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Limits to sustained energy intake

VIII. Resting metabolic rate and organ morphology of laboratory mice lactating at thermoneutrality

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Summary

We have previously shown that the food intake and milk production of MF1 laboratory mice lactating at 30°C, 21°C and 8°C increase as temperature declines. These data suggest that mice are not limited peripherally by the capacity of the mammary glands to produce milk but are limited by the capacity of the animal to dissipate body heat generated as a by-product of food processing and milk production. Here, we measure resting metabolic rate (*RMR*; prior to breeding and at peak lactation) and organ morphology (at peak lactation) in MF1 mice exposed to 30°C (thermoneutrality) and compare these traits with the same parameters measured previously in mice at 21°C and 8°C. The masses of visceral organs primarily responsible for energy flux (heart, lungs, stomach, small intestine, large intestine, liver, pancreas, spleen and kidneys) increased as temperature declined. The masses of all these organs differed between mice exposed to 8°C and 21°C, whereas only the masses of heart, liver and kidneys differed between mice at 21°C and 30°C. The increases in organ masses were paralleled by increases in *RMR* at peak lactation above the levels measured prior to breeding, with mice at 8°C and 21°C having significantly higher increases in *RMR* than mice at 30°C (29.6 kJ day^{-1}, 25.5 kJ day^{-1} and 8.1 kJ day^{-1}, respectively). The observed changes in visceral organs and *RMR* are consistent with both the heat dissipation and peripheral limit hypotheses. However, mice exposed to 8°C had substantially larger mammary glands than mice at 21°C or 30°C (2.450 g, 1.115 g and 0.956 g dry mass, respectively), which argues against the peripheral limitation hypothesis and is consistent with the heat dissipation limit hypothesis. In addition, cold exposure resulted in greater masses of brown adipose tissue, white adipose tissue, pelage and tail. We discuss these changes in the context of the potential thermoregulatory benefits from use of the heat generated as a by-product of milk synthesis.

Key words: resting metabolic rate, organ morphology, peripheral limit, heat dissipation limit, laboratory mouse, *Mus musculus*.

Introduction

During peak lactation, at least two strains of mice (MF1 and Swiss Webster) and a species of rat (hispid cotton rat *Sigmodon hispidus*) modulate their food intake in relation to the prevailing ambient temperature (Hammond et al., 1994; Rogowitz, 1998; Johnson and Speakman, 2001; Król and Speakman, 2003a). This was initially interpreted as a consequence of fixed limitation of the ability of small rodents to synthesise milk imposed at the mammary glands (peripheral limitation hypothesis; Hammond et al., 1994), in combination with increasing thermoregulatory demands as it gets colder. Therefore, at a constant ambient temperature, manipulations such as giving mice more pups to raise (Hammond and Diamond, 1992; Johnson et al., 2001a) or artificially extending lactation to 24 days (Hammond and Diamond, 1994) did not result in elevated maternal food intake but instead resulted in smaller pups – primarily because the mothers appeared unable to upregulate their milk production in response to elevated demands by the pups. Hence, eating more food would not resolve the problem posed by the manipulations. In the cold, however, the extra thermoregulatory demand placed on the mother does not require an elevation in milk production, so increasing food intake to meet these extra demands is a viable strategy. Consistent with the combined demands interpretation of the peripheral limits, Hammond et al. (1996) showed that surgical removal of half of the mammary glands did not result in compensatory increases in milk production by the remaining

glands. Furthermore, Rogowitz (1998) demonstrated in hispid cotton rats that milk production was relatively constant at warm and cold temperatures.

However, other observations are harder to reconcile with this framework. For example, mice at peak lactation that are simultaneously pregnant (Johnson et al., 2001c) or forced to exercise (Perrigo, 1987) do not eat more food than mice that are only lactating, despite the fact that these manipulations do not require elevations in milk energy output. In addition, in MF1 mice, milk production is not constant as a function of ambient temperature (Johnson and Speakman, 2001; Król and Speakman, 2003b) but rather closely mirrors changes in food intake. This pattern appears to be linked to the ability of mice to dissipate body heat generated as a by-product of processing food and producing milk (Król and Speakman 2003a,b). At lower temperatures, there is a greater driving gradient for heat loss, which permits the mice to increase their heat production, thereby allowing greater milk production and hence greater food intake (Król and Speakman, 2003a,b).

It has been widely suggested that the maximal capacity for daily energy expenditure (*DEE*) is regulated by the level of resting metabolic rate (*RMR*) (Drent and Daan, 1980; Peterson et al., 1990; Weiner, 1992). This might occur because *RMR* reflects the energy demands of sustaining the visceral organs that are responsible for most of the energy flux observed as *DEE* and hence food intake. The heat dissipation limit hypothesis (Król and Speakman, 2003a,b) suggests that the route of causality in these associations may be reversed. *DEE* may be limited by heat dissipation capacity, which defines the sizes of organs that will be necessary to supply this energy, and these organs in turn establish the rates of *RMR*. Hence, the heat dissipation model predicts that the components of morphology responsible for the energy flux through the body will be smaller at higher ambient temperatures and this will result in a smaller increase of *RMR* in lactation above the level observed prior to breeding. To test these ideas, we measured *RMR* (prior to breeding and at peak lactation) and organ morphology (at peak lactation) in MF1 laboratory mice exposed to 30°C (thermoneutrality) and compared these traits with the same parameters measured in mice at 21°C and 8°C (Johnson et al., 2001b; Johnson and Speakman, 2001).

Materials and methods

Animals

Experiments were conducted on female mice (*Mus musculus* L.: outbred MF1). We used the same individuals as in experiments presented in Król and Speakman (2003a): (1) 28 lactating (group B) and 15 non-reproductive mice for the resting metabolic measurements and (2) nine lactating mice (group C) for the organ morphology measurements. Housing, acclimation and breeding protocols are described in Król and Speakman (2003a).

Resting metabolic rate measurements

We assessed *RMR* from the rate of oxygen consumption at 30°C (constant-temperature incubator; model INL-401N-010; Gallenkamp, Loughborough, UK), measured during the light phase (between 10:00 h and 17:00 h) by an open-flow respirometry system connected to a paramagnetic oxygen analyser (Model 1100A; Servomex Ltd, Crowborough, UK). Individual mice were placed in a cylindrical Perspex respirometry chamber with rubber stoppers (volume 885 ml) for 3 h. The flow of air (dried with silica gel; BDH Laboratory Supplies, Poole, UK) was maintained by a diaphragm pump (Charles Austen Pumps Ltd, Byfleet, UK) and measured by a wet type laboratory gas flow meter (Model DM3A; G. H. Zeal Ltd, Alexander Wright Division, London, UK) upstream of the chamber. Flow rate was 426–702 ml min^{-1}. Gases leaving the chamber were dried (silica gel) and passed through the oxygen analyser at approximately 150 ml min^{-1}. Carbon dioxide was not absorbed, to maximise accuracy in the derived estimates of energy expenditure when the respiratory quotient (RQ) is not known (Koteja, 1996a; Speakman, 2000). Analyzer outputs were sampled at 30 Hz, averaged and recorded every 30 s by a PC equipped with an analogue-to-digital converter (PC-ADH24; Bede Technology Ltd, Jarrow, UK) and customised BASIC software. The ambient oxygen content of incurrent air was measured before and after each animal was placed in the chamber. These data were used to compensate for any drift in the ambient output of the analyser during each experiment. The rate of oxygen consumption was calculated by multiplying the incurrent flow rate (corrected to STPD) by the decrease in fractional oxygen content between ambient and excurrent flows (Speakman, 2000). *RMR* was estimated from the lowest rate of oxygen consumption over 5 min. The *RMR* data (ml O_2 min^{-1}) were converted to energy equivalents using an oxycalorific value of 21.117 J ml^{-1} O_2, derived from the Weir (1949) equation for an RQ of 1 (Speakman, 2000). Mean body mass was calculated from mass before and after each run.

The *RMR* of 43 adult females was measured both when the mice were virgins and 36–50 days later when 28 of the mice were at peak lactation (day 15 and 16 of lactation). The remaining 15 females were non-reproductive controls. All measurements at each time point were repeated for each animal on two consecutive days, to assess the repeatability of respirometry measurements, and then averaged for further analysis. Thus, for each reproducing female we measured *RMR* prior to breeding (RMR_{PB}) and at peak lactation (RMR_{L}). Non-reproductive females were characterised by $RMR_{NR\text{-}1}$ (measured at the same time as RMR_{PB}) and $RMR_{NR\text{-}2}$ (measured at the same time as RMR_{L}).

Organ morphology

On day 18 of lactation, nine females (litter size 8–12) were weighed, killed by cervical dislocation and immediately dissected. We removed brown adipose tissue, abdominal and mesenteric fat depots, brain, thyroid, heart, lungs, liver, spleen, pancreas, kidney, front mammary glands, rear mammary glands and uterus. The gut was cut at the pyloric and cardiac sphincters, the ileocaecal junction and the anus. The excised stomach and small and large intestines were cut

open longitudinally to remove any residual gut contents and mucous. The remaining body parts were divided into tail, pelage and carcass, including skeletal muscle and bone. We recorded wet mass of organs (±0.0001 g; Ohaus Analytical Plus), dried them in a convection oven at 60°C for 14 days (Król and Speakman, 1999) and re-weighed them to determine dry mass.

Statistics

Data are reported as means ± S.D. (N = sample size). For mice exposed to 30°C, the significance of changes in body mass and *RMR* over time was assessed by paired *t*-tests. The relationship between *RMR* and body mass was examined by least-squares linear regression analysis. The regression lines for lactating and non-reproductive mice were compared using analysis of covariance (ANCOVA). We calculated residuals for *RMR*, litter size, pup body mass, litter mass, litter mass increase and food intake from the least-squares regression lines on female body mass. Relationships between body masses of the same individuals measured on separate occasions, *RMR* measured prior to breeding and at peak lactation and *RMR* and life-history traits were described using Pearson product-moment correlation coefficients. We compared changes in maternal body mass, *RMR* and organ morphology following exposure to different temperatures (30°C, 21°C and 8°C) using analysis of variance (ANOVA). The Tukey *post-hoc* test was used when differentiation between the temperatures was required. The differences in *RMR* among the three temperatures were also examined by ANCOVA, with maternal body mass as a covariate. 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

Resting metabolic rate of mice exposed to 30°C

Screening of 172 respirometry files revealed that in 17 cases mice did not stop locomotory activity for longer than 1 min when in the respirometry chamber and therefore did not meet the criterion for measuring *RMR*. These measurements (2, 1, 4 and 10 files from $RMR_{NR\text{-}1}$, $RMR_{NR\text{-}2}$, RMR_{PB} and RMR_{L} groups, respectively) were omitted from subsequent analyses. As a consequence, 69 *RMR* data presented here are the mean of two measurements from the consecutive days, while 17 *RMR* data are based on single measurements. There was no difference between *RMR* measured on two consecutive days (paired $t=0.6$, $P=0.56$, $N=69$). The data from both days were highly correlated ($r=0.75$, $P<0.001$, $N=69$). The repeatability of respirometry measurements, calculated as coefficient of variation between two consecutive day replicates, averaged 7.7±0.8% ($N=69$).

Reproductive females increased their body mass from 28.6±1.6 g prior to breeding to 35.3±2.0 g at peak lactation (paired $t=17.1$, $P<0.001$, $N=28$). The correlation between pre-breeding and peak lactation body masses marginally failed to reach significance ($r=0.34$, $P=0.07$, $N=28$). The increase in body mass was accompanied by an increase in *RMR* from 17.9±1.6 kJ day^{-1} to 26.0±3.5 kJ day^{-1} (paired $t=13.0$, $P<0.001$, $N=28$). RMR_{PB} was not correlated with RMR_{L} ($r=0.32$, $P=0.10$, $N=28$). The mass-adjusted values (residuals) of RMR_{PB} and RMR_{L} were not correlated either ($r=0.06$, $P=0.77$, $N=28$). However, females with a greater increase in body mass between pre-breeding and peak lactation also had greater increases in *RMR* ($r=0.59$, $P=0.001$). Over the same period of time (36–50 days), the non-reproductive females also increased their body mass (from 30.2±2.4 g to 31.7±3.3 g; paired $t=4.1$, $P=0.001$, $N=15$), but the increase was much less than in reproductive females. The NR-1 and NR-2 body masses were highly correlated ($r=0.92$, $P<0.001$, $N=15$). Despite the increase in body mass, there was no significant difference between $RMR_{NR\text{-}1}$ (18.3±2.2 kJ day^{-1}) and $RMR_{NR\text{-}2}$ (18.9±2.1 kJ day^{-1}) (paired $t=1.1$, $P=0.30$, $N=15$). There was no correlation between $RMR_{NR\text{-}1}$ and $RMR_{NR\text{-}2}$ ($r=0.46$, $P=0.09$, $N=15$). Residual values of $RMR_{NR\text{-}1}$ and $RMR_{NR\text{-}2}$ were also not significantly correlated ($r=0.23$, $P=0.41$, $N=15$).

RMR increased with body mass in all groups (NR-1, NR-2, PB and L). As anticipated, there was no significant difference in *RMR* between mice that were destined to breed (PB) and those we selected not to (NR-1) (ANCOVA: interaction body mass × group, $P=0.67$; body mass effect, $F_{1,40}=11.8$, $P=0.001$; group effect, $F_{1,40}=0.4$, $P=0.55$). Pooling the data across both these groups ($N=43$), the relationship between RMR (kJ day^{-1}) and body mass (*BM*; g) was $RMR_{NR}=5.56+0.43BM$, with body mass explaining 22.8% of the individual variation in RMR_{NR} ($F_{1,41}=12.1$, $P=0.001$). The relationship between *RMR* and body mass was stronger at peak lactation ($RMR_{L}=-18.16+1.25BM$), with body mass explaining 52.0% of the variation in RMR_{L} ($F_{1,41}=28.1$, $P<0.001$; Fig. 1). The *RMR* of lactating females was compared with that of NR-1 and NR-2 groups separately. The slope of the regression line for lactating females was higher than for non-reproductive females from the NR-1 group (ANCOVA: interaction body mass × reproductive status, $F_{1,39}=4.8$, $P=0.034$) and the NR-2 group (ANCOVA: interaction body mass × reproductive status, $F_{1,39}=9.2$, $P=0.004$). This shows that the increase in *RMR* from a mean of 17.9 kJ day^{-1} (prior to breeding) to a mean of 26.0 kJ day^{-1} (peak lactation) was greater than expected from the increase in body mass (on average, 28.6 g prior to breeding and 35.3 g during lactation).

For reproductive females, neither *RMR* measured prior to breeding nor *RMR* at peak lactation were significantly correlated with any life-history traits (litter size, pup body mass, litter mass and litter mass increase), asymptotic food intake, residual life-history traits or residual asymptotic food intake (Table 1). Using residual RMR_{PB} and residual RMR_{L} yielded no significant correlations either. For non-reproductive females, neither $RMR_{NR\text{-}1}$ nor $RMR_{NR\text{-}2}$ was significantly correlated with food intake or with mass-adjusted food intake (Table 1). There was also no significant correlation when we used residual $RMR_{NR\text{-}1}$ and residual $RMR_{NR\text{-}2}$.

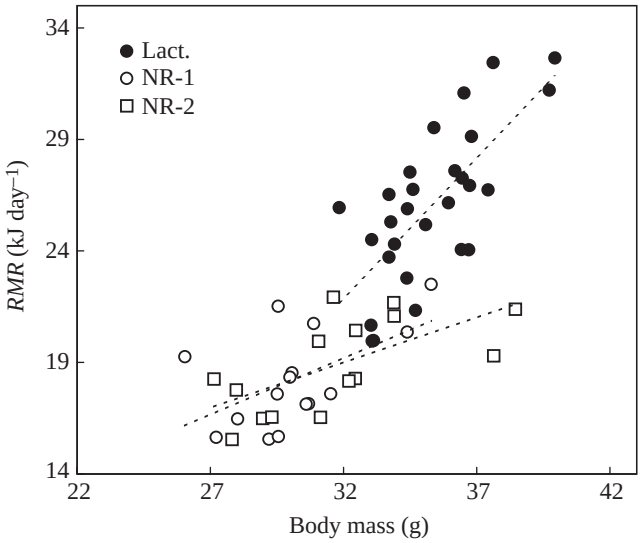

Fig. 1. Resting metabolic rate (*RMR*) as a function of body mass for lactating female mice (filled circles; y=–18.16+1.25x; N=28) and non-reproductive female mice (N=15) measured on two separate occasions: NR-1 (open circles; y=2.88+0.51x) and NR-2 (open squares; y=5.99+0.41x). Both lactating and non-reproductive mice were exposed to 30°C.

The effect of temperature on maternal RMR

We compared *RMR* and organ morphology of mice that were raising their first litters in hot (30°C; present study), warm (21°C; Johnson et al., 2001b) and cold (8°C; Johnson and Speakman, 2001) temperatures. The hot and the warm mice were exposed to 30°C and 21°C, respectively, through a two-week acclimation period (prior to breeding) as well as the whole course of pregnancy and lactation. The cold mice were maintained at 21°C until the pups had grown fur and were then exposed to 8°C from day 10 of lactation onwards.

All measurements of *RMR* were conducted at 30°C, using the same respirometry system and the same protocol. Pre-breeding measurements (RMR_{PB}) were taken at the end of the acclimation period to 30°C (hot mice) or 21°C (warm and cold mice). Peak lactation measurements (RMR_L) were taken on days 15–16 (hot mice) or 18 (warm and cold mice). Sample sizes for the hot, warm and cold groups were 28, 71 and 15, respectively.

Prior to breeding, the *RMR* of hot, warm and cold mice averaged 17.9±1.6 kJ day^{-1}, 21.5±6.1 kJ day^{-1} and 22.2±2.6 kJ day^{-1}, respectively (Table 2; Fig. 2). There was a significant difference between the groups (ANOVA, $F_{2,111}$=6.0, P=0.003), with hot mice having a lower RMR_{PB} than both warm and cold mice (Tukey pairwise comparisons, P<0.05). As expected, the RMR_{PB} of warm and cold mice did not differ (Tukey pairwise comparison, P>0.05), since at this stage both groups were kept at the same temperature (21°C). Hot mice still had a lower RMR_{PB} than warm or cold mice after adjusting for the differences in female body mass (ANCOVA: interaction body mass × temperature, P=0.83; body mass effect, $F_{1,110}$=9.8, P=0.002; temperature effect, $F_{2,110}$=8.6, P<0.001). At peak lactation, *RMR* also differed between the groups (ANOVA, $F_{2,111}$=35.0, P<0.001), with mice at 30°C

Table 1. *Pearson product-moment correlation coefficients between resting metabolic rate (*RMR*) and life-history traits and food intake for reproductive (*N*=28) and non-reproductive (*N*=15) female mice exposed to 30°C*

	RMR		Residual *RMR*	
Group/trait	PB/NR-1[a]	L/NR-2[b]	PB/NR-1[a]	L/NR-2[b]
Reproductive females				
Litter size[c]	0.20 (0.30)	–0.10 (0.60)	0.14 (0.49)	–0.04 (0.83)
Pup body mass[c]	–0.08 (0.69)	0.38 (0.05)	–0.09 (0.65)	0.30 (0.12)
Litter mass[c]	0.24 (0.21)	0.21 (0.29)	0.14 (0.49)	0.15 (0.45)
Litter mass increase[d]	0.43 (0.02)	–0.10 (0.62)	0.38 (0.04)	–0.13 (0.49)
Asymptotic food intake[e]	0.31 (0.10)	0.32 (0.09)	0.27 (0.17)	0.15 (0.46)
Residual litter size	0.18 (0.35)	–0.12 (0.55)	0.12 (0.54)	–0.03 (0.86)
Residual pup body mass	–0.11 (0.58)	0.35 (0.07)	–0.11 (0.56)	0.31 (0.11)
Residual litter mass	0.15 (0.44)	0.13 (0.51)	0.06 (0.75)	0.19 (0.34)
Residual litter mass increase	0.41 (0.03)	–0.12 (0.55)	0.36 (0.06)	–0.13 (0.52)
Residual food intake	0.13 (0.52)	0.16 (0.40)	0.12 (0.55)	0.24 (0.21)
Non-reproductive females				
Food intake[f]	0.53 (0.04)	0.08 (0.78)	0.50 (0.06)	–0.04 (0.88)
Residual food intake	0.08 (0.78)	0.01 (0.96)	0.02 (0.94)	–0.01 (0.98)

All residuals were calculated from regressions on female body mass.

After applying the Bonferroni correction to the significance level (0.05 divided by 10 and 2 comparisons for reproductive and non-reproductive females, respectively), none of the correlations were significant. The *P*-values for correlations are shown in parentheses.

[a]Measured prior to breeding (RMR_{PB}) or as RMR_{NR-1} in non-reproductive females; [b]measured at peak of lactation (RMR_L) or as RMR_{NR-2} in non-reproductive females; [c]day 14 of lactation; [d]between days 13 and 14 of lactation; [e]mean value for days 9–13 of lactation; [f]mean value for 8 days before RMR_{NR-2} was measured.

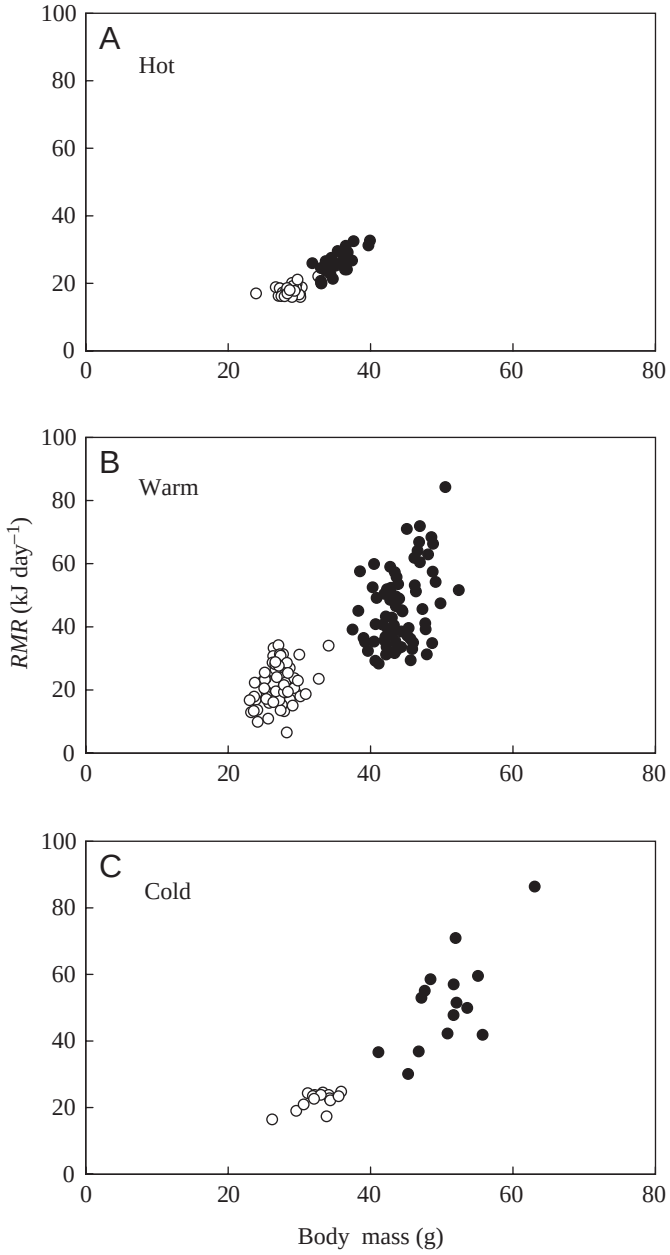

Fig. 2. Resting metabolic rate (*RMR*) prior to breeding (open circles) and at peak lactation (filled circles) as a function of body mass in mice exposed to (A) 30°C, (B) 21°C and (C) 8°C (*N* as in text; for statistical details, see Results).

having a lower RMR_L (26.0±3.5 kJ day^{-1}) than mice at 21°C (47.0±13.8 kJ day^{-1}; Tukey pairwise comparison, $P<0.05$) and mice at 8°C (51.8±14.3 kJ day^{-1}; Tukey pairwise comparison, $P<0.05$). After adjusting for the differences in maternal body mass, the effect of temperature on RMR_L was not significant (ANCOVA: interaction body mass × temperature, $P=0.82$; body mass effect, $F_{1,110}=38.3$, $P<0.001$; temperature effect, $F_{2,110}=2.5$, $P=0.07$). The ratios of mean asymptotic food intake (Table 2) to mean RMR_L in the hot, warm and cold mice were 7.5, 7.9 and 9.4, respectively.

Table 2. *Resting metabolic rate and food intake of mice exposed to hot (30°C,* N*=28), warm (21°C,* N*=71) and cold (8°C,* N*=15) temperatures*

	Mice		
Trait	Hot[a]	Warm[a]	Cold[b]
BM_{PB} (g)[c]	28.6±1.6	27.1±2.1	32.5±2.5
RMR_{PB} (kJ day^{-1})[d]	17.9±1.6	21.5±6.1	22.2±2.6
BM_L (g)[c]	35.3±2.0	44.3±3.2	50.8±5.2
RMR_L (kJ day^{-1})[d]	26.0±3.5	47.0±13.8	51.8±14.3
BM increase (g)[e]	6.7±2.1	17.1±2.8	18.3±4.2
RMR increase (kJ day^{-1})[f]	8.1±3.3	25.5±13.1	29.6±13.6
Food intake (kJ day^{-1})[g]	194.5±32.0	369.5±40.5	487.8±102.1

Values are means ± S.D.

[a]Hot/warm exposure included an acclimation period, pregnancy and the whole of lactation; [b]cold exposure started on day 10 of lactation, after an acclimation period, pregnancy and nine days of lactation at 21°C; [c]mean body mass during *RMR* measurements prior to breeding (BM_{PB}) or at peak lactation (BM_L); [d]resting metabolic rate prior to breeding (RMR_{PB}) or at peak lactation (RMR_L); [e]BM_L–BM_{PB}; [f]RMR_L–RMR_{PB}; [g]asymptotic food intake (hot mice, Król and Speakman, 2003a; warm mice, Johnson et al., 2001a; cold mice, Johnson and Speakman, 2001).

The increase in *RMR* at peak lactation above the level measured prior to breeding (RMR_L–RMR_{PB}) averaged 8.1±3.3 kJ day^{-1}, 25.5±13.1 kJ day^{-1} and 29.6±13.6 kJ day^{-1} in hot, warm and cold mice, respectively (Table 2). The difference between the groups was significant (ANOVA, $F_{2,111}=26.6$, $P<0.001$), with hot mice having a lower increase in *RMR* than both warm and cold mice (Tukey pairwise comparisons, $P<0.05$). However, when we adjusted for the differences in the increase in body mass (BM_L–BM_{PB}), the effect of temperature on the increase in *RMR* was not significant (ANCOVA: interaction body mass increase × temperature, $P=0.40$; body mass increase effect, $F_{1,110}=40.3$, $P<0.001$; temperature effect, $F_{2,110}=0.8$, $P=0.47$). Thus, the relatively small increase in *RMR* observed in mice at 30°C was associated with their relatively small changes in body mass (Fig. 3). The increase in body mass at peak lactation above the non-reproductive level averaged 6.7±2.1 g, 17.1±2.8 g and 18.3±4.2 g in hot, warm and cold mice, respectively. These values were significantly different (ANOVA, $F_{2,111}=146.9$, $P<0.001$), with hot mice having a smaller increase in body mass than both warm and cold mice (Tukey pairwise comparisons, $P<0.05$).

The effect of temperature on maternal organ morphology

To evaluate the effect of the hot, warm and cold temperature treatments on maternal morphology, we compared the dry masses of 15 organs: brown adipose tissue, heart, lungs, stomach, small intestine, large intestine, liver, pancreas, spleen, white adipose tissue (abdominal and mesenteric fat), mammary glands, uterus, tail, pelage and carcass. Since the kidneys were used for other analyses (M. S. Johnson and J. R.

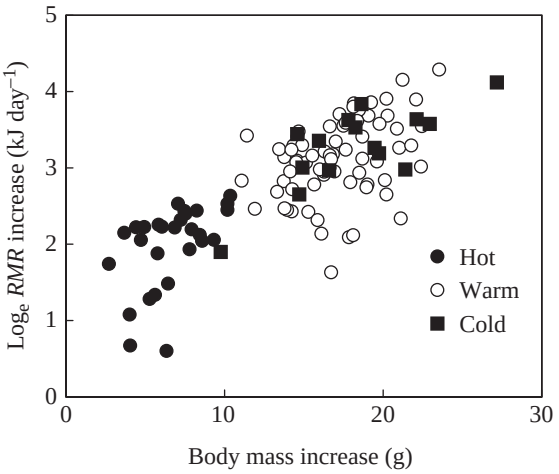

Fig. 3. Relationship between increase in resting metabolic rate (*RMR*) and increase in body mass at peak lactation above the levels measured prior to breeding, in mice exposed to hot (30°C), warm (21°C) and cold (8°C) temperatures (*N* as in text). The differences between the groups are not significant (for statistical details, see Results).

Speakman, manuscript in preparation), the comparison was made only between wet masses for this organ. All mice were dissected on day 18 of lactation, using the same protocol. The comparisons excluded females that had been previously milked (Król and Speakman, 2003b). For the warm and cold groups, dissections were performed on animals that had undergone measurements of *RMR*. No *RMR* measurements were taken for the mice dissected from the hot group. The sample sizes for the hot, warm and cold groups were 9, 16 and 15, respectively. The morphology data for the hot group are presented in Table 3.

On the day of dissection, the body masses of the hot, warm and cold mice averaged 36.6±3.0 g, 40.8±2.5 g and 51.0±5.5 g, respectively. The differences between the groups were significant (ANOVA, $F_{2,37}$=43.5, P<0.001; all Tukey pairwise comparisons, P<0.05). Since organ size frequently correlates with body mass (e.g. Selman et al., 2001), comparison of organ morphology usually requires corrections for the differences in body mass. In our study, however, the differences in body mass at peak lactation between the hot, warm and cold mice were a consequence of the exposure to different temperatures (Table 2). Therefore, correcting for differences in body mass would inevitably remove differences caused by the temperature treatment. To avoid this, we compared mean absolute masses of organs by ANOVA (Table 4).

The temperature to which the animals were exposed during lactation had a significant effect on masses of most of the organs (apart from the uterus), with mice at 8°C having heavier organs than mice at 21°C and 30°C (Table 4). The masses of heart, liver and kidneys in hot mice were significantly lower than those of the warm mice. The dry masses of brown adipose tissue, lungs, stomach, small intestine, large intestine, pancreas, spleen, white adipose tissue, mammary glands, tail, pelage and carcass did not differ significantly between the hot and warm mice.

Table 3. *Organ masses for lactating female mice (*N=*9) exposed to 30°C*

Organ	Organ mass (g)	
	Wet	Dry
Brown adipose tissue	0.149±0.063	0.060±0.026
Brain	0.438±0.031	0.096±0.006
Thyroid	0.212±0.066	0.050±0.015
Heart	0.218±0.047	0.044±0.010
Lungs	0.461±0.158	0.098±0.037
Stomach	0.362±0.055	0.085±0.011
Small intestine	1.295±0.290	0.282±0.068
Large intestine	0.611±0.061	0.120±0.015
Liver	2.148±0.288	0.599±0.070
Pancreas	0.458±0.052	0.113±0.015
Spleen	0.127±0.033	0.026±0.007
Kidneys	0.433±0.045	0.097±0.010
Abdominal fat	0.067±0.061	0.020±0.018
Mesenteric fat	0.075±0.030	0.014±0.008
Front mammary gland	1.932±0.435	0.520±0.116
Rear mammary gland	1.580±0.318	0.435±0.082
Uterus	0.169±0.032	0.035±0.006
Tail	0.946±0.119	0.358±0.035
Pelage	3.696±0.755	1.467±0.650
Carcass	14.836±0.858	4.331±0.276

Values are means ± S.D.

Discussion

We have already demonstrated that limits to sustained energy intake at peak lactation are likely to be imposed centrally by the capacity of the animal to dissipate heat (Król and Speakman, 2003a,b) rather than peripherally by the capacity of mammary glands (Hammond et al., 1994, 1996; Rogowitz, 1998). The main processes that contribute to heat load at peak lactation are digestion and milk production. Consequently, challenging MF1 laboratory mice with a reduced potential heat flow between the animal and the environment by exposing them to 30°C resulted in reduced food intake (Król and Speakman, 2003a) and reduced milk production (Król and Speakman, 2003b). When we released mice from the heat dissipation constraint by exposing them to 8°C, the animals were able to increase both food intake and milk production (Johnson and Speakman, 2001). It is well documented that maintaining the food intake at elevated levels requires enlarged organs to digest, absorb and process nutrients (gut and liver), deliver nutrients and oxygen to peripheral tissues (heart) and finally excrete the products of metabolism (kidneys; e.g. Toloza et al., 1991; Hammond et al., 1994; Konarzewski and Diamond, 1994; Koteja, 1996b; Speakman and McQueenie, 1996; Starck, 1999; Hammond and Kristan, 2000). Since the elevated food intake of mice exposed to sub-

Table 4. *Comparison of dry organ masses of lactating mice exposed to hot (30°C,* N=*9), warm (21°C,* N=*16) and cold (8°C,* N=*15) temperatures*

	Dry organ mass (g)			ANOVA	
Organ	Hot	Warm	Cold	$F_{2,37}$	*P*
Brown adipose tissue	0.060[a]	0.055[a]	0.136[b]	18.8	**<0.001**
Heart	0.044[a]	0.061[b]	0.078[c]	27.1	**<0.001**
Lungs	0.098[a]	0.081[a]	0.189[b]	13.8	**<0.001**
Stomach	0.085[a]	0.076[a]	0.328[b]	18.7	**<0.001**
Small intestine	0.282[a]	0.314[a]	0.398[b]	9.3	**0.001**
Large intestine	0.120[a]	0.158[a]	0.235[b]	28.9	**<0.001**
Liver	0.599[a]	0.859[b]	1.028[c]	31.3	**0.001**
Pancreas	0.113[a]	0.188[a]	0.292[b]	12.7	**<0.001**
Spleen	0.026[a]	0.022[a]	0.040[b]	18.4	**<0.001**
Kidneys (wet mass, g)	0.433[a]	0.557[b]	0.667[c]	40.3	**<0.001**
White adipose tissue	0.034[a]	0.050[a]	0.516[b]	10.5	**<0.001**
Mammary glands	0.956[a]	1.115[a]	2.450[b]	22.3	**<0.001**
Uterus	0.035	0.056	0.124	5.1	0.011
Tail	0.358[a]	0.324[a]	0.422[b]	28.5	**<0.001**
Pelage	1.467[a]	1.294[a]	1.847[b]	9.1	**0.001**
Carcass	4.331[a]	4.434[a]	5.463[b]	21.6	**<0.001**

Values are absolute means.

For organs with significant *P* values (bold type), different letters indicate significant differences between the groups, as assessed by the Tukey pairwise comparisons.

The *P* values in bold type are significant after Bonferroni correction (0.05 divided by 16 comparisons).

thermoneutral temperatures is predicted by the heat dissipation and peripheral limit hypotheses (Król and Speakman, 2003b), the expected changes in organ morphology would also be consistent with both hypotheses. However, in contrast to the peripheral limitation hypothesis, which predicts that the mammary glands would be at maximal size at peak lactation to sustain maximal milk production independent of the temperature, the heat dissipation limit hypothesis predicts that the changes in morphology of the visceral organs would be paralleled by differences in the size of the mammary glands. Furthermore, because of the high mass-specific metabolic rates of heart, liver, kidneys and intestine (e.g. Krebs, 1950; Martin and Fuhrman, 1955; Ferraris, 1994), morphological changes of these organs were anticipated to result in an increase of *RMR* at peak lactation above the level measured prior to breeding. It was also expected that the extent of this increase would be greater at lower temperatures.

We demonstrated that mice exposed to 30°C had significantly higher *RMR* at peak lactation than prior to breeding but that the values of RMR_{PB} and RMR_{L} (both absolute and mass-adjusted) were not correlated. An increase in *RMR* between pre-breeding and peak lactation conditions has been previously shown in MF1 laboratory mice at 21°C (Speakman and McQueenie, 1996; Johnson et al., 2001b) and 8°C (Johnson and Speakman, 2001) as well as in other small rodents (e.g. Garton et al., 1994; Künkele and Trillmich, 1997; Antinuchi and Busch, 2001). We found no evidence that individual variation in either RMR_{PB} or RMR_{L} was correlated with variation in litter size, litter mass, pup body mass or litter mass increase, for both absolute and residual values (Table 1). Previous studies have also shown no link between maternal *RMR* and life-history traits at temperatures of 20–22°C in MF1 mice (Johnson et al., 2001b), HSD/ICR mice (Hayes et al., 1992), deer mice (*Peromyscus maniculatus*; Earle and Lavigne, 1990) and hispid cotton rats (Derting and McClure, 1989). It would therefore appear that while the general pattern of increase in *RMR* during reproduction is compatible with the idea that these changes reflect changes in the capacity of the system to digest and process extra energy to support lactation, at an individual level this association breaks down. The reasons why no relationship between maternal *RMR* and reproductive performance was observed are unclear, since the repeatability of our *RMR* measurements was high (coefficient of variation=7.7%) when compared with the overall variation in *RMR* between individuals (20.0–32.6 kJ day^{-1}). Hence, the absence of a link between *RMR* and life-history traits was probably not because of errors inherent in the *RMR* estimate. Moreover, the estimates of *RMR* in the current study were predominantly the average of measures of *RMR* made on two consecutive days, further reducing variation attributable to analytical factors.

As predicted by both the heat dissipation and peripheral limit hypotheses, the increase in *RMR* at peak lactation above the level measured prior to breeding was significantly lower in mice exposed to 30°C than in mice at 21°C and 8°C (Table 2; Fig. 2). As temperature declined, increases in *RMR* were closely paralleled by changes in maternal body mass, and there was no independent temperature effect on *RMR* (Fig. 3). Examination of the morphological changes of mice at different temperatures revealed a progressive increase in mass as a function of the cold in several organs including heart, lungs, stomach, small intestine, large intestine, liver, pancreas, spleen, kidneys and the mammary glands. However, only the masses of the heart, liver and kidneys differed significantly between all three temperature groups (Table 4). These data indicate that the increased body mass and *RMR* were a consequence of the increases in the masses of the metabolically active organs – primarily involved in the energy flux through the body. Further tests of heat dissipation and peripheral limit hypotheses should involve measurements of organ safety margins (excesses of capacities over prevailing loads; e.g. Toloza et al., 1991; Diamond, 1998; Hammond, 1998). Since sustaining organ safety margins produces extra heat associated with tissue maintenance and enzyme biosynthesis, we expect these margins to be substantially reduced in mice at 30°C.

Milk energy output (*MEO*) in mice exposed to 21°C and 8°C was 90.1% and 228.4%, respectively, higher than the level measured in mice at 30°C (87.7 kJ day^{-1}; Król and Speakman, 2003b). The increase in *MEO* was paralleled by the increase

in the mass of mammary glands (16.6% in mice at 21°C and 156.3% in mice at 8°C), although the difference between the warm and hot groups was not significant (Table 4). Thus, the increase in milk production by 228.4% at 8°C was associated with a substantial increase in the mass of the mammary glands, whereas the 90.1% increase in *MEO* at 21°C was accommodated by mammary glands of a size similar to those at 30°C. These results suggest that mass might be a poor indicator of capacity of mammary glands to produce milk, especially when no adjustments are made for individual variation in organ composition such as lipid or connective tissue content. The use of techniques that measure the number of mammary secretory cells (e.g. the bromodeoxyuridine-labelling index; Capuco et al., 2002), the activity of the secretory cells (e.g. the explant method; Wilde et al., 1999) and the rate of their apoptosis (e.g. DNA laddering intensity; Wilde et al., 1997) could be more informative.

Although cold exposure enabled mice to increase milk production by releasing them from the heat dissipation constraint, there were some changes in the maternal organ morphology that suggest that cold was still a thermoregulatory burden. These include increases in the masses of brown adipose tissue, white adipose tissue, pelage and tail (Table 4). Changes in these organs are often observed in non-reproductive small rodents exposed to cold, since brown adipose tissue hypertrophy is associated with elevated non-shivering thermogenesis (e.g. Klaus et al., 1988), bigger white adipose tissue depots and heavier pelts provide better insulation (e.g. Heldmaier and Steinlechner, 1981), and increased vascularisation of peripheral tissue (e.g. tail and ears) prevents frostbite (e.g. Héroux, 1959). The fact that mice lactating at 8°C benefited from cold exposure in terms of reproductive performance but at the same time underwent morphological changes that increase heat production and improve heat retention has three possible explanations. First, heat generated *via* food processing and milk production might not be used to offset the costs of thermoregulation, either fully or partially. Such a lack of any level of compensation of thermoregulatory costs by the biochemical heat increment of feeding has been demonstrated in star-nosed moles (*Condylura cristata*; Campbell et al., 2000).

The second possibility is that heat produced from elevated food intake and milk production can substitute for active thermogenesis, but only partially. In this case, mice at 8°C would still need more brown adipose tissue, better insulation and increased vascularisation of peripheral tissue. This view is supported by a study of thermoregulation in Sprague-Dawley female rats (Eliason and Fewell, 1997). According to the data presented by Eliason and Fewell in fig. 3, *RMR* of non-reproductive rats averaged 72 ml O_2 kg^{-1} min^{-1} at 14°C and 22 ml O_2 kg^{-1} min^{-1} at 28°C (thermoneutrality), giving thermoregulatory costs of 50 ml O_2 kg^{-1} min^{-1}. However, when lactating rats (day 20 post partum) were measured at 14°C, their *RMR* averaged 60 ml O_2 kg^{-1} min^{-1}, i.e. 12 ml O_2 kg^{-1} min^{-1} below the level of non-reproductive individuals. These data suggest that the 24% reduction in thermoregulatory costs of lactating rats (12 of 50 ml O_2 kg^{-1} min^{-1}) could be attributed to the heat generated by lactogenesis. Since the measurements of *RMR* in the lactating rats were not paralleled by measurements of milk production, the proportion of heat used to compensate the cost of thermoregulation (12 ml O_2 kg^{-1} min^{-1}) to the total amount of heat produced by lactogenesis is unknown.

The third possibility is that heat generated *via* food processing and milk production could fully substitute for active thermogenesis. This would imply no need for bigger brown adipose tissue, white adipose tissue, pelage and tail, providing that the masses of these organs correlate with their function. It might be the case, however, that the mice lactating at 8°C increased the mass of brown adipose tissue, white adipose tissue, pelage and tail in anticipation of post-weaning thermoregulatory demands, so that the greater organ masses did not reflect increased function during lactation. Indeed, it has been demonstrated in Aston laboratory mice lactating at 23°C that the thermogenic capacity of brown adipose tissue (measured as the uncoupling protein content of the tissue) is as low as 8% of that of virgin mice, despite the morphological hypertrophy of the organ (Trayhurn and Jennings, 1987). Similar results have been obtained from measurements of the capacity for non-shivering thermogenesis following noradrenaline injections (Trayhurn, 1983). However, the changes in thermogenic capacity of brown adipose tissue during lactation at different temperatures remain unknown and would be a useful topic for future studies.

In summary, comparison of organ morphology in MF1 mice lactating at 30°C, 21°C and 8°C revealed that the masses of visceral organs responsible for energy flux increased as temperature declined. The differences in the organ masses between the cold and warm mice were all significant, whereas for warm and hot groups, only the masses of heart, liver and kidneys were significantly different. The increases in organ masses were paralleled by the increases in *RMR* above the levels measured prior to breeding, with warm and cold mice having significantly larger increases in *RMR* than hot mice. The observed changes in visceral organs and *RMR* are consistent with both the heat dissipation and peripheral limit hypotheses. However, mice exposed to 8°C had substantially bigger mammary glands than mice at 21°C and 30°C, which argues against the peripheral limitation hypothesis and supports the heat dissipation limit hypothesis. Cold exposure also resulted in greater masses of brown adipose tissue, white adipose tissue depots, pelage and tail, but the functional significance of these changes has yet to be established.

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