The Journal of Experimental Biology 206, 4255-4266

doi:10.1242/jeb.00674

Limits to sustained energy intake

VI. Energetics of lactation in laboratory mice at thermoneutrality

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Accepted 14 August 2003

Summary

The limits to sustained energy intake are important because of their implications for reproductive output, foraging behaviour and thermoregulatory capabilities. Recent attempts to elucidate the nature of the limits to sustained energy intake have focused on peak lactation, which is the most energetically demanding period for female mammals. The hypothesis that performance of lactating animals is limited peripherally by the capacity of mammary glands to produce milk has received the most attention. However, some empirical data cannot be explained by the peripheral limitation hypothesis. Here, we present a novel hypothesis that the limits to sustained energy intake at peak lactation are imposed by the capacity of the animal to dissipate body heat generated as a by-product of processing food and producing milk. To test the heat dissipation limit hypothesis we challenged reproducing MF1 laboratory mice (*N*=67) with a reduced potential heat flow between the animal and the environment by exposing them to 30°C (thermoneutral zone). We compared their food intake and reproductive output at peak lactation with animals studied previously at 21°C (*N*=71) and 8°C (*N*=15). Mice lactating at 30°C had a significantly lower mean asymptotic food intake (12.4 g day^{-1}) than those at 21°C (23.5 g day^{-1}) and 8°C (28.6 g day^{-1}). On average, mice at 30°C raised significantly fewer (9.8) and smaller (6.1 g) pups than those at 21°C (11.3 pups; 7.0 g per pup) and smaller pups than those at 8°C (9.6 pups; 7.3 g per pup). Consequently, mean litter mass at 30°C (56.0 g) was significantly lower than at 21°C (77.1 g) and at 8°C (68.7 g). The mean rate of litter mass increase at 30°C (2.1 g day^{-1}) was also lower than at 21°C (3.1 g day^{-1}). The reduced food intake and low reproductive output in mice lactating at 30°C are consistent with the heat dissipation limit hypothesis.

Key words: asymptotic food intake, digestibility, reproductive output, peripheral limit, heat dissipation limit, laboratory mouse, *Mus musculus*.

Introduction

The maximum sustained rate at which animals can process energy is an important parameter because it may provide an upper bound that constrains many aspects of animal performance, including reproductive output, foraging behaviour and thermoregulatory capabilities (Drent and Daan, 1980; Peterson et al., 1990; Weiner, 1992; Hammond and Diamond, 1997; Speakman, 2000). Considerable interest has been paid to the factors that might impose intrinsic physiological limits on this maximum. Historically, two different types of limitation have been distinguished. One type of limit is that imposed centrally by the energy-supplying machinery, i.e. the alimentary tract and associated organs such as the liver (e.g. Kirkwood, 1983; Perrigo, 1987; Hammond and Diamond, 1992, 1994; Koteja, 1996). The second type of limit is that imposed peripherally by the energy-consuming machinery, i.e. effector organs (e.g. Hammond et al., 1996; Rogowitz, 1998). These views have been called the 'central limitation hypothesis' and the 'peripheral limitation hypothesis', respectively. An alternative model is that all aspects of animal performance are optimally matched (i.e. evolutionarily coadjusted to operate up to a similar level) such that no single organ system is limiting, and the component systems have no excess capacity (the 'symmorphosis hypothesis'; Weibel, 1987; Weibel et al., 1991).

One system that has received considerable attention as a model for testing these ideas is the period of late lactation in small rodents (e.g. Perrigo, 1987; Weiner, 1987; Kenagy et al., 1989; Hammond and Diamond, 1992, 1994; Hammond et al., 1994, 1996; Rogowitz and McClure, 1995; Koteja, 1996; Speakman and McQueenie, 1996; Rogowitz, 1998; Hammond and Kristan, 2000; Johnson and Speakman, 2001; Johnson et al., 2001a,b,c). Increases in the mass of alimentary tract and liver at peak lactation, resulting in increased resting metabolic rate (*RMR*), and a constant ratio between daily energy intake

and *RMR* support the hypothesis that the limits in late lactation are imposed centrally (Speakman and McQueenie, 1996). Some experimental manipulations of mice during late lactation to increase the energy demands placed on the mother [enlarging litter size by cross-fostering (Hammond and Diamond, 1992; Johnson et al., 2001a), prolonging lactation to 24 days (Hammond and Diamond, 1994) and forcing animals to run to obtain their food (Perrigo, 1987)] have demonstrated a resistance to breach the upper limit of food intake established in unmanipulated mothers. This result is consistent with the central limitation hypothesis, since different manipulations might be anticipated to generate different peripheral combinations of energy requirements and hence no uniformity in the maximum food intake.

Yet further manipulations, however, have demonstrated that under certain conditions mice are able to increase their food intake beyond the apparent maximum sustained level of unmanipulated animals. In particular, exposing mice during late lactation to cold temperatures resulted in a significant elevation of their energy intake (Hammond et al., 1994; Johnson and Speakman, 2001), which is incompatible with the central limitation hypothesis. Consequently, Hammond et al. (1994) suggested that lactating mice are limited peripherally by the milk production capacity of the mammary glands and regulate their food intake to match this limit. Hence, when manipulations are performed that require the female to elevate this capacity (such as enlarging litter size or prolonging lactation) she is unable to respond because the mammary glands at peak lactation are already at maximal performance. Food intake does not increase in response to such manipulations because the extra food could not be converted into additional milk. However, when lactating animals are faced with an additional demand, which increases maternal maintenance expenditure but does not require elevated milk production, the animals demonstrate their capacity to process additional food (Kenagy et al., 1989; Hammond et al., 1994; Rogowitz, 1998; Hammond and Kristan, 2000; Johnson and Speakman, 2001). This combined demands explanation of the peripherally mediated limit at peak lactation seemed to be settled when Hammond et al. (1996) demonstrated that surgical removal of half of the mammary glands did not produce a compensatory response in the remaining tissue. In addition, Rogowitz (1998) demonstrated in the hispid cotton rat (*Sigmodon hispidus*) that milk energy output remained constant between warm and cold temperatures, suggesting independence of milk production and the expenditure on other components of the energy budget, also consistent with the combined demands interpretation.

Recent data, however, have cast doubt on this consensus opinion regarding the limits on food intake at peak lactation. In particular, Johnson et al. (2001c) found that when mice were made simultaneously pregnant during lactation, a manipulation that does not demand greater lactational output, the animals did not respond by elevating their food intake. More significantly, the combined demands interpretation suggests that the energy exported as milk should be fixed during late lactation. Yet Johnson and Speakman (2001) found elevated milk production in parallel with elevated food intake during cold exposure, suggesting that the mammary glands were not working at maximal capacity at 21°C and could not therefore be imposing a peripheral limit on maximal food intake.

Here, we propose a novel hypothesis that could explain these data and provide a test of this hypothesis. Rather than reflecting a combination of peripheral energy demands that are built up from lactation requirements (defined at the mammary glands) and thermoregulatory requirements (presumably set in part by heat production capacity of brown adipose tissue), we suggest that the level of food intake at peak lactation is set by a central process independent of the capacity of the alimentary tract. We suggest that this central limitation on food intake is the maximal capacity of the animal to dissipate body heat generated as a by-product of processing food and producing milk. It is well established that the capacity to dissipate heat depends on conductivity of the insulating surface and the difference between body temperature and ambient temperature. We suggest that at room temperature (21°C) food intake increases during lactation but reaches a plateau, because this is the point at which further intake of food and production of milk would generate so much heat that it would be beyond the capacity of the animal to dissipate it. This may explain why mice at room temperature faced with any additional demands at peak lactation – whether these require increases in milk energy output (e.g. enlarged litter size or prolonged lactation) or not (e.g. concurrent pregnancy or exercising to obtain their food) – do not breach the upper limit to food intake of unmanipulated animals. At lower ambient temperatures, however, this constraint is released because of the greater driving gradient permitting greater heat flow. This allows the animal to elevate its food intake, supporting greater lactational performance.

To test the heat dissipation limit hypothesis, we bred MF1 laboratory mice (*Mus musculus* L.) at 30°C, which we have shown previously to be in the thermoneutral zone of this strain (Speakman and Rossi, 1999). This is 9°C warmer than our measurements at 21°C, at which food intake appeared to be limited at approximately 23 g day^{-1} (Johnson et al., 2001a), and 22°C warmer than cold exposure, in which food intake appeared to be limited at around 32 g day^{-1} (Johnson and Speakman, 2001). The combined demands interpretation of the peripheral limitation hypothesis predicts that at 30°C the lower maternal thermoregulatory demands should result in a reduction in food intake. The heat dissipation limit hypothesis predicts the same response in food intake but for a different reason. The hypotheses differ, however, in their predicted effects on lactational performance. The combined demands/peripheral limitation hypothesis predicts that milk production and hence reproductive output should be unaffected by temperature, since the milk production is limited by the capacity of mammary glands, and the energy allocated to milk is additional to thermoregulatory requirements. By contrast, the heat dissipation limit hypothesis predicts that a reduced potential heat flow at 30°C should cause a reduction in milk

production and hence decrease in reproductive output because greater levels of milk production would lead to detrimentally prolonged maternal hyperthermia. To distinguish between the two hypotheses, we measured food intake and reproductive output (litter size, pup body mass, litter mass and litter mass increase) of mice lactating at thermoneutral temperature (30°C) and compared these traits with the same parameters measured in mice at 21°C (Johnson et al., 2001a) and 8°C (Johnson and Speakman, 2001).

Materials and methods

Animals and experimental protocol

Experiments were conducted on 95 virgin female mice (*Mus musculus* L.: outbred MF1). Mice were housed individually in shoebox cages (44 cm×12 cm×13 cm) containing sawdust and approximately 3 g of nesting material. They were provided with supplies of water and food *ad libitum* (CRM, Pelleted Rat and Mouse Breeder and Grower Diet; Special Diets Services, BP Nutrition, Witham, UK). The ambient temperature was regulated at 30°C (range 29–31°C), with a mean absolute humidity of 11.0 mg l^{-1} and a photoperiod of 16 h:8 h L:D (lights on 06:00 h).

The mice were 6 weeks old at the beginning of the 2-week acclimation period prior to the experimental conditions. After acclimation, 67 randomly selected females were paired with males for 7 days, after which the males were removed; the remaining 28 females were used as non-reproductive controls. The mice were checked twice a day to determine the day of parturition (day 0 of lactation). The reproductive females were divided into three groups: group A (N=12), for which body mass and food intake were measured during both pregnancy and lactation (days –7 to 17), group B (N=31), measured between days 0 to 17 of lactation, and group C (N=24), measured only at peak lactation (days 9–17). Litter size and mass were measured for all litters.

Body mass and food intake

The body mass of females, litter mass and the mass of food remaining in the food hoppers were measured (±0.01 g; Sartorius top-balance) daily, between 09:00 h and 11:00 h. The food hoppers were then refilled and reweighed. Food intake was calculated from the mass of food removed from the hopper each day. Sorting through the sawdust and nesting material of 93 cages (used in the digestibility measurements) revealed that spillage of food from the hoppers was negligible (0.7±0.4% of the food removed each day).

Digestibility of dry mass and energy

Digestibility measurements were conducted on 18 reproductive females (group B) during the last week of pregnancy, on day 6 of lactation and on day 13 of lactation. Simultaneous measurements of digestibility were also performed on 13 non-reproductive females. Digestibility was measured over 24 h by placing each non-lactating female or lactating female and her offspring in a cage with their nesting material and fresh sawdust and providing them with water and a weighed portion of food. Samples of the food were taken to determine dry mass content (94.4±0.3%; N=10). Uneaten food (including orts) and female faeces were separated manually from the nesting material and sawdust and dried at 60°C to a constant mass. The gross energy content of dry food (GE_{food}; 18.36±0.08 kJ g^{-1}; N=2) and of dry faeces (GE_{faeces}; kJ g^{-1}) from five reproductive and five non-reproductive females was measured by bomb calorimetry (Gallenkamp Autobomb Adiabatic Bomb Calorimeter; Rowett Research Institute Analytical Services, Aberdeen, UK).

We calculated female dry mass food intake for each trial (FI_{DM}; g) as:

$$FI_{DM} = (\text{mass food given} \times \text{dry mass content}) - (\text{dry mass uneaten food}) . \quad (1)$$

The apparent digestibilities of dry mass food (d_m;%) and energy (d_e;%) were calculated following Drożdż (1975) as:

$$d_m = 100 \times \left(\frac{FI_{DM} - DM_f}{Fl_{DM}} \right), \quad (2)$$

$$d_e = 100 \times \left(\frac{(FI_{DM} \times GE_{food}) - (DM_f \times GE_{faeces})}{FI_{DM} \times GE_{food}} \right), \quad (3)$$

where DM_f is dry mass of faeces (g).

Statistics

Data are reported as means ± S.D. (N = sample size). The significance of changes in body mass, food intake and digestibility over time was assessed by repeated measures analysis of variance (ANOVA). The Tukey *post-hoc* test was used when differentiation between days of reproduction was required. For percentage data (digestibility of dry mass and energy), arcsine-square-root transformations were performed prior to analysis (Zar, 1996). The relationships between energetic and reproductive parameters were examined by least-squares linear regression analysis. The regression lines were compared using analysis of covariance (ANCOVA). To test for differences in food intake, digestibility and energy content of faeces between reproductive and non-reproductive females, we used two-sample *t*-tests. The mass-adjusted values are residuals from the least-squares regression lines on female body mass. Relationships between the residuals were described using Pearson product-moment correlation coefficients. All statistical analyses were conducted using Minitab for Windows (version 13.31; Minitab Inc., State College, PA, USA; Ryan et al., 1985). Statistical significance was determined at P<0.05. All tests were two-tailed.

Results

Body mass

The body mass of reproductive female mice (group A) increased significantly during the last week of pregnancy (repeated measures ANOVA, $F_{6,77}$=164.3, P<0.001, N=12;

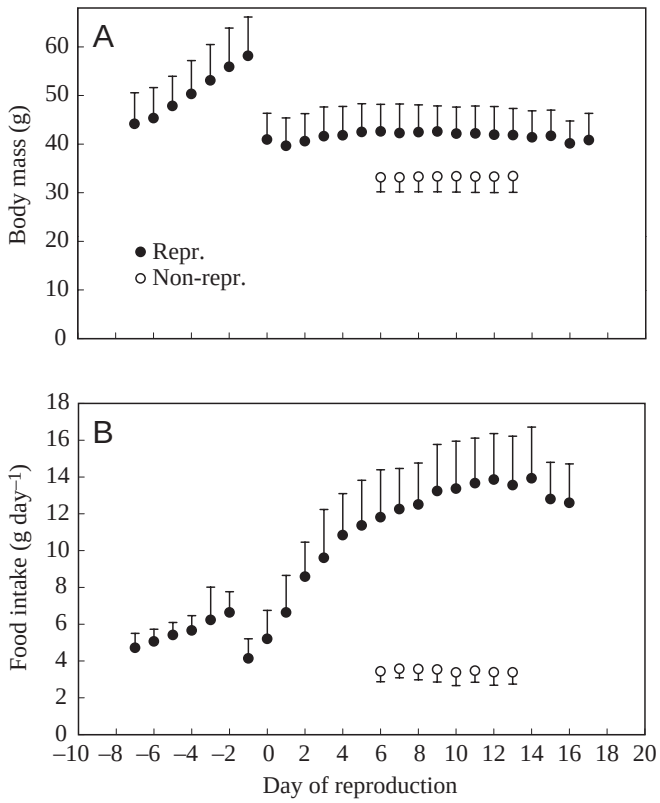

Fig. 1. Mean body mass (A) and food intake (B) of reproductive female mice (filled circles; N=12) throughout pregnancy and lactation at 30°C. Parturition is day 0. Data for non-reproductive females (open circles, N=28) are also shown. Error bars indicate 1 S.D.

Fig. 1A). Body mass of the females averaged 44.2±6.4 g on day –7 (where day 0 is the day of parturition), increasing to 58.2±8.0 g on day –1 (all Tukey pairwise comparisons amongst days –7 to –1, P<0.05).

There was significant day-to-day variation in body mass of the females during lactation (repeated measures ANOVA, $F_{17,198}$=4.1, P<0.001, N=12), but these changes were relatively minor compared with the changes during pregnancy. Female body mass remained stable between days 3–15 of lactation and averaged 42.1±5.5 g (all Tukey pairwise comparisons amongst days 3–15, P>0.05). There was a small but significant decrease in body mass on day 16 to a mean of 40.2±4.6 g (all Tukey pairwise comparisons between days 16 and 3–15, P<0.05), and this lower body mass was maintained on day 17 of lactation (Tukey pairwise comparison between days 16 and 17, P>0.05).

The mean body mass of non-reproductive females, measured between days 6 and 13 of lactation of the reproductive females, did not change significantly and averaged 33.3±3.1 g (repeated measures ANOVA, $F_{7,216}$=1.9, P=0.08, N=28; Fig. 1A).

Food intake

The mean food intake of reproductive females (group A) increased significantly during the last week of pregnancy (repeated measures ANOVA, $F_{6,77}$=13.5, P<0.001, N=12; Fig. 1B). On each day between –6 and –2, the females ate slightly but not significantly more food than on the previous day (all Tukey pairwise comparisons, P>0.05), reaching the maximum of 6.6±1.1 g on day –2 (all Tukey pairwise comparisons between days –2 and –7 to –4, P<0.05). Food intake decreased significantly to 4.1±1.1 g on day –1 (all Tukey pairwise comparisons between days –1 and –6 to –2, P<0.05).

Food intake of the mice increased significantly during lactation (repeated measures ANOVA, $F_{16,187}$=39.5, P<0.001, N=12), from a mean of 5.2±1.5 g on day 0 (parturition) to 12.5±2.3 g on day 8 (all Tukey pairwise comparisons between days 8 and 0–4, P<0.05). Over the next eight days (days 9–16 of lactation), food intake remained stable and averaged 13.4±2.1 g day^{-1} (all Tukey pairwise comparisons among days 9–16, P>0.05).

For reproductive females from groups B and C, the changes in food intake during lactation were similar to those described for females from group A. However, on day 14 of lactation, there was a small but significant decrease in food intake, which lasted till day 16. The decrease in food intake in these groups (B and C) may have been due to the doubly labelled water measurements or collection of milk samples on these days (Król and Speakman, 2003). However, similar but less noticeable changes were observed in the animals where these measurements were not made (group A), suggesting that our experimental procedures were only partly responsible for this effect. We therefore calculated asymptotic daily food intake from the mean food intake between days 9 and 13 for all groups.

The food intake of non-reproductive females, measured for eight consecutive days, remained constant at 3.5±0.5 g day^{-1} (repeated measures ANOVA, $F_{7,216}$=1.2, P=0.31, N=28; Fig. 1B). This value corresponds to 60.1±8.4 kJ day^{-1} gross energy intake (*GEI*; food intake multiplied by the gross energy content of food) and to 45.6±6.3 kJ day^{-1} metabolizable energy intake (*MEI*; *GEI* multiplied by apparent digestibility of energy, assuming that urinary energy loss is 3% of the energy digested). For the 17 non-reproductive females for which both food intake and daily energy expenditure (*DEE*) were measured (Król and Speakman, 2003), *GEI* was 1.3×*DEE* (range 1.1–1.5), while *MEI* was 1.0×*DEE* (range 0.8–1.1). For the 15 non-reproductive females for which both food intake and *RMR* were measured (Król et al., 2003), *GEI* and *MEI* were 3.3×*RMR* (range 2.5–3.8) and 2.5×*RMR* (range 1.9–2.8), respectively.

In both reproductive and non-reproductive groups of mice, heavier females ate more food (peak lactation, r^2=0.37, $F_{1,65}$=37.8, P<0.001; non-reproductive mice, r^2=0.50, $F_{1,26}$=26.2, P<0.001; Fig. 2). The interaction between body mass and reproductive status was significant (ANCOVA, $F_{1,91}$=6.2, P=0.015), indicating a steeper slope of the regression line for reproductive than for non-reproductive females. For a female mouse with a body mass of 37.5 g (mean value for both groups of mice), the predicted food intake would be 11.4 g day^{-1} and 4.0 g day^{-1} for reproductive and non-

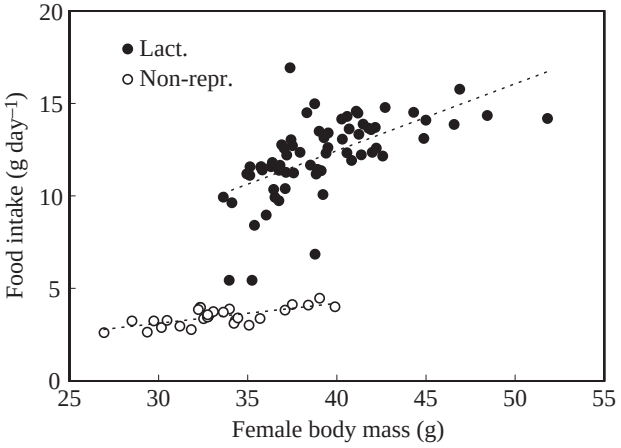

Fig. 2. Food intake as a function of body mass for lactating (filled circles, y=–2.11+0.36x; N=67) and non-reproductive (open circles, y=–0.15+0.11x; N=28) female mice at 30°C. Food intake is the mean value for days 9–13 of lactation (reproductive females) or the mean value for 8 consecutive days (non-reproductive females). Body masses are the mean values for the same days as for food intake.

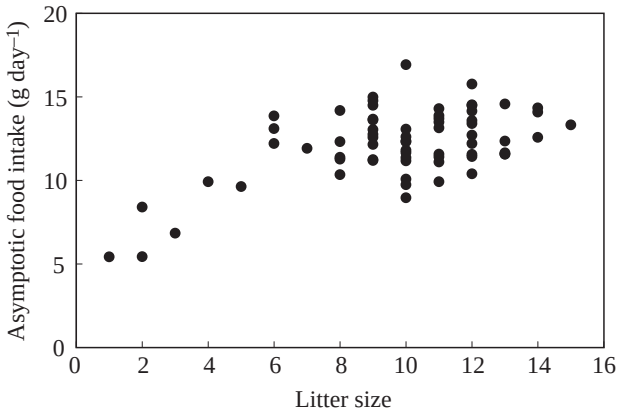

Fig. 3. Asymptotic food intake of lactating female mice exposed to 30°C (N=67) as a function of litter size. Asymptotic food intake is the mean value for days 9–13 of lactation. Litter size is that on day 14 of lactation. Females with 6 pups ate more food than those with 1–3 pups. For litter size increasing from 6 to 15, no further increase in asymptotic food intake was observed (for statistical details, see Results).

reproductive animals, respectively. Analyses of mass-adjusted food intake (the residuals from the regression lines on body mass presented in Fig. 2, added to the values of predicted mean food intake) showed that reproductive females at peak lactation ate significantly more food than non-reproductive mice (t_{77}=34.0, P<0.001).

The asymptotic food intake of lactating females was related to litter size on day 14 of lactation (ANOVA, $F_{14,52}$=5.0, P<0.001, N=67). Food intake at peak lactation increased significantly as litter size increased from 1 to 6 pups (all Tukey pairwise comparisons, P<0.05; Fig. 3). No further increase in asymptotic food intake was observed as litter size increased from 6 to 15 (all Tukey pairwise comparisons amongst litter sizes 6–15, P>0.05). The mean asymptotic food intake for females raising 6–15 pups was 12.6±1.6 g day^{-1} (N=61). This value corresponds to 218.7±27.0 kJ day^{-1} *GEI* and to 163.7±20.2 kJ day^{-1} *MEI*. For the 24 females for which both food intake and *DEE* were measured at peak lactation (Król and Speakman, 2003), the asymptotic *GEI* was 2.9×*DEE* (range 1.9–4.1), while the asymptotic *MEI* was 2.2×*DEE* (range 1.4–3.1). For the 28 females for which both food intake and *RMR* were measured at peak lactation (Król et al., 2003), the asymptotic *GEI* was 7.5×*RMR* (range 4.0–10.1) and the asymptotic *MEI* was 5.6×*RMR* (range 3.0–7.5).

Digestibility of dry mass and energy

The apparent digestibility of dry mass (d_m) during reproduction decreased from a mean of 77.9±2.1% during the last week of pregnancy to 76.6±1.9% on day 6 of lactation and 74.9±2.5% on day 13 (repeated measures ANOVA, $F_{2,51}$=18.2, P<0.001, N=18; all three means significantly different, Tukey pairwise comparisons, P<0.05; Table 1). The three corresponding estimates of dry mass digestibility did not differ in non-reproductive females (76.9±2.2%, 76.2±2.8% and 76.1±1.9%, respectively; repeated measures ANOVA, $F_{2,36}$=1.0, P=0.38, N=13). Dry mass digestibility measured simultaneously in reproductive and non-reproductive females did not differ between the two groups (pregnancy, t_{25}=1.3, P=0.19; day 6 of lactation, t_{20}=0.4, P=0.68; day 13 of lactation, t_{28}=1.4, P=0.16). In reproductive females (N=18), faecal production (g dry mass day^{-1}) was positively related to food intake (g dry mass day^{-1}) during the last week of pregnancy (y=0.05+0.21x, r^2=0.75, $F_{1,16}$=47.0, P<0.001), on day 6 of lactation (y=–0.56+0.29x, r^2=0.71, $F_{1,16}$=39.9, P<0.001) and on day 13 of lactation (y=–0.20+0.27x, r^2=0.69, $F_{1,16}$=34.9, P<0.001). In non-reproductive females, for which we randomly assigned one of the three estimates of dry mass

Table 1. *Digestibility of dry mass (d_m), gross energy content of faeces (GE_{faeces}) and digestibility of energy (d_e) measured during 24-h feeding trials in reproductive and non-reproductive female mice at 30°C*

Trait/female group	N	Last week of pregnancy	Day 6 of lactation	Day 13 of lactation
d_m (%)				
Reproductive	18	77.9±2.1	76.6±1.9	74.9±2.5
Non-reproductive[a]	13	76.9±2.2	76.2±2.8	76.1±1.9
GE_{faeces} (kJ g^{-1} dry mass)				
Reproductive	5	16.82±0.25	16.67±0.14	16.64±0.32
Non-reproductive[a]	5	16.85±0.29	16.77±0.16	16.71±0.25
d_e (%)				
Reproductive	18	79.9±1.9	78.7±1.7	77.1±2.2
Non-reproductive[a]	13	78.9±2.0	78.3±2.5	78.2±1.8

Values are means ± S.D.
[a]Measured on the same days as reproductive females.
N, number of females.

digestibility, the relationship between the faecal production and the food intake was also highly significant (y=–0.02+0.24x, r^2=0.96, $F_{1,11}$=233.5, P<0.001, N=13).

The gross energy content of the faeces (GE_{faeces}) of reproductive females did not differ between pregnancy, day 6 of lactation and day 13 of lactation (repeated measures ANOVA, $F_{2,12}$=1.1, P=0.38, N=5; Table 1). There was also no difference between the three estimates of energy content of faeces of non-reproductive females (repeated measures ANOVA, $F_{2,12}$=1.1, P=0.36, N=5) or between reproductive females and non-reproductive individuals measured at the same time (pregnancy, t_7=0.2, P=0.87; day 6 of lactation, t_7=1.0, P=0.34; day 13 of lactation, t_7=0.4, P=0.71). We therefore used the mean gross energy content of faeces for all females (16.74±0.15 kJ g^{-1} dry mass, N=10) to calculate the digestibility of energy.

Since the equations for calculating apparent digestibility of dry mass and energy differ only in the two constants (gross energy content of food and faeces), the digestibility of dry mass and energy were closely correlated (Table 1). Therefore, the statistics performed on the estimates of energy digestibility gave similar results to those on digestibility of dry mass. There was a decrease in the apparent digestibility of energy during reproduction (repeated measures ANOVA, $F_{2,51}$=18.1, P<0.001, N=18; the means for pregnancy, day 6 of lactation and day 13 of lactation were 79.9±1.9, 78.7±1.7 and 77.1±2.2%, respectively; all three means significantly different, Tukey pairwise comparison, P<0.05). The three estimates of the digestibility of energy in non-reproductive females (78.9±2.0%, 78.3±2.5% and 78.2±1.8%) did not differ (repeated measures ANOVA, $F_{2,36}$=1.0, P=0.38, N=13). The digestibility of energy did not differ between reproductive and non-reproductive females measured simultaneously (pregnancy, t_{25}=1.3, P=0.20; day 6 of lactation, t_{20}=0.4, P=0.71; day 13 of lactation, t_{28}=1.4, P=0.16).

Reproductive output

For six reproductive females, we recorded high mortality of pups (three or more pups dead) within 48 h of parturition. Consequently, the females raising these litters (in which only 1–5 pups remained) had lower asymptotic food intake than females raising 6–15 pups (Fig. 3). The data for litters consisting of 1–5 pups are presented together with data from larger litters in Figs 4–6 but were excluded from further analyses. On day 14 of lactation, the mean litter size of females (N=61) raising 6–15 pups was 10.4±2.0, with a mean pup body mass of 5.9±1.3 g and a mean litter mass of 59.3±8.5 g. The rate of litter mass increase between days 13 and 14 of lactation averaged 2.2±0.8 g day^{-1}.

In all analyses presented below, litter size and mass as well as pup body mass refer to day 14 of lactation, while maternal body mass and asymptotic food intake are the mean values for days 9–13 of lactation. All analyses were performed on the 61 lactating females and their litters. Litter mass was positively related to litter size (r^2=0.20, $F_{1,59}$=14.9, P<0.001; Fig. 4A). Pup mass decreased with increasing litter size (r^2=0.51, $F_{1,59}$=60.3, P<0.001; Fig. 4B). Litter size was not related to maternal body mass (r^2=0.001, $F_{1,59}$=0.1, P=0.79; Fig. 5A); however, heavier females were associated with larger litter mass (r^2=0.29, $F_{1,59}$=24.2, P<0.001; Fig. 5B) and greater pup body mass (r^2=0.18, $F_{1,59}$=13.0, P=0.001; Fig. 5C). Both litter mass (r^2=0.63, $F_{1,59}$=99.2, P<0.001) and pup body mass (r^2=0.20, $F_{1,59}$=14.9, P<0.001) were positively related to asymptotic food intake (Fig. 6). Since litter mass, pup body mass and asymptotic food intake were all related to maternal body mass, we calculated their residual values from the regression lines shown on Figs 2, 5B,C. Both residual litter mass (r=0.70, P<0.001) and residual pup body mass (r=0.27, P=0.033) were significantly correlated with residual asymptotic food intake (Fig. 7).

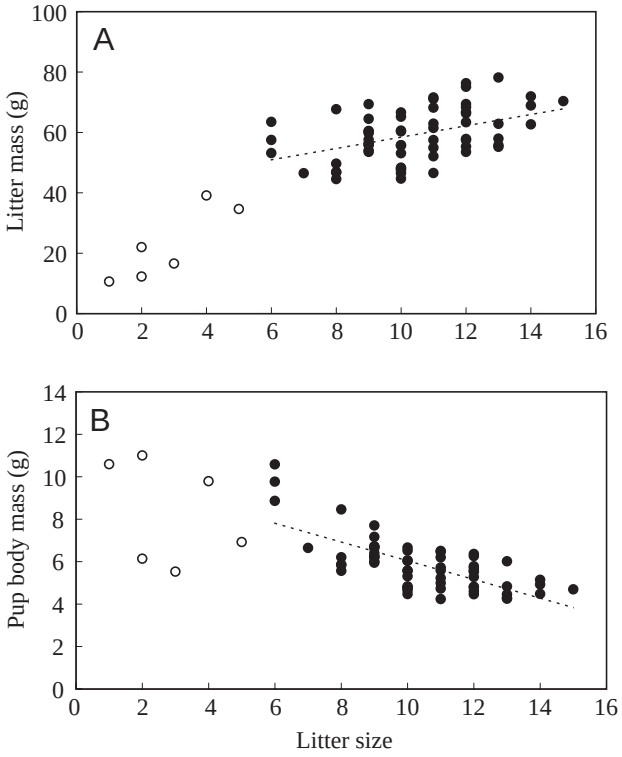

Fig. 4. Litter mass (A) and pup body mass (B) as a function of litter size for mice at 30°C raising 6–15 pups (filled circles; N=61). Data for mice raising 1–5 pups are also shown (open circles; N=6). All parameters are those on day 14 of lactation. Pup body mass is the litter mass divided by litter size. The relationships are described by y=39.58+1.89x (A) and y=10.48–0.44x (B).

The effect of temperature on maternal body mass, food intake and reproductive output

We compared the body mass, food intake and reproductive output of mice that were exposed to 30°C (present study), 21°C (Johnson et al., 2001a) and 8°C (Johnson and Speakman, 2001). Unless stated otherwise, the sample sizes for the hot, warm and cold groups were 67, 71 and 15, respectively. All females were raising their first litters. The hot and the warm mice were exposed to 30°C and 21°C, respectively, prior to

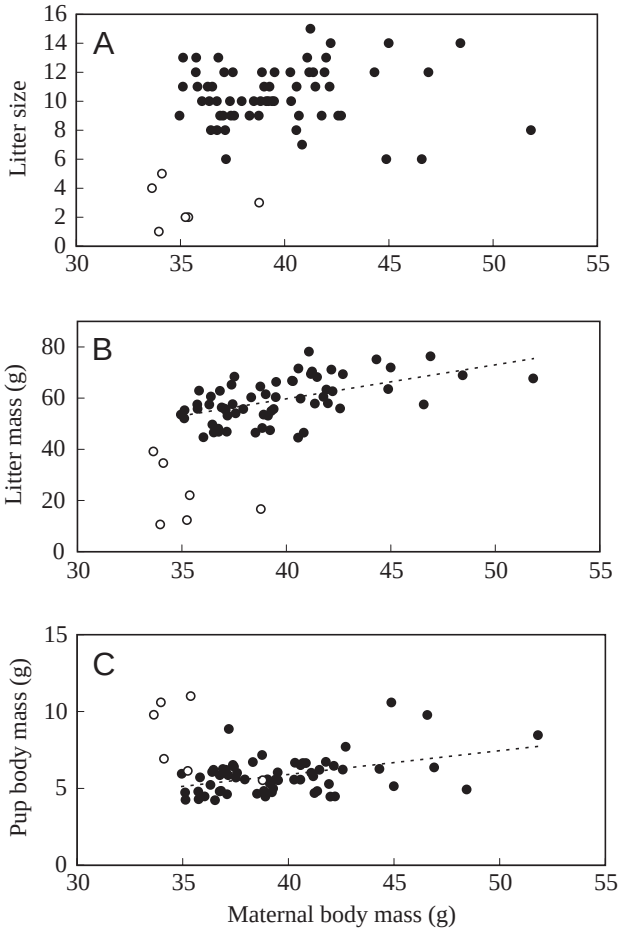

Fig. 5. Litter size (A), litter mass (B) and pup body mass (C) as a function of maternal body mass for mice at 30°C raising 6–15 pups (filled circles; N=61). Data for mice raising 1–5 pups are also shown (open circles; N=6). Litter size, litter mass and pup body mass are those on day 14 of lactation. Pup body mass is the litter mass divided by litter size. Maternal body mass is the mean value for days 9–13 of lactation. The relationships are described by y=6.40+1.33x (B) and y=–0.31+0.15x (C).

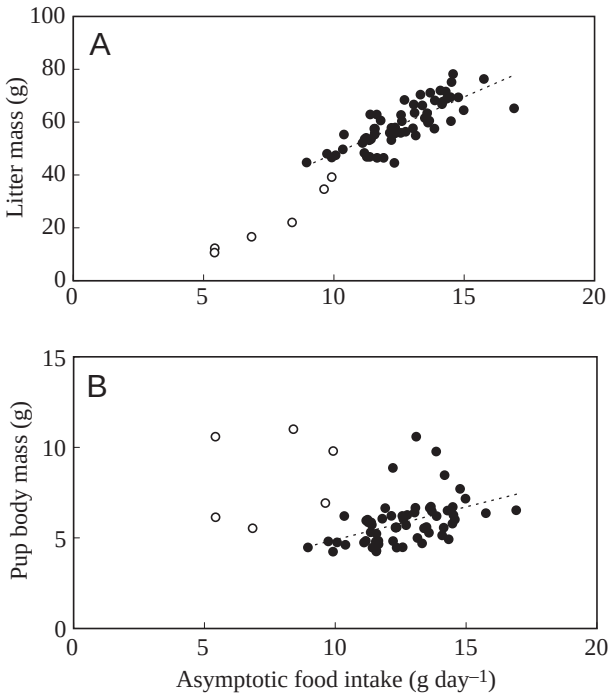

Fig. 6. Litter mass (A) and pup body mass (B) as a function of asymptotic food intake for mice at 30°C raising 6–15 pups (filled circles; N=61). Data for mice raising 1–5 pups are also shown (open circles; N=6). Litter mass and pup body mass refer to day 14 of lactation. Pup body mass is the litter mass divided by litter size. Asymptotic food intake is the mean value for days 9–13 of lactation. The relationships are described by y=4.58+4.33x (A) and y=1.25+0.36x (B).

breeding, and they were kept at those temperatures through the whole course of pregnancy and lactation. The mice in the cold group were maintained at the warm temperature until the pups had grown fur and were then exposed to 8°C from day 10 of lactation onwards.

Mean maternal body mass on day 0 of lactation differed significantly between the three groups (ANOVA, $F_{2,126}$=10.8, P<0.001), with the cold mice being slightly heavier (41.8±3.8 g) than both the hot (38.0±3.7 g, N=43) and the warm mice (37.8±3.8 g) (Fig. 8A). This difference in body mass was not related to the temperature, since at this stage the cold mice were still housed at 21°C. However, temperature did have a significant effect on the increase in body mass between days 0 and 13 of lactation (ANOVA, $F_{2,126}$=100.9, P<0.001). Over this time, the hot mice increased their mass by 0.8±2.2 g (N=43), while the warm and the cold mice increased their masses by 6.7±2.3 g and 7.3±2.4 g, respectively (Fig. 8B). As a result of the differences in body mass increase, in addition to the differences of body mass on day 0, body mass on day 13 of lactation differed significantly between the three groups (ANOVA, $F_{2,150}$=68.7, P<0.001) and averaged 39.0±3.6 g in the hot mice, 44.5±3.5 g in the warm mice and 49.1±3.5 g in the cold mice (Fig. 8C). At peak lactation, the body mass of mice exposed to all three temperatures remained stable (Fig. 1A, present study; fig. 1A in Johnson and Speakman, 2001), indicating that mice were in energy balance and responded to the increased energy demand of lactation by increasing food intake.

The asymptotic food intake in warm and cold mice in the previous papers was calculated from the mean food intake between days 13–16 of lactation. By contrast, since the hot mice may have responded to the doubly labelled water measurements or collection of milk samples (started on day 14 of lactation; Król and Speakman, 2003), we calculated their asymptotic food intake for days 9–13 of lactation. To facilitate comparison between the three groups, we used food intake measured on day 13 of lactation. The three groups differed significantly in their food intake on day 13 of lactation (ANOVA, $F_{2,150}$=260.8, P<0.001). The hot mice ate significantly less food than the warm mice (12.4±2.5 g day^{-1} and 23.5±3.3 g day^{-1}, respectively), while the cold mice, after

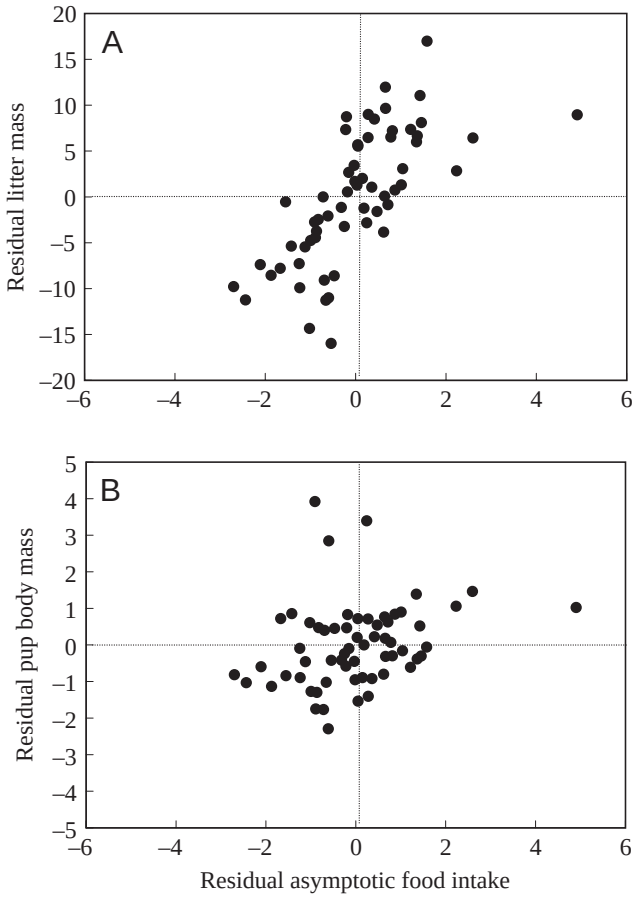

Fig. 7. Residual litter mass (A) and residual pup body mass (B) as a function of residual asymptotic food intake for mice at 30°C raising 6–15 pups (N=61). Litter mass (g) and pup body mass (g) refer to day 14 of lactation. Pup body mass is the litter mass divided by litter size. Asymptotic food intake (g day^{-1}) is the mean value for days 9–13 of lactation. Both correlations (A and B) are significant (for statistical details, see Results).

three days of exposure to 8°C, increased their food intake to 28.6±5.8 g day^{-1} (Fig. 8D). The effect of temperature on food intake remained significant after adjusting for the differences in maternal body mass (ANCOVA: interaction body mass × temperature, P=0.87; body mass effect, $F_{1,149}$=13.1, P<0.001; temperature effect, $F_{2,149}$=111.3, P<0.001).

Since the food intake of non-reproductive mice averaged 3.5 g day^{-1} at 30°C, 5.2 g day^{-1} at 21°C and 7.8 g day^{-1} at 8°C, the limit on the sustained food intake in mice lactating at these temperatures occurred at 3.5×, 4.5× and 3.7× non-reproductive intake, respectively. Assuming that non-reproductive food intake accounted for most of the maternal maintenance expenditure, the maximal amount of ingested food available for milk production was only 8.9 g day^{-1} in the hot mice, 18.3 g day^{-1} in the warm mice and 20.8 g day^{-1} in the cold mice. Thus, the exposure of mice to 30°C (compared with 21°C and 8°C) resulted in a substantial decrease in the amount of energy allocated for reproduction.

We assessed the reproductive output of mice exposed to hot, warm, and cold temperatures by comparing litter size, pup body mass and litter mass (all on day 14 of lactation) as well as the rate of litter mass increase (between days 13 and 14 of lactation). The comparison included all litter sizes.

Mean litter size differed significantly between the three groups (ANOVA, $F_{2,150}$=6.9, P=0.001), with the warm mice raising more pups (11.3±2.0) than both the hot (9.8±2.9) and the cold (9.6±3.2) mice (Fig. 9A). The effect of temperature on litter size remained significant after adjusting for the differences in maternal body mass (ANCOVA: interaction body mass × temperature, P=0.98; body mass effect, $F_{1,149}$=12.3, P=0.001; temperature effect, $F_{2,149}$=6.3, P=0.002).

Mean pup body mass differed significantly between the three groups (ANOVA, $F_{2,150}$=11.6, P<0.001), with pups in the hot temperature (6.1±1.5 g) being smaller than those from the warm (7.0±1.1 g) and from the cold (7.3±1.1 g) conditions (Fig. 9B). Pup body mass across temperature was not affected by maternal body mass (ANCOVA, P=0.98). The significant effect of temperature on pup body mass remained after adjusting for the differences in litter size (ANCOVA: interaction litter size × temperature, P=0.13; litter size effect, $F_{1,149}$=184.0, P<0.001; temperature effect, $F_{2,149}$=49.4, P<0.001).

The three groups also differed significantly in their litter mass (ANOVA, $F_{2,150}$=48.8, P<0.001), for which the hot, warm and cold mice averaged 56.0±13.7 g, 77.1±9.8 g and 68.7±18.1 g, respectively (Fig. 9C). The effect of temperature on litter mass was also significant when we adjusted for the differences in maternal body mass (ANCOVA: interaction body mass × temperature, P=0.09; body mass effect, $F_{1,149}$=24.0, P<0.001; temperature effect, $F_{2,149}$=24.0, P<0.001) and litter size (ANCOVA: interaction litter size × temperature, P=0.10; litter size effect, $F_{1,149}$=205.9, P<0.001; temperature effect, $F_{2,149}$=60.6, P<0.001).

The rate of increase in litter mass varied across temperature treatments (ANOVA, $F_{2,150}$=13.6, P<0.001), with litters in the warm condition growing faster (3.1±1.0 g day^{-1}) than litters in both hot (2.1±0.9 g day^{-1}) and cold (2.4±2.2 g day^{-1}) conditions (Fig. 9D). The effect of temperature on the litter mass increase was significant when adjusted for the differences in maternal body mass (ANCOVA: interaction body mass × temperature, P=0.08; body mass effect, $F_{1,149}$=10.8, P=0.001; temperature effect, $F_{2,149}$=8.6, P<0.001). The rate of litter mass gain across temperature was not affected by litter size (ANCOVA, P=0.32).

Discussion

In this paper, we have presented a novel hypothesis concerning the limits to sustained energy intake at peak lactation. We suggest that lactating mice are not limited peripherally by the capacity of the mammary glands for milk production (Hammond et al., 1994, 1996; Rogowitz, 1998) but that the limits are imposed by the capacity of the animal to dissipate heat generated as a by-product of processing food and

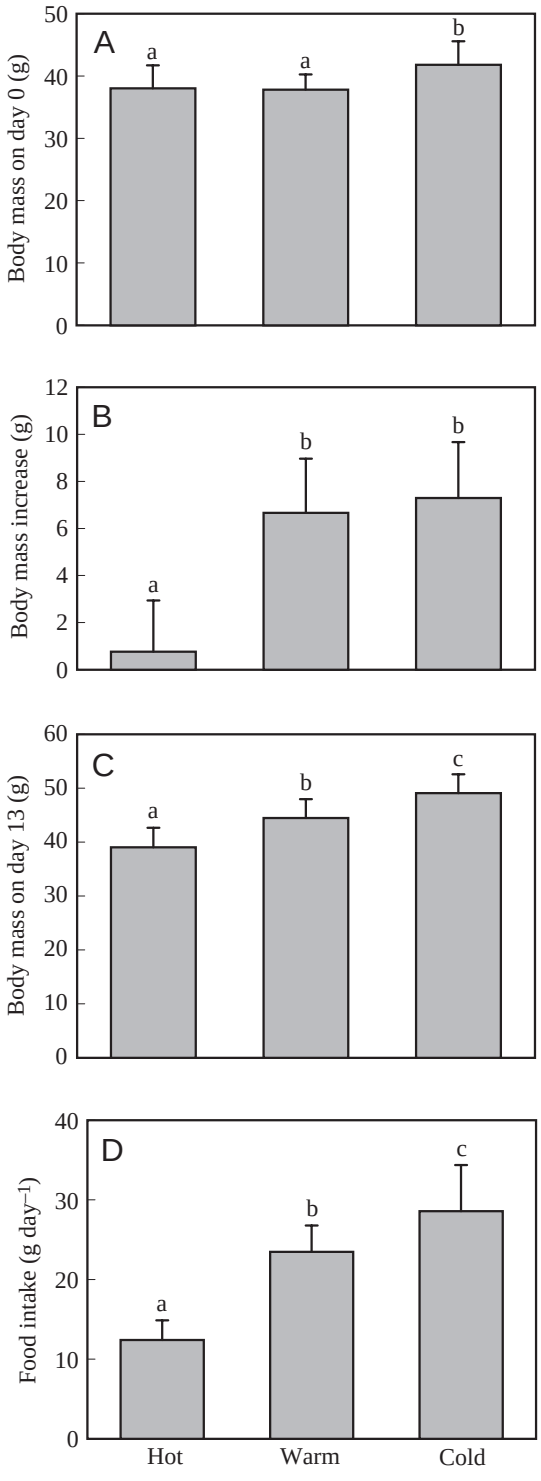

Fig. 8. Maternal body mass on day 0 of lactation (A), increase in maternal body mass over days 0–13 of lactation (B), maternal body mass on day 13 of lactation (C) and maternal food intake on day 13 of lactation (D) in mice at hot (30°C), warm (21°C) and cold (8°C) temperatures. The cold group was transferred from 21°C to 8°C on day 10 of lactation. Bars are means + 1 S.D. (*N* as in text). Different letters above bars indicate significant differences between the temperatures ($P<0.05$), as assessed by ANOVA followed by Tukey pairwise comparisons.

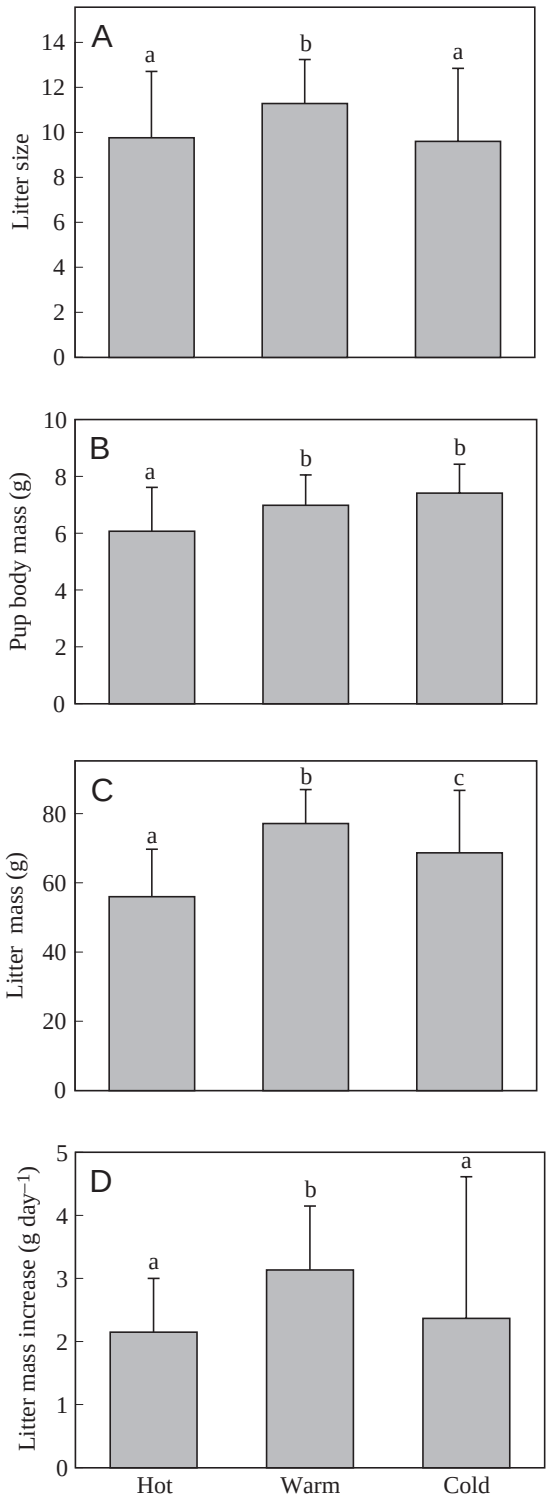

Fig. 9. Litter size (A), pup body mass (B), litter mass (C) and litter mass increase between days 13 and 14 of lactation (D) in mice lactating at hot (30°C), warm (21°C) and cold (8°C) temperatures. Litter size, pup body mass and litter mass refer to day 14 of lactation. Bars are means + 1 S.D. (*N* as in text). Different letters above bars indicate significant differences between the temperatures ($P<0.05$), as assessed by ANOVA followed by Tukey pairwise comparisons.

producing milk. The main processes that contribute to the metabolic heat production at peak lactation are the increased heat increment of feeding at elevated level (Webster, 1981; Blaxter, 1989) and the exothermic process of milk synthesis (Adels and Leon, 1986). Furthermore, it has been shown that maternal levels of progesterone and corticosterone are elevated (Stern et al., 1973). These hormones are known to have thermogenic effects and therefore may also contribute to heat production (Woodside et al., 1981). Most likely as a consequence of the increased obligatory heat production, thermogenic capacity of the brown adipose tissue in mice lactating at room temperature is suppressed (Trayhurn et al., 1982; Trayhurn, 1989). This suppression provides further support for the hypothesis that lactating females obtain their required heat from lactation.

To test the heat dissipation limit hypothesis, we studied energetics of MF1 mice lactating at 30°C (thermoneutrality) and compared their body masses, food intake and reproductive output (litter size, pup body mass, litter mass and the rate of litter mass increase) with those of the mice lactating at 21°C (Johnson et al., 2001a) and 8°C (Johnson and Speakman, 2001). We conducted the present experiment at 30°C, since this temperature provides a much lower gradient between body temperature and environment than our previous experiments at 21°C and 8°C and thus greatly reduces potential heat flow. Another consequence of breeding mice at 30°C is that it reduces the maternal thermoregulatory demands to a minimum. The peripheral limitation hypothesis predicts that mice lactating at 30°C would have milk production and therefore reproductive output similar to those at 21°C and 8°C, because the mammary glands would be expected to work at maximal capacity regardless of ambient temperature. However, the mice would have lower food intake, because of the lower maternal maintenance expenditure. The heat dissipation limit hypothesis predicts that mice lactating at 30°C would have reduced milk production (and therefore lower reproductive output) as well as reduced food intake, since both these processes contribute to the maternal heat burden.

Comparison of the energetics of mice lactating at hot (this study), warm (Johnson et al., 2001a) and cold (Johnson and Speakman, 2001) temperatures showed that the females exposed to 30°C had a smaller increase in body mass over days 0–13 of lactation (Fig. 8B), and consequently lower body mass on day 13 of lactation (Fig. 8C). The hot mice had a substantially lower asymptotic food intake (Fig. 8D). They raised fewer pups than the warm mice (Fig. 9A). Furthermore, the mean pup body mass (Fig. 9B), litter mass (Fig. 9C) and the rate of litter mass increase over days 13–14 of lactation (Fig. 9D) were also reduced. Thus, mice lactating at 30°C had a lower food intake and lower reproductive output than mice lactating at 21°C and 8°C. These data are consistent with the heat dissipation limit hypothesis.

The capacity to dissipate heat depends not only on the difference between body temperature and ambient temperature (the manipulation used in our experiment) but also on conductivity of the insulating surface (Holman, 1986). It has been shown that dietary-induced obesity reduces milk production in rats (Rolls et al., 1983). This observation is consistent with the heat dissipation limit hypothesis, since large amounts of adipose tissue might provide elevated thermal insulation that may prevent heat flow and therefore impair milk synthesis, but it is difficult to reconcile with the other hypotheses.

Increased obligatory heat production during lactation, combined with a decreased ability to dissipate heat as a result of mother–pup contact (Adels and Leon, 1986; Scribner and Wynne-Edwards, 1994a), may also contribute to a chronic maternal hyperthermia. This phenomenon is well documented in laboratory rodent species (e.g. Jans and Leon, 1983; Kittrell and Satinoff, 1988; Scribner and Wynne-Edwards, 1994b – but see Stern and Azzara, 2002) as well as livestock (e.g. Elmasry and Marai, 1991; Ulmershakibaei and Plonait, 1992; Silanikove, 2000). There has been some dispute as to whether maternal hyperthermia occurs because heat production is higher than the rate at which it can be dissipated or because the CNS temperature set point is elevated (Gordon, 1983; Adels and Leon, 1986; Eliason and Fewell, 1997). To address this question, non-pregnant, pregnant and lactating Sprague-Dawley rats were presented with a choice of ambient temperature between 12°C and 36°C (Eliason and Fewell, 1997). Non-pregnant and pregnant rats selected a temperature of 24–25°C, whereas lactating rats chose a substantially cooler temperature (14–15°C). The fact that lactating rats selected the temperature that promoted elevated heat flow from the body to the environment suggests that maternal hyperthermia involves a failure of homeostasis rather than a regulated response.

When maternal hyperthermia approaches the upper lethal body temperature, lactating females are forced to interrupt pup contact and leave the nest area to dissipate heat (Croskerry et al., 1978; Adels and Leon, 1986; Scribner and Wynne-Edwards, 1994a). As ambient temperature increases, nest bout termination increases in frequency (Leon et al., 1978). At the same time, warmer ambient temperatures provide a smaller gradient for the heat flow and, therefore, increase the duration of each nest absence (Scribner and Wynne-Edwards, 1994a). Frequent and prolonged maternal nest absence would affect the suckling behaviour involved in stimulation of milk production (Epstein, 1978; Russel, 1980; Knight et al., 1986). Consequently, the amount of milk produced would decrease. Maternal nest absence resulting from the heat stress may explain the low reproductive output of mice lactating at 30°C (Fig. 9) and the slow pup growth rate of Djungarian hamsters (*Phodopus campbelli*) reported at 23°C (Walton and Wynne-Edwards, 1998). Similarly, cool ambient temperatures would decrease the frequency and duration of maternal absences and therefore improve pup growth and survival, as observed in mice lactating at 21°C (Fig. 9) and in Djungarian hamsters lactating at 18°C (Walton and Wynne-Edwards, 1998).

In conclusion, we have demonstrated that MF1 mice lactating at 30°C had lower asymptotic food intake and lower reproductive output than mice lactating at cooler ambient temperatures (Johnson et al., 2001a; Johnson and Speakman,

2001). The current results, along with experiments showing the behavioural responses of rats and hamsters to maternal hyperthermia (Croskerry et al., 1978; Adels and Leon, 1986; Scribner and Wynne-Edwards, 1994a), are consistent with the heat dissipation limit hypothesis. Finally, our hypothesis can also explain the lack of changes in food intake in mice lactating at 21°C that have to run to obtain their food (Perrigo, 1987) or that are simultaneously pregnant (Johnson et al., 2001c) as well as the higher milk energy output in mice lactating in the cold compared with in the warm (Johnson and Speakman, 2001).

We thank Shona Fleming and Duncan Wood for assistance with animal care. Sally Ward, Maria Johnson and Dave McNeill provided helpful suggestions and discussion about many aspects of the study. Critical comments from Henk Visser and an anonymous referee greatly improved the manuscript. E.K. was supported by a Royal Society Postdoctoral Fellowship and BBSRC grant 1/S12830.

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