity varied significantly with day (repeated-measures ANOVA, $F_{36,825}=14.01$, $P<0.001$). Physical activity was significantly increased on all 7 days of baseline compared with lactating days (DOL 1–20, Tukey pairwise comparisons, $P<0.05$). There was no difference in the level of daily activity throughout lactation (Tukey pairwise comparisons, $P>0.05$). In addition, the daily activity level in the post-lactation period remained as low as that in lactation (Tukey pairwise comparisons, $P>0.05$).

Circadian patterns in activity were calculated for each phase (baseline, lactation and post-lactation) by calculating the mean activity counts for each hour of the day (Fig. 6A). Physical activity varied significantly with time of day and among these periods (ANOVA, time, $F_{22,1518}=46.65$, $P<0.001$, period, $F_{2,1518}=125.24$, $P<0.001$, interaction of time and period, $F_{44,1518}=10.51$, $P<0.001$; Fig. 6A). A clear circadian pattern in activity was seen during baseline, with the highest levels of activity occurring during the night and the lowest during the day. At baseline, the highest and lowest hourly activity counts were 58.47±31.43 counts h^{-1} at 20:00 h and 5.14±2.57 counts h^{-1} at 15:00 h, respectively. During lactation, the lowest activity during the day was 9.11±2.11 counts h^{-1} at 12:00 h, which was significantly higher than the lowest value during baseline (Tukey pairwise comparisons, $P<0.01$). In contrast, the highest activity in lactation, 17.08±6.39 counts h^{-1} at 20:00 h, was significantly lower than that of baseline (Tukey pairwise comparisons, $P<0.01$). Mean hourly activity in lactation was significantly lower than baseline between 19:00 and 02:00 h at night,
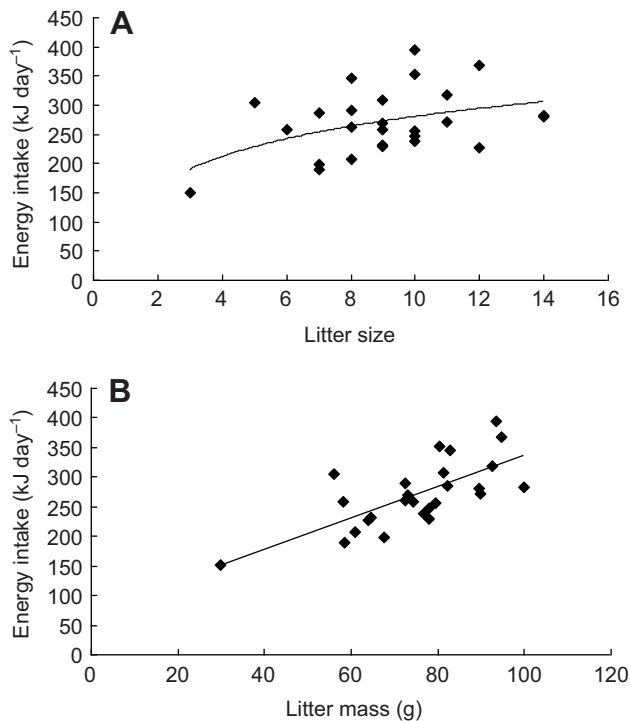
Fig. 3. Relationships between energy intake and litter size or litter mass during DOL 13–17. All data were averaged for individual litters across days between DOL 13 and 17 (N=26). (A) The relationship between litter size and maternal energy intake. The regression is described by $y=75.26\log x+107.6$ ($R^2=0.184$, $P=0.029$). (B) The relationship between litter mass and maternal energy intake. The regression is described by $y=2.63x+73.004$ ($R^2=0.497$ $P<0.001$).
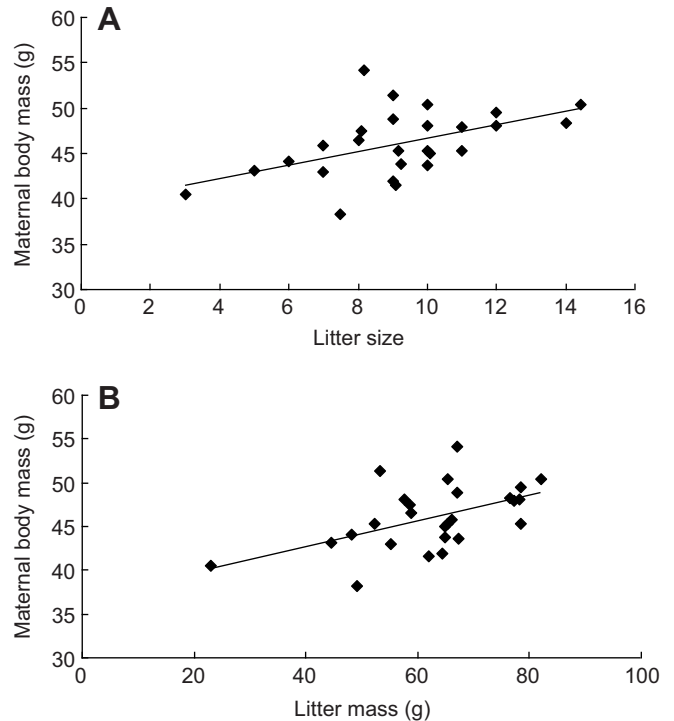

Fig. 4. Relationships between maternal body mass and litter size or litter mass during DOL 13–17. All data were averaged for individual litters across days between DOL 13 and 17 (N=26). (A) The relationship between litter size and maternal body mass. The regression is described by $y=0.666x+40.876$ ($R^2=0.203$, $P=0.021$). (B) The relationship between litter mass and maternal body mass. The regression is described by $y=0.122x+37.765$ ($R^2=0.18$, $P=0.033$).

and did not differ significantly between lactation and baseline between 03:00 and 18:00 h. During lactation, a circadian pattern in activity could still be observed as females were more active during the night between 19:00 and 22:00 h than during the daytime at 11:00, 12:00 and 14:00 h (Tukey pairwise comparisons, $P<0.05$). Night-time activity during post-lactation was intermediate between baseline and lactation. The highest and lowest hourly values were 34.7 ± 20.19 at 20:00 h and 4.1 ± 1.28 at 13:00 h, respectively. Comparing baseline and post-lactation, only the highest values at 20:00 h differed significantly (Tukey pairwise comparisons, $P<0.01$). Compared with lactation, activity in post-lactation was significantly higher between 19:00 and 03:00 h (Tukey pairwise comparisons, $P<0.05$).

To explore the patterns of activity in lactation more closely, the lactation period was divided into four separate 5-day periods: DOL 1–5, DOL 6–1, DOL 11–15 and DOL 16–20. Circadian patterns in activity levels of these four periods were compared (Fig. 6B). Activity counts differed among time of day and period (ANOVA, time, $F_{22,2277}=14.76$, $P<0.001$, period, $F_{3,2277}=34.09$, $P<0.001$, interaction of time and period, $F_{66,2277}=1.46$, $P=0.01$). In early lactation (DOL 1–5) and late lactation (DOL 16–20), there was a clear circadian rhythm in activity levels; i.e. activity was significantly increased during the night (at 20:00 h) compared with the lowest activity during daytime (Tukey pairwise comparisons, $P<0.05$). During DOL 6–10 and DOL 11–15 there were no statistically significant changes of activity over time of day. In addition, the activity level at 20:00 h was significantly higher in late lactation (DOL 16–20) compared with that in DOL 6–10 and DOL 11–15

(Tukey pairwise comparisons, $P<0.05$), but similar to that in early lactation of DOL 1–5 (Tukey pairwise comparisons, $P>0.05$).

Body temperature

Daily means of active and inactive T_b are illustrated from baseline to post-lactation (Fig. 7A). Active T_b differed significantly with day (ANOVA, $F_{36,825}=23.21$, $P<0.001$). Mean active T_b was $37.63\pm0.23°C$ during baseline and was significantly increased during lactation (DOL 2–19, Tukey pairwise comparisons, $P<0.01$). From DOL 25 during post-lactation, mean T_b was significantly lower than that in lactation and not significantly different to the baseline values.

Inactive T_b also varied with day (ANOVA, $F_{36,825}=73.83$, $P<0.001$). During baseline, inactive T_b was lower than that in the whole of lactation (DOL 1–20, Tukey pairwise comparisons, $P<0.01$). Compared with $37.4\pm0.27°C$ on DOL 0, inactive T_b increased significantly from DOL 5 until DOL 17 (Tukey pairwise comparisons, $P<0.01$). Inactive T_b declined after DOL 17 until it reached a level not statistically different to baseline on DOL 21. Differences between the active and inactive T_b are illustrated in Fig. 7B. Through all days in baseline, lactation DOL 0–10 and DOL 18–20 and all days of post-lactation, active T_b was significantly higher than inactive T_b (Tukey pairwise comparisons, $P<0.05$). However, the difference between inactive and active T_b became substantially compressed during the latter stages of lactation (DOL 11–17), reflecting the large increase in the inactive T_b.

A significant circadian pattern in T_b was observed but this pattern differed between the different periods (two-way ANOVA,
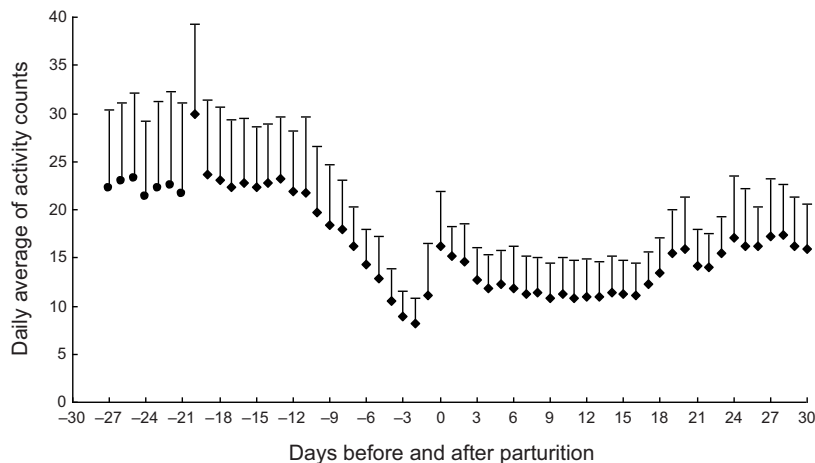
Fig. 5. Activity levels over baseline (days –27 to –21), pregnancy (days –15 to –1), lactation (days 0 to 20) and post-lactation (days 21 to 30). The data are expressed as means + s.d.

time, $F_{22,1518}$=92.84, P<0.001, period, $F_{2,1518}$=979.36, P<0.001, interaction of time and period, $F_{44,1518}$=6.17, P<0.001; Fig. 8A). No differences in T_b were found between baseline and post-lactation at any time of day. In contrast, mean T_b during lactation was higher during most of the day than at baseline (except from 20:00 to 23:00h) and post-lactation (except at 00:00 and 01:00h, Tukey pairwise comparisons, P<0.01). Thus, it appeared that lactating females experienced elevated T_b throughout both the day and night. Mean T_b recovered immediately after weaning to the pattern at baseline (Fig. 8A). The highest and lowest mean T_b within lactation was 38.35±0.18°C at 21:00h and 37.61±0.17°C at 12:00h, respectively. As mean T_b was constantly high, the range of mean T_b was smaller during lactation than in either baseline or post lactation periods.

Mean T_b changes over the day were also compared among four different stages of lactation (Fig. 8B). Mean T_b varied significantly with time and with period (two-way ANOVA, time, $F_{22,2277}$=76.79, P<0.001, period, $F_{3,2277}$=65.34, P<0.001, interaction of time and period, $F_{66,2277}$=2.95, P<0.001). During the daytime, mean T_b over DOL 16–20 was lower than that in the earlier periods. Especially at 12:00 and 14:00h, the mean T_b during DOL 16–20 was significantly lower than that of DOL 6–10 and DOL 11–15 (Tukey pairwise comparisons, P<0.01). There was no difference in mean T_b between DOL 6–10 and DOL 11–15. During the night-time, the earliest lactation period (DOL 1–5) had the lowest temperatures, and the mid-lactation (DOL 11–15) had the highest temperatures. Comparing these two periods, mean T_b differed significantly between 19:00 and 23:00h (Tukey pairwise comparisons, P<0.05).
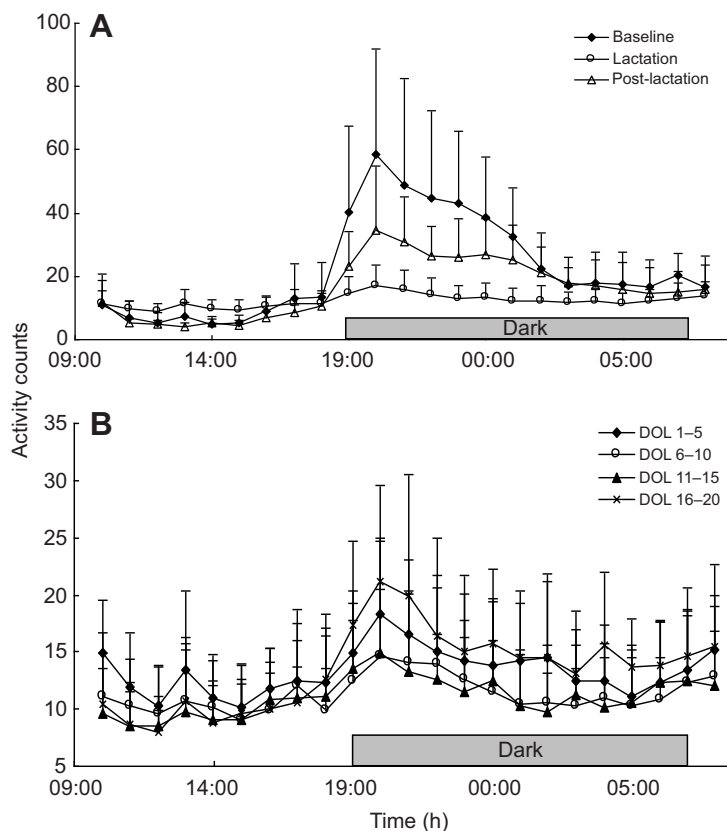
Fig. 6. (A) Activity levels through the day during baseline, lactation and post-lactation. Closed diamonds, open circles and open triangles show hourly mean activity counts in baseline, lactation and post-lactation, respectively. All data are expressed as means + s.d. (N=26 for baseline and lactation, and N=17 for post-lactation). Grey bar indicates the dark phase. (B) Activity changes throughout the day at different stages of lactation. Closed diamonds, open circles, closed triangles and crosses show hourly mean activity counts over DOL 1–5, 6–10, 11–15 and 16–20, respectively. All data are expressed as means + s.d. Sample sizes are 26 for DOL 1–5, 6–10 and 11–15, and 25 for DOL 16–20. Grey bar indicates the dark phase.
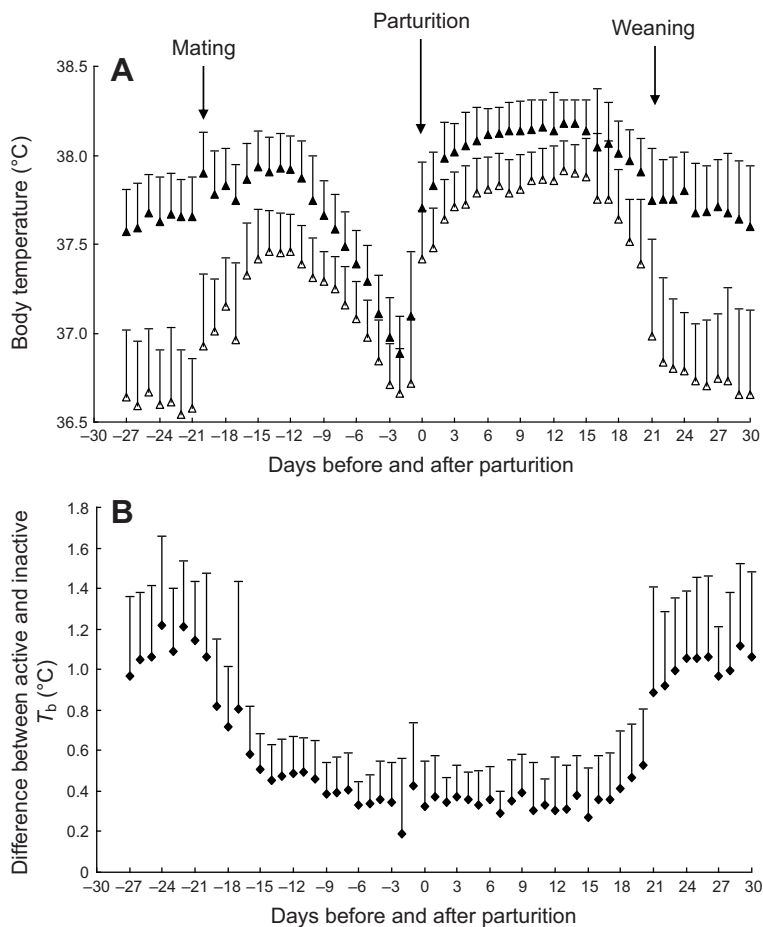
Fig. 7. (A) Daily changes of active and inactive body temperature (T_b) in baseline, mating, pregnancy, lactation and post-lactation. Closed and open triangles represent active and inactive T_b, respectively. Baseline was days –27 to –21, mating days –20 to –16, pregnancy days –15 to –1, lactation days 0 to 20 and post-lactation days 21 to 30. (B) Difference between active and inactive T_b in relation to the day of reproduction. All data are expressed as means + s.d.

Relationships between activity, mean T_b, energy intake and body mass

Relationships between physical activity and mean T_b, energy intake and body mass were analysed during lactation and the post-lactation period. The data for each parameter were averaged for each mouse for the 5 days approximating the peak lactation period (DOL 13–17; N=26) and during post-lactation over the whole 10 days after weaning (N=17). The relationship between physical activity and energy intake at peak lactation marginally failed to reach significance (P=0.051; Fig. 9). When the female with the smallest litter was removed from the analysis, the relationship was significant (y=–6.52x+349.55, R^2=0.25, $F_{1,23}$=7.56, P=0.011). Mothers that had higher activity ingested less energy at peak lactation across the natural litter sizes of 5–14. The relationship between physical activity and energy intake was not significant during post-lactation (P=0.78). Activity levels were also not related to maternal body mass at peak lactation (P=0.29) or during the post-lactation period (P=0.33). Energy intake was not significantly related to maternal T_b during peak lactation (P=0.54) or during the post-lactation period (P=0.88). The relationships between litter size or litter mass and maternal T_b were both not significant (litter size: P=0.25; litter mass: P=0.20).

DISCUSSION
Energy intake and body mass during lactation

Energy intake during lactation reached a plateau on day 13, consistent with previous data in the same strain of mouse lactating at the same ambient temperature (Johnson et al., 2001a; Vaanholt et al., 2013). However, the asymptotic net energy intake (270.3 kJ day^{-1}) in the present study was lower than that reported

previously in MF1 mice raising natural sized litters [310.2 kJ day^{-1} (Johnson et al., 2001a)] and where litter size was experimentally increased [314.4 kJ day^{-1} (Duah et al., 2013)]. Nevertheless, it was similar to the asymptotic net energy intake in the unmanipulated litters observed by Vaanholt et al. (Vaanholt et al., 2013) (265.8 kJ day^{-1}) and a group where litter sizes had been manipulated (both up and down), which averaged 273.3 kJ day^{-1} (Johnson et al., 2001b). The differences between the studies that contributed to the different asymptotic intakes are unclear. Although in the present experiment, the average litter size at weaning was slightly lower (mean=8.9) than in the previous experiments [11.2 (Duah et al., 2013); 12.0 (Johnson et al., 2001a); and 12.2 (Vaanholt et al., 2013)], this is unlikely to have been important because above a litter size of around 6 there was no association between food (energy) intake and litter size in either the previous or present studies. As individual asymptotic intakes are highly variable and heritable (Vaanholt et al., 2013), it is possible that the differences between studies reflect random variations across samples, perhaps linked to genetic differences over time (the different studies were carried out over a period of 10 years).

Similar to energy intake, body mass gradually increased until DOL 13, which was consistent with previous reports in rats (*Rattus norvegicus*) (Strubbe and Gorissen, 1980), field voles (*Microtus agrestis*) (Simons et al., 2011) and mice (Duah et al., 2013; Johnson et al., 2001a; Johnson et al., 2001b; Vaanholt et al., 2013; Zhao et al., 2013b). Maternal body mass was correlated with the asymptotic energy intake, as previously reported in Brandt's voles (Wu et al., 2009) and mice (Johnson et al., 2001a; Johnson et al., 2001b; Vaanholt et al., 2013). Across the entire range of litter sizes, both
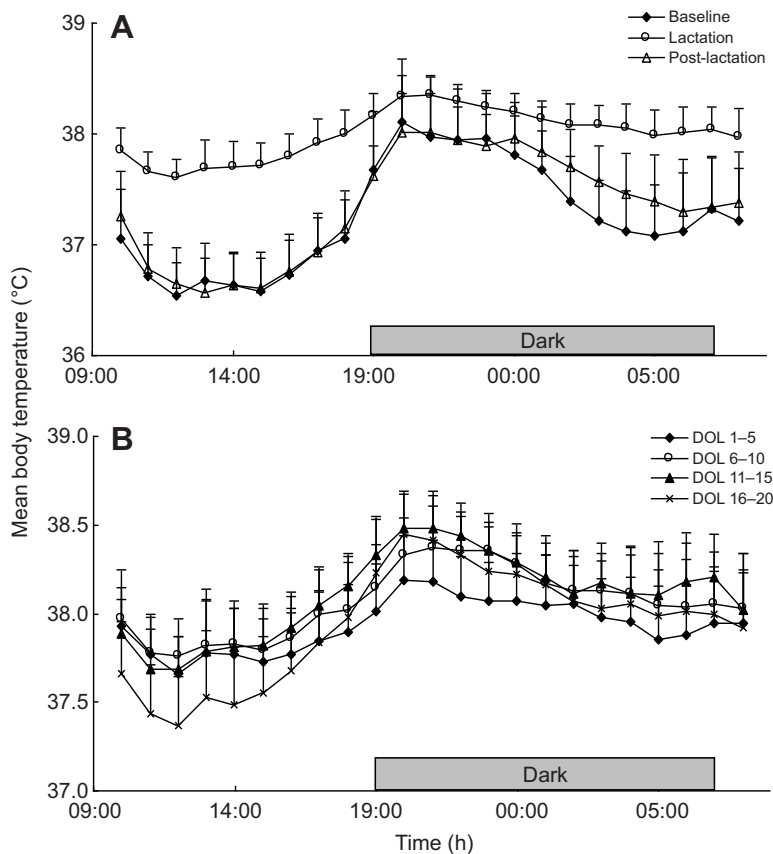
Fig. 8. (A) Mean T_b changes within a day of baseline, lactation and post-lactation. Closed diamonds, open circles and open triangles show hourly mean T_b in baseline, lactation and post-lactation, respectively. All data are expressed as means + s.d. (N=26 for baseline and lactation, and 17 for post-lactation). Grey bar indicates the dark phase. (B) Mean T_b throughout the day at different stages of lactation. Closed diamonds, open circles, closed triangles and crosses show hourly mean activity counts over DOL 1–5, 6–10, 11–15 and 16–20, respectively. All data are expressed as means + s.d. Sample sizes are 26 for DOL 1–5, 6–10 and 11–15, and 25 for DOL 16–20. Grey bar indicates the dark phase.

litter size and litter mass were correlated with the asymptotic energy intake and maternal body mass during DOL 13–17. However, there was no correlation between asymptotic food intake and litter size over the range of litter sizes from 5 to 15, which was similar to the non-significant effects of litter size on asymptotic intake over natural litter size ranges from 7 to 13 pups (Johnson et al., 2001a; Johnson et al., 2001b) and 4 to 18 pups (Vaanholt et al., 2013). In contrast, total litter mass was more closely correlated with maternal energy intake and maternal body mass than litter size, as has also been reported previously (Duah et al., 2013; Johnson et al., 2001a; Johnson et al., 2001b; Vaanholt et al., 2013).

Together, the pooled data across these studies suggests that in these mice litter mass was a consequence of maternal investment, but that mothers were largely unresponsive to variations in litter size, leading to the negative relationship between individual pup mass and litter size. Consistent with this interpretation of a lack of sensitivity to litter size, asymptotic food intake did not increase in response to experimental increases in litter size in mice (Duah et al., 2013; Hammond and Diamond, 1992; Johnson et al., 2001a) or guinea pigs (*Cavia porcellus*) (Laurien-Kehnen and Trillmich, 2003). However, in other species of small mammal asymptotic intake at peak lactation was significantly related to differences in litter size [field vole for litters below N=6 (Simons et al., 2011); brown hare (Valencak et al., 2010); Brandt's vole (Wu et al., 2009)]. The main difference between these two types of study, where lactation investment was, and was not, responsive to litter size, appears to be the sizes of the litters. Where litters are small (<6), the limits on maternal performance may be imposed by growth capacity of the offspring. In contrast, where litters are large, the limit may be more dependent on maternal energy intake and lactation capacity. Indeed, when litters were experimentally reduced in mice there was a significant relationship between litter size and intake (Johnson et

al., 2001a; Zhao et al., 2013b). The main exception to this rule appears to be the guinea pig, where investment was unrelated to litter size despite their small litters. This may be reflective of the precocial nature of guinea pig offspring and their high milk demands over a relatively short lactation. The differences resulting from litter size effects have probably contributed to differing interpretations in the past about the factors that limit lactation performance and asymptotic lactation food intake. A key question therefore is whether lactating mice are unusual because they have been selected to raise artificially large litter sizes. More studies of small mammals raising small litter sizes are required to answer this question.

In the present study, at the same time that maternal energy intake reached a plateau, the growth rate of offspring fell to almost half that observed in early lactation. This slow growth was unlikely to have been caused by the lowered ability of offspring to grow, as they returned to the same rate observed in early lactation a few days later when they obtained solid food by themselves at the end of lactation. This supports the interpretation that at peak lactation, at 21°C, when these mice are raising natural litter sizes, the lactating mother–offspring unit is limited by maternal energy intake, rather than by the growth potential of the offspring.

Physical activity and body temperature in lactating mice
Physical activity decreased significantly from parturition to DOL 18. This was similar to the decline in voluntary wheel-running activity shown in lactating hamsters (*Phodopus campbelli* and *P. sungorus*) (Scribner and Wynne-Edwards, 1994) and mice (Schubert et al., 2009; Zhao et al., 2013a), and to the reduced levels of general activity observed in lactating mice (Speakman et al., 2001). Reduced physical activity in lactation may have several benefits: it may release time for feeding or suckling (Speakman et al., 2001), it may save energy during lactation, which can then be allocated to reproduction (Schubert
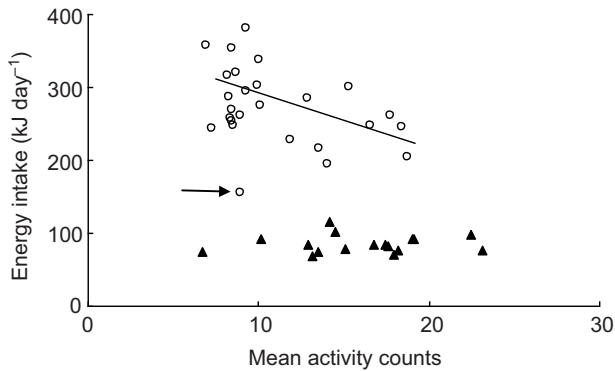
Fig. 9. Relationships between energy intake and activity level averaged for individual mice during peak lactation (DOL 13–17; N=26 mice) and the whole post-lactation (N=17 mice). Open circles and closed triangles represent lactation and post-lactation, respectively. The fitted line is the least squares regression for the lactation data (y=−6.515x+349.55, R^2=0.247, $F_{1,23}$=7.56, P=0.011) after removing the data for the smallest litter (arrow).

et al., 2009), and it may reduce competitive heat production (Zhao et al., 2013a). Consistent with the latter effect, we found that physical activity was negatively associated with energy intake levels at peak lactation. This suggests that those mice with greater energy intake reduced their physical activity the most to facilitate the greater intake. Previous observations suggested the major reduction in locomotor (wheel running) activity in lactation occurred at night, in both mice and hamsters (Schubert et al., 2009; Scribner and Wynne-Edwards, 1994; Zhao et al., 2013a), and we observed a similar effect with respect to voluntary physical activity. In contrast to what happened at night, voluntary activity in the light phase increased during lactation compared with that during baseline. Previous studies have also shown that the frequencies of feeding and drinking behaviours progressively increased during the day in lactating mice (Speakman et al., 2001), rats (Kittrell and Satinoff, 1988; Strubbe and Gorissen, 1980) and dwarf hamsters (Scribner and Wynne-Edwards, 1994). This suggests that non-reproductive animals can rest during the daytime, but lactating mothers may need to be more active during daytime because of their elevated food and water requirements that cannot be satisfied by feeding at night alone. There may also be a disruption of sleep, because of disturbance by their offspring (Young et al., 1998; Waterhouse et al., 2001).

Lactating mice were chronically hyperthermic. This observation accords with more sporadic measurements of T_b made in other lactating small rodents such as Mongolian gerbils (*Meriones unguiculatus*) (Weinandy and Gattermann, 1995), dwarf hamsters (*Phodopus campbelli* and *P. sungorus*) (Scribner and Wynne-Edwards, 1994) and Sprague-Dawley rats (*Rattus norvegicus*) (Fewell, 1995). The period when T_b was highest was between DOL 13 and 17, which was equivalent to the time when asymptotic daily energy intake occurred. The fact that chronic hyperthermia matched to the period of maximal sustained energy intake is consistent with the HDL hypothesis that peak energy intake may be constrained by the ability of mothers to dissipate heat at peak lactation (Król and Speakman, 2003a; Król and Speakman, 2003b; Król et al., 2003; Król et al., 2007; Speakman and Król, 2010).

Potential drivers of the elevated T_b during lactation include the heat associated with digestion of the elevated levels of food intake, and the heat generated as a byproduct of milk synthesis and the resting metabolic rate. Previous estimates of resting metabolic rate

in lactation in this strain indicate that it is dependent on body mass and averages $40\,\text{kJ}\,\text{day}^{-1}$ (Johnson et al., 2001b). Hence resting metabolic rate was on average 14.8% of the gross energy intake, and 33% of the total heat burden (net energy intake minus energy exported as milk). Both food intake and milk production increased between parturition and peak lactation, and T_b increased in parallel with these changes as would be predicted. However, we did not find any significant association between T_b and food intake at peak lactation across the individuals. This could be because heat generated from milk synthesis is far more significant. Milk energy output is equivalent to 44% of gross energy intake at 21°C (Johnson et al., 2001a) and milk production is only approximately 60% efficient, leading to a large heat burden when compared with the 3–6% of gross energy intake that appears as the heat increment of feeding (Secor, 2009). Yet we also did not find any significant association across the individual mice between maternal T_b at peak lactation and litter mass, which we have previously shown to be strongly correlated with milk production (Johnson et al., 2001a; Król and Speakman, 2003b; Vaanholt et al., 2013). These negative relationships do not, however, mean that food intake and milk production at peak lactation do not contribute to elevated heat production, because the mice may have modulated their intake and milk production in relation to their individual capacities to dissipate body heat to keep T_b constant.

Alternatively, or additionally, the elevation in T_b might be associated indirectly with the hormonal secretions that induce and maintain milk production such as prolactin (Svennersten-Sjaunja and Olsson, 2005) and other hormonal changes that are linked to lactation such as the reduced levels of circulating leptin (Cui et al., 2011; Król et al., 2011). In fact, prolactin appears to be strongly positively correlated with T_b during exertional heat stress (Pitsiladis et al., 2002; Wright et al., 2012), although in this case it appears the causal relationship is that elevated temperature stimulates prolactin release (Low et al., 2005) and not the reverse. In non-reproductive gerbils, peripheral administration of prolactin significantly reduced T_b (Yang et al., 2013), perhaps mediated *via* a reduction in the activity of BAT (Król et al., 2011). Prolactin-releasing peptide administered by intracerbroventricular (ICV) injection resulted in increased core T_b (Ellacott et al., 2002), but the relationship of this peptide to prolactin release, despite its name, is uncertain. Hence, the relationship of prolactin to T_b remains unclear. Leptin, in contrast, consistently increases T_b when administered either by ICV injection or peripherally (Luheshi et al., 1999). Leptin was originally discovered as the mutated gene in a mutant mouse called the ob/ob mouse, which is characterised by having extreme hyperphagia and profound obesity (Zhang et al., 1994). Ob/ob mice are unable to produce functional leptin because of a mutation that leads to a premature stop codon and hence a truncated protein. Ob/ob mice that lack functional leptin have lowered T_b, which can be reversed by leptin administration (Harris et al., 1998). Hence lowered leptin levels, in lactation, seem an unlikely candidate to stimulate T_b. However, T_b was significantly suppressed by the removal of the adrenal glands and the ovaries from female rats (Leon et al., 1978; Leon et al., 1985; Marrone et al., 1976), pointing to additional hormonal drivers that could be involved.

Finally, mothers might have dissipated heat inefficiently, as a large portion of the surface area of the maternal body was surrounded by growing pups during suckling bouts. This may have elevated heat retention, and therefore T_b, as female rats (*Rattus norvegicus*) terminate contact with their pups, and leave their nest to avoid a risk of developing hyperthermia, at a high ambient temperature (Adels and Leon, 1986; Croskerry et al., 1978). Both mice and gerbils

significantly thin their pelage during lactation (Zhao et al., 2013a; Yang et al., 2013) and this may offset some of the problem of being unable to dissipate heat because the suckling pups clustered around the female.

Post-lactation

Consistent with the previous reports in rats (Strubbe and Gorissen, 1980) and hamsters (*Mesocricetus auratus*) (Fleming, 1978), the decline in maternal energy intake was immediate after lactation ended, and was reduced to levels not significantly different from those at baseline pre-reproduction. Nevertheless, in MF1 mice (present study) and rats (Strubbe and Gorissen, 1980), post-lactation body mass remained much heavier than that at baseline pre-reproduction. This disconnect between energy intake and body mass between pre- and post-lactation periods might be explained by the reduction in physical activity at night during the post-lactation period, suggesting that mice recovering from lactation decreased their energy demands by lowering energy expenditure for physical activity rather than by losing body mass.

In summary, we used implanted transponders to monitor the T_b of mice at 1 min intervals throughout lactation, and compared these values with those from the same individuals during both pre-reproduction and post-lactation periods to test predictions of the HDL theory. The main finding was that mice during lactation were chronically hyperthermic relative to their own T_b measured pre- and post-reproduction. This hyperthermia persisted through both day and night, but was most marked relative to during the daytime, when T_b of lactating mice was on average 1.1°C hotter than during the baseline pre-reproduction period and post-lactation period. The extent of hyperthermia was greatest during the period of peak lactation. These observed patterns were consistent with the expectations from the HDL theory. The lactation hyperthermia developed despite activity levels in lactation being reduced to approximately half the baseline levels. Moreover, the reduction in activity was negatively associated with peak lactation energy intake – suggesting that the reduction in activity primarily served to reduce competitive heat production, and was therefore downregulated the most in those mice with the greatest increase in energy intake. The mechanisms contributing to the lactational hyperthermia remain unclear, but it was unrelated to variations in either food intake or litter size/mass at peak lactation. Finally, offspring growth was suppressed during the period of peak lactation, relative to the immediately preceding and following periods, suggesting that maternal performance at peak lactation was the primary limit on the mother–offspring system, not offspring growth capacity.

AUTHOR CONTRIBUTIONS

J.R.S. and Y.G. designed the study. Y.G., C.T., S.E.M. and C.H. collected the data. Y.G., L.M.V. and J.R.S. analysed the data. J.R.S. and Y.G. wrote the paper, and C.H., S.E.M. and L.M.V. contributed to improving it for publication.

COMPETING INTERESTS

No competing interests declared.

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Table S1. Energy intake and body mass in non-reproductive and reproductive mice during lactation and post lactation. The data are expressed as means ± S.D. Sample size (n) is 8 for energy intake and body mass in non-reproductive mice.

| | DOL | Non-reproductive mice (n=8) | | Reproductive mice | | | |
		Energy intake (kJ day^{-1})	Body mass (g)	Energy intake (kJ day^{-1})	(n)	Body mass (g)	(n)
Lactation	1	71.10 ± 14.66		145.01 ± 44.40	26		
	2	73.58 ± 11.45	34.74 ± 2.82	183.01 ± 37.91	26	41.60 ± 3.41	26
	3	73.29 ± 13.23	34.85 ± 2.91	201.66 ± 46.94	26	42.63 ± 3.12	26
	4	76.25 ± 11.49	34.82 ± 2.90	222.98 ± 48.26	26	42.93 ± 3.48	26
	5	66.96 ± 10.55	34.86 ± 2.56	234.11 ± 47.33	26	44.18 ± 3.41	26
	6	77.00 ± 17.63	34.93 ± 2.80	240.99 ± 48.00	26	44.67 ± 3.69	26
	7	72.47 ± 19.80	34.96 ± 3.06	245.55 ± 51.35	26	44.89 ± 3.71	26
	8	75.62 ± 16.46	35.17 ± 3.18	246.94 ± 53.80	26	45.29 ± 3.80	26
	9	72.84 ± 13.73	35.31 ± 3.08	254.32 ± 55.93	26	45.51 ± 4.05	26
	10	76.89 ± 13.99	35.56 ± 2.86	263.70 ± 53.94	26	45.61 ± 3.94	26
	11	68.81 ± 13.64	35.56 ± 3.18	267.93 ± 55.43	25	46.08 ± 3.63	26
	12	71.24 ± 18.40	35.75 ± 3.35	273.99 ± 56.79	26	46.12 ± 4.01	26
	13	64.62 ± 17.88	35.88 ± 3.25	271.23 ± 49.46	26	47.00 ± 4.01	26
	14	61.68 ± 9.45	35.78 ± 3.30	280.55 ± 62.08	26	46.76 ± 3.76	26
	15	68.58 ± 11.38	35.60 ± 3.29	268.85 ± 57.62	26	47.11 ± 3.83	26
	16	68.95 ± 13.58	35.69 ± 3.61	266.90 ± 65.20	26	47.16 ± 3.65	26
	17	75.18 ± 24.77	35.45 ± 3.25	263.84 ± 65.35	26	46.58 ± 4.07	26
	18	68.38 ± 11.65	36.05 ± 3.90	284.41 ± 63.11	25	46.23 ± 3.86	26
	19	69.95 ± 10.57	36.17 ± 3.82	307.08 ± 70.64	23	44.76 ± 3.88	25
	20	68.77 ± 10.56	36.35 ± 3.68	341.85 ± 82.99	21	44.19 ± 4.39	23
Recovery	21	62.93 ± 18.91	36.17 ± 3.89	122.76 ± 33.33	18	44.20 ± 4.05	18
	22	66.98 ± 11.81	36.26 ± 3.78	96.79 ± 20.96	18	44.47 ± 3.04	18
	23	74.18 ± 13.11	36.35 ± 3.84	96.33 ± 27.82	18	43.22 ± 2.27	18
	24	73.36 ± 18.36	36.42 ± 3.83	89.54 ± 25.51	18	41.92 ± 1.96	18
	25	64.60 ± 10.83	36.52 ± 3.96	89.03 ± 24.08	17	41.36 ± 1.87	18
	26	73.39 ± 15.44	36.64 ± 3.82	82.87 ± 15.34	17	41.36 ± 1.98	18
	27	69.10 ± 9.39	36.62 ± 4.07	83.45 ± 15.46	17	41.62 ± 1.96	17
	28	77.57 ± 17.45	36.75 ± 4.11	79.82 ± 13.72	16	41.67 ± 1.99	17
	29	65.83 ± 20.30	36.51 ± 4.49	78.35 ± 11.60	16	41.88 ± 2.06	17
	30	73.32 ± 15.44	36.64 ± 4.20	80.54 ± 12.29	16	41.94 ± 2.05	17

Table S2. Daily activity counts, mean T_b, active T_b and Inactive T_b during lactation and post lactation. The data are expressed as means ± S.D.

	DOL	Sample size	Activity	Mean T_b	Active T_b	Inactive T_b
Lactation	0	26	16.25 ± 5.64	37.65 ± 0.27	37.70 ± 0.26	37.41 ± 0.27
	1	26	15.17 ± 3.14	37.77 ± 0.19	37.83 ± 0.19	37.48 ± 0.22
	2	26	14.64 ± 3.96	37.92 ± 0.21	37.98 ± 0.20	37.64 ± 0.22
	3	26	12.72 ± 3.31	37.95 ± 0.16	38.01 ± 0.17	37.71 ± 0.20
	4	26	11.84 ± 3.47	37.98 ± 0.19	38.05 ± 0.19	37.72 ± 0.22
	5	26	12.22 ± 3.57	38.02 ± 0.19	38.08 ± 0.19	37.79 ± 0.19
	6	26	11.88 ± 4.33	38.05 ± 0.15	38.11 ± 0.15	37.81 ± 0.18
	7	26	11.25 ± 3.95	38.05 ± 0.16	38.12 ± 0.15	37.83 ± 0.18
	8	26	11.34 ± 3.75	38.06 ± 0.15	38.14 ± 0.16	37.79 ± 0.19
	9	26	10.77 ± 3.64	38.05 ± 0.16	38.14 ± 0.16	37.80 ± 0.20
	10	26	11.21 ± 3.87	38.07 ± 0.16	38.14 ± 0.17	37.85 ± 0.17
	11	26	10.87 ± 3.85	38.09 ± 0.15	38.16 ± 0.15	37.86 ± 0.17
	12	26	10.91 ± 3.94	38.07 ± 0.21	38.14 ± 0.22	37.86 ± 0.20
	13	26	10.94 ± 3.66	38.11 ± 0.13	38.18 ± 0.14	37.91 ± 0.17
	14	26	11.43 ± 3.75	38.12 ± 0.12	38.18 ± 0.13	37.90 ± 0.16
	15	25	11.21 ± 3.47	38.10 ± 0.16	38.14 ± 0.17	37.88 ± 0.22
	16	25	11.14 ± 3.36	38.01 ± 0.33	38.05 ± 0.32	37.75 ± 0.37
	17	25	12.20 ± 3.47	38.03 ± 0.23	38.07 ± 0.23	37.75 ± 0.33
	18	25	13.37 ± 3.67	37.96 ± 0.17	38.01 ± 0.18	37.64 ± 0.29
	19	24	15.54 ± 4.50	37.89 ± 0.17	37.97 ± 0.17	37.51 ± 0.23
	20	22	15.93 ± 5.45	37.79 ± 0.21	37.90 ± 0.19	37.39 ± 0.36
Post-lactation	21	17	14.14 ± 3.86	37.52 ± 0.34	37.79 ± 0.36	37.10 ± 0.61
	22	17	13.98 ± 3.51	37.40 ± 0.32	37.75 ± 0.23	36.84 ± 0.47
	23	17	15.42 ± 3.86	37.43 ± 0.28	37.75 ± 0.24	36.80 ± 0.39
	24	17	17.14 ± 6.40	37.45 ± 0.23	37.80 ± 0.22	36.79 ± 0.33
	25	17	16.22 ± 5.98	37.32 ± 0.27	37.68 ± 0.28	36.73 ± 0.32
	26	17	16.16 ± 4.10	37.29 ± 0.30	37.68 ± 0.26	36.71 ± 0.37
	27	17	17.26 ± 5.96	37.34 ± 0.30	37.71 ± 0.27	36.75 ± 0.36
	28	17	17.37 ± 5.32	37.31 ± 0.43	37.67 ± 0.34	36.73 ± 0.52
	29	17	16.21 ± 5.07	37.24 ± 0.43	37.64 ± 0.33	36.66 ± 0.48
	30	17	15.91 ± 4.67	37.22 ± 0.44	37.60 ± 0.34	36.66 ± 0.47

Table S3. Activity levels over the day during baseline, lactation and post lactation. The data are expressed as means ± S.D.

Time (h)	Baseline	(n)	Lactation	(n)	Post lactation	(n)
10	11.12 ± 9.76	(26)	11.58 ± 3.82	(26)	11.89 ± 6.68	(17)
11	6.78 ± 5.29	(26)	9.84 ± 2.33	(26)	5.13 ± 1.52	(17)
12	5.39 ± 2.98	(26)	9.11 ± 2.11	(26)	5.08 ± 1.14	(17)
13	7.34 ± 4.65	(26)	11.26 ± 4.45	(26)	4.12 ± 1.28	(17)
14	4.90 ± 2.40	(26)	9.79 ± 2.70	(26)	5.19 ± 1.32	(17)
15	5.14 ± 2.57	(26)	9.43 ± 3.06	(26)	4.49 ± 1.73	(17)
16	9.00 ± 4.27	(26)	10.53 ± 3.22	(26)	6.73 ± 2.06	(17)
17	12.82 ± 10.98	(26)	11.45 ± 4.31	(26)	8.72 ± 2.33	(17)
18	13.24 ± 11.05	(26)	11.43 ± 4.14	(26)	10.38 ± 4.11	(17)
19	40.07 ± 27.27	(26)	14.49 ± 5.33	(26)	23.28 ± 11.01	(17)
20	58.47 ± 33.54	(26)	17.08 ± 6.39	(26)	34.69 ± 20.19	(17)
21	48.85 ± 33.52	(26)	15.80 ± 6.22	(26)	30.82 ± 14.15	(17)
22	44.57 ± 27.75	(26)	14.43 ± 4.96	(26)	26.34 ± 9.30	(17)
23	42.95 ± 22.94	(26)	13.19 ± 3.98	(26)	26.19 ± 11.87	(17)
0	38.43 ± 19.21	(26)	13.31 ± 4.53	(26)	26.90 ± 11.29	(17)
1	32.48 ± 15.67	(26)	12.22 ± 4.07	(26)	25.14 ± 11.20	(17)
2	22.47 ± 11.34	(26)	12.24 ± 4.68	(26)	21.14 ± 8.33	(17)
3	17.23 ± 8.83	(26)	11.70 ± 3.51	(26)	17.89 ± 4.94	(17)
4	17.88 ± 9.68	(26)	12.19 ± 4.07	(26)	17.08 ± 8.07	(17)
5	17.54 ± 10.17	(26)	11.33 ± 3.30	(26)	15.70 ± 8.70	(17)
6	16.79 ± 8.54	(26)	12.27 ± 3.38	(26)	14.70 ± 7.95	(17)
7	20.16 ± 7.26	(26)	13.19 ± 4.56	(26)	15.10 ± 6.43	(17)
8	16.79 ± 9.83	(26)	13.82 ± 4.66	(26)	15.82 ± 7.76	(17)

Table S4. Activity levels over a day during the four stages of lactation. The data are expressed as means ± S.D.

Time (h)	Lactation			
	DOL 1-5 (n)	DOL 6-10 (n)	DOL 11-15 (n)	DOL 16-20 (n)
10	14.87 ± 4.67 (26)	11.17 ± 5.55 (26)	9.64 ± 3.98 (26)	10.44 ± 4.44 (25)
11	11.93 ± 4.78 (26)	10.35 ± 4.08 (26)	8.54 ± 2.98 (26)	8.70 ± 3.57 (25)
12	10.33 ± 3.31 (26)	9.63 ± 4.21 (26)	8.59 ± 2.53 (26)	7.97 ± 1.91 (25)
13	13.47 ± 6.84 (26)	10.70 ± 4.97 (26)	9.73 ± 5.49 (26)	10.69 ± 5.64 (25)
14	10.99 ± 3.81 (26)	10.18 ± 4.02 (26)	9.01 ± 3.02 (26)	8.85 ± 3.59 (25)
15	10.13 ± 3.72 (26)	9.12 ± 3.13 (26)	9.02 ± 3.65 (26)	9.59 ± 4.43 (25)
16	11.75 ± 3.61 (26)	9.90 ± 4.21 (26)	10.80 ± 4.45 (26)	10.06 ± 3.02 (25)
17	12.50 ± 5.00 (26)	12.00 ± 6.69 (26)	10.93 ± 5.05 (26)	10.59 ± 3.07 (25)
18	12.37 ± 4.76 (26)	9.91 ± 3.64 (26)	11.11 ± 5.30 (26)	12.65 ± 5.61 (25)
19	14.86 ± 5.46 (26)	12.53 ± 5.29 (26)	13.53 ± 5.78 (26)	17.41 ± 7.24 (25)
20	18.32 ± 6.61 (26)	14.66 ± 5.77 (26)	14.92 ± 9.72 (26)	21.09 ± 8.44 (25)
21	16.53 ± 6.54 (26)	14.03 ± 6.37 (26)	13.34 ± 6.68 (26)	19.96 ± 10.53 (25)
22	15.04 ± 5.55 (26)	14.02 ± 7.73 (26)	12.54 ± 3.98 (26)	16.44 ± 8.50 (25)
23	14.18 ± 5.85 (26)	12.54 ± 5.23 (26)	11.46 ± 4.82 (26)	15.02 ± 6.72 (25)
0	13.85 ± 5.52 (26)	11.56 ± 4.42 (26)	12.53 ± 7.16 (26)	15.67 ± 6.60 (25)
1	14.29 ± 6.07 (26)	10.39 ± 4.79 (26)	10.29 ± 4.43 (26)	14.51 ± 4.70 (25)
2	14.46 ± 7.35 (26)	10.57 ± 5.01 (26)	9.81 ± 3.34 (26)	14.52 ± 6.57 (25)
3	12.44 ± 4.56 (26)	10.30 ± 3.25 (26)	11.26 ± 4.32 (26)	13.10 ± 5.44 (25)
4	12.52 ± 4.88 (26)	10.97 ± 4.73 (26)	10.20 ± 4.34 (26)	15.54 ± 6.44 (25)
5	11.13 ± 4.46 (26)	10.23 ± 4.27 (26)	10.63 ± 4.54 (26)	13.63 ± 4.33 (25)
6	12.37 ± 5.32 (26)	10.90 ± 3.62 (26)	12.34 ± 5.45 (26)	13.78 ± 3.89 (25)
7	13.48 ± 5.11 (26)	12.32 ± 6.44 (26)	12.46 ± 5.70 (26)	14.70 ± 5.97 (25)
8	15.14 ± 7.52 (26)	12.84 ± 6.15 (26)	12.10 ± 4.73 (26)	15.41 ± 4.55 (25)

Table S5. Mean T_b throughout a day during baseline, lactation and post lactation. The data are expressed as means ± S.D.

Time (h)	Baseline	(n)	Lactation	(n)	Post lactation	(n)
10	37.05 ± 0.45	(26)	37.85 ± 0.21	(26)	37.25 ± 0.41	(17)
11	36.71 ± 0.29	(26)	37.66 ± 0.19	(26)	36.78 ± 0.33	(17)
12	36.55 ± 0.29	(26)	37.61 ± 0.17	(26)	36.65 ± 0.31	(17)
13	36.68 ± 0.34	(26)	37.69 ± 0.25	(26)	36.56 ± 0.31	(17)
14	36.64 ± 0.27	(26)	37.71 ± 0.22	(26)	36.64 ± 0.29	(17)
15	36.58 ± 0.30	(26)	37.71 ± 0.21	(26)	36.61 ± 0.31	(17)
16	36.73 ± 0.31	(26)	37.80 ± 0.20	(26)	36.76 ± 0.33	(17)
17	36.94 ± 0.34	(26)	37.92 ± 0.22	(26)	36.93 ± 0.31	(17)
18	37.06 ± 0.34	(26)	38.00 ± 0.22	(26)	37.15 ± 0.33	(17)
19	37.68 ± 0.52	(26)	38.16 ± 0.20	(26)	37.62 ± 0.26	(17)
20	38.11 ± 0.55	(26)	38.34 ± 0.18	(26)	38.01 ± 0.35	(17)
21	37.98 ± 0.53	(26)	38.35 ± 0.18	(26)	38.02 ± 0.35	(17)
22	37.94 ± 0.46	(26)	38.29 ± 0.15	(26)	37.95 ± 0.30	(17)
23	37.95 ± 0.41	(26)	38.24 ± 0.15	(26)	37.89 ± 0.28	(17)
0	37.81 ± 0.35	(26)	38.20 ± 0.16	(26)	37.95 ± 0.32	(17)
1	37.67 ± 0.36	(26)	38.13 ± 0.16	(26)	37.83 ± 0.40	(17)
2	37.40 ± 0.39	(26)	38.09 ± 0.18	(26)	37.71 ± 0.33	(17)
3	37.22 ± 0.36	(26)	38.08 ± 0.19	(26)	37.57 ± 0.33	(17)
4	37.13 ± 0.36	(26)	38.05 ± 0.21	(26)	37.46 ± 0.36	(17)
5	37.08 ± 0.46	(26)	37.99 ± 0.24	(26)	37.39 ± 0.41	(17)
6	37.12 ± 0.53	(26)	38.02 ± 0.23	(26)	37.30 ± 0.47	(17)
7	37.33 ± 0.45	(26)	38.04 ± 0.20	(26)	37.34 ± 0.46	(17)
8	37.22 ± 0.47	(26)	37.97 ± 0.25	(26)	37.38 ± 0.46	(17)

Table S6. Mean T_b throughout the day during the four stages of lactation. The data are expressed as means ± S.D.

Time (h)	Lactation			
	DOL 1-5 (n)	DOL 6-10 (n)	DOL 11-15 (n)	DOL 16-20 (n)
10	37.93 ± 0.22 (26)	37.97 ± 0.28 (26)	37.89 ± 0.19 (26)	37.66 ± 0.29 (25)
11	37.77 ± 0.22 (26)	37.78 ± 0.20 (26)	37.69 ± 0.23 (26)	37.44 ± 0.28 (25)
12	37.66 ± 0.21 (26)	37.76 ± 0.22 (26)	37.69 ± 0.18 (26)	37.37 ± 0.27 (25)
13	37.78 ± 0.30 (26)	37.82 ± 0.32 (26)	37.79 ± 0.28 (26)	37.53 ± 0.37 (25)
14	37.77 ± 0.26 (26)	37.83 ± 0.25 (26)	37.81 ± 0.22 (26)	37.48 ± 0.32 (25)
15	37.73 ± 0.24 (26)	37.79 ± 0.26 (26)	37.82 ± 0.21 (26)	37.55 ± 0.31 (25)
16	37.77 ± 0.26 (26)	37.86 ± 0.23 (26)	37.92 ± 0.20 (26)	37.68 ± 0.33 (25)
17	37.85 ± 0.28 (26)	37.99 ± 0.27 (26)	38.04 ± 0.21 (26)	37.84 ± 0.28 (25)
18	37.90 ± 0.26 (26)	38.02 ± 0.27 (26)	38.16 ± 0.18 (26)	37.98 ± 0.34 (25)
19	38.01 ± 0.27 (26)	38.15 ± 0.24 (26)	38.33 ± 0.20 (26)	38.23 ± 0.32 (25)
20	38.18 ± 0.25 (26)	38.33 ± 0.21 (26)	38.48 ± 0.19 (26)	38.44 ± 0.25 (25)
21	38.18 ± 0.23 (26)	38.37 ± 0.23 (26)	38.48 ± 0.21 (26)	38.42 ± 0.25 (25)
22	38.10 ± 0.22 (26)	38.36 ± 0.21 (26)	38.44 ± 0.18 (26)	38.33 ± 0.21 (25)
23	38.07 ± 0.22 (26)	38.36 ± 0.16 (26)	38.36 ± 0.21 (26)	38.24 ± 0.25 (25)
0	38.07 ± 0.27 (26)	38.28 ± 0.16 (26)	38.28 ± 0.23 (26)	38.22 ± 0.23 (25)
1	38.05 ± 0.28 (26)	38.17 ± 0.22 (26)	38.21 ± 0.23 (26)	38.16 ± 0.17 (25)
2	38.05 ± 0.26 (26)	38.13 ± 0.22 (26)	38.11 ± 0.24 (26)	38.07 ± 0.20 (25)
3	37.98 ± 0.22 (26)	38.13 ± 0.21 (26)	38.17 ± 0.23 (26)	38.03 ± 0.26 (25)
4	37.95 ± 0.25 (26)	38.12 ± 0.26 (26)	38.11 ± 0.27 (26)	38.05 ± 0.27 (25)
5	37.85 ± 0.31 (26)	38.05 ± 0.29 (26)	38.10 ± 0.30 (26)	37.99 ± 0.27 (25)
6	37.88 ± 0.28 (26)	38.04 ± 0.36 (26)	38.18 ± 0.27 (26)	38.01 ± 0.29 (25)
7	37.94 ± 0.30 (26)	38.06 ± 0.29 (26)	38.20 ± 0.25 (26)	38.00 ± 0.27 (25)
8	37.95 ± 0.30 (26)	38.03 ± 0.32 (26)	38.02 ± 0.31 (26)	37.92 ± 0.31 (25)

Table S7. Active T_b throughout the day during baseline, lactation and post lactation. The data are expressed as means ± S.D.

Time (h)	Baseline	(n)	Lactation	(n)	Post lactation	(n)
10	37.48 ± 0.51	(26)	37.91 ± 0.22	(26)	37.52 ± 0.39	(17)
11	36.96 ± 0.36	(26)	37.73 ± 0.18	(26)	37.10 ± 0.33	(17)
12	36.75 ± 0.38	(26)	37.67 ± 0.15	(26)	36.89 ± 0.34	(17)
13	36.87 ± 0.32	(26)	37.77 ± 0.25	(26)	36.78 ± 0.31	(17)
14	36.85 ± 0.28	(26)	37.78 ± 0.22	(26)	36.88 ± 0.30	(17)
15	36.78 ± 0.28	(26)	37.79 ± 0.20	(26)	36.82 ± 0.31	(17)
16	36.96 ± 0.30	(26)	37.86 ± 0.20	(26)	36.99 ± 0.29	(17)
17	37.19 ± 0.33	(26)	37.97 ± 0.22	(26)	37.16 ± 0.29	(17)
18	37.29 ± 0.30	(26)	38.06 ± 0.22	(26)	37.37 ± 0.28	(17)
19	37.83 ± 0.40	(26)	38.21 ± 0.19	(26)	37.79 ± 0.23	(17)
20	38.20 ± 0.44	(26)	38.38 ± 0.18	(26)	38.17 ± 0.31	(17)
21	38.11 ± 0.40	(26)	38.38 ± 0.18	(26)	38.14 ± 0.31	(17)
22	38.05 ± 0.37	(26)	38.33 ± 0.14	(26)	38.09 ± 0.26	(17)
23	38.06 ± 0.33	(26)	38.29 ± 0.15	(26)	38.07 ± 0.27	(17)
0	37.96 ± 0.28	(26)	38.25 ± 0.16	(26)	38.08 ± 0.30	(17)
1	37.89 ± 0.24	(26)	38.19 ± 0.16	(26)	38.03 ± 0.31	(17)
2	37.66 ± 0.31	(26)	38.14 ± 0.18	(26)	37.90 ± 0.30	(17)
3	37.61 ± 0.29	(26)	38.12 ± 0.19	(26)	37.82 ± 0.29	(17)
4	37.48 ± 0.32	(26)	38.09 ± 0.21	(26)	37.72 ± 0.27	(17)
5	37.40 ± 0.35	(26)	38.03 ± 0.24	(26)	37.63 ± 0.36	(17)
6	37.45 ± 0.42	(26)	38.07 ± 0.23	(26)	37.59 ± 0.34	(17)
7	37.54 ± 0.38	(26)	38.10 ± 0.19	(26)	37.54 ± 0.43	(17)
8	37.48 ± 0.51	(26)	37.94 ± 0.25	(26)	37.62 ± 0.36	(17)

Table S8. Active T_b throughout the day during the four stages of lactation. The data are expressed as means ± S.D.

Time (h)	Lactation							
	DOL 1-5	(n)	DOL 6-10	(n)	DOL 11-15	(n)	DOL 16-20	(n)
10	37.95 ± 0.23	(26)	38.01 ± 0.28	(26)	37.94 ± 0.22	(26)	37.67 ± 0.30	(25)
11	37.80 ± 0.20	(26)	37.86 ± 0.21	(26)	37.71 ± 0.23	(26)	37.46 ± 0.29	(25)
12	37.72 ± 0.20	(26)	37.81 ± 0.22	(26)	37.70 ± 0.18	(26)	37.42 ± 0.28	(25)
13	37.81 ± 0.26	(26)	37.87 ± 0.30	(26)	37.84 ± 0.29	(26)	37.51 ± 0.32	(25)
14	37.82 ± 0.26	(26)	37.88 ± 0.24	(26)	37.85 ± 0.23	(26)	37.51 ± 0.34	(25)
15	37.80 ± 0.22	(26)	37.85 ± 0.24	(26)	37.86 ± 0.21	(26)	37.59 ± 0.31	(25)
16	37.84 ± 0.26	(26)	37.91 ± 0.23	(26)	37.96 ± 0.19	(26)	37.71 ± 0.30	(25)
17	37.89 ± 0.30	(26)	38.05 ± 0.26	(26)	38.07 ± 0.20	(26)	37.86 ± 0.28	(25)
18	37.96 ± 0.25	(26)	38.05 ± 0.26	(26)	38.17 ± 0.18	(26)	38.01 ± 0.32	(25)
19	38.05 ± 0.25	(26)	38.18 ± 0.24	(26)	38.35 ± 0.20	(26)	38.24 ± 0.31	(25)
20	38.21 ± 0.26	(26)	38.35 ± 0.20	(26)	38.49 ± 0.19	(26)	38.45 ± 0.25	(25)
21	38.21 ± 0.23	(26)	38.39 ± 0.23	(26)	38.50 ± 0.21	(26)	38.44 ± 0.25	(25)
22	38.14 ± 0.22	(26)	38.39 ± 0.20	(26)	38.46 ± 0.18	(26)	38.35 ± 0.22	(25)
23	38.11 ± 0.21	(26)	38.38 ± 0.15	(26)	38.38 ± 0.20	(26)	38.26 ± 0.24	(25)
0	38.10 ± 0.25	(26)	38.31 ± 0.14	(26)	38.31 ± 0.22	(26)	38.26 ± 0.22	(25)
1	38.09 ± 0.27	(26)	38.21 ± 0.21	(26)	38.26 ± 0.21	(26)	38.17 ± 0.17	(25)
2	38.09 ± 0.25	(26)	38.16 ± 0.22	(26)	38.13 ± 0.22	(26)	38.11 ± 0.21	(25)
3	38.01 ± 0.20	(26)	38.15 ± 0.21	(26)	38.19 ± 0.22	(26)	38.05 ± 0.27	(25)
4	37.98 ± 0.24	(26)	38.14 ± 0.26	(26)	38.15 ± 0.23	(26)	38.07 ± 0.28	(25)
5	37.88 ± 0.31	(26)	38.10 ± 0.29	(26)	38.13 ± 0.29	(26)	38.00 ± 0.27	(25)
6	37.89 ± 0.30	(26)	38.07 ± 0.34	(26)	38.21 ± 0.27	(26)	38.03 ± 0.30	(25)
7	37.97 ± 0.29	(26)	38.11 ± 0.26	(26)	38.24 ± 0.24	(26)	38.02 ± 0.27	(25)
8	37.98 ± 0.29	(26)	38.07 ± 0.31	(26)	37.96 ± 0.57	(26)	37.93 ± 0.31	(25)

Table S9. Inactive T_b throughout the day during baseline, lactation and post-lactation. The data are expressed as means ± S.D.

Time (h)	Baseline	(n)	Lactation	(n)	Post-lactation	(n)
10	36.77 ± 0.38	(26)	37.70 ± 0.18	(26)	36.90 ± 0.31	(17)
11	36.53 ± 0.32	(26)	37.55 ± 0.21	(26)	36.64 ± 0.33	(17)
12	36.42 ± 0.27	(26)	37.50 ± 0.20	(26)	36.52 ± 0.31	(17)
13	36.52 ± 0.34	(26)	37.61 ± 0.30	(26)	36.48 ± 0.32	(17)
14	36.51 ± 0.30	(26)	37.60 ± 0.21	(26)	36.49 ± 0.32	(17)
15	36.48 ± 0.30	(26)	37.58 ± 0.23	(26)	36.52 ± 0.31	(17)
16	36.53 ± 0.29	(26)	37.67 ± 0.23	(26)	36.58 ± 0.33	(17)
17	36.63 ± 0.35	(26)	37.77 ± 0.23	(26)	36.68 ± 0.31	(17)
18	36.78 ± 0.37	(26)	37.83 ± 0.24	(26)	36.90 ± 0.37	(17)
19	37.01 ± 0.48	(26)	37.99 ± 0.24	(26)	37.07 ± 0.42	(17)
20	37.13 ± 0.68	(16)	38.18 ± 0.21	(26)	37.28 ± 0.51	(16)
21	37.11 ± 0.62	(18)	38.21 ± 0.22	(26)	37.36 ± 0.52	(17)
22	37.11 ± 0.47	(18)	38.14 ± 0.21	(26)	37.39 ± 0.43	(17)
23	37.11 ± 0.42	(20)	38.11 ± 0.19	(26)	37.23 ± 0.47	(17)
0	36.87 ± 0.45	(24)	38.01 ± 0.20	(26)	37.29 ± 0.40	(16)
1	36.87 ± 0.48	(24)	37.96 ± 0.18	(26)	37.14 ± 0.55	(17)
2	36.77 ± 0.51	(26)	37.88 ± 0.19	(26)	37.13 ± 0.43	(17)
3	36.73 ± 0.55	(25)	37.90 ± 0.19	(26)	36.92 ± 0.54	(17)
4	36.55 ± 0.42	(26)	37.94 ± 0.24	(26)	36.74 ± 0.59	(17)
5	36.49 ± 0.59	(25)	37.84 ± 0.23	(26)	36.80 ± 0.52	(17)
6	36.57 ± 0.54	(26)	37.84 ± 0.25	(26)	36.77 ± 0.50	(17)
7	36.66 ± 0.47	(26)	37.84 ± 0.30	(26)	36.82 ± 0.41	(17)
8	36.77 ± 0.44	(26)	37.68 ± 0.35	(26)	36.81 ± 0.48	(17)

Table S10. Inactive T_b throughout the day during the four stages of lactation. The data are expressed as means ± S.D.

Time (h)	Lactation			
	DOL 1-5 (n)	DOL 6-10 (n)	DOL 11-15 (n)	DOL 16-20 (n)
10	37.76 ± 0.24 (26)	37.85 ± 0.32 (26)	37.84 ± 0.25 (26)	37.59 ± 0.31 (25)
11	37.63 ± 0.27 (26)	37.60 ± 0.28 (26)	37.62 ± 0.23 (26)	37.41 ± 0.28 (25)
12	37.56 ± 0.23 (26)	37.58 ± 0.20 (26)	37.62 ± 0.22 (26)	37.28 ± 0.30 (25)
13	37.65 ± 0.33 (26)	37.70 ± 0.38 (26)	37.72 ± 0.29 (25)	37.50 ± 0.51 (25)
14	37.62 ± 0.29 (26)	37.66± 0.26 (26)	37.74 ± 0.21 (26)	37.43 ± 0.35 (25)
15	37.54 ± 0.25 (26)	37.65 ± 0.28 (26)	37.73 ± 0.25 (26)	37.46 ± 0.35 (25)
16	37.65 ± 0.30 (26)	37.72 ± 0.28 (26)	37.78 ± 0.22 (26)	37.59 ± 0.42 (25)
17	37.67 ± 0.26 (26)	37.85 ± 0.32 (26)	37.95 ± 0.25 (26)	37.74 ± 0.32 (25)
18	37.70 ± 0.36 (26)	37.89 ± 0.29 (26)	38.05 ± 0.22 (26)	37.82 ± 0.42 (25)
19	37.84 ± 0.32 (26)	37.97 ± 0.32 (26)	38.23 ± 0.31 (26)	38.09 ± 0.37 (25)
20	38.07 ± 0.31 (26)	38.24 ± 0.36 (26)	38.41 ± 0.30 (25)	38.32 ± 0.28 (23)
21	38.02 ± 0.31 (25)	38.29 ± 0.29 (26)	38.38 ± 0.27 (25)	38.23 ± 0.38 (25)
22	37.95 ± 0.37 (26)	38.22 ± 0.30 (25)	38.30 ± 0.32 (26)	38.12 ± 0.42 (25)
23	37.91 ± 0.36 (25)	38.27 ± 0.24 (26)	38.29 ± 0.36 (26)	38.13 ± 0.43 (24)
0	37.94 ± 0.32 (26)	38.18 ± 0.23 (26)	38.11 ± 0.36 (25)	38.01 ± 0.41 (25)
1	37.85 ± 0.35 (26)	38.04 ± 0.29 (26)	38.03 ± 0.28 (26)	38.08 ± 0.32 (25)
2	37.80 ± 0.29 (26)	37.99 ± 0.27 (25)	38.07 ± 0.34 (26)	37.89 ± 0.30 (25)
3	37.73 ± 0.27 (26)	38.00 ± 0.32 (26)	38.11 ± 0.30 (26)	37.94 ± 0.28 (25)
4	37.87 ± 0.37 (25)	38.02 ± 0.28 (25)	38.06 ± 0.36 (26)	37.85 ± 0.37 (23)
5	37.67 ± 0.28 (25)	37.89 ± 0.30 (26)	38.00 ± 0.36 (25)	37.90 ± 0.28 (25)
6	37.70 ± 0.28 (25)	37.90 ± 0.39 (26)	38.04 ± 0.34 (25)	37.93 ± 0.32 (25)
7	37.80 ± 0.38 (25)	37.86 ± 0.43 (25)	38.02 ± 0.29 (26)	37.73 ± 0.34 (24)
8	37.77 ± 0.31 (26)	37.87 ± 0.36 (25)	37.86 ± 0.36 (26)	37.74 ± 0.35 (25)