

## RESEARCH ARTICLE

# Energy budget during lactation in striped hamsters at different ambient temperatures

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### SUMMARY

The combination of two stressors, lactation and cold, is suggested to be an excellent model for testing the factors limiting sustained energy intake (SusEI). Limits to SusEI during peak lactation may be imposed peripherally by the capacity of mammary glands to produce milk or may be driven by the ability of animals to dissipate body heat. To distinguish between the two mechanisms, body mass change, food intake, reproductive output (using litter size and mass) and serum prolactin (PRL) levels were measured in striped hamsters lactating at 23, 30 and 5°C. Resting metabolic rate (RMR) during late lactation was also measured. Female hamsters lactating at 5°C showed significantly lower change in body mass, but had higher food intake and RMR than females at 23 and 30°C. Asymptotic food intake averaged  $14.6 \pm 0.4$ ,  $14.5 \pm 0.7$  and  $16.2 \pm 0.5 \text{ g d}^{-1}$  for females at 23, 30 and 5°C, respectively. The females at 5°C had 11.4% higher asymptotic food intake than females at 23 and 30°C ( $F_{2,51}=3.3$ ,  $P<0.05$ , Tukey's HSD,  $P<0.05$ ). No significant differences in litter size and PRL levels were observed between the three groups; however, litter mass at 5°C was lower by 19.7 and 19.8% than litter mass at 23 and 30°C on day 19 of lactation ( $F_{2,51}=3.5$ ,  $P<0.05$ , Tukey's HSD,  $P<0.05$ ). Differences in the above parameters between 23 and 30°C were not significant. Litter mass was positively correlated with asymptotic food intake (23°C,  $r=0.60$ ,  $P<0.05$ ; 30°C,  $r=0.94$ ,  $P<0.01$ ; 5°C,  $r=0.77$ ,  $P<0.01$ ). These data suggested that females lactating at cold temperatures increased food intake to compensate for additional energy demands for thermogenesis, but they might not be capable of exporting more energy as milk to the pups, indicating a possible consistency with the peripheral hypothesis. However, the present results do not considerably distinguish the peripheral limitation hypothesis from the heat dissipation limits hypothesis.

Key words: energy budget, lactation, prolactin, resting metabolic rate, striped hamster, sustained energy intake.

### INTRODUCTION

The maximal rates of energy intake and expenditure that animals can sustain for protracted periods of days and weeks [sustained energy intake (SusEI) or sustained metabolic rate] are very important because they define upper energetic limits to the ability of animals to distribute, survive and reproduce (Karasov, 1986; Root, 1988; Bozinovic and Rosenmann, 1989; Peterson et al., 1990; Thompson, 1992; Hammond and Diamond, 1997; Bryan and Bryant, 1999; Speakman, 2000; Johnson and Speakman, 2001; Johnson et al., 2001a; Johnson et al., 2001b; Johnson et al., 2001c; Speakman and Król, 2005; Zhao and Cao, 2009a). It is widely believed that SusEI is constrained intrinsically by some aspects of physiology (Daan et al., 1990; Peterson et al., 1990; Weiner, 1992; Hammond and Diamond, 1997; Speakman, 2000; Speakman and Król, 2005; Speakman and Król, 2010). For example, SusEI limitation is suggested to be set by the expenditure capacities of the energy-consuming organs, such as skeletal muscle during physical exercise, brown adipose tissue and muscles during cold exposure, and the mammary glands during lactation, i.e. the peripheral limitation hypothesis (Hammond and Diamond, 1997; Speakman and Król, 2005; Speakman, 2007; Speakman, 2008; Zhao and Cao, 2009a; Zhao et al., 2010a; Zhao, 2010). Alternatively, SusEI limitation may be driven by the capacity of animals to dissipate heat, i.e. the heat dissipation limits hypothesis (Król and Speakman, 2003a; Król and Speakman, 2003b; Speakman and Król, 2005; Król et al., 2007).

Lactation is the most energetically demanding period encountered by small mammals (Thompson and Nicol, 1986; Hammond and

Diamond, 1997; Johnson et al., 2001a; Johnson et al., 2001b; Speakman and Król, 2005; Speakman, 2007). In general, mammals exposed to cold have to increase their energy demands for additional thermogenesis to maintain constant thermoregulation (Heldmaier et al., 1982; Wang and Wang, 1989; Wang and Wang, 1990; Bozinovic et al., 2004; Li and Wang, 2005; Wang et al., 2006; Zhang and Wang, 2007). Wild animals may experience lactation at cold temperature; thus, the combination of the two stressors is an excellent model with which to study factors limiting SusEI (Hammond and Diamond, 1997; Johnson and Speakman, 2001; Speakman and Król, 2005; Zhang and Wang, 2007). A previous study showed that Swiss mice lactating at cold increased their food intake and did not raise heavier litters compared with mice lactating at normal temperatures, suggesting that limits on SusEI were not driven by the capacity of the gut, but were likely imposed by the capacity of mammary tissue to produce milk (Hammond et al., 1994). Additionally, during lactation, cotton rats (*Sigmodon hispidus*) did not increase milk energy output compared with controls lactating in warm conditions (Rogowitz, 1998). Zhang and Wang also reported similar results in cold-exposed lactating Brandt's vole (*Lasiopodomys brandtii*) (Zhang and Wang, 2007). These studies provide support for the peripheral limitation hypothesis (Zhang and Wang, 2007).

However, MF1 mice (*Mus musculus*) lactating at cold temperatures (8°C) not only increased their food intake but also exported more milk energy than lactating females kept under normal conditions (21°C), which was inconsistent with the expectation of

the peripheral limitation hypothesis (Johnson and Speakman, 2001). Król and Speakman compared food intake and reproductive output during peak lactation between MF1 mice at 30, 21 and 8°C, and found that females lactating at 30°C had a lower food intake and raised fewer and smaller pups than those lactating at 21 and 8°C (Król and Speakman, 2003a). Moreover, MF1 mice with their dorsal fur removed showed higher food intake and raised heavier litters than non-shaved controls (Król et al., 2007). Similar results were also found in Brandt's voles (Wu et al., 2009). These studies suggested that limits to SusEI during lactation were imposed by the capacity of an animal to dissipate heat (Król and Speakman, 2003a; Król and Speakman, 2003b; Król et al., 2003; Król et al., 2007; Speakman and Król, 2010). Zhao and Cao (Zhao and Cao, 2009a) repeated the shaving experiment performed by Król et al. (Król et al., 2007), but instead used Swiss mice, and found that fur shaving did increase thermal conductance but had no effect on reproductive output (Zhao and Cao, 2009a; Zhao, 2010; Zhao et al., 2010a). These studies suggested that limits on lactating performance might be set at different levels in different strains or species. Further work in a range of additional species or strains is necessary to establish the more normal condition, i.e. whether SusEI in animals during late lactation is set by peripheral demands or heat dissipation capacity (Zhao and Cao, 2009a).

It has been advocated that changes in resting metabolic rate (RMR) are potential factors affecting the reproductive performance of female animals (Thompson and Nicoll, 1986; Thompson, 1992; Johnson et al., 2001b). Animals with higher RMRs may have a greater capacity for absorbing energy and, therefore, may be able to devote more energy to reproduction (Thompson, 1992; Johnson et al., 2001b). Alternatively, they may have less energy remaining to allocate to reproduction, as they have to spend additional energy on the increased rate of metabolism (Gadgil and Bossert, 1970; Johnson et al., 2001b). RMR has also been found to be influenced by environmental variations, especially changes in ambient temperature (Thompson and Nicoll, 1986; Daan et al., 1990; Garton et al., 1994; Król and Speakman, 2003a; Bozinovic et al., 2004; Speakman, 2007; Speakman, 2008). The measurement of RMR would provide insight into the energy budgets for animals lactating at different temperatures. During lactation, the suckling stimulus feeds back to prolactin (PRL) release, thereby regulating milk production (Speakman and Król, 2005). According to the peripheral hypothesis, milk secretion may not be changed in the females during peak lactation, regardless of the PRL levels produced. However, the heat dissipation limits hypothesis predicts that the suckling unit of mother and pup(s) may generate heat that leads to maternal hyperthermia and forces the female to discontinue suckling, ultimately resulting in lower levels of PRL produced and lower reproductive output (Croskerry et al., 1978; Speakman and Król, 2005). Thus, the measurements of PRL levels and the analysis of correlations between PRL and food intake or reproductive output may be helpful to distinguish the peripheral hypothesis from the heat dissipation limits hypothesis.

The striped hamster, *Cricetulus barabensis* (Pallas 1773), is a major rodent in northern China and is also distributed in Russia, Mongolia and Korea (Zhang and Wang, 1998). This species is granivorous, nocturnal and stores food in winter (Zhang and Wang, 1998; Song and Wang, 2003; Zhao and Cao, 2009b; Zhao et al., 2010b). There are seasonal variations in population dynamics in wild hamsters and two peaks usually occur in April and August (Zhu and Qin, 1991). Reproductive periods of hamsters are reported to be 10 months (ranging from February to November), during which

this species has two reproductive peaks, one in spring and another in autumn (Xing et al., 1991; Zhu and Qin, 1991; Bao et al., 2001; Wang et al., 2003). Striped hamsters used in the present study were wild-captured on farmland in the North China Plain. The climate is arid and characterized by warm and dry summers (extreme maximum temperatures of 42.6°C) and cold winters (low-temperature months range from November to February, and extreme minimum temperatures are below -20°C) (Zhao and Cao, 2009b; Zhao et al., 2010c). Thus the species must experience great seasonal fluctuations in environmental temperature (Zhang and Wang, 1998). In the present study, the effects of different ambient temperatures on energy budget during lactation in striped hamsters were examined. Maternal body mass, food intake, litter size, litter mass and serum PRL levels were measured in hamsters lactating at 5, 23 and 30°C. Effects of different temperatures on the RMR during late lactation were also investigated.

## MATERIALS AND METHODS

### Animals and experiment protocol

Striped hamsters were obtained from our laboratory breeding colony, which started with animals that were initially trapped from farmland at the center of the Hebei province (115°13'E, 38°12'S), North China Plain. This breeding colony was maintained under a 12h:12h light:dark photoperiod (lights on at 08:00h), and room temperature was kept at 23±1°C. Food (standard rodent chow, Beijing KeAo Feed Co., Beijing, China) and water were provided *ad libitum*.

Eighty-six virgin female hamsters aged 3.5–4 months were paired with males for 11 days, after which the males were removed; 54 of the females became pregnant. Following parturition (day 0 of lactation), lactating females with their pups were randomly assigned to one of three experimental groups (23°C, *N*=17; 30°C, *N*=17; or 5°C, *N*=20). All females with their pup(s) were kept at 23°C at first, and then (those that were allocated to the 30 and 5°C groups) transferred to either 30 or 5°C on day 7 of lactation until their pups were weaned on day 19 of lactation.

### Body mass and food intake

Females were weighed on days 3–19 of lactation on a daily basis, during which food intake of females was also measured. Food intake was calculated as the mass of food missing from the hopper every day, subtracting orts mixed in the bedding. As no significant difference was found in daily food intake between days 16 and 18 of lactation by repeated measurements, the asymptotic food intake during peak lactation was calculated as the mean daily food intake over this period (Zhao et al., 2010a). Additionally, litter size and mass were recorded during days 3–19 of lactation.

### Resting metabolic rate

Maternal RMR was measured on day 19 of lactation using a closed-circuit respirometer as described previously (Gorecki, 1975; Wang et al., 2000). Briefly, the metabolic chamber size was 3.6l, and chamber temperature was controlled within ±0.5°C by a water bath. KOH and silica gel were used to absorb CO<sub>2</sub> and water in the metabolic chamber, respectively. RMR was determined at 29±0.5°C within the thermal neutral zone of this species (27–30°C) (Song and Wang, 2003; Zhao et al., 2010b). Animals were in the chambers for ~60 min to stabilize before metabolic measurement; after this period, oxygen consumption was recorded for 60 min at 5 min intervals. Two continuous stable minimum recordings were taken to calculate RMR, which was corrected to standard temperature and air pressure conditions. All measurements were made between 10:00 and 15:00h.

### Serum prolactin

On day 19 of lactation, females were killed by decapitation between 15:00 and 17:00h. Trunk blood was collected for serum PRL measurement. Serum was separated from each blood sample by centrifugation and stored at  $-75^{\circ}\text{C}$ . Serum PRL levels were quantified by radioimmunoassay using RIA kits (Beijing North Institute of Biological Technology, Beijing, China). Intra- and inter-assay coefficients of variation were less than 10% for PRL. According to the standard kit instructions, this RIA kit was validated for use with striped hamsters.

### Statistical analysis

Data were analyzed using SPSS 13.0 statistical software (IBM, Somers, NY, USA). Differences in maternal body mass, food intake, litter size and mass between 5, 23 and  $30^{\circ}\text{C}$  groups on any given lactation day were examined by one-way ANOVA, followed by Tukey's honestly significant difference (HSD) *post hoc* tests where appropriate. Changes in body mass, food intake and litter mass throughout lactation in each group were analyzed using repeated-measures ANOVA. Effects of temperature on PRL and RMR were examined using a one-way ANOVA or analysis of covariance (ANCOVA) with body mass as a covariate. Pearson's correlation was performed to determine the relationships between litter mass, change in body mass, and asymptotic food intake, as well as body mass, litter mass and serum PRL levels. Correlations between asymptotic food intake, litter mass and RMR were examined using partial correlation, with body mass as a covariate. Data are presented as means  $\pm$  s.e.m. Statistical significance was assumed at  $P < 0.05$ .

## RESULTS

### Body mass

Maternal body mass decreased significantly over the period of lactation, during which body mass declined from  $31.4 \pm 0.9$  g on day 3 to  $26.2 \pm 0.7$  g on day 19 in the females lactating at  $23^{\circ}\text{C}$  (16.6% decline,  $F_{16,256} = 91.5$ ,  $P < 0.001$ ), from  $30.8 \pm 0.8$  to  $26.5 \pm 0.8$  g in females at  $30^{\circ}\text{C}$  (14.2% decline,  $F_{16,256} = 67.2$ ,  $P < 0.001$ ) and from  $32.0 \pm 0.7$  to  $25.7 \pm 0.6$  g for females at  $5^{\circ}\text{C}$  (19.7% decline,  $F_{16,304} = 179.8$ ,  $P < 0.001$ ; Fig. 1A). Temperature had no significant effect on maternal body mass on any given day throughout the lactation (day 3,  $F_{2,24} = 0.6$ ,  $P > 0.05$ ; day 19,  $F_{2,24} = 0.4$ ,  $P > 0.05$ ; Fig. 1A). However, a significant difference in change in body mass was observed on day 12 and thereafter (day 12,  $F_{2,51} = 3.4$ ,  $P < 0.05$ ; day 19,  $F_{2,51} = 8.0$ ,  $P < 0.01$ ; Fig. 1B); body mass was significantly lower in the  $5^{\circ}\text{C}$  group than in the 23 and  $30^{\circ}\text{C}$  groups (Tukey's HSD, day 12,  $P < 0.05$ ; day 19,  $P < 0.05$ ) whereas the differences between the 23 and  $30^{\circ}\text{C}$  groups were not significant (Tukey's HSD, day 12,  $P > 0.05$ ; day 19,  $P > 0.05$ ; Fig. 1B).

### Food intake

There was no significant difference in food intake during early lactation (day 3,  $F_{2,51} = 0.4$ ,  $P > 0.05$ ; day 6,  $F_{2,51} = 0.7$ ,  $P > 0.05$ ; Fig. 1C). Females lactating at  $5^{\circ}\text{C}$  consumed significantly more food than females at 23 and  $30^{\circ}\text{C}$  on day 7 and thereafter, except for days 16 and 19 (day 7,  $F_{2,51} = 4.1$ ,  $P < 0.05$ ; day 16,  $F_{2,51} = 1.6$ ,  $P > 0.05$ ; day 19,  $F_{2,51} = 0.1$ ,  $P > 0.05$ ). Food intake of females at  $30^{\circ}\text{C}$  was not different from that of females at  $23^{\circ}\text{C}$  throughout lactation (Tukey's HSD, day 7,  $P > 0.05$ ; day 19,  $P > 0.05$ ). Asymptotic food intake between days 16 and 18 of lactation averaged  $14.6 \pm 0.4$ ,  $14.5 \pm 0.7$  and  $16.2 \pm 0.5$  g day $^{-1}$  for the females at 23, 30 and  $5^{\circ}\text{C}$ , respectively. Females at  $5^{\circ}\text{C}$  showed 11.4% higher asymptotic food intake than females at 23 and  $30^{\circ}\text{C}$  ( $F_{2,51} = 3.3$ ,  $P < 0.05$ ; Tukey's HSD,  $P < 0.05$ ),

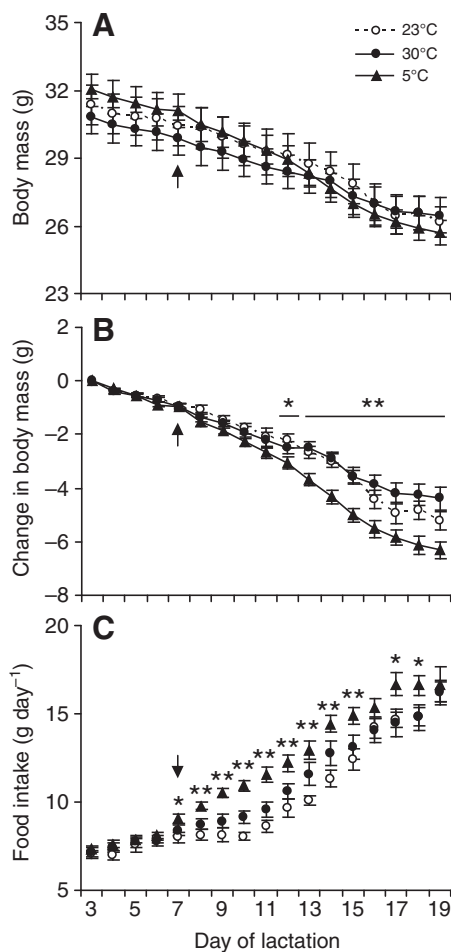


Fig. 1. (A) Maternal body mass, (B) change in body mass and (C) food intake throughout the lactation period in striped hamsters exposed to 23, 30 or  $5^{\circ}\text{C}$  on day 7 of lactation (arrows) and thereafter. Change in body mass was significantly lower in the  $5^{\circ}\text{C}$  group than in the 23 and  $30^{\circ}\text{C}$  groups on days 12–19 of lactation. Females lactating at  $5^{\circ}\text{C}$  had significantly higher food intake than other two groups on day 7 and thereafter, except for days 16 and 19. \*, significant effects of temperature ( $P < 0.05$ ); \*\*,  $P < 0.01$ . Values are means  $\pm$  s.e.m.

whereas there was no difference in food intake between females at 23 and  $30^{\circ}\text{C}$  (Tukey's HSD,  $P > 0.05$ ).

### Litter size and litter mass

On day 3 of lactation, litter size was  $4.6 \pm 0.2$  (range=3–6),  $5.1 \pm 0.3$  (range=3–7) and  $4.9 \pm 0.3$  (range=3–7) for the 23, 30 and  $5^{\circ}\text{C}$  groups, respectively ( $F_{2,51} = 0.8$ ,  $P > 0.05$ ; Fig. 2A). Litter size decreased significantly throughout the lactation period and by 5.0, 9.2 and 17.5% for females lactating at 23, 30 and  $5^{\circ}\text{C}$ , respectively ( $23^{\circ}\text{C}$ ,  $F_{16,256} = 3.1$ ,  $P < 0.01$ ;  $30^{\circ}\text{C}$ ,  $F_{16,256} = 5.3$ ,  $P < 0.01$ ;  $5^{\circ}\text{C}$ ,  $F_{16,304} = 4.3$ ,  $P < 0.01$ ). No significant difference in litter size was found between the three groups on day 6 ( $F_{2,51} = 0.3$ ,  $P > 0.05$ ) or after exposure to 30 or  $5^{\circ}\text{C}$  (day 7,  $F_{2,51} = 0.2$ ,  $P > 0.05$ ; day 19,  $F_{2,51} = 1.2$ ,  $P > 0.05$ , Fig. 2A).

Litter mass was not significantly different between the three groups during early lactation (day 3,  $F_{2,51} = 1.0$ ,  $P > 0.05$ , day 6,  $F_{2,51} = 0.6$ ,  $P > 0.05$ ; Fig. 2B). There was significant increase in litter mass throughout lactation; it increased by 253, 230 and 157% in the 23, 30 and  $5^{\circ}\text{C}$  groups, respectively (days 3–19,  $F_{16,256} = 300.3$ ,

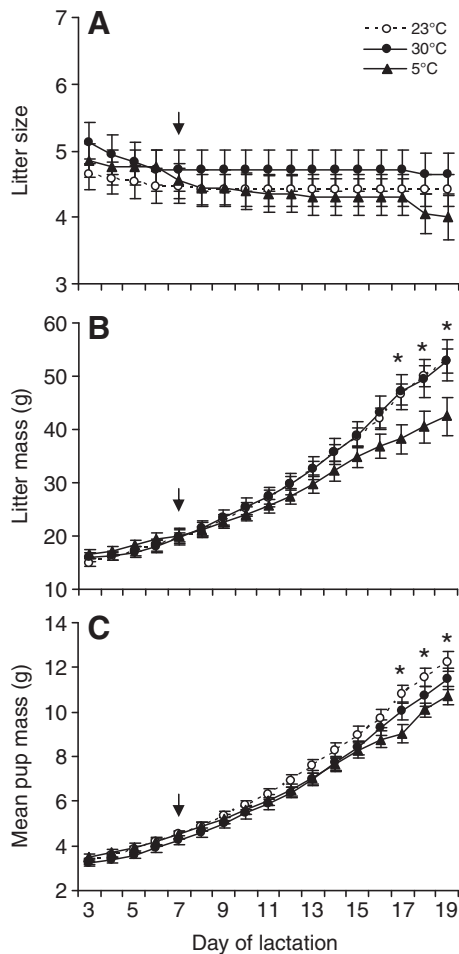


Fig. 2. (A) Litter size, (B) litter mass and (C) mean pup mass throughout the lactation period in striped hamsters exposed to 23, 30 or 5°C on day 7 of lactation (arrow) and thereafter. On days 17–19 of lactation, litter mass and mean pup mass were significantly lower in the 5°C group than in the 23°C and 30°C groups. \*, significant effects of temperature ( $P < 0.05$ ). Values are means  $\pm$  s.e.m.

$P < 0.01$ ; days 3–19, 30°C,  $F_{16,256} = 137.0$ ,  $P < 0.01$ ; 5°C,  $F_{16,304} = 66.6$ ,  $P < 0.01$ ). During late lactation, litter mass was significantly lower in the 5°C group than in the 23 and 30°C groups (day 17,  $F_{2,51} = 3.6$ ,  $P < 0.05$ ). At weaning, litter masses were  $52.9 \pm 2.2$ ,  $53.0 \pm 3.8$  and  $42.5 \pm 3.6$  g for the 23, 30 and 5°C groups, and litter mass of the 5°C group was 19.7 and 19.8% lower than that of the 23 and 30°C groups (day 19,  $F_{2,51} = 3.5$ ,  $P < 0.05$ ; Tukey's HSD,  $P < 0.05$ ) but litter mass did not differ between the 23 and 30°C groups (Tukey's HSD,  $P > 0.05$ ; Fig. 2B).

Mean pup mass was not significantly different between the three groups during early and mid lactation (day 3,  $F_{2,51} = 1.2$ ,  $P > 0.05$ ; day 6,  $F_{2,51} = 0.9$ ,  $P > 0.05$ ; day 16,  $F_{2,51} = 1.4$ ,  $P > 0.05$ ; Fig. 2C). On day 17 and thereafter, there was a significant effect of temperature; mean pup mass was significantly lower in the 5°C group than in the 23°C group (day 17,  $F_{2,51} = 4.8$ ,  $P < 0.05$ , Tukey's HSD,  $P < 0.05$ ; day 19,  $F_{2,51} = 3.2$ ,  $P < 0.05$ , Tukey's HSD,  $P < 0.05$ ) but the difference between the 23 and 30°C groups was not significant (Tukey's HSD,  $P > 0.05$ , Fig. 2C).

There was a negative correlation between litter mass and change in body mass in females lactating at 5°C ( $r = -0.58$ ,  $P < 0.01$ ; Fig. 3A), whereas this correlation was not significant in the 23 or 30°C groups

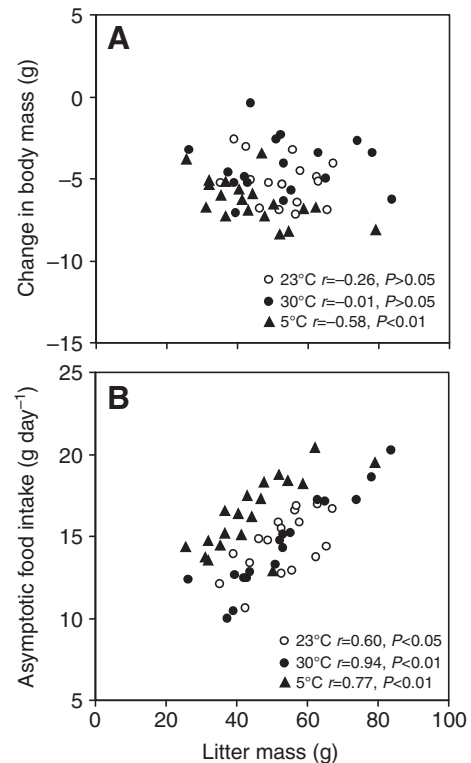


Fig. 3. Correlations between litter mass and (A) change in body mass and (B) asymptotic food intake during late lactation in striped hamsters lactating at 23, 30 or 5°C.

(23°C,  $r = -0.26$ ,  $P > 0.05$ ; 30°C,  $r = -0.01$ ,  $P > 0.05$ ). Litter mass was positively correlated with asymptotic food intake in females lactating at 23, 30 and 5°C (23°C,  $r = 0.60$ ,  $P < 0.05$ ; 30°C,  $r = 0.94$ ,  $P < 0.01$ ; 5°C,  $r = 0.77$ ,  $P < 0.01$ ; Fig. 3B).

#### Resting metabolic rate

Temperature had a significant effect on maternal RMR: females lactating at 5°C had 15 and 22% higher RMRs than females lactating at 23 and 30°C, respectively ( $F_{2,50} = 22.5$ ,  $P < 0.01$ ; Tukey's HSD,  $P < 0.05$ ; Fig. 4). RMR in the 23°C group did not differ from that of the 30°C group (Tukey's HSD,  $P > 0.05$ ). RMR was positively correlated with asymptotic food intake in females lactating at 23°C ( $r = 0.49$ ,  $P = 0.05$ ; Fig. 5A) and 5°C ( $r = 0.63$ ,  $P < 0.01$ ) whereas the correlation was not significant for females at 30°C ( $r = 0.33$ ,  $P > 0.05$ ). RMR was also positively correlated with litter mass in the 23°C ( $r = 0.63$ ,  $P < 0.01$ ) and 5°C ( $r = 0.62$ ,  $P < 0.01$ ) groups, but not in the 30°C group ( $r = 0.29$ ,  $P > 0.05$ ; Fig. 5B).

#### Serum PRL

Serum PRL levels averaged  $179.7 \pm 10.5$ ,  $176.2 \pm 10.6$  and  $191.0 \pm 9.7$   $\mu\text{ml}^{-1}$  in females lactating at 23, 30 and 5°C, respectively, but the differences between groups was not significant ( $F_{2,50} = 0.6$ ,  $P > 0.05$ ). There was no correlation between serum PRL level and body mass in females lactating at 23°C ( $r = 0.03$ ,  $P > 0.05$ ), 30°C ( $r = 0.08$ ,  $P > 0.05$ ) or 5°C ( $r = 0.15$ ,  $P > 0.05$ ; Fig. 6A). Neither 23°C nor 5°C females showed correlations between serum PRL levels and asymptotic food intake (23°C,  $r = 0.20$ ,  $P > 0.05$ ; 5°C,  $r = 0.28$ ,  $P > 0.05$ ) whereas the correlation was significant in females at 30°C ( $r = 0.50$ ,  $P < 0.05$ , Fig. 6B). Serum PRL levels were significantly positively correlated with litter size (23°C,  $r = 0.75$ ,  $P < 0.01$ ; 30°C,

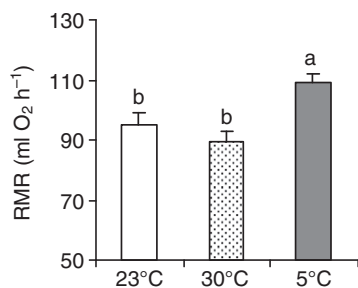


Fig. 4. Maternal resting metabolic rate (RMR) in striped hamsters lactating at 23, 30 or 5°C. Different letters (a and b) above the columns indicate a significant difference between the groups ( $P < 0.05$ ). Values are means  $\pm$  s.e.m.

$r = 0.85$ ,  $P < 0.01$ ; 5°C,  $r = 0.81$ ,  $P < 0.01$ ; Fig. 6C). Serum PRL levels were also significantly positively correlated with litter mass in the three groups (23°C,  $r = 0.55$ ,  $P < 0.05$ ; 30°C,  $r = 0.57$ ,  $P < 0.05$ ; 5°C,  $r = 0.57$ ,  $P = 0.01$ ; Fig. 6D).

### DISCUSSION

This study showed that striped hamsters lactating at 5°C had a significantly higher food intake but a lower change in body mass than hamsters lactating at 23 and 30°C. During late lactation, litters raised by females at 5°C had significantly lower mass compared with the other two groups. RMR was significantly higher in females exposed to 5°C than females exposed to 23 and 30°C. Neither cold nor hot exposure imposed a significant effect on serum PRL levels.

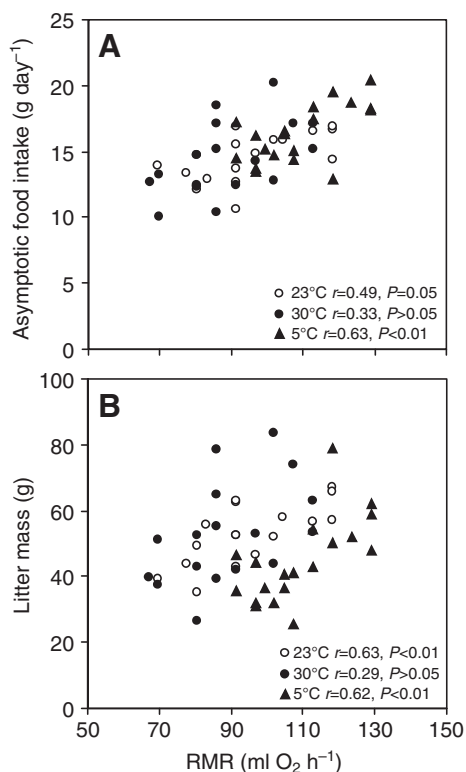


Fig. 5. Correlations between resting metabolic rate (RMR) and (A) asymptotic food intake and (B) litter mass during late lactation in striped hamsters lactating at 23, 30 or 5°C.

Differences in any variables mentioned above were not significant between females lactating at 23 and 30°C.

The fact that peak lactation is the most energetically demanding period encountered by small mammals has been well established (Thompson and Nicol, 1986; Hammond and Diamond, 1997; Johnson et al., 2001a; Johnson et al., 2001b; Speakman and Król, 2005; Speakman, 2007). In the present study, body mass decreased significantly throughout the lactation period, i.e. by 16.6, 14.2 and 19.7% in hamsters lactating at 23, 30 and 5°C, respectively (late lactation relative to early lactation). Decreases in body mass during lactation have been consistently found in cotton rats (Rogowitz, 1996) and Brandt's voles (Zhang and Wang, 2007). However, Swiss mice have shown stable body mass throughout lactation (49.6 g on day 3 to 49.5 g on day 17 of lactation) (Zhao and Cao, 2009a) and MF1 mice increased their body mass from 37.8 g on parturition to 44.2 g on day 17 of lactation (increased by 17%) (Johnson et al., 2001c). This suggests that different species might vary in their patterns of changes in body mass during lactation. It has been reported that maternal food intake and weight loss provide metabolizable energy that is allocated for maternal use or exported as milk to maintain offspring (Rogowitz, 1996). For Swiss mice and MF1 mice, previous studies have shown that there is no limitation on the capacity of the gastrointestinal tract to process ingested food and make its nutrients available for use, allowing the two species to compensate for the highest energetic demands during lactation and maintain constant body mass or even increase their body mass by late lactation (Hammond and Diamond, 1992; Hammond et al., 1994; Hammond et al., 1996; Hammond and Diamond, 1997; Hammond and Kristan, 2000; Johnson and Speakman, 2001; Johnson et al., 2001c; Speakman et al., 2001; Król et al., 2003; Speakman and Król, 2005; Król et al., 2007; Speakman, 2007; Speakman, 2008; Zhao and Cao, 2009a). However, for striped hamsters in the present study, body mass loss increased throughout lactation, which indicates that fat stores were greatly mobilized over this period of high energetic demand. This suggests that the increased food intake was not able to completely compensate for the energy exported as milk to maintain offspring.

Over the period of lactation, food intake increased significantly in the hamsters at 23, 30 and 5°C, and reached a maximum during peak lactation. In addition, hamsters lactating at 5°C had a significantly higher food intake than hamsters at 23 and 30°C, indicating a significant impact of temperature on food intake. Furthermore, asymptotic food intake in hamsters at 5°C increased beyond that observed in females lactating at 23 and 30°C, suggesting that an increase in food intake was employed to meet the combined energy demands of lactation and cold exposure. Similar results were also found in cold-exposed Swiss mice (Hammond et al., 1994), MF1 mice (Johnson and Speakman, 2001) and Brandt's voles (Zhang and Wang, 2007). Consistent with previous studies on Swiss mice (Hammond and Diamond, 1992; Hammond et al., 1994; Hammond et al., 1996; Hammond and Diamond, 1997; Zhao and Cao, 2009a), MF1 mice (Johnson and Speakman, 2001; Johnson et al., 2001c; Speakman et al., 2001; Speakman and Król, 2005; Król et al., 2007; Speakman, 2007; Speakman, 2008), deer mice *Peromyscus maniculatus* (Hammond and Kristan, 2000), cotton rats (Rogowitz, 1998) and Brandt's voles (Zhang and Wang, 2007), in striped hamsters in the present study, limits to SusEI are not centrally driven by the capacity of the gastrointestinal tract to process ingested food.

It has been well established that additional energy demands are required of animals exposed to cold temperature (Heldmaier et al., 1982; Wang and Wang, 1989; Wang and Wang, 1990; Bozinovic

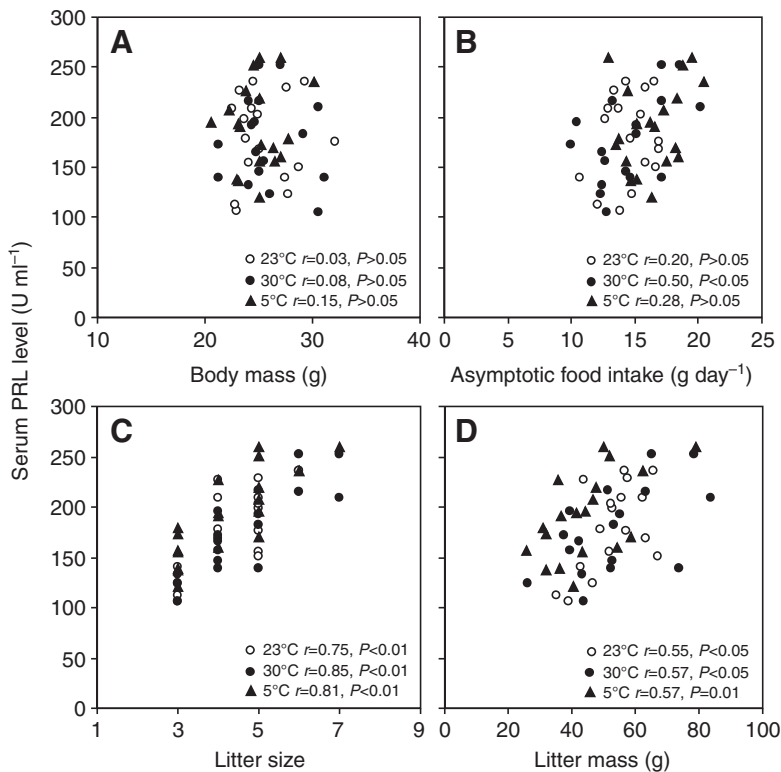


Fig. 6. Correlations between serum prolactin (PRL) levels and (A) body mass, (B) asymptotic food intake, (C) litter size and (D) litter mass during late lactation in striped hamsters lactating at 23, 30 or 5°C.

et al., 2004; Chi and Wang, 2005; Li and Wang, 2005; Wang et al., 2006; Zhang and Wang, 2007). In the present study, the litter size raised by females at 23°C was similar to that of females in the 30 and 5°C groups; however, significantly lower litter mass and mean pup mass was observed in the 5°C group. Johnson and Speakman found that total litter mass decreased significantly in MF1 female mice exposed to cold temperatures (Johnson and Speakman, 2001). Zhang and Wang also observed a significantly lower weight gain in Brandt's vole lactating at cold temperatures (Zhang and Wang, 2007). There are two possible explanations for the lower litter mass raised in the 5°C group of hamsters in the present study. First, milk production could not be upregulated in the cold because the mammary glands were already working at capacity, which might be consistent with the peripheral limitation hypothesis. A second possibility is that the pups were extremely inefficient at growing in the cold because of their own elevated demands for energy. Hence the females might have increased milk production when they were placed in the cold, as predicted by the alternative heat dissipation limits hypothesis. Additionally, in the present study, female hamsters lactating at 30°C (the thermoneutral zone of this species) (Song and Wang, 2003; Zhao et al., 2010b) did not show significant differences in energetic parameters from females lactating at 23°C. If the mice were limited by the peripheral capacity of the mammary gland or by their capacity to dissipate heat, then it would be anticipated that at 30°C their intake would go down either because their thermogenic demands are lower or because their heat dissipation capacity is lower. If it is assumed that the milk production levels were the same at 23 and 30°C, then one would predict that the pups at 30°C would grow better because they would have lower thermogenic demands, yet this was not observed. Alternatively, the heat dissipation limits hypothesis would predict that the pups should grow less well because the mothers would reduce their milk production at 30°C to avoid hyperthermia; however, this also was not observed, i.e. there was no statistical significant difference between the two groups in either

litter mass (52.9±2.2 g at 23°C vs 53.0±3.8 g at 30°C; Tukey's HSD,  $P>0.05$ ) or mean pup mass (12.3±0.4 g at 23°C vs 11.5±0.5 g at 30°C; Tukey's HSD,  $P>0.05$ ). This comparison does not provide strong support for either hypothesis.

PRL is undoubtedly an essential hormone for lactogenesis and maintenance of milk production in mammals (Tucker, 1985; Farmer et al., 1999). In the present study, serum PRL levels were positively correlated with litter size and mass, but no significant differences in PRL levels between female hamsters lactating at 23, 30 and 5°C were found. This suggests that the exposure to hot (30°C) and cold (5°C) had negligible effects on PRL secretion by the anterior pituitary gland. It should be pointed out that a drawback of the present study was the lack of milk production measurements; therefore, more studies should be performed to carefully address this issue.

RMR has been previously shown to increase in animals exposed to cold or in females during peak lactation (Garton et al., 1994; Spaaij et al., 1994; Speakman and McQueenie, 1996; Johnson et al., 2001b). In the present study, an increase in RMR in female hamsters lactating at 5°C was observed. Positive correlations between RMR and litter mass or asymptotic food intake during peak lactation in females at 5 or 23°C were also observed, suggesting that females with higher RMRs may have a greater capacity for absorbing energy and, therefore, be able to devote more energy to reproduction (Thompson, 1992; Johnson et al., 2001b). The increased RMR was accompanied by an increase in food intake in females lactating at 5°C. Food intake also increased to a similar extent in non-reproductive hamsters after exposure to the cold (Z.-J.Z. and J. Cao, unpublished data). These data suggest that increased food intake of females lactating at cold might compensate for the additional energy demands of maternal thermogenesis at the expense of the maintenance of normal offspring growth.

The study in MF1 mice also showed that fur-removal induced increases in food intake and reproductive output during lactation,

which was consistent with the heat dissipation limits hypothesis (Król et al., 2007). However, fur-removal failed to affect reproductive output in Swiss mice (Zhao and Cao, 2009a; Zhao, 2010; Zhao et al., 2010a). These results suggest that the factors limiting SusEI might be species-specific (Hammond and Diamond, 1992; Hammond et al., 1994; Hammond and Diamond, 1997; Rogowitz, 1998; Hammond and Kristan, 2000; Johnson and Speakman, 2001; Johnson et al., 2001b; Johnson et al., 2001c; Speakman et al., 2001; Król and Speakman, 2003a; Król and Speakman, 2003b; Król et al., 2003; Speakman and Król, 2005; Król et al., 2007; Speakman, 2007; Zhang and Wang, 2007; Speakman, 2008; Zhao and Cao, 2009a; Wu et al., 2009; Speakman and Król, 2010; Zhao, 2010; Zhao et al., 2010a). In addition, the inconsistent results from different rodent species also suggest that the factors limiting SusEI may be dependent on the conditions that animals are subjected to. For striped hamsters in the present study, limitation on SusEI might be consistent with the peripheral hypothesis, yet the present data did not provide strong support for this. Peripheral limitations on SusEI during lactation have previously been reported in cotton rats (Rogowitz, 1998) and Swiss mice (Hammond and Diamond, 1992; Hammond et al., 1994; Hammond and Diamond, 1997; Hammond and Kristan, 2000; Zhao and Cao, 2009a; Zhao, 2010; Zhao et al., 2010a), indicating that there might be a general pattern at least in some rodent species. In contrast to laboratory animals, like mice and rats, wild hamsters show seasonal variations in reproduction, suggesting potential effects of seasonal environmental factors on reproductive output. There is also a possibility that limitations on SusEI are associated with seasonal reproductive performance or may be affected by environmental factors, such as seasonal changes in temperature, food quality and availability.

In summary, the present study showed that striped hamsters lactating at 5°C had significantly higher food intake and energy expended on RMR but raised significantly lower litter mass compared with hamsters lactating at 23°C. No group differences were observed in serum PRL levels. The differences in food intake and reproductive output between females lactating at 23 and 30°C were not significant. These data suggest that the increased food intake of females lactating at 5°C was likely to compensate for additional energy demands for thermogenesis. For striped hamsters in this study, limitation on SusEI was inconsistent with the heat dissipation limits hypothesis but might be consistent with the peripheral limitation hypothesis. However, because the present study did not measure milk production, it failed to provide strong support for either the peripheral limitation or the heat dissipation limits hypotheses.

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