

RESEARCH ARTICLE

Not that hot after all: no limits to heat dissipation in lactating mice selected for high or low BMR

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ABSTRACT

Heat dissipation has been suggested as a limit to sustained metabolic effort, e.g. during lactation, when overheating is a possible risk. We tested this hypothesis using mice artificially selected for either high or low BMR that also differed with respect to parental effort. We used fixed size cross-fostered families and recorded litter mass daily until the 14th day of lactation. Midway through the experiment (day 8) half of the mothers from each group had fur from the dorsal body surface removed to increase their thermal conductance and facilitate heat dissipation. Our results showed that neither high nor low BMR mouse lines benefited from increasing their thermal conductance at peak lactation. On the contrary, growth of the litters reared by the low BMR females was compromised. Thus, our results do not support the heat dissipation limitation hypothesis.

KEY WORDS: Heat dissipation, BMR, Experimental selection, Growth rate

INTRODUCTION

Identification of limits to individual performance remains one of the top problems of physiological ecology. From an evolutionary standpoint, reproduction is crucial for fitness; therefore, considerable attention has been paid to factors that limit reproductive output. Two major concepts have been extensively studied and discussed. The first, called the ‘central limitation hypothesis’, invokes a limit to the energy acquisition rate (Weiner, 1992; Koteja, 1996; Hammond et al., 1994; Hammond and Kristan, 2000; Zhao, 2012). According to this idea, a lactating female is constrained by the capacity of the alimentary tract to intake, digest and absorb food (Weiner, 1992; Koteja, 1996; Hammond et al., 1994; Hammond and Kristan, 2000; Zhao, 2012). So, in theory, a lactating mother would produce more (or higher-quality) milk if she was able to acquire the nutrients at a sufficient rate. This hypothesis has been at least partially debunked by multiple studies demonstrating that exposing a lactating female to lower ambient temperature triggers an increase in food intake to compensate for the additional costs of thermoregulation (Hammond et al., 1994; Hammond and Kristan, 2000; Zhao, 2012). Therefore, a second hypothesis, the ‘peripheral limitations hypothesis’, proposed a limit on expending already acquired energy (Hammond et al., 1994, 1996; McDevitt and Speakman, 1994). According to this hypothesis, the limitations are set not by energy acquisition rate but by the output of organs and tissues, such as muscles or mammary tissue that are limited by their peak capacity to process energy (Hammond et al.,

1996; Rogowitz, 1998; Speakman et al., 2001). However mammary output has been shown not to be an exclusive limiting factor (Hammond et al., 1996).

A different peripheral constraint restricting reproductive performance, maternal hyperthermia during lactation, was studied in the early 1980s (Leon et al., 1978, 1983, 1985, 1990; Croskerry et al., 1978; Woodside et al., 1980; Jans and Leon, 1983a,b; Leon and Woodside, 1983) and has gained attention more recently as the ‘heat dissipation limit’ (HDL) hypothesis (Hammond et al., 1996; Król and Speakman, 2003a,b; Król et al., 2007; Speakman and Król, 2010, 2011; Ohrnberger et al., 2016; Sadowska et al., 2016). It promotes the idea that a lactating mother’s performance is bound by her ability to dissipate excessive amounts of heat generated during milk synthesis and energy turnover (Speakman and Król, 2010, 2011). If the limitation on heat dissipation was to be removed, a lactating mother would increase her food intake and milk production, which would allow a higher reproductive output in terms of larger litter size or larger offspring. Experimental results thus far have been equivocal. A number of studies reported elevated milk production and heavier litters in mothers placed in colder environments (Speakman et al., 2001; Król and Speakman, 2003b; Valencak et al., 2010; Valencak et al., 2013) as well as in mothers with thermal conductance enhanced by shaving (Król et al., 2007; Sadowska et al., 2016). Other papers, however, failed to demonstrate any effects of shaving or placing the lactating mother at lower ambient temperature on her parental effort (Zhao and Cao, 2009; Zhao, 2011, 2012).

Heat limitation should be a particularly relevant limiting factor in individuals with inherently high metabolic heat production, i.e. with an intrinsically high basal metabolic rate (H-BMR). In our previous work on a unique model, mice from two line types, one selected for H-BMR and the other for low basal metabolic rate (L-BMR), we were able to demonstrate a significant positive correlation between parental effort (quantified as pup growth rate and milk output; Sadowska et al., 2013) with BMR (Sadowska et al., 2015a). The differences between the two line types were, however, most prominent when animals at peak lactation were exposed to a temperature lower than that of normal housing conditions (17°C versus 23°C). These earlier studies were not designed to determine whether the higher parental effort of the H-BMR mice was in any way enabled by alleviated heat dissipation limitation, or was simply a manifestation of their higher capabilities of coping with a combined burden of lactation and cold exposure. Here, we designed an experiment that allowed us to increase the mothers’ heat dissipation abilities during the most energetically demanding phase of lactation, while not exposing the pups to low ambient temperatures and thus hampering their growth. To do this, we created experimental litters for each mother by cross-fostering pups to achieve a fixed size of eight pups per litter (with four from the H-BMR and four from L-BMR line type), which allowed us to discriminate between the effects of parental effort of mothers from

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both line types. This also equalized the maternal effect and equalized the load set on lactating mothers, since pups from both line types may differ in suckling abilities. For the second more demanding week of lactation, we removed the fur from mothers' dorsal body surface. This would allow the mothers from both line types to: (1) achieve higher thermal conductance even at normal housing temperature (23°C), (2) avoid overheating while increasing food intake and milk production, as assumed by the HDL hypothesis, and (3) raise heavier pups compared with a control group of non-shaved mice (Król et al., 2007; Zhao, 2012; Sadowska et al., 2016). Conversely, if there is no HDL, the increased thermal conductance would impose an additional cost to the mothers, which should be especially noticeable in the L-BMR mice because of their lower capabilities to sustain a high parental effort (Sadowska et al., 2013, 2015a).

MATERIALS AND METHODS

Selection of animals

We used Swiss Webster (*Mus musculus*) mice from the 50th generation of a long-term selection experiment for divergent levels of BMR. Selection was designed to produce two line types: the H-BMR and L-BMR line type. In each generation, depending on the current reproductive success, we maintain 45–50 families per line type. BMR measurements were carried out at 12 weeks of age. Briefly, BMR was measured in an open respirometry system (after 4 h fasting; FC-10 Oxygen Analyzer, Sable Systems, Las Vegas, NV, USA), during the final 2 h of a 3 h trial at 32°C, a temperature falling within the thermoneutral zone of our mice. The lowest oxygen concentration that did not change by more than 0.01% for at least 4 min was used for BMR calculation. No more than three individuals per family with the highest (H-BMR line) and lowest (L-BMR line) body mass-corrected BMR were chosen as progenitors for further selection and mated outside their families (for details, see Książek et al., 2004; Książek et al., 2009; Gębczyński and Konarzewski, 2009).

Because our selection line types are not replicated, there is always a possibility that the between-line type differences in the traits of interest may be due to genetic drift rather than a genuine effect of artificial selection. To rule out such a possibility, we analysed the between-line type difference in BMR of our mice according to Henderson's guidelines, assuming narrow sense heritability of $h^2=0.4$ for BMR and inbreeding coefficient of $F=0.3$ for the effective population size of generation F50 (for details see Konarzewski et al., 2005; Sadowska et al., 2015b). BMR of females from H-BMR and L-BMR line types of the F50 generation averaged 69.11 ± 1.83 ml O₂ h⁻¹ and 40.77 ± 1.69 O₂ h⁻¹, respectively. The between-line type difference in BMR did exceed that expected to arise from genetic drift alone (the difference expressed as a multiple of phenotypic s.d., $D=7.82$, was much higher than that expected under genetic drift, $D_{\text{drift}}=1.47$).

For this study, 79 females (36 H-BMR and 43 L-BMR) that successfully reared one litter were paired outside their families for the second time and placed with males in plastic cages for a 2 week period. Second lactation has been shown to be more effective in terms of milk production; thus, we used animals in their second lactation for this study (Sadowska et al., 2015a). When pregnancy was detectable by an increase in body mass, the males were removed.

Experimental design

All procedures were approved by the Local Ethical Committee on Testing Animals at the Medical University of Białystok (permit no. 65/2015). The experiment lasted for 14 days, from birth to day 14, when pups rely solely on maternal milk for nutrition. Two-day-old pups were switched between mothers so that each mother fostered a

litter of eight pups, with four of them originating from the H-BMR and the four others from the L-BMR line type, none of which was her own offspring. This design enabled us to: (1) discriminate between the effect of parental effort of mothers from both line types, (2) equalize the maternal effect and (3) equalize the load on lactating mothers, since pups from both line types may differ in suckling abilities. We used a litter size of eight because it reflects the mean rearing abilities of our selected line types and provides a sufficient energy load on the mother (Hammond et al., 1996). The families were placed in cages with sawdust bedding, at 23°C and 12 h:12 h light:dark photoperiod, the standard housing conditions in our mouse colony. Each pup in a litter was individually marked and weighed every other day to determine its growth rate for the entire experimental period. On the 8th day of lactation, 17 randomly chosen mothers from the H-BMR and 23 from the L-BMR line type had fur from their dorsal surface removed (FR group thereafter) with a mild hair removal cream (Veet, RB Healthcare, UK). This procedure increased thermal conductance (C) and freed mothers of the putative heat dissipation constraint. The remaining animals consisted of the control group. We chose the second week of lactation for fur removal as this is the more demanding period, approaching the peak of lactation at days 14–15, when pups are not only bigger but also end the period when they rely solely on milk for nutrition (Sadowska et al., 2013, 2015a).

Thermal conductance was measured on day 14 using the same open flow respirometry system as described above, during a 1 h period at 24.5°C in non-fasted and anaesthetized (ketamine 100 mg kg⁻¹) animals. Anaesthetics were used as a part of milk collection protocol of the concurrent study. Rectal temperature was recorded to the nearest 0.1°C using a thermocouple digital thermometer (model BAT-12, Physitemp Instruments, Clifton, NJ). Thermal conductance was calculated as $C=BMR/(T_b-T_a)$, where BMR is expressed in J and calculated assuming that consumption of 1 ml of O₂ equals 20.1 kJ of energy (RQ=0.85), T_b is rectal body temperature and T_a denotes ambient temperature. We successfully completed measurements for 66 females (FR: 12 H-BMR and 22 L-BMR animals; Control: 16 H-BMR and 16 L-BMR females).

Energy assimilation rate of mother mice and litter energy content

We calculated food consumption for each dam over two consecutive days (day 12–14) of lactation as the mass of food disappearing from

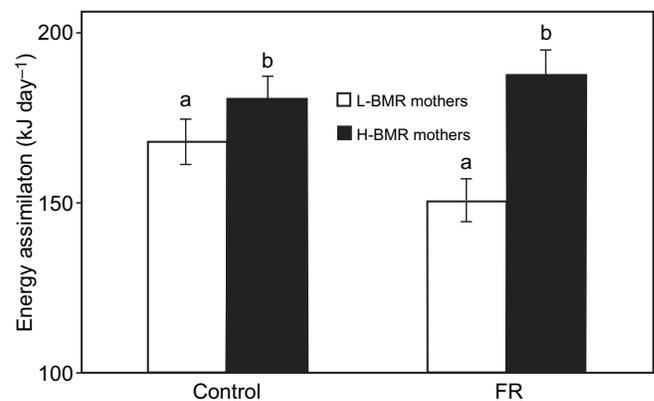


Fig. 1. Daily body mass-corrected energy assimilation at peak lactation in H-BMR and L-BMR mice with and without fur removal. Data are presented as means \pm s.e.m. Different letters denote significant between-line type differences by Tukey *post hoc* test at $P=0.05$. FR, fur removal; H-BMR, high metabolic rate; L-BMR, low metabolic rate.

Table 1. ANCOVA results and mean±s.e.m. values for organ masses of H-BMR and L-BMR mothers from control and shaved (FR) groups

	Line type	Experimental group	Carcass mass	Line type× Experimental group	Mass (g)			
					FR H-BMR	FR L-BMR	Control H-BMR	Control L-BMR
Liver	$F_{1,61}=25.50$ $P<0.001$	$F_{1,12}=0.11$ $P=0.748$	$F_{1,12}=10.11$ $P=0.008$	$F_{1,11}=0.03$ $P=0.870$	3.284±0.100	2.686±0.100	3.235±0.100	2.671±0.095
Kidney	$F_{1,61}=73.92$ $P<0.001$	$F_{1,12}=1.95$ $P=0.188$	$F_{1,12}=7.44$ $P=0.018$	$F_{1,12}=0.13$ $P=0.723$	0.690±0.018	0.505±0.018	0.659±0.019	0.487±0.018
Intestine	$F_{1,61}=79.74$ $P<0.001$	$F_{1,12}=0.14$ $P=0.712$	$F_{1,12}=10.60$ $P=0.007$	$F_{1,12}=0.01$ $P=0.993$	2.765±0.099	1.849±0.087	2.733±0.094	1.849±0.087

Significant effects are indicated in bold.

the food dispenser minus food remaining. For this period, families were placed in cages equipped with plastic grids. The grids allowed faeces and food remains to fall freely to the bottom of the cage. Water content of the murine chow was also determined by drying pre-weighed food samples to a constant mass at 60°C. After completion of food consumption measurements, faeces collected from each mother as well as food samples were re-dried, and their caloric content was measured with an oxygen bomb calorimeter (IKA Werke 7000 calorimeter, Germany). We calculated individual daily energy assimilation rate (A) as [(mass of food consumed×caloric value of food)–(mass of faeces×caloric value of faeces)]/2.

At the end of the experiment, all litters were killed by CO₂ exposure. Whole pup carcasses were dried at 60°C to a constant mass, homogenized with an electric mill, pelleted, re-dried, weighed and their caloric value was measured via oxygen bomb calorimetry in duplicate. We then used the caloric equivalent of each pup to calculate the total litter energy content.

Statistics

Maternal organ masses and thermal conductance were analysed by means of ANCOVA with the line type and experimental group as main factors, female line affiliation nested within the line type as a random factor and carcass mass (body mass minus all organ masses) for the organ masses and body mass for thermal conductance as covariates. Energy assimilation rate was analysed by means of ANCOVA with female line type and experimental groups as main factors, female family affiliation nested within the line type, and body mass as a covariate. Maternal body mass changes during the second week of lactation (day 8–14) were defined as the slope of a linear regression of the changes in body mass against the day of lactation and analysed with ANOVA with female line type and experimental group affiliation as main factors, body mass at day 8 as a covariate and family affiliation nested within the line type of origin as a random factor.

Individual pup growth rate was defined as the slope of a linear regression of the changes in body mass against the age of the pup. We analysed the growth rate of the pups for the second week of the experiment using a mixed ANCOVA with the foster female line type, experimental group (FR versus control), pup line type as fixed factors, the pup family affiliation nested within pup line type, and

female family affiliation nested within the line type as random factors, and the individual pup starting body mass (at day 8) as a covariate.

Litter energy content was analysed by means of ANCOVA with female line type, experimental group as main factors, female family affiliation nested within the female line type and combined litter mass from the 8th experimental day as a covariate. Mass from the 8th experimental day was used, because this was the day of implementing the experimental protocol (i.e. fur removal). All statistical analyses were performed with SAS 9.3 software (SAS Institute, Cary, NC, USA).

RESULTS

Maternal thermal conductance, morphometrics and energy assimilation

Fur removal significantly increased thermal conductance (C) of lactating mothers by 16% and 10% in the H-BMR and L-BMR mice, respectively ($F_{1,61}=4.74$; $P=0.033$; H-BMR: control=209.36±13.49 J h⁻¹ K⁻¹, FR=246.49±26.58 J h⁻¹ K⁻¹; L-BMR: control=194.86±12.66 J h⁻¹ K⁻¹, FR=214.02±9.23 J h⁻¹ K⁻¹). C was not affected by the line type ($F_{1,61}=2.08$; $P=0.154$), the line type×experimental protocol interaction ($F_{1,61}=0.42$; $P=0.516$) or body mass ($F_{1,61}=1.21$; $P=0.275$). Fur removal did not affect daily energy assimilation rate A ($F_{1,13}=0.64$; $P=0.439$), with no line type×experimental group interaction ($F_{1,13}=3.48$; $P=0.085$). However A differed between the line types ($F_{1,61}=10.65$; $P=0.002$), with H-BMR females acquiring more energy (Fig. 1). Body mass was at the verge of significance as a covariate ($F_{1,13}=4.52$; $P=0.053$). All metabolically active organs (liver, kidneys, intestine) were significantly heavier in the H-BMR mothers without any effect of fur removal (Table 1). Body mass changes during the second week of lactation were significantly affected by the line type affiliation ($F_{1,14}=23.77$; $P<0.001$) and the initial body mass (at day 8; $F_{1,14}=11.27$; $P=0.004$) and were higher in the L-BMR type, as these mice lost more body mass (L-BMR FR: $-1.03±0.10$ g day⁻¹; L-BMR control: $-0.61±0.10$ g day⁻¹; H-BMR FR: $-0.20±0.11$ g day⁻¹; H-BMR control: $-0.27±0.10$ g day⁻¹). The experimental protocol showed no effect ($F_{1,14}=2.97$; $P=0.107$); however, there was a significant line type experimental protocol interaction ($F_{1,14}=5.34$; $P=0.366$).

Table 2. ANCOVA results for pup growth rate in the second week of the experiment analysed within shaved (FR) and control mice groups

Experimental group	Mother line	Pup line	Body mass	Pup line×Mother line
FR	$F_{1,29}=19.01$ $P<0.001$	$F_{1,46}=0.50$ $P=0.484$	$F_{1,218}=1.17$ $P=0.281$	$F_{1,218}=0.18$ $P=0.676$
Control	$F_{1,27}=3.11$ $P=0.089$	$F_{1,50}=1.82$ $P=0.184$	$F_{1,223}=0.27$ $P=0.605$	$F_{1,223}=7.78$ $P=0.006$

Significant effects are indicated in bold.

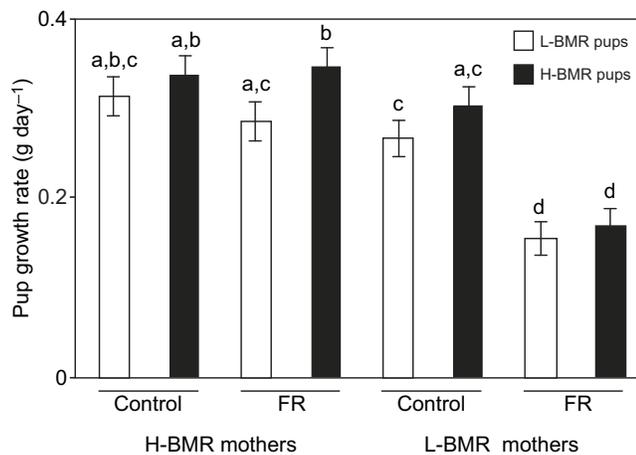


Fig. 2. Growth rate of pups originating from both line types raised by H-BMR and L-BMR mothers with and without fur removal. Data are presented as means±s.e.m. Different letters denote significant between-line type differences by Tukey *post hoc* test at $P=0.05$.

Pup growth rate and litter energy content

Pup growth rate was affected both by the experimental protocol ($F_{1,469}=17.15$; $P=0.001$) and mother line type ($F_{1,61}=26.19$; $P<0.001$) as well as the pup's own line of origin ($F_{1,64}=4.73$; $P=0.033$). We found no effect of a pup's initial body mass on growth rate ($F_{1,469}=2.44$; $P=0.119$) and no pup line×mother line interaction ($F_{1,469}=1.44$; $P=0.231$). However, since pup growth rate showed a significant pup line×mother line×experimental group ($F_{1,469}=6.04$; $P=0.001$) interaction, we analysed the data separately for each experimental group and each female line type. Within the FR group, the pups reared by the L-BMR mothers grew at slower rate (Table 2; Fig. 2). This result has been further corroborated by the analyses carried out within the line types. Growth rate of the pups reared by the FR L-BMR mothers was slower than that of pups reared by the control L-BMR dams. In contrast, fur removal did not affect pups reared by the H-BMR mothers (Table 3, Fig. 2).

The effect of fur removal on pup growth rate resulted in differences in litter energy content ($F_{1,72}=5.53$; $P=0.021$). Mother line type ($F_{1,72}=16.67$; $P<0.001$), litter mass ($F_{1,72}=96.78$; $P<0.001$) and mother line×experimental group interaction ($F_{1,72}=10.22$; $P<0.002$) were also significant. A separate analysis carried out within control and FR groups revealed that in the FR group, the L-BMR mothers raised litters of lower energy content (Fig. 3, $F_{1,35}=71.66$; $P<0.001$).

DISCUSSION

We demonstrated that heat dissipation is not a limiting factor in lactating mother mice from the line types with inherently high or low BMR. In previous studies we found that a significant difference in parental effort between the two line types is most prominent when selected mice are lactating at an ambient temperature of 17°C,

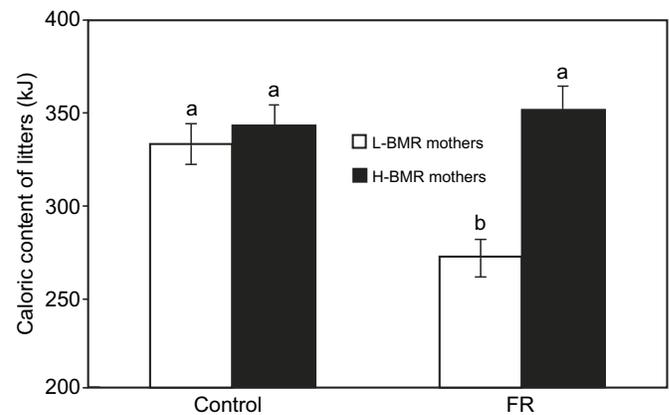


Fig. 3. Caloric content of litters reared by shaved (FR) and control mothers from the L-BMR and H-BMR line types. Data are presented as means±s.e.m. Different letters denote significant between-line type differences by Tukey *post hoc* test at $P=0.05$.

i.e., significantly lower than the housing temperature of 23°C. This difference was manifested both in higher pup growth rate (Sadowska et al., 2013) and higher milk production in the H-BMR mice (Sadowska et al., 2015a). Since higher rates of BMR are associated with high heat production, it is tempting to attribute higher reproductive output of the H-BMR type mice at low ambient temperatures to a release from the heat constraint. Thus, if heat transfer were to be a limiting factor, one should also observe a similar response when the fur removal protocol was applied at 23°C.

Our results did not support the above prediction. In the second week of lactation, the most energetically demanding phase, we did not find a significant difference between reproductive output of the H-BMR mice with or without fur. Furthermore, growth rate of the pups and energy content of litters raised by the L-BMR mothers from the FR group was lower compared with that in their depilated H-BMR counterparts. Also, the FR L-BMR mothers raised pups that were smaller than those nursed by the H-BMR mothers and those raised by the control L-BMR mice. Taken together, these results show that an increased thermal conductance achieved by fur removal did not bring a release from thermal constraints but rather incurred additional thermoregulatory costs. This resulted in the reduction of parental investment of the L-BMR mothers, while seemingly not affecting the H-BMR mothers.

A number of studies tested the HDL hypothesis by either placing lactating animals and their offspring at low ambient temperature or applying the fur removal protocol to allow higher rates of heat dissipation (Leon and Woodside, 1983; Johnson and Seapkman, 2001; Król et al., 2007; Wu et al., 2009; Zhao, 2011; Valencak et al., 2013; Yang et al., 2013; Ohrnberger et al., 2016; Sadowska et al., 2016). All of these studies found increased rates of food consumption and/or higher energy assimilation, which is consistent with the HDL hypothesis, but could also be a response to the additional costs of thermoregulation associated with higher

Table 3. ANCOVA results for pup growth rate in the second week of the experiment analysed within each of the line types

Mother line	Experimental group	Pup line	Body mass	Pup line×Experimental group
H-BMR	$F_{1,200}=1.40$ $P=0.238$	$F_{1,47}=4.35$ $P=0.042$	$F_{1,200}=1.49$ $P=0.224$	$F_{1,200}=14.93$ $P<0.001$
	$F_{1,242}=46.55$ $P<0.001$	$F_{1,56}=0.25$ $P=0.616$	$F_{1,242}=0.56$ $P=0.456$	$F_{1,242}=3.24$ $P=0.073$

Significant effects are indicated in bold.

thermal conductance. Król et al. (2003) demonstrated that a group of MF1 strain dams lactating in the cold (8°C) had larger internal organs compared with the group lactating at 30°C. Mice lactating at 8°C also increased brown fat depots, white fat and pelage mass, all suggestive of the lactogenic heat not being sufficient for meeting the mothers' thermoregulatory needs. Despite this, most of the studies still found higher energy transfer via milk in the cold (Ohrnberger et al., 2016; Sadowska et al., 2016; Johnson and Speakman, 2001; Wu et al., 2009; Zhao, 2011; Yang et al., 2013; Valencak et al., 2013). In an earlier study, however, Johnson and Speakman (2001) reported increased pup mortality and reduced body mass in mouse litters transferred to 8°C for peak lactation. Likewise, cold-exposed lactating Swiss mice increased food intake but weaned smaller litters of lighter pups compared with mice maintained at 23°C (Zhao, 2012). Król et al. (2007) demonstrated higher growth rate of pups reared by shaved MF1 mice compared with non-shaved mothers, while in Swiss mice, no positive effects of shaving on parental investment were found, either in terms of the pup growth rate or milk energy output (Zhao and Cao, 2009; Zhao et al., 2010).

Surprisingly, except for Zhao and Cao (2009), none of the above cited studies examined whether fur removal significantly increased thermal conductance and therefore, heat dissipation. The effectiveness of such manipulation was only indirectly inferred from an increase in food consumption or energy expenditure observed after protocol application (Szafrńska et al., 2014). Nonetheless methodological integrity requires direct validation of the applied manipulation. We were able to demonstrate that our protocol was indeed effective in increasing thermal conductance. Despite this, the maternal effort of lactating H-BMR mothers did not increase, which is clearly at odds with predictions of the HDL hypothesis. Conversely, compromised growth of pups reared by the L-BMR mothers indicates that they were not able to meet the new demands imposed by increased thermal conductance, when simultaneously challenged with lactation, which probably resulted in less energy available for milk investment.

The H-BMR selected mice had larger internal organs associated with sustained metabolic effort – small intestines, liver and kidneys – all contributing to elevated BMR (Table 1; Konarzewski and Diamond, 1995). These animals also showed a higher food consumption compared with the L-BMR mice, most likely to fuel their higher basal upkeep (Sadowska et al., 2015a,b; Książek et al., 2004; Gębczyński and Konarzewski, 2009; Brzęk et al., 2007). During reproduction, the larger organs may put them in an advantageous position, allowing higher rates of energy assimilation and transfer via milk to offspring (Sadowska et al., 2015a). Energy assimilation from food (kJ day⁻¹) was higher in the H-BMR mothers from both groups with and without fur (Fig. 3). This result is mirrored both in the growth rate of pups nursed by H-BMR mothers as well as the caloric content of litters (Figs 2 and 3). This essentially means that thanks to larger internal organs they were capable of fuelling their own physiological needs, including costs of thermoregulation incurred by fur removal, while also maintaining a sufficient energy transfer to the offspring. The L-BMR mice were most likely struggling to cope with the combined high load of lactation and thermoregulatory demands, and were therefore unable to upregulate their thermoregulatory and energy acquisition mechanisms within a short experimental time period. Phenotypic flexibility of internal organs in these mice is largely determined by their size and/or mass (Książek et al., 2009). This, in turn, may limit an immediate spare capacity of the alimentary tract and other organs to respond to a sudden energy demand imposed by fur removal. This is a most likely explanation, considering that the L-BMR mice in our experiment

reduced body mass during the second week of lactation (after the implementation of the experimental protocol).

In conclusion, our experiment did not provide support for the HDL hypothesis as neither of the line types benefitted from increasing their thermal conductance at peak lactation. Quite the contrary, the L-BMR mothers manifested reduced body mass, possibly using up their fat stores for thermoregulatory purposes because of the higher energy demand, while their litters showed compromised growth. Between-line type differences reported herein are in agreement with our previous studies demonstrating a positive correlation between BMR and the ability to cope with metabolic challenges thanks to increased capacity for sustaining high energy turnover rates.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: J.S., A.K.G.; Methodology: J.S., A.K.G., M.L.; Formal analysis: J.S., A.K.G.; Investigation: M.L.; Data curation: J.S., A.K.G., M.K.; Writing - original draft: J.S.; Writing - review & editing: J.S., A.K.G., M.K.; Project administration: J.S.

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References

- Brzęk, P., Bielawska, K., Książek, A. and Konarzewski, M. (2007). Anatomic and molecular correlates of divergent selection for basal metabolic rate in laboratory mice. *Physiol. Biochem. Zool.* **80**, 491-499. doi:10.1086/520617
- Croskerry, P. G., Smith, G. K. and Leon, M. (1978). Thermoregulation and the maternal behaviour of the rat. *Nature* **273**, 299-300. doi:10.1038/273299a0
- Gębczyński, A. K. and Konarzewski, M. (2009). Locomotor activity of mice divergently selected for basal metabolic rate: a test of hypotheses on the evolution of endothermy. *J. Evol. Biol.* **22**, 1212-1220. doi:10.1111/j.1420-9101.2009.01734.x
- Hammond, K. A. and Kristan, D. M. (2000). Responses to lactation and cold exposure by deer mice (*Peromyscus maniculatus*). *Physiol. Biochem. Zool.* **73**, 547-556. doi:10.1086/317757
- Hammond, K. A., Konarzewski, M., Torres, R. M. and Diamond, J. (1994). Metabolic ceilings under a combination of peak energy demands. *Physiol. Zool.* **67**, 1479-1506. doi:10.1086/physzool.67.6.30163908
- Hammond, K. A., Lloyd, K. C. K. and Diamond, J. (1996). Is mammary output capacity limiting to lactational performance in mice? *J. Exp. Biol.* **199**, 337-349.
- Jans, J. E. and Leon, M. (1983a). Determinants of mother-young contact in Norway rats. *Physiol. Behav.* **30**, 919-935. doi:10.1016/0031-9384(83)90258-5
- Jans, J. E. and Leon, M. (1983b). The effects of lactation and ambient temperature on the body temperature of female Norway rats. *Physiol. Behav.* **30**, 959-961. doi:10.1016/0031-9384(83)90261-5
- Johnson, M. S. and Speakman, J. R. (2001). Limits to sustained energy intake. V. Effect of cold exposure during lactation in *Mus musculus*. *J. Exp. Biol.* **204**, 1967-1977.
- Konarzewski, M. and Diamond, J. (1995). Evolution of Basal Metabolic Rate and organ masses in laboratory mice. *Evolution* **49**, 1239-1248. doi:10.1111/j.1558-5646.1995.tb04450.x
- Konarzewski, M., Książek, A. and Łapo, I. B. (2005). Artificial selection on metabolic rates and related traits in rodents. *Integr. Comp. Biol.* **45**, 416-425. doi:10.1093/icb/45.3.416
- Koteja, P. (1996). Limits to the energy budget in a rodent, *Peromyscus maniculatus*: the central limitation hypothesis. *Physiol. Zool.* **69**, 981-993. doi:10.1086/physzool.69.5.30164242
- Król, E. and Speakman, J. R. (2003a). Limits to sustained energy intake. VI. Energetics of lactation in laboratory mice at thermoneutrality. *J. Exp. Biol.* **206**, 4255-4266. doi:10.1242/jeb.00674
- Król, E. and Speakman, J. R. (2003b). Limits to sustained energy intake. VII. Milk energy output in laboratory mice at thermoneutrality. *J. Exp. Biol.* **206**, 4267-4281. doi:10.1242/jeb.00675
- Król, E., Johnson, M. S. and Speakman, J. R. (2003). Limits to sustained energy intake. VIII. Resting metabolic rate and organ morphology of laboratory mice lactating at thermoneutrality. *J. Exp. Biol.* **206**, 4283-4291. doi:10.1242/jeb.00676

- Król, E., Murphy, M. and Speakman, J. R. (2007). Limits to sustained energy intake X. Effects of fur removal on reproductive performance in laboratory mice. *J. Exp. Biol.* **210**, 4233-4243. doi:10.1242/jeb.009779
- Książek, A., Konarzewski, M. and Łapo, I. B. (2004). Anatomic and energetic correlates of divergent selection for basal metabolic rate in laboratory mice. *Physiol. Biochem. Zool.* **77**, 890-899. doi:10.1086/425190
- Książek, A., Czerniecki, J. and Konarzewski, M. (2009). Phenotypic flexibility of traits related to energy acquisition in mice divergently selected for basal metabolic rate (BMR). *J. Exp. Biol.* **212**, 808-814. doi:10.1242/jeb.025528
- Leon, M. and Woodside, B. (1983). Energetic limits on reproduction: maternal food intake. *Physiol. Behav.* **30**, 945-957. doi:10.1016/0031-9384(83)90260-3
- Leon, M., Croskerry, P. G. and Smith, G. K. (1978). Thermal control of mother-young contact in rats. *Physiol. Behav.* **21**, 793-811. doi:10.1016/0031-9384(78)90021-5
- Leon, M., Fischette, C., Chee, P. and Woodside, B. (1983). Energetic limits on reproduction: interaction of thermal and dietary factors. *Physiol. Behav.* **30**, 937-943. doi:10.1016/0031-9384(83)90259-7
- Leon, M., Adels, L. and Coopersmith, R. (1985). Thermal limitation of mother-young contact in Norway rats. *Dev. Psychobiol.* **18**, 85-105. doi:10.1002/dev.420180202
- Leon, M., Coopersmith, R., Beasley, L. J. and Sullivan, R. M. (1990). Thermal aspects of parenting. In *Mammalian Parenting, Biochemical, Neurobiological, and Behavioral Determinants* (ed. N. A. Krasnegor and R. S. Bridges), pp. 400-415. New York: Oxford University Press.
- McDevitt, R. M. and Speakman, J. R. (1994). Central limits to sustainable metabolic rate have no role in cold acclimation of the short-tailed field vole (*Microtus agrestis*). *Physiol. Zool.* **67**, 1117-1139. doi:10.1086/physzool.67.5.30163885
- Ohmberger, S. A., Monclús, R., Rödel, H. G. and Valencak, T. G. (2016). Ambient temperature affects postnatal litter size reduction in golden hamsters. *Front. Zool.* **13**, 51. doi:10.1186/s12983-016-0183-8
- Rogowitz, G. L. (1998). Limits to milk flow and energy allocation during lactation of the hispid cotton rat (*Sigmodon hispidus*). *Physiol. Zool.* **71**, 312-320. doi:10.1086/515923
- Sadowska, J., Gębczyński, A. K. and Konarzewski, M. (2013). Basal metabolic rate is positively correlated with parental investment in laboratory mice. *Proc. Biol. Sci.* **280**, 20122576. doi:10.1098/rspb.2012.2576
- Sadowska, J., Gębczyński, A. K., Paszko, K. and Konarzewski, M. (2015a). Milk output and composition in mice divergently selected for basal metabolic rate. *J. Exp. Biol.* **218**, 249-254. doi:10.1242/jeb.111245
- Sadowska, J., Gębczyński, A. K. and Konarzewski, M. (2015b). Effect of reproduction on the consistency of the between-line type divergence in laboratory mice selected on basal metabolic rate. *Physiol. Biochem. Zool.* **88**, 328-335. doi:10.1086/680167
- Sadowska, E. T., Król, E., Chrzascik, K. M., Rudolf, A. M., Speakman, J. R. and Koteja, P. (2016). Limits to sustained energy intake. XXIII. Does heat dissipation capacity limit the energy budget of lactating bank voles? *J. Exp. Biol.* **219**, 805-815. doi:10.1242/jeb.134437
- Speakman, J. R. and Król, E. (2010). Maximal heat dissipation capacity and hyperthermia risk: neglected key factors in the ecology of endotherms. *J. Anim. Ecol.* **79**, 726-746. doi:10.1111/j.1365-2656.2010.01689.x
- Speakman, J. R. and Król, E. (2011). Limits to sustained energy intake. XIII. Recent progress and future perspectives. *J. Exp. Biol.* **214**, 230-241. doi:10.1242/jeb.048603
- Speakman, J. R., Gidney, A., Bett, J., Mitchell, I. P. and Johnson, M. S. (2001). Limits to sustained energy intake: IV. Effect of variation in food quality on lactating mice, *Mus musculus*. *J. Exp. Biol.* **204**, 1957-1965.
- Szafrańska, P. A., Zub, K., Wiczorek, M., Książek, A., Speakman, J. R. and Konarzewski, M. (2014). Shaving increases daily energy expenditure in free-living root voles. *J. Exp. Biol.* **217**, 3964-3967. doi:10.1242/jeb.103754
- Valencak, T. G., Hacklander, K. and Ruf, T. (2010). Peak energy turnover in lactating European hares: a test of the heat dissipation limitation hypothesis. *J. Exp. Biol.* **213**, 2832-2839. doi:10.1242/jeb.040238
- Valencak, T. G., Wright, P., Weir, P., Mitchell, S. E., Vaanholt, L. M., Hambly, C., Król, E. and Speakman, J. R. (2013). Limits to sustained energy intake. XXI. Effect of exposing the mother, but not her pups, to a cold environment during lactation in mice. *J. Exp. Biol.* **216**, 4326-4333. doi:10.1242/jeb.092023
- Weiner, J. (1992). Physiological limits to sustainable energy budgets in birds and mammals: ecological implications. *TREE* **7**, 384-388. doi:10.1016/0169-5347(92)90009-Z
- Woodside, B., Pelchat, R. and Leon, M. (1980). Acute elevation of the heat load of mother rats curtails maternal nest bouts. *J. Comp. Physiol. Psychol.* **94**, 61. doi:10.1037/h0077653
- Wu, S.-H., Zhang, L.-N., Speakman, J. R. and Wang, D.-H. (2009). Limits to sustained energy intake. XI. A test of the heat dissipation limitation hypothesis in lactating Brandt's voles (*Lasiopodomys brandtii*). *J. Exp. Biol.* **212**, 3455-3465. doi:10.1242/jeb.030338
- Yang, D.-B., Li, L., Wang, L.-P., Cho, Q.-S., Hambly, C., Wang, D.-H. and Speakman, J. R. (2013). Limits to sustained energy intake. XIX. A test of the heat dissipation limitation hypothesis in Mongolian gerbils (*Meriones unguiculatus*). *J. Exp. Biol.* **216**, 3358-3368. doi:10.1242/jeb.085233
- Zhao, Z.-J. (2011). Energy budget during lactation in striped hamsters at different ambient temperatures. *J. Exp. Biol.* **214**, 988-995. doi:10.1242/jeb.049395
- Zhao, Z.-J. (2012). Effect of cold exposure on energy budget and thermogenesis during lactation in Swiss mice raising large litters. *Biol. Open* **1**, 397-404. doi:10.1242/bio.2012661
- Zhao, Z.-J. and Cao, J. (2009). Effect of fur removal on the thermal conductance and energy budget in lactating Swiss mice. *J. Exp. Biol.* **212**, 2541-2549. doi:10.1242/jeb.029603
- Zhao, Z.-J., Chi, Q.-S. and Cao, J. (2010). Milk energy output during peak lactation in shaved Swiss mice. *Physiol. Behav.* **101**, 59-66. doi:10.1016/j.physbeh.2010.04.017