

## RESEARCH ARTICLE

# Limits to sustained energy intake. XXXII. Hot again: dorsal shaving increases energy intake and milk output in golden hamsters (*Mesocricetus auratus*)

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## ABSTRACT

Golden hamsters have four times the body size of mice, raise very large litters and are required to produce large quantities of milk during the 18-day lactation period. We have previously proposed that they may be prone to being limited by their heat dissipation capacity. Studies where lactating females are shaved to elevate their heat dissipation capacity have yielded conflicting data so far. With their short pregnancy of ~18 days, the large litters and the reported high skin temperatures, they may serve as an ideal model to elucidate the role of epilation for energy budgets in lactating mammals. We shaved one group of lactating females dorsally on the sixth day of lactation, and tested if the elevated heat dissipation capacity would enable them to have higher energy intakes and better food-to-milk conversion rates. Indeed, we observed that females from the shaved group had 6% higher body mass and 0.78°C lower skin temperature than control females during lactation. When focusing on the phase of peak lactation, we observed significantly higher (10%) gross energy intake of food and 23.4% more milk energy output in the shaved females, resulting in 3.3 g higher individual pup weights. We conclude that shaving off the females' fur, even though restricted to the dorsal surface, had large consequences on female energy metabolism in lactation and improved milk production and pup growth in line with our previous work on heat dissipation limitation. Our new data from golden hamsters confirm heat dissipation as a limiting factor for sustained metabolic rate in lactation in some small mammals and emphasise the large effects of a relatively small manipulation such as fur removal on energy metabolism of lactating females.

**KEY WORDS:** Golden hamster, Heat dissipation limitation, Milk production, Pup growth, Shaving, Subcutaneous temperature, Sustained energy intake

## INTRODUCTION

It is widely accepted that maximum rates of energy turnover, i.e. food intake, over protracted periods can only be sustained by allowing a certain level of overheating (reviewed in Speakman and

Król, 2010). Therefore, unsurprisingly, female rodents nursing their young are usually observed to have increased body temperatures of 39°C compared with 37°C in the non-reproductive state (Gamo et al., 2013a,b; Ohrnberger et al., 2018a; Valencak et al., 2013).

Undoubtedly, lactation is the most energetically demanding phase in life for females and increases in body temperature may reflect the high energy expenditures. Similar increases in body temperatures up to 45°C were observed in birds having high energy expenditures during long-distance flights while having no stopovers but facing high ambient temperatures in their habitat (Nilsson and Nord, 2018).

In the case of lactation, the higher body temperature leads to a temperature conflict between the mother and her pups: while maternal performance is improved at lowered ambient temperature, pup growth rates are smaller (Simons et al., 2011). Conceivably, mothers are trying to avoid hyperthermia as much as possible or are seeking cooler environments (Valencak et al., 2013), which may, however, lead to lower growth rates in the pups (mice: Valencak et al., 2013; golden hamsters: Ohrnberger et al., 2018a). When trying to understand the physiological processes underlying the intrinsic limit on energy intake during lactation in the females, manipulations facilitating heat loss for the females while keeping the environment constant for the young seem most meaningful.

A large body of work in rodents, lagomorphs and some other mammalian taxa has been collected over the past two decades to identify experimental situations where females, despite being pushed to the apparent physiological limits of food intake, are able to raise their energy intake and synthesise more milk for their offspring. Similarly, a test for a physiological limitation imposed by heat was previously performed in passerine birds (Nilsson and Nord, 2018). Initial studies, for example, showed that when females were exposed to the cold (5°C), they could elevate their food intake above the level established at higher temperatures but they were found to be unresponsive to other manipulations (Hammond and Diamond, 1992, 1997; Hammond et al., 1994; Hammond and Kristan, 2000; Johnson and Speakman, 2001; Johnson et al., 2001). This led to the notion that capacity to dissipate heat might be a key constraining factor. For example, female laboratory mice from the MF1 strain, shaved dorsally along the body length axis, thereby removing parts of their insulation, consumed 15% more food and correspondingly weaned offspring 15% heavier (Król et al., 2007). These data therefore supported the heat dissipation limitation hypothesis, suggesting that the increased energy intake becomes possible when the females can more easily dissipate body heat to the environment (Speakman and Król, 2010).

Several attempts have been made to replicate these observations, with varying success. In Swiss mice, shaving at 21°C led to greater thermal conductance and elevated food intake, but the mice did not produce more milk or heavier litters (Zhao and Cao, 2009;

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**List of symbols and abbreviations**

BMR	basal metabolic rate
DEE	daily energy expenditure
DLW	doubly labelled water
GEI	gross energy intake
$M_b$	body mass
MEI	metabolisable energy intake
MEO	milk energy output
$T_b$	subcutaneous body temperature

Zhao et al., 2010). Similarly, studies of shaved oriental voles (*Eothenomys miletus*) from the Qinghai Tibetan plateau in lactation also indicated increased food intake with no effects on litter growth (Zhu et al., 2016). However, the milk energy output in these studies was measured from an energy budget approach assuming constant milk utilisation efficiency of the offspring, which has subsequently been shown to be incorrect (Speakman et al., 2001). In bank voles (*Myodes glareolus*), shaving females at peak lactation resulted in elevated food intake, metabolisable energy intake and elevated litter growth (Sadowska et al., 2016). This was linked to increased milk output that was measured using an isotope-based methodology that is robust to the assumption of constant offspring milk conversion efficiency (Sadowska et al., 2016). Recently, Sadowska et al. (2019) reported that mice selected for either high or low basal metabolic rate (BMR) that had their fur removed with a hair removal cream during lactation showed significant differences in their thermal conductance. Yet, contrasting previous studies where small mammals were shaved, they did not increase their daily energy assimilation rates (Sadowska et al., 2019). Litters were experimentally stabilised at a litter size of eight pups, consisting of four 'own' offspring and four 'fostered' pups from the other genetic background (either high BMR or low BMR) (Sadowska et al., 2019). While total milk energy output (MEO) was not determined in that study, pup growth rates more closely followed the genetic origin of the mothers than having been affected by the hair removal (Sadowska et al., 2019). Thus, lowered thermal conductance owing to hair removal did not allow the females to elevate their intake or raise heavier litters (Sadowska et al., 2019). In view of the diversity of study outcomes in response to the experimental manipulation of depilation during lactation, new experiments are needed to clarify its role. All studies thus far show that shaving results in elevated thermal conductance. All studies with one exception have shown increased food intake, but the effects of shaving on milk production and pup growth varied most between the studies. Part of the variation in MEO estimates may stem from the method used to quantify it. Some methods are sensitive to assumptions of offspring utilisation efficiency for milk intake and hence may fail to detect any impact of the shaving, while more direct isotope-based methods may provide a more robust quantification. Another reason for the different responses is that different strains and species are differentially affected by the limits imposed by heat dissipation capacity, or that such limits apply over different temperature ranges in different strains and species (Speakman and Król, 2010; Wen et al., 2017; Yang et al., 2013).

The golden hamster (*Mesocricetus auratus* Waterhouse 1839) provides a promising model system to test the role of depilation during lactation. If certain mammalian species are more likely to be constrained by heat dissipation during lactation, we hypothesised it will be those females that have a larger body size than mice (hence less favourable surface-to-volume ratio) but at the same time equally

large litters, very altricial young and thus a quick raising and swiftly maturing offspring.

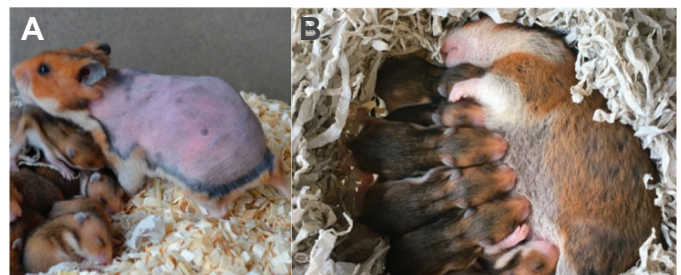
We have shown previously that golden hamsters significantly reduce litter size when nursing young at ambient temperatures of 30°C (Ohrnberger et al., 2016) and that energy intake at 30°C cannot be increased to the levels observed in lactating females at normal temperatures (Ohrnberger et al., 2016). Subsequently, we observed that cold temperatures of 8°C led to elevated gross energy intakes (GEI) and MEO (Ohrnberger et al., 2018a), thereby indicating that this species is also constrained by heat dissipation, and is affected by overheating and higher body temperatures during lactation (Ohrnberger et al., 2018a). If these responses really reflect heat dissipation limits, then we predict that this species should respond to shaving by elevating food intake, MEO and pup growth. Golden hamsters have the shortest gestation among placental mammals (16–18 days) (Fritzsche et al., 2006) and give birth to large litters of up to 16 pups. Their lactational energy costs and level of required sustained energy intake during nursing of the offspring is therefore similar to that of laboratory mice, reaching six to eight times resting metabolic rate during peak lactation (Ohrnberger et al., 2018a).

To test the prediction that increasing thermal conductance in lactating female golden hamsters would alleviate maternal heat load and enable them to increase their GEI and MEO, and hence offspring growth, we conducted a shaving experiment (Fig. 1). Apart from time courses of GEI in the females, we measured body masses, pup growth rates, MEO using doubly labelled water, and subcutaneous body temperatures in shaved experimental and control hamsters (unshaved group).

**MATERIALS AND METHODS****Animals and maintenance**

All experiments described below were approved by the ethics committee of the University of Veterinary Medicine Vienna and then by the Austrian Ministry of Science (approval numbers GZ 68.205/0028-WF/V/3b/2016 and GZ 68.205/0047-WF/V/3b/2017). Thus, all necessary actions were taken according to the Animal Experiments Act (Tierversuchsgesetz 2012) in Austria.

Eight laboratory golden hamsters were obtained from Charles River Laboratories (Sulzfeld, Germany). Using these animals, we started a breeding stock of golden hamsters in our laboratory. From this colony we used a total of 33 lactating females and 16 male golden hamsters for the shaving experiment. Males and females were between 90 and 300 days old and were kept on a 16 h:8 h light:dark photoperiod at 22±2°C. Animals were housed individually in polycarbonate cages (595 mm×380 mm×200 mm; Eurostandard Type IV, Techniplast, Germany) with autoclaved wood shavings (Abedd; Ssniff, Soest, Germany) as described in Ohrnberger et al.



**Fig. 1. Lactating female golden hamsters with their litters.** (A) Shaved and (B) unshaved golden hamster females with their litters.

(2018a). Golden hamsters were randomly assigned to one of the two experimental groups and remained in this assigned group for potential, consecutive lactational events. Females were regularly paired with males and allowed to raise litters consecutively (one to four litters per individual female). In total, we collected data from 18 litters in the shaved group, and 22 litters in the unshaved group. Three individual females in the shaved experimental group were observed during two separate, consecutive breeding events and four females from the unshaved group were observed twice. Once assigned to one experimental group (shaved or unshaved), the animals remained in this category throughout the experiment. By using repeated measurements ANOVA and including the random factor 'individual', we accounted for the repeated observation of the same individuals (see 'Statistical analysis' section below). To ensure that all females became pregnant, they were paired with males for 4 days, after which the males were removed. Females were paired with the same males in case of successful pregnancy and lactation and the same males were used for pairing in both groups. Pregnancy was observed by an increase in body mass over 7 days following the mating. On day 19 after parturition, litters were separated from their mothers, and body masses were measured. During the following 3-week experimental break, the females' fur fully re-grew until the next pairing. All animals had *ad libitum* access to food and water throughout the experiment. They received commercial hamster diet V 2144 (Ssniff) during the experiment and had their food intake measured daily by weighing the amount of food left on the hopper each day and subtracting it from the previous day, as given in Ohrnberger et al. (2018a). Metabolisable energy intake (MEI) was computed as daily food intake, i.e. GEI (indicated as dry food consumption in  $\text{g day}^{-1} \times \text{food energy content in kJ g}^{-1}$  dry mass) subtracted by defecated energy corrected for urinary protein losses owing to nitrogen excretion. Urinary energy loss was assumed to be 3% of the digestible energy intake (Drozd, 1975). Thus, digestive efficiency was determined as a percentage of GEI digested, as described in more detail in Ohrnberger et al. (2018a). To obtain faecal energy content, we used a bomb calorimeter (IKA, Königswinter, Germany) (see Ohrnberger et al., 2018a). The animal room where the experiment took place was isolated from outside and accessible only to four people, following strict hygiene protocols. Twice per year, the hygiene status of the colony was determined according to Felasa recommendations by AnLab (Prague, Czech Republic) and the results can be released upon request to the lead author.

### Shaving

To shave the females, we used a Wella Contura hair clipper (Wella, Darmstadt, Germany). On day 6 of lactation, females were briefly taken out of their cages, separated carefully from the pups, and shaved dorsally (Fig. 1) to manipulate heat dissipation of the females around the time of asymptotic food intake but to minimise the stress around day 1 of lactation. They were then immediately returned to the nest. The animals were previously handled daily, so removal for shaving caused minimal stress to the individual animals. However, to control for this disturbance, unshaved females were also taken out of the nest, handled for 3–4 min (without shaving but with the clipping device on and making the noise), and then carefully returned to the nest.

### Subcutaneous body temperature and use of the infrared camera

At 8 weeks of age, all females had a passive integrated transponder implanted subcutaneously, which allowed daily measurements of

subcutaneous body temperature (IPTT-300, BioMedic Data Systems). The device was factory calibrated according to <http://www.bmds.com/products/transponders/iptt-300/specs>. For the implantation process, the female was briefly taken out of the cage, held between the shoulders, and the transmitter was carefully implanted with a syringe in the subcutaneous tissue over the animal's lumbar vertebrae (to avoid the interscapular brown adipose tissue as well as the mammary glands where the suckling pups could affect skin temperature). The use of the IPTT-300 allowed us not only to identify the animal even when it was co-housed with the male, but also to obtain recordings of subcutaneous body temperature with the help of a hand-held reading device (DAS-7006/7s, BioMedic Data Systems). No anaesthesia was needed for implantation of the transponders, as the severity of the subcutaneous implantation was minor and restricted to less than 3 min, with the females used to being handled.

To show the altered circulation of heat over the body surface in the females and to identify the hottest body parts, we took pictures with an infrared camera (VarioCAM; InfraTec, Dresden, Germany) with its analysis software IRBIS (version 3) with an accuracy of  $\pm 1^\circ\text{C}$ .

### Milk energy output

Daily energy expenditure (DEE) was determined to compute milk production of females between days 12 and 14 of lactation using the doubly labelled water (DLW) method (Butler et al., 2004). Briefly, females were injected intraperitoneally with  $\sim 0.2$  ml of DLW of known mass and characterised isotopic enrichment ( $\sim 329,000$  ppm  $^{18}\text{O}$ ,  $\sim 186,000$  ppm  $^2\text{H}$ ) on day 12 of lactation. The exact dose was quantified by weighing the syringe to the nearest 0.0001 g before and after administration. An initial blood sample of 100  $\mu\text{l}$  was collected 1 h after the injection from the lateral saphenous vein and stored in glass capillaries that were immediately flame-sealed with a blowtorch. The female was immediately returned to her cage and litter. Forty-nine hours after the injection a second and final blood sample was collected, timed to minimise the effects of diurnal variation in activity (Speakman and Racey, 1987). Ten blood samples of additional hamsters (that had no litter) and had not been injected with DLW were collected to assess the natural background abundances of  $^2\text{H}$  and  $^{18}\text{O}$  in the body water pools of the animals (Speakman and Racey, 1987). Capillaries that contained the blood samples were vacuum distilled while water from the resulting distillate was used to produce  $\text{CO}_2$  and  $\text{H}_2$  (Vaanholt et al., 2013). The isotope ratios  $^{18}\text{O}:^{16}\text{O}$  and  $^2\text{H}:^1\text{H}$  were analysed using gas source isotope ratio mass spectrometry (IRMS) (Micromass Optima, Isochrom  $\mu\text{G}$ , Manchester, UK) (Speakman et al., 1990). Samples were run alongside three laboratory standards for each isotope (calibrated to international standards) to correct delta values to ppm (Vaanholt et al., 2013). Energy expenditure was calculated using a single pool model as recommended for animals of this size (Speakman, 1993) and is shown to be superior in validation studies (Król and Speakman, 2003).

### Data collection

All measurements were taken daily between 08:00 and 11:00 h. The morning when pups were found was considered as the day of parturition, referred to as day 0 of lactation. To minimise disturbances, all measurements were suspended on the day of parturition. From day 1 of lactation onwards, we measured female body masses ( $M_b$ ) and subcutaneous body temperatures ( $T_b$ ), food intake (FI), pup number and pup masses ( $M_{\text{pup}}$ ) all together on a daily basis until weaning (day 19). Daily food intake (in grams) was continuously monitored except during the mating period (when they

were housed with males). To assess faecal energy content, we collected faeces of each female over a 3-day period, dried the faeces to constant mass (Heraeus drying oven T 5042, Hanau, Germany) and determined its energy content with a bomb calorimeter (C 5000 control calorimeter, IKA).

### Statistical analysis

All statistical analyses were conducted in R version 3.5.3 (<https://www.r-project.org/>). Data on GEI, MEI, average daily  $M_{\text{pup}}$ , DEE and  $T_b$  were obtained repeatedly from the same females and therefore with a repeated measures design. We thus used linear mixed effect models (package nlme; <https://CRAN.R-project.org/package=nlme>) to analyse the dataset. We included  $M_b$  of the female, experimental group (shaved or unshaved) and day of lactation as factors and the identity (ID) of each female as a 'random' factor to fit separate intercepts for each animal. Energy metabolism data from peak lactation (i.e. days 10–12 of lactation when food intake reached the well-known asymptote) along with data for DEE and MEO were analysed separately with multivariate linear regression models ('lm' models), with each female going into the dataset once as we obtained faecal samples and DLW data only once per lactational event from each female. In these models, the dependent variables were  $M_b$ , subcutaneous  $T_b$ , GEI,  $M_{\text{pup}}$  and MEO. Similarly, any potential two-way interactions between the experimental group (shaved–unshaved) and female age were

computed for all the models calculated. Graphs were prepared in GraphPad Prism 7 with all values used in the graphs presented as means with standard errors of the mean (s.e.m.). We also used linear mixed-effects models to assess differences between the experimental groups of  $M_b$ , food intake, GEI during pregnancy and baseline period.

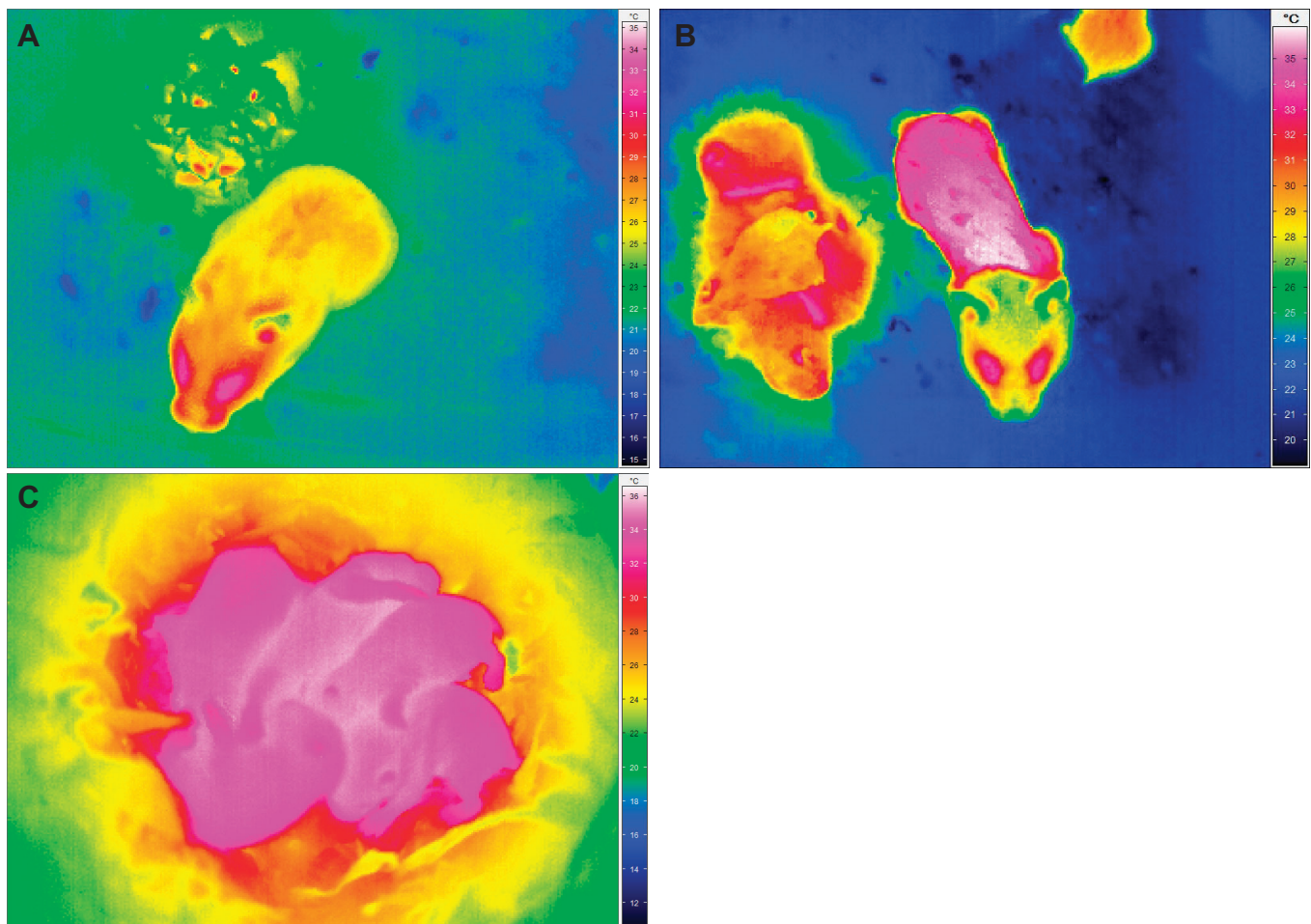
### RESULTS

Fig. 2 shows the experimental effect of shaving on the heat dissipation over the body surface of the female, as well as of the heat production caused by huddling of the furred pups.

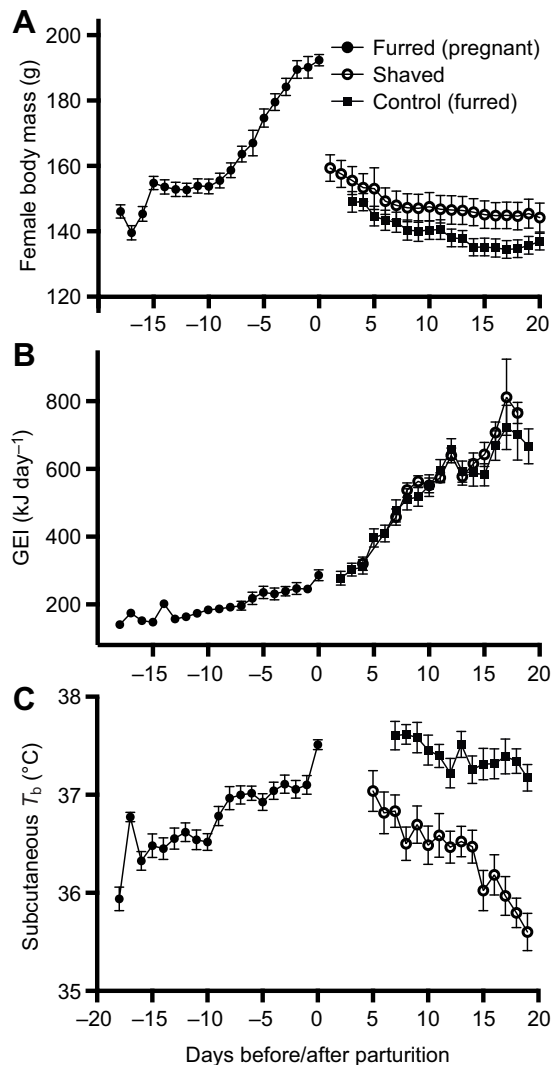
#### Overall gestation and lactation

As expected, female body mass increased over the course of gestation ( $F_{1,656}=1150.39$ ,  $P<0.001$ ; Fig. 3A), as did GEI ( $F_{1,412}=57.94$ ,  $P<0.001$ ; Fig. 3B). Older females had higher body mass increases in gestation ( $F_{1,656}=40.71$ ,  $P<0.001$ ). Individual age of the females had no influence on GEI or subcutaneous temperatures during pregnancy (partial for GEI:  $F_{1,412}=0.173$ ,  $P=0.7$ ). Subcutaneous body temperatures increased during gestation ( $F_{1,643}=231.4$ ,  $P<0.0001$ ; Fig. 3C).

Time courses of  $M_b$ , GEI and subcutaneous temperatures prior to and after parturition are given in Fig. 3. Overall, we observed that  $M_b$  differed significantly between day of lactation ( $F_{1,834}=494.08$ ,  $P<0.0001$ ; Fig. 3A) and shaved females were heavier than unshaved females ( $F_{1,834}=494.08$ ,  $P<0.0001$ ;



**Fig. 2.** Heat dissipation over the body surface monitored with an infrared thermocamera. Heat dissipation in lactating female golden hamsters shown by an infrared camera in (A) the unshaved group, (B) the shaved group and (C) in their litter.



**Fig. 3. Body mass, gross energy intake and subcutaneous body temperature throughout gestation and lactation of female golden hamsters.** (A) Body mass, (B) GEI and (C) subcutaneous body temperature ( $T_b$ ). Please note that the filled circles reflect the furred state in all females. From day 6 of lactation onwards, females in the shaved group ( $N=18$ ) had their fur removed dorsally, whereas unshaved females ( $N=22$ ) had intact fur. Values are given as means $\pm$ s.e.m.

Fig. 3A). Shaved and unshaved females had mean  $M_b$  of  $149.2\pm 4.5$  and  $135.98\pm 2.7$  g, respectively (Fig. 3A, Table 1). Females raising larger litters were also heavier ( $F_{1,834}=197.5$ ,  $P<0.0001$ ). GEI differed between days of lactation ( $F_{1,788}=684.82$ ,  $P<0.0001$ ; Fig. 3B) and we observed that older, heavier females had higher GEI ( $F_{1,788}=44.38$ ,  $P<0.0001$ ; Fig. 3B).

As expected, subcutaneous temperatures differed between shaved and unshaved females ( $F_{1,834}=60.8$ ,  $P<0.0001$ ; Fig. 4C). We observed fluctuating temperatures with day of lactation ( $F_{1,834}=233.1$ ,  $P<0.0001$ ; Fig. 4C). Especially late in lactation, when pups were picking up solid food, the subcutaneous temperatures in the shaved group were clearly lower than earlier in lactation and lower than in controls (Fig. 5C).  $M_{pup}$  during lactation was significantly influenced by maternal body mass ( $F_{1,828}=395.2$ ,  $P<0.0001$ ), day of lactation ( $F_{1,826}=4045.5$ ,  $P<0.0001$ ) and litter size ( $F_{1,826}=9.5$ ,  $P=0.002$ ), with pups from larger litters being lighter.  $M_{pup}$  was significantly higher in shaved females ( $F_{1,826}=76.02$ ,  $P<0.0001$ ; Fig. 5A).

**Table 1. Body mass and energy metabolism at peak lactation (days 10–12 of lactation) in shaved and unshaved lactating golden hamsters**

	Shaved	Unshaved
Number of litters	18	22
$M_b$ (g)	$149.2\pm 4.5$	$135.98\pm 2.7$
GEI ( $\text{kJ day}^{-1}$ )	$650.53\pm 19.97$	$592.12\pm 20.43$
Asymptotic MEI ( $\text{kJ day}^{-1}$ )	$581.79\pm 21.5$	$521.05\pm 11.5$
DEE ( $\text{kJ day}^{-1}$ )	$224.55\pm 7.6$	$231.47\pm 11.1$
MEI ( $\text{kJ day}^{-1}$ )	$581.79\pm 21.5$	$521.05\pm 11.5$
MEO ( $\text{kJ day}^{-1}$ )	$357.23\pm 22.9$	$289.6\pm 8.4$
AE (%)	$86.74\pm 1.7$	$87.2\pm 3.2$
Individual $M_{pup}$ (g)	$17.2\pm 1.9$	$13.9\pm 1.5$
Litter size	$8.6\pm 0.6$	$9.9\pm 0.5$
Subcutaneous $T_b$ ( $^{\circ}\text{C}$ )	$36.58\pm 0.14$	$37.32\pm 0.11$

$M_b$ , body mass;  $M_{pup}$ , pup mass; GEI, gross energy intake; MEI, metabolisable energy intake; DEE, daily energy expenditure; MEO, milk energy output. Data are given as means $\pm$ s.e.m.

### Peak lactation (days 10–12 of lactation) and milk production

Body masses differed significantly between shaved and control females at peak lactation, with shaved individuals being on average 13.3 g heavier ( $F_{1,36}=9.92$ ,  $P=0.003$ ; Table 1, Fig. 4A). Older and experienced golden hamster females had higher  $M_b$  at peak lactation ( $F_{1,36}=5.42$ ,  $P=0.03$ ). Finally,  $M_b$  was affected by litter size during peak lactation ( $F_{1,36}=13.5$ ,  $P=0.0008$ ), with females raising large litters being heavier. We observed no interaction between female age and litter size ( $F_{1,35}=0.19$ ,  $P=0.67$ ).

Expectedly, subcutaneous  $T_b$  were lower in the shaved group ( $F_{1,35}=18.14$ ,  $P=0.0001$ ; Table 1, Fig. 4C). Shaved females had a  $T_b$  of  $36.6\pm 0.14^{\circ}\text{C}$  on average, and females with intact fur had a  $T_b$  of  $37.3\pm 0.11^{\circ}\text{C}$  (Table 1). Subcutaneous  $T_b$  was not influenced by female age ( $F_{1,6}=1.98$ ,  $P=0.21$ ) or litter size ( $F_{1,6}=2.48$ ,  $P=0.17$ ).

GEI differed significantly between shaved and unshaved golden hamsters at peak lactation (partial effect:  $F_{1,36}=5.52$ ,  $P=0.02$ ; Table 1). Conceivably, GEI was independently influenced by litter size, with higher GEI in the large litters ( $F_{1,36}=14.06$ ,  $P=0.0006$ ). Female age did not have an influence on GEI at peak lactation ( $F_{1,36}=1.4$ ,  $P=0.25$ ). Similarly, MEI was higher in the shaved group (partial effect:  $F_{1,36}=6.74$ ,  $P=0.014$ ; Table 1, Fig. 4B) at peak lactation. Maternal age and litter size did not affect MEI at peak lactation ( $P>0.05$ ; results not shown). We observed no interaction between age and experimental group ( $F_{1,35}=0.04$ ,  $P=0.84$ ).

Individual  $M_{pup}$  at peak lactation differed significantly between litters from shaved females and controls ( $F_{1,36}=37.89$ ,  $P<0.0001$ ; Fig. 5A, Table 1).

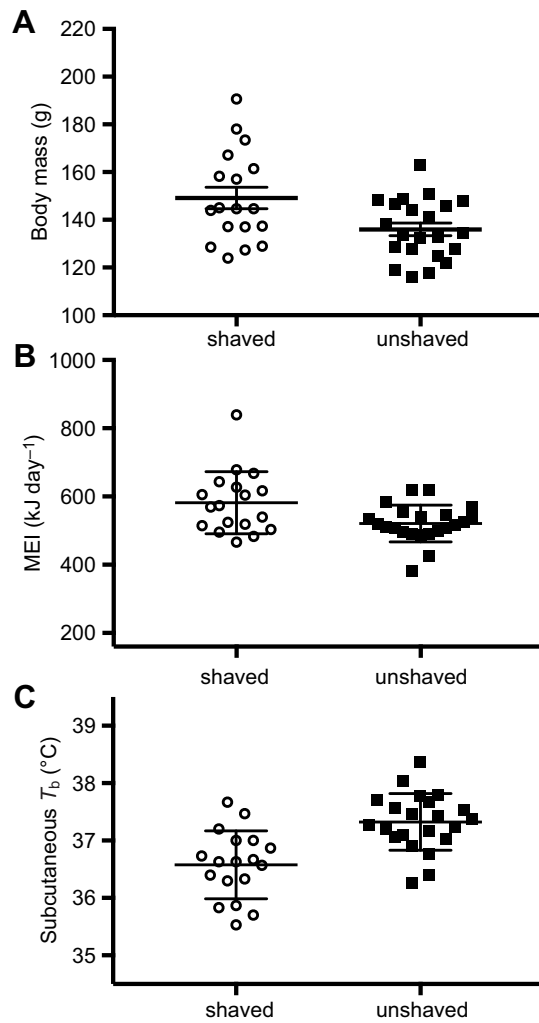
$M_{pup}$  was not influenced by maternal age ( $F_{1,36}=0.4$ ,  $P=0.5$ ). When in a larger litter, individual  $M_{pup}$  values were lower ( $F_{1,36}=4.8$ ,  $P=0.04$ ).

MEO was higher in shaved golden hamsters ( $F_{1,36}=8.7$ ,  $P=0.006$ ; Fig. 5C, Table 1). MEO was not independently related to mother's age ( $F_{1,36}=0.02$ ,  $P=0.9$ ) or litter size ( $F_{1,36}=1.0$ ,  $P=0.3$ ). DEE did not differ between shaved and unshaved females ( $F_{1,35}=1.05$ ,  $P=0.3$ ; Table 1), and was not affected by age ( $F_{1,35}=1.6$ ,  $P=0.22$ ) or litter size ( $F_{1,35}=0.5$ ,  $P=0.47$ ). There was no significant interaction between female age and experimental group ( $F_{1,35}=2.3$ ,  $P=0.14$ ).

## DISCUSSION

### Hot again: heat dissipation limitation in lactating golden hamsters

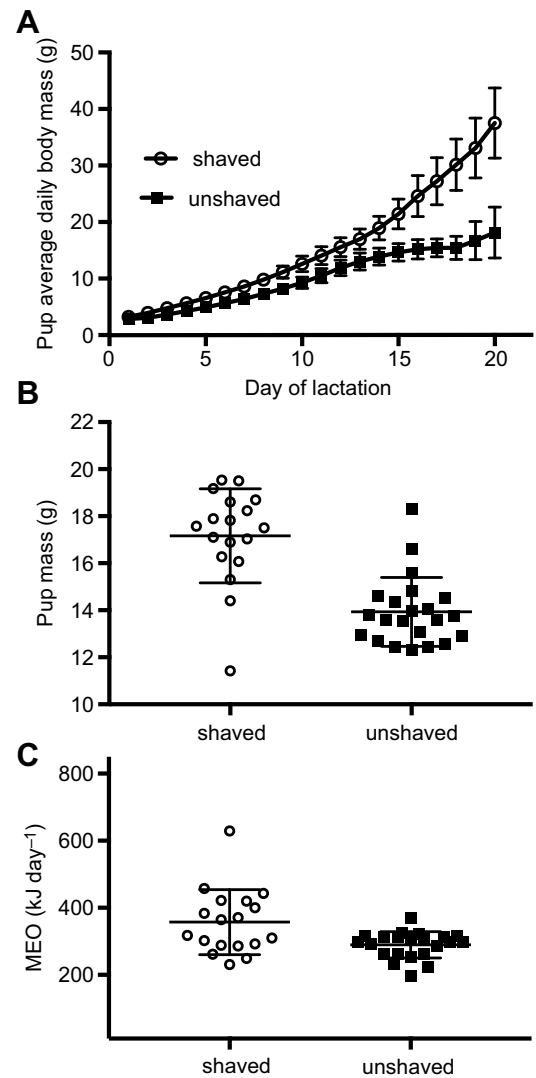
Shaving golden hamsters dorsally gave rise to increased milk production while keeping the thermal budgets for the offspring constant at the same time. Golden hamsters are not limited centrally



**Fig. 4. Differences between shaved and unshaved females at peak lactation.** (A) Body mass, (B) metabolisable energy intake (MEI) and (C) subcutaneous temperature ( $T_b$ ) at peak lactation (days 10–12 of lactation) from lactating female golden hamsters either from the shaved ( $N=18$ ) or the unshaved ( $N=22$ ) group. Values are given as means $\pm$ s.e.m.

by the alimentary tract or peripherally by the energy-spending machinery, but female energy intake is dictated by heat dissipation at peak lactation, as shown in Ohrnberger et al. (2018a) when nursing females were exposed to 8, 22 and 30°C. Female GEI and MEO were significantly higher in cold (8°C) exposed animals, while remaining intermediate at room temperature and were reduced in hot (30°C) conditions (Ohrnberger et al., 2018a). Even in the non-reproductive state, Kauffman et al. (2003) observed a 22% increase in food intake in furless Siberian hamsters (*Phodopus sungorus*) at cold conditions of 5°C.

However, lowering ambient temperatures during lactation has negative consequences on pup growth rates owing to the increased energy costs for thermoregulation (Ohrnberger et al., 2018a). Thus, in the current experiment, we set out to experimentally manipulate female thermal conductance by shaving, keeping constant thermal conditions for the litter. Indeed, shaved lactating golden hamster females showed higher GEI, MEI and MEO with strong effect sizes (Figs 4 and 5). These observations only allow the interpretation that shaved females were released from heat stress by getting rid of body heat through the body surface as visible in Fig. 2B. Compared with their unshaved counterparts, shaved golden hamsters successfully



**Fig. 5. Differences in offspring-related parameters between litters from shaved and unshaved females.** Time courses of (A) pup average daily body mass, (B) mean pup mass in both experimental groups, and (C) milk energy output at peak lactation (days 10–12 of lactation) from lactating female golden hamsters either from the shaved ( $N=18$ ) or the unshaved ( $N=22$ ) group. Values are given as means $\pm$ s.d.

release heat along the dorsal axis (Fig. 2B) and interestingly, their shaved body parts show similar surface heat as their litter huddling together (Fig. 2C). By releasing heat along the shaved body parts (contrary to the unshaved ones in Fig. 2A), they were able to significantly improve their milk conversion rate and produce more milk and heavier pups (Fig. 5). Impressively, their GEI when shaved was increased to 650.5 kJ day<sup>-1</sup> (i.e. by only 9%; Table 1) compared with controls, whereas it was increased by 29% in lactating females exposed to 8°C (Ohrnberger et al., 2018a). Their MEO, however, was increased over 20% compared with unshaved controls, from 289.6 to 357.2 kJ day<sup>-1</sup> (Table 1). Previously, we have shown that the release from heat stress is visible in lower faecal cortisol metabolites in the shaved females (Ohrnberger et al., 2018b), also suggesting alleviation from heat stress.

To our knowledge, few studies have studied the effects of heat dissipation limitation on animals outside the laboratory, in a natural setting. On particularly hot days, male weasels (*Mustela nivalis*) were previously observed to have decreased activity and daily

energy expenditure, well in line with facing heat stress in their environment (Zub et al., 2013).

In migrating eider ducks (*Somateria mollissima*), Guillemette et al. (2016) showed that increased body temperatures in flying birds led to a termination of the flight so that total time spent flying and maximum flight duration were related to turnover of body heat. Birds combine higher and more variable body temperatures than mammals, so it has been suggested that they might indeed be limited by heat dissipation (Grémillet et al., 2012). Following that, Nilsson and Nord (2018) showed that manipulating brood size and increasing parental workload during the nestling feeding time in marsh tits (*Poecile palustris*) caused higher body temperatures in both males and females, even exceeding 45°C (Nilsson and Nord, 2018). Recently, Tapper et al. (2020) trimmed the ventral feathers of tree swallows (*Tachycineta bicolor*) to provide a thermal window and showed that trimmed females fed their nestlings at higher rates. Yet Andreasson et al. (2020) observed in ventrally trimmed blue tits (*Cyanistes caeruleus*) that the manipulated females fed less frequently but had a higher innate immune function than controls, suggesting that the reduced constraints of overheating allowed for an improved self-maintenance function in the females. In conclusion, there is growing recent experimental evidence from breeding birds that the energy budgets and immune system are significantly affected by feather removal and heat loss, and that these manipulations even lead to heavier offspring (Nord and Nilsson, 2018), partly mimicking the observations in shaved mammals during lactation.

By showing higher milk production in the shaved golden hamster females, we confirm the large energy burden imposed on them: solitarily living animals have the shortest known gestation period known from placental mammals (less than 18 days), at the same time producing large litters of up to 16 pups (Fritzsche et al., 2006). However, litter size seems very plastically adjusted according to the thermal conditions encountered during reproduction, with a large number of pups disappearing from the litters at 30°C (Ohrnberger et al., 2016). Traditionally, these litter size reductions were attributed to maternal distress owing to lactation and mostly were identified as infanticide, although siblicide would also be possible (Ohrnberger et al., 2016). Yet we have argued that, if thermal conditions are too hot, litter size reductions probably represent one possible way for the females to rescue reproductive success by investing in a small number of pups only at conditions when GEI cannot be increased and milk conversion rates are minimal (Ohrnberger et al., 2016, 2018b).

To maximise GEI during lactation, the aspect of time required to grow and enlarge the gastrointestinal tract to allow for daily increased energy intake, is important (Valencak and Ruf, 2009). Our new study in golden hamsters adds that if females are shaved and relieved of heat stress on day 6 of lactation, they swiftly but efficiently improve their lactational output (Fig. 5C). Shaved golden hamsters thus benefited from the improved conductance around peak lactation by being able to ingest more food when pup demand was at its peak around day 10 of lactation (Fig. 3B). On day 10 of lactation, pups still rely completely on milk but have already grown to an individual body size of over 10 g, thereby requiring more milk from the mother than during the first week of lactation (Fig. 5A). Shaved females seemed to have an extra advantage in this situation by utilising the improved conductance for improving their food-to-milk conversion rates during the critical phase of peak lactation (Table 1, Fig. 3). This effect is underlined by the fact that DEE did not differ between shaved and unshaved females and by the increased growth curves of the pups from shaved mothers later in lactation when the pups independently pick up food (Fig. 5A,

Table 1). Our new data thus confirm that heat dissipation is limiting maternal energy metabolism in lactating golden hamsters. The data also highlight the aspect of time because the seemingly non-invasive dorsal shaving on day 6 of lactation led to improved milk production at, impressively, similar GEI.

Recently, Sadowska et al. (2019) rejected the heat dissipation limitation hypothesis when considering different mouse strains selected for high and low BMR, and rather suggested that sustained high energy turnover rates were associated with a positive correlation between BMR and the ability to cope with metabolic challenges. Although Sadowska et al. (2019) did not quantify milk energy output, which would be necessary to completely dispute the heat dissipation limit hypothesis, their rejection of this model clearly pertains only to the strains and circumstances under which they were measured, i.e. at 21°C. Similarly, Zhao and Cao (2009) and Zhao et al. (2010) rejected the heat dissipation limit idea in Swiss mice at 21°C, but later showed that at 30°C, this strain of mouse in fact is limited by heat dissipation (Zhao et al., 2016). In Mongolian gerbils (*Meriones unguiculatus*), it was also shown that at high temperatures lactation was probably heat limited, but at low temperature probably was not (Yang et al., 2013).

With a body mass of ~150 g (Fig. 3A), golden hamsters weigh over four times that of an average laboratory mouse, and at the same time have enlarged maternal demands as litter size compares with mice, and their young are equally altricial at birth. Golden hamsters nurse their young throughout the day and night (S. A. Ohrnberger personal observation) and face temperatures above thermoneutrality in the nest (Fig. 2). Our previous data for these rodents lactating at temperatures between 8 and 30°C are clearly consistent with the heat dissipation limit concept (Speakman and Król, 2010). Here we confirm this interpretation experimentally by increasing heat dissipation capacity by shaving. One difference between the study of Sadowska et al. (2019) and other depilation studies was the mode of hair removal. Whether this matters for the results needs to be elucidated in future studies. Sadowska et al. (2019) used a hair removal cream on mice on day 8 of lactation and achieved 16% increased thermal conductance in the mice selected for a high BMR. By shaving the fur off completely dorsally by following the protocols from Król et al. (2007) (Figs 1 and 2), we may have achieved an even higher thermal conductance (Fig. 2).

However, the different body size allometries between laboratory mice and golden hamsters need to be considered. We have previously indicated the potential role of fat reserves in having an influence on the limit to sustained energy intake in mammals (Valencak et al., 2009), and our new data from shaved golden hamsters are well in line with this suggestion. While we are aware that the capacity to store body fat reserves may be somewhat limited in laboratory mice owing to their energy metabolism, mammals with larger body sizes may have increased capacity to store and accumulate white adipose tissue well ahead of the time when the actual peak energy expenditures occur. This may avoid the energy demands of lipogenesis and milk synthesis in a similar way as has been proposed for mice eating high-fat diets (Huang et al., 2020; Kagya-Agyemang et al., 2018). To cover the high lactational energy costs, females of larger body size than mice therefore may benefit from previously built-up white adipose tissue depots by metabolising them during the phase of lactation. The consistent influence of maternal age, which ranged between 3 and 10 months on the measured parameters in our study, further underlines this argument. Older, heavier individuals might indeed have a higher potential to successfully raise large litters by producing more milk under *ad libitum* fed conditions.

## Conclusions

By shaving lactating females dorsally and observing increased rates of MEO and pup growth, we have shown that heat dissipation limits drive maternal food intake and metabolism in support of the heat dissipation limit hypothesis in a different model system: the golden hamster. These solitary animals not only have large litters and a short gestation time, but they succeed in weaning viable offspring within 18 days post-parturition. Our experiment performed in an animal with four times larger body size than a mouse yet with similar reproductive performance brought up numerous interesting effects, and allows the conclusion that shaving females dorsally lowered maternal heat stress, thus enabling increased milk production while keeping the thermal environment for the pups constant and allowing continuous growth. Confirming earlier studies, the thermal conditions during the phase while asymptotic GEI occurs are critical for both maternal milk output and the growth curve of the pups. Those pups, optimally supplied with milk around peak lactation, are the ones which swiftly mature to pick up solid food themselves, thereby lowering the maternal demand. While we are aware that this solid food compensation in the pups only takes place in a laboratory setting, we suggest that offspring growth rates in wild-reared litters might be compromised even more, when mothers encounter too high ambient temperatures in lactation.

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## Competing interests

The authors declare no competing or financial interests.

## Author contributions

Conceptualization: J.R.S., T.G.V.; Methodology: S.A.O., C.H.; Investigation: S.A.O., C.H., J.R.S.; Writing - original draft: S.A.O., T.G.V.; Writing - review & editing: J.R.S., T.G.V.; Visualization: S.A.O., T.G.V.; Supervision: T.G.V.; Project administration: S.A.O.; Funding acquisition: T.G.V.

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