

## RESEARCH ARTICLE

# Limits to sustained energy intake. XV. Effects of wheel running on the energy budget during lactation

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### SUMMARY

The capacity of animals to dissipate heat may constrain sustained energy intake during lactation. We examined these constraints at peak lactation in MF1 mice that had *ad libitum* access to food, or that had to run a pre-set target on running wheels to obtain *ad libitum* access to food. The voluntary distance run decreased sharply during pregnancy and peak lactation. When lactating females were provided with 80% of their estimated food requirements, and had to run pre-set distances of 2, 4 or 6 km before given access to additional *ad libitum* food, most of them did not complete the running target during late lactation and the mice with the highest targets failed to reach their targets earlier in lactation. There were consequently significant group differences in asymptotic food intake (2 km,  $16.97 \pm 0.40 \text{ g day}^{-1}$ ; 4 km,  $14.29 \pm 0.72 \text{ g day}^{-1}$ ; and 6 km,  $12.65 \pm 0.45 \text{ g day}^{-1}$ ) and weaned litter masses (2 km,  $71.11 \pm 2.39 \text{ g}$ ; 4 km,  $54.63 \pm 4.28 \text{ g}$  and 6 km,  $47.18 \pm 2.46 \text{ g}$ ). When the females did run sufficiently to gain *ad libitum* food access, their intake did not differ between the different distance groups or from controls that were not required to run. Thus, despite being physically capable of running the distances, mice could not exercise sufficiently in lactation to gain regular *ad libitum* access to food, probably because of the risks of hyperthermia when combining heat production from exercise with thermogenesis from lactation.

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### INTRODUCTION

The maximal rates of energy metabolism that animals can sustain for protracted periods provide an upper limit constraining many aspects of animal performance (Drent and Daan, 1980; Hammond and Diamond, 1997; Piersma and van Gils, 2011; Ruf and Graf, 2010; Speakman, 2000; Speakman, 2008; Thompson, 1992; Weiner, 1992). Maximal rates of energy intake and expenditure are probably limited by aspects of an animal's physiology (Hammond and Diamond, 1997; Kirkwood, 1983; Koteja, 1996; Perrigo, 1987; Speakman et al., 2004; Speakman and Król, 2005; Speakman and Król, 2010a). Late lactation is the most energetically demanding period during the life cycle of altricial mammals (Gittleman and Thompson, 1988; Millar, 1977; Thompson, 1992) and has been increasingly used as a model for testing potential factors that limit sustained energy intake and expenditure (Hammond and Diamond, 1992; Hammond et al., 1994; Hammond et al., 1996; Hammond and Diamond, 1997; Johnson and Speakman, 2001; Johnson et al., 2001a; Johnson et al., 2001b; Johnson et al., 2001c; Król and Speakman, 2003a; Król and Speakman, 2003b; Król et al., 2003; Król et al., 2007; Piersma and van Gils 2011; Speakman and McQueenie, 1996; Speakman et al., 2001; Valencak and Ruf, 2009; Valencak et al., 2010; Wu et al., 2009; Zhang and Wang, 2007; Zhao and Cao, 2009a; Zhao et al., 2010). Sustained energy intake (SusEI) during late lactation has been previously suggested to be limited centrally by the energy-

supplying machinery, i.e. the alimentary tract and associated organs, which has been called the 'central limitation hypothesis' (Denis et al., 2003; Drent and Daan, 1980; Drummond et al., 2000; Hackländer et al., 2002; Hammond and Diamond, 1992; Hammond and Diamond, 1994; Hammond et al., 1996; Hammond and Diamond, 1997; Johnson et al., 2001a; Johnson et al., 2001c; Kirkwood, 1983; Knight et al., 1986; Koteja, 1996; Meyer et al., 1985; Perrigo, 1987; Rogowitz, 1998; Schubert et al., 2009; Speakman et al., 2001; Hammond and Diamond, 1997; Kirkwood, 1983; Weiner, 1992). This idea was consistent with observations that mice given additional tasks to perform during peak lactation, such as running for their food (Perrigo, 1987; Schubert et al., 2009), being simultaneously pregnant when lactating (Johnson et al., 2001c) and being given extra pups to raise (Hammond et al., 1996; Johnson et al., 2001a; Knight et al., 1986; Zhao and Cao, 2009a) did not elevate their food intake or milk production above the levels observed in unmanipulated females: perhaps reflecting the central processing capacity of the alimentary tract. Failure to increase milk production when given more pups to raise has also been reported in laboratory rats (Denis et al., 2003), cotton rats (*Sigmodon hispidus*) (Rogowitz, 1998), rabbits (*Oryctolagus cuniculus*) (Drummond et al., 2000) and dogs (*Canis familiaris*) (Meyer et al., 1985). Moreover, manipulations of the energy density of the food were also consistent with a central processing limit in the alimentary tract (Hackländer et al., 2002; Speakman et al., 2001).

The notion of a central processing limit, however, has been disproved by repeated observations that when lactating rodents are exposed to the cold they are able to elevate their food intake above levels that at room temperature appeared to be limiting on performance (Hammond et al., 1994; Hammond and Kristan, 2000; Johnson and Speakman, 2001; Rogowitz, 1998; Zhang and Wang, 2007). It was consequently suggested that the sustained energy intake may rather reflect the summed requirements of the energy-consuming machinery, each of which may be working to capacity in different circumstances. Hence, in lactation the mammary glands may be working at capacity. Manipulations, such as increasing the number of pups, therefore did not result in elevated food intake because the female could not translate this extra intake into more milk. However, when lactating females were exposed to cold they did increase their intake because they could utilise the extra ingested energy to facilitate thermoregulation. This idea was called the 'peripheral limitation hypothesis'. Hammond and colleagues (Hammond et al., 1996) provided strong experimental support for this hypothesis by surgically reducing the number of mammary glands in lactating mice, and demonstrating that the residual tissue was unable to compensate by increasing milk production, confirming the mammary glands were indeed working at capacity. This hypothesis was further confirmed by observations that in the cold some lactating rodents did not significantly elevate their milk production (Rogowitz, 1998; Zhao and Cao, 2009a; Zhao et al., 2010; Zhao, 2011).

Other data, however, are inconsistent with the peripheral limitation hypothesis. For example, it is not clear why rodents that have to run to obtain food did not increase their intake in late lactation above those of non-runners, but rather culled their offspring to reduce demand, or postponed weaning their pups (Perrigo, 1987; Schubert et al., 2009). In addition it was shown that MF1 mice did elevate their milk production when temperatures were reduced (Król and Speakman, 2003a; Król and Speakman, 2003b; Speakman and Król, 2005), even though they would not elevate their milk production when given additional pups to raise (Johnson et al., 2001a). To explain these anomalous data it was suggested that the key limit on performance is the capacity of an animal to dissipate body heat (Król and Speakman, 2003a; Speakman and Król, 2010a; Speakman and Król, 2010b). Hence, manipulations made at a fixed ambient temperature (such as forcing animals to run for their food, making them simultaneously pregnant or giving them more pups to raise) did not result in elevated intake because animals were still constrained by their heat dissipation capacity. In contrast, when animals were exposed to the cold, this relaxed the heat dissipation limit, allowing the animals to elevate both their food intake and energy output as milk production (Johnson and Speakman, 2001; Król and Speakman, 2003a; Król and Speakman, 2003b; Speakman and Król, 2005). Król and colleagues (Król et al., 2007) showed that removing the dorsal fur from MF1 female mice during lactation significantly increased food intake beyond that observed in normal lactation, allowing the animals to generate more milk and raise heavier litters, strongly supporting the heat dissipation limit (HDL) theory (Król et al., 2007). However, levels of milk production in cotton rats (Rogowitz, 1998), Swiss mice (Zhao et al., 2010) and striped hamsters (Zhao, 2011) exposed to the cold are not elevated, consistent with the mammary glands working at maximal capacity. Moreover, removal of the dorsal fur increased food intake at peak lactation in Swiss mice (Zhao and Cao, 2009a) and in Siberian hamsters (Paul et al., 2010) but had no significant effect on the weaned litter masses. These data are not consistent with the HDL theory but rather support the peripheral limitation hypothesis [but

see Speakman and Król (Speakman and Król, 2011) for alternative explanations of the Paul et al. (Paul et al., 2010) results]. The current data are consequently inconsistent.

One data set that does not support the peripheral limitation hypothesis, but is consistent with the HDL theory, is the observation that mice forced to run for their food do not elevate their intake to accommodate the extra demands of running, but rather scale back their demands by reducing the number of pups they are raising, or slowing their growth and extending lactation (Perrigo, 1987; Schubert et al., 2009). One potential reason why this result does not support the peripheral limitation hypothesis might be the experimental design. In these experiments mice must run a certain distance on a running wheel to obtain small pellets of food. There is consequently a fixed relationship between the amount of running an animal does, and the amount of food it can obtain. It is not possible in this design for the mouse to elevate its intake without performing more running. This link between intake and expenditure may be a realistic reflection of natural foraging behaviour (Schubert et al., 2009), but may make it impossible for the mice to elevate their intake sufficiently to cover both lactation and running demands because they reach a peripheral limit in their muscle capacity to perform exercise.

To explore this phenomenon further, we designed a protocol that dissociates the tight linkage between how much running is performed and how much food is obtained. In detail, we gave lactating mice 80% of their estimated total daily energy requirements and then required them to run a fixed distance that was lower than the distance they could run when not lactating (hence below any peripheral limit in their running capacity) to gain access to *ad libitum* food supplies. In this design, therefore, we broke the tight linkage between exercise level and intake that has characterised the previous experiments in this area (Perrigo, 1987; Schubert et al., 2009). In this situation we predicted that if the peripheral limitation hypothesis was correct, the mice would run to the fixed criterion level to obtain *ad libitum* food supplies, and then they would eat whatever food was necessary to cover both the costs of lactation and the costs of running. In contrast, if the heat dissipation limit theory was correct (and mice do not modulate their thermal conductance when forced to run), the mice running during lactation would have significant problems reaching the criterion distances to obtain *ad libitum* food because of the additional heat burden of running combined with lactation (assuming thermal conductance of the pelage is not modulated). Hence, we predicted the mice would often fail to obtain *ad libitum* food, and even when they did so, they would still face a heat dissipation limit and might be unable to ingest more food than that observed in mice that were not forced to exercise for food. Consequently, they would be obliged to reduce their milk production and raise lighter litters (comprising smaller individual offspring or smaller litter sizes). One assumption we made when deriving the predictions of the different hypotheses was that the thermal conductance of the fur was not altered in animals that had to run for their food. In the present study we also tested the effect of running on the thermal conductance of the fur.

## MATERIALS AND METHODS

We performed four different experimental studies. In the first experiment we observed the pattern of activity in reproducing mice given access to running wheels, but not required to run to obtain access to food. This experiment allowed us to determine the levels of activity that the mice could sustain and hence the criterion distances to obtain access to food during lactation. In the second experiment we gave mice running wheels but this time during lactation we required them

to run a fixed distance (either 2, 4 or 6 km each day) to obtain *ad libitum* access to food above 80% of their requirements, which we provided whether they ran or not. One assumption we made when deriving the predictions of the different hypotheses was that the thermal conductance of the fur was not altered in animals that had to run for their food. The third experiment was designed to directly test this assumption by investigating the effect of wheel-running on conductance of the fur. Finally, the effects of the running behaviour on the food intake of the mice might come about not because of limits reflecting heat dissipation but because mice that have to run for a substantial period each day are unable to do anything else, like feeding, or suckling their pups, during the time that they are running (about 3 h per day). To investigate this possibility, we performed a fourth experiment where we manipulated different groups of lactating female mice by preventing them from (a) feeding, (b) suckling, (c) feeding and suckling and (d) feeding, suckling and sleeping, for 3 h each day (six half-hour periods). In these groups, we compared the asymptotic food intake at peak lactation, and growth of the offspring, with that of unmanipulated mice.

### Animals

Virgin female mice (outbred MF1), 8–10 weeks of age, were housed individually in plastic cages with clean sawdust and paper bedding and maintained at  $21 \pm 1^\circ\text{C}$  on a 12 h:12 h light:dark cycle (lights on at 07:00 h). In experiment 1, rodent chow (CRM, Pelleted Rat and Mouse Breeder and Grower Diet, Special Diets Services, BP Nutrition, Witham, Essex, UK) and water were available *ad libitum*. In experiments 2, 3 and 4, food ( $16.17 \text{ kJ g}^{-1}$ , 67.3% carbohydrate, 19.2% protein, 4.3% fat; produced by Research Diets, Inc., New Brunswick, NJ, USA: open source diet code D12450B) and water were available in relation to running performance (experiment 2) or *ad libitum* (other experiments).

### Experiment 1

To evaluate changes in wheel-running activity of female mice during the different stages of reproduction, 15 females were placed separately in cages with access to a running wheel that was linked to a computer system, which monitored the number of wheel revolutions and the times at which these occurred in the daily cycle. In detail, a wheel (15 cm diameter and 10 cm width) was fixed using an axle on the wall inside the cage. The lower side of the wheel was 1 cm above the bottom of the cage, giving females easy access to the wheel. The wheel carried a small magnet that operated a counting device. All the wheel revolution counters were linked into a computer that monitored the wheel-running activity on a minute by minute basis (number of wheel revolutions). This computer also operated the hoppers based on the number of recorded revolutions. After 1 week of baseline measurements, a male was introduced to the cage for mating and was removed 11 days later. This period is normally sufficient to ensure the female becomes pregnant (Król and Speakman, 2003). After parturition (day 0 of lactation), litter size and mass were measured first on day 3 of lactation and then daily until weaning on day 21 of lactation. In our previous work on this strain, we have normally weaned mice at age 18 or 19 days. Because we were unsure how they would respond to having a wheel in the cage and one potential response would be to extend lactation, we used 21 days as the weaning date in experiment 1. Based on the data from experiment 1, it was clear the mice did not extend lactation and hence in the following experiments we reverted to a weaning date of day 19. The females were then monitored for an additional 8 days after lactation. Over the baseline, mating, pregnant, lactating and post-lactating periods, wheel-running behaviour was monitored

at 1 min intervals, and summary data on activity time (minutes containing wheel activity) and distance run were summarised each day (09:00 h to 09:00 h).

### Experiment 2

To test the effects of wheel running on maternal body mass, food intake, litter mass and litter size in lactating mice, 57 females were transferred into cages with monitored running wheels. Body mass, food intake and wheel-running activity were measured for 3 days. Then the females were provided with 80% of their individual *ad libitum* food intake. The running wheels were connected to a computer-controlled food dispenser. When the mice reached a target distance the computer would open the food dispenser, giving the animals *ad libitum* access to additional food. The target distance that the mice had to run to obtain the food was  $3.5 \text{ km day}^{-1}$  (equal  $7500 \text{ revolutions day}^{-1}$ ) for the first 2 days,  $5.2 \text{ km day}^{-1}$  ( $11,000 \text{ revolutions day}^{-1}$ ) for the next 2 days and  $7.1 \text{ km day}^{-1}$  ( $15,000 \text{ revolutions day}^{-1}$ ) for the next 2 days. From experiment 1, we knew that non-reproductive mice ran on average  $7.25 \text{ km day}^{-1}$  so the criteria distances to obtain access to *ad libitum* food were within their potential performance limits. We defined this period as the baseline training stage. After this period, 57 males were transferred into the cages to mate with the females and were removed after 11 days during which both females and males had free access to food and water and the running wheel. After the males were removed, females were given 80% of their estimated food requirements with a target distance to run to obtain additional *ad libitum* food of  $0.4 \text{ km day}^{-1}$  until the day of parturition. This very low target was designed to be easily achievable by pregnant mice but kept the mice aware of the requirement to run to obtain *ad libitum* food. The measurements in this stage were termed 'pregnancy'. We provided 80% of their required food calculated from a regression equation linking food intake and body mass of pregnant MF1 mice derived from data in the same strain of mouse (Gamo et al., 2013) ( $N=112$ ,  $y=0.096x+1.110$ ,  $R^2=0.316$ ,  $P<0.001$ ). No measurements were carried out from parturition (day 0 of lactation) until day 2 of lactation. On day 3 of lactation, all lactating females were randomly divided into three groups, balanced for litter size. Females in the three groups were given 80% of their estimated food requirement and then required to run a pre-set 0.2, 0.4 and  $0.6 \text{ km day}^{-1}$ , respectively, to obtain additional food. The running target of these three groups was increased by 0.2, 0.4 and  $0.6 \text{ km per day}$ , and reached 2, 4 and  $6 \text{ km day}^{-1}$ , respectively, on day 12 of lactation. It was then maintained at this same level until weaning. These groups are hereafter referred to as 2 km, 4 km and 6 km mice, respectively. Eleven females did not become pregnant and were set the same running targets as the 6 km mice. On day 3 until day 19 of weaning, body mass and food intake of females and litter mass and size were measured daily. The 80% food requirement on days 3–5 of lactation was calculated according to the regression equation between food intake and body mass ( $N=40$ ,  $y=0.509x-9.230$ ,  $R^2=0.407$ ,  $P<0.001$ ), and the 80% food requirement on days 6–18 was calculated based on the regression equation between food intake and litter mass derived from the data in a previous study (Gamo et al., 2013) ( $N=234$ ,  $y=0.114x+8.486$ ,  $R^2=0.547$ ,  $P<0.001$ ). In addition to monitoring food intake and body mass of the mother each day, we also measured the mass and number of offspring. If the number of offspring was discrepant between days we assumed that mortality had occurred. Because we did not record actual deaths we do not know whether offspring died or were killed by the mother. The mothers often ate the missing pups so there was no trace of them. The raw data on the daily pattern of running activity for mice are available for collaborative exploitation.



### Experiment 3

To test for wheel-running effects on the thermal conductance of fur, 10 females were housed individually in cages with free access to running wheels. Another 10 animals were housed individually in cages without running wheels. Both running and non-running females had *ad libitum* access to food and water. After 3 weeks these animals were killed by CO<sub>2</sub> overdose, and the whole pelage except for the head, limbs and tail was immediately removed. The pelage was stitched around a 20 ml glass pot containing water. The pot was warmed to 45°C and then transferred to an incubator at 4°C. Water temperature change was monitored using a transmitter (Vital view transponder, Mini Mitter, Bend, OR, USA) in the water pot and automatically recorded and stored in the computer at 30 s intervals. Ambient temperature outside the pot was also continuously monitored. After the temperature of the pot declined to around 19°C, the pelage was removed, carefully shaved and then replaced on the pot. The pot was re-warmed to 45°C then transferred to the 4°C incubator and allowed to cool again. Finally, the temperature changes of the water pot alone, without any surrounding pelage were monitored as it cooled from 45°C in the 4°C environment. The intact pelage, shaved skin and removed fur were all weighed (to 0.0001 g).

### Experiment 4

To test for time restriction effects on food intake and litter mass in lactating mice, 33 MF1 female mice were randomly assigned to one of the following groups: control mice ( $N=5$ ) underwent a normal lactation; group 1 ( $N=6$ ), females were prevented from feeding for 3 h day<sup>-1</sup> (food deprived: FD); group 2 ( $N=6$ ), females were separated from their pups but retained access to food for 3 h day<sup>-1</sup> (pup deprived: PD); group 3 ( $N=8$ ), females were separated from the pups and at the same time prevented from feeding for 3 h day<sup>-1</sup> (food and pup deprived: FPD); or group 4 ( $N=8$ ), as group 3 but additionally the females were not allowed to sleep when the food and pups were removed (food, pup and sleep deprived: FPSD). To prevent sleep, the females were continuously observed and if they attempted to settle down and rest, the cage was gently tapped to prevent them so doing. In all these manipulations except for group 4, the female could sleep if she chose to. The mice in groups 1–4 were manipulated for six half-hour periods during the day (07:30 h–08:00 h, 09:30 h–10:00 h, 11:30 h–12:00 h, 13:30 h–14:00 h, 15:30 h–16:00 h, 17:30 h–18:00 h) making a total of 3 h of disruption, which was the same time spent running by the long distance (6 km) group in experiment 2. Females were paired with males for 11 days to become pregnant. The above manipulations were performed daily from day 3 of lactation until day 19 when the offspring were weaned. Food intake and litter mass were measured on a daily basis over the entire lactation period.

### Statistics

Data analysis was carried out using SPSS package (13.0). Distributions of all variables were tested for normality using the Kolmogorov–Smirnov test. In experiment 1, the change in wheel-running activity was analysed using repeated measures ANOVA. The relationship between wheel-running activity and litter mass or size was tested by Pearson's correlation. In experiment 2, the changes in wheel-running activity, food intake, litter mass and pup mortality were analysed by repeated measures ANOVA. The difference in wheel-running activity, food intake, litter mass and pup mortality between the 2 km, 4 km and 6 km groups was tested using one way ANOVA followed by Tukey's HSD *post hoc* tests. Pearson's correlation was performed to determine the correlation between litter mass and percentage of animals successfully opening

the food hoppers. In experiment 3, the differences in mass of the intact pelage, shaved skin and removed fur as well as cooling slope between non-running and running groups were examined using independent *t*-tests. In experiment 4, the restriction effects on female food intake and litter mass were tested using one-way ANCOVA with litter size as a covariate. Statistical significance was determined at  $P<0.05$ . Values are means  $\pm$  s.e.m.

All procedures concerning animal care and treatment were approved by the ethical committee for the use of experimental animals of the University of Aberdeen, and were licensed by the UK Home Office and performed under PPL 60/3705.

## RESULTS

### Experiment 1

#### Running activity and the time spent wheel running

When given free access to the running wheel, the females showed significant changes in running activity over the first 4 days (days 1–4 of baseline,  $F_{3,39}=7.059$ ,  $P<0.01$ ; Fig. 1) as they became used to the wheels, and then had a relatively stable activity, running on average  $7.25\pm0.54$  km day<sup>-1</sup> on days 5–7 of baseline (days 5–7,  $F_{2,28}=0.581$ ,  $P>0.05$ ; Fig. 1) and for  $330.62\pm15.60$  min. The time and distance spent wheel running increased on the day that the male was placed in the cage. For the remainder of the time the male was present, the running time was approximately double that when the female was in the cage alone (averaging  $548.64\pm19.04$  min) but the total distance run each day was no different from when only the female was in the cage. At this time there were two mice in the cage and it was not possible to identify the separate contributions to this running activity. After the male was removed, the distance run immediately dropped to  $3.58\pm0.26$  km day<sup>-1</sup> (on day -8 of pregnancy) and the time spent running similarly fell to  $203.10\pm7.87$  min day<sup>-1</sup>. Both these parameters continued to decrease significantly as pregnancy progressed and reached a minimum on day -2 of pregnancy that averaged  $0.35\pm0.10$  km day<sup>-1</sup> and  $50.70\pm7.36$  min day<sup>-1</sup> (days -8 to 0 of pregnancy: distance,  $F_{8,176}=29.35$ ,  $P<0.001$ ; time,  $F_{8,176}=22.49$ ,  $P<0.001$ ). The females ran  $1.10\pm0.25$  km day<sup>-1</sup> on the day of parturition and ran less during the whole of lactation, averaging  $0.51\pm0.08$  km day<sup>-1</sup> on day 15 of lactation (days 0–15 of lactation,  $F_{15,210}=3.095$ ,  $P<0.001$ ) taking on average  $63.13\pm7.37$  min. On day 18, and thereafter, wheel-running activity slightly increased and reached  $2.14\pm0.28$  km day<sup>-1</sup> on day 20 of lactation (Fig. 1). After the offspring were weaned, wheel-

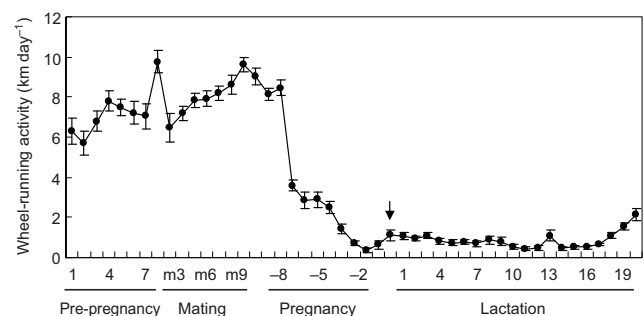


Fig. 1. Wheel-running activity over the pre-pregnancy, mating, pregnancy, parturition and lactation period (days) in voluntarily running MF1 mice. The distance run averaged  $7.25\pm0.54$  km day<sup>-1</sup> in pre-pregnant mice, decreased sharply during pregnancy and reached a minimum of  $0.51\pm0.08$  km day<sup>-1</sup> at peak lactation. The arrow indicates parturition day. The third, sixth and ninth days when the male was present are indicated as m3, m6 and m9, respectively. Values are means  $\pm$  s.e.m.

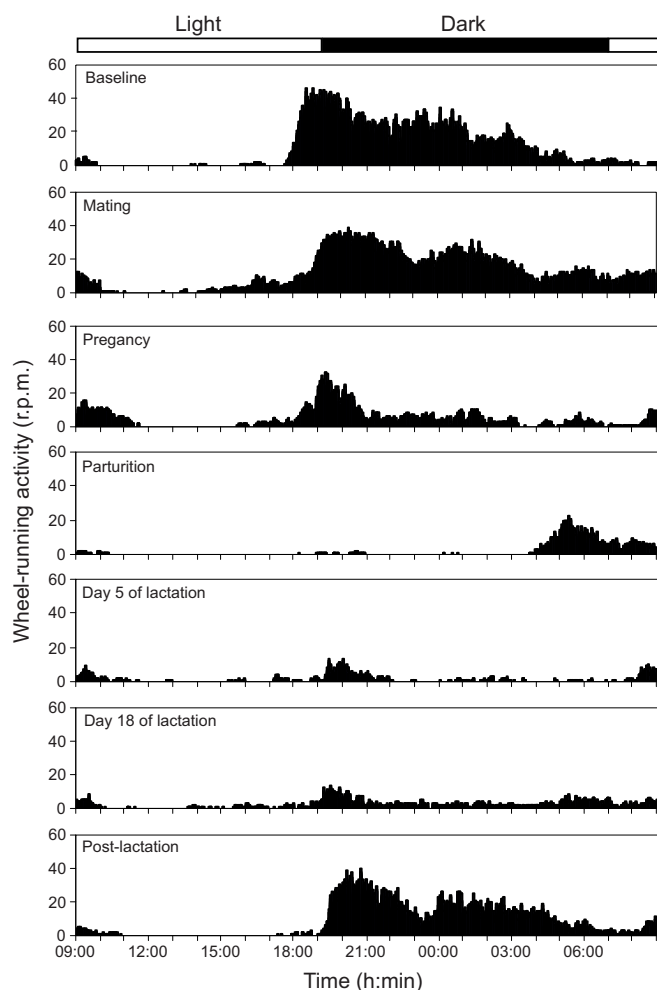


Fig. 2. Daily pattern of voluntary wheel running activity of MF1 mice on seven representative days for the baseline, mating, pregnancy, parturition, lactation and post-lactation periods. Values are means.

running activity of females increased significantly to  $5.01 \pm 0.55 \text{ km day}^{-1}$  on day 1 of post-lactation, and reached a plateau ( $5.38 \pm 0.51 \text{ km day}^{-1}$ ) that was similar to that of pre-pregnancy (days 1–8 of post-lactation,  $F_{7,84}=1.781$ ,  $P>0.05$ ). The time spent running similarly recovered to  $318.03 \pm 25.89 \text{ min day}^{-1}$ . The detailed daily patterns of wheel-running activity averaged across individuals in the five different reproductive periods are included in supplementary material Fig. S1. The raw data used to compile these plots are also available for collaborative use (contact the corresponding author). Seven representative days for the baseline, mating, parturition, pregnancy, lactation and post-lactation periods are included in Fig. 2. These figures clearly illustrate the precipitous decline in wheel running that occurred in pregnancy and lactation. During baseline, the running activity was mostly nocturnal and involved a peak of activity after lights out of between 40 and 60 r.p.m.

## Experiment 2

### Running distance

Prior to mating, the distance run did not differ significantly between non-reproductive mice and reproductive mice allocated to the 2 km, 4 km or 6 km groups; the distance run during the baseline period was  $7.70 \pm 0.50$ ,  $8.23 \pm 0.82$ ,  $7.52 \pm 0.67$  and  $7.31 \pm 0.58 \text{ km day}^{-1}$  in non-reproductive, 2 km, 4 km and 6 km groups, respectively ( $F_{3,48}=0.356$ ,

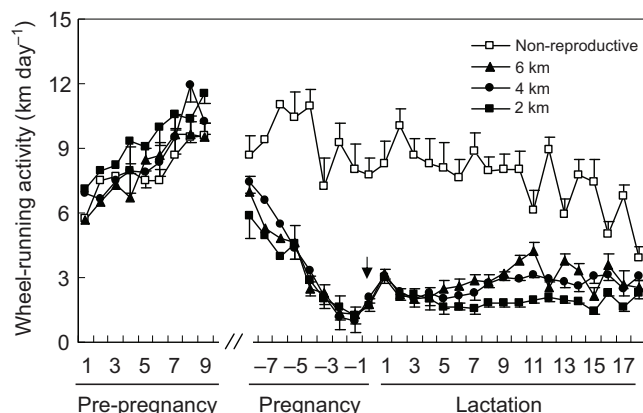


Fig. 3. Voluntary wheel-running activity of MF1 mice during pre-pregnancy, pregnancy, parturition and lactation (days). Mice were non-reproductive or had to run 2, 4 and 6 km  $\text{day}^{-1}$  at peak lactation to obtain access to *ad libitum* food (2 km, 4 km and 6 km groups). Arrow indicates parturition day. Values are means  $\pm$  s.e.m.

$P>0.05$ ; Fig. 3, Table 1). When the mice were on the restricted diet (80% of food intake on days 1–3) and were required to run 3.5, 5.2 and then  $7.1 \text{ km day}^{-1}$  to obtain access to *ad libitum* food, wheel-running distance increased significantly (days 4–9,  $F_{5,210}=11.062$ ,  $P<0.001$ ; Table 1) and averaged  $9.58 \pm 0.90$ ,  $11.53 \pm 1.03$ ,  $10.25 \pm 0.55$  and  $9.52 \pm 0.73 \text{ km day}^{-1}$  for non-reproductive, 2 km, 4 km and 6 km groups, respectively, on day 9 of baseline.

During pregnancy, wheel-running distances of 2 km, 4 km and 6 km mice decreased significantly (days –8 to –1,  $F_{7,259}=82.56$ ,  $P<0.001$ ) and reached a minimum on day –1 (Table 1) that averaged  $1.25 \pm 0.13$ ,  $0.97 \pm 0.15$  and  $1.28 \pm 0.24 \text{ km day}^{-1}$ , respectively. This was again substantially more than the  $0.4 \text{ km day}^{-1}$  they were required to run to get *ad libitum* food access. On day 3 of lactation and thereafter, wheel-running activity of both 4 km and 6 km mice increased in line with the increasing daily target (4 km,  $F_{15,180}=2.538$ ,  $P<0.01$  and 6 km,  $F_{15,150}=3.424$ ,  $P<0.001$ ; Fig. 3, Table 1). The distance run averaged  $2.04 \pm 0.34$  and  $2.05 \pm 0.31 \text{ km day}^{-1}$  on day 3, increasing to a maximum on day 11 that averaged  $3.15 \pm 0.27$  and  $4.26 \pm 0.36 \text{ km day}^{-1}$  for 4 km and 6 km mice, respectively. However, wheel-running distance of the 2 km mice did not show a significant increase over the lactation period ( $F_{15,165}=1.601$ ,  $P>0.05$ ) and the average distance run was  $1.95 \pm 0.24 \text{ km day}^{-1}$ . The wheel-running distance was significantly greater in 6 km mice than in 2 km mice on day 9, 10, 13 and 15 of lactation (day 9,  $F_{3,48}=64.572$ ,  $P<0.001$ ). Among the four groups, non-reproductive mice ran far more than the pregnant and lactating mice on day –7 of pregnancy to day 17 of lactation (day –7,  $F_{3,48}=9.572$ ,  $P<0.01$  and day 17,  $F_{3,48}=16.485$ ,  $P<0.001$ ; Fig. 3).

### Food intake

Mean food intake was not significantly different between non-reproductive mice and 2 km, 4 km and 6 km mice ( $4.94 \pm 0.23$ ,  $4.66 \pm 0.23$ ,  $4.63 \pm 0.11$  and  $4.30 \pm 0.18 \text{ g day}^{-1}$ , respectively) at the start of the experiment during baseline ( $F_{3,49}=1.784$ ,  $P>0.05$ ; Fig. 4, Table 1). After 6 days of running training with 80% food, the food intake of all mice increased significantly by 15%, 21%, 14% and 14% and averaged  $5.67 \pm 0.32$ ,  $5.64 \pm 0.22$ ,  $5.30 \pm 0.28$  and  $4.91 \pm 0.28 \text{ g day}^{-1}$  in non-reproductive controls and 2 km, 4 km and 6 km mice ( $F_{11,539}=8.716$ ,  $P<0.01$ ), but no group differences were found at the end of the baseline training period (day 12 of baseline,  $F_{3,49}=2.003$ ,  $P>0.05$ ; Table 1). During pregnancy, the food intake

Table 1. Summary of mean data for experiment 2

Group	NR	R2	R4	R6
Distance run (km day <sup>-1</sup> )				
Baseline	7.70±0.50	8.23±0.82	7.52±0.67	7.31±0.58
When forced to run	9.58±0.90	11.53±1.03	10.25±0.55	9.52±0.73
Pregnant day -1	NA	1.25±0.13	0.97±0.15	1.28±0.24
Lactation day 3	NA	1.95±0.24	2.04±0.34	2.05±0.31
Lactation day 11	NA	1.95±0.24	3.15±0.27	4.26±0.36
Food intake (g day <sup>-1</sup> )				
Baseline	4.94±0.23	4.66±0.23	4.63±0.11	4.30±0.18
When forced to run	5.67±0.32	5.64±0.22	5.30±0.28	4.91±0.28
Maximum intake lactation	NA	17.20±0.63	15.13±1.21	13.86±0.76
Asymptotic intake lactation	NA	16.97±0.40	14.29±0.72	12.65±0.45
Intake when hoppers open	NA	18.07±0.36	17.60±0.60	16.80±0.58
Day of maximum intake	NA	13	12	8
Litter mass (g)				
Lactation day 3	NA	18.47±1.37	17.67±1.81	19.19±1.47
Lactation day 19	NA	72.31±2.40	55.46±4.26	48.57±2.52

Data (means ± s.e.m., except for day of maximum intake) are shown for distance run, food intake and litter mass for non-reproductive (NR) and reproducing animals that had to run 2, 4 and 6 km each day to open the food hoppers (R2, R4 and R6, respectively).

Baseline refers to the period prior to any manipulation.

'When forced to run' refers to the period prior to breeding when the mice had to run to open the food hoppers.

'Asymptotic intake' refers to average intake over days 9–14 of lactation, independent of whether the hoppers were open or not.

'Intake when hoppers open' refers only to days when the mice ran sufficient distances to open the hoppers.

NA, not applicable.

of pregnant mice increased significantly and was higher than that of non-reproductive mice on days -5 to -2 (day -2,  $F_{3,49}=3.360$ ,  $P<0.05$ ). After parturition, all the lactating mice ate more food than non-reproductive mice on days 2–18 of lactation (day 18 of lactation,  $F_{3,49}=81.226$ ,  $P<0.001$ ; Fig. 4).

Daily food intake of the three lactating groups increased significantly over time in lactation (days 3–18 of lactation,  $F_{15,690}=8.057$ ,  $P<0.001$ ). The maximum intake averaged  $17.20\pm0.63$  g day<sup>-1</sup> in 2 km mice on day 13,  $15.13\pm1.21$  g day<sup>-1</sup> in 4 km mice on day 12 and  $13.86\pm0.76$  g day<sup>-1</sup> in 6 km mice on day 8. Among the three lactating groups, the food intake of 6 km mice was significantly lower than that of 2 km mice on days 9–12, and days 15–18 of lactation (day 9,  $F_{2,38}=8.506$ ,  $P<0.01$ ; day 18,  $F_{2,38}=15.918$ ,  $P<0.01$ ). The asymptotic food intake (averaged over days 9–14) was  $16.97\pm0.40$ ,  $14.29\pm0.72$  and  $12.65\pm0.45$  g day<sup>-1</sup> in 2 km, 4 km and 6 km mice, respectively. The asymptotic food intake of the 6 km mice was significantly lower by 25% than that of the 2 km mice ( $F_{2,38}=14.800$ ,  $P<0.001$ ; Fig. 4, Table 1). On the days

when the mice ran sufficient distance to gain access to *ad libitum* food, the asymptotic food intake (daily food intake between days 11 and 18) was not significantly different between the females that had to run 2, 4 or 6 km to obtain food ( $18.07\pm0.36$ ,  $17.60\pm0.60$  and  $16.80\pm0.58$  g day<sup>-1</sup>,  $P<0.05$ ; Fig. 5, Table 1).

#### Percentage of open hoppers

The 2 km mice were able to run the pre-set target distance and open hoppers every day until day 11 of lactation. The percentage of mice that ran the target distance was 100% before day 9 in the 4 km mice, and 100% before day 6 in the 6 km mice. The decreasing percentage of mice that ran sufficiently to open the hoppers (Fig. 6A) indicated that more and more mice in both the 4 km and 6 km groups were unable to run the target distance as lactation progressed. On the day of weaning (day 19), the percentage of open hoppers was 50%, 30% and 0% for the 2 km, 4 km and 6 km mice, respectively. There were

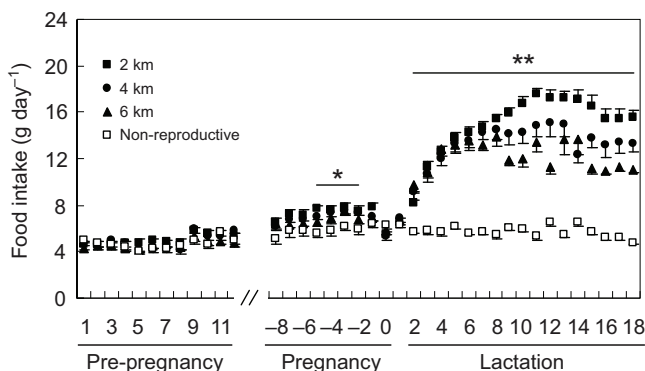


Fig. 4. Food intake of MF1 mice during pre-pregnancy, pregnancy, parturition and lactation (days). Mice were non-reproductive or had to run 2, 4 and 6 km day<sup>-1</sup> at peak lactation to obtain access to *ad libitum* food (2 km, 4 km and 6 km groups). \* $P<0.05$ , \*\* $P<0.01$ . Values are means ± s.e.m.

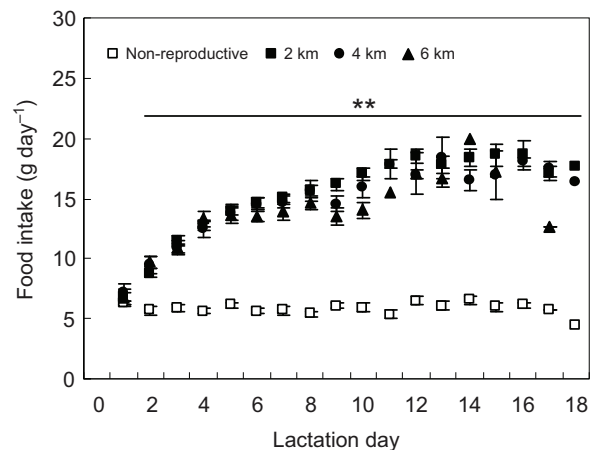


Fig. 5. Food intake of female mice on the days that they ran to target throughout lactation. Mice were non-reproductive or had to run 2, 4 and 6 km day<sup>-1</sup> at peak lactation to obtain access to *ad libitum* food (2 km, 4 km and 6 km groups). \*\* $P<0.01$ . Values are means ± s.e.m.

positive correlations between weaned litter mass and the percentage of opened hoppers for 4 km mice ( $y=0.59x+17.52$ ,  $P<0.05$ ) and 6 km mice ( $y=0.59x+20.30$ ,  $P<0.001$ ), but not for 2 km mice ( $y=0.23x+53.81$ ,  $P>0.05$ ; Fig. 6B). No significant relationships ( $P>0.05$ ) were observed between female body mass on day 2 of lactation and the accumulated days on which the mice ran sufficiently to open the hoppers during mid- and late lactation (days 8–17) for the 2 km, 4 km or 6 km group.

#### Daily pattern of running activity

The average daily pattern of running activity for mice in the three distance groups for all days are available as supplementary material Figs S2, S3 and S4 for 2 km, 4 km and 6 km mice, respectively. The raw data on which these plots are based are also available for collaborative use. Example days for early (day 5) and late (day 18) lactation for all three groups are presented in Fig. 7. The most striking difference between these patterns and the patterns of voluntary running during baseline (see Fig. 2 and supplementary material Fig. S1) is that when lactating mice had to run to get access to *ad libitum* food, they distributed their activity much more equally between the light and dark cycles. Moreover, although there was a noticeable increase in activity after lights out, the maximum running distance was always less than 40 r.p.m., contrasting the much greater running speeds (40–60 r.p.m.) at baseline.

#### Litter mass

Litter mass averaged  $18.47\pm1.37$ ,  $17.67\pm1.81$  and  $19.19\pm1.47$  g for 2 km, 4 km and 6 km mice on day 3 of lactation ( $F_{2,38}=0.230$ ,  $P>0.05$ ; Fig. 8A) and increased significantly over the lactation period (days 3–19,  $F_{16,608}=141.163$ ,  $P<0.001$ ). From day 13 until weaning, litter mass in the 2 km group was significantly greater than that in the other two groups (day 13,  $F_{2,38}=3.455$ ,  $P<0.01$ ). The weaned litter mass of the 2 km mice was 30.4% and 48.9% heavier than the mass of the weaned litters of the 4 km and 6 km mice, respectively (day 19,  $72.31\pm2.40$ ,  $55.46\pm4.26$  and  $48.57\pm2.52$  g for 2 km, 4 km and 6 km mice,  $F_{2,38}=13.531$ ,  $P<0.001$ ; Fig. 8A). Pup mortality of 2 km mice averaged  $2.17\pm1.14\%$  by day 4 of lactation, and averaged  $4.48\pm2.70\%$  on day 19 of weaning ( $F_{15,165}=1.397$ ,  $P>0.05$ ; Fig. 8B). However, both 4 km and 6 km mice showed an increasing mortality between days 4 and 13 (4 km mice,  $F_{9,108}=6.291$ ,  $P<0.001$  and 6 km mice,  $F_{9,99}=9.489$ ,  $P<0.001$ ) and then a stable mortality between days 14 and 19 (4 km,  $F_{5,60}=1.000$ ,  $P>0.05$ ; 6 km,  $F_{5,55}=1.730$ ,  $P>0.05$ ). During the increasing phase on days 4–13, pup mortality increased from  $2.88\pm1.59\%$  to  $24.17\pm5.78\%$  and 0 to  $26.20\pm6.50\%$  in 4 km and 6 km mice, respectively. Mortality of 4 km and 6 km mice was 5.7 and 6.2 times higher than that of 2 km mice during days 14–19, which was significant from day 12 onwards (day 12,  $F_{2,36}=3.658$ ,  $P<0.001$ ; Fig. 8B).

#### Experiment 3

##### Mass and cooling rate of pelage and fur

There was no significant difference in the mass of the intact pelage between non-running and running mice (Table 2). In addition, the shaved pelage and removed fur showed no significant group differences in mass. Between  $39^{\circ}\text{C}$  and  $18^{\circ}\text{C}$ , the cooling rate of the pots surrounded by the intact pelage from non-running mice was not significantly different to that when surrounded by the pelage of running mice. The slopes of the exponential cooling curves were  $-0.037\pm0.001$  and  $-0.037\pm0.001$  in non-runners and runners, respectively ( $t_{18}=0.474$ ,  $P>0.05$ ). The cooling rate of the pots alone was not significantly faster than when surrounded by the pelage and also did not show any group difference (non-runners,  $-0.072\pm0.003$ ; runners,  $-0.077\pm0.005$ ;  $t_{18}=0.904$ ,  $P>0.05$ ). As expected, shaved

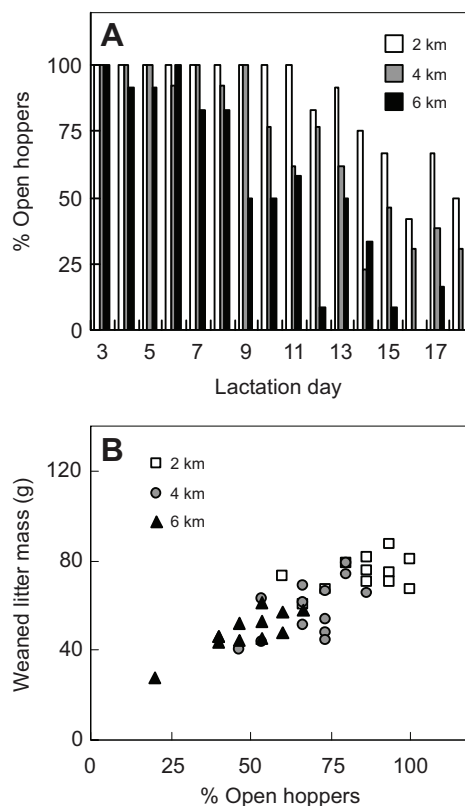


Fig. 6. (A) The percentage of opening hoppers over the lactation period and (B) the correlation between litter mass on weaning day and percentage of open hoppers in MF1 mice voluntarily running to obtain *ad libitum* access to food; 2 km, 4 km and 6 km indicate mice whose targets were set at 2, 4 and 6 km day<sup>-1</sup>, respectively. There was a positive correlation between litter mass and percentage of open hoppers in 4 km mice ( $N=13$ ,  $y=0.59x+17.52$ ,  $P<0.05$ ) and 6 km mice ( $N=12$ ,  $y=0.59x+20.30$ ,  $P<0.001$ ).

skin retarded heat loss less efficiently than the intact pelage. The cooling rate of shaved skin from the runners was significantly faster than that of non-runners (slope of non-runner,  $-0.058\pm0.002$ ; runner,  $-0.063\pm0.001$ ;  $t_{18}=2.109$ ,  $P<0.05$ ; Fig. 9). These data suggest that 37–40% of the resistance to heat flow of the intact pelage was provided by the skin and 60–63% by the fur.

#### Experiment 4

##### Food intake

Mean food intake on day 3 of lactation (the day the manipulations began) was  $11.01\pm1.19$  g day<sup>-1</sup> in controls,  $9.69\pm1.24$  g day<sup>-1</sup> in the FD group,  $10.05\pm0.77$  g day<sup>-1</sup> in the PD group,  $11.12\pm0.64$  g day<sup>-1</sup> in the FPD group and  $11.06\pm0.57$  g day<sup>-1</sup> in the FPSD group and no significant differences were found between these groups ( $F_{4,25}=0.973$ ,  $P>0.05$ ; Fig. 10). The group differences on any day throughout lactation were not significant when litter size was included as a covariate in the analysis (e.g. day 20: control,  $21.98\pm2.17$  g day<sup>-1</sup>; FD,  $16.25\pm1.89$  g day<sup>-1</sup>; PD,  $16.27\pm1.67$  g day<sup>-1</sup>; FPD,  $19.07\pm1.08$  g day<sup>-1</sup>; and FPSD,  $19.72\pm0.90$  g day<sup>-1</sup>;  $F_{4,25}=2.715$ ,  $P>0.05$ ).

##### Litter size

Over the lactation period (days 3–20), litter mass increased from  $24.67\pm4.17$  g on day 3 to  $100.46\pm10.15$  g on day 20 in controls,  $15.60\pm2.69$  g to  $75.83\pm8.93$  g in the FD group,  $18.10\pm3.71$  g to



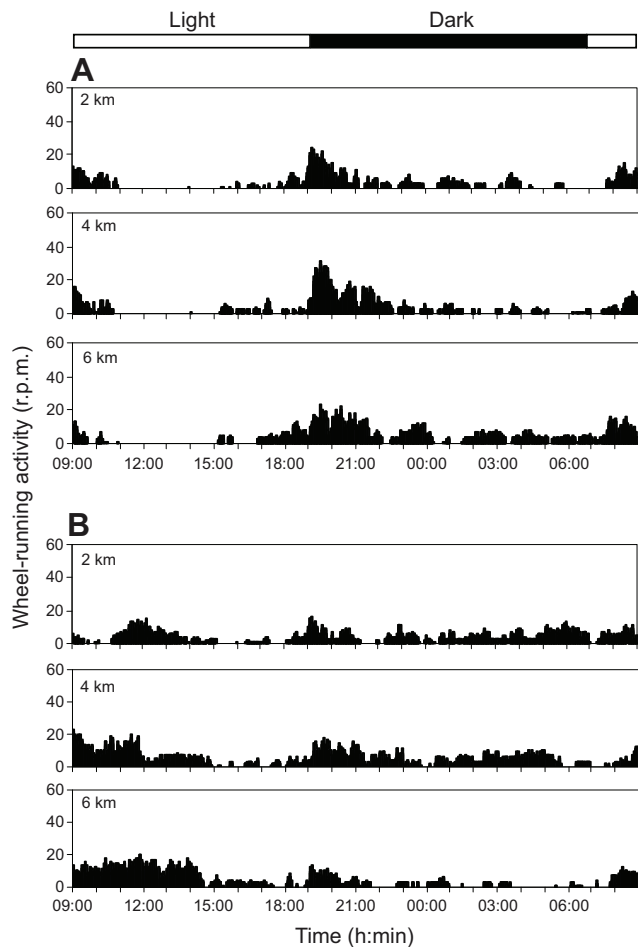


Fig. 7. Daily pattern of voluntary wheel-running activity during early (day 5, A) and late (day 18, B) lactation in MF1 mice that had to run a set target of 2, 4 or 6 km to obtain *ad libitum* access to food. Values are means.

77.33±10.47 g in the PD group, 23.87±2.04 g to 85.45±6.10 g in the FPD group and 22.87±1.08 g to 93.84±2.77 g in the FPSD group. There were no significant differences between these groups in litter mass on any day in lactation (e.g. day 3,  $F_{4,25}=1.380$ ,  $P>0.05$ ; day 20,  $F_{4,25}=2.122$ ,  $P>0.05$ ; Fig. 10).

### DISCUSSION

During the baseline period, prior to reproduction, the mice voluntarily ran on average 7.25 km day<sup>-1</sup>. This is at the lower end of the distances that rodents have been reported to voluntarily run in wheels. For example, the distance run per day can be as far as 43 km for rats (Richter, 1927), 31 km for white footed mice *Peromyscus leucopus* (Kavanau and Brant, 1965; Kavanau, 1967),

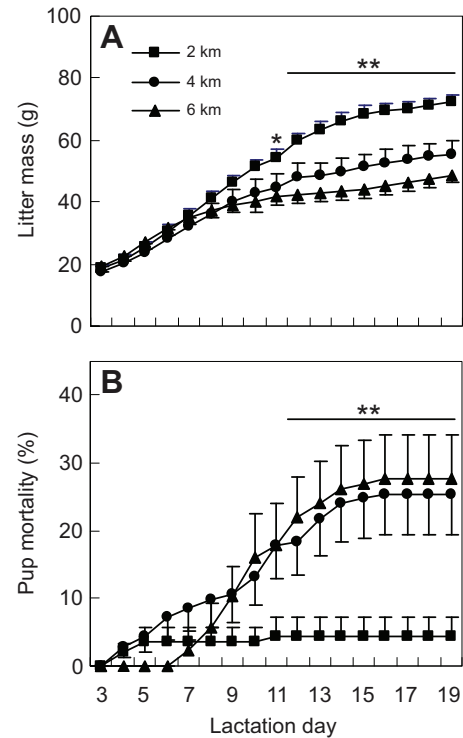


Fig. 8. Changes in litter mass (A) and pup mortality (B) throughout lactation in MF1 mice that had to run a set target of 2, 4 or 6 km to obtain *ad libitum* access to food. Values are means ± s.e.m. \* $P<0.05$ , \*\* $P<0.01$ .

19 km for lemmings *Lemmus lemmus* (de Kock and Rohn, 1971), 16 km for laboratory mice (Festing and Greenwood, 1976; Morgan et al., 2003), 9 km for golden hamsters *Mesocricetus auratus* (Richards, 1966) and 8 km for Mongolian gerbils *Meriones unguiculatus* (Roper, 1976; Roper, 1978). When mice had to run pre-set revolutions per pellet, they increased wheel-running activity from an average of 10–20 km day<sup>-1</sup> (Vanholt et al., 2007). The maximum distance that laboratory mice could run under this training was around 23 km day<sup>-1</sup> (Vanholt et al., 2007). Similarly, when the mice in our study were food restricted to 80% of their normal intake and required to run to obtain *ad libitum* access to food, they greatly increased their wheel-running distances from on average 7.25 to around 11.5 km day<sup>-1</sup>. This was despite the fact that they first needed to run only 3.5 km and later 7.1 km to obtain *ad libitum* food access, distances that they had covered voluntarily prior to any restriction. Consequently, the animals had no need to increase their activity but they did so anyway. Increased physical activity of mice under caloric restriction is a well-established phenomenon (reviewed in Speakman and Mitchell, 2011) and is presumed to reflect a drive to search for food when under restriction,

Table 2. Mass of skin with fur, shaved skin and fur in non-running and running MF1 mice

	Non-runner	Runner	<i>t</i>	<i>P</i>
Mass of skin with fur (g)	2.6562±0.0857	2.6533±0.1106	0.021	n.s.
Mass of shaved skin (g)	0.2540±0.0078	0.2613±0.0077	0.663	n.s.
Mass of shaved fur (g)	2.2315±0.0972	2.2148±0.0975	0.121	n.s.
Fur as % of all skin	9.6220±0.3475	9.9300±0.3097	0.662	n.s.
Ratio of fur to shaved skin	0.1140±0.0048	0.1180±0.0047	0.600	n.s.

Values are means ± s.e.m. n.s. indicates no significant difference between non-runners and runners,  $P>0.05$ .



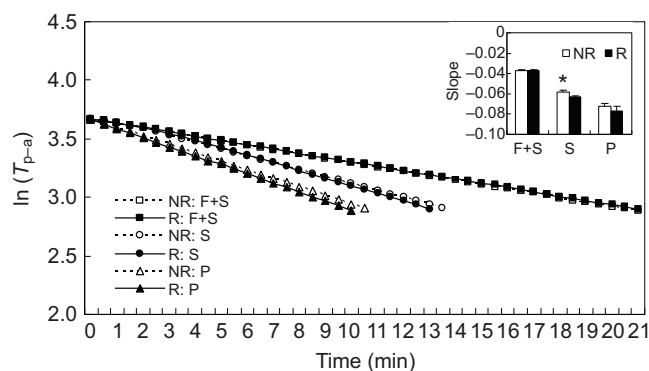


Fig. 9. Cooling time and slopes of cooling curves (inset) measured in pots surrounded by fur and skin (F+S) or shaved skin (S), and for the pot only (P) in non-running (NR) and running (R) MF1 females.  $T_{p-a}$  indicates the temperature of the pot water minus ambient temperature (in the incubator). Values are means  $\pm$  s.e.m. There was no significant effect of wheel running on the slope of cooling time of the fur and skin ( $t_{18}=0.474$ ,  $P>0.05$ ). \*The cooling rate of the shaved skin from the runners was significantly greater than that of non-runners ( $P<0.05$ ).

or anticipatory behaviour waiting for the daily food delivery (called food anticipatory activity) (Zhao and Cao, 2009b). Paradoxically, this leads to an exacerbated energy deficit, which for rats maintained under restriction can ultimately be fatal (Even and Nicolaïdis, 1993; Selman et al., 2005). In the case of our mice, the consequence of the increased activity was only that their food intake increased to cover the costs of the elevated activity.

During pregnancy and lactation, voluntary wheel running activity fell enormously. This was similar to the decline in wheel running reported previously in pregnant and lactating rats (Slonaker, 1924; Wang, 1924) and also the decline in general activity in this strain of mouse during reproduction (Speakman et al., 2001). The pattern of wheel-running distance (and time) revealed here is an exaggerated version of the general activity patterns. This decline in wheel-running activity during lactation may have several contributory causes. First, the time available to run may be reduced because the animals spend large amounts of time suckling their offspring and also an increased time spent feeding (Speakman et al., 2001). Second, the energy that would have been devoted to physical activity may be diverted to support the export of milk. Finally, by reducing activity the animals can avoid hyperthermia, which might occur for animals combining the high heat load of lactation (Król and Speakman, 2003a; Król and Speakman, 2003b) with the heat generated by physical activity, in a situation where their total capacity to dissipate heat is limited. The large decline in the levels of wheel-running activity in lactation was consequently consistent with both the HDL theory and the resource allocation hypothesis that mice prioritise energy use between activities when under energetic stress.

When mice had to run during lactation to obtain *ad libitum* access to food, they significantly increased their levels of activity above the voluntary levels observed in experiment 1, but these levels remained much lower than those of non-reproductive individuals on the same reward schedule, and lower than their own activity prior to reproduction. As lactation progressed, the females increasingly failed to reach the pre-set target that would allow them *ad libitum* access to the food. This was despite the fact that the target for all three groups was well below the levels that the mice were clearly capable of running based on their own performances prior to mating, and the simultaneous activity of non-reproducing mice. The failure to run these distances was therefore not because

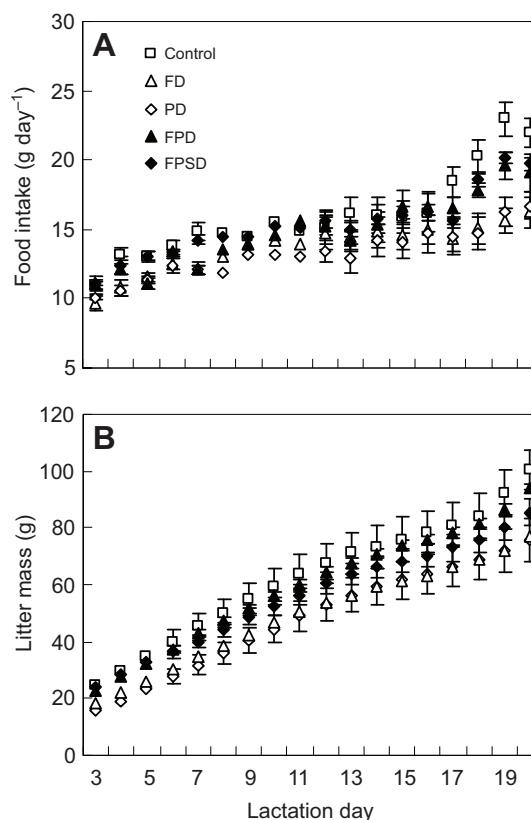


Fig. 10. Food intake (A) and litter mass (B) during lactation in the MF1 females deprived of food (FD), pups (PD), both food and pups (FPD) or food, pups and sleep (FPSD). Values are means  $\pm$  s.e.m.

the mice were being asked to perform a task beyond their physiological capabilities. We suggest the most likely reason for the failure to run the required distance to open the food hoppers was that the mice were limited in their capacity to run because of the risks of hyperthermia when combining physical activity with lactation. The greater the required distance, the less likely the mice were to be able to run this distance without overheating. Supporting this interpretation, the body temperatures of mice with implanted transmitters were higher when they were physically active than when at rest (Gamo et al., 2013).

The detailed daily patterns of activity of the running animals (Fig. 7) strongly support this interpretation, in that the mice that had to run in lactation never ran at the same high revolution rates that were observed in non-reproductive mice (Fig. 2), and their running was more spread out across the day and more fragmented in nature. This is consistent with mice attempting to run the target distance but limiting their exposure to hyperthermia risk by restricting their maximum running speed, making shorter running bouts and therefore extending their activity over a longer period.

The direct consequence of failing to reach the target running distance was that the asymptotic level of food intake of mice at peak lactation was inversely related to the target distance. The further direct consequence of these asymptotic intakes was that the weaned litter mass in the 2 km, 4 km and 6 km females was lower by 18%, 37% and 46% than the previously reported  $86.7 \pm 1.41$  g litter mass in normal lactating females (Johnson et al., 2001a). A major contributory factor to the low food intake and hence impaired litter mass accumulation was the fact the mice failed to open the hoppers and therefore only obtained 80% of their estimated food energy

requirements on these days. On the days that the mice did open the hoppers they had *ad libitum* access to food. In theory, therefore, they could have ingested food on these days to cover not only the costs of lactation and running on those days, but also to compensate for the energy shortfalls on the days that the hoppers were not opened. The peripheral limitation hypothesis predicted that on these days, when the hoppers were opened, the mice would ingest food in direct proportion to the summed demands of their peripheral tissues. Hence, it would be predicted that the food intake would increase from 2 km to 4 km and 6 km mice. However, this was not the case, and the actual intake did not differ significantly across the groups. Between days 11 and 18 the average *ad libitum* intake on days that the hoppers were opened was 18.1 g for the 2 km mice, 17.3 g for the 4 km mice and 16.5 g for the 6 km mice. These were all significantly lower than the previously reported 23.1 g day<sup>-1</sup> food intake of MF1 mice that had *ad libitum* access to food during lactation (Johnson et al., 2001a). However, this previous mass of food intake refers to a different diet (CRM pellets) which has a lower absorption efficiency – hence, a greater mass of food must be ingested to obtain the same energy. The average asymptotic food intake of lactating MF1 mice fed *ad libitum* in lactation, feeding on the same diet as used here was 18.2 g day<sup>-1</sup> (Gamo et al., 2013). This was not significantly different from the intake reported here on the days the mice ran sufficiently to successfully gain access to *ad libitum* food. Hence, the data do not support the predictions of the peripheral limitation hypothesis, but strongly support the heat dissipation limitation hypothesis, which predicts a constraint imposed on the food intake of the mice by the capacity to dissipate heat. Our direct measurements of the pelages of mice that ran, and mice that did not run, indicated that the heat dissipation capacity of the pelage was not affected by whether the mice ran or not. However, runners had shaved skin that conducted more heat than for non-runners, suggesting some acclimation at this level to improve heat dissipation. Moreover, we cannot eliminate the possibility that mice were able to modulate their heat loss in other ways – by, for example, vasodilation.

Our data are similar to those reported previously using a different method linking food intake to physical activity (Perrigo, 1987), in which forced running deer mice and house mice did not exhibit higher food intake compared with control females fed *ad libitum*. Both maternal energy intake and expenditure declined with increasing foraging costs in house mice, in which milk energy output showed a relative decline with rising foraging costs, both at peak lactation and over the entire reproductive period (Schubert et al., 2009).

In the present study, the females that had to run to obtain *ad libitum* access to food spent about 3 h in running wheels, which was about six times higher than voluntary running females during lactation. This required time spent running may have resulted in the mice spending less and more fragmented time suckling their young (or feeding and sleeping). It has been well established that suckling is one of the primary factors stimulating oxytocin and prolactin release and milk let down, thereby influencing milk production (Speakman and Król, 2005). As running activity might interfere with the suckling stimulus, this could lead to the reduced milk production and consequently the lower litter mass in females that had to run a pre-set distance to obtain access to food. Thus, the failure to open the hoppers might not be because of a heat constraint but because, by running, the suckling stimulus was disrupted and the demands of the pups were therefore reduced, and hence the females had less need to run. However, when lactating females were prevented from accessing their pups, or from feeding and sleeping for the same duration as the time spent on running wheels in 6 km runners, they

did not have significantly altered food intake or litter mass from each other, or from non-manipulated control females. Consequently, it is unlikely that the effect of wheel running on the time–activity distribution during lactation was the primary factor mediating the limitation on sustained energy intake.

Although the present data are consistent with the heat dissipation limit theory, it is important to recognise that other factors may also be important in the decision making of the female about how hard she will work in lactation. Additional factors may be the consequences of reproduction for oxidative stress (Costantini, 2008; Speakman, 2008; Dowling and Simmons, 2009; Monaghan et al., 2009; Selman et al., 2012) (but see Speakman and Selman, 2011) and immune function (Christe et al., 2000; Christe et al., 2011; Drazen et al., 2003). Among mammals, studies of the impact of reproduction on oxidative stress have generated mixed results, with some field studies, using serum or plasma, indicating increased damage as a consequence of reproduction (Bergeron et al., 2011; Fletcher et al., 2013). In contrast, laboratory-based studies have indicated that in some tissues, notably the liver, oxidative damage is actually reduced in reproducing compared with non-reproducing females (Garratt et al., 2011; Oldakowski et al., 2012). The cause of this difference between studies remains unclear. Perhaps in the laboratory environment where animals are fed high-quality foods with abundant antioxidants they are able to offset any increase in oxygen radical production, but in the field such foods are not available, exposing the trade-off between reproduction and oxidative damage. Alternatively, the difference between laboratory and field studies may be confounded by the different tissues used for analysis and this may then be an analytical artifact. Current data are insufficient to separate these ideas.

In summary, this study has shown that the voluntary wheel-running activity significantly decreased during pregnancy and was maintained at a low level during lactation, before recovering during post-lactation to baseline pre-reproduction levels. This could reflect avoidance of hyperthermia or reallocation of energy devoted to exercise into milk production. When the females were provided with 80% of their estimated food requirements and had to run a pre-set 2, 4 or 6 km distance before being given *ad libitum* access to additional food, most of them did not complete the running target during late lactation. Food intake and litter mass were significantly lower in the females running to obtain *ad libitum* access to food compared with those reported previously (Johnson and Speakman, 2001; Johnson et al., 2001a; Johnson et al., 2001b; Johnson et al., 2001c; Speakman et al., 2001). The running distance attained before reproduction (and in non-reproductive animals on the same reward schedule) were much greater than the target distances required to obtain *ad libitum* food access, suggesting that the failure to reach the target was not due the limited capacity of the leg muscles to perform work. The detailed patterns of activity in running females in lactation compared with baseline activity strongly suggested that the primary reason for the failure to reach the target was that the animals were avoiding the risk of hyperthermia, due to the combined heat stress of running and lactation. When the females did run sufficiently to gain *ad libitum* food access, their intake did not differ between the different distance groups or from controls that were not required to run. These last data also support the heat dissipation limit theory idea but are inconsistent with the peripheral limitation hypothesis.

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### AUTHOR CONTRIBUTIONS

Z.-J.Z. co-conceived and co-designed all four experiments and conducted the laboratory work for the first three experiments. E.K. assisted with the work in experiments 2 and especially experiment 3. S.M. and Y.G. performed experiment 4. J.R.S. co-conceived and co-designed all four experiments. J.R.S. and Z.-J.Z. co-wrote the manuscript, which was then commented on by E.K., S.M. and Y.G.

### COMPETING INTERESTS

No competing interests declared.

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