© 2020. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2020) 223, jeb228809. doi:10.1242/jeb.228809

CORRECTION

Correction: Exposure to hot temperatures during lactation in Swiss mice stunts offspring growth and decreases future reproductive performance of female offspring

Meng-Huan Bao, Li-Bing Chen, Catherine Hambly, John R. Speakman and Zhi-Jun Zhao

There was an error published in the online full-text and PDF versions of *Journal of Experimental Biology* (2020) **223**, jeb223560 (doi:10.1242/jeb.223560).

The common name of the study organism was originally given as striped hamsters in the title of the article. The correct name appears in the title above, and both the online full-text and PDF versions of the article have been updated to reflect that the research was carried out with Swiss mice, as stated elsewhere in the article.

The authors apologise to readers for this error.

© 2020. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2020) 223, jeb223560. doi:10.1242/jeb.223560

RESEARCH ARTICLE

Exposure to hot temperatures during lactation in Swiss mice stunts offspring growth and decreases future reproductive performance of female offspring

Meng-Huan Bao[1], Li-Bing Chen[1], Catherine Hambly[2], John R. Speakman[2,3,4,*] and Zhi-Jun Zhao[1,*]

ABSTRACT

Exposure to high temperatures (heatwaves) is rapidly emerging as an important issue of climate change, in particular for female mammals during lactation. High temperatures adversely affect the ability to dissipate heat, which has negative effects on reproductive output. The cumulative effects on growth of F1 offspring after weaning, and future reproductive performance of offspring, remain uncertain. In this study, F1 mice weaned from mothers lactating at 21 and 32.5°C were housed at 21°C from day 19 until day 56 of age, during which food intake and body mass were measured. The F1 adult females that were weaned at the two temperatures were bred and then exposed to 32.5°C during lactation. Energy intake and milk output, and litter size and mass, were determined. The F1 adults weaned at 32.5°C consumed less food and had lower body mass than their counterparts weaned at 21°C. Several visceral organs or reproductive tissues were significantly lower in mass in F1 weaned at 32.5°C than at 21°C. The exposure to 32.5°C significantly decreased energy intake, milk output and litter mass in F1 adult females during lactation. The F1 adult females weaned at 32.5°C produced less milk and raised lighter pups than those previously weaned at 21°C. The data suggest that transient exposure to hot temperatures during lactation has long-lasting impacts on offspring, including stunted growth and decreases in future reproductive performance when adult. This indicates that the offspring of females previously experiencing hot temperatures have a significant fitness disadvantage.

KEY WORDS: Hot temperature, Heat dissipation limit, Lactation, Offspring, Reproductive performance, Swiss mice

INTRODUCTION

Life on Earth is facing increasing temperatures and increasing variability in climate (IPCC Working Group 1, 2014). Animals that are affected by climate change may alter their distribution and adapt through changes in morphology, physiology, behavior and life history (Hoffmann and Sgrò, 2011; Mifsud et al., 2011; Stawski and Geiser, 2012; Lovegrove et al., 2014; Smith et al., 2014; Godde et al., 2019; Radchuk et al., 2019). Exposure to periods of high temperature (heatwaves) due to climate change is rapidly emerging as an important threat to a variety of animals (Martin et al., 2018). High temperatures adversely affect the body by interfering with its ability to dissipate heat and thermoregulate (Quiniou and Noblet, 1999). Many animals show chronic hyperthermia during lactation (Ulmershakibaei and Plonait, 1992; Speakman and Król, 2005a). This is because heat generated as a by-product of processing food and producing milk increases considerably in lactating females compared with that in non-lactating subjects (Król and Speakman, 2003a,b). It has been proposed that females during lactation are capable of dissipating heat, but the maximal capacity of an animal to dissipate body heat is fixed (Speakman and Król, 2005a, 2011). Maximal heat dissipation capacity therefore imposes a limitation on sustained energy intake and milk energy output, i.e. the heat dissipation limit (HDL) hypothesis (Król and Speakman, 2003a,b). Consistent with the HDL hypothesis, mice selected for high heat loss produced more milk and raised heavier litters than low heat loss mice (McDonald and Nielsen, 2006, 2007). Król et al. (2007) dorsally shaved MF1 mice lactating at 21°C to reduce their external insulation and thereby elevate their capacity to dissipate body heat. These shaved females had a greater food intake, exported more energy as milk, and raised heavier litters at weaning than unshaved females (Król et al., 2007). Other studies, however, have failed to replicate this shaving effect (Zhao and Cao, 2009a; Zhao et al., 2010; Sadowska et al., 2019).

Nevertheless, consistent with the HDL theory, exposure to high ambient temperature has been reported to decrease food intake and milk yield in a variety of lactating animals, including laboratory mice (*Mus musculus*; Król and Speakman, 2003a,b; Wen et al., 2017), rats (*Rattus norvegicus*; Morag et al., 1969; Leon and Woodside, 1983; Jansen and Binard, 1991), Brandt's voles (*Lasiopodomys brandtii*; Wu et al., 2009), common voles (*Microtus arvalis*; Simons et al., 2011), Mongolian gerbils (*Meriones unguiculatus*; Yang et al., 2013), European brown hares (*Lepus europaeus*; Valencak et al., 2010), striped hamsters (*Cricetulus barabensis*; Zhao, 2011) and the golden hamster (*Mesocricetus auratus*; Ohrnberger et al., 2018). Reductions in milk output at high temperatures were also observed in other large animals such as dairy cattle (*Bos taurus*; Cobble and Herman, 1951; Brody et al., 1958), sheep (*Ovis aries*; Abdalla et al., 1993) and pigs (*Sus scrofa*; Black et al., 1993; Quiniou and Noblet, 1999; Renaudeau and Noblet, 2001; Renaudeau et al., 2003). Consequently, litter size, litter mass, or both, are significantly reduced during lactation and maternal reproductive values considerably decrease, as a result of a direct effect of high ambient temperature on milk production (Król and Speakman, 2003a,b; Wen et al., 2017).

Consequently, pups raised by mothers at hot temperatures are generally weaned smaller, and would potentially have a fitness disadvantage (Speakman and Król, 2005a, 2011). It has been reported that in some instances smaller offspring undergo

[1]College of Life and Environmental Science, Wenzhou University, Wenzhou 325035, China. [2]Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen AB24 2TZ, UK. [3]State Key Laboratory of Molecular Developmental Biology, Chinese Academy of Sciences, Bei Chen Xi Lu, Chaoyang, Beijing 100101, People's Republic of China. [4]CAS Center of Excellence in Animal Evolution and Genetics, Kunming 650223, People's Republic of China.

*Authors for correspondence (zhaozj@wzu.edu.cn; j.speakman@abdn.ac.uk)

Z.-J.Z., 0000-0001-8745-2330

Received 17 February 2020; Accepted 31 March 2020

Journal of Experimental Biology (2020) 223, jeb223560. doi:10.1242/jeb.223560

subsequent catch-up growth (Rauw et al., 1999; Metcalfe and Monaghan, 2001, 2003; Speakman and Król, 2011). This catch-up growth might attenuate the impact of weaning at a smaller mass, but might bring its own problems (Metcalfe and Monaghan, 2001). Alternatively, smaller F1 offspring might not undergo catch-up growth and would therefore have a smaller body size when adult. Smaller individuals fare less well in aggressive interactions with larger offspring, and hence would be expected to be less competitive for food and mates (Wolff and Sherman, 2007; Speakman and Król, 2011), resulting in a fitness disadvantage. However, smaller F1 adults may have less heat dissipation difficulties at hot temperatures as they have a higher surface-to-volume ratio than females with larger body size. Hence, smaller F1 female adults born to hot temperature-exposed mothers might show greater reproductive performance, if they themselves experienced hot temperatures during lactation. To date, such knock-on impacts of temperature manipulations on lactating females have not been extensively studied.

We hypothesized that F1 offspring weaned at hot temperatures would have a smaller weaning body size, and that growth after weaning would be stunted, resulting in smaller F1 adults compared with those weaned at cool temperatures. These smaller F1 adult females weaned at hot temperatures, however, may exhibit greater reproductive performance, and therefore have a fitness advantage. In the present study, female Swiss mice were lactating at room temperature (21°C) or exposed to hot temperature (32.5°C) during lactation. F1 females and males were maintained at 21°C after weaning, and growth was monitored from day 18 until day 56 of age. The F1 female adults that previously weaned at the two temperatures were then bred and exposed to a hot temperature (32.5°C) during lactation, during which maternal energy intake and milk output, and litter size and mass were examined.

MATERIALS AND METHODS

Animals

Experimental subjects were the offspring of a breeding colony of Swiss mice maintained at Wenzhou University, Wenzhou, China. Animals were housed individually in plastic cages (29×18×16 cm) with sawdust bedding, and were provided *ad libitum* with food (D12450B; Research Diets, Inc., New Brunswick, NJ, USA) and water. Animals were kept under a 12 h:12 h light:dark cycle (lights on at 08:00 h) at a constant temperature of 21±1°C. This experiment was approved by the Wenzhou University Animal Care and Use Committee (WU-ACUC), and all experimental procedures complied with guidelines of the WU-ACUC.

Virgin female mice (9–10 weeks of age) were paired with males for 11 days after which the males were removed. Fifty females subsequently became pregnant and gave birth. On day 1 of lactation, pups were removed from their mothers, and mixed with pups from other litters born on the same day (±1 day). Females were allocated 12 pups on day 1. The females and their offspring were randomly assigned to either a 21°C (n=21) or a 32.5°C (n=29) group. The females in the 21°C group were kept at 21°C throughout lactation, while the females in the 32.5°C group were maintained at 21°C on days 1 to 6, and were then transferred to 32.5°C on day 7 until day 18. All pups were weaned on day 18 of lactation. Four female and four male pups were then randomly selected from each litter (each mother) at 21 and 32.5°C, respectively (hereafter referred to as F1). In total, 96 F1 females were selected in the 21 and 32.5°C groups (W21°C, weaned at 21°C, n=48; W32.5°C, weaned at 32.5°C, n=48); and 31 F1 males were selected in the two temperatures (W21°C, n=15; W32.5°C, n=16). The pups from the 21°C and 32.5°C groups were kept at 21°C after weaning (days 19–56 of age), during which body mass and food intake were measured.

Milk intake of pups

The milk intake of pups was estimated from the milk energy output (MEO) of mothers during lactation. As described previously, MEO was assessed from the energy budget of litters (Król and Speakman, 2003b; Wen et al., 2017). This is because the energy available to pups is obtained only from their mother's milk, which can be calculated as the sum of energy allocated to the growth of new tissue and daily energy expenditure (DEE) of the pups (Zhao et al., 2010, 2013). DEE was predicted from pup body mass on the basis of the relationship between resting metabolic rate (RMR) and body mass under the assumption that DEE=1.4×RMR, to take into account the energetic costs of pups' activity. The equation used was (Król and Speakman, 2003b):

$$\mathrm{MEO} = \left[(7.28 + 0.71 \times M_{\mathrm{L}}) \times \mathrm{CF} + M_{\mathrm{L,inc}} \times \mathrm{GE}_{\mathrm{pups}}\right] \times 100/d_{\mathrm{milk}}, \quad (1)$$

where MEO (kJ day^{-1}) is milk energy output, M_{L} (g) is the litter mass, CF is the correction factor (CF=1.4, the mean ratio of daily energy expenditure to RMR) and $\mathrm{GE}_{\mathrm{pups}}$ (kJ g^{-1} wet mass) is pups' gross energy content. The mean $\mathrm{GE}_{\mathrm{pups}}$ values used in this formula for the two treatment groups were determined using an IKA C2000 oxygen bomb calorimeter. $M_{\mathrm{L,inc}}$ (g day^{-1}) is the increase in litter mass between two days of lactation, and d_{milk} is the apparent digestibility of milk (d_{milk}=96%) (Król and Speakman, 2003b). As offspring could access the diet and started to eat on day 16 and thereafter, the milk intake of pups was presented as the MEO per pup (kJ pup^{-1} day^{-1}), i.e. MEO/litter size, on days 2 to 15 of lactation.

Body mass of F1 pups during lactation and post-lactation

Body mass of F1 pups was estimated from litter mass on a daily basis over the period of lactation (days 2–18 of lactation). Pup mass did not differ significantly between female and male pups during lactation, therefore pup body mass of both sexes was pooled, estimated from litter mass divided by litter size (g pup^{-1} day^{-1}). Both F1 females and males were weighed at 2-day intervals after they were weaned on day 18 of age until day 56 of age (F1 females: W21°C, n=15; W32.5°C, n=16; F1 males: W21°C, n=15; W32.5°C, n=16). Food intake was calculated as the mass of food missing from the hopper every 3 days, subtracting orts mixed in the bedding (Johnson et al., 2001; Zhao and Cao, 2009b).

Organ mass and sexual hormone concentrations of F1 female and male adults

On day 58 of age, 16 F1 females (W21°C, n=8; W32.5°C, n=8) and 31 F1 males (W21°C, n=15; W32.5°C, n=16) were randomly selected from both groups, and euthanized by decapitation. Brain, liver, heart, lung, spleen and kidneys were removed from each animal. The ovaries, oviduct (including uterine horns), uterus of the females, and testis, epididymis, cord and seminal vesicle of males, were carefully separated. All the organs were weighed immediately to determine wet mass (to 1 mg). The carcass, excluding the organs listed above, was also weighed (to 1 mg). Trunk blood was collected and serum was separated from each blood sample by centrifugation and stored at −80°C for sex hormone measurements. The concentrations of serum estradiol and estriol of females, and testosterone of males, were quantified by ^{125}I radioimmunoassay

(RIA), using RIA kits (Beijing North Biological Technical Research Institute, Beijing, China). The detection range of serum estradiol was 4–4000 pg ml^{-1}, and intra- and inter-assay coefficients of variation were less than 10 and 15%, respectively. The minimum detection was 5 ng ml^{-1}, and intra- and inter-assay coefficients of variation were less than 15% for estriol. The minimum detection for serum testosterone was 0.1 ng ml^{-1}, and the intra- and inter-assay coefficients of variation were less than 10 and 15%, respectively.

At 9 weeks of age, F1 adult females in the W21°C and W32.5°C groups were paired with males as described previously. The F1 females that subsequently became pregnant and gave birth were randomly assigned to either 21°C or 32.5°C groups, i.e. F1 weaned 21°C: two treatments, lactated (L) at 21°C and lactated at 32.5°C; F1 weaned 32.5°C: two treatments, lactated at 21°C and lactated at 32.5°C. Therefore, there were four groups in total: W21°C–L21°C (*n*=16), W21°C–L32.5°C (*n*=14), W32.5°C–L21°C (*n*=15) and W32.5°C–L32.5°C (*n*=15). The F1 females in the W21°C–L21°C and W32.5°C–L21°C groups were lactating at 21°C throughout lactation, while the females in the W21°C–L32.5°C and W32.5°C–L32.5°C groups were lactating at 21°C on days 1 to 6, and transferred to 32.5°C on day 7 and thereafter. As described earlier, females were allocated 12 pups on day 1 of lactation. Body mass, food intake, litter size and litter mass were measured daily over the course of lactation.

Energy intake and digestibility of F1 lactating females

Gross energy intake (GEI), digestive energy intake (DEI) and digestibility were measured between days 13 and 15 of lactation (W21°C–L21°C, *n*=16; W21°C–L32.5°C, *n*=14; W32.5°C–L21°C, *n*=15; W32.5°C–L32.5°C, *n*=15). As described previously (Grodzinski and Wunder, 1975; Wen et al., 2018a, 2018b), a known quantity of food was provided at 16:00 h on day 13, and any uneaten food and orts mixed with the bedding material were collected, along with feces from each animal on day 15. Food and feces were separated manually after drying at 60°C to constant mass. Gross energy content of food and feces were determined using an IKA C2000 oxygen bomb calorimeter (IKA, Königswinter, Germany). GEI, DEI, digestibility and gross energy of feces (GEF) were calculated as described previously (Zhao et al., 2014a,b; Wen et al., 2017). Urinary energy loss (UEL) was estimated at 3% of the digestible energy intake (Drozdz, 1975). Metabolic energy intake (MEI) was calculated as follows: MEI=DEI–(DEI×3%) (Vaanholt et al., 2013).

Daily energy expenditure and milk energy output of F1 lactating females

Daily energy expenditure (DEE) was measured on days 13–14 of lactation (W21°C–L21°C, *n*=16; W21°C–L32.5°C, *n*=14; W32.5°C–L21°C, *n*=15; W32.5°C–L32.5°C, *n*=15), using the doubly labeled water (DLW) technique (Lifson et al., 1955; Speakman, 1998). As described previously (Król et al., 2007), females were weighed (±0.01 g) and injected intraperitoneally with approximately 0.2 g of water containing enriched ^{18}O (27.8×atom%) and ^{2}H (15.9×atom%). Initial blood samples were taken 1 h after the injection via the tail tip (Król and Speakman, 1999; Visser et al., 2000a), and final blood samples were collected 24 h later to estimate isotope elimination rates (Speakman and Racey, 1988b). Blood samples were also collected from unlabeled animals to estimate the background isotope enrichments. Each blood sample was stored in a glass capillary that was immediately flame-sealed with a torch. Glass capillaries containing blood samples were vacuum distilled, and water from the resulting distillate was used to produce CO_2 (see Speakman et al., 1990) and H_2 (see Speakman and Król, 2005b). The isotope ratios ^{18}O:^{16}O and ^{2}H:^{1}H were analysed using gas source isotope ratio mass spectrometry (Optima, Micromass IRMS and Isochrom μG, Manchester, UK). Samples were run alongside three laboratory standards for each isotope (calibrated to international standards) to correct delta values to p.p.m. Isotope enrichments were converted to values of DEE using a single pool model as recommended for this size of animal (Speakman, 1993; Vaanholt et al., 2013). MEO was calculated from the difference between MEI and DEE (for a full description of the method, see Król and Speakman, 2003b; Vaanholt et al., 2013).

Organ mass of F1 lactating females

The F1 females were euthanized by decapitation on day 17 of lactation (W21°C–L21°C, *n*=16; W21°C–L32.5°C, *n*=14; W32.5°C–L21°C, *n*=15; W32.5°C–L32.5°C, *n*=15). The organs, including liver, heart, lung, spleen, kidneys and digestive tract were removed as described above. The stomach, small and large intestine, and caecum were separated, and the contents of the tracts were removed. In addition, the mammary glands and fur were separated and weighed (to 1 mg). The tail was removed and its length (to 1 mm) and weight (to 1 mg) were also recorded.

Statistics

Data are expressed as means±s.e.m. and were analysed using SPSS 21.0 statistical software. All variables were tested for normality using the Kolmogorov–Smirnov test. The differences in milk intake, body mass and food intake of pups between W21°C and W32.5°C groups were examined using independent *t*-tests. Organ mass and sexual hormone concentrations of adults were also examined using independent *t*-tests. Litter size and litter mass, and maternal body mass, food intake, energy intake, digestibility, DEE and MEO, and body composition were examined using two-way ANOVA (temperature at weaning×temperature for F1 when lactating), followed by Tukey's *post hoc* tests where required. In addition, the changes over a time period were assessed using repeated measures ANOVA, where appropriate. Correlations between asymptotic food intake (AFI) and MEO and litter mass, as well as between AFI, GEI, DEI, MEO and litter mass and body composition, were examined using Pearson's correlation analysis. The tests were two-tailed, and the level of significance was set at $P<0.05$.

RESULTS

Milk intake of F1 pups during lactation

The milk energy intake of F1 pups, estimated by the milk energy output of their mothers, was not different between the two groups during early lactation (day 2, t_{48}=0.80, $P>0.05$; day 6, t_{48}=1.14, $P>0.05$; Fig. 1A). The pups that were raised at 21°C considerably increased milk intake during the period of lactation; milk intake increased by 23.5% on day 15 compared with that on day 6 (repeated measures across days 6–15, $F_{9,180}$=37.15, $P<0.01$). The pups in the 32.5°C group significantly decreased their milk intake following exposure to hot temperature; intake was reduced by 17.2% on day 15 compared with day 6 (repeated measures across days 6–15, $F_{9,252}$=17.14, $P<0.01$). Additionally, milk intake in the 32.5°C group was lower by 25.1% on day 7 and by 30.6% on day 15 than that of the 21°C group (day 7, t_{48}=6.49, $P<0.01$; day 15, t_{48}=7.83, $P<0.01$; Fig. 1A).

Body mass of F1 pups during lactation

No significant difference was observed in pup body mass between the two groups during early lactation (day 2, t_{48}=0.04, $P>0.05$; day 7, t_{48}=1.52, $P>0.05$; Fig. 1B). The exposure to hot

Journal of Experimental Biology (2020) 223, jeb223560. doi:10.1242/jeb.223560

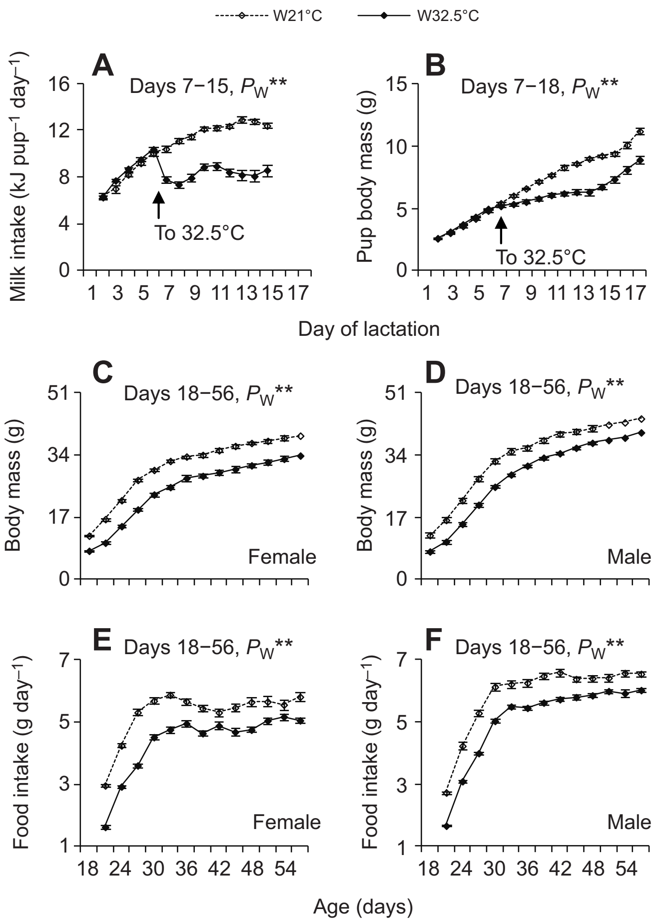

Fig. 1. Milk intake and pup body mass during lactation, and food intake and body mass during post-lactation. Milk intake (A) and body mass (B) of F1 pups raised by the mothers at 21°C and 32.5°C (W21°C, *n*=21; W32.5°C, *n*=29), and body mass (C,D) and food intake (E,F) during growth of F1 female and male mice that previously weaned at 21°C and 32.5°C (F1 females, 21°C, *n*=15; 32.5°C, *n*=16; F1 males, 21°C, *n*=15; 32.5°C, *n*=16). Data are means±s.e.m. P_W**, significant difference between W21°C and W32.5°C groups (P<0.01). The arrow indicates that the mother and F1 pups were exposed to 32.5°C on day 7 until day 18 of lactation.

temperature had a significant effect on pup body mass, which was significantly lower in the 32.5°C group than that in the 21°C group from day 8 onwards (day 8, t_{48}=4.00, P<0.01). The pups that were weaned at 32.5°C showed 20.4% lower mass relative to their counterparts weaned in at 21°C (day 18, t_{48}=5.21, P<0.01; Fig. 1B).

Body mass and food intake of F1 females and males after weaning

The females of the W32.5°C group were weaned significantly lighter than those of the W21°C group (7.8±0.3 versus 12.0±0.2 g, t_{29}=11.24, P<0.01; Fig. 1C). The females showed considerable increases in body mass from 18 to 56 days of age, whereas the growth of F1 females in the W32.5°C group was attenuated compared with the W21°C group. On day 56 of age, body mass of F1 females in the W32.5°C group was lower by 13.8% than that of the W21°C group (t_{29}=5.56, P<0.01). Consistently, body mass of males was significantly lower in the W32.5°C group than that in the W21°C group on any day over the period of growth, and it was lower by 8.6% in the W32.5°C group at day 56 (t_{29}=4.34, P<0.01; Fig. 1D).

Food intake of both females and males was lower in the W32.5°C group than that in the W21°C group on any day between days 19 and 56 of age. The females of the W32.5°C group consumed 44.9% less food on day 21 of age, and 13.0% less food on day 56 than those in the W21°C group (day 21, t_{29}=20.21, P<0.01; day 56, t_{29}=4.57, P<0.01; Fig. 1E). The males of the W32.5°C group consumed 39.0 and 9.9% less food on days 21 and 56 of age, respectively, than those of the W21°C group (day 21, t_{29}=20.14, P<0.01; day 56, t_{29}=5.01, P<0.01; Fig. 1F).

Organs of F1 adults

Most of the F1 female organs were lighter in the W32.5°C group than in the W21°C group (see Table S1). For example, the mass of carcass was lower by 15.0% in the W32.5°C group than in the W21°C group, and the brain, liver, lung, spleen and kidneys were lower by 7.5, 22.2, 22.9, 25.5 and 14.0%, respectively, in the W32.5°C group than in the W21°C group (Table S1). The reproductive organs, such as ovary, oviduct and uterus, were not different in mass between the two groups (Table 1). In addition, the two groups did not differ significantly in the levels of estrogenic hormones (Table 1).

The males of the W32.5°C group had significantly lighter carcasses than those of the W21°C group (Table S1). The masses of heart, liver and kidney were significantly lower in the W32.5°C group than in the W21°C group (Table S1). Importantly, the masses of testis, epididymis and seminal vesicle were lower by 9.2, 38.8 and 30.7% in the W32.5°C group than in the W21°C group (Table 1). Serum testosterone level of the W32.5°C group also decreased by 25.2% compared with the W21°C group; however, the difference between the two groups was not statistically significant (Table 1).

Body mass of F1 female adults during lactation

Body mass of F1 female adults during lactation was significantly affected by the hot temperature at which they had weaned, with the F1 females weaned at 32.5°C being significantly lighter than the females weaned at 21°C (day 2, $F_{1,56}$=30.54, P<0.01; day 16, $F_{1,56}$=12.59, P<0.01; Fig. 2A). Body mass of F1 female adults in the W21°C and W32.5°C groups decreased significantly following the exposure to 32.5°C, and was decreased by 19.9 and 15.6% in the W21°C–L32.5°C and W32.5°C–L32.5°C groups, respectively, on day 16 compared with day 6 (repeated measures across days 6–16,

Table 1. Reproductive organ mass and sexual hormone concentration of F1 adult female and male mice previously weaned at 21 and 32.5°C

	W21°C	W32.5°C	*t*	*P*
F1 females	*n*=8	*n*=8		
Ovary (g)	0.065±0.009	0.084±0.009	1.58	n.s.
Oviduct (including uterine horns) (g)	0.121±0.016	0.115±0.011	0.34	n.s.
Uterus (g)	0.092±0.008	0.075±0.005	1.80	n.s.
Estradiol (pg ml^{-1})	30.99±5.76	26.33±2.89	0.753	n.s.
Estriol (ng ml^{-1})	9.31±0.95	8.60±0.84	0.563	n.s.
F1 males	*n*=15	*n*=16		
Testis (g)	0.273±0.007	0.248±0.005	2.88	**
Epididymis (g)	0.400±0.044	0.245±0.021	3.23	**
Cord (g)	0.245±0.024	0.232±0.038	0.27	n.s.
Seminal vesicle (g)	0.062±0.005	0.043±0.002	3.48	**
Testosterone (ng ml^{-1})	8.21±1.71	6.14±1.56	0.93	n.s.

Mothers were maintained at 21°C throughout lactation (W21°C) or exposed to 32.5°C on days 7–18 of lactation (W32.5°C), and all F1 females and males were housed at room temperature (21°C) from age day 18 until day 56. Data are means±s.e.m. **Significant difference between the two groups (P<0.01). n.s., not significant.

Journal of Experimental Biology (2020) 223, jeb223560. doi:10.1242/jeb.223560

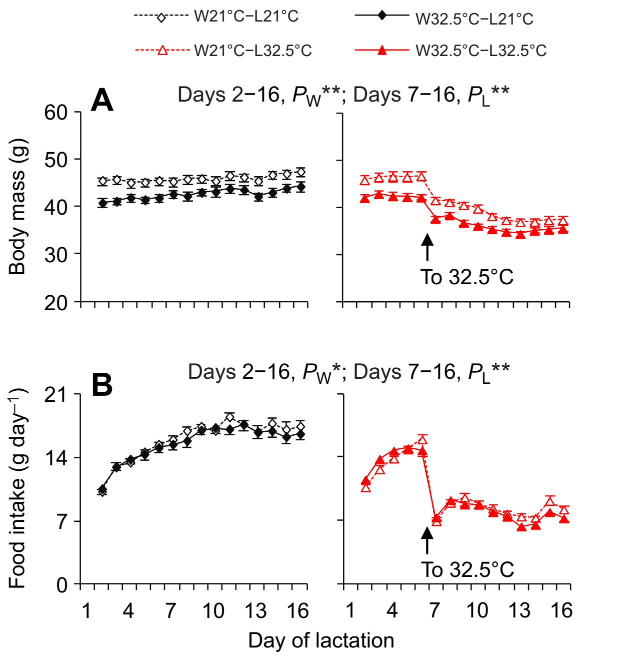

Fig. 2. Body mass and food intake during lactation in F1 offspring. Body mass (A) and food intake (B) at 21 and 32.5°C during lactation in F1 Swiss mice. F1 weaned 21°C: two treatments, lactated at 21°C (W21°C–L21°C, *n*=16) and lactated at 32.5°C (W21°C–L32.5°C, *n*=14); F1 weaned 32.5°C: two treatments, lactated at 21°C (W32.5°C–L21°C, *n*=15) and lactated at 32.5°C (W32.5°C–L32.5°C, *n*=15). Data are means±s.e.m.; *P*-values indicate that all of the treatments were assessed together. P_W, effect of hot temperature on F1 pups weaned at 32.5°C; P_L, effect of hot temperature on F1 adult females lactating at 32.5°C. **P*<0.05; ***P*<0.01.

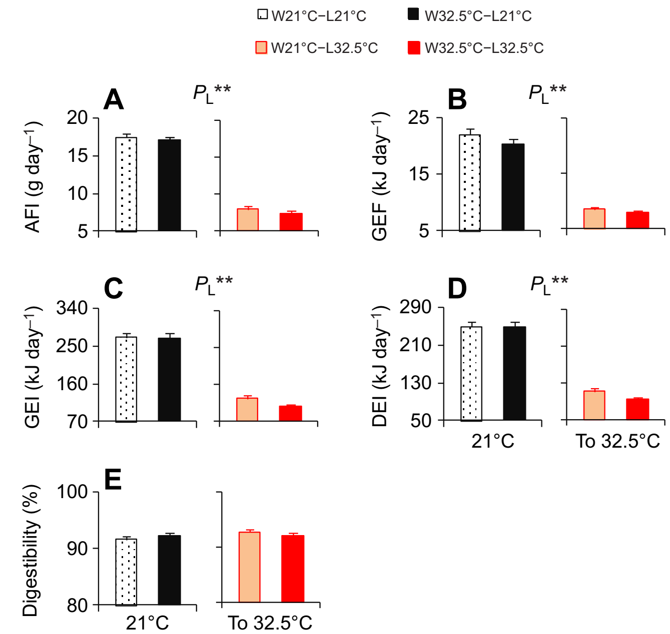

Fig. 3. Asymptotic food intake (AFI), energy intake and digestibility during lactation in F1 offspring. AFI (A), gross energy of feces (GEF; B), gross energy intake (GEI; C), digestive energy intake (DEI; D) and digestibility (E) at 21°C and 32.5°C during lactation in F1 Swiss mice. F1 weaned 21°C: two treatments, lactated at 21°C (W21°C–L21°C, *n*=16) and lactated at 32.5°C (W21°C–L32.5°C, *n*=14); F1 weaned 32.5°C: two treatments, lactated at 21°C (W32.5°C–L21°C, *n*=15) and lactated at 32.5°C (W32.5°C–L32.5°C, *n*=15). Data are means±s.e.m.; *P*-values indicate that all of the treatments were assessed together. P_L, the effect of the hot temperature of the F1 adult females lactating at 32.5°C. ***P*<0.01.

W21°C, $F_{10,130}$=48.91, *P*<0.01; W32.5°C, $F_{10,140}$=36.85, *P*<0.01; Fig. 2A). The interaction effect on body mass was not statistically significant (day 16, $F_{1,56}$=0.65, *P*>0.05).

Food intake of F1 female adults during lactation

The F1 females of the W21°C group did not differ in food intake on days 2 to 15 of lactation from that observed in the W32.5°C group (day 2, $F_{1,56}$=1.72, *P*>0.05; day 15, $F_{1,56}$=2.45, *P*>0.05; Fig. 2B). On day 16, the females weaned at 32.5°C consumed less food than their counterparts weaned at 21°C (day 16, $F_{1,56}$=4.17, *P*<0.05). Food intake was considerably decreased following exposure to 32.5°C, and that of the W21°C–L32.5°C and W32.5°C–L32.5°C groups decreased by 56.7 and 50.3%, respectively, on day 7 compared with that on day 6 (Fig. 2B). The AFI was not significantly different between the females previously weaned at 21 and 32.5°C ($F_{1,56}$=2.01, *P*>0.05), but decreased considerably after exposure to 32.5°C, i.e. it decreased more in the W32.5°C–L32.5°C group than in the W21°C–32.5°C group ($F_{1,56}$=863.78, *P*<0.01; Fig. 3A). The interaction effect on AFI was not statistically significant ($F_{1,56}$=0.04, *P*>0.05).

Energy intake and digestibility of F1 female adults during lactation

The F1 females of the W32.5°C group did not differ in feces production at peak lactation (days 13–14) from the females of the W21°C group ($F_{1,56}$=2.31, *P*>0.05; Fig. 3B). The females produced considerably less feces after exposure to 32.5°C ($F_{1,56}$=271.77, *P*<0.01), while the difference between the W21°C–L32.5°C and W32.5°C–L32.5°C groups was not statistically significant (*post hoc*, *P*>0.05; Fig. 3B). Neither GEI nor DEI at peak lactation was different between the F1 females weaned at 21 and 32.5°C (GEI, $F_{1,56}$=1.18, *P*>0.05, Fig. 3C; DEI, $F_{1,56}$=0.93, *P*>0.05, Fig. 3D). GEI decreased considerably after exposure to 32.5°C, and was lower by 55.8 and 62.2% in the W21°C–L32.5°C and W32.5°C–L32.5°C groups, respectively, than in their counterparts lactating at 21°C ($F_{1,56}$=344.27, *P*<0.01; *post hoc*, *P*<0.05). Consistently, the hot temperature had a significant effect on DEI, and was lower in the W21°C–L32.5°C and W32.5°C–L32.5°C groups than in the W21°C and W32.5°C groups ($F_{1,56}$=271.21, *P*<0.01; *post hoc*, *P*<0.05). Digestibility at peak lactation did not differ between W21°C and W32.5°C groups ($F_{1,56}$=0.01, *P*>0.05), and was also not affected by hot temperature ($F_{1,56}$=0.87, *P*>0.05; Fig. 3E). There were no significant interaction effects on GEF ($F_{1,56}$=0.51, *P*>0.05), GEI ($F_{1,56}$=0.91, *P*>0.05), DEI ($F_{1,56}$=1.07, *P*>0.05) or digestibility ($F_{1,56}$=2.46, *P*>0.05).

DEE and MEO of F1 female adults during lactation

DEE at peak lactation (days 13–14) did not differ between the F1 females previously weaned at 21 and 32.5°C ($F_{1,56}$=2.31, *P*>0.05; Fig. 4A). Hot temperature decreased DEE significantly, and it was lower by 47.1 and 52.8% in W21°C–L32.5°C and W32.5°C–L32.5°C groups, respectively, compared with their counterparts lactating at 21°C ($F_{1,56}$=449.62, *P*<0.01; *post hoc*, *P*<0.05). The interaction effect on DEE was not statistically significant ($F_{1,56}$=2.13, *P*>0.05). The milk output at peak lactation differed significantly between the F1 females weaned at 21 and at 32.5°C, and it was lower by 20.4% in the W32.5°C–L32.5°C group than the W21°C–L32.5°C group ($F_{1,56}$=5.04, *P*<0.05; *post hoc*, *P*<0.05; Fig. 4B). The hot temperature significantly decreased milk

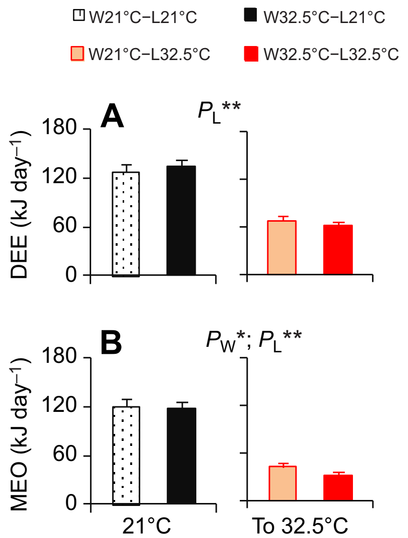

Fig. 4. Daily energy expenditure (DEE) and milk energy output (MEO) during lactation in F1 offspring. DEE (A) and MEO (B) at 21°C and 32.5°C during lactation in F1 Swiss mice. F1 weaned 21°C: two treatments, lactated at 21°C (W21°C–L21°C, *n*=16) and lactated at 32.5°C (W21°C–L32.5°C, *n*=14); F1 weaned 32.5°C: two treatments, lactated at 21°C (W32.5°C–L21°C, *n*=15) and lactated at 32.5°C (W32.5°C–L32.5°C, *n*=15). Data are means±s.e.m.; *P*-values indicate that all of the treatments were assessed together. P_W, effect of hot temperature on F1 pups weaned at 32.5°C; P_L, effect of hot temperature on F1 adult females lactating at 32.5°C. *$P<0.05$; **$P<0.01$.

production of F1 females, and the decreased MEO was more considerable in the W32.5°C–L32.5°C mice than in the W21°C–L32.5°C mice ($F_{1,56}$=123.08, $P<0.01$). The interaction effect on MEO was not statistically significant ($F_{1,56}$=0.40, $P>0.05$). There were positive correlations between AFI and MEO in the F1 females lactating at 21°C (r=0.85, $P<0.01$) and at 32.5°C (r=0.58, $P<0.01$; Fig. 5A).

Litter size and litter mass of F2 offspring

Litter size did not differ significantly between the F1 females weaned at 21 and 32.5°C (day 16, $F_{1,56}$=0.03, $P>0.05$), and it was also not affected by hot temperature (day 16, $F_{1,56}$=0.01, $P>0.05$; Fig. 6A). Litter size of both 21 and 32.5°C groups did not change significantly throughout lactation (repeated measures across days 2–16, $F_{14,784}$=0.60, $P>0.05$). Litter mass considerably increased over the period of lactation, and was increased by 90.9 and 82.9% in the W21°C and W32.5°C groups, respectively, on day 16 relative to day 6 (repeated measures across days 6–16, W21°C, $F_{10,150}$=388.77, $P<0.01$; W32.5°C, $F_{10,140}$=280.80, $P<0.01$; Fig. 6B). The increase in litter mass was considerably attenuated after exposure to the hot temperature, and it increased only by 39.2 and 27.2% in W21°C–L32.5°C and W32.5°C–L32.5°C groups, respectively (repeated measures across days 6–16, W21°C–L32.5°C, $F_{10,130}$=40.30, $P<0.01$; W32.5°C–L32.5°C, $F_{10,140}$=40.39, $P<0.01$). Litter mass was significantly lower on days 15 and 16 in the females weaned at 32.5°C than those weaned at 21°C (day 15, $F_{1,56}$=4.44, $P<0.05$; day 16, $F_{1,56}$=4.34, $P<0.05$). Litter mass of the groups lactating at 32.5°C was significantly lower than that of the groups lactating at 21°C on days 8 to 16 (day 8, $F_{1,56}$=10.03, $P<0.01$; day 16, $F_{1,56}$=66.12, $P<0.01$; Fig. 6B). Litter mass was positively correlated with AFI in the F1 females lactating at 21°C (r=0.78, $P<0.01$) and at 32.5°C (r=0.56, $P<0.01$; Fig. 5B). Consistently, the pups raised by the F1 females weaned at 32.5°C

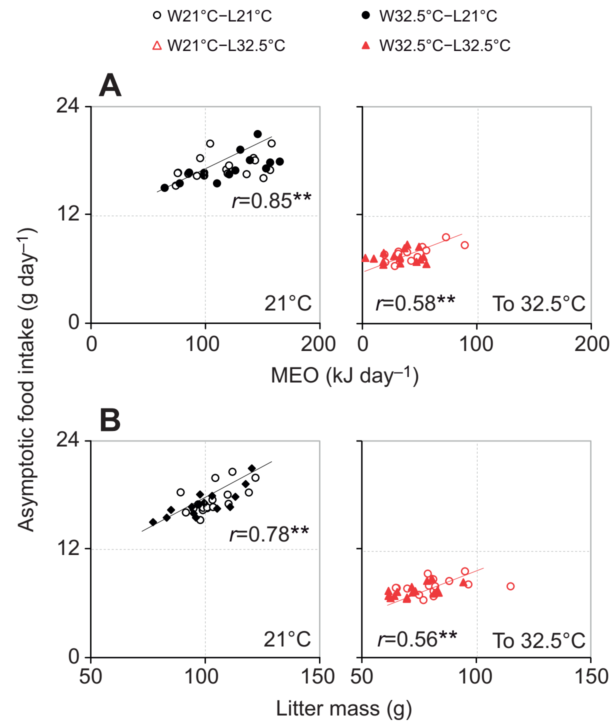

Fig. 5. Relationship between asymptotic food intake and milk energy output and litter mass. The coefficient of relationship between AFI and MEO (A) and litter mass (B) at 21 and 32.5°C during lactation in F1 Swiss mice. F1 weaned 21°C: two treatments, lactated at 21°C (W21°C–L21°C, *n*=16) and lactated at 32.5°C (W21°C–L32.5°C, *n*=14); F1 weaned 32.5°C: two treatments, lactated at 21°C (W32.5°C–L21°C, *n*=15) and lactated at 32.5°C (W32.5°C–L32.5°C, *n*=15). **Significant correlations ($P<0.01$).

were significantly lighter on days 14 to 16 than those in the females weaned at 21°C (day 14, $F_{1,56}$=3.69, $P<0.05$; day 15, $F_{1,56}$=5.23, $P<0.05$; day 16, $F_{1,56}$=4.69, $P<0.05$; Fig. 6C). The mean pup mass was significantly lower in the females lactating at 32.5°C than that at 21°C (day 8, $F_{1,56}$=22.71, $P<0.01$; day 16, $F_{1,56}$=75.12, $P<0.01$). There were no significant interaction effects on litter size (day 16, $F_{1,56}$=0.33, $P>0.05$), litter mass (day 16, $F_{1,56}$=0.78, $P>0.05$) or pup mass (day 16, $F_{1,56}$=0.54, $P>0.05$).

Mass in F1 female adults at weaning

On day 17 of lactation, F1 females previously weaned at 32.5°C showed lower body mass and carcass mass than those weaned at 21°C (Table S2). Exposure to hot temperature during lactation significantly decreased body mass and carcass mass (Table S2). The F1 females previously weaned at 32.5°C had significantly lighter livers, hearts and kidneys than those weaned at 21°C (Table S2). Exposure to hot temperature during lactation significantly decreased the masses of visceral organs, including liver, heart, lung, spleen and kidneys, as well as the digestive tract (Table S2). In addition, the masses of mammary glands and fur of the F1 females weaned at 32.5°C were similar to that of the females weaned at 21°C (Table 2; Table S2). However, F1 females lactating at 32.5°C had significantly lower masses of mammary glands and fur than those lactating at 21°C.

Correlations between asymptotic food intake, energy output and body composition

Almost all the correlation data were mixed together between the two groups that were lactating at 21°C, as well as between the

Journal of Experimental Biology (2020) 223, jeb223560. doi:10.1242/jeb.223560

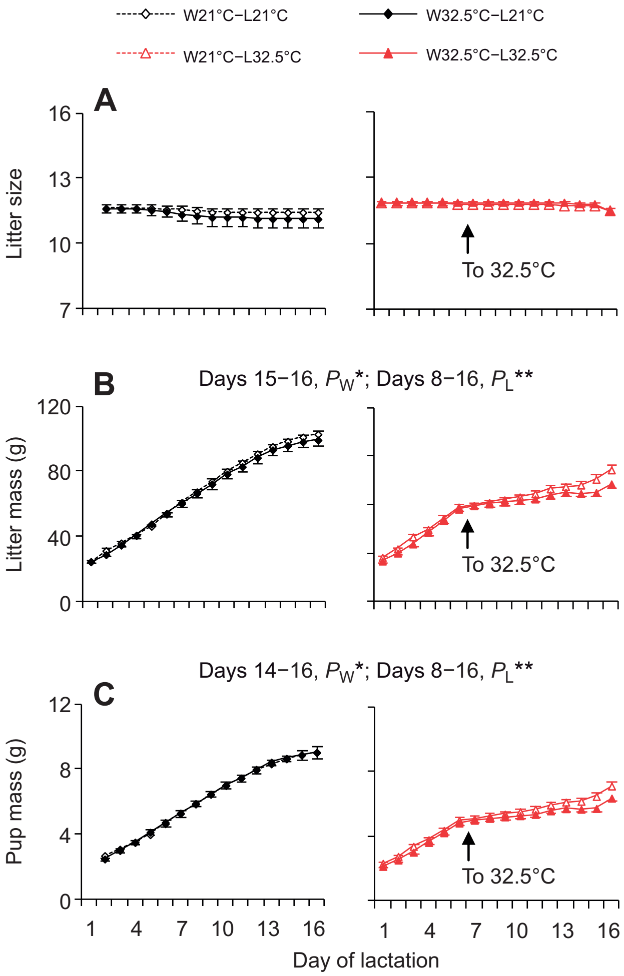

Fig. 6. Litter size, litter mass and pup mass during lactation in F1 offspring. Litter size (A), litter mass (B) and pup mass (C) at 21°C and 32.5°C during lactation in F1 Swiss mice. F1 weaned 21°C: two treatments, lactated at 21°C (W21°C–L21°C, *n*=16) and lactated at 32.5°C (W21°C–L32.5°C, *n*=14); F1 weaned 32.5°C: two treatments, lactated at 21°C (W32.5°C–L21°C, *n*=15) and lactated at 32.5°C (W32.5°C–L32.5°C, *n*=15). Data are means±s.e.m.; *P*-values indicate that all of the treatments were assessed together. P_W, effect of hot temperature on F1 pups weaned at 32.5°C; P_L, effect of hot temperature on F1 adult females lactating at 32.5°C. **P*<0.05; ***P*<0.01.

two groups that were exposed to 32.5°C (Fig. S1). The correlation data in the two groups lactating at 32.5°C were considerably different from the two groups lactating at 21°C. For example, the correlation data between body composition and energy intake, including AFI, GEI and DEI, in the females lactating at 32.5°C were notably shifted downward compared with that in the females lactating at 21°C. The correlation data between body composition and energy output, indicated by litter mass and MEO, were also lower in the groups exposed to 32.5°C (Fig. S1). Consistently, the correlation data of both energy intake and output and digestive tracts in the groups lactating at 21°C were located on the upper right and that of the groups exposed to 32.5°C was shifted to the lower left (Fig. S2). These findings indicate that considerable effects of hot temperature exposure were observed on energy budgets and body composition, i.e. the groups exposed to 32.5°C consumed less food, produced less milk and had lower body mass, compared with their counterparts lactating at 21°C.

DISCUSSION

Reproduction is the period of highest energy demand for small mammals, during which females must increase food intake considerably to meet the energy requirements of raising their offspring (Kenagy et al., 1989, 1990; Hammond and Diamond, 1994; Koteja, 1996a,b; Hammond and Diamond, 1997; Rogowitz, 1998; Johnson et al., 2001; Valencak et al., 2010, 2013; Sadowska et al., 2016; Kagya-Agyemang et al., 2018; Ohrnberger et al., 2018). In the present study, litter size of both 21 and 32.5°C groups did not change throughout lactation, and did not differ between the two temperatures. The milk intake of the pups that were raised by the mothers at 21°C increased considerably throughout lactation, whereas it decreased significantly following exposure to the hot conditions. This suggested that milk energy output was limited in the females lactating at the hot temperature potentially by their capacity to dissipate heat. Consistent with these data, it has been widely reported previously that exposure to high ambient temperature resulted in a considerable reduction of milk output in small rodents (Morag et al., 1969; Leon and Woodside, 1983; Jansen and Binard, 1991; Król and Speakman, 2003a,b; Wu et al., 2009; Simons et al., 2011; Yang et al., 2013; Wen et al., 2017) and large mammals (Cobble and Herman, 1951; Abdalla et al., 1993; Renaudeau et al., 2003). It has been previously observed that in the same strain of mouse the pup growth in the females raising small litters at hotter temperatures was similar to that in cooler conditions (Wen et al., 2017). We exposed the pups only to a hotter condition and observed that they showed consistent growth rates with that of pups housed at cooler conditions (Zhao et al., 2016). This indicates that the capacity of pup growth rate was probably unchanged at hot temperatures compared with that at room temperature (Zhao et al., 2016; Wen et al., 2017).

In the present study, milk output of the mothers was assessed from the energy budget of litters. As mentioned above, it is calculated as the sum of energy allocated to the growth of new tissue and DEE of the pups; and DEE is estimated by 1.4×RMR (Król and Speakman, 2003b). Based on personal observation, RMR of pups is lower at 32.5°C than 21°C; in addition, the general activity of pups is a little lower at 32.5°C, making the correction factor lower at 32.5°C than that at 21°C. Therefore, the actual milk output at 32.5°C is probably a little lower than that estimated from the equation used in this study. The fact that pup body mass weaned at 32.5°C was significantly lower than that at 21°C might also indicate that the mothers at hot temperatures were not capable of producing enough milk to meet offspring energy requirements. It has been also observed in other studies that litter growth rate declined as a direct result of high ambient temperature on milk production of mothers (Król and Speakman, 2003a,b; Wen et al., 2017).

In the present study, F1 females and males that previously weaned at 32.5°C consumed significantly less food and had lower body mass than their counterparts weaned at 21°C. This is inconsistent with some animals subjected to food shortage, where body mass usually decreases as a result of food shortage, and increases considerably when the food shortage ends, i.e. compensatory growth of body mass (Baker, 1955; Brownlow et al., 1993; Rozen et al., 1994; Zhao and Cao, 2009b; Zhao et al., 2014b; Kliewer et al., 2015). Compensatory growth is also observed in the same strain of mouse, being lighter by about 40% under food restriction, and undergoing subsequent catch-up growth when food restriction ends (Zhao et al., 2009). Here, F1 females and males weaned at 32.5°C showed significantly lower body mass after weaning (days 17 to 56 of age), and did not show catch-up growth relative to their counterparts weaned at 21°C. This indicates that the

Journal of Experimental Biology (2020) 223, jeb223560. doi:10.1242/jeb.223560

Table 2. Mass of digestive tracts and mammary glands in the F1 adult females

	W21°C–L21°C	W21°C–L32.5°C	W32.5°C–L21°C	W32.5°C–L32.5°C	P_W	P_L
	n=16	*n*=14	*n*=15	*n*=15		
Stomach (g)	0.411±0.021	0.339±0.016	0.386±0.021	0.277±0.014	*	**
Small intestine (g)	1.171±0.151	0.880±0.088	1.637±0.233	0.849±0.093	n.s.	**
Large intestine (g)	0.367±0.024	0.260±0.010	0.341±0.013	0.254±0.018	n.s.	**
Caecum (g)	0.168±0.015	0.128±0.011	0.180±0.015	0.112±0.008	n.s.	**
Mammary gland (g)	6.138±0.431	2.669±0.241	5.474±0.503	2.839±0.258	n.s.	**

F1 weaned 21°C: two treatments, lactated at 21°C (W21°C–L21°C) and lactated at 32.5°C (W21°C–L32.5°C); F1 weaned 32.5°C: two treatments, lactated at 21°C (W32.5°C–L21°C) and lactated at 32.5°C (W32.5°C–L32.5°C). Data are means±s.e.m.; *P*-values indicate that all of the treatments were assessed together. P_W, effect of hot temperature on F1 pups weaned at 32.5°C; P_L, effect of hot temperature on F1 adult females lactating at 32.5°C. *P<0.05; **P<0.01.; n.s., not significant.

short exposure to hot conditions for mothers during lactation had profound effects on F1 growth rate capacity.

It is known that some individuals would have a fitness disadvantage if they were weaned smaller and were not able to undergo subsequent catch-up growth (Speakman and Król, 2005a, 2011; Metges et al., 2015). Here we observed that there was significantly lower mass of the carcass, brain, liver, lung, spleen and kidneys in F1 female adults that were previously weaned at 32.5°C, and lower mass of carcass, liver, heart and kidneys in F1 male adults. Importantly, testis, epididymis and seminal vesicle mass of F1 males previously weaned at 32.5°C were lower by 9.2, 38.8 and 30.6% in mass, respectively, than those of F1 males weaned at 21°C. These findings suggest that the negative impact of hot temperature during lactation was not only observed on the capacity of milk production of female mice and therefore F1 pup growth during lactation, but also had effects on offspring growth after they were weaned. However, the organs involved in reproduction (Table 1) did not significantly differ in F1 adult females weaned at 32.5°C from those weaned at 21°C, suggesting that the reproductive organs may have 'caught up' growth. Moreover, the organs most involved in absorbing dietary nutrients and the organs involved in producing milk (Table 2) were not different significantly in lactating F1 weaned at 32.5°C from those weaned at 21°C, indicating that these organs may be recovered from weaning at the hot temperature (32.5°C). Even so, the F1 weaned at 32.5°C were unable to perform better at high temperature than those weaned at 21°C, which might be the consequence of the other organs (liver, spleen, kidneys) that never recovered from the weaning treatment.

In the present study, the smaller F1 offspring previously weaned at 32.5°C did not undergo catch-up growth, and consequently developed smaller adult body size. It is known that smaller offspring would be less competitive for natural resources such as territory, food and mates (Wolff and Sherman, 2007; Speakman and Król, 2011). However, there may be compensatory effects. For example, the F1 female adults would have greater surface-to-volume ratios because of smaller body size, and consequently would have less heat dissipation difficulties than female adults with larger body size. In the present study, food intake, digestive energy intake, milk energy output or litter mass did not differ between F1 female adults that previously weaned at 32.5 and 21°C when they were lactating at 21°C. Consistent with these data, Swiss mice artificially selected for either high or low basal metabolic rate, which had had fur from the dorsal body surface removed to increase their thermal conductance and facilitate heat dissipation, did not benefit from increasing their thermal conductance at peak lactation (Sadowska et al., 2019). This is also consistent with previous data which indicate at 21°C that this strain is probably not limited by the capacity for heat dissipation (Wen et al., 2017). Interestingly, when they were exposed to hot temperature during lactation, F1 female adults weaned at 32.5°C produced significantly less milk than those previously weaned at 21°C. As the energy available to pups is obtained only from their mother's milk, the pups probably had lower milk intake at 32.5°C compared with pups raised at 21°C, resulting in a lower rate of pup growth.

As mentioned above, mice with lower body mass would have a higher surface-to-volume ratio, leading to a relaxed heat dissipation limitation, which may allow mothers to eat more food and produce more milk (Speakman and Król, 2005a, 2011). In the present study, F1 female adults previously weaned at 32.5°C had significantly lower body mass, but did not do better in lactation when they were adults than those previously weaned at 21°C. The relationships between AFI, MEO and litter mass did not seem to change significantly in the F1 weaned at 32.5°C compared with those weaned at 21°C, while the correlation locations were changed considerably in the F1 lactating at 32.5°C compared with those lactating at 21°C. The findings of this study therefore do not support the predictions we established *a priori*. Clearly in both lactations there was a strong negative impact of high lactation temperature consistent with the HDL hypothesis. Exposure to hot conditions caused significant decreases in food intake, digestive energy intake and milk energy output, which was consistent with other studies performed in a variety of animals that were lactating at hot temperatures (Król and Speakman, 2003a,b; Renaudeau et al., 2003; Wu et al., 2009; Simons et al., 2011; Yang et al., 2013). However, the impact of high ambient temperature in the second lactation was not ameliorated in those that had previously weaned at high temperatures and were smaller. The reasons for this effect remain unclear.

In contrast with studies that were previously performed in the same strain of mouse (Zhao et al., 2016; Wen et al., 2017), the findings of this study indicate that the limitation on mothers impacted by the HDL at hotter conditions probably has profound effects on organ development and future reproductive performance of F1 females. The data provide support for the hypothesis that F1 offspring weaned at 32.5°C had smaller body size, and the growth after weaning would be stunted, resulting in smaller F1 adults compared with those weaned at 21°C. On the contrary, the findings do not seem consistent with the hypothesis that smaller F1 adult females weaned at 32.5°C would exhibit greater reproductive performance, and therefore have a fitness advantage.

Heat, generated as a by-product of processing food and producing milk, increases considerably in lactating females, making them dangerously hyperthermic (Quiniou and Noblet, 1999; Król and Speakman, 2003a,b). As the difference between body temperature and ambient temperature considerably decreased with increasing ambient temperature, females were more likely to develop chronic hyperthermia (Ulmershakibaei and Plonait, 1992; Speakman and Król, 2005a). It is known that the maximal capacity of the body to dissipate heat is fixed, and females lactating at hot temperatures may have less capacity for heat dissipation (Speakman and Król, 2005a, 2011; Wen et al., 2017). Therefore, heat dissipation limits are likely to be more severe on food intake and milk output in females lactating at hot temperatures, resulting in worse reproductive performance at current lactation in the mothers and also for future lactation in F1 offspring. Finally, these findings suggest that hot temperatures decrease reproductive value at the current stage, stunt offspring growth, and also decrease the future reproductive value of F1 female offspring, providing strong evidence that females previously exposed to hot temperatures would have a significant fitness disadvantage. Moreover, exposure to periods of high temperature (heatwaves) is an important threat, not only to the females themselves, but also to their offspring, indicating a lasting effect over a long period of time.

Acknowledgements
We thank Jing Cao, Wenzhou University, for her assistance with animal care, and Peter Thomson and Marina Stamatiou from the University of Aberdeen for technical assistance with isotope analysis.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Methodology: M.-H.B., L.-B.C., C.H., Z.-J.Z.; Formal analysis: M.-H.B., C.H., J.R.S., Z.-J.Z.; Data curation: M.-H.B., C.H.; Writing - original draft: M.-H.B., J.R.S., Z.-J.Z.; Writing - review & editing: J.R.S., Z.-J.Z.; Supervision: J.R.S., Z.-J.Z.; Funding acquisition: Z.-J.Z., J.R.S.

Funding
This work was partly supported by grants (no. 31670417 and no. 31870388) from the National Natural Science Foundation of China and the Chinese Academy of Sciences Strategic program (XDB13030100).

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.223560.supplemental

References
Abdalla, E. B., Kotby, E. A. and Johnson, H. D. (1993). Physiological-responses to heat-induced hyperthermia of pregnant and lactating ewes. *Small Ruminant Res.* **11**, 125-134. doi:10.1016/0921-4488(93)90145-8

Baker, R. A. (1955). The effects of repeated deprivation experience on feeding behavior. *J. Comp. Physiol. Psychol.* **48**, 37-42. doi:10.1037/h0048051

Black, J. L., Mullan, B. P., Lorschy, M. L. and Giles, L. R. (1993). Lactation in the sow during heat stress. *Livest. Prod. Sci.* **35**, 153-170. doi:10.1016/0301-6226(93)90188-N

Brody, S., Ragsdale, A. C., Yeck, R. G. and Worstell, D. (1958). Environmental physiology and shelter engineering with special reference to domestic animals XXXIII. Milk production, feed and water consumption and body weight of Jersey and Holstein cows in relation to several diurnal temperature rhythms. *Mo. AES Res. Bull* **1958**, 578.

Brownlow, B. S., Park, C. R., Schwartz, R. S. and Woods, S. C. (1993). Effect of meal pattern during food restriction on body weight loss and recovery after refeeding. *Physiol. Behav.* **53**, 421-424. doi:10.1016/0031-9384(93)90133-Z

Cobble, J. W. and Herman, H. A. (1951). The influence of environmental temperature on the composition of milk of the dairy cow. *Mo. AES Res. Bull* **1951**, 485.

Drozdz, A. (1975). Metabolic cages for small mammals. In *Methods for Ecological Bioenergetics, International Biological Programme Handbook No. 24* (ed. W. Grodzinski, R. Z. Klekowski and A. Duncan), pp. 346-351. Oxford: Blackwell Scientific Publications.

Godde, C., Dizyee, K., Ash, A., Thornton, P., Sloat, L., Roura, E., Henderson, B. and Herrero, M. (2019). Climate change and variability impacts on grazing herds: insights from a system dynamics approach for semi-arid Australian rangelands. *Glob. Chang. Biol.* **25**, 3091-3109. doi:10.1111/gcb.14669

Grodzinski, W. and Wunder, B. A. (1975). Ecological energetics of small mammals. In *Small Mammals: Their Productivity and Population Dynamics* (ed. F. B. Golley, K. Petrusewicz and L. Ryszkowski), pp. 173-204. Cambridge: Cambridge Univ. Press.

Hammond, K. and Diamond, J. (1994). Limits to dietary nutrient intake and intestinal nutrient uptake in lactating mice. *Physiol. Zool.* **67**, 282-303. doi:10.1086/physzool.67.1.30163847

Hammond, K. A. and Diamond, J. (1997). Maximal sustained energy budgets in humans and animals. *Nature* **386**, 457-462. doi:10.1038/386457a0

Hoffmann, A. A. and Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature* **470**, 479-485. doi:10.1038/nature09670

IPCC (2014). Summary for policymakers. In *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (ed. C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, et al.), pp. 1-32. Cambridge University Press.

Jansen, G. R. and Binard, R. (1991). Effect of dietary protein and environmental factors on lactation performance in rats. *Physiol. Behav.* **50**, 297-304. doi:10.1016/0031-9384(91)90069-Z

Johnson, M. S., Thomson, S. C. and Speakman, J. R. (2001). Limits to sustained energy intake. I. Lactation in the laboratory mouse *Mus musculus*. *J. Exp. Biol.* **204**, 1925-1935.

Kagya-Agyemang, J. K., Vaanholt, L. M., Hambly, C., Król, E., Mitchell, S. E. and Speakman, J. R. (2018). Limits to sustained energy intake. XXVIII. Beneficial effects of high dietary fat on lactation performance in mice. *J. Exp. Biol.* **221**, jeb180828. doi:10.1242/jeb.180828

Kenagy, G., Stevenson, R. and Masman, D. (1989). Energy requirements for lactation and postnatal growth in captive golden-mantled ground squirrels. *Physiol. Zool.* **62**, 470-487. doi:10.1086/physzool.62.2.30156180

Kenagy, G., Masman, D., Sharbaugh, S. and Nagy, K. (1990). Energy expenditure during lactation in relation to litter size in free-living golden-mantled ground squirrels. *J. Anim. Ecol.* **59**, 73-88. doi:10.2307/5159

Kliewer, K. L., Ke, J. Y., Lee, H. Y., Stout, M. B., Cole, R. M., Samuel, V. T., Shulman, G. I. and Belury, M. A. (2015). Short-term food restriction followed by controlled refeeding promotes gorging behavior, enhances fat deposition, and diminishes insulin sensitivity in mice. *J. Nutr. Biochem.* **26**, 721-728. doi:10.1016/j.jnutbio.2015.01.010

Koteja, P. (1996a). Limits to the energy budget in a rodent, *Peromyscus maniculatus*: does gut capacity set the limit? *Physiol. Zool.* **69**, 994-1020. doi:10.1086/physzool.69.5.30164243

Koteja, P. (1996b). Limits to the energy budget in a rodent, *Peromyscus maniculatus*: the central limitation hypothesis. *Physiol. Zool.* **69**, 981-993. doi:10.1086/physzool.69.5.30164242

Król, E. and Speakman, J. R. (1999). Isotope dilution spaces of mice injected simultaneously with deuterium, tritium and oxygen-18. *J. Exp. Biol.* **202**, 2839-2849.

Król, E. and Speakman, J. R. (2003a). Limits to sustained energy intake VI. Energetics of lactation in laboratory mice at thermoneutrality. *J. Exp. Biol.* **206**, 4255-4266. doi:10.1242/jeb.00674

Król, E. and Speakman, J. R. (2003b). Limits to sustained energy intake VII. Milk energy output in laboratory mice at thermoneutrality. *J. Exp. Biol.* **206**, 4267-4281. doi:10.1242/jeb.00675

Król, E., Murphy, M. and Speakman, J. R. (2007). Limits to sustained energy intake. X. Effects of fur removal on reproductive performance in laboratory mice. *J. Exp. Biol.* **210**, 4233-4243. doi:10.1242/jeb.009779

Leon, M. and Woodside, B. (1983). Energetic limits on reproduction: maternal food intake. *Physiol. Behav.* **30**, 945-957. doi:10.1016/0031-9384(83)90260-3

Lifson, N., Gordon, G. B. and Mcclintock, R. (1955). Measurement of total carbon dioxide production by means of D2O18. *J. Appl. Physiol.* **7**, 704-710. doi:10.1152/jappl.1955.7.6.704

Lovegrove, B. G., Canale, C., Levesque, D., Fluch, G., Reháková-Petrů, M. and Ruf, T. (2014). Are tropical small mammals physiologically vulnerable to Arrhenius effects and climate change? *Physiol. Biochem. Zool.* **87**, 30-45. doi:10.1086/673313

Martin, J. M., Mead, J. I. and Barboza, P. S. (2018). Bison body size and climate change. *Ecol. Evol.* **8**, 4564-4574. doi:10.1002/ece3.4019

Mcdonald, J. M. and Nielsen, M. K. (2006). Correlated responses in maternal performance following divergent selection for heat loss in mice. *J. Anim. Sci.* **84**, 300-304. doi:10.2527/2006.842300x

McDonald, J. M. and Nielsen, M. K. (2007). Renewed selection for heat loss in mice: direct responses and correlated responses in feed intake, body weight, litter size, and conception rate. *J. Anim. Sci.* **85**, 658-666. doi:10.2527/jas.2006-465

Metcalfe, N. B. and Monaghan, P. (2001). Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* **16**, 254-260. doi:10.1016/S0169-5347(01)02124-3

Metcalfe, N. B. and Monaghan, P. (2003). Growth versus lifespan: perspectives from evolutionary ecology. *Exp. Gerontol.* **38**, 935-940. doi:10.1016/S0531-5565(03)00159-1

Metges, C. C., Görs, S., Martens, K., Krueger, R., Metzler-Zebeli, B. U., Nebendahl, C., Otten, W., Kanitz, E., Zeyner, A., Hammon, H. M. et al. (2015). Body composition and plasma lipid and stress hormone levels during 3 weeks of feed restriction and refeeding in low birth weight female pigs. *J. Anim. Sci.* **93**, 999-1014. doi:10.2527/jas.2014-8616

Mifsud, K. R., Gutièrrez-Mecinas, M., Trollope, A. F., Collins, A., Saunderson, E. A. and Reul, J. M. H. M. (2011). Epigenetic mechanisms in stress and adaptation. *Brain Behav. Immun.* **25**, 1305-1315. doi:10.1016/j.bbi.2011.06.005

Morag, M., Kali, J. and Furman, M. (1969). The effect of a desert climate on the lactation and on the fertility of the nursing rat. *Int. J. Biometeorol.* **13**, 61-68. doi:10.1007/BF02329579
Ohrnberger, S. A., Hambly, C., Speakman, J. R. and Valencak, T. G. (2018). Limits to sustained energy intake. XXIX. The case of the golden hamster (*Mesocricetus auratus*). *J. Exp. Biol.* **221**, jeb183749. doi:10.1242/jeb.183749
Quiniou, N. and Noblet, J. (1999). Influence of high ambient temperatures on performance of multiparous lactating sows. *J. Anim. Sci.* **77**, 2124-2134. doi:10.2527/1999.7782124x
Radchuk, V., Reed, T., Teplitsky, C., Van De Pol, M., Charmantier, A., Hassall, C., Adamík, P., Adriaensen, F., Ahola, M. P., Arcese, P. et al. (2019). Adaptive responses of animals to climate change are most likely insufficient. *Nat. Commun.* **10**, 3109. doi:10.1038/s41467-019-10924-4
Rauw, W. M., Luiting, P., Beilharz, R. G., Verstegen, M. W. A. and Vangen, O. (1999). Selection for litter size and its consequences for the allocation of feed resources: a concept and its implications illustrated by mice selection experiments. *Livest. Prod. Sci.* **60**, 329-342. doi:10.1016/S0301-6226(99)00104-9
Renaudeau, D. and Noblet, J. (2001). Effects of exposure to high ambient temperature and dietary protein level on sow milk production and performance of piglets. *J. Anim. Sci.* **79**, 1540-1548. doi:10.2527/2001.7961540x
Renaudeau, D., Noblet, J. and Dourmad, J. Y. (2003). Effect of ambient temperature on mammary gland metabolism in lactating sows. *J. Anim. Sci.* **81**, 217-231. doi:10.2527/2003.811217x
Rogowitz, G. L. (1998). Limits to milk flow and energy allocation during lactation of the hispid cotton rat (*Sigmodon hispidus*). *Physiol. Biochem. Zool.* **71**, 312-320. doi: 10.1086/515923
Rozen, R., Brigant, L. and Apfelbaum, M. (1994). Effects of cycles of food restriction followed by ad libitum refeeding on body composition and energy expenditure in obsess rats. *Am. J. Clin. Nutr.* **59**, 560-565. doi:10.1093/ajcn/59.3.560
Sadowska, E. T., Król, E., Chrzascik, K. M., Rudolf, A. M., Speakman, J. R. and Koteja, P. (2016). Limits to sustained energy intake. XXIII. Does heat dissipation capacity limit the energy budget of lactating bank voles? *J. Exp. Biol.* **219**, 805-815. doi:10.1242/jeb.134437
Sadowska, J., Gębczyński, A. K., Lewoc, M. and Konarzewski, M. (2019). Not that hot after all: no limits to heat dissipation in lactating mice selected for high or low BMR. *J. Exp. Biol.* **222**, jeb204669. doi:10.1242/jeb.204669
Simons, M. J., Reimert, I., van der Vinne, V., Hambly, C., Vaanholt, L. M., Speakman, J. R. and Gerkema, M. P. (2011). Ambient temperature shapes reproductive output during pregnancy and lactation in the common vole (*Microtus arvalis*): a test of the heat dissipation limit theory. *J. Exp. Biol.* **214**, 38-49. doi:10.1242/jeb.044230
Smith, F. A., Murray, I. W., Harding, L. E., Lease, H. M. and Martin, J. (2014). Life in an extreme environment: a historical perspective on the influence of temperature on the ecology and evolution of woodrats. *J. Mamm.* **95**, 1128-1143. doi:10.1644/13-MAMM-S-070
Speakman, J. R. (1993). How should we calculate CO2 production in doubly labeled water studies of animals. *Funct. Ecol.* **7**, 746-750.
Speakman, J. R. (1998). The history and theory of the doubly labeled water technique. *Am. J. Clin. Nutr.* **68**, 932S-938S. doi:10.1093/ajcn/68.4.932S
Speakman, J. R. and Król, E. (2005a). Limits to sustained energy intake IX: a review of hypotheses. *J. Comp. Physiol. B* **175**, 375-394. doi:10.1007/s00360-005-0013-3
Speakman, J. R. and Król, E. (2005b). Comparison of different approaches for the calculation of energy expenditure using doubly labeled water in a small mammal. *Physiol. Biochem. Zool.* **78**, 650-667. doi:10.1086/430234
Speakman, J. R. and Król, E. (2011). Limits to sustained energy intake. XIII. Recent progress and future perspectives. *J. Exp. Biol.* **214**, 230-241. doi:10.1242/jeb.048603
Speakman, J. R., Nagy, K. A., Masman, D., Mook, W. G., Poppitt, S. D., Strathearn, G. E. and Racey, P. A. (1990). Interlaboratory comparison of different analytical techniques for the determination of O-18 abundance. *Anal. Chem.* **62**, 703-708. doi:10.1021/ac00206a011
Speakman, J. R. and Racey, P. A. (1988b). Consequences of non steady-state CO2 production for accuracy of the doubly labeled water technique – the importance of recapture interval. *Comp. Biochem. Physiol.* **90A**, 337-340. doi:10.1016/0300-9629(88)91125-5
Stawski, C. and Geiser, F. (2012). Will temperature effects or phenotypic plasticity determine the thermal response of a heterothermic tropical bat to climate change? *PLoS ONE* **7**, e40278. doi:10.1371/journal.pone.0040278
Ulmershakibaei, C. and Plonait, H. (1992). Studies of lactational hyperthermia in sows. *Tierarztliche Umschau* **47**, 605-611.
Valencak, T. G., Hacklander, K. and Ruf, T. (2010). Peak energy turnover in lactating European hares: a test of the heat dissipation limitation hypothesis. *J. Exp. Biol.* **213**, 2832-2839. doi:10.1242/jeb.040238
Valencak, T. G., Wright, P., Weir, A., Mitchell, S. E., Vaanholt, L. M., Hambly, C., Król, E. and Speakman, J. R. (2013). Limits to sustained energy intake. XXI. Effect of exposing the mother, but not her pups, to a cold environment during lactation in mice. *J. Exp. Biol.* **216**, 4326-4333. doi:10.1242/jeb.092023
Vaanholt, L. M., Sinclair, R. E. and Speakman, J. R. (2013). Limits to sustained energy intake. XIV. Heritability of reproductive performance in mice. *J. Exp. Biol.* **216**, 2308-2315. doi:10.1242/jeb.078394
Visser, G. H., Dekinga, A., Achterkamp, B. and Piersma, T. (2000a). Ingested water equilibrates isotopically with the body water pool of a shorebird with unrivaled water fluxes. *Am. J. Physiol.* **279**, C1795-C1804. doi:10.1152/ajpcell.2000.279.6.C1795
Wen, J., Tan, S., Qiao, Q.-G., Fan, W.-J., Huang, Y.-X., Cao, J., Liu, J.-S., Wang, Z.-X. and Zhao, Z.-J. (2017). Sustained energy intake in lactating Swiss mice: a dual modulation process. *J. Exp. Biol.* **220**, 2277-2286. doi:10.1242/jeb.157107
Wen, J., Tan, S., Qiao, Q., Shi, L., Huang, Y. and Zhao, Z. (2018b). The strategies of behavior, energetic and thermogenesis of striped hamsters in response to food deprivation. *Integr. Zool.* **13**, 70-83. doi:10.1111/1749-4877.12259
Wen, J., Tan, S., Wang, D. H. and Zhao, Z. J. (2018a). Variation of food availability affects male striped hamsters (*Cricetulus barabensis*) with different levels of metabolic rate. *Integr. Zool.* **13**, 769-782. doi:10.1111/1749-4877.12337
Wolff, J. O. and Sherman, P. W. (2007). *Rodent Societies: an Ecological and Evolutionary Perspective*. Chicago, IL: The University of Chicago Press.
Wu, S. H., Zhang, L. N., Speakman, J. R. and Wang, D. H. (2009). Limits to sustained energy intake. XI. A test of the heat dissipation limitation hypothesis in lactating Brandt's voles (*Lasiopodomys brandtii*). *J. Exp. Biol.* **212**, 3455-3465. doi:10.1242/jeb.030338
Yang, D.-B., Li, L., Wang, L.-P., Chi, Q.-S., Hambly, C., Wang, D.-H. and Speakman, J. R. (2013). Limits to sustained energy intake. XIX. A test of the heat dissipation limitation hypothesis in Mongolian gerbils (*Meriones unguiculatus*). *J. Exp. 698 Biol.* **216**, 3358-3368. doi:10.1242/jeb.085233
Zhao, Z.-J. (2011). Energy budget during lactation in striped hamsters at different ambient temperatures. *J. Exp. Biol.* **214**, 988-995. doi:10.1242/jeb.049395
Zhao, Z.-J. and Cao, J. (2009a). Effect of fur removal on the thermal conductance and energy budget in lactating Swiss mice. *J. Exp. Biol.* **212**, 2541-2549. doi:10.1242/jeb.029603
Zhao, Z.-J. and Cao, J. (2009b). Plasticity in energy budget and behavior in Swiss mice and striped hamsters under stochastic food deprivation and refeeding. *Comp. Biochem. Physiol.* **154A**, 84-91. doi:10.1016/j.cbpa.2009.05.004
Zhao, Z.-J., Cao, J. and Chi, Q.-S. (2010). Milk energy output during peak lactation in shaved Swiss mice. *Physiol. Behav.* **101**, 59-66. doi:10.1016/j.physbeh.2010.04.017
Zhao, Z.-J., Chen, K.-X., Liu, Y.-A., Wang, C.-M. and Cao, J. (2014b). Decreased circulating leptin and increased neuropeptide Y gene expression are implicated in food deprivation-induced hyperactivity in striped hamsters, *Cricetulus barabensis*. *Horm. Behav.* **65**, 355-362. doi:10.1016/j.yhbeh.2014.03.001
Zhao, Z. J., Chi, Q. S., Liu, Q. S., Zheng, W. H., Liu, J. S. and Wang, D.-H. (2014a). The shift of thermoneutral zone in striped hamster acclimated to different temperatures. *PLoS ONE* **9**, e84396. doi:10.1371/journal.pone.0084396
Zhao, Z.-J., Li, L., Yang, D.-B., Chi, Q.-S., Hambly, C. and Speakman, J. R. (2016). Limits to sustained energy intake XXV: milk energy output and thermogenesis in Swiss mice lactating at thermoneutrality. *Sci. Rep.* **6**, 31626. doi:10.1038/srep31626
Zhao, Z.-J., Song, D.-G., Su, Z.-C., Wei, W.-B., Liu, X.-B. and Speakman, J. R. (2013). Limits to sustained energy intake. XVIII. Energy intake and reproductive output during lactation in Swiss mice raising small litters. *J. Exp. Biol.* **216**, 2349-2358. doi:10.1242/jeb.078436
Zhao, Z. J., Wang, R.-R., Cao, J. and Pei, L. Y. (2009). Effect of random food deprivation and refeeding on energy budget and development in mice. *Zool. Res.* **30**, 534-538. doi:10.3724/SP.J.1141.2009.05534