RESEARCH ARTICLE

Exposure to artificial wind increases energy intake and reproductive performance of female Swiss mice (*Mus musculus*) in hot temperatures

Guang-Min Deng, Jing-Xin Yu, Jia-Qi Xu, Yu-Fan Bao, Qian Chen, Jing Cao and Zhi-Jun Zhao*

ABSTRACT

High temperatures and heatwaves are rapidly emerging as an important threat to many aspects of physiology and behavior in females during lactation. The body's capacity to dissipate heat is reduced by high ambient temperatures, increasing the risk of hyperthermia. Exposure to wind, a pervasive environmental factor for most terrestrial animals, is known to increase heat loss, but its effects on the reproductive performance of small mammals remains unclear. In the present study, the effects of wind on the energy budgets, resting metabolic rate and milk energy output (MEO) were measured in lactating Swiss mice at 21 and 32.5°C. Females kept at 32.5°C had a significantly lower resting metabolic rate, food intake and MEO, and lighter offspring, than those kept at 21°C. However, exposure to wind increased the asymptotic food intake of females kept at 32.5°C by 22.5% ($P<0.01$), their MEO by 20.7% ($P<0.05$) and their litter mass by 17.6% ($P<0.05$). The body temperature of females kept at 32.5°C was significantly higher during lactation than that of females kept at 21°C, but this difference was reduced by exposure to wind. These findings suggest that exposure to wind considerably improves reproductive performance, increasing the fitness of small mammals while undergoing hot temperatures during heatwaves.

KEY WORDS: Energy budget, Heat dissipation, Hot temperature, Lactation, Reproductive performance, Swiss mice, Wind

INTRODUCTION

Energy is generally considered a key resource, and limits on energy intake and output are thought to have had profound effects on the evolution of many morphological, physiological and behavioral traits (Feder et al., 1987; Speakman and McQueenie, 1996). These limits usually occur in animals during periods of high sustained energy demand, such as the demand of physical activity, heat production and lactation (Hammond et al., 1996). Lactation is the most energetically demanding activity performed by small mammals (Thompson and Nicol, 1986; Thompson, 1992; Speakman and Król, 2005, 2011). The mothers must increase their food intake to meet the increasing energy demand for milk production for the growing pups, while probably approaching a ceiling, and resulting in a failure to raise additional pups during lactation (Hammond and Diamond, 1992; Hammond et al., 1994, 1996; Johnson et al., 2001; Zhao et al., 2016). The limitations on

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maximum energy budget are important because they define an envelope within which all the competing biological functions are constrained (Speakman and Król, 2005, 2011). The extrinsic and intrinsic factors that possibly impose the limitations have been extensively examined, among which ambient temperature appears to be one of the most important factors (Hammond and Diamond, 1992, 1997; Hammond and Kristan, 2000; Król and Speakman, 2003a,b; Wu et al., 2009; Simons et al., 2011; Speakman and Król, 2011; Valencak et al., 2013; Wen et al., 2017).

It is generally accepted that global temperatures are both increasing and becoming more variable (IPCC, 2014). Exposure to the unusually high temperatures that occur during heatwaves is rapidly emerging as a major threat to the survival of a variety of animals (Hoffmann and Sgrò, 2011; Mifsud et al., 2011; Stawski and Geiser, 2012; Lovegrove et al., 2014; Martin et al., 2018; Godde et al., 2019; Radchuk et al., 2019). Studies on a number of mammals demonstrate that the maximum energy budgets during lactation are also considerably affected by ambient temperature. For example, lactating laboratory MF1 mice kept under hot temperature conditions produced less milk and had slower-growing pups (Król and Speakman, 2003a) than those kept at cooler temperatures (Król and Speakman, 2003b). Exposure to high ambient temperatures has consistently been found to decrease food intake and milk output during lactation, in small mammals and medium and large domestic animals (Cobble and Herman, 1951; Brody et al., 1958; Morag et al., 1969; Leon and Woodside, 1983; Jansen and Binard, 1991; Abdalla et al., 1993; Renaudeau and Noblet, 2001; Król and Speakman, 2003a,b; Renaudeau et al., 2003; Wu et al., 2009; Valencak et al., 2010, 2013; Simons et al., 2011; Zhao, 2011; Zhao et al., 2013; Yang et al., 2013; Wen et al., 2017; Ohrnberger et al., 2018; Bao et al., 2020).

These adverse effects of high temperatures probably occur because it is harder to dissipate heat when it is hot (Quiniou and Noblet, 1999). Lactating females maximize their energy intake and also increase heat production, generated as a by-product of processing food and producing milk, increasing the risk of chronic hyperthermia (Ulmershakibaei and Plonait, 1992; Król and Speakman, 2003a,b; Speakman and Król, 2005). This is because it is possible that the maximal capacity to dissipate body heat is fixed in lactating females, which may impose a limitation on sustained energy intake and milk output, i.e. the heat dissipation limit hypothesis (Król and Speakman, 2003a,b; Speakman and Król, 2005, 2011). If so, hot temperatures should adversely affect lactating females more than non-lactating females and any factor that increases heat dissipation should increase reproductive performance. For example, Król et al. (2007) found that partially shaving lactating mice improved their ability to dissipate body heat and allowed them to significantly increase their energy intake and milk production compared with unshaved control mice (Król et al.,

2007; Sadowska et al., 2019). However, whether environmental factors that increase heat loss also allow lactating females to increase their food intake and milk production, have not, to the best of our knowledge, been investigated.

Wind is a pervasive environmental factor that promotes heat dissipation by convection and evaporation (Porter and Gates, 1969; Winne et al., 2001; Tracy and Christian, 2005; Kearney and Porter, 2009; Ortega et al., 2017). Exposure to wind would therefore be expected to have a non-trivial effect on the reproductive performance of small mammals. This paper reports the results of experiments designed to examine the effect of wind on the energy budget and lactation of Swiss mice kept at either 21 or 32.5°C. The mass of body organs that are associated with energy intake, and the relationship between organ mass and MEO, were measured and compared with the goal of testing the hypothesis that exposure to wind improves reproductive performance in lactating small mammals, particularly at hot ambient temperatures.

MATERIALS AND METHODS
Animals
Female Swiss mice (*Mus musculus*) were obtained from the breeding colony maintained in the animal house of Wenzhou University, Wenzhou, China. Animals were housed individually in plastic cages (29 cm×18 cm×16 cm) with sawdust bedding and were kept at constant temperatures of 21±1°C under a 12 h:12 h (light:dark, lights on at 08:00 h) photoperiod. Food (rodent chow, D12450B; Research Diets Inc., New Brunswick, NJ, USA) and water were provided *ad libitum*. All experimental procedures complied with the Wenzhou University Animal Care and Use Committee's (WU-ACUC) guidelines and were approved by the WU-ACUC.

One hundred and thirty-two female mice were paired with males for 11 days after which the males were removed. One hundred and twenty-seven females subsequently became pregnant and gave birth. Pups were transferred between females on the day of parturition so that each female ultimately had 12 pups to raise. The day on which litter size was equalized for all females became day 0 of the experiment. Females and their pups were kept at 21°C between days 0 and 6. On day 7, females were randomly assigned to one of four treatment groups: a 21°C no-wind group (21NW, $N=30$), a 21°C wind group (21W, $N=36$), a 32.5°C no-wind group (32.5NW, $N=28$) and a 32.5°C wind group (32.5W, $N=33$). Lactating females in the 21°C groups continued to be kept at 21°C, and those in the 32.5°C groups were transferred to a room with an ambient temperature of 32.5±1°C. The females and pups in the wind groups were exposed to simulated wind generated with an electric fan placed 30 cm away from each cage (AUX FS1605; AUX Electrical Appliances Co. Ltd, Ningbo, China). The simulated wind speed averaged 2 m s^{-1}, and ranged from 1.6 to 3.3 m s^{-1} (anemometer, Testo 405-V1; Testo Instruments International Trading Ltd, Titisee-Neustadt, Germany). Females and pups in wind treatment groups were exposed to wind 24 h a day from day 7 until the end of the experiment on day 16.

Body mass and food intake
The body mass and food intake of females (21NW, $N=11$; 21W, $N=14$; 32.5NW, $N=11$; 32.5W, $N=12$) were measured daily from day 1 to day 16. Food intake was calculated as the difference between the mass of the food provided and that of the uneaten food on the following day, minus any food residues mixed with bedding material. Asymptotic food intake during the peak of lactation was calculated from the food intake from day 10 until day 16. Litter size and litter mass were measured daily from day 1 to day 16.

Energy intake and digestibility
Gross energy intake (GEI) and digestibility (21NW, $N=11$; 21W, $N=14$; 32.5NW, $N=11$; 32.5W, $N=12$) were measured between days 13 and 14 of the experiment using the food balance method described previously (Grodzinski and Wunder, 1975; Wen et al., 2017). In brief, a known quantity of food was provided, and any uneaten food and orts mixed with the bedding material were collected, together with feces, every 24 h. Food and feces were separated manually after being dried to a constant mass at 60°C, after which their gross energy content was determined using an IKA C2000 oxygen bomb calorimeter (IKA, Königswinter, Germany). GEI, gross energy of feces (GEF), digestive energy intake (DEI) and digestibility were calculated using equations described previously (Wen et al., 2017).

Resting metabolic rate
The resting metabolic rate (RMR), quantified as the rate of oxygen consumption, was measured in the females on day 17 (21NW, $N=11$; 21W, $N=14$; 32.5NW, $N=11$; 32.5W, $N=12$), with an open-flow respirometry system (PhenoMaster/LabMaster; TSE Systems, Bad Homburg, Germany). As described previously (Wen et al., 2018a,b), air was pumped through a cylindrical sealed Perspex chamber at a rate of 1000 ml min^{-1} at a temperature of 30±0.5°C (the thermal neutral zone of the mouse). Gases leaving the chamber were directed through the oxygen analyser at a flow rate of 380 ml min^{-1}. Data were collected every 10 s and analysed using standard software (TSE Systems). RMR was measured for 2.5 h. Pups were left alone for the duration of these measurements after which females were returned to their litters. RMR was calculated from the consecutive minimum rate of oxygen consumption over 10 min, corrected to standard temperature and air pressure (STP) conditions and expressed as ml O$_2$ h^{-1}.

Milk energy output of lactating females
MEO of females on days 13–14 of lactation (21NW, $N=11$; 21W, $N=14$; 32.5NW, $N=11$; 32.5W, $N=12$) was assessed from the energy budget of litters, as described previously (Król and Speakman, 2003b). As pups obtain all their energy from their mother's milk, total energy was calculated as the sum of the energy allocated for the pups' daily energy expenditure (DEE) and the growth of new tissue (Zhao et al., 2010). DEE was predicted from pup body mass on the basis of the relationship between RMR and body mass, under the assumption that DEE=1.4×RMR, to take into account the energetic costs of the pups' activity.

The equation used was (Król and Speakman, 2003b):

$$\text{MEO} = \left[(7.28 + 0.17 \times M_{\text{L}}) \times \text{CF} + M_{\text{L,inc}} \times \text{GE}_{\text{pups}}\right] \times 100/d_{\text{milk}},$$

where M_{L} (g) is the litter mass on day 13, CF is the correction factor (CF=1.4, the mean ratio of DEE to RMR) and GE$_{\text{pups}}$ (kJ g^{-1} wet mass) is the gross energy content of the pups. The mean GE$_{\text{pups}}$ values used in this formula were determined using an IKA C2000 oxygen bomb calorimeter. $M_{\text{L,inc}}$ (g day^{-1}) was the increase in litter mass between days 13 and 14, and d_{milk} was the apparent digestibility of milk (d_{milk}=96%) (Król and Speakman, 2003b; Zhao et al., 2010; Wen et al., 2017).

Body temperature of lactating females
Subcutaneous and intraperitoneal body temperature (T_{b}) were measured daily from day 4 until day 16 with encapsulated thermosensitive passive transponders (diameter 2 mm and length 14 mm; Destron Fearing, South St Paul, MN, USA).

The transponders were implanted subcutaneously in the dorsolateral hip region of females on day 4 (21NW, N=10; 21W, N=8; 32.5NW, N=10; 32.5W, N=9), according to the manufacturer's instructions. In addition, 42 females also had intraperitoneal transponders implanted (21NW, N=9; 21W, N=14; 32.5 NW, N=7; 32.5W, N=12). T_b was measured with a Pocket Reader which did not touch the females, and did not affect the behavior of the females or their pups.

Body parts

The lactating females were killed by inhaling a CO_2 overdose at the end of the experiments. Tails were removed and the length (to 0.1 cm) and weight (to 0.001 g) of each tail were recorded. The fresh pelt of each female was then removed, except for the head, limbs and tail. The liver, heart, lung, spleen, kidneys and mammary glands were also removed, as were the stomach, small and large intestine and caecum, minus their contents. All organs were weighed immediately after removal (to 0.001 g). The remaining carcass was then weighed to determine its wet mass (to 0.001 g).

Statistics

Data were analysed using SPSS statistical software (version 20.0). All variables were tested for normality with the Kolmogorov–Smirnov test, which confirmed that all data, excluding litter size, were normally distributed and were therefore appropriate for ANOVA. The effects of temperature and wind on the body mass, food intake, RMR and energy parameters, and body parts of lactating females (as well as their litter mass) were examined using a two-way ANOVA (temperature×wind) or ANCOVA with body mass or carcass mass as covariates where appropriate. ANOVA was followed by Tukey's *post hoc* tests where required. Correlation coefficients between different variables were estimated using Pearson's correlation coefficient. All data are presented as means± s.e.m. All tests were two-tailed and P-values less than 0.05 were considered statistically significant.

RESULTS

Body mass

Exposure to simulated wind did not significantly affect body mass (day 7, $F_{1,44}$=0.69, P>0.05; day 16, $F_{1,44}$=1.19, P>0.05) but the body mass of females exposed to 32.5°C was significantly lower than that of those kept at 21°C from day 7 to day 16 (day 7, $F_{1,44}$=29.99, P<0.01; day 16, $F_{1,44}$=100.34, P<0.01; Fig. 1A). There was no significant interaction effect of temperature and wind on body mass (day 16, $F_{1,44}$=2.32, P>0.05; Fig. 1A).

Food intake

Temperature had a significant effect on food intake from day 7 to day 16; lactating females at 32.5°C consumed significantly less food than those at 21°C (day 7, $F_{1,44}$=748.01, P<0.01; day 16, $F_{1,44}$=25.88, P<0.01; Fig. 1B). Temperature also significantly affected asymptotic food intake, which was 50% lower in the 32.5NW group, and 44% lower in 32.5W group, than in the respective 21°C control groups (21NW, 18.1±0.4 g day^{-1}; 21W, 21.4±0.3 g day^{-1}; 32.5NW, 9.1±0.3 g day^{-1}; 32.5W, 11.9± 0.2 g day^{-1}; $F_{1,44}$=988.29, P<0.01; Fig. 2A). Wind significantly affected food intake between days 7 and 16; females exposed to simulated wind consumed more food than those that were not (day 7, $F_{1,44}$=58.19, P<0.01; day 16, $F_{1,44}$=6.12, P<0.01; Fig. 1B). Asymptotic food intake was also significantly affected by exposure to wind, being 22.5% higher, on average, in females that were exposed to simulated wind than in those that were not ($F_{1,76}$=108.13, P<0.01; Fig. 2A). There was, however, no
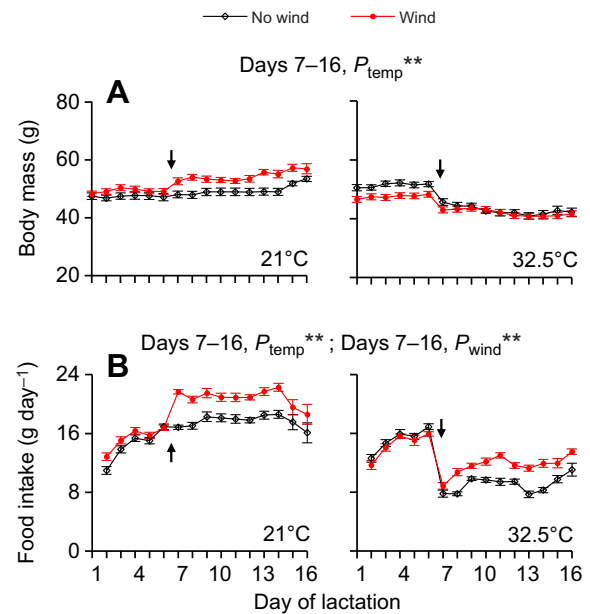

Fig. 1. Effect of a high temperature (32.5°C) and exposure to artificial wind on the body mass and food intake of lactating Swiss mice, *Mus musculus*. (A) Body mass; (B) food intake. Experimental groups: 21°C no wind, N=11; 21°C wind, N=14; 32.5°C no wind, N=11; 32.5°C wind, N=12. Arrows indicate when the temperature or wind treatments began (day 7). Wind was simulated with an electric fan; 'No wind' denotes no electric fan; **P<0.01.

significant interaction between temperature and wind with respect to either food intake (day 16, $F_{1,44}$=0.01, P>0.05; Fig. 1B) or asymptotic food intake ($F_{1,44}$=0.39, P>0.05; Fig. 2A).

Gross energy intake and digestibility

GEI was significantly affected by temperature, with that of females at 32.5°C being 51.6% lower, on average, than those kept at 21°C ($F_{1,44}$=452.24, P<0.01; Fig. 2B). GEI was also affected by wind; GEI of females exposed to wind was 23.1% higher, on average, than that of those that were not ($F_{1,44}$=39.85, P<0.01; Fig. 2B). DEI was significantly lower in females at 32.5°C ($F_{1,44}$=396.36, P<0.01; Fig. 2C), but was significantly increased by exposure to wind ($F_{1,44}$=38.16, P<0.01; Fig. 2C). The GEF of females at 32.5°C was significantly lower than that of those kept at 21°C ($F_{1,44}$=550.38, P<0.01), but was also significantly increased by exposure to wind ($F_{1,44}$=16.36, P<0.01; Fig. 2D). Digestibility was significantly higher in females exposed to wind than in those that were not ($F_{1,44}$=5.59, P<0.05; Fig. 2E). The interaction between temperature and wind had no significant effect on GEI ($F_{1,44}$=0.64, P>0.05), DEI ($F_{1,44}$=0.68, P>0.05), GEF ($F_{1,44}$=0.57, P>0.05) or digestibility ($F_{1,44}$=0.34, P>0.05).

Resting metabolic rate and milk energy output

RMR was significantly affected by temperature; that of the females kept at 32.5°C was 36.7% lower, on average, than that of those kept at 21°C ($F_{1,44}$=57.43, P<0.01; Fig. 3A). RMR was not significantly affected by wind ($F_{1,44}$=2.82, P>0.05), or by the interaction between temperature and wind ($F_{1,44}$=0.41, P>0.05).

MEO was significantly affected by temperature; that of females at 32.5°C was 25.7% lower, on average ($F_{1,44}$=74.21, P<0.01; Fig. 3B) than that of those kept at 21°C. MEO was also significantly affected by wind ($F_{1,44}$=4.32, P<0.05), and by the interaction between temperature and wind ($F_{1,44}$=6.71, P<0.05).
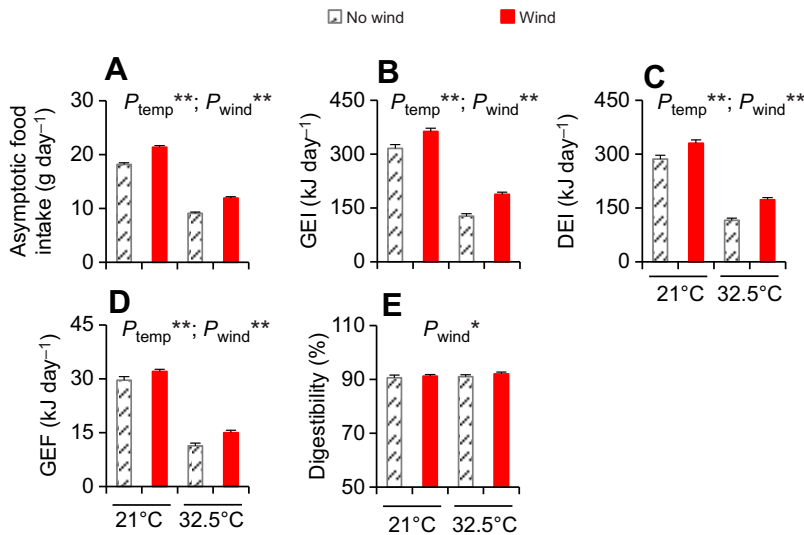
Fig. 2. Asymptotic food intake, gross energy intake, digestive energy intake, gross energy of feces and digestibility of female Swiss mice during peak lactation. (A) Asymptotic food intake; (B) Gross energy intake (GEI); (C) digestive energy intake (DEI); (D) gross energy of feces (GEF); (E) digestibility. Experimental groups: 21°C no wind, N=11; 21°C wind, N=14; 32.5°C no wind, N=11; 32.5°C wind, N=12. Wind was simulated by an electric fan; 'No wind' denotes no electric fan; *P<0.05; **P<0.01.

Although exposure to wind had no significant effect on MEO at 21°C (*post hoc*, P>0.05), it increased the MEO of females kept at 32.5°C by 20.7% (*post hoc*, P<0.05). MEO was positively correlated with body mass in both females that were exposed to wind and those that were not (Fig. 4A). There were significant positive correlations between MEO and asymptotic food intake, and between MEO and RMR (Fig. 4B,C).

Body temperature of lactating females
Intraperitoneal T_b was significantly affected by ambient temperature; that of females kept at 32.5°C was significantly higher from day 11 until day 16 than that of those kept at 21°C (day 11, $F_{1,38}$=22.28, P<0.01; Fig. 5A). Wind had a significant effect on intraperitoneal T_b from day 10 until day 12, during which females exposed to wind had lower intraperitoneal T_b than those that were not (day 10, $F_{1,38}$=11.86, P<0.05). Mean intraperitoneal T_b from day 10 to day 16 was significantly higher at 32.5°C and significantly lower in females exposed to wind (temperature, $F_{1,38}$=29.66, P<0.01; wind, $F_{1,38}$=11.04, P<0.01; Fig. 5B). The subcutaneous T_b of females kept at 32.5°C was significantly higher from day 7 to day 16 than that of those kept at 21°C (day 7, $F_{1,33}$=27.22, P<0.01; Fig. 5C). The subcutaneous T_b of females that were exposed to wind was significantly lower on day 16 than that of females that were not exposed to wind (day 16, $F_{1,33}$=4.44, P<0.05). Furthermore, the mean subcutaneous T_b of females kept at 32.5°C

was significantly higher than that of those kept at 21°C during the peak of lactation ($F_{1,33}$=264.39, P<0.01), but this difference was significantly attenuated by exposure to wind ($F_{1,33}$=7.26, P<0.01; Fig. 5D). There was no significant interaction between temperature and wind with respect to mean intraperitoneal ($F_{1,38}$=2.98, P=0.09) or subcutaneous T_b ($F_{1,33}$=3.76, P=0.07).

Litter size and litter mass
Litter size did not vary after it was equalized to 12 on day 1 (Fig. 6A). Litter mass was significantly affected by temperature from day 8 until day 16; litters raised at 32.5°C were lighter than those raised at 21°C (day 8, $F_{1,44}$=7.90, P<0.01; Fig. 6B). Litter mass at 32.5°C on day 16 was 17.0% lower, on average, than that at 21°C (day 16, $F_{1,44}$=47.59, P<0.01). Litter mass was also affected by wind between days 11–16 (day 11, $F_{1,44}$=8.76, P<0.01), and by the interaction of temperature and wind, between days 12–16
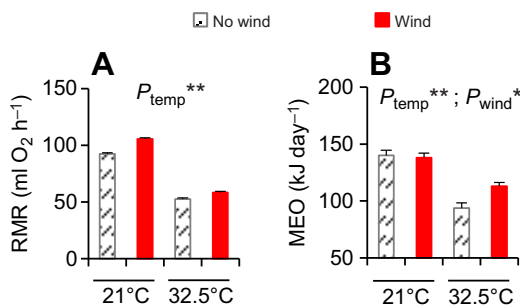

Fig. 3. Resting metabolic rate and milk energy output of lactating Swiss mice. (A) Resting metabolic rate (RMR); (B) milk energy output (MEO). Experimental groups: 21°C no wind, N=11; 21°C wind, N=14; 32.5°C no wind, N=11; 32.5°C wind, N=12. Wind was simulated by an electric fan; 'No wind' denotes no electric fan; *P<0.05; **P<0.01.
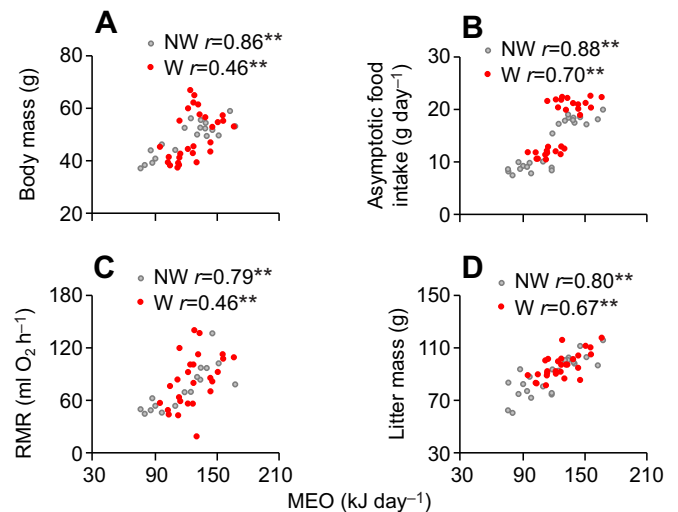

Fig. 4. Effect of exposure to artificial wind on the relationship between maternal milk energy output and body mass, asymptotic food intake, resting metabolic rate and litter mass of lactating Swiss mice. (A) Body mass; (B) asymptotic food intake; (C) resting metabolic rate (RMR); (D) litter mass. W, artificial wind simulated with an electric fan from day 7 to day 16 of the experiment (W, N=26); NW, no electric fan throughout lactation (NW, N=22); r is Pearson's correlation coefficient; **P<0.01.
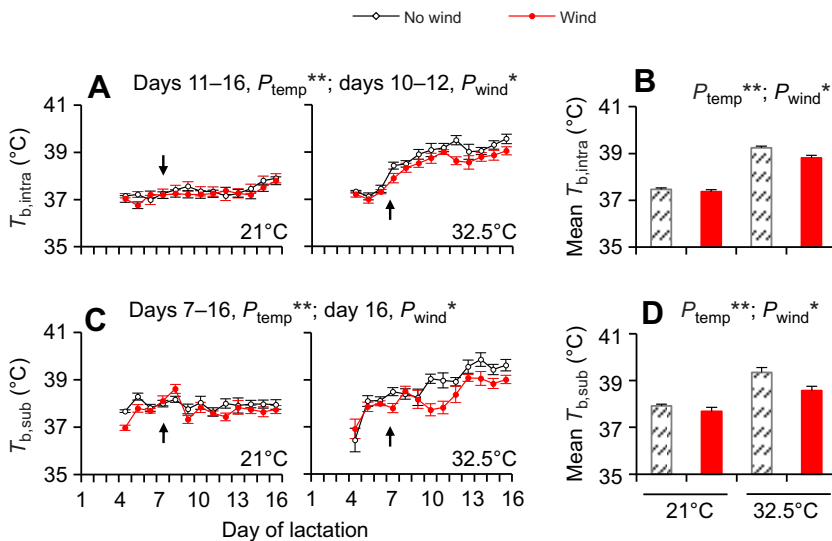
Fig. 5. Effect of high temperature (32.5°C) and exposure to artificial wind on the intraperitoneal and subcutaneous body temperatures of lactating Swiss mice. (A,B) Intraperitoneal body temperature ($T_{b,intra}$); experimental groups: 21°C no wind, N=9; 21°C wind, N=14; 32.5°C no wind, N=7; 32.5°C wind, N=12. (C,D) Subcutaneous body temperature ($T_{b,sub}$); experimental groups: 21°C no wind, N=10; 21°C wind, N=8; 32.5°C no wind, N=10; 32.5°C wind, N=9. Arrows indicate when the temperature or wind treatments began (day 7). Wind was simulated with an electric fan; 'No wind' denotes no electric fan; *P<0.05; **P<0.01.

(day 12, $F_{1,44}$=6.07, P<0.05; Fig. 6B). Interestingly, although exposure to wind did not significantly affect the litter mass of females at 21°C (*post hoc*, P>0.05), it did significantly affect that of females kept at 32.5°C; litter mass of these females was significantly greater if they were exposed to wind (*post hoc*, P<0.05). Indeed, exposure to wind increased the litter mass of the 32.5°C group by 17.6% by day 16, (*post hoc*, P<0.05). Litter mass

was positively correlated with MEO in both females that were exposed to wind and those that were not (Fig. 4D).

Pup mass was significantly affected by temperature from day 8 to day 16 (day 8, $F_{1,44}$=9.18, P<0.01); pup growth was significantly slower at 32.5°C than at 21°C (day 16, $F_{1,44}$=33.19, P<0.01; Fig. 6C). Pup mass was also significantly affected by wind between days 11 and 16 (day 11, $F_{1,44}$=5.19, P<0.05), and by the interaction of temperature and wind between days 13 and 15 (day 13, $F_{1,44}$=5.21, P<0.05). The rate of pup growth at 32.5°C was higher in the wind group than that in the no-wind groups, and pup mass on day 16 was 15.3% higher in the wind group (day 16, $F_{1,44}$=4.10, P<0.05, *post hoc*; Fig. 6C).

Body parts of the females

The carcass mass was not affected by either temperature or wind (Table S1). Fresh pelt mass was significantly affected by temperature, but not by wind; that of females kept at 32.5°C was significantly lower than that of those kept at 21°C (Table S1). Neither temperature nor wind significantly affected tail mass or length (Table S1).

The mammary gland mass was significantly affected by temperature, with that of females kept at 32.5°C being 67.2% lower, on average, compared with that of those kept at 21°C (Table S1). The mammary gland mass of females exposed to wind was 15.3% higher at 21°C, and 19.9% higher at 32.5°C, than that of females that were not exposed to wind, but this difference is not statistically significant (Table S1). The masses of the liver, heart, spleen and kidneys were significantly affected by temperature, being, on average, 29.3% (P<0.01), 12.5% (P<0.01), 34.4% (P<0.01) and 23.3% (P<0.01) lower, respectively, in females kept at 32.5°C than in those kept at 21°C (Table S1). The masses of the stomach and the small and large intestine were also affected by temperature, being, on average, 14.6%, 36.7% and 35.1%, lower, respectively, in females kept at 32.5°C than in those kept at 21°C (Table S1). The mass of the gastrointestinal tract was not, however, significantly affected by wind.

Asymptotic food intake was positively correlated with the mass of most organs associated with energy intake and milk output (Fig. S1) and exposure to wind did not change the relationship between food intake and the mass of most organs or body parts. MEO was significantly and positively correlated with the mass of certain organs, including the fresh pelt, mammary glands, liver, spleen,
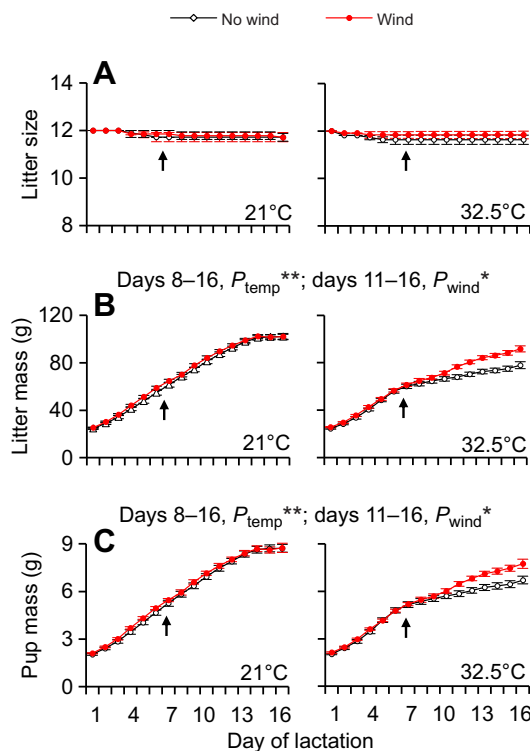

Fig. 6. Effect of high temperature (32.5°C) and exposure to artificial wind on the litter size, litter mass and mean pup mass of lactating Swiss mice. (A) Litter size; (B) litter mass; (C) mean pup mass. Experimental groups: 21°C no wind, N=11; 21°C wind, N=14; 32.5°C no wind, N=11; 32.5°C wind, N=12. Arrows indicate when the temperature or wind treatments began (day 7). Wind was simulated with an electric fan; 'No wind' denotes no electric fan; *P<0.05; **P<0.01.

kidneys and gastrointestinal tract (Fig. S2). These correlations were not significantly changed by exposure to wind.

DISCUSSION

It is generally accepted that exposure to high temperature (heatwaves) has an adverse impact on maximum energy intake and MEO in a variety of animals. Our results show that female Swiss mice lactating at 32.5°C consumed significantly less food and produced less milk, and consequently raised lighter offspring than those kept at 21°C, indicating that the limitations on sustained energy intake and reproductive output were more severe and considerable at the hot temperature. Interestingly, we observed that exposure to wind improved the reproductive performance of lactating mice, in particular those kept at 32.5°C. This suggests that wind, a pervasive environmental factor, could significantly ameliorate the reduction in food intake and milk production caused by high ambient temperatures.

We exposed both lactating females and their pups to the artificial wind 24 h a day to maximize their ability to lose heat through convection and evaporation and thereby any consequent wind effects on energy intake and milk output. Lactating females exposed to wind consumed significantly more food than those that were not; the asymptotic food intake of females exposed to artificial wind was 22.5% (*P*<0.01) greater than that of controls, and females exposed to wind also had higher gross and digestive energy intake and produced more feces. Wind promotes heat dissipation by convection and evaporation (Porter and Gates, 1969; Winne et al., 2001; Tracy and Christian, 2005; Kearney and Porter, 2009; Ortega et al., 2017), allowing females lactating under hot ambient temperatures to dissipate more body heat. Exposure to wind could therefore decrease the risk of hyperthermia, thereby allowing females to consume more food and produce more milk.

The increased energy intake of lactating females is mainly to compensate for MEO (Hammond and Diamond, 1992, 1997; Speakman and Król, 2005, 2011; Sadowska et al., 2016; Kagya-Agyemang et al., 2018). We found that the MEO of females lactating at 32.5°C was considerably lower than at 21°C. The reduced milk production of females at 32.5°C resulted in these females having significantly lighter litters than females kept at 21°C. Reduced milk production has been also observed in many other mammals lactating under warm or hot ambient temperatures, including laboratory mice (*Mus musculus*; Król and Speakman, 2003a,b), 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), the golden hamster (*Mesocricetus auratus*; Ohrnberger et al., 2018), 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). This suggests that the capacity to dissipate body heat is significantly reduced by higher ambient temperatures resulting in considerable reductions of both energy intake and milk output.

We found that exposure to wind increased the MEO of females at 32.5°C by 20.7% (*post hoc*, *P*<0.05) and that the litters of females exposed to wind were 17.6% heavier (*post hoc*, *P*<0.05) than those of females that were not. Because both females and their pups were exposed to artificial wind throughout lactation, any effect on maternal energy budgets could have been due to increased heat

dissipation by their young. In other words, pups exposed to artificial wind may have increased their milk intake to compensate for greater heat loss. Because we calculated the MEO of lactating females from the energy budget of their pups, we may have underestimated the MEO of pups exposed to wind. Therefore, the actual MEO of females exposed to wind could be slightly higher than that estimated from the energy budget of their litter. Our results suggest that exposure to wind improves the capacity to dissipate body heat, thereby allowing females to consume more food and increase milk production under a relatively hot ambient temperature. It appears that the exposure to wind has positive effects on reproductive performance, and could potentially increase the fitness of females lactating under hot ambient temperatures.

Unlike females kept at 32.5°C, those that were exposed to wind at 21°C did not significantly change their MEO. One possible explanation for this is that 21°C constitutes not thermoneutrality but cold exposure for mice. Thus, if additionally exposed to cold wind, females may face an energy allocation problem. If more energy is required for thermogenesis (they had indeed higher food intake) less can be allocated to milk production. Another possible explanation for this is provided by the peripheral limitation hypothesis which proposes that milk production is limited by the capacity of the mammary glands to produce milk (Hammond and Diamond, 1992, 1997; Hammond et al., 1996; Hammond and Kristan, 2000; Speakman and Król, 2005, 2011; Zhao et al., 2010). From this perspective, the mammary glands of female mice could probably already have been operating at maximum capacity at 21°C, making it impossible for females to further increase their milk production (Hammond et al., 1996; Speakman and Król, 2011; Wen et al., 2017). This suggests that the effect of wind on reproductive performance could be temperature dependent.

We found that the mass of most organs was positively correlated with asymptotic food intake and milk output. This suggests that the morphology of the alimentary tract and associated organs changed to accommodate the increased food intake and energy output required for lactation (Speakman and McQueenie, 1996). We also found that most organs of females kept at 32.5°C were significantly lighter than those of females kept at 21°C. This is consistent with the reduced food intake and MEO of females at 32.5°C. Unexpectedly, the mass of most body parts was not affected by wind; the relationship between the mass of most body parts and asymptotic food intake or MEO was not significantly different between mice that were exposed to wind and those that were not. This indicates that exposure to wind does not affect the role of the alimentary tract and associated organs in balancing the energy budget during lactation.

Conclusion

Our results demonstrate that the asymptotic food intake and MEO of female Swiss mice were considerably lower at 32.5°C than at 21°C. Litters raised at 32.5°C were also significantly lighter than those raised at 21°C. This suggests that high ambient temperatures have adverse impacts on maximum energy budget of lactating small mammals by reducing their capacity to dissipate body heat, providing support for the heat dissipation limit hypothesis. Exposure to wind considerably increased the reproductive performance of lactating mice, particularly at 32.5°C. This suggests that wind improves the capacity to dissipate heat at higher ambient temperatures, allowing the females to consume more food and produce more milk under these otherwise adverse conditions. Exposure to wind could therefore significantly improve the reproductive output and fitness of female mammals under hot

ambient temperatures, potentially counteracting the adverse effects of global warming on milk production and litter mass.

Supplementary information

Supplementary information available online at
https://jeb.biologists.org/lookup/doi/10.1242/jeb.231415.supplemental

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Supplementary materials

Table S1 Mass of selected body parts of lactating Swiss mice (*Mus musculus*) exposed to 32.5°C and a simulated wind treatment.

	21°C		32.5°C		P_{tem}	P_{wind}	P_{tem} x P_{wind}
	No-Wind	Wind	No-Wind	Wind			
Carcass (g)	18.930±0.528	19.490±0.459	19.304±0.588	17.771±0.463	NS	NS	NS
Fresh pelt (g)	4.245±0.091	4.489±0.154	3.322±0.144	3.082±0.084	**	NS	NS
Tail length (cm)	9.994±0.296	10.279±0.198	8.914±0.628	10.338±0.231	NS	NS	NS
Tail mass (g)	0.831±0.030	0.864±0.028	0.891±0.046	0.888±0.037	NS	NS	NS
MG (g)	6.766±0.839	7.804±0.473	2.173±0.184	2.605±0.214	**	NS	NS
Liver (g)	4.115±0.166	4.429±0.124	2.894±0.163	2.461±0.077	**	NS	NS
Heart (g)	0.299±0.013	0.327±0.015	0.281±0.018	0.230±0.008	**	NS	NS
Lung (g)	0.270±0.011	0.324±0.017	0.296±0.024	0.296±0.049	NS	NS	NS
Spleen (g)	0.291±0.027	0.279±0.018	0.170±0.022	0.122±0.018	**	NS	NS
Kidneys (g)	0.647±0.016	0.745±0.011	0.546±0.011	0.492±0.015	**	NS	**
Stomach (g)	0.403±0.018	0.438±0.017	0.305±0.018	0.307±0.019	**	NS	NS
SI (g)	2.244±0.209	2.451±0.133	1.340±0.121	1.210±0.095	**	NS	NS
LI (g)	0.530±0.047	0.595±0.040	0.315±0.029	0.276±0.017	**	NS	NS
Caecum (g)	0.251±0.038	0.264±0.031	0.208±0.042	0.180±0.036	NS	NS	NS

Females were exposed to 32.5°C and wind treatment from Day 7 to 16 of lactation (21°C-NW, *n*=11; 21°C-W, *n*=14; 32.5°C- NW, *n*=11; 32.5°C-W, *n*=12). MG = mammary glands; SI = small intestine; LI = large intestine. Data are means ± s.e.m. P_{tem} = the effect of temperature; P_{wind} = the effect of wind; *P<0.05; **P<0.01; NS = not significant (P>0.05).
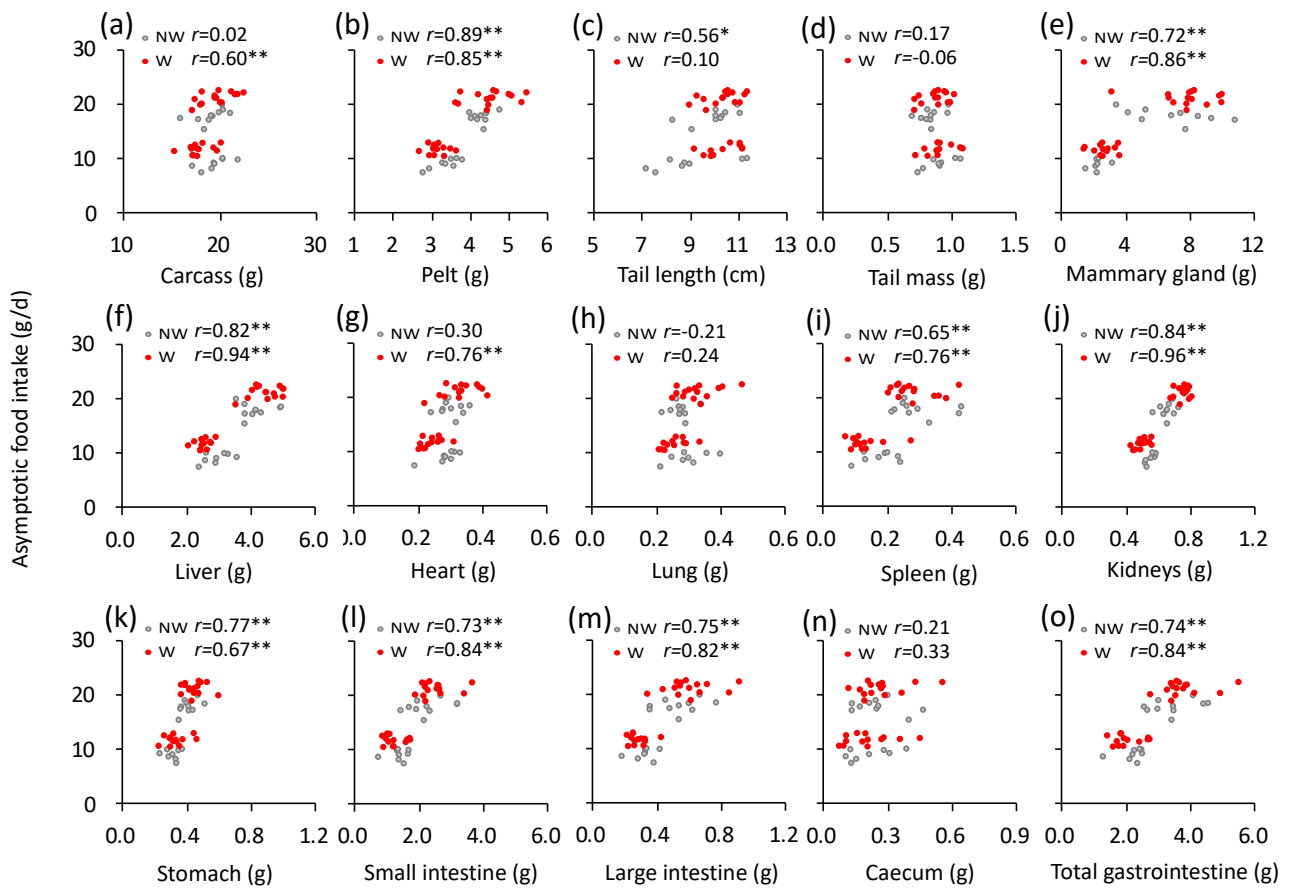
Figure S1 Relationships between asymptotic food intake and carcass mass (a), fresh pelt mass (b), tail length (c), tail mass (d), mammary gland mass (e), liver mass (f), heart mass (g), lung mass (h), spleen mass (j) kidney mass (i), stomach mass (k), small and large intestine mass (l and m), caecum mass (n) and total gastrointestinal mass (o), of lactating Swiss mice (21°C-NW, *n*=11; 21°C-W, *n*=14; 32.5°C- NW, *n*=11; 32.5°C-W, *n*=12). W = artificial wind simulated with an electric fan from Day 7 to 16; NW = no artificial wind treatment. *P<0.05; **P<0.01.
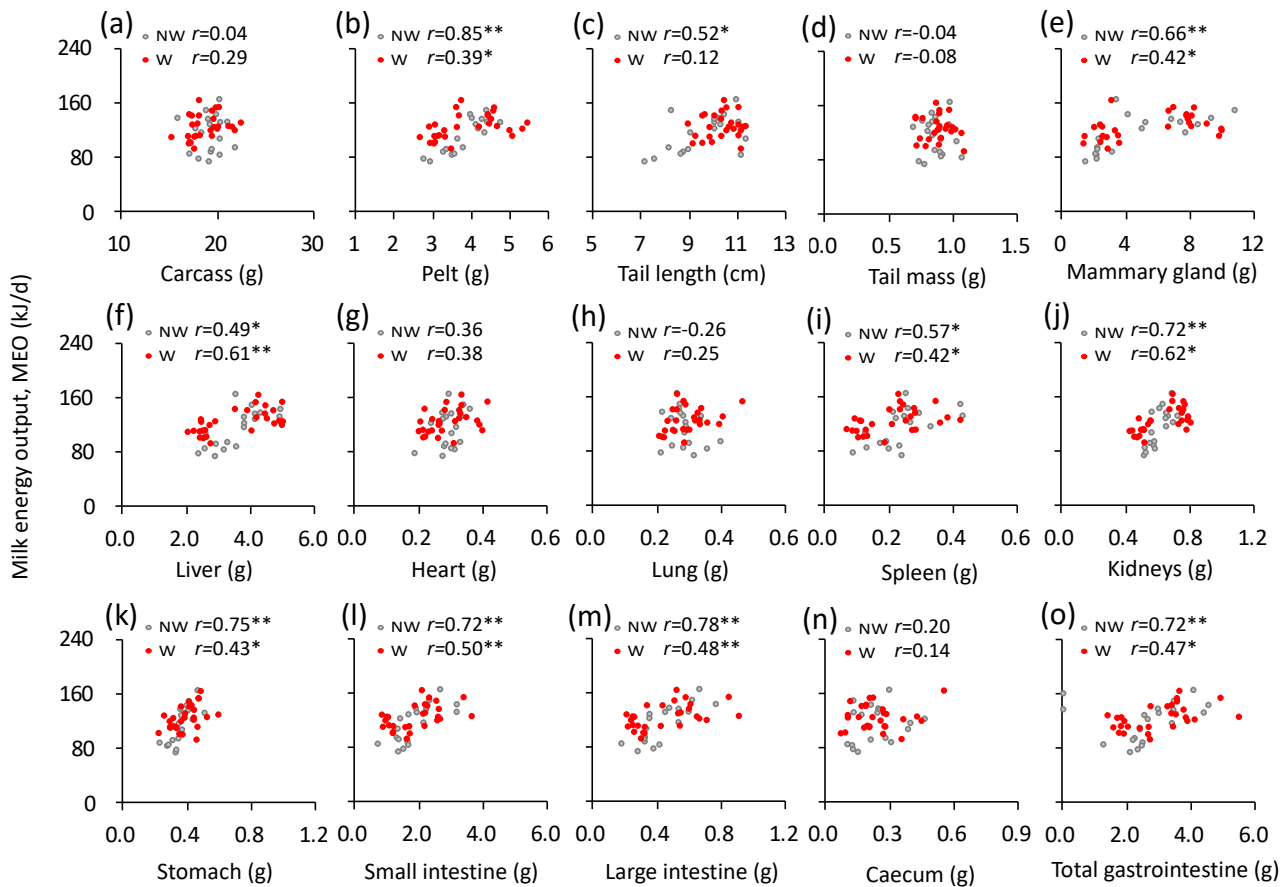
Figure S2 Relationships between milk energy output (MEO) and carcass mass (a), fresh pelt mass (b), tail length (c), tail mass (d), mammary gland mass (e), liver mass (f), heart mass (g), lung mass (h), spleen mass (j) kidney mass (i), stomach mass (k), small and large intestine mass (l and m), caecum mass (n) and total gastrointestinal mass (o), of lactating Swiss mice (21°C-NW, n=11; 21°C-W, n=14; 32.5°C- NW, n=11; 32.5°C-W, n=12). W = artificial wind simulated with an electric fan from Day 7 to 16; NW = no artificial wind treatment. *P<0.05; **P<0.01.