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MICE SELECTIVELY BRED FOR HIGH WHEEL RUNNING ACTIVITY HAVE INCREASED GROWTH EFFICIENCY DURING LACTATION

I. Jónás, K. A. Schubert, T. Garland Jr, A. J.W. Scheurink, C. Nyakas, and G. van Dijk

Summary

We hypothesized that high voluntary physical activity would negatively affect growth efficiency (GE) during lactation in mice. We assessed mother and litter characteristics during lactation under low fat (LF) and high fat (HF) dietary feeding conditions in mice selectively bred for high wheel-running behavior (lab-designated lines 7 and 8) and in a non-selected control line (line 2). At birth, litter weights were lower in HF feeding mothers irrespective of line, and litter sizes were larger in line 7 and 8 relative to line 2, irrespective of diet. Over the course of lactation, all females lost pups, but HF feeding line 7 females lost significantly more than others. At peak lactation (post-gestational days 13-16), litter weights but not maternal body masses of HF feeding mothers became increased above LF ones. GE (calculated as weight gain of mothers and litters divided by absorbed energy of mothers) during peak-lactation was higher in HF feeding than LF feeding mothers, and statistically interacted with line, with both line 7 and 8 higher than line 2 in the HF diet condition. Regression analysis revealed that this HF-diet effect to increase GE was particularly the case at large litter sizes. It is concluded that a trait for increased physical activity augments GE during HF diet feeding, but may have disadvantageous effects on pup survival.

1. Introduction

Lactation is an energetically demanding occupation for mammals. In human beings, the incremental energy cost of lactation adds approximately 25% to the normal energy requirements, and this is met by an increase in food intake, mobilization of body fat reserves, and an increase in metabolic efficiency (reviewed by (Butte and King 2005). In rodents, the energy cost of lactation is estimated to increase even four-fold above normal energy requirements (Johnson, Thomson, and Speakman 2001a). The maximum energy consumption and expenditure - termed sustainable energy intake (SusEI) and sustainable metabolic rate (SusMR), respectively - during this period have been suggested to be limited intrinsically by aspects of physiology, and this concept has been outlined in a number of mouse studies by Krol and Speakman (Krol and Speakman 2003; Speakman and Krol 2005; Krol, Murphy, and Speakman 2007) and earlier workers.

Over the course of lactation, laboratory house mice first show a linear increase in energy intake and then it stabilizes to a maximum intake between days 13 and 16. This period has been termed “peak lactation” (Thompson and Nicoll 1986). At peak lactation, Johnson et al observed that the amount of ingested food correlates positively with litter size as well as litter mass ((Johnson, Thomson, and Speakman 2001a). Furthermore, resting metabolic rate (RMR) and daily energy expenditure (DEE) of lactating dams also increase with increasing litter size (Johnson, Thomson, and Speakman 2001a; Johnson, Thomson, and Speakman 2001b). Thus, litter size may be important to determine the mother’s energy procurement to her pups, as variation in litter mass was observed to be linearly related with litter size, without affecting individual pup mass (Johnson, Thomson, and Speakman 2001a). However, when litter size increases above a certain number, SusEI becomes limited, which subsequently causes a reduction in average pup mass. One hypothesis by which SusEI is maximized states that a lactating animal has the risk of lethal overheating when it is feeding litters that are too large (Krol and Speakman 2003). Thus, the maximum capacity to dissipate heat would then limit individual pup growth above a certain litter size.

In the current study, the relation between maternal body weight, SusEI and resting metabolic rate, litter size, litter weight, and individual pup growth was investigated during lactation of female mice that were selectively bred for high wheel-running activity and in non-selected controls. Female mice from the breeding line for increased running wheel activity have low body masses together with a high level of energy turn-over (Vaanholt et al. 2008). On a high-fat/high-sucrose "Western"-type diet they are markedly hyperphagic, but resistant to becoming obese due to increased spontaneous activity and elevated heat production. In contrast, control mice on this diet suppress their food intake, but have dramatically increased growth efficiency (van Dijk et al. in preparation), and thus become obese (Vaanholt et al. 2008).

The major aim of the present study was to investigate whether the increased level of performance (see also (Meek et al. 2009)) of highly active mice alters afore-mentioned relationships between SusEI and litter characteristics during lactation when feeding a standard low-fat laboratory (LF) diet or a high fat/high sucrose (HF) diet. The one study reporting on

reproductive success and litter characteristics of these highly-active female mice from an earlier generation showed that they do not differ in reproductive output, litter size, and litter mass from control lines (Girard et al. 2002). When body masses of lactating females were taken into account, selected mice tended to wean relative heavier litters than control females. Therefore, it may be predicted that energy procurement to offspring of highly-active mice would be higher and/or more efficient, particularly on a HF diet. Alternatively, it may be predicted that individual pup growth becomes limited by activity of muscular tissue of highly-active mice feeding a HF diet, which drains energy away from milk production, thus leading to a trade-off. This has been mentioned by Krol et al. as a “peripheral” limiting factor to offspring development (Krol, Johnson, and Speakman 2003). The likelihood of such a trade-off is supported by the observation that, in a previous study, males from one of the selected lines did not exhibit an increased maximum workload when mice were forced to work (via wheel running) for food (Vaanholt et al. 2007).

2. Materials and methods

2.1. Animals and housing

Mice used in this study were selectively bred for voluntary wheel-running behavior (the base population was the Hsd:ICR strain) over 49 generations and were obtained from T. Garland Jr, Riverside, CA. In the original selection protocol, eight lines of mice were created (4 selected and 4 control) (Swallow, Carter, and Garland, Jr. 1998). Breeding lines were maintained at our facilities in Haren without further selection for wheel-running activity. Mice were typically housed in standard cages (Macrolon Type II, UNO Roestvaststaal BV, Zevenaar, NL) in the same room with *ad libitum* access to water and a low-fat (LF) standard laboratory mouse chow (3.8 kcal/g; 58 % carbohydrate, 6 % fat, 22 % protein; RMH-B 2181, HopeFarms BV, Woerden, NL) at an ambient temperature of 22 ± 1 °C, and maintained on a 12:12 light-dark cycle with lights on at 8 am. Pine shavings and EnviroDry® were used as bedding material. All methods were approved by, and are in agreement with the regulations of the Institutional Animal Use and Care Committee of the University of Groningen. These regulations are consistent with the guidelines for the care and use of laboratory animals as described by the U.S. National Institutes of Health.

From two selection lines (lab designation line 7, n=26; and line 8, n=26) and one control line (line 2, n=24), virgin female mice at the age of 4.5 months were paired with males of equal line. Two weeks before pairing, about half of the mice from each selection/control group were switched from the LF diet to a high-fat (HF) diet, additionally containing sucrose (4.7 kcal/g; 30 % carbohydrate, 45 % fat, 18 % protein; AB Animal Diets, Woerden, NL). The others of each group were left on the LF diet. After three weeks of pairing, males were removed and females were left to deliver and raise their litters until weaning (see Table 1).

Table 1. The number of paired females per line either feeding a low-fat (LF) or high-fat (HF) diet, successful deliveries/weanings and the total pup counts at these time points.

Line	2 Control		7 Selected		8 Selected	
Diet	LF	HF	LF	HF	LF	HF
Females (n)						
paired	13	11	12	14	12	14
successful deliveries	13	9	10	13	11	11
successfully weaned nests	13	9	9	10	10	11
Pups alive (n)						
at birth	117	65	116	123	116	121
at weaning	114	62	77	73	95	119

2.2. Mother and offspring characteristics from parturition to weaning

Maternal body mass was assessed once prior to breeding and after parturition, and daily throughout the period of lactation. Food intake was also measured daily throughout lactation. Bedding was also daily checked for spilled or crumbled food (Koteja et al. 2003). At peak lactation (between day 13-16), besides assessing food intake, feces produced by the female and her offspring over these 3 days were collected. The energy content in dried, homogenized feces and food was determined using a bomb calorimeter (CBB 330, standard benzoic acid 6320 cal/g, BCS-CRM No.90N). After day 16 (before offspring started to eat from food hoppers), mice feeding the HF diet were switched back to feeding the LF diet.

The absorbed energy of the mothers at peak lactation was calculated from the difference between energy intake and energy content of the feces, and subsequently used to calculate efficiency of body weight gain of mother and pups (i.e., expressed as mg body weight gain/kJ food absorbed). On the last day of peak lactation (day 16), animals were subjected to indirect calorimetry to assess resting metabolic rate (RMR, kJ h⁻¹) (Johnson, Thomson, and Speakman 2001b). Each animal was placed in a 2 L Plexiglas metabolic chamber at thermoneutrality (30±1°C) for 4 hours. Home cage bedding was provided to reduce stress of novelty. During this period, females were separated from their litters (which remained in their home cage) and did not have access to food. Specifically, measurements included O₂ consumption (VO₂, ml h⁻¹) and CO₂ production (VCO₂, ml h⁻¹) with an open air flow system (Oklejewicz et al. 1997) with inlet airflow set at 20 l hr⁻¹ (Brooks Type 5850 mass flow controller, Rijswijk, NL). Channels were sampled in sequence, with each measurement lasting one minute, and a reference channel measured at least once every five minutes. Inlet and outlet air were dried (3 Å molecular sieve drying beads, Merck, Darmstadt, Germany) and analyzed for gas concentrations with a paramagnetic oxygen analyzer (Servomex Xentra 4100, Crowborough, UK) and infrared gas analyzer for CO₂ (Servomex 1440). The system recorded the differentials in oxygen and carbon dioxide between dried reference air and dried air from the metabolic cages. Oxygen consumption was calculated using equation 2 of

Hill (Hill 1972) and expressed at standard temperature and pressure. We calculated the respiratory quotient (RQ) as VCO_2/VO_2 . Heat production (HP, kJ h^{-1}) was calculated from the equation formulated by (Romijn and Lokhorst 1961):

$$HP = 16.18 * VO_2 + 5.02 * VCO_2$$

We defined the RMR at peak lactation as the lowest value of a 20-minute running mean of HP, typically the average of three consecutive measurement points which corresponded when the animals were fully resting, as detected with Passive InfraRed sensors (PIRs). Metabolic rate tended to decrease over time and reach a stable low level. Animals usually reached RMR after 3 hours in the measurement chamber. Following parturition, the number of pups and the total mass of the litters were assessed and mean pup mass was calculated. Measurements were done at birth (day 0-1), 13, 16 and 21 (weaning).

2.3. Statistical analysis

GLM Repeated Measures was used to analyze differences in body mass and food intake of females through lactation. GLM Univariate Analysis was used to analyze differences in energetic measures taken at peak lactation (food intake, RMR, PIR, energy content of feces, calculated absorbed energy and growth efficiency) and for differences in litter characteristics. Multiple regression analysis was performed to assess correlations between litter characteristics, as well as between litter size and mother characteristics at peak lactation to reveal line and diet effects. A value of $p < 0.05$ (for 2-tailed tests) was considered significant for all tests.

3. Results

3.1. Mother characteristics

Before pregnancy, body weight of line 2 females was 22 % higher than that of line 7 and line 8 females ($F(2,55)=24.2$; $p < 0.001$), irrespective of diet (Fig. 1). On the last day of pregnancy, a doubling was observed in body weights of females relative to the day of conception ($F(2,55)=15.01$; $p < 0.001$), without any significant difference in body weight gain among lines or between diets. After delivery, body weight of line 2 females was approximately 15 % higher compared to line 7 and 8 females ($F(2,56)=15.09$; $p < 0.001$), irrespective of diet. Within-subject repeated measures analysis over days 1 through 16 revealed that body weight of line 8 females increased significantly more (5.2 ± 0.5 g) than that of line 2 females (1.4 ± 0.8 g), while that of line 7 females gained intermediate weight (3.4 ± 0.5 g) (line*time interaction: $F(30,840)=2.96$; $p < 0.001$). Furthermore, as can be seen in Figure 1, females fed the HF diet gained significantly less weight, irrespective of line, than those fed the LF diet (diet*time interaction: $F(15,840)=1.73$; $p < 0.05$).

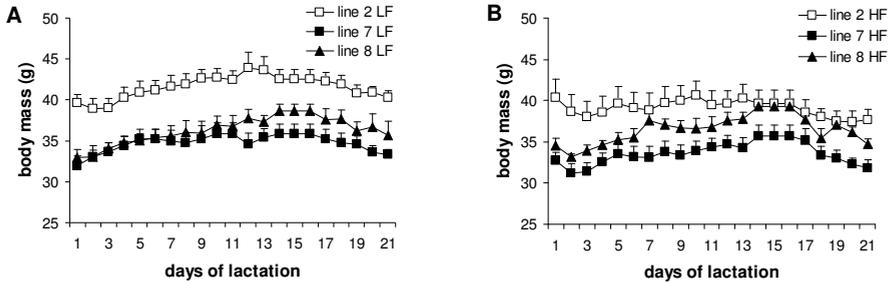


Figure 1. Body weight of line 2 (control line), line 7, and line 8 (selected lines) mothers fed a low-fat (LF, panel A) or a high-fat (HF, panel B) diet during lactation.

Within-subject repeated measures analysis over days 1 through 16 revealed that food intake (Fig. 2) of lactating females was increasing over time ($F(14,770)=46.18$; $p<0.001$). This effect interacted with line (line*time: ($F(28,770)=2.01$; $p<0.01$), but not with diet. Specifically, line 2 mothers ate 3 times more over the 16-day period, whereas line 7 and line 8 mothers ate only 2.3 times more over the period of lactation. When GLM Univariate Analysis was performed for each day, line 8 mothers were found to eat the most on the first and second day after delivery in the HF diet group only (day 2: $F(2,60)=3.55$; $p<0.05$). Post-hoc differences were lost after day 2.

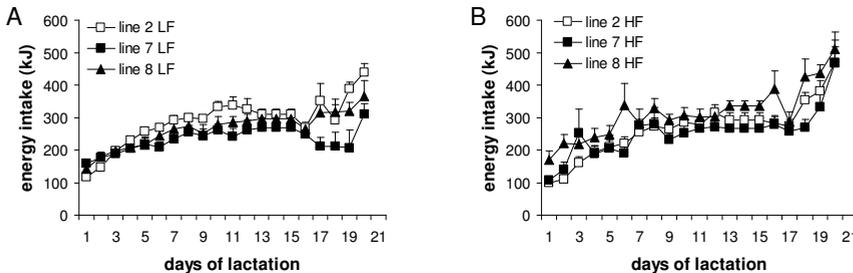


Figure 2. Energy intake of line 2 (control line), line 7, and line 8 (selected lines) mothers fed a low-fat (LF, panel A) or a high-fat (HF, panel B) diet during lactation.

3.2. Litter characteristics

At birth, litter sizes of line 7 and line 8 females were significantly larger ($F(2,61)=7.24$; $p<0.01$) than those of line 2 (see Table 2). Over the course of lactation, pups were found missing in all lines, but, on average, line 7 females lost significantly more pups per litter than line 2 and 8 females, irrespective of diet ($F(2,61)=8.68$; $p<0.001$). Litter weight at birth was lower in females fed the HF diet ($F(1,60)=4.73$; $p<0.05$), and litter weights of line 2 and 7 females contributed mostly to this effect. At day 13, however, litter weight of HF-fed mothers became higher

($F(1,56)=4.36$; $p<0.05$) compared to litters of LF-fed mothers. This effect was particularly observed in line 8, which persisted until day 16 ($F(1,56)=6.65$; $p<0.05$). At day 13, litter weight was also influenced by line ($F(2,56)=5.20$; $p<0.01$); i.e., litter weight of line 7 was smaller than litter weight of line 2 and line 8 females, which remained at day 16 ($F(2,55)=5.45$; $p<0.01$) and day 21 ($F(2,55)=7.88$; $p<0.001$). On day 21, effects of diet were lost, an effect presumably caused by the fact that mothers were switched back to the LF diet (see table 2).

Mean individual pup mass at birth was 14% lower in line 7 and 12% lower in line 8 than that of line 2 ($F(2,60)=14.87$; $p<0.001$), irrespective of diet. From day 13 onwards, these differences became larger; mean pup mass of line 7 was 29 % lower and line 8 was 27 % lower than that of line 2 ($F(2,54)=29.48$; $p<0.001$). Furthermore, mean pup mass became increased by diet irrespective of line from day 13 onwards ($F(1,56)=23.92$; $p<0.001$) (see table 2).

Table 2. Litter characteristics of line 2 (control line), line 7, and line 8 (selected lines) mothers fed a low-fat (LF) or a high-fat (HF) diet.

	2 Control		7 Selected		8 Selected	
	LF	HF	LF	HF	LF	HF
Litter size						
day 1	9.0 ± 0.8	7.2 ± 1.0	11.6 ± 0.5 #	9.5 ± 0.8	10.5 ± 0.6	11 ± 0.8
day 13	8.8 ± 0.8	6.9 ± 1.0	8.1 ± 1.3	5.8 ± 1.3	8.6 ± 1.3	10.9 ± 0.8
day 16	8.8 ± 0.8	6.9 ± 1.0	7.8 ± 1.3	5.6 ± 1.3	8.6 ± 1.3	10.8 ± 0.7
day 21	8.8 ± 0.8	6.9 ± 1.0	7.7 ± 1.3	5.6 ± 1.3	8.6 ± 1.3	10.8 ± 0.7
Litter weight						
day 1	16.1 ± 1.2	12.5 ± 1.6	17.8 ± 0.7	14.3 ± 1.2*	16.4 ± 0.7	17.4 ± 1.0
day 13	68.4 ± 3.8	71.4 ± 7.1	48.2 ± 5.9 ##	53.8 ± 8.1	54.4 ± 5.2 #	76.8 ± 6.1*
day 16	75.7 ± 4.4	82.5 ± 8.1	53.1 ± 6.3 ##	61.5 ± 10.0	62.3 ± 5.2	89.9 ± 6.2**
day 21	103 ± 6.4	104.8 ± 10.6	65.6 ± 7.9 ##	74.3 ± 12.4	83.8 ± 8.3	113.6 ± 8.5
Mean pup mass						
day 1	1.8 ± 0.1	1.8 ± 0.0	1.5 ± 0.0 ##	1.5 ± 0.0	1.6 ± 0.0 ##	1.6 ± 0.1
day 13	8.3 ± 0.5	10.9 ± 0.5**	5.5 ± 0.4 ##	7.2 ± 0.5*	5.9 ± 0.3 ##	7.1 ± 0.4*
day 16	9.1 ± 0.5	12.6 ± 0.7**	6.3 ± 0.4 ##	8.8 ± 0.5**	6.9 ± 0.4 ##	8.4 ± 0.5*
day 21	12.3 ± 0.6	16 ± 0.8**	7.9 ± 0.5 ##	10.4 ± 0.8*	9.4 ± 0.6 ##	10.6 ± 0.6

denotes significant difference with line 2 (line effect) (#, $p<0.05$; ##, $p<0.01$). *denotes significance difference with LF diet (diet effect) (*, $p<0.05$; **, $p<0.01$).

3.3. Peak lactation

3.3.1. Maternal energetic characteristics

Table 3 shows maternal energetics during peak lactation (day 13-16). The total weight of food eaten during peak lactation was significantly influenced by diet ($F(1,59)=12.93$; $p<0.001$). Specifically, HF-fed mothers ate 18.2 % less than LF-fed females, without any effects of line. Conversion to energy content of the food revealed that energy intake (in kJ) was not significantly

altered by diet or line. Resting metabolic rate (RMR assessed on day 16 of lactation) was significantly lower in females fed the HF diet ($F(1,52)=19.18$; $p<0.001$) than that in females fed the LF diet. No line or interaction effect between line and diet was observed. The activity of lactating females (measured by PIR detection) was affected by line ($F(2,52)=5.98$; $p<0.01$) with line 7 being more active than line 2, and line 8 being intermediate. The total dry weight of feces during peak lactation was 42.7 % lower in females on the HF diet ($F(1,59)=41.64$; $p<0.001$) than those on the LF diet, without a line or interaction effect. The total energy content of feces assessed by bomb calorimetry was 35.3 % lower in HF-fed females than in LF-fed females ($F(1,59)=26.81$; $p<0.001$), but no line or interaction effect was observed. Furthermore, the relative energy content per feces mass was 10.5 % higher in HF-fed females than in LF-fed females ($F(1,59)=79.46$; $p<0.001$), again without a line or interaction effect. The absorbed energy was neither affected by diet nor line. However, the efficiency of absorption was significantly increased in mothers fed HF diet ($F(1,53)=44.38$; $p<0.001$), without any line differences.

Table 3. Energetics during peak lactation (day 13-16) of line 2 (control line), line 7 and line 8 (selected lines) mothers fed a low-fat (LF) or a high-fat (HF) diet.

	2 Control		7 Selected		8 Selected	
	LF	HF	LF	HF	LF	HF
Food intake (g)	60.1 ± 2.8	44.5 ± 3.6**	50.6 ± 3.2	40.6 ± 5.0	56.1 ± 3.5	51.2 ± 2.6
Food intake (kJ)	954.8 ± 45.3	875.1 ± 70.6	805.0 ± 50.5	797.6 ± 98.9	891.5 ± 55.7	1007.0 ± 51.0
RMR (kJ/h)	2.1 ± 0.1	1.6 ± 0.1**	1.9 ± 0.2	1.4 ± 0.1*	1.9 ± 0.1	1.7 ± 0.0
PIR (#/min)	1.6 ± 0.4	1 ± 0.2	3.7 ± 1.3	4 ± 0.8	2.9 ± 0.9	1.8 ± 0.9
Feces						
total dry weight (g)	7.9 ± 0.5	4.3 ± 0.5**	7.0 ± 0.6	3.6 ± 0.5**	7.5 ± 0.9	5.0 ± 0.6*
total energy content (kJ)	140.0 ± 7.3	83.4 ± 9.4**	124.0 ± 10.1	72.7 ± 10.2**	132.7 ± 15.1	101.6 ± 12.3
energy density (kJ/g)	17.7 ± 0.2	19.3 ± 0.3**	17.8 ± 0.2	19.6 ± 0.4**	17.9 ± 0.2	20.0 ± 0.2**
Absorbed energy (kJ)	814.8 ± 44.2	791.7 ± 62.4	681.0 ± 44.6	722.5 ± 89.2	758.8 ± 50.7	892.6 ± 46.4
Absorption efficiency (%)	85.0 ± 1.0	90.6 ± 0.5**	84.5 ± 1.0	90.7 ± 0.5**	85.1 ± 1.5	90.0 ± 1.1*

* denote significant effect of diet (*, $p<0.05$; **, $p<0.01$).

3.3.2. Growth efficiency of mothers and litters

Calculating weight change of the mothers combined with weight change of their respective litters over the course of peak lactation divided by the absorbed maternal energy over this period yields “growth efficiency” (GE) of mothers and pups. In Figure 3, GE is shown as averages in the different line and diet groups. GE was influenced by line ($F(2,49)=5.23$; $p<0.01$), diet ($F(1,49)=41.21$; $p<0.001$), and by an interaction between line and diet ($F(2,49)=4.53$; $p<0.05$). Thus, whereas effect of line on GE was variable when mothers were feeding the LF diet (i.e., with GE 24 % lower in line 7 and 21 % higher in line 8 relative to line 2), GE was significantly higher in line 7 and 8 animals feeding the HF diet than line 2 animals feeding the HF diet.

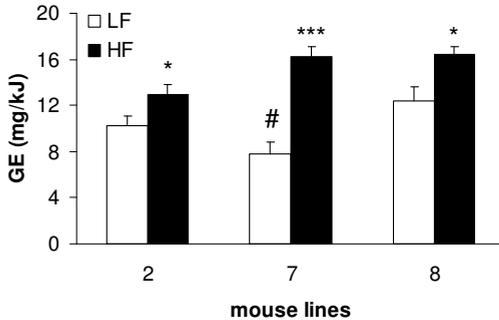


Figure 3. Growth efficiency (GE) of line 2 (control line), line 7, and line 8 (selected lines) mothers and their offspring fed a low-fat (LF) or a high-fat (HF) diet at peak lactation. # denotes significant line effect ($p < 0.05$). *denotes significance difference with LF diet (diet effect) (*, $p < 0.05$; ***, $p < 0.001$).

3.4. Multiple regression analysis

It was reported previously that litter size is an important factor influencing (negatively) individual pup mass (e.g., Hayes et al., 1992), but it also might influence maternal investments in the offspring and therefore could affect ingestive behavior of the mother (Johnson, Thomson, and Speakman 2001a). Using multiple regression analysis, we investigated whether the relation between litter size and maternal and/or offspring traits were influenced by line and diet.

3.4.1. Litter size and mean pup mass

As expected and consistent with previous work of Johnson and colleagues (Johnson, Thomson, and Speakman 2001a), mean pup mass was correlated in a negative direction to litter size at birth. Within this correlation, pups of line 7 and line 8 weighed significantly less than those of line 2 at each corresponding litter size. HF diet did not cause any change in mean pup mass at birth at each corresponding litter sizes. From day 13 onwards, however, HF diet increased mean pup mass compared to those of LF-fed mothers at each corresponding litter size, and this effect was still observed at day 21.

Litter size remained negatively correlated to mean pup mass at day 13, 16, and 21. Furthermore, at these time points, line 7 and line 8 pup masses interacted positively with litter size, meaning that line 7 and line 8 pup masses were only smaller than those of line 2 pups in relatively small but not in large litters independent of diet. The interactions are shown for day 16 (peak lactation) in figure 4, which are exemplary for days 13 and 21. For intercepts, slopes, and p-values at the four time points of the lactation period, see Table 4.

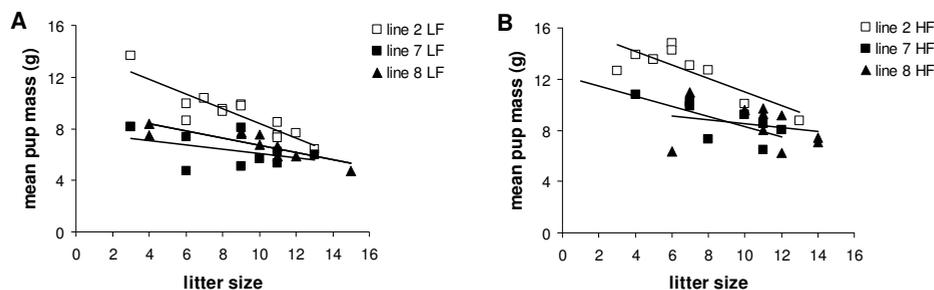


Figure 4. Relations between litter size and mean pup mass of line 2 (control line), line 7, and line 8 (selected lines) mothers fed a low-fat (LF, panel A) or a high-fat (HF, panel B) diet at peak lactation. Least-squares linear regressions are shown overall in LF diet condition: $R^2=0.31$, $p=0.001$; and in HF diet condition: $R^2=0.49$, $p<0.001$.

Table 4. Multiple regression analysis of litter size in relation to mean pup mass and litter weight at day 1 (birth), day 13, day 16, and day 21 (weaning) in interaction with line and diet.

	day 1		day 13		day 16		day 21	
	B	p	B	p	B	p	B	p
MEAN PUP MASS								
Intercept	2.21	<0.001	12.76	<0.001	13.94	<0.001	19.42	<0.001
littersize	-0.04	<0.001	-0.50	<0.001	-0.55	<0.001	-0.79	<0.001
Diet	-0.26	0.040	1.52	<0.001	2.50	<0.001		
Line 7	-0.17	<0.001	-6.22	<0.001	-5.37	<0.001	-8.71	<0.001
Line 8			-6.06	<0.001	-4.89	<0.001	-6.57	<0.001
Line 7 x Diet								
Line 8 x Diet								
Line 7 x Litter size			0.39	0.001	0.29	0.028	0.47	0.001
Line 8 x Litter size	-0.02	0.001	0.41	0.002	0.32	0.010	0.42	0.031
Diet x Litter size							0.27	<0.001
Line 7 x Diet x Litter size								
Line 8 x Diet x Litter size								
LITTER WEIGHT								
Intercept	1.55	0.038	29.90	<0.001	29.23	<0.001	41.62	<0.001
littersize	1.58	<0.001	4.44	<0.001	5.33	<0.001	7.00	<0.001
Diet								
Line 7			-21.70	<0.001	-22.52	<0.001	-35.07	<0.001
Line 8	3.24	0.023	-18.15	<0.001				
Line 7 x Diet								
Line 8 x Diet								
Line 7 x Litter size								
Line 8 x Litter size	-0.46	0.001			-1.85	<0.001		
Diet x Litter size			1.56	<0.001	2.19	<0.001	2.17	<0.001
Line 7 x Diet x Litter size								
Line 8 x Diet x Litter size								

Intercept (B) is shown by main effects and the interaction terms between line and diet; slope is shown by the two- and three-way interaction terms with litter size. P indicates the level of significance.

3.4.2. Litter size and litter weight

If mean pup mass contributes to the weight of a litter, then one would expect that litter size is positively correlated to litter weight, and that was indeed the case at birth as well as during other time points over the period of lactation. Line 7 had smaller litter weights than line 2 at each corresponding litter size from day 13 onwards. Line 8 also had smaller litter weights than line 2 in interaction with litter size at birth and at day 16, meaning that litter weights of line 8 were smaller only at large litter sizes. Furthermore, at day 13, litter weights of line 8 were smaller than those of line 2 at each corresponding litter size. At day 21, differences between lines were lost. Diet alone did not contribute to this model, but there was a significant interaction between diet and litter size from day 13 onwards (see Fig. 5 and Table 4).

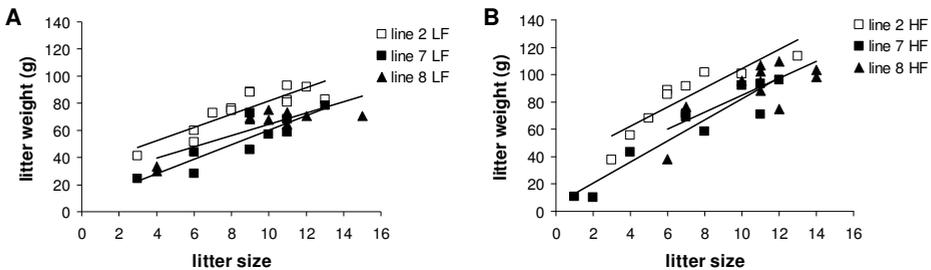


Figure 5. Relationship between litter size and litter weight of line 2 (control line), line 7 and line 8 (selected lines) mothers fed a low-fat (LF, panel A) or a high-fat (HF, panel B) diet at peak lactation. Least-squares linear regressions are shown overall in LF diet condition: $R^2=0.53$, $p<0.001$; and in HF diet condition: $R^2=0.69$, $p<0.001$.

3.4.3. Litter size and maternal energy absorption at peak lactation

As mentioned earlier, SusEI is maximized during the period of peak lactation, and the effect of energy procurement to each pup thus depends on litter size. The finding that mean pup mass was smaller only at small litter sizes but not at large ones in the selection lines makes it of interest to test relationships between litter size and absorbed energy. As expected, multiple regression analysis revealed a positive relation between absorbed energy and litter size (Table 5 and Fig. 6). Within this relation, feeding the HF diet caused the slope of the regression line to be more positive than for mice feeding the LF diet, which indicates that the absorbed energy was increased only at large litter sizes. Line 7 mice had significantly smaller absorption than line 2 at each corresponding litter size. Absorbed energy of line 8 was similar to that of line 2 in relation to litter size.

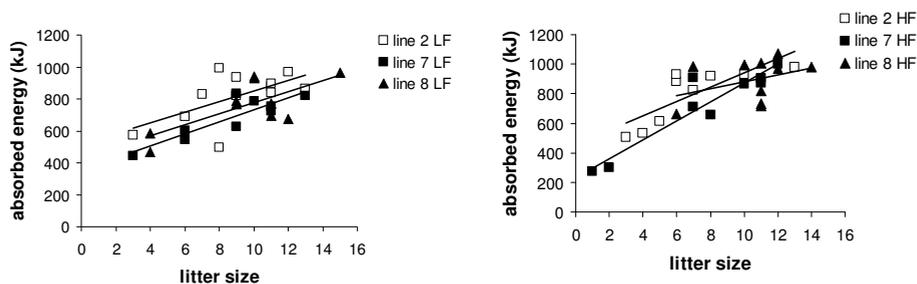


Figure 6. Relations between litter size and maternal absorbed energy of line 2 (control line), line 7 and line 8 (selected lines) mothers fed a low-fat (LF, left panel) or a high-fat (HF, right panel) diet at peak lactation. Least-squares linear regressions are shown overall in LF diet condition: $R^2=0.48$, $p<0.001$; and in HF diet condition: $R^2=0.62$, $p<0.001$.

Table 5. Multiple regression analysis between litter size and maternal/offspring traits (absorbed energy, combined weight gain of litter and mother, growth efficiency (GE) and Resting Metabolic Rate (RMR) during peak lactation in interaction with line and diet.

	Absorbed energy		Comb. weight gain		GE		RMR	
	B	p	B	p	B	p	B	p
Intercept	484.39	<0.001	5.76	<0.001	10.18	<0.001	1.54	<0.001
littersize	36.00	<0.001					0.07	<0.001
Diet							-0.38	<0.001
Line 7	-206.79	0.024			-2.43	0.029		
Line 8					10.42	<0.001	-0.24	0.025
Line 7 x Diet					4.93	0.002		
Line 8 x Diet								
Line 7 x Litter size							-0.03	0.025
Line 8 x Litter size					-0.85	<0.001		
Diet x Litter size	10.08	0.003	0.76	0.002	0.42	<0.001		
Line 7 x Diet x Litter size								
Line 8 x Diet x Litter size								

Intercept (B) is shown by main effects and the interactions term between line and diet; slope is shown by the two- and three-way interaction terms with litter size. P indicates the level of significance.

3.4.4. Interaction between litter size and combined weight gain of litter and mother

Litter size was not correlated with the combined weight gain of litter and mother over the course of peak lactation in the LF feeding condition (Table 5 and Fig. 7). However, the HF diet increased combined weight gain of litter and mother in interaction with litter size, meaning that combined weight gain was increased in the HF diet condition compared to the LF diet condition

only at large litter sizes. Combined weight gain of line 7 and line 8 was not different from that of line 2.

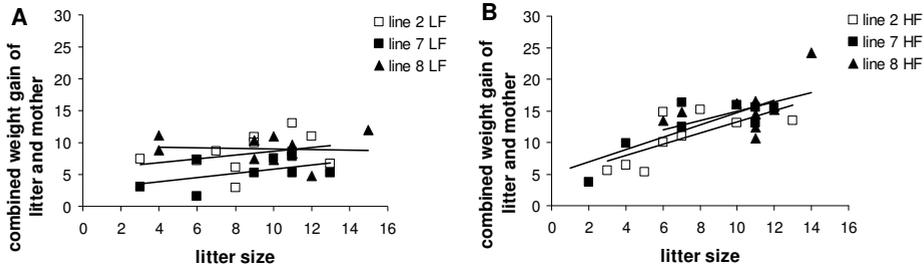


Figure 7. Relations between litter size and combined weight gain of line 2 (control line), line 7, and line 8 (selected lines) mothers and their litters fed a low-fat (LF, panel A) or a high-fat (HF, panel B) diet at peak lactation. Least-squares linear regressions are shown overall in LF diet condition: $R^2=0.06$, $p=0.186$; and in HF diet condition: $R^2=0.57$, $p<0.001$.

3.4.5. Interactions between litter size and GE at peak lactation

Litter size was not correlated with GE when animals fed the LF diet (Table 5 and Fig. 8). However, viewing lines separately, we observed that line 8 had a negative relationship with litter size, meaning that GE of line 8 was increased only at small litter sizes relative to that of line 2. Line 7 had smaller GE than line 2 at each corresponding litter size when fed a LF diet. Compared to the LF diet condition, HF diet caused an increase in GE only at large litter sizes, although the overall correlation was weak. Furthermore, HF diet caused line 7 to increase their GE to a larger extent than line 2 females and pups at each corresponding litter size.

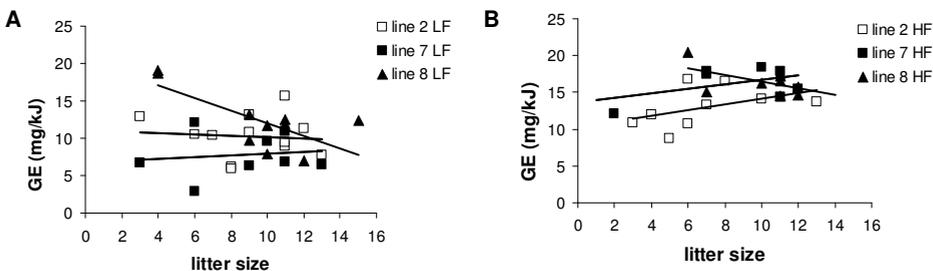


Figure 8. Relationship between litter size and GE of line 2 (control line), line 7 and line 8 (selected lines) mothers and litters fed a low-fat (LF, panel A) or a high-fat (HF, panel B) diet at peak lactation. Least-squares linear regressions are shown overall in LF diet condition: $R^2=0.05$, $p=0.26$; and in HF diet condition: $R^2=0.17$, $p=0.039$.

3.4.6. Interactions between litter size and RMR

Litter size was positively correlated with maternal RMR (Table 5 and Fig. 9). Line 7 showed an interaction with litter size, meaning that at large litter sizes RMR of line 7 mothers was lower than that of line 2 when fed the LF diet. Line 8 mothers fed the LF diet had lower RMR relative to line 2 at each corresponding litter size. HF diet decreased RMR at each corresponding litter size compared to the LF condition.

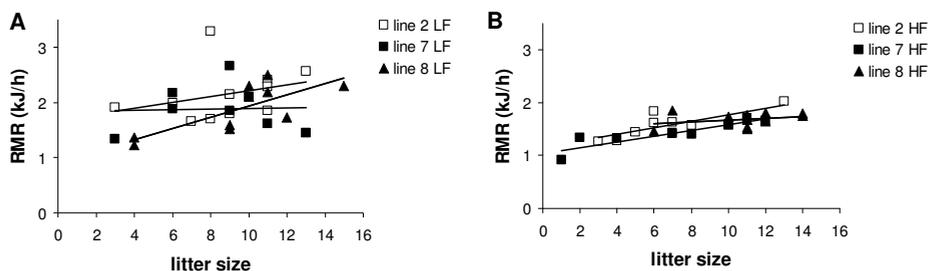


Figure 9. Relationship between litter size and resting metabolic rate (RMR) of line 2 (control line), line 7 and line 8 (selected lines) mothers fed a low-fat (LF, panel A) or a high-fat (HF, panel B) diet at peak lactation. Least-squares linear regressions are shown overall in LF diet condition: $R^2=0.14$, $p=0.049$; and in HF diet condition: $R^2=0.57$, $p<0.001$.

4. Discussion

The capacity to turn-over energy efficiently and at a high rate is an important asset to maintain a high level of physical activity over a long period. Finding nutrients under conditions of (anticipated) famine, or escaping increased levels of predation, for example, may rely on this capacity, and probably served as selective factors for many species. In many species, reproduction is a condition during which energy absorption and metabolic efficiency are increased, and often maximized. Rodents, for example, give birth to relatively large litters with a high growth rate, and subsequently face enormous energetic challenges during lactation (Johnson, Thomson, and Speakman 2001a; Johnson, Thomson, and Speakman 2001b). In the present study, we tested the hypothesis that a high capacity for physical activity and energy turn-over negatively affects a number of lactation characteristics, which we investigated in two mouse lines (designated line 7 and line 8) selectively bred for high voluntary wheel-running behavior and in a randomly bred control line (line 2) (see Swallow et al. 1998a) under conditions of feeding a low-fat (LF) or a high-fat/high-sucrose (HF) diet.

During pregnancy, line 7 and line 8 females were smaller than line 2 females - as under non-reproductive conditions - and also delivered pups with a lower birth weight. The number of newborn pups per litter, however, was increased in line 7 and 8 compared to line 2. While this

difference is largely consistent with a previous report on these activity-selected mice (Girard et al. 2002), a reduction in individual pup mass may be explained by energetic limitations intrinsically imposed by the size of the litter the pups were born in. As expected according to the work of Hayes et al. (1992), Johnson et al, and others, (Johnson, Thomson, and Speakman 2001a), mean pup mass was indeed correlated in a negative direction to litter size at birth in the present study. Within this correlation, however, pups of line 7 and line 8 weighed significantly less than those of line 2 at each corresponding litter size. Thus, selective breeding for increased physical activity has caused animals in these two lines to be smaller per sé - perhaps as an adaptation to limit incremental cost of locomotion (Rezende et al. 2006b) - rather than being the consequence of higher litter sizes in line 7 and line 8 mice relative to line 2. Diet effects on birth characteristics were less conspicuous, although a tendency was observed for lower litter sizes and weights in the HF diet condition in lines 2 and 7, but not in line 8, compared to the LF diet condition. Differences in vulnerability to HF-diet induced lipotoxicity may have caused line-specific effects (McCurdy et al. 2009).

Over the course of lactation, litter weight and individual pup mass became increased in the HF diet condition irrespective of line relative to the LF condition, despite the fact that absorbed energy was indistinguishable between diets and lines. As resting metabolic rate (RMR) of the HF diet feeding mothers was lower than of the LF feeding mothers at corresponding litter sizes, it is conceivable that more nutrients became available for lactation. This could have subsequently increased the flow of nutrients derived from dietary fat, presumably without first being processed by de novo lipogenesis in the condition of the LF diet (Rudolph, Neville, and Anderson 2007; Neville and Picciano 1997). Secondly, dietary fat causes lower thermic effects of ingestion than carbohydrate (Donato 1987), which allows more energy to be available for production of milk as well, and refined sugars are more easily absorbed. A consequence of this is that sustainable energy intake (SusEI) could have been elevated more easily without running the risk of lethal overheating, as suggested by the heat dissipation hypothesis of Krol and Speakman and colleagues (Krol and Speakman 2003). In line with this is the notion that above-mentioned effects of the HF diet to increase energy absorption and litter weight gain occurred specifically at large litter sizes, i.e., when SusEI would have been maximized sooner in the LF condition than in the HF condition.

When maternal energy absorption was taken into account during peak lactation (i.e., when energy intake by the mother reached a plateau between days 13 and 16), also growth efficiency (GE) of mother and pups was higher in the HF diet condition than in the LF condition. Interestingly, this effect appeared significantly stronger in both of the high-activity selected lines as compared with the control line. These data therefore imply that a trait for increased voluntary activity increases efficiency by which lactating females can procure energy for growth. At this point, we do not know whether the HF diet-induced exaggeration of GE in the high-activity lines was due to a more efficient mobilization of fuels for production of milk in the mammary tissue of the lactating mothers, to increased growth efficiency of line 7 and line 8 pups,

or both. RMR of the mother assessed by indirect calorimetry during peak lactation did not seem to play a role in the observed effects since the average values were affected by diet, but not by an interaction between line and diet. Furthermore, viewing RMR in relation to litter size only revealed differences between lines in the LF, but not in the HF diet condition, thus ruling out a potential role of this trait in the observed differences in GE. Under non-reproductive conditions, a physically active state is associated with increased energy requirements and fast nutrient mobilization and utilization (Hamilton and Booth 2000). This has also been supported by studies in selection lines having increased metabolic turn-over (Vaanholt et al. 2008) and increased aerobic capacity (Swallow et al. 1998). To facilitate these processes, there may be muscular enzymatic changes, which promote aerobic capacity (Rezende et al. 2006a; Vaanholt et al. 2008; Wong et al. 2009; Houle-Leroy et al. 2000). Provided that these differences exist in the reproductive state between the highly active animals and their controls, this might fuel mammary glands more optimally in the selected mothers, particularly in the HF condition. Studies in humans have shown that physical exercise can increase fertility and reproduction success by causing an increase in placental growth and vascularization only in the condition of obesity, i.e., a situation which might share some homology with the HF diet condition in the present study (Rich-Edwards et al. 2002; Weissgerber et al. 2006). Thus, animals with a trait for increased physical activity may redirect their high level of fat turn-over during the non-reproductive phase towards offspring development during lactation. This, however, does not rule out the possibility that line 7 and line 8 pups are more efficiently utilizing the nutrients obtained from the mother and add significantly to the increased growth efficiency of line 7 and line 8 animals relative to the line 2 animals.

Besides similarities in birth characteristics and GEs during peak lactation, a major difference between the two high-activity lines was that line 7 mothers, but not line 8 mothers, lost 37% of their offspring irrespective of diet. Perhaps the suppression of behavioral hyperactivity normally seen during lactation (Karasawa, Suwa, and Kimura 1981; Collier et al. 1984) was less effective in line 7 mothers than in line 8 and line 2 mothers, and this is indeed consistent with the higher PIR count during indirect calorimetry in the line 7 mothers. Physically active lactating mothers may be draining fuels away from milk production towards muscular activity (Speakman and Krol 2005). Such a mechanism is supported by our most recent observation that animals forced to work during lactation had increased pup loss and pups with stunted pup growth. One of the main differences among that studies and the current one is that selected animals in the present study were not forced to be active. We do not know at this point whether the increased voluntary and/or forced locomotor activity in these and other studies interfered directly with maternal care. At least, assessment of maternal care of these activity-selected mouse strains at generation 21 did not reveal differences in maternal care, but neither was there a difference in locomotor activity relative to the control lines when dams were housed without wheel access (Girard et al. 2002). Further studies must be performed to unravel the mechanisms of pup loss by line 7 mothers, as opposed to the other lines.

In summary, selection for high voluntary wheel-running activity generally favored increased growth efficiency (GE) when the mice were feeding a HF diet during pregnancy and lactation, but had variable effects when they were feeding a LF. The effects of the HF diet to promote GE occurred irrespective of reproductive success, as pup loss was variable among lines. It may be argued that the increased GE is adaptive for animals that need to cover a large habitat to fulfill nutritional requirements.

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