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RESEARCH ARTICLE

Limits to sustained energy intake. XXXI. Effect of graded levels of dietary fat on lactation performance in Swiss mice

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ABSTRACT

The heat dissipation limit theory predicts that lactating female mice consuming diets with lower specific dynamic action (SDA) should have enhanced lactation performance. Dietary fat has lower SDA than other macronutrients. Here we tested the effects of graded dietary fat levels on lactating Swiss mice. We fed females five diets varying in fat content from 8.3 to 66.6%. Offspring of mothers fed diets of 41.7% fat and above were heavier and fatter at weaning compared with those of 8.3 and 25% fat diets. Mice on dietary fat contents of 41.7% and above had greater metabolizable energy intake at peak lactation (8.3%: 229.4±39.6; 25%: 278.8±25.8; 41.7%: 359.6±51.5; 58.3%: 353.7±43.6; 66.6%: 346±44.7 kJ day^{-1}), lower daily energy expenditure (8.3%: 128.5±16; 25%: 131.6±8.4; 41.7%: 124.4±10.8; 58.3%: 115.1±10.5; 66.6%: 111.2±11.5 kJ day^{-1}) and thus delivered more milk energy to their offspring (8.3%: 100.8±27.3; 25%: 147.2± 25.1; 41.7%: 225.1±49.6; 58.3%: 238.6±40.1; 66.6%: 234.8± 41.1 kJ day^{-1}). Milk fat content (%) was unrelated to dietary fat content, indicating that females on higher fat diets (>41.7%) produced more rather than richer milk. Mothers consuming diets with 41.7% fat or above enhanced their lactation performance compared with those on 25% or less, probably by diverting dietary fat directly into the milk, thereby avoiding the costs of lipogenesis. At dietary fat contents above 41.7% they were either unable to transfer more dietary fat to the milk, or they chose not to do so, potentially because of a lack of benefit to the offspring that were increasingly fatter as maternal dietary fat increased.

KEY WORDS: Asymptotic food intake, Graded dietary fat levels, Heat dissipation limitation, Laboratory mouse, Milk production

INTRODUCTION

The maximum rate of energy intake that animals can sustain over protracted periods of time (also called sustained energy intake, SusEI) plays a key role in setting physiological upper boundaries that affect many aspects of animal and human performance, including reproductive output and thermoregulatory capabilities (Drent and Daan, 1980; Peterson et al., 1990; Weiner, 1992; Hammond and Diamond, 1997; Speakman and Król, 2005b; Thurber et al., 2019). Lactation is the most energetically expensive period for female mammals, particularly in smaller species (Speakman, 2008). Limits to SusEI at peak lactation are important because they may determine the total investment that females can contribute to their offspring, and may therefore define maximum litter sizes and pup growth (Johnson et al., 2001a,b).

Explanations of the limits on female lactation performance are disputed. The 'central limitation' hypothesis suggests that the limits are imposed by the uptake capacity of the energy-supplying machinery (such as the alimentary tract and associated organs) (Perrigo, 1987; Hammond and Diamond, 1992, 1994; Koteja, 1996; Thurber et al., 2019; Sadowska et al., 2019). More recent evidence in small mammals tends to support the 'peripheral limitation' or 'heat dissipation limitation' (HDL) theories. The 'peripheral limitation' hypothesis suggests that the capacities of the mammary gland to produce milk set the limitation (Hammond et al., 1996; Rogowitz, 1998; Hammond and Kristan, 2000). The HDL theory suggests that females are constrained by the maximal capacity to dissipate body heat generated as a by-product of processing food and producing milk (Wu et al., 2009; Simons et al., 2011; Yang et al., 2013; Sadowska et al., 2016). One reason why lactating females may face problems in dissipating heat is because of the surrounding pups when they are nursing. Both the pups and the nest may affect their ability to dissipate heat as suggested in lactating rats (Croskerry et al., 1978; Leon et al., 1978). However, this effect appears to be unimportant in mice (Gamo et al., 2016). Furthermore, an interaction of heat dissipation and peripheral limitation was also supported by several studies, suggesting that the limitation is dominated by different factors under different ambient temperature conditions (Speakman and Król, 2011; Zhao et al., 2016; Wen et al., 2017). An alternative 'trade-off' idea suggests that mammals may not maximise their lactation performance under all conditions, particularly if maximizing performance during the present reproduction would have a detrimental effect on their future reproductive performance or survival (Speakman and Król, 2005b; Piersma, 2011; Vaanholt et al., 2018).

Previous studies in MF1 mice showed that milk production and pup growth were enhanced at cold ambient temperatures and reduced at 30°C, strongly supporting the HDL theory (Johnson and Speakman, 2001; Krol and Speakman, 2003; Krol et al., 2007). However, Swiss mice, Brandt's voles and Mongolian gerbils did not show the same response under cold conditions, supporting the peripheral limitation idea (Zhang and Wang, 2007; Zhao and Cao, 2009; Zhao et al., 2010, 2013; Yang et al., 2013). In hot conditions, reproductive performance in Swiss mice is probably also constrained by the capacity of body heat dissipation, suggesting that the constraints seem to change with the ambient temperature in this strain (Zhao et al., 2016). Moreover, surgically removing half of the mammary glands in Swiss mice had an impact on pup growth

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List of symbols and abbreviations

BAT	brown adipose tissue
DLW	doubly labelled water
DEE	daily energy expenditure
FI	food intake
GE_{feces}	the gross energy lost in feces
GE_{food}	the gross energy content of the food
GLM	general linear models
HDL	heat dissipation limitation
HF	high fat
LF	low fat
MEI	metabolizable energy intake
MEO	milk energy output
MF	medium fat
M_{feces}	dry mass of feces produced
M_B	maternal body mass
M_{food}	mass of food intake
M_L	litter mass
M_{pup}	pup mass
RM GLM	repeated measures general linear models
SDA	specific dynamic action
SUB	subcutaneous fat
SusEI	sustained energy intake

(Hammond et al., 1996), which also supports the idea that the mammary gland imposes the limit on milk production capacity at room temperature in this strain. Another study in artificially selected Swiss mice (high and low basal metabolic rate lines) showed that the lactation performance of both lines did not benefit from increasing their thermal conductance at peak lactation by fur removal (Sadowska et al., 2019). This also suggested that the limit was not imposed by heat dissipation capacity, but rather by the spare capacity of the alimentary tract and other organs to respond to such sudden energy demand (Sadowska et al., 2019).

Overall, the current data suggest that different species and strains are probably impacted by different limitations at different ambient temperatures. Contrasting the situation in Swiss mice, MF1 mice probably have higher maximum milk production capacity relative to their capacity to dissipate body heat, resulting in a consistent limitation by their heat dissipation capacity, and hence when the ability to dissipate heat was elevated by cold exposure, milk production was increased (Speakman and Król, 2011).

Elevated heat production during lactation may stem from two main sources: the processes associated with digestion, assimilation and biosynthesis [specific dynamic action (SDA)], and heat generated during milk synthesis (Kagya-Agyemang et al., 2018). Diets with different macronutrient contents have different SDA (Secor, 2009; Kagya-Agyemang et al., 2010). High-carbohydrate and high-protein content diets have higher SDA than high-fat content diets (Kagya-Agyemang et al., 2010). A previous study in MF1 mice showed that milk production and pup growth at room temperature (21°C) was enhanced when the mothers were fed diets with 45 and 60% fat compared with those fed 10% fat (Kagya-Agyemang et al., 2018).

It was suggested that these MF1 mice were able to overcome the heat dissipation limit at 21°C because they were able to transfer fatty acids from the high-fat diets directly into the milk, thereby avoiding the heat generated from lipogenesis. As Swiss mice at the same temperatures are suggested to be limited by capacity of the mammary glands (Hammond et al., 1996; Zhao et al., 2016), the effects of dietary fat may be different in this strain. In this study therefore, we aimed to evaluate the impact of diets differing in fat content on lactating performance in Swiss mice at 23°C.

MATERIALS AND METHODS

Animals and experimental design

All animal experiments were approved by the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences (IGDB-CAS) Institutional Animal Care and Use Committee (IACUC) (approval number: AP2016018). Female and male Swiss mice were purchased at 6 weeks of age (Charles River, Beijing, China). All animals were housed in rooms kept at 23±1°C with a 12 h:12 h dark:light cycle (lights on at 07.30 h). Female mice were housed (five mice per cage) together until 9 weeks old and then singly housed for 1 week before males were introduced. Females were fed with a standard low-fat chow diet (crude fat ≥4% by weight, crude protein ≥20% by weight; Huafukang Bioscience, Beijing, China) before the controlled diets were introduced. Five batches of female mice (n=14 per batch) were randomly allocated into five dietary groups (n=14 per group initially): 8.3% energy from fat (D14071619, Research Diets, New Brunswick, NJ, USA), 25% energy from fat (D14071620), 41.7% energy from fat (D14071622), 58.3% energy from fat (D14071623) and 66.6% energy from fat (D14071624). All diets had constant contents of cellulose (5% by weight), sucrose (5% by energy) and protein (25% by energy). The source of fat was a mix of cocoa butter, coconut oil, menhaden oil, palm oil and sunflower oil (for further details, see Hu et al., 2018). The protein source was casein, and the balance was made up by carbohydrate (corn starch and maltodextrin 10). All diets were supplemented with a standard vitamin and mineral mix. Throughout pregnancy, mice continued to feed on the baseline diet. Seven females did not become pregnant, reducing the final sample sizes to 13, 12, 14, 11 and 13 in each group, respectively. Litter size was manipulated on lactation day 1 (the day after birth; Johnson et al., 2001a) to 10 pups per litter, with all the pups in each litter cross-fostered among different dams, to reduce the variation due to litter size effects. Previous work suggests that at litter sizes below 10, females do not work at the sustained maximal limit (Johnson et al., 2001a). The experimental diets were introduced on lactation day 1. Maternal body mass (M_B) and food intake (FI) were measured daily from the point the males were removed. The litter mass (M_L) and litter size were measured daily from lactation day 1.

Body fat content

The total *in vivo* body fat content of the females was evaluated by magnetic resonance spectroscopy (EchoMRI, Houston, TX, USA) the day before males were introduced, on lactation days 1 and 10 and on the weaning day (around days 17–22 of lactation depending on pup size). The total *in vivo* body fat content of the litters was also evaluated on the weaning day.

Daily energy expenditure and milk energy output

Feces produced by female mice during lactating days 14–16 were collected, separated from the bedding manually and oven-dried at 60°C to a constant mass (14 days). The calorific values of feces were determined by a Parr 1281 oxygen bomb calorimeter (Parr Instrument Company, Moline, IL, USA). Samples of each diet were also weighed and dried to a constant mass to obtain dry mass. The water content of the diets was measured to correct the FI. Metabolizable energy intake (MEI) was calculated as below (Kagya-Agyemang et al., 2018):

$$MEI=(M_{food}\times GE_{food})-(M_{feces}\times GE_{feces}),$$

where M_{food} is the dry mass of FI in g day^{-1}, M_{feces} is the dry mass of feces produced in g day^{-1}, GE_{Food} is the gross energy content of the food (KJ g^{-1}) and GE_{feces} is the gross energy lost in feces (KJ g^{-1}).

Journal of Experimental Biology (2020) 223, jeb221911. doi:10.1242/jeb.221911

The doubly labelled water (DLW) method (Butler et al., 2004) was used to measure daily energy expenditure (DEE) from the elimination rates of ^{2}H (deuterium) and ^{18}O in lactating females during peak lactation. Measures of DEE were made to determine the milk energy output (MEO) from the difference between MEI and DEE (Krol and Speakman, 2003). The DLW measurements were conducted on days 14–16 of lactation. In our previous studies we have shown that FI increases until about day 10–11, and then reaches an asymptote (Johnson et al., 2001a). After about day 17, the pups start to access the solid food and so days 14–16 represent peak lactation where the animals are working at their sustainable maximum. Individual mice were weighed to ±0.01 g using a balance (BSA2202S; Sartorius, Göttingen, Germany) and labelled with an intraperitoneal injection of approximately 0.1 g of water containing enriched ^{2}H (36.3 atoms%) and ^{18}O (59.9 atoms%). Syringes used to inject the DLW were weighed (±0.001 g; JA2003N; Hangping, Shanghai, China) immediately before and after the injection to provide an accurate measurement of the amount of isotope injected. Mice were placed in their cages during the 1 h equilibration period. An initial 30–80 µl blood sample was collected by tail tipping 1 h after the injection (Krol and Speakman, 1999). Blood samples were immediately flame-sealed into pre-calibrated 50 µl capillaries. A final blood sample was collected 48 h after the initial blood sample to estimate isotope elimination rates. Samples of blood in capillaries were vacuum-distilled (Nagy, 1983). A liquid water analyser (Berman et al., 2013; Los Gatos Research, Mountain View, CA, USA) was used to analyse the isotope ratios of ^{18}O:^{16}O and ^{2}H:^{1}H. The samples were run alongside a range of international and in-house standards that were used to correct the raw data for daily machine variation. For each lactating mouse, initial ^{2}H and ^{18}O dilution spaces were calculated by the intercept method and then converted to mass, assuming a molecular mass of body water of 18.02 and expressed as a percentage of body mass before injection. The intercept method was used as the actual body water pool estimated by desiccation using the intercept method is more accurate than the plateau method in small mammals (Speakman and Król, 2005a). The final ^{2}H and ^{18}O dilution spaces were inferred from the final body mass, assuming the same percentage of body mass as measured for the initial dilution spaces. For calculation of DEE based on CO_2 production, single pool model Eqn 7.17 (Speakman, 1997) was used as recommended for small mammals (Speakman, 1993). Energy equivalents of rates of CO_2 production were calculated using a conversion factor of 24.03 J ml^{-1} CO_2, derived from the Weir equation (Weir, 1949). Female total water turnover was calculated by multiplying the fractional turnover rate by the total body water ($k_d \times N_d$). It was assumed that 25% of the water leaving the body was fractionated (Speakman, 1997). Therefore, a fractionation factor of 0.9366 was applied for deuterium turnover (Speakman, 1997). This approach assumes that rates of water influx and efflux are constant, so the water turnover rate r (H_2O)=total water influx=total water efflux (Nagy and Costa, 1980).

Milk collection and milk fat extraction

Milk was collected from each female on day 17 of lactation. After separating from pups for approximately 3 h, the female was injected with 0.2 ml of oxytocin (20 USP ml^{-1} i.p.) and was anaesthetized with light isoflurane. A 100 µl capillary tube was used to collect 150–200 µl milk per mouse. Milk was placed in a 1.5 ml centrifuge tube after collection and stored at −80°C until further analysis. Milk crude fat was measured based on a miniaturized Röse-Gottlieb method (Görs et al., 2009). A 100 µl sample of milk (samples below 100 µl were pooled, leading to the final samples size of 5, 6, 8, 7 and 8 in each group) was weighed and diluted with 900 µl ddH_2O in 15 ml pre-combusted glass tubes. Subsequently, 200 µl NH_3 solution (25–28%), 1 ml ethanol, 3 ml diethyl ether, 3 ml petroleum ether (boiling point 30–60°C) and 800 µl ddH_2O were added, and each step was shaken vigorously for 30 s. After standing for 30 min and complete separation, the lipid layer was measured and 4 ml of the supernatant was transferred into a pre-combusted and pre-weighed glass vial and evaporated by a boiling water bath. The residue was dried for 2 h at 105°C, cooled and weighed to determine the fat percentage. All the samples were weighed in triplicate on a ±0.0001 g balance (Mettler Toledo ME204, Zurich, Switzerland)

Organ morphology

After weaning (around days 17–22 of lactation depending on pup sizes), the animals (mother, one male pup and one female pup from each litter) were fasted for 3–4 h and sacrificed by CO_2 overdose. The brain, intrascapular brown adipose tissue (BAT), subcutaneous fat (SUB) with mammary gland, mesenteric fat, gonadal fat, retroperitoneal fat, heart, liver, kidneys, pancreas, stomach, spleen, small intestine, caecum, colon, uterus and ovaries for mothers were immediately dissected and weighed on a ±0.001 g balance (JA2003N; Hangping). The brain, BAT, SUB, mesenteric fat, heart, liver, lungs, kidneys, pancreas, stomach, spleen, small intestine, caecum and colon were also removed and weighed for male and female pups.

Behavior observations

Behavior observations were conducted on individual mothers during early lactation (days 4–6), mid-lactation (days 8–10) and late lactation (days 12–14), and classified into seven activities: climbing (C), drinking (D), eating (E), general activities (GA), resting (R), grooming (G) and feeding pups (FP) (Gamo et al., 2016). Feeding pups was when the pups were attached to the mother inside or outside the nest. It was common to observe the mother feeding the pups and conducting other activities simultaneously, such as eating or grooming. This was most common for eating, hence we created a new activity denoting feeding the pups and simultaneously eating (FP/E). General activity was considered as any other physical activity different from the previous mentioned behaviors. Lactating mice were housed in transparent cages and visually observed on one occasion during the specified time window for early, mid- or late lactation. Observations were conducted for 10 s each minute for 100 min per day during the light phase. The activity first observed during the 10 s was recorded: focal time sampling.

Statistical analyses

Differences in M_B, FI, litter/pup mass and maternal body fat content during experiments were tested using repeated measures general linear models (RM GLM) with maternal diet as the fixed factor, and day of lactation as the repeated factor. Body fat content of weaned offspring was tested using GLM with maternal diet as a fixed factor. Changes in MEI, DEE and MEO between dietary groups were compared using GLM with diet as fixed factor and M_B as a covariate (Tschöp et al., 2012); interaction between the fixed factor and the covariate was also tested. Organ morphology changes between dietary groups were also conducted using GLM (fixed factor: diet, covariate: M_B). If the result showed no significant effects while including the interaction or covariate effect, significance analysis of the fixed factor was analysed individually. If found, the effects by

Journal of Experimental Biology (2020) 223, jeb221911. doi:10.1242/jeb.221911

the interaction or covariate would be taken into consideration. Where significant effects of diet were found, *post-hoc* Tukey's tests were used to assess differences between groups. Data are represented as means±s.d. All data were tested for normality prior to analysis; if not normally distributed, Kruskal–Wallis tests with Boniferroni correction were performed. All statistical analyses were performed using IBM SPSS Statistics for Macintosh (version 24).

RESULTS

Maternal body mass and food intake

There were no significant differences observed between maternal M_B of the five dietary groups before mating (ANOVA, $F_{4,58}$=2.395, P=0.061), during pregnancy (15 days before parturition) (RM GLM, $F_{4,58}$=0.727, P=0.577) and during lactation days 1–9 (RM GLM, $F_{4,58}$=2.17, P=0.084) (Fig. 1A; Table 1). A highly significant effect of day of lactation (RM GLM, $F_{6,346}$=5.193, P<0.001) and diet ($F_{4,58}$=13.648, P<0.001) on maternal M_B was observed during lactating days 10–17 (Fig. 1A). The females fed diets with 41.7% fat and above had significantly higher M_B than those fed a 8.3% fat diet, and the mice fed a 66.6% fat diet had significantly higher M_B than those fed a 25% fat diet (*post-hoc* Tukey's test: P<0.05).

A significant difference between dietary groups was observed in the maternal gross FI before mating (ANOVA, $F_{4,58}$=4.66, P=0.002), but this difference disappeared during pregnancy (7 days before parturition) (RM GLM, $F_{4,58}$=2.142, P=0.087) (Fig. 1B). RM GLM over lactating days 1–17 showed that there was a highly significant effect of day of lactation ($F_{15,860}$=79.284, P<0.001), day×diet ($F_{59,860}$=22.909, P<0.001) and diet ($F_{4,58}$=11.259, P<0.001) on maternal gross FI. Between days 1 and 11 of lactation, FI increased steadily in all the dietary groups and reached an asymptote over the next 6 days (days 12–17) (Fig. 1B).

Gross energy of food ingested (GE_{food}) was 15.88, 17.56, 19.23, 21.74 and 23.00 KJ g^{-1} for 8.3, 25, 41.7, 58.3 and 66.6% fat diets, respectively. Significant effects of day of lactation (RM GLM, $F_{14,836}$=81.611, P<0.001), day×diet ($F_{58,836}$=4.979, P<0.001) and diet ($F_{4,58}$=32.537, P<0.001) were also observed in GE_{food} over days 1–17 of lactation. Mothers fed a 41.7% fat diet and above had higher daily energy intake than those of 25% fat diets and below. No significant differences were observed between the groups fed the 41.7% fat diet and above, or 25% fat diet and below. The asymptotic energy intake level in females fed 8.3, 25 and 41.7% fat diets were significantly increased in line with their fat levels, but there was no further increase in the groups fed 41.7% fat and above (ANOVA, $F_{4,58}$=41.837, P<0.001) (Fig. 1C; Table 1).

Litter mass and pup mass

Despite the female mice occasionally culling pups during lactation, mice on all diets weaned a similar number of pups (ANOVA, $F_{4,58}$=1.371, P=0.225) (Table 1). RM GLM over lactating days 1–17 showed there were significant differences in litter mass (M_L) between maternal dietary groups: the pups raised by mothers fed 41.7% fat diet and above had significantly larger M_L than those fed 25% fat diet and below (diet: $F_{4,58}$=21.72, P<0.001; day of

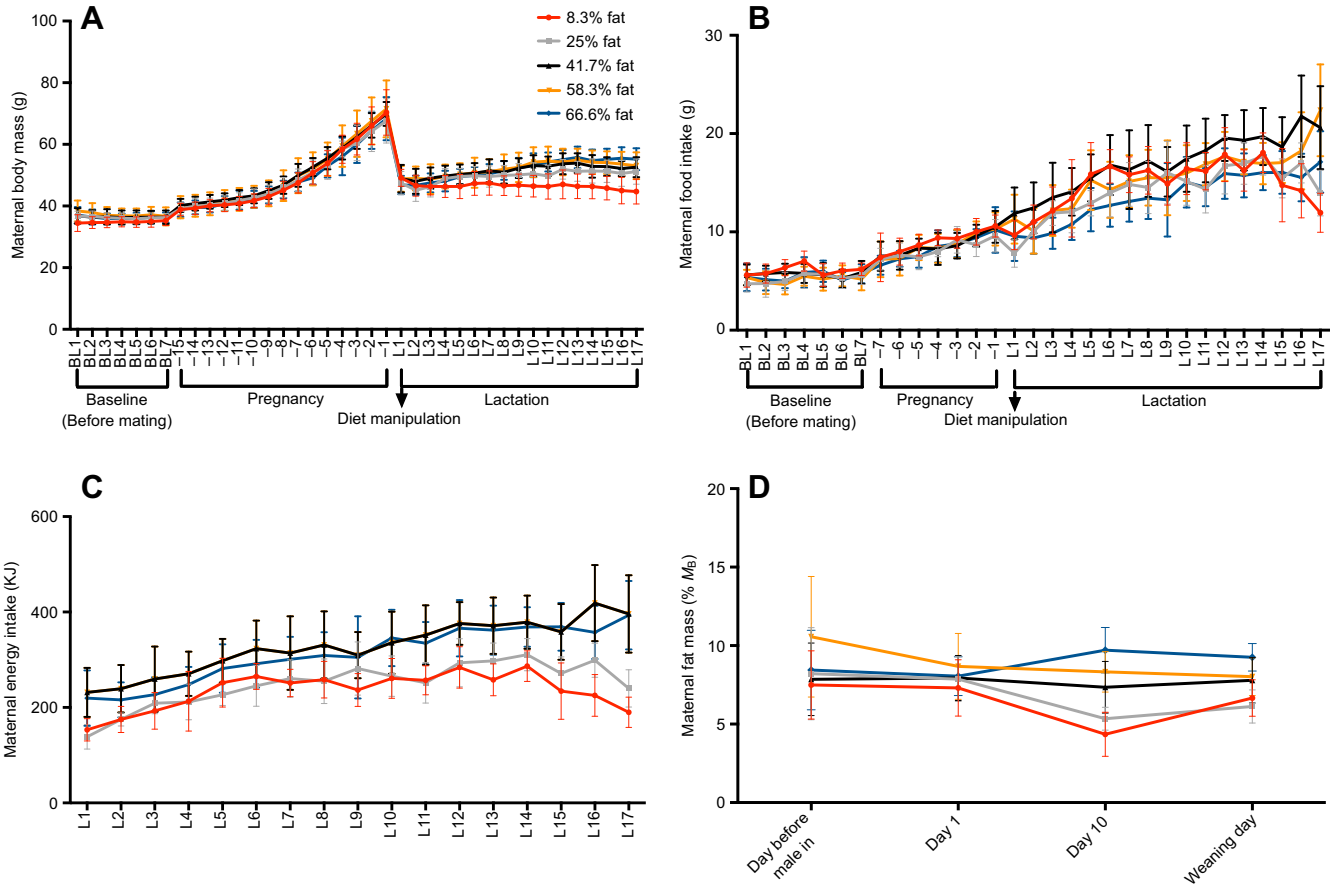

Fig. 1. Maternal body mass, food and energy intake and fat mass in different dietary fat groups during lactation. Values are means±s.d. in maternal body mass (A), food intake (B), energy intake (C) and fat mass (D) in 8.3% (n=13), 25% (n=12), 41.7% (n=14), 58.3% (n=11) or 66.6% (n=13) fat dietary groups.

Journal of Experimental Biology (2020) 223, jeb221911. doi:10.1242/jeb.221911

Table 1. Descriptive statistics for traits measured in lactating Swiss mice fed diets with different fat content

Items	8.3% fat	25% fat	41.7% fat	58.3% fat	66.6% fat	*F*	*P*
Maternal body mass before mating (g)	34.8±1.7	36.3±1.4	36.4±2.2	37.2±2.7	36.3±1.8	2.395	0.061
Maternal body mass at parturition (g)	49.2±2.8	47.4±3.8	48.9±4.5	48.8±4.2	46.8±3.0	1.052	0.389
Maternal body fat content at weaning (%)	6.66±1.16^{a,b}	6.12±1.06^{a}	7.79±1.45^{b,c}	8.12±1.2^{c,d}	9.26±0.88^{d}	14.201	<0.001
Litter size at weaning	9.8±1.3	8.9±1.1	10±1.4	10.3±2.5	10.2±1.6	1.371	0.225
Litter mass (g, day 1 of lactation)	20.4±1.6	19.9±1.9	20.5±2.3	20.8±4.3	19.4±2.3	0.543	0.704
Litter mass (g, weaning day)	66.9±15.6^{a}	78.6±10.8^{a}	128.2±14.1^{bc}	132.1±16.7^{c}	116.1±10.1^{b}	59.572	<0.001
Pup mass (g, day 1 of lactation)	2.0±0.2	1.9±0.2	1.9±0.2	1.9±0.2	1.8±0.1	2.336	0.066
Pup mass (g, weaning day)	6.9±1.9^{a}	8.9±1.6^{b}	12.9±1.3^{c}	13.3±2.6^{c}	11.6±1.4^{c}	29.471	<0.001
Litter fat content at weaning (%)	5.2±1.2^{a}	8.0±1.1^{b}	10.9±2.1^{c}	12.7±2.8^{c,d}	13.2±2.1^{d}	36.058	<0.001
Asymptotic energy intake (kJ day^{-1})	246.1±32.1^{a}	286.7±17.9^{b}	379.6±47.6^{c}	395.8±33.7^{c}	370.8±38.8^{c}	41.837	<0.001
Metabolizable energy intake (kJ day^{-1})	229.4±39.6^{a}	278.8±25.8^{a,b}	359.6±51.5^{c}	353.7±43.6^{c}	346.0±44.7^{b,c}	8.743	<0.001
Daily energy expenditure (kJ day^{-1})	128.5±16.0^{c}	131.6±8.4^{b,c}	124.4±10.8^{a,b}	115.1±10.5^{a}	111.2±11.5^{a}	14.451	<0.001
Milk energy output (kJ day^{-1})	100.8±27.3^{a}	147.2±25.1^{b}	225.1±49.6^{c}	238.6±40.1^{c}	234.8±41.1^{c}	32.047	<0.001
Milk fat content (%)	23.1±2.1	21.6±2.2	20.8±3.5	19.3±4.1	22.1±3.9	1.126	0.326

Descriptive statistics for lactating mice fed diets with 8.3% (*n*=13), 25% (*n*=12), 41.7% (*n*=14), 58.3% (*n*=11) or 66.6% (*n*=13) fat contents from lactating day 1 to the weaning day. Values shown are means±s.d. Significant effects of diet are indicated using superscript a–d; i.e. groups that have the same letter did not differ significantly, and groups with a different letter differed significantly (P<0.05).

lactation: $F_{2,121}$=2347.732, P<0.001; day×diet: $F_{8,121}$=47.055, P<0.001), whereas the M_L of offspring raised by mothers fed 41.7, 58.3 or 66.6% fat diets did not differ significantly from each other (Fig. 2A). Similarly, pup mass (M_{pup}) of offspring raised by mothers fed 41.7, 58.3 or 66.6% fat diet was significantly increased over lactating days 1–17 compared with those fed 8.3 or 25% fat diet. The masses of the pups from the 58.3% fat diet mothers were larger than those from the 66.6% fat diet (RM GLM: diet: $F_{4,58}$=37.652, P<0.001; day of lactation: $F_{2,99}$=2144.384, P<0.001; day×diet: $F_{7,99}$=32.668, P<0.001) (Fig. 2B). Final M_L and M_{pup} at weaning did not show the same patterns of significance in different dietary groups (Table 1); there was no significant difference in pup masses between the dietary groups of 41.7% fat and above, while significantly reduced litter masses in 66.6% fat-fed groups were observed compared with the 58.3% fat groups (maybe due to the variations in litter size), but both the litters and pups from the mothers fed 41.7% fat diet or above were significantly larger than those from 8.3 and 25% fat diets.

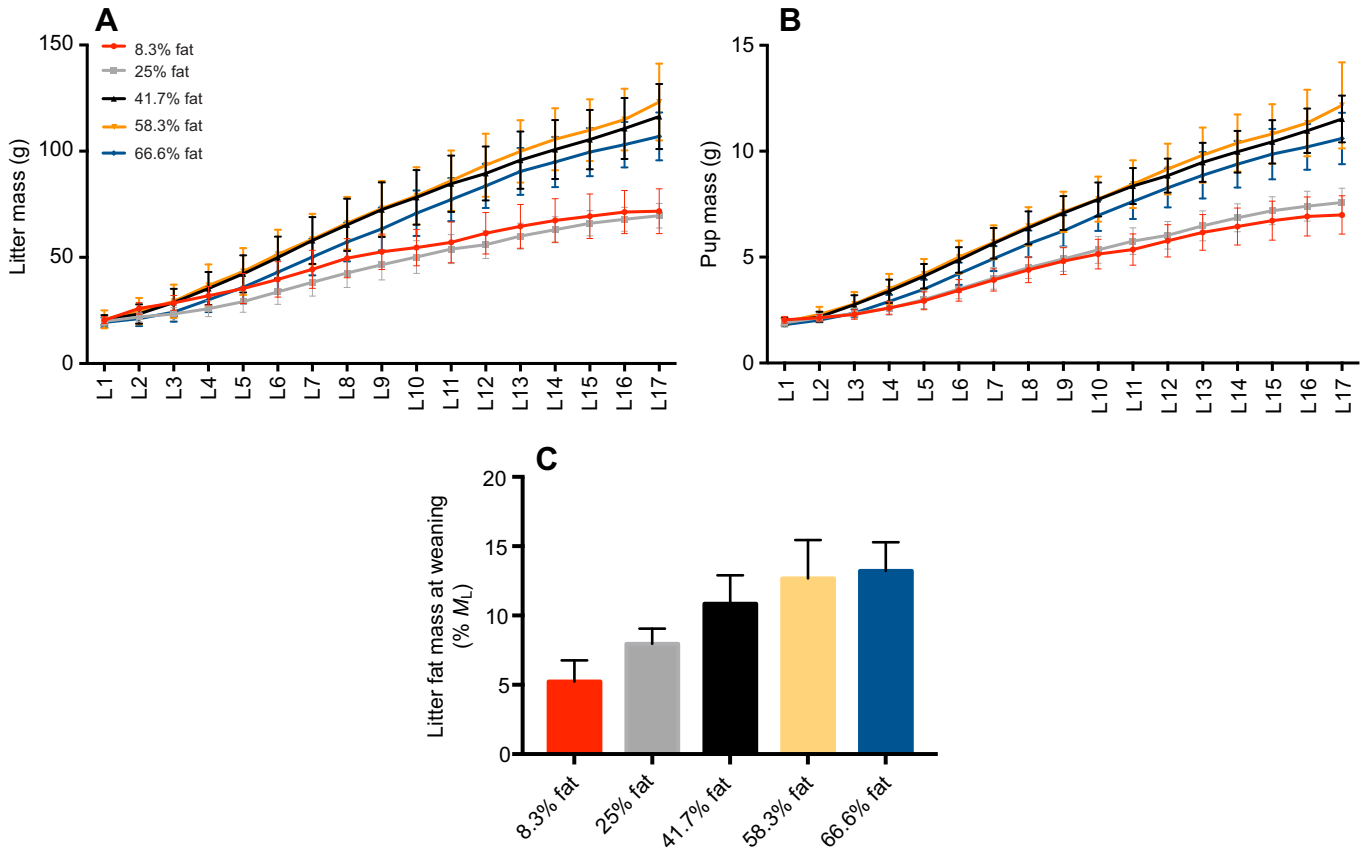

Fig. 2. Litter and pup mass and litter fat mass at weaning in different dietary fat groups. Values are means±s.d. in litter mass (A) and pup mass (B) over lactating day 1 to the weaning day throughout lactation as well as weaned litter fat mass (C) in 8.3% (*n*=13), 25% (*n*=12), 41.7% (*n*=14), 58.3% (*n*=11) or 66.6% (*n*=13) fat dietary groups.

Journal of Experimental Biology (2020) 223, jeb221911. doi:10.1242/jeb.221911

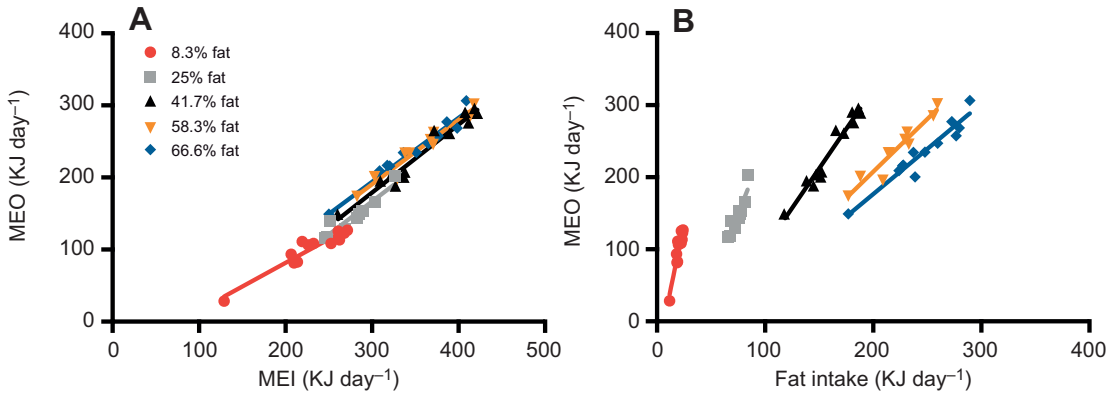

Fig. 3. Linear regression between metabolizable energy intake (MEI), milk energy output (MEO) and fat intake. (A) Relationship between MEI and MEO; 8.3%: R^2=0.899, y=0.656x–49.717, P<0.001; 25%: R^2=0.881, y=0.922x–109.859, P<0.001; 41.7%: R^2=0.952, y=0.941x–103.259, P<0.001; 58.3%: R^2=0.938, y=0.893x–77.267, P<0.001; 66.6%: R^2=0.93, y=0.889x–72.985, P<0.001. (B) Relationship between fat intake and MEO; 8.3%: R^2=0.87, y=7.484x–50.477, P<0.001; 25%: R^2=0.818, y=3.793x–135.331, P<0.001; 41.7%: R^2=0.95, y=2.189x–116.298, P<0.001; 58.3%: R^2=0.918, y=1.463x–85.128, P<0.001; 66.6%: R^2=0.91, y=1.252x–73.752, P<0.001. MEI, MEO and fat intake were calculated over lactating days 14–16. R^2 is adjusted R^2. Sample sizes were 12, 10, 12, 10 and 12 for 8.3, 25, 41.7, 58.3 and 66.6% fat groups, respectively.

Metabolizable daily energy intake, daily energy expenditure and milk energy output

DEE measured over lactating days 14–16 was significantly different between dietary groups (GLM, diet: $F_{4,50}$=14.451, P<0.001; M_B: $F_{1,50}$=23.168, P<0.001) (Table 1). Generally, females had the trend of gradually lower DEE with increasing of the fat levels. Females fed 58.3 and 66.6% fat diet had the lowest DEE (115.1±10.5 and 111.2±11.5 KJ day^{-1}, respectively), while those fed 8.3% fat diet had the highest (128.5±16.0 KJ day^{-1}), and 11.64 and 15.56% higher than the 58.3 and 66.6% fat mothers, respectively. The mothers fed 41.7% fat diet and above also had significantly higher MEI (GLM, diet: $F_{4,50}$=8.743, P<0.001; M_B: $F_{1,50}$=8.239, P=0.006) and MEO (GLM, diet: $F_{4,51}$=32.047, P<0.001) than those fed a 25% fat diet or lower. Compared with females fed 8.3 and 25% fat diets, the MEO from the mice fed 41.7% fat diet and above were increased by approximately 123.31, 136.71 and 132.94% more than the 8.3% group, as well as 52.92%, 62.09% and 59.51% compared with the 25% group, respectively (Table 1). Linear regression revealed a highly significant relationship between MEI and MEO, as well as between MEO and fat intake from the diets (Fig. 3). However, significant associations between MEO and litter mass growth, as well as between litter mass growth and fat intake, were only observed in 8.3 and 41.7% dietary fat groups (Fig. 4). No significant differences were observed between different dietary fat fed groups in water turnover. However, there was a significant but weak positive relationship between the MEO and water turnover (adjusted R^2=0.092, y=0.002x+2.291, P=0.013) (Fig. 5).

Milk fat content

There were no significant differences in milk fat content between different maternal dietary groups (ANOVA, $F_{4,29}$=1.216, P=0.326) (Table 1). The milk fat contents were 23.1±2.1, 21.6±2.2, 20.8±3.5, 19.3±4.1 and 22.1±3.9% for the mothers fed with 8.3, 25, 41.7, 58.3 and 66.6% fat diets, respectively.

Effect of diets on body composition of mothers

The maternal body fat content showed no significant differences between dietary groups before mating (ANOVA, $F_{4,58}$=2.167, P=0.084) and at day 1 of lactation (ANOVA, $F_{4,58}$=1.138, P=0.348). RM GLM over lactating day 10 to weaning revealed

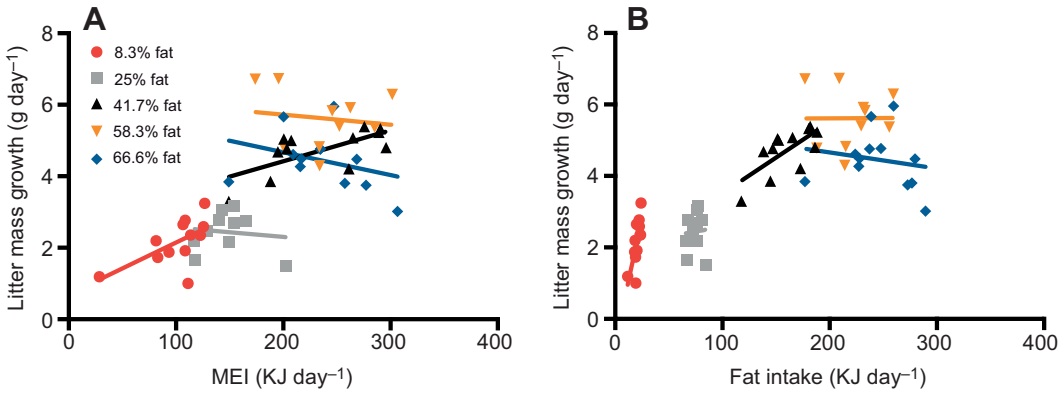

Fig. 4. Linear regression between MEI, litter mass growth and fat intake. (A) Relationship between MEI and litter mass growth; 8.3%: R^2=0.305, y=0.014x+0.701, P=0.037; 25%: R^2=0, y=–0.003x+2.835, P=0.741; 41.7%: R^2=0.403, y=0.009x+2.689, P=0.016; 58.3%: R^2=0, y=–0.003x+6.288, P=0.709; 66.6%: R^2=0.014, y=–0.006x+5.95, P=0.308. (B) Relationship between fat intake and litter mass growth; 8.3%: R^2=0.506, y=0.141x–0.69, P=0.006; 25%: R^2=0, y=0.005x+2.046, P=0.874; 41.7%: R^2=0.434, y=0.02x+1.517, P=0.012; 58.3%: R^2=0, y=0x+5.576, P=0.987; 66.6%: R^2=0, y=–0.005x+5.563, P=0.588. MEI and fat intake were calculated over lactating days 14–16, and litter mass growth was calculated over lactating days 10–17. R^2 is adjusted R^2; 0 would be used to replace the value when it is below 0. Sample sizes were 12, 10, 12, 10 and 12 for 8.3, 25, 41.7, 58.3 and 66.6% fat groups, respectively.

Journal of Experimental Biology (2020) 223, jeb221911. doi:10.1242/jeb.221911

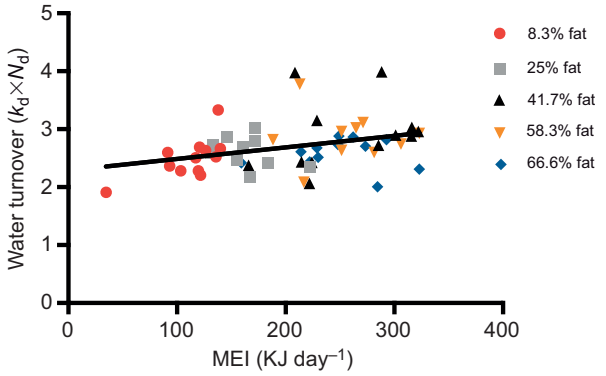

Fig. 5. Linear regression between MEI and water turnover in lactating female mice fed different dietary fat diets. R^2=0.092, y=0.002x+2.291, P=0.013. R^2 is adjusted R^2. Sample sizes were 12, 10, 12, 10 and 12 for 8.3, 25, 41.7, 58.3 and 66.6% fat groups, respectively.

that the females fed 41.7% fat diet and above had significantly higher body fat content than those fed 25% fat diet and below [day ($F_{1,58}$=9.916, P=0.003), day×diet ($F_{4,58}$=8.103, P<0.001), diet ($F_{4,58}$=33.956, P<0.001)]. At weaning, the 66.6% fat fed females had 1.47, 3.14 and 2.6% higher body fat content than those fed 41.7, 25 and 8.3% fat, respectively. No significant differences were shown between 8.3/25%, 8.3/41.7% and 41.7/58.3% fat fed females, but 1.67 and 2% higher body fat content was observed between 41.7/58.3% and 25% fat fed females (Fig. 1D; Table 1).

To evaluate the effects of five dietary treatments on morphology of mothers and offspring, the masses of organs were compared. There were significant differences between the mothers in the masses of mammary gland (with SUB), mesenteric fat, gonadal fat, retroperitoneal fat, liver, spleen, kidney, uterus and ovaries (Table S1a; Fig. 6). Generally, females fed 41.7% fat diet and above deposited more fat than those fed 8.3% and/or 25% fat diets, but no significant differences in fat deposition were observed between the mother fed 41.7% fat diet and above.

Linear regression between MEI and organ masses in heart, liver, spleen, kidneys, stomach, intestine, colon and caecum were conducted. Significant effects between MEI and the masses in liver and colon were observed in 8.3% fat fed mothers, but no significant associations were observed in the rest of the dietary groups (Fig. S1).

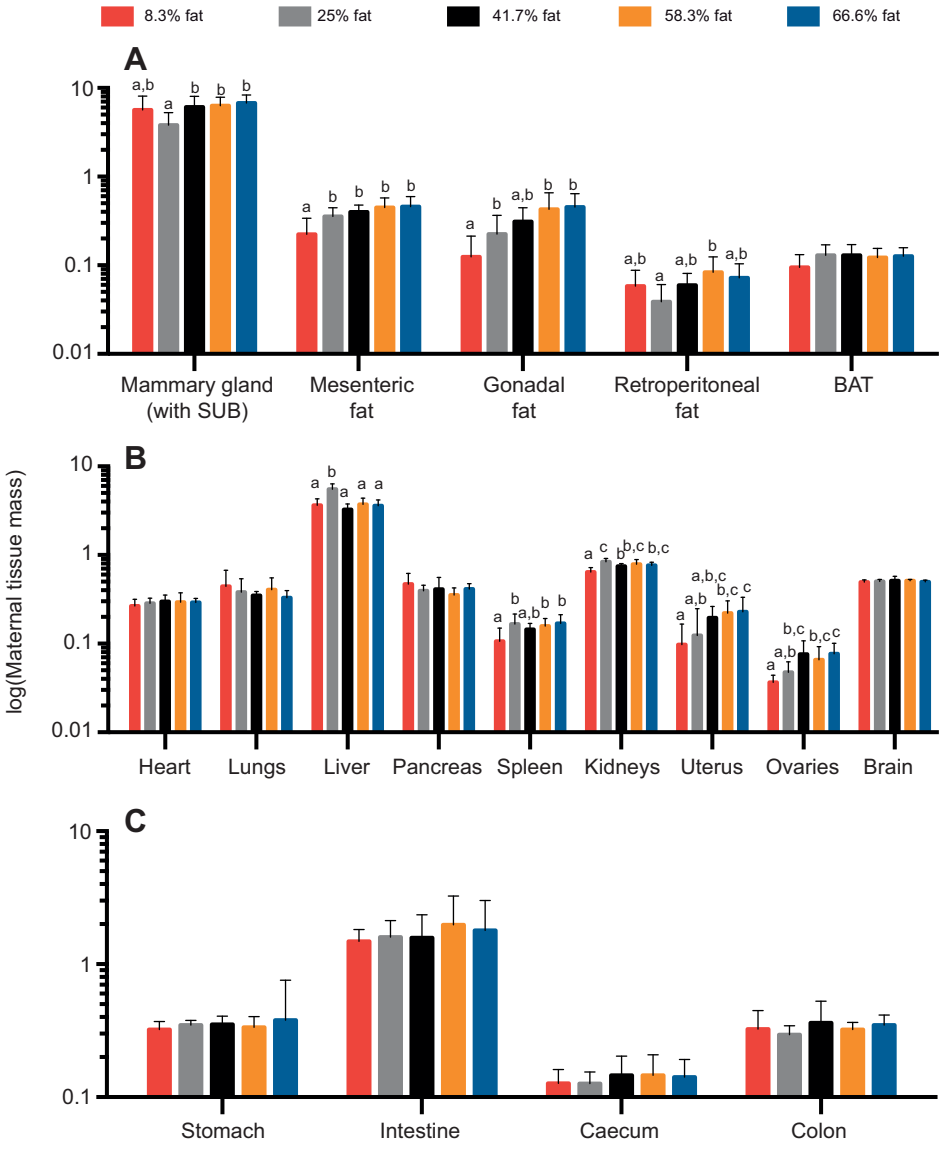

Fig. 6. Organ masses in lactating female mice fed on diets with graded fat levels at weaning. (A) Organ masses in mammary gland (with SUB), mesenteric fat, gonadal fat, retroperitoneal fat and BAT; (B) organ masses in heart, lungs, liver, pancreas, spleen, kidneys, uterus, ovaries and brain; (C) organ masses in stomach, intestine, caecum and colon. Significant effects of diets are indicated using superscript a, b and c; i.e. groups that have the same letter did not differ significantly, and groups with a different letter differed significantly (P<0.05). Values are means±s.d.; sample sizes were 13, 12, 14, 11 and 13 for 8.3, 25, 41.7, 58.3 and 66.6% fat groups, respectively.

Journal of Experimental Biology (2020) 223, jeb221911. doi:10.1242/jeb.221911

Effect of maternal diets on body composition of offspring

The fat contents of litters were also compared between dietary groups at weaning. Generally, the fat contents of the litters were gradually increased in line with the maternal fat levels (ANOVA, $F_{4,58}$=36.058, P<0.001), and the litters from 66.6% fat fed mothers had 2.37, 5.27 and 7.98% higher fat content than those fed 41.7, 25 and 8.3% fat, respectively (Fig. 2C), exhibiting much more fat deposition than their mothers under the maternal high fat (HF) exposure during lactation.

Significant group effects were observed in the masses of mesenteric fat, BAT, heart, lungs, liver, pancreas, spleen, kidneys, brain, stomach and colon in female pups; after correcting for M_B, significant effects disappeared in the masses of BAT, stomach, kidneys and brain (Table S1b; Fig. 7). Significant group effects were also observed in the masses of subcutaneous fat, mesenteric fat, BAT, heart, lungs, liver, pancreas, spleen, kidneys, brain, stomach, caecum and colon in male pups; after calibrating with M_B, significant effects disappeared except the masses of mesenteric fat, heart, liver and kidneys (Table S1c; Fig. 8). Pups raised by mothers fed 41.7 or 58.3% fat diets generally had heavier organ masses than those fed 25% fat diet and below, and those fed 41.7% fat diet had highest masses in most organs. The fat deposition in mesenteric fat in both male and female pups raised by 41.7% fat-fed mothers was higher than those in 8.3% and/or 25% fat-fed mothers. The reason we failed to collect the gonadal fat and retroperitoneal fat was due to some pups (such as pups raised by 8.3 and 25% fat-fed mothers) having no fat in those tissues. The inconsistency of fat deposition between the organ masses (not significant in the masses of subcutaneous in female pups) and the body fat content indicated that the fat that contributed to the significant difference between groups might stem from the gonadal fat or retroperitoneal fat.

Behavior observations

On average for all diets, climbing (χ^2_2=2.539, P=0.281), drinking (χ^2_2=5.302, P=0.071), eating (χ^2_2=5.142, P=0.076), grooming (χ^2_2=5.157, P=0.076), general activities (χ^2_2=6.468, P=0.039, not significant after *post hoc* Bonferroni correction), resting (χ^2_2=1.983, P=0.371) and feeding the pups (χ^2_2=4.345, P=0.114) did not significantly change over time between the three lactation periods. Mothers increased the time they spent FP/E over the time (χ^2_2=30.578, P<0.001). Mothers had to combine activities and spent more time eating while still feeding the pups, which continued

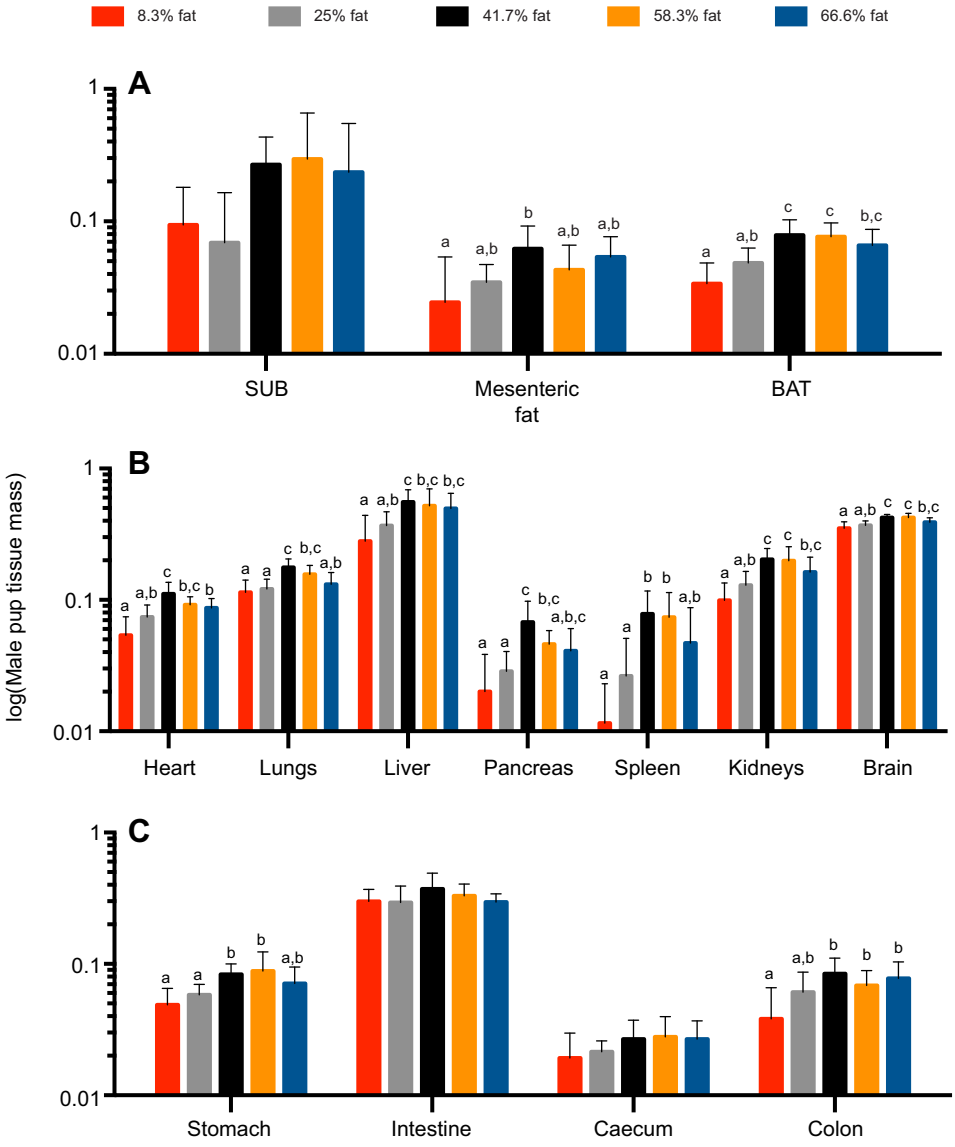

Fig. 7. Organ masses in female pups raised by mothers fed on diets with graded fat levels at weaning. (A) Organ masses in subcutaneous fat (SUB), mesenteric fat and brown adipose tissue (BAT); (B) organ masses in heart, lungs, liver, pancreas, spleen, kidneys and brain; (C) organ masses in stomach, intestine, caecum and colon. Significant effects of diets are indicated using superscript a, b and c; i.e. groups that have the same letter did not differ significantly, and groups with a different letter differed significantly (P<0.05). Values are means± s.d.; sample sizes were 13, 12, 14, 11 and 13 for 8.3, 25, 41.7, 58.3 and 66.6% fat groups, respectively.

Journal of Experimental Biology (2020) 223, jeb221911. doi:10.1242/jeb.221911

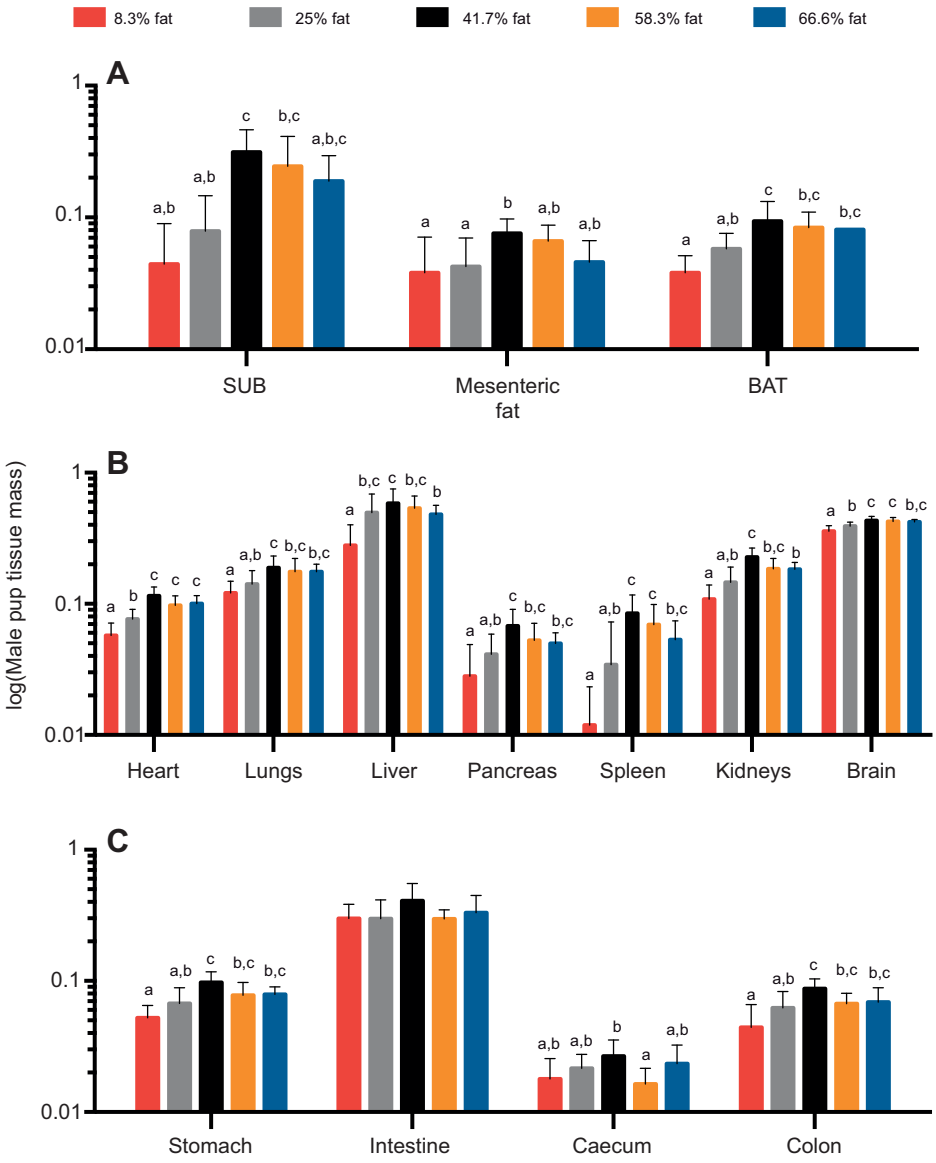

Fig. 8. Organ masses in male pups raised by mothers fed on diets with graded fat levels at weaning. (A) Organ masses in SUB, mesenteric fat and BAT; (B) organ masses in heart, lungs, liver, pancreas, spleen, kidneys and brain; (C) organ masses in stomach, intestine, caecum and colon. Significant effects of diets are indicated using superscript a, b and c; i.e. groups that have the same letter did not differ significantly, and groups with a different letter differed significantly (P<0.05). Values are means±s.d.; sample sizes were 13, 12, 14, 11 and 13 for 8.3, 25, 41.7, 58.3 and 66.6% fat groups, respectively.

into late lactation, probably because the pups were more active and able to follow their mother around the cage. The dominant activity during all three periods was feeding the pups. Mothers spent, on average across the five diets, 69±28% of the observed time feeding the pups during early lactation, and 65±22 and 65±23% for mid- and late lactation, respectively. During the three periods, eating behavior occupied 13% of the total time, and mothers spent only about 10% of their time on general activities. A smaller proportion of their time was spent either climbing (0.33±2%), drinking (1±2%), grooming (3±4%), resting (2±6%) or feeding the pups/eating (2±6%) (Table S2; Figs S2–S4).

Dietary fat had no impact on the amount of time that mice spent climbing (χ^2_4=3.343, P=0.502), drinking (χ^2_4=7.404, P=0.116), grooming (χ^2_4=4.412, P=0.353), general activities (χ^2_4=1.891, P=0.756) or resting (χ^2_4=4.634, P=0.327) in early lactation. Mothers spent more time eating when they were on the 8.3% fat diet than any other diet (χ^2_4=12.030, P=0.017), but this was only significant compared with the 25% fat diet (P=0.025). In contrast, mothers who were fed with diets between 25 and 66.6% fat spent 70–80% of the observed time feeding the pups, while 8.3% fat diet mothers spent only about half of that time (χ^2_4=12.426, P=0.014), but again, it was only found to be significant between 8.3 and 25% (P=0.045) and 41.7% (P=0.035) groups. FP/E was not observed in early lactation (Table S2; Fig. S2).

Similarly, mothers did not exhibit any significant behavior differences between dietary groups for climbing (χ^2_4=1.403, P=0.844), drinking (χ^2_4=1.783, P=0.776), grooming (χ^2_4=4.832, P=0.305), general activities (χ^2_4=1.861, P=0.761), resting (χ^2_4=1.304, P=0.861) or FP/E (χ^2_4=3.597, P=0.463) during mid-lactation, with the exception of eating (χ^2_4=21.751, P<0.001) and feeding the pups (χ^2_4=13.399, P=0.009). During mid-lactation, mothers fed with 8.3% fat diet spent about four times more time on eating compared with those fed the 41.7% (P=0.002), 58.3% (P=0.002) and 66.6% fat diets (P<0.001), but only about 19% more time than when fed with 25% fat diet (P=0.803). No differences were found in eating behavior between the 25, 41.7, 58.3 and 66.6% fat diets (P<0.05 for all). This result was exacerbated when we added the time that mice spent eating and FP/E together (χ^2_4=23.781, P<0.001). Females fed with 8.3% fat diet increased their eating time approximately six times more than those fed 41.7% (P<0.001), 58.30% (P<0.001) and 66.6% (P<0.001) fat diets. However, when the time that mice spent FP/E and feeding the pups

Journal of Experimental Biology (2020) 223, jeb221911. doi:10.1242/jeb.221911

were combined, the differences in feeding the pups were then no longer significant (χ^2_4=10.160, P=0.038, not significant after a Bonferroni correction) (Table S2; Fig. S3).

During late lactation, mothers spent similar percentage of time climbing (χ^2_4=3.105, P=0.540), drinking (χ^2_4=1.622, P=0.805), grooming (χ^2_4=3.734, P=0.443), general activities (χ^2_4=8.998, P=0.061), resting (χ^2_4=4.478, P=0.345), feeding the pups (χ^2_4=9.095, P=0.059) and FP/E (χ^2_4=6.124, P=0.190) between diets, and only significant differences were found in the time spent eating (χ^2_4=14.341, P=0.006). Mothers fed with 8.3% fat diet spent between three and four times more time eating compared with those fed 41.7% (P=0.026) and 58.3% (P=0.032) fat diets. When we add the time eating and FP/E together, the differences remain (χ^2_4=26.874, P<0.001). Similar to the situation during mid-lactation, when we added the time spent eating and FP/E together, significant differences were found in eating between 8.3% fat diet and the diets with 41.7% fat and above (P<0.05 for all), as well as between 25% fat and 58.3% fat diets (P=0.048). Mice fed with 8.3% fat diet spent more than 30% of the time eating (eating and FP/E) compared with those fed 41.7, 58.3 and 66.6% fat diet who spend less than 10% of the time eating. No differences were found when feeding the pups and FP/E were added together (χ^2_4=3.922, P=0.417) (Table S2; Fig. S4).

DISCUSSION

Previous work in Swiss mice suggested that the limitations imposed on the SusEI during lactation are constrained by both peripheral and heat dissipation limitations, and that the dominant process is ambient temperature dependent (Zhao et al., 2016; Wen et al., 2017). It has been suggested that peripheral limitation is more dominant at temperatures below room temperature (21–23°C), while heat dissipation is more significant at hotter temperatures (Zhao et al., 2016; Wen et al., 2017). MF1 mice, in contrast, appear to be limited by heat dissipation down to 8°C (Johnson and Speakman, 2001; Krol et al., 2003). We previously showed that when MF1 mice are fed diets high in fat (45 and 60% by energy) at 22°C they are able to circumvent the heat dissipation limit because they diverted fat directly from the diet into the milk, reducing heat generation associated with lipogenesis (Kagya-Agyemang et al., 2018). The motivation of the present study was to see if feeding Swiss mice high-fat diets would have a similar impact. At 23°C, Swiss mice have been previously suggested to be limited by the capacity of their mammary glands to synthesize milk (Hammond and Diamond, 1992, 1997, 1994; Zhao and Cao, 2009; Zhao et al., 2010; Zhao, 2012), so rather than being limited by heat dissipation capacity, they might be unable to take advantage of the fats from the diet in the same way as MF1 mice can, and hence milk production might be independent of dietary fat composition.

The responses of Swiss and MF1 mice to alterations in dietary fat content are summarized in Fig. 9. We found that at 23°C, metabolizable energy intake and milk energy output increased as the fat content of the diet increased from 8.3 to 41.7% fat. Mice may enhance milk delivery by changing either the amount of milk or the fat content (richness). The fat content of the milk showed no significant differences between different dietary fat groups. This would suggest that the mothers on the higher fat diets were delivering more milk to their pups. However, overall there was only a very weak relationship between the milk energy export and water turnover, and mothers feeding on higher fat diets did not have significantly higher values of water turnover as might be anticipated if milk production was higher. This was potentially because they compensated their water budget elsewhere to allow greater milk

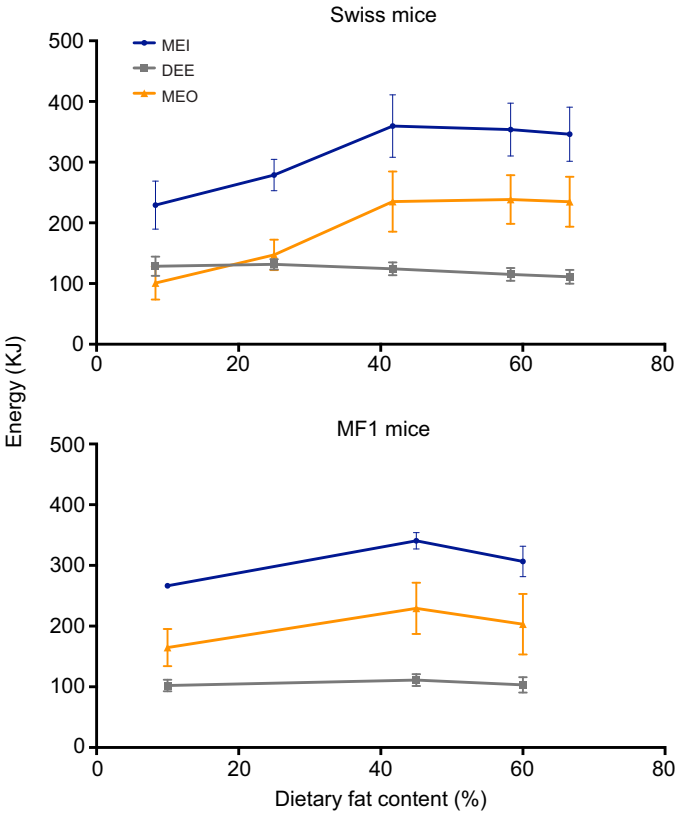

Fig. 9. Comparison of MEI, DEE and MEO between Swiss mice and MF1 mice under different dietary fat levels in female mice during lactation. Five dietary fat groups in Swiss mice were used: 8.3% fat, 25% fat, 41.7% fat, 58.3% fat and 66.6% fat; three dietary fat groups in MF1 mice were used: 10% fat, 45% fat and 60% fat (data from Kagya-Agyemang et al., 2018).

export, but we have no data with respect to that. The higher levels of milk export were not associated with higher levels of DEE. This suggests that the excess fat export was not being directly synthesized and was likely therefore directly transferred into the milk from the diet, thereby avoiding any costs of lipogenesis and coincident heat production. The same effect was observed previously in MF1 mice (Kagya-Agyemang et al., 2018). However, lactation performances were not further enhanced at dietary fat levels above 41.7% fat, suggesting that there is potentially a limit in the capacity to transfer fats from the diet into milk. Similar to the present finding, there were no significant differences in MEO and litter/pup masses between the HF and MF groups in MF1 mice (Kagya-Agyemang et al., 2018). The patterns of change in energy intake, milk production and energy expenditure were remarkably similar between the two strains (Fig. 9), despite the suggestion that they are limited by different factors at this temperature.

The reason why females did not generate even more milk as dietary fat increased above 41.7% may be related to the impacts of this extra milk on pup growth and body composition. Already by 41.7% fat in the maternal diet the pups were substantially fatter than pups fed diets with 8.3 and 25% fat (Fig. 2). By diverting even more fat from the diet into the milk the offspring would presumably become even fatter. Fatter pups may have advantages during weaning as they would have a greater reserve of energy on which to draw if the transition to self-feeding was in any way interrupted. However, the benefits of this fat store may be limited, and beyond a point greater fat deposits may not generate any greater advantage. Hence females may not transfer more fat into the milk as dietary fat

increases above 41.7% not because there are limits in the fat transfer process, but rather because there are no additional benefits in doing so. This may then also explain why metabolizable energy intake actually declines slightly at the highest fat levels (which was observed in both strains; see Fig. 9).

Increased food intake requires enlarged organs to digest, absorb and process the nutrients, and deliver nutrients and oxygen to peripheral tissues (Toloza et al., 1991; Hammond et al., 1994; Konarzewski and Diamond, 1994; Koteja, 1996; Speakman and McQueenie, 1996; Starck, 1999; Hammond and Kristan, 2000; Krol et al., 2003). No significant differences in the masses of the alimentary tract and associated organs (i.e. small intestine, caecum and colon), and no significant relationship was found between MEI and organ masses of the heart, liver, spleen, kidneys and digestive tracts, even after they ate massively more food in the HF dietary groups, suggesting that the limitation was not likely to be imposed by 'central limitation'. Growth of pups may not only depend on milk delivery but also on the behavior of the mothers. We were therefore interested in whether the dietary fat levels had an impact on maternal behavior. This could happen for example because the higher energy content of the high-fat diets might make the time spent on eating lower, and this would release time to engage in other things. However, the eating and other behaviors of the females during early, mid- and late lactation was unrelated to the dietary fat content.

Generally, the M_B and body fat content of mothers were increased in line with the increasing fat intake levels; as a result, the higher fat intake during lactation could also pre-dispose the mothers to deposit more fat, even under a lower MEI level (66.6% fat group versus 41.7% fat group), suggesting that the elevated high-fat feeding would not be more beneficial to the mother either. Strangely, despite the masses of mammary gland with SUB, maternal mesenteric fat, retroperitoneal fat and gonadal fat did differ significantly, the patterns of fat deposition were not completely consistent with the observed patterns of body fat content changes in the HF groups (diets of 41.7% fat and above), the fat deposition in mothers fed 41.7% fat and above did not increase in line with the dietary fat levels, and the reason is unknown. In the case of MF1 mice, significant differences were only observed in the gonadal fat, stomach and liver (Kagya-Agyemang et al., 2018), indicating that the female Swiss mice were more sensitive to higher dietary fat, and hence they made more morphological changes to cope with it.

Beneficial effects of HF feeding on reproductive performance have previously been observed also in sows and rats (Del Prado et al., 1997; Averette et al., 1999; Van den Brand et al., 2000; Loh et al., 2002). Dietary high fat increased milk fat and energy concentration and a higher piglet body fat concentration in sows, but no MF groups were set up in these studies (Averette et al., 1999; Van den Brand et al., 2000). In the two studies in Sprague–Dawley rats, one showed that milk lipid concentration and daily output of fat were higher in the HF (20 g fat/100 g diet) fed group compared with the low fat (LF) (2.5 g fat/100 g diet) group (Del Prado et al., 1997). Another study was performed in rats fed LF (25 g fat/kg diet), medium fat (MF) (75 g fat/kg diet) and HF (150 g fat/kg diet) diets during both pregnancy and lactation. Significant differences in milk fat concentration at lactating days 10 and 15 were observed between LF and HF groups, yet there were no significant differences between HF and MF, or LF and MF groups. The pups raised by mothers fed HF diets had significantly higher M_B than those fed LF and MF diets (Loh et al., 2002). No limitation of lactation performance was observed in all the studies above, probably not because there are no limits in this strain, but as a result of the rather limited setting of the diets. For example, the energy from fat in LF, MF and HF groups from the Sprague–Dawley rat study were 2.12, 5.27 and 9.65 KJ g^{-1}, respectively (table 1 from Loh et al., 2002). However, in our study, the energy from fat in the 8.3, 25, 41.7, 58.3 and 66.6% dietary groups were 1.32, 4.39, 8.02, 12.67 and 15.32 KJ g^{-1}, respectively. As a result, the fat energy of MF group was basically equal with our 25% fat group, and the fat energy from their HF group was more or less between our 41.7 and 58.3% fat groups, which means it was not possible to figure out whether the lactation performance would be limited in higher dietary fat groups.

In conclusion, HF feeding during lactation facilitated greater milk production and generated heavier litters in Swiss mice, yet the lactation performance was not further enhanced in line with the elevated dietary fat intake when fat exceeded 41.7% of the diet. This may be limited by the ability of the mothers to transfer additional dietary fat to the milk. Alternatively, they may not do this because elevated fat transfer would not be more beneficial to the pups. Despite the suggestion that two mouse strains (Swiss and MF1) are limited by different factors, the impact of high-fat diets on their performance at 22–23°C were remarkably similar.

Acknowledgements
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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: J.R.S.; Methodology: J.R.S.; Formal analysis: Y.H., J.O.; Investigation: Y.H., J.O., C.H., B.L., Z.J., L.L., M.M., S.H.; Writing - original draft: Y.H., J.O.; Writing - review & editing: Y.H., C.H., J.R.S.; Supervision: J.R.S.; Project administration: J.R.S.

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Supplementary information

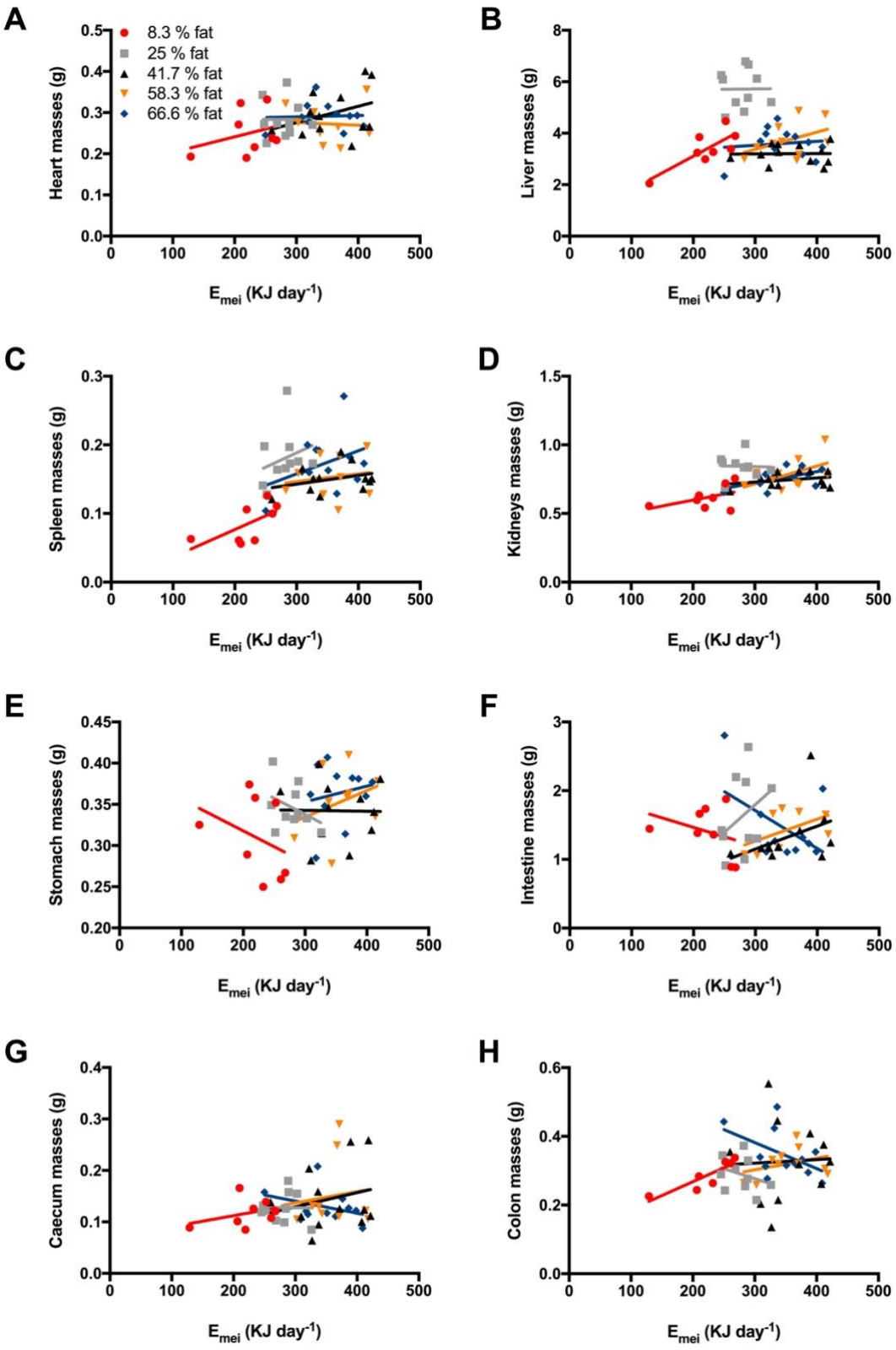

Fig. S1. Linear regression between metabolizable energy intake (E_{mei}) and organ

masses in lactating female mice fed different dietary fat diets. (A) Relationship between E_{mei} and heart. 8.3%: R_2=0, y=0x+0.166, P=0.463; 25%: R_2=0, y=(4.109E-7)x+0.284, P=0.999; 41.7%: R_2=0.034, y=0x+0.157, P=0.265; 58.3%: R_2=0, y=(-6.579E-5)x+0.296, P=0.87; 66.6%: R_2=0, y=(3.19E-5)x+0.28, P=0.895; (B) Relationship between E_{mei} and liver. 8.3%: R_2=0.572, y=0.013x+0.522, P=0.018; 25%: R_2=0, y=0x+5.652, P=0.983; 41.7%: R_2=0, y=0x+3.158, P=0.958; 58.3%: R_2=0.086, y=0.007x+1.365, P=0.212; 66.6%: R_2=0, y=0.002x+3.068, P=0.725; (C) Relationship between E_{mei} and spleen. 8.3%: R_2=0.3, y=0x-0.004, P=0.092; 25%: R_2=0, y=0x+0.063, P=0.465; 41.7%: R_2=0.014, y=0x+0.104, P=0.307; 58.3% R_2=0, y=0x+0.113, P=0.653; 66.6%: R_2=0.046, y=0x+0.057, P=0.245; (D) Relationship between E_{mei} and kidneys. 8.3%: R_2=0.08, y=0.001x+0.424, P=0.252; 25%: R_2=0, y=(-9.743E-5)x+0.871, P=0.932; 41.7%: R_2=0, y=0x+0.638, P=0.347; 58.3%: R_2=0.174, y=0.001x+0.328, P=0.127; 66.6%: R_2=0.205, y=0.001x+0.479, P=0.078; (E) Relationship between E_{mei} and stomach. 8.3%: R_2=0, y=0x+0.395, P=0.401; 25%: R_2=0.028, y=0x+0.455, P=0.295; 41.7%: R_2=0, y=(-1.098E-5)x+0.346, P=0.966; 58.3%: R_2=0.014, y=0x+0.238, P=0.319; 66.6%: R_2=0, y=0x+0.296, P=0.592; (F) Relationship between E_{mei} and intestine. 8.3%: R_2=0, y=-0.003x+2.012, P=0.426; 25%: R_2=0.021, y=0.008x-0.62, P=0.307; 41.7%: R_2=0.065, y=0.003x+0.146, P=0.226; 58.3%: R_2=0.157 y=0.003x+0.31, P=0.18; 66.6%: R_2=0.152, y=-0.006x+3.365, P=0.129; (G) Relationship between E_{mei} and caecum. 8.3%: R_2=0, y=0x+0.069, P=0.393; 25%: R_2=0, y=(-1.487E-5)x+0.132, P=0.971; 41.7%: R_2=0, y=0x+0.046, P=0.475; 58.3%: R_2=0, y=0x+0.072, P=0.687; 66.6%: R_2=0.028, y=0x+0.211, P=0.278; (H) Relationship between E_{mei} and colon. 8.3%: R_2=0.732, y=0.001x+0.107, P=0.009; 25%: R_2=0, y=-0.001x+0.452, P=0.391; 41.7%: R_2=0, y=0x+0.291, P=0.899; 58.3%: R_2=0, y=0x+0.201, P=0.346; 66.6%: R_2=0.166, y=-0.001x+0.609, P=0.104. E_{mei} was calculated over lactating days 14–16. R_2 is adjusted R_2. 0 would be used to replace the value when it is below 0. Sample sizes were 8, 10, 12, 10 and 12 for 8.3%, 25%, 41.7%, 58.3% and 66.6% fat groups, respectively.

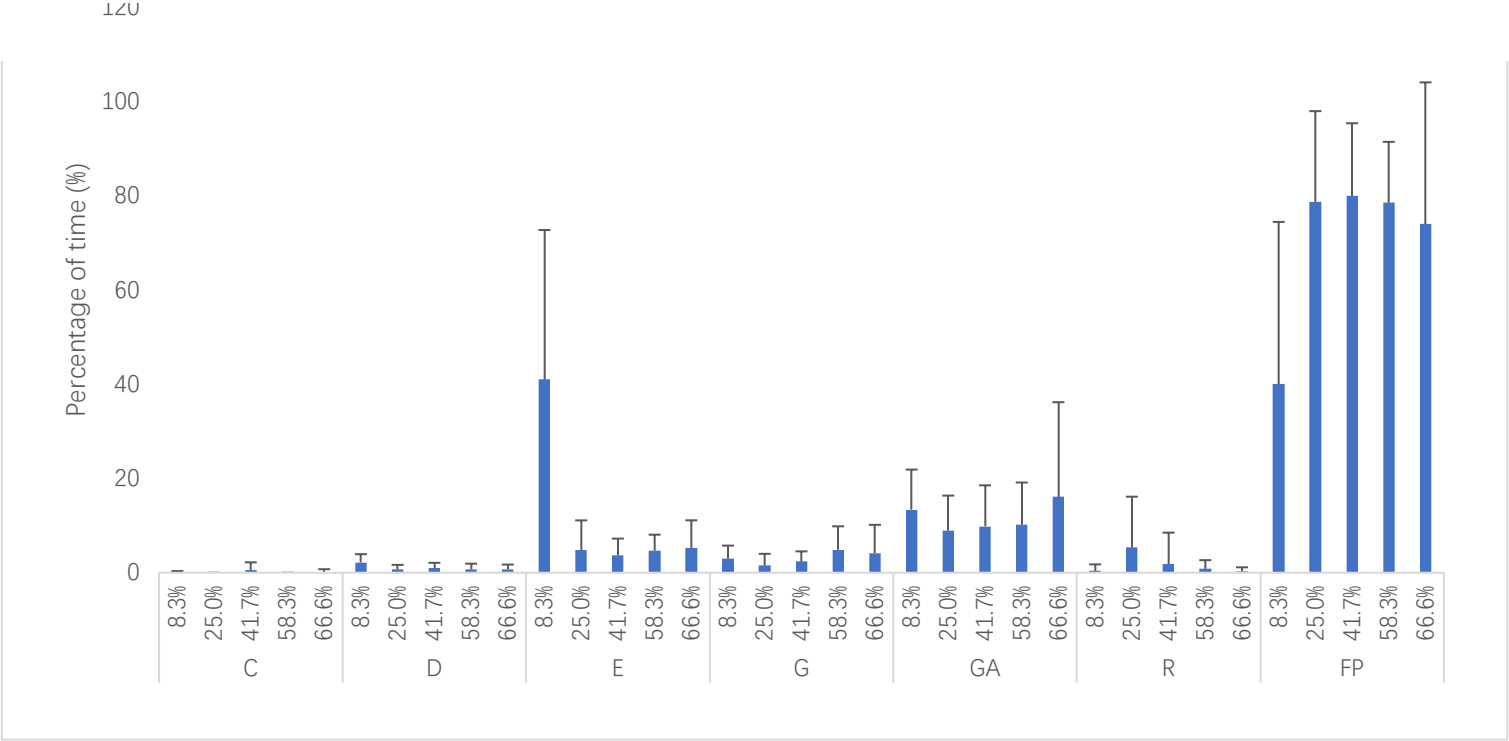

Figure S2. Percentage of time that mothers spent on each activity: climbing (C), drinking water (D), eating (E), grooming (G), general activities (GA), resting (R) and feeding pups (FP) in early lactation (day 4–6 of lactation) while fed with 8.3%, 25%, 41.7%, 58.3% and 66.6% of fat diet during the light phase (Means±s.d.). Sample sizes were 13, 12, 14, 11 and 13for 8.3%, 25%, 41.7%, 58.3% and 66.6% fat groups, respectively.

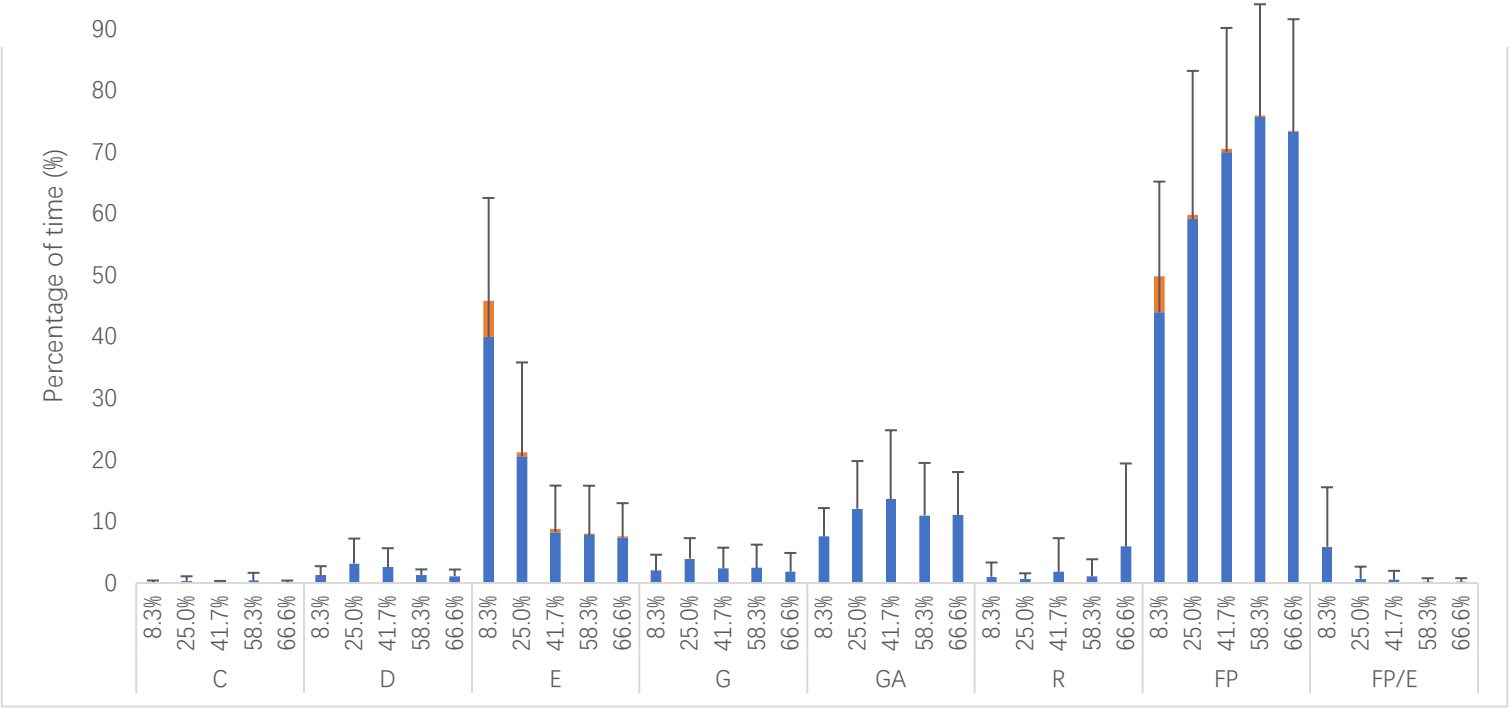

Figure S3. Percentage of time that mothers spent on each activity: climbing (C), drinking water (D), eating (E), grooming (G), general activities (GA), resting (R), feeding pups (FP) and feeding pups/eating (FP/E) in mid-lactation (day 8–10 of lactation) while fed with 8.3%, 25%, 41.7%, 58.3% and 66.6% of fat diet during the light phase (Means±s.d.). Sample sizes were 13, 12, 14, 11 and 13for 8.3%, 25%, 41.7%, 58.3% and 66.6% fat groups, respectively. Orange bars represent the time spent eating while simultaneously feeding the pups.

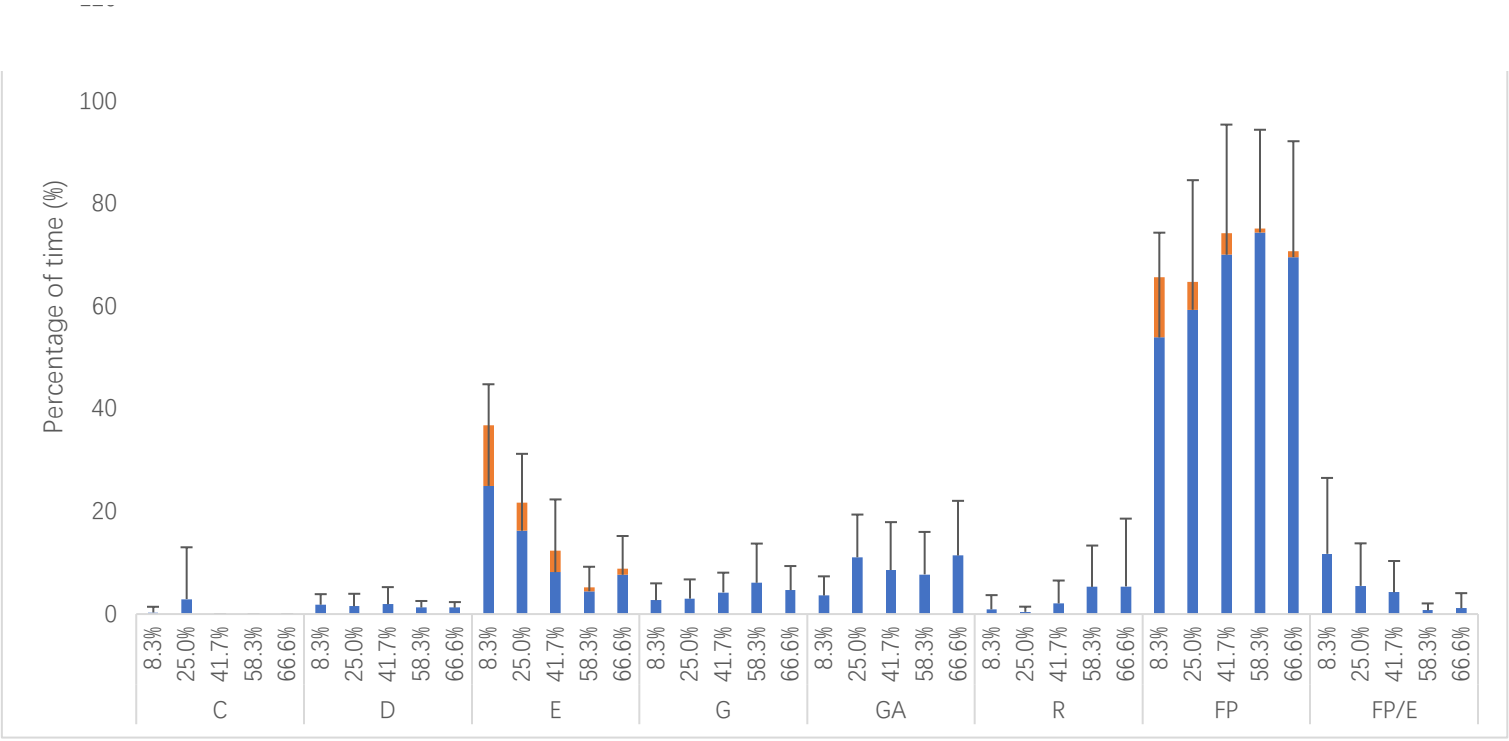

Figure S4. Percentage of time that mothers spent in each activity: climbing (C), drinking water (D), eating (E), grooming (G), general activities (GA), resting (R), feeding pups (FP) and feeding pups/eating (FP/E) in late lactation (day 12–14 of lactation) while fed with 8.3%. 25%, 41.7%, 58.3% and 66.6% of fat diet during the light phase (Means±s.d.). Sample sizes were 13, 12, 14, 11 and 13 for 8.3%, 25%, 41.7%, 58.3% and 66.6% fat groups, respectively. Orange bars represent the time spent eating while simultaneously feeding the pups.

Table S1a. Organ masses in female mice fed on diets with graded fat levels at weaning.

Organ	8.3% fat	25% fat	41.7 % fat	58.3 % fat	66.6 % fat	ANOVA Diet		GLM (BM covariate) Diet		BM	
						F	P	F	P	F	P
mammary gland with Subcutaneous fat	5.662±2.413$_{ab}$	3.802±1.462$_{a}$	6.102±1.940$_{b}$	6.299±1.568$_{b}$	6.741±1.607$_{b}$	4.648	**0.003**	5.836	**0.001**	50.349	< 0.001
Mesenteric fat	0.223±0.115$_{a}$	0.356±0.09$_{b}$	0.399±0.077$_{b}$	0.448±0.126$_{b}$	0.457±0.137$_{b}$	9.338	**< 0.001**	6.236	**< 0.001**	1.789	0.186
Gonadal fat	0.124±0.089$_{a}$	0.224±0.142$_{a}$	0.310±0.135$_{ab}$	0.428±0.229$_{b}$	0.454±0.186$_{b}$	6.961	**< 0.001**	5.170	**0.001**	0.162	0.689
Retroperitoneal fat	0.058±0.030$_{ab}$	0.038±0.022$_{a}$	0.060±0.022$_{ab}$	0.083±0.041$_{b}$	0.072±0.032$_{ab}$	3.331	**0.017**	2.847	**0.033**	3.020	0.088
Brown adipose tissue	0.095±0.037	0.129±0.040	0.130±0.041	0.122±0.033	0.126±0.031	2.075	0.096	1.854	0.131	0.012	0.914
Heart	0.266±0.050	0.285±0.041	0.299±0.054	0.271±0.044	0.291±0.032	1.135	0.350	0.699	0.596	4.124	0.047
Lungs	0.357±0.066	0.339±0.071	0.350±0.037	0.368±0.074	0.329±0.068	0.629	0.644	0.482	0.749	0.827	0.367
Liver	3.654±0.659$_{a}$	5.517±0.839$_{b}$	3.263±0.499$_{a}$	3.728±0.641$_{a}$	3.598±0.583$_{a}$	23.366	**< 0.001**	32.615	**< 0.001**	16.496	< 0.001
Pancreas	0.469±0.150	0.395±0.062	0.411±0.146	0.354±0.070	0.412±0.062	1.753	0.151	1.883	0.126	0.576	0.451
Spleen	0.106±0.043$_{a}$	0.167±0.049$_{b}$	0.144±0.024$_{ab}$	0.158±0.034$_{b}$	0.169±0.043$_{b}$	5.448	**0.001**	3.643	**0.010**	5.545	0.022
Stomach	0.322±0.048	0.348±0.030	0.341±0.035	0.352±0.040	0.366±0.036	2.231	0.077	0.727	0.578	7.002	0.011

Intestine	1.483±0.335	1.598±0.527	1.442±0.590	1.424±0.275	1.452±0.051	0.245	0.912	0.278	0.891	0.172	0.680
Caecum	0.127±0.035	0.127±0.028	0.146±0.058	0.146±0.063	0.141±0.051	0.509	0.729	0.500	0.736	0.065	0.799
Colon	0.292±0.043	0.295±0.049	0.329±0.118	0.322±0.042	0.348±0.066	1.402	0.245	0.730	0.575	0.949	0.334
Kidneys	0.642±0.080$_{a}$	0.839±0.077$_{c}$	0.747±0.052$_{b}$	0.786±0.104$_{bc}$	0.763±0.067$_{bc}$	11.235	**< 0.001**	10.355	**< 0.001**	6.502	0.013
Uterus	0.097±0.069$_{a}$	0.124±0.085$_{ab}$	0.195±0.067$_{abc}$	0.220±0.083$_{bc}$	0.229±0.104$_{c}$	5.103	**0.002**	3.681	**0.011**	0.217	0.643
Ovaries	0.037±0.007$_{a}$	0.048±0.015$_{ab}$	0.076±0.031$_{bc}$	0.066±0.027$_{bc}$	0.077±0.024$_{c}$	6.246	**0.001**	5.062	**0.002**	0.198	0.659
Brain	0.493±0.031	0.502±0.027	0.497±0.028	0.512±0.018	0.497±0.022	0.916	0.461	0.863	0.492	0.451	0.505

Table S1b. Weaning organ masses in the female pup of lactating female mice fed on diets with graded fat levels.

Organ	8.3% fat	25% fat	41.7 % fat	58.3 % fat	66.6 % fat	ANOVA		GLM (BM covariate)			
						Diet		Diet		BM	
						F	P	F	P	F	P
Subcutaneous fat	0.094±0.087	0.069±0.096	0.269±0.164	0.294±0.363	0.235±0.313	1.312	0.282	2.083	0.101	92.628	< 0.001
Mesenteric fat	0.025±0.029$_{a}$	0.035±0.096$_{ab}$	0.062±0.03 $_{b}$	0.043±0.023$_{ab}$	0.054±0.023$_{ab}$	3.831	**0.009**	4.123	**0.007**	66.15	< 0.001
Brown adipose tissue	0.034±0.015$_{a}$	0.049±0.014$_{ab}$	0.079±0.024$_{c}$	0.077±0.021$_{c}$	0.066±0.021$_{bc}$	12.259	**< 0.001**	1.183	0.328	132.616	< 0.001
Heart	0.056±0.021$_{a}$	0.074±0.017$_{ab}$	0.111±0.025$_{c}$	0.092±0.014$_{bc}$	0.087±0.016$_{b}$	15.149	**< 0.001**	4.340	**0.004**	90.485	< 0.001
Lungs	0.114±0.027$_{a}$	0.121±0.022$_{a}$	0.177±0.029$_{c}$	0.156±0.027$_{bc}$	0.132±0.030$_{ab}$	10.994	**< 0.001**	3.465	**0.014**	28.626	< 0.001
Liver	0.280±0.161$_{a}$	0.366±0.100$_{ab}$	0.556±0.132$_{c}$	0.520±0.181$_{bc}$	0.497±0.151$_{bc}$	7.795	**< 0.001**	4.835	**0.002**	241.79	< 0.001
Pancreas	0.020±0.018$_{a}$	0.029±0.012$_{ab}$	0.063±0.025$_{c}$	0.046±0.012$_{bc}$	0.041±0.019$_{abc}$	9.765	**< 0.001**	3.237	**0.02**	56.042	< 0.001
Spleen	0.012±0.011$_{a}$	0.026±0.025$_{a}$	0.078±0.039$_{b}$	0.074±0.040$_{b}$	0.047±0.040$_{ab}$	9.496	**< 0.001**	4.479	**0.004**	202.777	< 0.001
Stomach	0.049±0.016$_{a}$	0.058±0.012$_{a}$	0.083±0.017$_{b}$	0.088±0.035$_{b}$	0.071±0.024$_{ab}$	7.069	**< 0.001**	0.178	0.949	91.338	< 0.001
Intestine	0.300±0.070	0.294±0.098	0.372±0.120	0.329±0.076	0.296±0.046	1.852	0.132	0.839	0.507	4.558	0.037
Caecum	0.020±0.010	0.021±0.005	0.027±0.010	0.028±0.012	0.027±0.010	1.756	0.152	0.266	0.898	11.402	0.001
Colon	0.038±0.028$_{a}$	0.061±0.026$_{ab}$	0.084±0.026$_{b}$	0.068±0.021$_{b}$	0.077±0.026$_{b}$	6.217	**< 0.001**	3.203	**0.021**	52.096	< 0.001
Kidneys	0.099±0.035$_{a}$	0.129±0.035$_{ab}$	0.203±0.043$_{c}$	0.199±0.056$_{c}$	0.163±0.049$_{bc}$	12.803	**< 0.001**	0.784	0.540	218.097	< 0.001
Brain	0.351±0.042$_{a}$	0.367±0.030$_{ab}$	0.423±0.024$_{c}$	0.424±0.032$_{c}$	0.390±0.032$_{bc}$	11.491	**< 0.001**	1.026	0.403	53.262	< 0.001

Table S1c. Weaning organ masses in the male pup of lactating female mice fed on diets with graded fat levels.

Organ	8.3% fat	25% fat	41.7 % fat	58.3 % fat	66.6 % fat	ANOVA		GLM (BM covariate)			
						Diet		Diet		BM	
						F	P	F	P	F	P
Subcutaneous fat	0.044±0.126$_{a}$	0.079±0.067$_{ab}$	0.313±0.149$_{c}$	0.244±0.168$_{bc}$	0.189±0.106 $_{abc}$	7.056	**< 0.001**	0.443	0.777	37.909	< 0.001
Mesenteric fat	0.042±0.028$_{a}$	0.042±0.028$_{a}$	0.076±0.021$_{b}$	0.066±0.021$_{ab}$	0.046±0.021$_{ab}$	4.615	**0.003**	4.433	**0.004**	69.84	< 0.001
Brown adipose tissue	0.038±0.013$_{a}$	0.058±0.018$_{ab}$	0.094±0.038$_{c}$	0.084±0.026$_{bc}$	0.081±0.015$_{bc}$	9.944	**< 0.001**	0.226	0.923	33.128	< 0.001
Heart	0.057±0.018$_{a}$	0.077±0.014$_{b}$	0.115±0.019$_{c}$	0.097±0.018$_{c}$	0.100±0.015$_{c}$	23.362	**< 0.001**	3.923	**0.007**	32.962	< 0.001
Lungs	0.121±0.037$_{a}$	0.141±0.038$_{ab}$	0.189±0.043$_{c}$	0.176±0.046$_{bc}$	0.176±0.025$_{bc}$	6.783	**< 0.001**	0.189	0.943	22.144	< 0.001
Liver	0.279±0.122$_{a}$	0.495±0.191$_{bc}$	0.640±0.115$_{c}$	0.537±0.128$_{bc}$	0.481±0.084$_{b}$	11.459	**< 0.001**	9.597	**< 0.001**	169.192	< 0.001
Pancreas	0.028±0.020$_{a}$	0.041±0.017$_{ab}$	0.064±0.017$_{c}$	0.053±0.018$_{bc}$	0.050±0.010$_{bc}$	7.136	**< 0.001**	1.133	0.351	62.248	< 0.001
Spleen	0.012±0.014$_{a}$	0.034±0.038$_{ab}$	0.085±0.032$_{c}$	0.069±0.030$_{c}$	0.053±0.021$_{bc}$	12.041	**< 0.001**	0.758	0.557	103.061	< 0.001
Stomach	0.052±0.015$_{a}$	0.067±0.022$_{ab}$	0.097±0.020$_{c}$	0.078±0.020$_{bc}$	0.079±0.011$_{bc}$	10.755	**< 0.001**	2.226	0.078	59.808	< 0.001
Intestine	0.299±0.108	0.299±0.116	0.408±0.145	0.297±0.051	0.331±0.116	2.276	0.074	1.192	0.326	6.975	0.328
Caecum	0.018±0.008$_{ab}$	0.022±0.006$_{ab}$	0.027±0.009$_{b}$	0.016±0.005$_{a}$	0.023±0.009$_{ab}$	3.233	**0.02**	2.551	0.051	0.143	0.707
Colon	0.044±0.022$_{a}$	0.062±0.021$_{ab}$	0.087±0.016$_{c}$	0.067±0.014$_{bc}$	0.069±0.020$_{bc}$	8.555	**< 0.001**	1.884	0.127	19.349	< 0.001
Kidney	0.108±0.034$_{a}$	0.146±0.044$_{ab}$	0.227±0.039$_{c}$	0.185±0.037$_{bc}$	0.184±0.023$_{b}$	18.764	**< 0.001**	3.248	**0.018**	131.015	< 0.001
Brain	0.357±0.104$_{a}$	0.391±0.028$_{b}$	0.439±0.019$_{c}$	0.424±0.031$_{c}$	0.422±0.018$_{bc}$	15.754	**< 0.001**	0.988	0.422	76.499	< 0.001

Organ masses (g) were shown as means±s.d. Female mice were fed 8.3 % fat (n=13), 25 % fat (n=12), 41.7 % fat (n=14), 58.3 % fat (n=11) or 66.6 % fat (n=13) diets during lactation. Differences between dietary groups were analysed separately using ANOVA and GLM with body mass as a covariate. For organs with significant P values (bold type), different letters indicate significant differences between the groups, as assessed by the Tukey post-hoc.

Table S2. Behavior observations of mothers during early lactation (day 4–6 of lactation), mid-lactation (da late lactation y 8–10 of lactation) and (day 12–14 of lactation).

	DIET/ ACTIVITY	Climbing	Drinking	Eating	Grooming	General activities	Resting	Feeding pups	Feeding pups/eating
Early lactation period	**8.3% Fat**	0.07±0.27	2.19± 1.73	41.00±31.72$_a$	2.95±2.79	13.37±8.52	0.38±1.38	40.01±34.44$_a$	0.00±0.00
	25% Fat	0.00±0.00	0.67±0.99	4.80±6.30$_b$	1.52±2.47	8.93±7.43	5.35±10.78	78.71±19.24$_b$	0.00±0.00
	41.7% Fat	0.53±1.66	0.95±1.14	3.7±03.53$_{ab}$	2.36±2.16	9.84±8.70	1.84±6.65	79.95±15.43$_b$	0.00±0.00
	58.3% Fat	0.00±0.00	0.73±1.19	4.66±3.40$_{ab}$	4.86±4.97	10.20±8.93	0.82±1.83	78.52±12.92$_{ab}$	0.00±0.00
	66.6% Fat	0.16±0.57	0.75±0.97	5.25±4.87$_{ab}$	4.12±6.03	16.08±20.10	0.34±0.80	73.99±30.06$_{ab}$	0.00±0.00
	Average	**0.16±0.82**	**1.08±1.34**	**12.37±21.11**	**3.12±3.97**	**11.70±11.59**	**1.74±5.89**	**69.76±28.18**	**0.00±0.00**
Mid lactation period	**8.3% Fat**	0.10±0.31	1.31±1.43	39.93±22.63$_a$	2.01±2.59	7.66±4.62	1.01±2.33	43.97±21.24$_a$	5.9±9.66
	25% Fat	0.35±0.75	3.18±4.04	20.59±15.24$_{ab}$	3.95±3.34	12.07±7.74	0.68±1.89	59.14±24.06$_{ab}$	0.66±2
	41.7% Fat	0.07±0.27	2.57±3.08	8.25±7.57$_b$	2.37±3.37	13.64±11.18	1.82±5.47	69.98±20.20$_{ab}$	0.53±1.45
	58.3% Fat	0.38±1.28	1.31±0.90	7.79±8.01$_b$	2.44±3.79	11.00±8.51	1.09±2.77	75.77±18.26$_b$	0.18±0.60
	66.6% Fat	0.09±0.31	1.13±1.06	7.38±5.59$_b$	1.85±3.03	11.06±6.97	5.91±13.51	73.33±18.27$_b$	0.17±0.62

	Average	**0.18±0.67**	**1.89±2.44**	**15.60±17.35**	**2.46±3.21**	**11.23±8.29**	**2.22±7.06**	**65.45±22.64**	**1.36±4.59**
Late lactation period	**8.3% Fat**	0.30±1.10	1.82±2.05	25.00±19.79$_{a}$	2.68±3.29	3.60±3.74	0.91±2.77	53.92±20.43	11.73±14.80
	25% Fat	2.91±10.10	1.52±2.43	16.25±14.98$_{ab}$	2.99±3.75	11.06±8.33	0.42±1.01	59.33±25.26	5.48±8.29
	41.7% Fat	0.00±0.00	1.91±3.33	8.16±14.18$_{b}$	4.21±3.85	8.57±9.34	2.07±4.46	70.05±25.37	4.24±6.10
	58.3% Fat	0.00±0.00	1.27±1.27	4.45±4.75$_{b}$	6.10±7.63	7.73±8.28	5.29±8.06	74.41±20.00	0.72±1.34
	66.6% Fat	0.00±0.00	1.24±1.08	7.65±7.56$_{ab}$	4.65±4.70	11.40±10.66	5.38±13.21	69.60±22.58	1.14±2.93
	Average	**0.61±4.43**	**1.56±2.18**	**12.41±15.05**	**4.8±4.77**	**8.46±8.63**	**2.76±7.39**	**65.35±23.45**	**4.72±8.95**

Percentage of time (means±s.d.) that female mice fed 8.3 % fat (n=13), 25 % fat (n=12), 41.7 % fat (n=14), 58.3 % fat (n=11) or 66.6 % fat (n=13) diets spend in each activity. Activities with significant differences are highlighted in grey. Different letters indicate significant differences between diets after a pairwise comparation with Bonferroni correction.