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RESEARCH ARTICLE

Limits to sustained energy intake. XXIX. The case of the golden hamster (*Mesocricetus auratus*)

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ABSTRACT

Golden hamster females have the shortest known gestation period among placental mammals, and at the same time raise very large litters of up to 16 offspring, which are born in a naked and blind state and are only able to pick up food from days 12 to 14 onwards. We quantified energy metabolism and milk production in female golden hamsters raising offspring under cold (8°C), normal (22°C) and hot (30°C) ambient temperature conditions. We monitored energy intake, subcutaneous body temperature, daily energy expenditure, litter size and pup masses over the course of lactation. Our results show that, in line with the concept of heat dissipation limitation, female golden hamsters had the largest energy intake under the coldest conditions and a significantly lower intake at 30°C (partial for influence of ambient temperature: $F_{2,403}$=5.6; P=0.004). Metabolisable energy intake as well as milk energy output showed the same pattern and were significantly different between the temperatures (partial for milk energy production: $F_{1,40}$=86.4; P<0.0001), with consistently higher subcutaneous temperatures in the reproductive females ($F_{1,813}$=36.77; P<0.0001) compared with baseline females. These data suggest that raising offspring in golden hamsters comes at the cost of producing large amounts of body heat up to a level constraining energy intake, similar to that observed in some laboratory mice. Notably, we observed that females seemed to adjust litter size according to their milk production, with the smallest litters (3.4±0.7 pups) being raised by hot-exposed mothers. Future research is needed to unravel the mechanism by which females assess their own milk production capabilities and how this may be linked to litter size at different ambient temperatures. Golden hamsters reach 8–10 times resting metabolic rate when raising offspring under cold conditions, which is compatible with the findings from laboratory mice and other rodents.

KEY WORDS: Resting metabolic rate, Litter size, Heat dissipation limitation, Subcutaneous temperature, Milk production

INTRODUCTION

Mammalian reproduction is the most energy expensive endeavour for female mammals (Gittleman and Thompson, 1988; Glazier, 1985; Loudon and Racey, 1987; Millar, 1977) and is possibly limited by the female's ability to manage heat loss during lactation (Król and Speakman, 2003a; Speakman and Król, 2010; Sadowska et al., 2016). These high energetic demands, together with the risk of overheating, influence aspects of social structure, group size, breeding strategies and life history strategies of nursing females (Speakman and Król, 2011; Thompson, 1992).

It is widely accepted that the energetic costs of reproduction reach a peak during lactation, with pregnancy leading to relatively modest costs of 3–4 times basal metabolism (Johnson and Speakman, 2001; Thompson, 1992). Yet, from an ecological viewpoint, pregnant females may more easily become subject to predation, and may have difficulties when foraging owing to impaired locomotion (Heldstab et al., 2017). Peak rates of energy use by pregnant females typically occur a few days before parturition, and the energetic costs of pregnancy may influence the ability of a female to expend energy during early lactation (Gittleman and Thompson, 1988; Duah et al., 2013). Female energy and fat reserves may also affect intrinsic physiological limitations on the energy budgets during lactation, and may allow for larger litters at constant size of the abdominal enlargement. Because the fetal growth is exponential, even a small shortening of the gestation period may allow for disproportionate increases in litter size.

Often, rodents give birth to neurobehaviourally underdeveloped neonates, termed 'altricial offspring', which are born after a relatively short gestation time in a helpless state involving no vision and sense of hearing and very limited ability to locomote and thermoregulate (Anderson et al., 2015; Lonstein et al., 2015). Based on their developmental stage, one might expect that maternal care is frequent and protracted when raising altricial young, but this impression turns out to be an over-simplification. Animals such as rabbits (*Oryctolagus cuniculus*) raise altricial young with a minimal nursing effort confined to just a couple of minutes each day (Drummond et al., 2000; Martínez-Gómez et al., 2004). Summarising, there are no simple relationships between the duration of gestation, later maternal milk investment and the development of the young. At the same time, these associations may be very important to consider when investigating the proximate factors causing the upper limits to sustained energy intake in lactation. These have been previously studied in a range of small rodents including various strains of mice and voles (Weiner, 1989; Hammond et al., 1994; Hammond et al., 1996; Koteja, 1996). In early work, the debate focused on whether the upper physiological limit of energy intake may be imposed 'centrally', by the capacity of nutrient-processing visceral organs (i.e. intestines, liver, kidneys) or 'peripherally', by the effectiveness of the mammary gland (Koteja, 1996). Later work showed that the capacity to dissipate heat [the heat dissipation limitation (HDL) theory] might provide an additional explanation as in most rodent models females consistently increased their food intake during peak energy demands around days 11–14 when exposed to temperatures below thermoneutral (Johnson et al., 2001; Król and Speakman, 2003b). In other words, the females appeared constrained in their sustained

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List of symbols and abbreviations

AE	assimilation efficiency
BAT	brown adipose tissue
DEE	daily energy expenditure
DLW	doubly labelled water
GEI	gross energy intake
HDL	heat dissipation limitation
MEI	metabolisable energy intake
MEO	milk energy output
RMR	resting metabolic rate
T_{subcut}	subcutaneous body temperature

food intake by their heat dissipation capacity, which in turn is driven by ambient temperature. Experimental interventions in female rodents exposed to normal (22°C), cold (8°C) and hot (30°C) conditions while raising offspring shed light on the energetic turnover rates and the underlying physiological limitations (Hammond et al., 1994; Johnson et al., 2001).

We chose to work with golden hamsters (*Mesocricetus auratus* Waterhouse 1839) owing to their suitability for testing the HDL concept in a model with very interesting reproductive biology. They have the shortest gestation period (16 days) among placental mammals, give birth to altricial young and have litter sizes of up to 16 pups (Hindle, 1934; Ohrnberger et al., 2016). Having a short gestation may permit such large litters, but this then creates a potential issue during lactation, when demands placed on the female reach their peak. How do golden hamsters cope with such high maternal demands in the lactation period spanning 19 days? More specifically, may they be equally limited by the capacity to dissipate excess body heat as mice (*Mus musculus*) (Johnson et al., 2001; Król and Speakman, 2003b), common voles (*Microtus arvalis*) (Simons et al., 2011), Brandt's voles (*Lasiopodomys brandtii*) (Wu et al., 2009) and Mongolian gerbils (*Meriones unguiculatus*) (Yang et al., 2013)? Or are they somehow able to circumvent such limits, allowing them to successfully raise such large litters?

We aimed to study the questions presented above owing to the short gestation time, large litter size and high maternal demand caused by the altricial young in golden hamsters. In their natural habitat, golden hamsters regularly encounter temperatures that exceed 30°C and fall below 8°C (Gattermann et al., 2001), so the manipulations we performed were inside the normal range of temperatures experienced by this species. We exposed lactating female golden hamsters to cold, normal and hot ambient conditions (8, 22 and 30±2°C, respectively) and assessed time courses of energy intake, energy expenditure by doubly labelled water (Butler et al., 2004), milk energy output, body masses and subcutaneous body temperatures in the individuals. On a daily basis, we also measured litter size, litter mass and pup growth over the lactation period. From a large body of work undertaken over the past two decades, lactation costs in cold-exposed lactating mice from the MF1 strain were reported to rise up to 7.9 and 9.4 times resting metabolism, depending on whether gross energy intake or metabolisable energy intake was used for calculation (Speakman et al., 2004). Therefore, we quantified the total lactation costs in golden hamsters at three different temperature conditions of 8, 22 and 30°C to evaluate whether they matched or exceeded such limits.

MATERIALS AND METHODS

Animals and maintenance

Golden hamsters (n=8) were obtained from Charles River Laboratories (Sulzfeld, Germany). Using these animals, we started a breeding stock in our laboratory and used 32 female and 10 male individuals. Animals were housed individually in polycarbonate cages (Eurostandard Type IV, 595×380×200 mm, Techniplast, Germany) with autoclaved wood shavings (Abedd, Ssniff, Soest, Germany). Cages were cleaned once a week except if it coincided with the day of parturition, and special care was taken with late pregnant and early lactating females. All animals were kept on a 16 h:8 h light:dark photoperiod. Before and during the pairing, all animals were kept at 22°C. Because of the solitary behaviour of golden hamsters, they were housed together only during the pairings and during lactation, when females were housed with their litters. To allow several consecutive litters, females were regularly paired with males and were allowed to raise those litters, leading to a reproductive rate of one to four litters per female. To ensure that all females, regardless of their oestrus cycle, became pregnant, they were paired with males for 4 days, after which the males were removed. Pregnancy was observed by an increase in body mass over 7 days following the mating. On day 7 after mating, i.e. days 7–11 of pregnancy (depending on individual oestrus), pregnant hamsters were randomly assigned to one of three treatment groups with different temperature settings of 8, 22 and 30°C, and remained under these different temperature regimes from the late pregnancy phase until the end of lactation on postnatal day 19. Females were exposed to the different temperatures well before parturition on day ca. 10 of pregnancy not only to separate the behavioural effects of temperature acclimation from the stress around parturition but also to allow energy intake, thermoregulation and membrane composition to stabilise well before the actual rise in energy metabolism imposed by lactation itself. On postnatal day 19, litters were separated from their mothers and all females were returned to 22°C. To expose hamster females to 8°C, their cages were put in a refrigerated cabinet (Zoin Refrigerazione, Padova, Italy), as used previously (Valencak et al., 2013). This unit provides constant temperature regulation throughout a 24 h day, has very limited noise production to which the animals easily habituate and is open on one side to allow air to flow freely. To generate the 30°C environment, a separate animal room was heated up to 30°C. To monitor stability of ambient temperatures at both 8 and 30°C, we used temperature loggers (DS1921G-F5, Thermochron iButton, Maxim Integrated, USA) with an accuracy of ±1°C that were placed in each female's cage (away from the nest but in the bedding). Measurements were taken every 3 h throughout lactation, revealing constant temperatures (±2°C) under all three ambient temperature conditions.

At an age of 8 weeks, well before the onset of the measurements, all 32 females had a temperature-sensitive transponder implanted subcutaneously, which provided each female with a unique ID and allowed measurement of subcutaneous body temperature (IPTT-300, BioMedic Data Systems®). All transponders were factory calibrated and gave an accurate temperature reading with the help of a hand-held reading device (http://www.bmds.com/products/transponders/iptt-300/specs, DAS-7006/7s, BioMedic Data Systems®). To implant the transponder, the female was briefly taken out of the cage, held between the shoulders, and the transponder was carefully implanted sub-dorsally. This was done by gently forming a skin triangle, and injecting the needle into it swiftly and with the help of the pre-loaded, sterilised needle (BioMedic Data Systems®). By pushing the transponder to the end of the injection canal, it was carefully but safely implanted and stayed in the animal for the entire duration of the study. For implantation of the transponder, no anaesthesia, incision or glue were required. The transponder was located over the animal's lumbar vertebrae to avoid the ventral area with the

mammary glands and prevent any misleading warming up effects of the suckling pups on maternal skin temperature. To avoid any potential interference by the interscapular brown adipose tissue (BAT), the transponder was implanted in the hip region, where no BAT depot is found.

Data collection and hygiene protocols

All measurements were taken daily between 08:00 h and 11:00 h. The day when pups were found was considered as the day of parturition, referred to as day 0 of lactation (following Johnson et al., 2001a). From day 1 of lactation onwards, we measured female body masses and subcutaneous body temperatures, food intake, pup number and pup masses on a daily basis until weaning (day 19). Females had *ad libitum* access to food and water throughout the experiment under all ambient temperature conditions. Daily food intake (in g) was continuously monitored except during the mating period (when they were housed with males). From the pairing onwards, females were provisioned with a fixed amount of pelleted food during lactation. Fresh supply was provided and weighed in the morning if necessary. The remaining pellets from the previous day were weighed and the difference (i.e. the consumption) was noted. We carefully checked the cage floor and bedding for pieces of uneaten food, which were also weighed and put back into the cage so as not to disturb the female's food hoarding behaviour. To reduce potential handling stress in the females and allow mothers to establish bonds with their offspring, all measurements were suspended for 1 day around parturition. All animals received the same diet during the experiment (commercial hamster diet, Ssniff). The rooms where the experiments took place were accessible only to four people, who followed very strict hygiene protocols. The colony was free from specific pathogens as testified every 6 months by Anlab (Prague, Czech Republic).

Milk transfer

We quantified daily energy expenditure (DEE) to compute milk energy output (MEO) indirectly in the females between days 12 and 14 (Król and Speakman, 2003b) using the doubly labelled water (DLW) method (Butler et al., 2004). Briefly, females were injected intraperitoneally with ∼0.2 ml of DLW of known mass and characterized isotopic enrichment (ca. 329,000 ppm ^{18}O, ca. 186,000 ppm ^{2}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 µl 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). Twenty 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}H and ^{18}O in the body water pools of the animals (Speakman and Racey, 1987; Method C). Capillaries that contained the blood samples were vacuum distilled while water from the resulting distillate was used to produce CO_2 and H_2 (Vaanholt et al., 2013). The isotope ratios ^{18}O:^{16}O and ^{2}H:^{1}H were analysed using an off-axis laser spectroscopy liquid water isotope analyser (Los Gatos Research) (Berman et al., 2012, 2015). Samples were run alongside international laboratory standards of known enrichment (Speakman and Hambly, 2016) for standardisation. To derive the isotope dilution spaces (N_O and N_H for oxygen and hydrogen, respectively) and the isotope washout constants (k_O and k_H for oxygen and hydrogen, respectively), we log transformed the excess isotope enrichments. The back-extrapolated intercepts were used to evaluate the dilution spaces as this maximises precision and accuracy in DLW studies of small rodents (Speakman and Król, 2005b). Isotope enrichments were converted to values of DEE using a single pool model as recommended for this size of animal (Speakman, 1993). For the treatment of evaporative water loss in the calculation, we followed the assumption of a fixed evaporation of 25% of the water flux (eqn 7.17 from Speakman, 1997), as already successfully applied in lactation experiments (Król et al., 2003).

Metabolisable energy intake

To assess metabolisable energy intake (MEI), we collected and pooled faeces of each female over a 3-day period (at 22 and 8°C) and over a 7-day period (for females at 30°C owing to the lower faecal production). We dried the faeces to constant mass (Haereus, Germany) and determined the energy content with a bomb calorimeter (IKA Calorimeter, C 5000 control, IKA Germany). MEI was computed as daily food intake, i.e. gross energy intake (GEI) (indicated as dry food consumption in g day^{-1} × food energy content in kJ g^{-1} dry mass kJ per gram) subtracted by defecated energy corrected for urinary protein losses. Urinary energy loss was assumed to be 3% of the digestible energy intake, and thus digestive efficiency was determined as a percentage of GEI digested (Drozdz, 1975). MEO calculation was performed after Król and Speakman (2003b). Assimilation efficiency (AE, %) was calculated as (MEI/GEI)×100.

All experiments described above were approved by the ethics committee of the University of Veterinary Medicine Vienna and the Austrian Ministry of Science (GZ 68.205/0035-WF/II3b/2014), and thus all necessary actions were undertaken according to the Animal Experiments Act (Tierversuchsgesetz 2012-TVG 2012) in Austria.

Statistical analysis

Data on GEI, MEI, average daily pup mass, DEE and subcutaneous body temperature were obtained repeatedly from the same females (repeated measures design). We used 32 females for our study and analysed 48 litters from them. Some females therefore contributed to the study twice in the same temperature group. No female was switched between groups. We used linear mixed-effect models (lme models in R package nlme) to analyse the dataset by taking the individual into account. We included body mass of the female, age, experimental group (cold, normal, hot), day of lactation, parity and litter size as fixed factors and entered the ID of the female as a 'random' factor to fit separate intercepts for each animal. AE and DEE data were analysed in a separate data sheet with simple linear regression models (lm models in R package nlme), with each female going into the dataset once as we obtained faecal samples as well as milk production data only once from each female. In the same way, we analysed peak lactation data in the females, which was determined as the day at which mean food intake was at a maximum during lactation. For all response variables, we computed linear two-way interactions between day of lactation and thermal environment group. Graphs were prepared in GraphPad Prism 7 (GraphPad Software), with all values presented as means with s.e.m. or in their original form with a line representing the means. We used linear mixed-effects models to assess differences between the experimental groups in body mass, food intake and GEI during pregnancy and the baseline period. Baseline females were body mass-matched females of the same age that were paired but did not become pregnant, e.g. in the first pairing, but were kept as controls at

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the same temperature conditions to allow comparison. All statistical analyses were conducted in RStudio (version 0.99.489 RStudio, Inc.).

RESULTS

Body mass and energy metabolism

In non-reproductive (baseline) females, body masses were 142.5 ±3.2, 142.2±2.3 and 144.4±2.8 g in cold-, normal- and hot-exposed females, respectively, and were not significantly different from each other ($F_{2,112}$=1.54, P=0.218; Fig. 1A, Table 1). GEI in baseline females was on average 168.3±10.5 at cold, 140.83±6.5 at normal and 125.2±7.6 kJ day^{-1} at hot temperatures (Fig. 1B, Table 1), with this difference being significant between the groups ($F_{2,111}$=13.3, P<0.0001).

During pregnancy, body mass increased as expected in all females (partial for day of pregnancy: $F_{2,689}$=1660.1, P<0.0001; Fig. 1). Owing to our temperature acclimatisation protocol, females were moved to either hot or cold conditions 7 days before the expected parturition day and then stayed at the respective temperatures until weaning of the pups on day 19 of lactation. Their body masses differed between experimental groups during pregnancy (partial for group: $F_{2,689}$=64.4, P<0.0001), with the hot-exposed females having the heaviest pregnancy weights (Table 1, Fig. 2A). The body mass increase in pregnancy did not show the same slopes in the three groups (interaction: $F_{2,689}$=9.9, P<0.0001). GEI was influenced by day of pregnancy (partial for day of pregnancy: $F_{2,403}$=79.8, P<0.0001) and experimental group (partial for experimental group: $F_{2,403}$=5.6, P=0.004), with the cold-exposed pregnant females having the highest GEIs (Table 1). Individual body mass also partially affected GEI during pregnancy (partial for body mass: $F_{2,403}$=10.1, P=0.005; Table 1), with the cold-exposed females ingesting the largest amounts of food (Table 1). Again, we found a significant interaction between day of pregnancy and experimental group (day of pregnancy×group: $F_{2,403}$=10.1, P=0.0001).

With respect to time courses of body mass over the entire experiment, we observed no interaction between female reproductive state (pregnancy, lactation) and thermal environment during lactation (reproductive state × thermal environment: $F_{2,1591}$=0.51, P=0.6). During lactation, GEI was dependent on body mass of the female ($F_{1,798}$=21.95, P<0.0001) and on day of lactation ($F_{1,798}$=282.8, P<0.0001). The highest GEIs were observed in the cold-exposed females (Table 1, Fig. 1B). Asymptotic GEI (days 10 to 14) was twice as high in the cold-exposed group than in the hot group ($F_{2,193}$=95.0, P<0.0001; Table 1) whereas MEI was 144.7% higher ($F_{1,42}$=78.8, P<0.0001; Table 1, Fig. 2A).

The efficiency of energy assimilation (AE) was 94.8±0.1% in the cold-exposed group, 94.0±0.1% in the normal temperature group and 91.7±0.4% in the hot-exposed group ($F_{1,42}$=43.95, P<0.0001; Table 1).

Milk energy transfer, pup growth and litter size

MEO, as assessed from the difference between MEI and DEE, was highest in the cold-exposed individuals ($F_{1,40}$=86.4, P<0.0001; Table 1, Fig. 2B). Further, as litter size varied between thermal environments (Table 1), we identified an interaction between thermal environment of the females and litter size (interaction: $F_{1,40}$=4.4, P=0.042), with the cold-exposed females exhibiting greater milk production at a given number of pups (Fig. 3B,C, Table 1), and we suspect this was due to the largest sample size of 23 litters at 22°C and the smallest variance in this group. As can be seen from Fig. 3A, pups in all three experimental groups rapidly increased their body mass, with pups from the cold-exposed group being heaviest around the time of female asymptotic GEI, i.e. peak lactation. We observed a significant interaction between pup masses from different thermal environments and day of lactation, as shown by the different slopes in pup growth between the three groups ($F_{2,852}$=76.7, P<0.0001; Fig. 3A). Note that pups under hot conditions grew larger and faster as soon as they started to feed

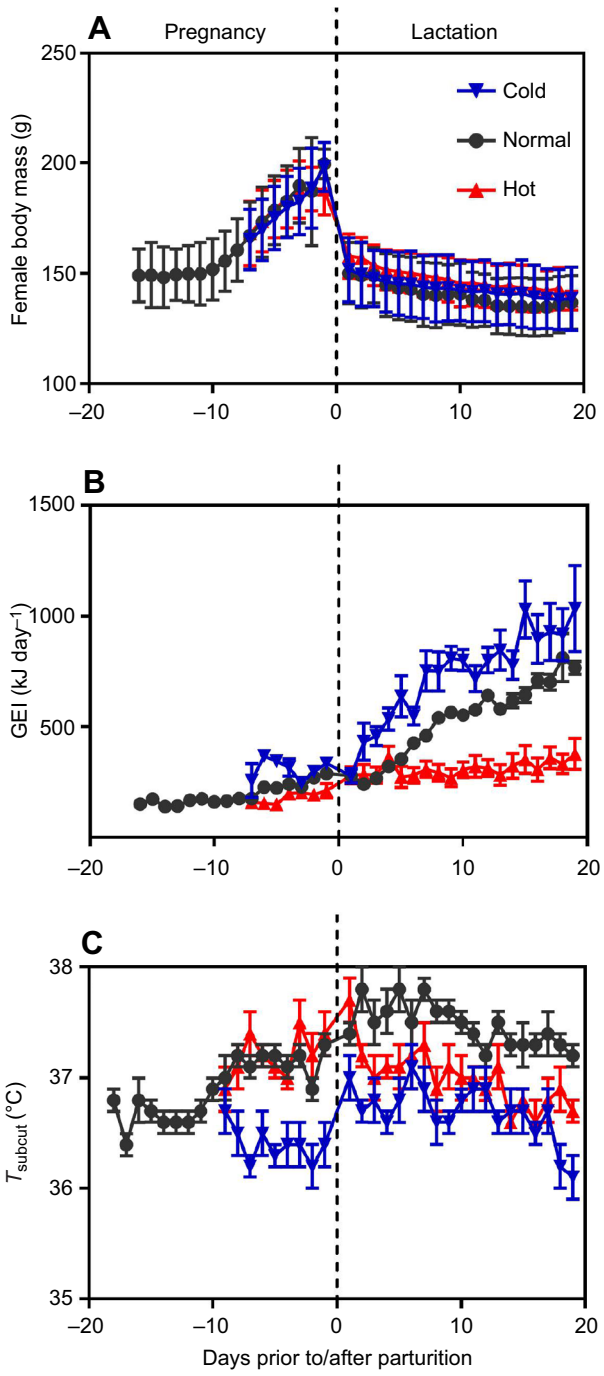

Fig. 1. Time courses of female body mass, gross energy intake and subcutaneous body temperature. (A) Body mass, (B) gross energy intake (GEI) and (C) subcutaneous body temperatures (T_{subcut}) throughout gestation and lactation of female golden hamsters exposed to cold (8±2°C), normal (22±2°C) and hot (30±2°C) ambient temperature conditions. Data are means± s.e.m. (sample sizes in Table 1).

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Table 1. Body masses and energy metabolism during pregnancy and lactation of all three groups of female golden hamsters

	Cold	Normal	Hot
No. of individuals	15	23	10
Baseline non-reproducing			
Body mass (g)	142.5±3.2	142.2±2.3	144.4±2.8
GEI (kJ d^{-1})	168.3±10.5	140.8±6.5	125.2±7.6
T_{subcut} (°C)	35.9±0.2	36.2±0.2	36.7±0.2
Pregnancy			
Body mass (g)	161.7±4.9	159.6±4.3	163.8±5.8
GEI (kJ d^{-1})	211.9±27.3	183.6±15.3	182.4±23.0
T_{subcut} (°C) on day 16 of pregnancy	36.4±0.2	37.1±0.1	37.0±0.1
Lactation			
Body mass (g)	140.2±3.7	140.7±3.2	147.5±3.3
Mean GEI (kJ d^{-1})	749.3±50	533.1±49	308.7±44.7
Asymptotic GEI (kJ d^{-1})	768.5±39.4	556.5±11.4	327.3±25.9
T_{subcut} (°C)	36.7±0.2	37.5±0.2	37.0±0.2
Asymptotic MEI (kJ d^{-1})	729.1±38.3	523±11.1	301.2±25.1
AE (%)	94.8±0.1	94.0±0.1	91.7±0.4
DEE (kJ day^{-1})	297.9±11.2	231.5±10.6	150.6±12.6
MEO (kJ day^{-1})	431.2±29.4	289.6±38.5	147.3±18.9
Litter size at birth	9.9±0.7	11.9±0.5	9.1±0.9
Litter size at weaning	6.9±0.7	9.4±0.6	3.4±0.7
Pup mass at weaning (g)	23.3±2.2	18.2±0.9	28.1±1.0

Data are means±s.e.m. AE, assimilation efficiency; DEE, daily energy expenditure; GEI, gross energy intake; MEI, metabolisable energy intake; MEO, milk energy output; T_{subcut}, subcutaneous body temperature.

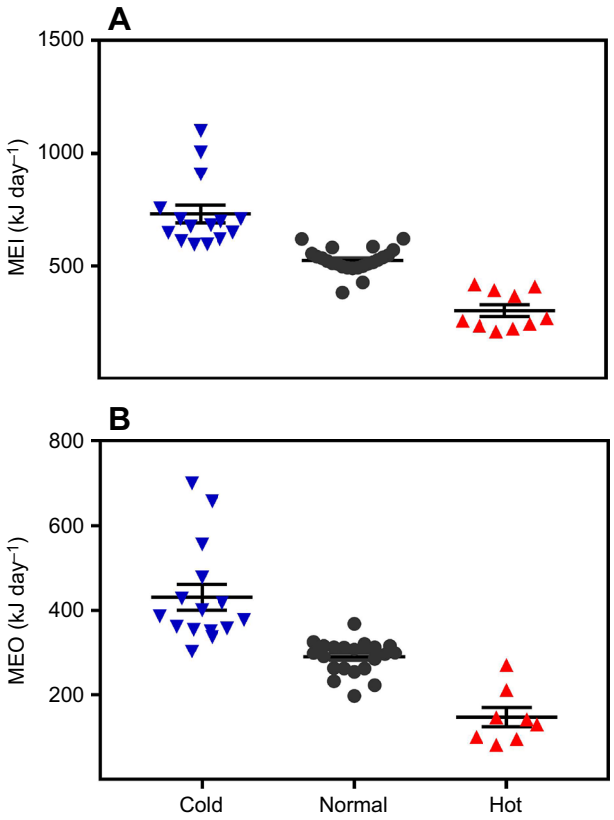

Fig. 2. Metabolisable energy intake and milk energy output. (A) Metabolisable energy intake and (B) milk energy output from lactating female golden hamsters exposed to cold, normal and hot ambient temperature conditions. Each data point represents data from one female with the line indicating the mean.

independently, as can be seen from the shape of the pup mass curve (Fig. 3A). Mean pup mass was influenced by individual female body mass ($F_{1,852}$=901.7, P<0.0001) and by thermal environment of the mothers ($F_{2,852}$=158.4, P<0.0001; Fig. 3B,C). Average pup weaning masses were highest in the hot-exposed hamsters and lowest in the normal-exposed hamsters ($F_{1,42}$=9.5, P=0.00039; Fig. 3A, Table 1).

All 32 females gave birth approximately 18–19 days after introduction of the males (with the variation being due to the 4-day oestrus cycle of the females), consistent with a 16-day gestation period in golden hamsters. Litter sizes at birth were 9.9±0.7 at 8°C, 11.9±0.5 at 22°C and 9.1±0.9 at 30°C, and showed a tendency to be higher in the females at normal temperature (general linear model with litter size and experimental group: $F_{2,45}$=35.9, P=0.041; Fig. 3B). Mean litter size at peak lactation (days 10–14 of lactation) was 7.2±0.6 at 8°C, 10.1±0.5 at 22°C and 4.5±0.7 at 30°C, and differed significantly between the groups ($F_{2,42}$=30.6, P<0.0001; Fig. 3B). Mean litter size at weaning across all temperatures was 7.6±0.5 (343 pups from 32 females). Litter size averaged 6.9±0.7 at 8°C, 9.4±0.6 at 22°C and 3.9±0.8 at 30°C and differed significantly across the experimental groups ($F_{2,42}$=47.1, P<0.0001; Table 1). As can be seen from Fig. 3B, the most apparent losses in pup numbers occurred in the hot-exposed group between days 5 and 13, whereas losses in the cold-exposed and normal temperature group were lower and occurred predominantly in the first few days (Fig. 3B).

Skin (subcutaneous) temperature

Subcutaneous temperatures in pregnant females increased over the course of pregnancy in all three experimental groups (partial for day of pregnancy: $F_{1,679}$=94.5, P<0.0001; Table 1, Fig. 1C). Individual body mass did not affect skin temperature during pregnancy ($F_{1,679}$=3.3, P=0.07). We observed significant differences in skin subcutaneous temperature between the three experimental groups during pregnancy (partial for group: $F_{1,679}$=51.5, P<0.0001; Fig. 1C, Table 1). The increase in subcutaneous temperatures of pregnant females towards parturition was not similar in all three experimental groups, as reflected by Fig. 1C and a significant two-way interaction between day of pregnancy and group ($F_{2,679}$=10.4, P<0.0001).

During lactation, females significantly increased their subcutaneous body temperature by almost 1.3°C (partial for day of lactation: $F_{1,813}$=36.77, P<0.0001; Fig. 1C, Table 1). The implanted thermosensitive transmitter was located subcutaneously so we observed an influence of the thermal environment of the females (partial $F_{2,813}$=58.5, P=0<0.0001). Age of the females also had a partial effect (partial $F_{1,813}$=23.97, P=<0.0001), with the older ones having lower subcutaneous body temperatures. Body mass of the females did not affect subcutaneous temperature (partial $F_{1,813}$=1.25, P=0.26), nor did we observe an interaction between the day of lactation and the thermal environment of the mothers (partial $F_{2,813}$=0.15, P=0.86).

DISCUSSION

Heat dissipation limitation in lactating golden hamsters

By exposing female golden hamsters to three different ambient temperatures while nursing and caring for young, we manipulated their GEI, MEO and overall expenses of reproduction as indexed by the multiples of resting metabolic rate (RMR) at peak lactation. Our experimentally induced cold exposure of lactating females induced metabolic rates exceeding 10 times RMR in four out of 15 of the mothers (Fig. 4) and ranged between 6 and 7.8 times RMR in lactating golden hamsters at room temperature compared with non-reproducing golden hamsters at thermoneutrality. To allow this

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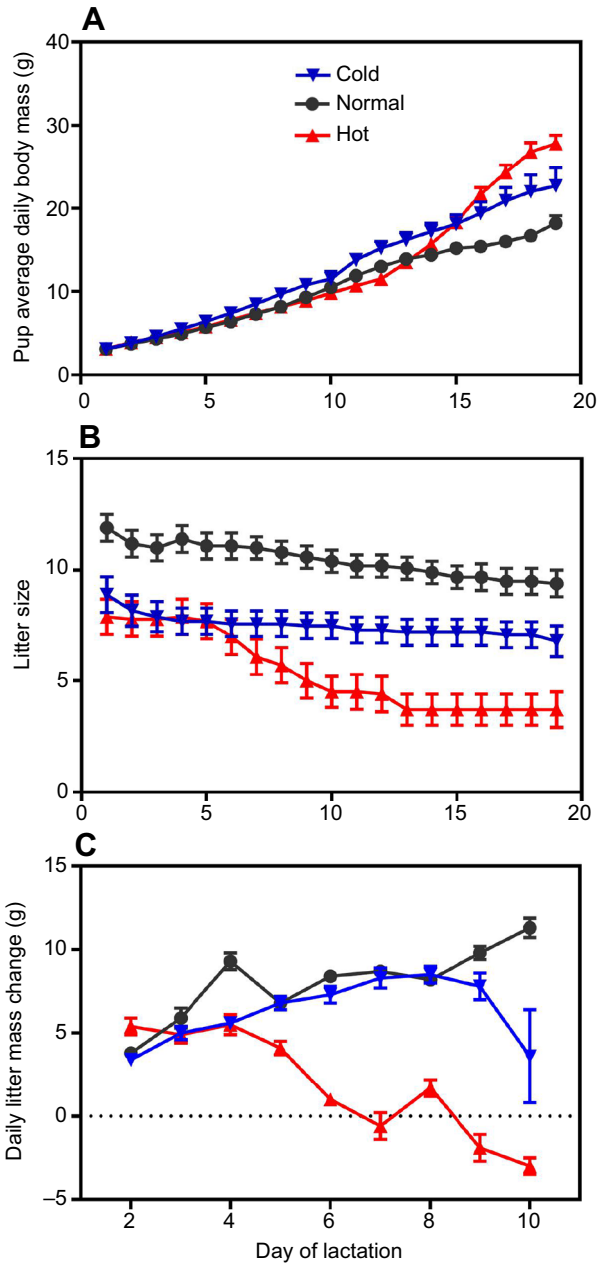

Fig. 3. Reproductive output from lactating golden hamsters exposed to cold, normal and hot ambient temperature conditions. (A) Pup mean (±s.e.m.) daily body mass (g), (B) litter size (mean±s.e.m.) and (C) daily change in litter mass (g) during the first 10 days of lactation, when growth is driven solely by maternal milk production.

comparison and compute multiples of RMR, we divided mass-specific MEI by 147 ml O_2 h^{-1}, the RMR of non-reproducing golden hamsters at thermoneutrality as given in White and Seymour, (2003). Our data on multiples of RMR compare closely with the data obtained from other studies on cold-exposed MF1 mice ranging between 7.9 and 9.4 times RMR (Johnson et al., 2001; Speakman et al., 2004) and cold-exposed European hares with 9 times RMR (Valencak et al., 2009). Only the very precocial guinea pigs, which are very independent from birth onwards, were reported to have much lower lactation costs in the range of 4 times RMR (Künkele and Trillmich, 1997).

Female golden hamsters ingested the most energy, achieved the highest metabolic rates and produced the most milk when exposed

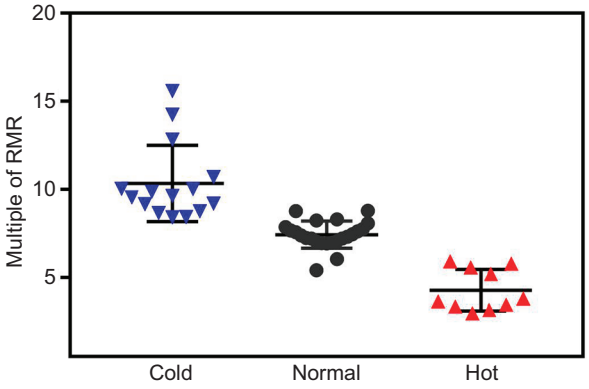

Fig. 4. Total lactation costs as given in multiples of resting metabolic rate (RMR) in female golden hamster mothers exposed to cold (*n*=15), normal (*n*=23) and hot ambient temperature conditions (*n*=10). To compute multiples of RMR, we divided mass-specific metabolisable energy intake by 147 ml O_2 h^{-1}, the RMR of non-reproducing golden hamsters at thermoneutrality as given in White and Seymour (2003).

to 8°C, in line with the concept of heat dissipation. Only alleviation of the heat burden by cold exposure seems to enable females to ingest more food and transfer this extra food energy into milk (Król et al., 2003, 2007; Speakman and Król, 2005a). Our study shows that golden hamsters were limited in their food/energy intake when maintained at 30°C and also partly when maintained at 22°C (Fig. 1B). Only cold-exposed hamsters performed at their best and reached the highest multiples of RMR (10.3 times). Although ambient temperatures of 8°C are 22°C below the thermoneutral temperature of hamsters, they are well in the range of temperatures that golden hamsters experience, both in the wild and when they are kept as pets.

The shape of the curve representing GEI largely resembled that obtained from other rodents, increasing very rapidly over the first 10 days of lactation and reaching an asymptote at peak lactation (Fig. 1B), with the exception of the 30°C-exposed group. In that group, GEIs were elevated over baseline levels but did not show an increase culminating in an asymptote as observed particularly in MF1 mice and some other rodents (Fig. 1B). At the same time, we observed that between days 5 and 13, many pups disappeared (presumably cannibalized) from the nest, so litter size was drastically reduced during the course of lactation (Fig. 3B; Ohrnberger et al., 2016). Rather than displaying the same well-described GEI asymptote, GEIs in lactating golden hamster females at 30°C increased shallowly in comparison with the two other groups (Fig. 1B). This contrasts the findings of Król and Speakman (2003a), who observed similar shapes of the GEI curve at different ambient temperatures but at a lower (quantitative) level. In MF1 mice lactating at 30°C, females succeeded in raising large litters of up to 9.8 pups (Król and Speakman, 2003a), a litter size that golden hamsters could not achieve when exposed to the same temperature (also thermoneutrality in both species). We interpret that golden hamsters may be even more heat sensitive and physiologically limited in lactation than laboratory mice from the MF1 strain. This may be partly due to their insulating fur or to their only very small body appendages compared with muroids, which have long ears and a tail that facilitates heat loss (Al-Hilli and Wright, 1983; Gordon, 2012; Gordon et al., 2014; Serrat, 2014; Serrat et al., 2008).

Pup weight gain and milk intake clearly differ in magnitude between laboratory mice and golden hamsters. Laboratory mice are born with a body size of 1–2 g (Valencak et al., 2013) and

golden hamsters similarly weigh 2–3 g. Weaning masses, however, amount to 30 g in golden hamsters (Fig. 3A) but only ca. 8–10 g in MF1 mice (Guerra and Nunes, 2001; Valencak et al., 2013). Mass-specific MEO is 4 kJ g^{-1} day^{-1} in MF1 mice (Valencak et al., 2013) and 2 kJ g^{-1} day^{-1} in golden hamsters (present study). Importantly, when starting to consume solid food, juvenile golden hamsters grow very quickly and often compensate for temperature-driven availability of milk energy (Fig. 3A). Therefore, early maturation, i.e. opening of the eyes, independent thermoregulation, etc., may be advantageous for survival under conditions when maternal milk output is suboptimal owing to overheating.

Our observation of heat limitation in golden hamsters therefore has two major implications. By observing that golden hamsters had drastically reduced litter sizes of fewer than five pups at 30°C (Fig. 3B), we conclude that they are heat limited during lactation, similar to lactating MF1 mice. Our data underline that the female golden hamster at high ambient temperatures inefficiently dissipates heat to the environment during lactation, and that females avoid complete litter loss by modifying their litter mass, both of which will reduce the demand for milk and also reduce the required food intake. Unfortunately, we cannot elucidate whether the pups at 30°C were dying of hunger under the low milk supply owing to the siblings committing siblicide or the mother killing and removing pups. As extensively discussed in Ohrnberger et al. (2016), however, we speculate that the females might 'tune' their maternal effort and litter size according to the ambient temperature conditions. Further behavioural observations are needed to determine why and how pups go missing from the nest and how this may relate to the heat dissipation limit. We observed that golden hamster females quickly consume the placentas when giving birth (S.A.O., personal observation); also, we found partially consumed pups at 30°C, so it is very likely that they benefit at least in part from the energy content of the tissues. However, because the pups are >80% water, and they were not always consumed by the mother, their contribution to the maternal energy budget was small. Moreover, consuming the pups would not allow females to evade the heat dissipation limit. They may therefore be eaten primarily for hygiene considerations in the nest. We did not include the dead pups into the estimates of MEI in our study because we did not accurately know the mass of ingested tissue.

Our data show that exposure of lactating golden hamsters to thermoneutral conditions largely reduces survival of the young. However, the literature on maintenance conditions of laboratory rodents in the biomedical field has suggested keeping laboratory rodents at thermoneutrality (Cannon and Nedergaard, 2011; Even and Nadkarni, 2012; Gordon, 2017; Lodhi and Semenkovich, 2009; Overton, 2010). It is argued that when kept at room (or lower) temperatures, small rodents, such as mice, continuously produce heat by non-shivering thermogenesis in the BAT and that this situation might influence the data obtained under such conditions (Cannon and Nedergaard, 2011; Even and Nadkarni, 2012; Gordon, 2017; Lodhi and Semenkovich, 2009; Overton, 2010). As reviewed in Speakman and Keijer (2013), this is a very debatable concept and has a huge impact on stress and well-being of the female mothers. In a parallel study, we recently showed that female golden hamsters shaved dorsally in lactation had lower stress than mothers with intact fur (Ohrnberger et al., 2018). In light of our new data on lactation energetics in nursing golden hamsters, we caution against too high maintenance temperatures in breeding golden hamsters in accordance with the lower recommendation for mice (Speakman and Keijer, 2013).

Subcutaneous body temperature elevation in golden hamsters nursing pups

In our study, we chose not to implant transmitters into the body cavity, as done in some other studies (Gamo et al., 2016; Valencak et al., 2013), and instead used thermosensitive passive integrated transponders, which allowed daily non-invasive measurements of the subcutaneous body temperature of the females. By comparing the time courses of subcutaneous body temperatures over pregnancy and lactation as well as in non-reproductive females, we found that body temperature is elevated in nursing females (Fig. 1C), similar to MF1 mice (Gamo et al., 2016), but with the restriction that our absolute numbers may not reflect body core temperature but instead only a correlate of it owing to the location in the subcutis, below the fur coat but still more exposed to the environment than any surgically implanted device. This probably also explains why the subcutaneous temperature curves were consistently lower in the 8°C-exposed females compared with the 22 and 30°C animals (Fig. 1C). Our data suggest that golden hamsters lactating at 22°C had the highest skin temperatures, even higher than those of the females at 30°C (Table 1, Fig. 1C). So far, the body temperature elevation in lactating females was observed to be similar in different experimental situations (Gamo et al., 2016; Valencak et al., 2013). Judging from the temperature curves measured with the passive integrated transponder tags, lactating hamsters at 22°C had peak heat production, which is reinforced by the fact that litter size was significantly larger than at 30°C (see below).

Pup growth rates in golden hamsters exposed to different environmental temperatures

Golden hamster pups very quickly doubled birth weights and interestingly showed similar growth curves in all three groups until around day 10, which is approaching peak lactation or the time span of asymptotic energy intake. During this time window between days 10 and 14 (Fig. 3A), pups from 8°C-exposed mothers grew quicker, although they clearly had higher energetic maintenance and growth costs owing to the lower temperature. The long-term effects of this growth advantage are so far only a matter for speculation. Certainly, being mature enough to independently pick up solid pellets is very beneficial for the survival of the hamster pups, but in our study, we were unable to follow metabolism of the golden hamster pups after weaning. Similar to our observed pup growth curves, Król and Speakman (2003a) also found that around peak lactation, the cold-exposed pups had the highest body masses compared with pups at room temperature or pups at 30°C. This observation among different families of rodents is a consequence of the larger quantities of milk that are transferred from the females at lower ambient temperatures (Król and Speakman, 2003b; Valencak et al., 2013).

Regarding weaning masses, we observed the largest weaning masses in the hot group, and we attribute this finding to the small competition for milk in the 1–2 pup litters on the one hand, and to the lowered thermoregulatory costs of the pups at 30°C on the other hand. The lowest weaning masses were found in the 22°C group; again, here we would like to bring up the litter size argument, as this group had 9.4 pups on average and competition within the litter supposedly was largest. At 22°C, lactating females were probably suffering most from the heat burden as they had higher skin temperatures and raised the largest litters, and pup demand might have been at its peak as well. By collecting data on golden hamsters in southern Turkey, Gattermann et al. (2008) showed not only that naturally encountered temperatures ranged between 6 and 32°C but

also that the hamsters are diurnal outside the laboratory, with peak foraging activities in the morning and the evening hours while avoiding both nocturnal predators and high mid-day surface temperatures (Gattermann et al., 2008).

Conclusions

We conclude from our study that golden hamster females allowed to raise natural litters at 8, 22 and 30°C greatly differed in their energy metabolism. Although all lactating females were observed to have higher subcutaneous body temperatures than non-reproductive controls, similar to what was observed in laboratory mice, golden hamsters did not reach maximal GEI when nursing young at 30°C, and consequently could hardly raise their offspring. In contrast, MEO was highest at 8°C, so we interpret that any decrease in ambient temperature will lead to an increase in milk production in lactating female golden hamsters. Pup growth was highest around the time of asymptotic GEI in the 8°C group, most likely owing to the greater milk production rates. We conclude that golden hamsters are limited by heat production when achieving maximum rates of metabolism. They may need to balance quantitative aspects of milk production and litter size with the costs of overheating and hyperthermia.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

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

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