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RESEARCH ARTICLE

Limits to sustained energy intake. XIV. Heritability of reproductive performance in mice

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SUMMARY

Limits to sustained energy intake (SusEI) are important because they constrain many aspects of animal performance. Individual variability in SusEI may be imposed by genetic factors that are inherited from parents to offspring. Here, we investigated heritability of reproductive performance in MF1 mice. Food intake, milk energy output (MEO) and litter mass were measured in mothers (F_0) and daughters (F_1) that were raising litters of 10 pups. Cross-fostering was designed so that half of each litter consisted of biological offspring and the rest came from one unrelated female (i.e. fostered pups). Food intake increased linearly during early lactation and reached a plateau during late lactation (day 9–13, called the asymptotic food intake, FI_{AS}, equivalent to SusEI). Parent–offspring regression showed that FI_{AS}, MEO and litter mass were all positively and significantly related between mothers and their biological daughters, but no significant relationships were found between the same traits for mothers and fostered daughters. FI_{AS} at peak lactation was significantly correlated to adult food intake and body mass when the mice were 6 months old and not lactating. In conclusion, a large part of the variation in FI_{AS} could be explained by genetic variation or maternal effects in pregnancy whereas non-genetic maternal effects in lactation were negligible. As a consequence, biological daughters of mothers with high reproductive performance (i.e. high milk production and hence higher litter mass at weaning) had a better reproductive performance themselves, independent of the mother that raised them during lactation.

Key words: milk energy output, heritability, reproduction, lactation.

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INTRODUCTION

Sustained energy intake (SusEI) is the maximum rate of energy intake that animals can sustain over sufficiently long periods (days to weeks) so that their energy demands are met by food intake, rather than by depletion of body reserves (e.g. adipose tissue) (Drent and Daan, 1980; Peterson et al., 1990; Weiner, 1992). SusEI is an important parameter because it may provide an upper bound that constrains many aspects of animal performance, like reproductive output, migration behaviour and thermoregulatory capabilities (Drent and Daan, 1980; Hammond and Diamond, 1997; Johnson et al., 2001a; Piersma, 2011; Speakman and Król, 2005a). To date, much research has focused on finding the factors that might impose intrinsic physiological limits on maximum SusEI. The 'central limitation hypothesis' suggests that these limits are imposed centrally by the energy-supplying machinery, e.g. the alimentary tract (Hackländer, 2002; Hammond et al., 1994; Perrigo, 1987; Speakman and Król, 2005a), while the 'peripheral limitation hypothesis' states that the limit is imposed peripherally by the energy-consuming machinery, e.g. the muscles or the mammary glands (Hammond et al., 1996; Zhao and Cao, 2009; Zhao et al., 2010). More recently, the 'heat dissipation limit theory' has been proposed, which suggests that animals may be limited by the capacity to dissipate heat generated as a by-product of processing food and producing milk (Król and Speakman, 2003a; Król et al., 2007; Speakman and Król, 2010; Speakman and Król, 2011). Although these experiments have greatly contributed to our understanding of the physiological mechanisms involved in imposing limits on SusEI, the individual variability in these limits has been largely ignored.

The period of late lactation in small rodents has been intensively studied when investigating limits to SusEI as it is one of the most demanding periods in a small mammal's life (Bergeron et al., 2011; Gittleman and Thompson, 1988; McNab, 2002; Speakman, 2008; Thompson and Nicoll, 1986). During early lactation, food intake increases sharply to meet the increasing demands of the pups; however, during late lactation a plateau is reached (Johnson et al., 2001a; Speakman and Król, 2005a; Speakman, 2008). This asymptotic food intake (FI_{AS}=SusEI) varies considerably between individuals, but the primary physiological or morphological features that drive this variability remain uncertain (Hackländer et al., 2002; Hammond et al., 1996; Johnson et al., 2001b; Król et al., 2003; Speakman et al., 2004a). Individual variability in FI_{AS} is not driven by variation in pup demand during lactation, as it is largely unrelated to litter size and unresponsive to experimental increases in litter size (Hammond and Diamond, 1992; Johnson et al., 2001a). In the present study, we experimentally investigated whether the individual variability in SusEI is heritable. It is well known that milk yield in dairy cows is a heritable trait and dairy cows have been selected for high milk yields for many years. Heritability of milk yield in dairy cows has been estimated to be between 0.04 and 0.67 (Dechow and Norman, 2007; Lee, 1997; Schneider and Vanvleck, 1986; Vanvleck and Bradford, 1964; Veerkamp, 1998) and milk yield is generally found to be highly associated with food

intake (Veerkamp, 1998). To test the hypothesis that SusEI is a heritable trait, we bred two generations of MF1 mice and monitored their food intake, milk energy output (MEO) and the mass of their litters throughout lactation. Litter sizes of all litters were adjusted to 10 pups to ensure that all mothers were exposed to similar pup demands and energetic challenge. In addition, pups were experimentally cross-fostered to enable us to distinguish between non-genetic lactation maternal effects and genetic effects (combined with maternal effects in pregnancy).

MATERIALS AND METHODS

Animals and housing

Male and female MF1 mice (*Mus musculus*, *N*=26 for each sex) were obtained from Harlan UK Ltd (Bicester, UK) at 4 weeks of age. Mice were housed in plastic cages (12×13×44 cm) with sawdust and paper shreds for bedding and a red dome house for enrichment. Mice had *ad libitum* access to food [Standard chow, CRM(P), Special Diets Services, BP Nutrition, Witham, UK] and water. Mice were maintained in a temperature-controlled room (21±1°C) under a 12 h:12 h light:dark cycle, with lights on at 05:00 h and a 'dawn/dusk' period of 20 min at either end of the light period. At 10 weeks of age all animals were mated (one female with one male). Males stayed with the females for 10 days (Król and Speakman, 2003a). To reduce grinding of food (Cameron and Speakman, 2010), mice were fed on a different diet [10% of energy (kcal) from fat; Open source diet, 1245OB, Research Diets, New Brunswick, NJ, USA) from day 1 of lactation onwards (Cameron and Speakman, 2010). Animals were acclimated to the new diet by offering both diets [CRM(P) and 12450B] from the time the males were removed until day 1 of lactation. Twenty-one out of 26 females gave birth (date of birth defined as day 0) (Johnson et al., 2001a) to 12.2±2.5 pups on average (mean ± s.d.; F_0 generation). The remaining five females did not become pregnant. On day 2 of lactation, litter size was adjusted to 10 pups and cross-fostering took place so that all females had 5±2 of their own pups and 5±2 pups from one unrelated mother. All pups were marked daily with animal identification pens (Vet Tech Solutions Ltd, Congleton, UK) to enable identification of pups from different mothers within each litter. Pups were weaned on day 22 of lactation, and from each litter we kept two female and two male offspring (one pup of each sex from both the natural and foster mothers, total *N*=42 for each sex) that were housed in standard cages until they were also mated at the age of 10 weeks (preventing mating of siblings, F_1 generation). Of the 42 F_1 females, 33 gave birth to 12.2±2.9 pups. Litter size was adjusted to 10 on day 2 and cross-fostering took place as described above; one female from this group received offspring from two instead of one unrelated mother. Of the remaining nine females, one female gave birth but did not care for her litter, and was thus removed from the experiment, and the other eight did not become pregnant. All procedures concerning animal care and treatment were approved by the ethical committee for the use of experimental animals of the University of Aberdeen, and were licensed by the UK Home Office and performed under PPL 60/3705.

Experimental procedure

Body mass, food intake, litter size and litter mass were measured every day throughout lactation in both generations (F_0 and F_1). On day 12 of lactation, mice received a clean cage, and bedding was collected 48 h later to determine digestive efficiency (DE). A known amount of food was also collected to determine the water content of the food. Daily energy expenditure (DEE, kJ day^{-1}) was measured by the doubly labelled water (DLW) technique from day 14 to day 16 of lactation (over 48 h) (Butler et al., 2004; Lifson et al., 1955). This method has been previously validated by comparison to indirect calorimetry in a range of small mammals (Speakman and Król, 2005b). In short, mice were injected intraperitoneally with ~0.2 ml of DLW of known mass and characterised isotopic enrichment (*ca*. 329,000 p.p.m. ^{18}O, *ca*. 186,000 p.p.m. ^{2}H). The exact dose was quantified by weighing the syringe to the nearest 0.0001 g before and after administration. An initial blood sample was collected 1 h after the injection *via* the tail tip and stored in a glass capillary that was immediately flame-sealed with a torch. The mouse was then returned to its home cage. A final blood sample was collected 49 h after the injection, timed to minimise the effects of diurnal variation in activity (Speakman and Racey, 1988). Blood samples of three mice (that failed to have litters) that had not been injected with DLW were collected to assess the natural background abundance of ^{2}H and ^{18}O in the body water pools of the animals [Method C of Speakman and Racey (Speakman and Racey, 1987)]. After weaning (day 22 of lactation), all pups were housed individually, and average adult food intake and body mass were measured over 1 week when animals were 6 months of age to evaluate whether food intake in non-breeding adults was related to food intake at peak lactation. Previous studies have suggested that food intake at baseline prior to reproduction is not related to the asymptotic intake (reviewed in Speakman and Król, 2005a) but in these studies food intake was measured before rather than after reproduction. It is potentially the case that the effects of reproduction on the alimentary tract (Hammond and Diamond, 1992; Speakman and McQueenie, 1996; Weiner, 1992) subsequently affect food intake; hence, a relationship between sustained maximum intake in reproduction and that in non-reproductive animals after, rather than before, reproduction might be significant.

DEE

Glass capillaries containing the blood samples were vacuum distilled, and water from the resulting distillate was used to produce CO_2 (see Speakman et al., 1990) and H_2 (see Speakman and Król, 2005b). The isotope ratios ^{18}O:^{16}O and ^{2}H:^{1}H were analysed using gas source isotope ratio mass spectrometry (Optima, Micromass IRMS and Isochrom μG, Manchester, UK). Samples were run alongside three lab standards for each isotope (calibrated to international standards) to correct delta values to p.p.m. Isotope enrichments were converted to values of DEE using a single pool model as recommended for this size of animal (Speakman, 1993). There are several alternative approaches for the treatment of evaporative water loss in the calculation (Visser and Schekkerman, 1999). We chose the assumption of a fixed evaporation of 25% of the water flux [see eqn 7.17 of Speakman (Speakman, 1997)], which has been established to minimise error in a range of conditions (Visser and Schekkerman, 1999).

Metabolisable energy intake and MEO

Metabolisable energy intake (MEI, kJ day^{-1}) at peak lactation was estimated from individual measurements of FI_{AS} (day 9–13 of lactation) (Król and Speakman, 2003b), dry mass of food (FI_{DM}=91% of wet mass), gross energy content of the food (FI_{GE}=18.24 kJ g^{-1} dry mass, determined by bomb calorimetry; Parr 6200 calorimeter with semi-micro oxygen bomb 1109A, Scientific and Medical Products Ltd, Cheadle, UK) and individual DE (%). To determine DE, faeces were manually separated from the bedding that had been collected from day 12 to day 14 of lactation for each female. The collected faeces were then dried to constant mass at 60°C and weighed (dry mass of faeces, F_{DM} in g). Gross energy

content of dried faeces, F_{GE} (kJ g^{-1}) was then measured by bomb calorimetry. Urinary energy loss (UEL) was estimated at 3% of the digestible energy intake (Drozdz, 1975). DE and MEI were calculated as follows (Król and Speakman, 2003a; Król and Speakman, 2003b):

$$DE = 100 \times (FI_{DM} \times FI_{GE} - F_{DM} \times F_{GE}) / FI_{DM} \times FI_{GE}\,, \quad (1)$$

$$MEI = FI_{DM} \times FI_{GE} \times DE/100 \times (100 - UEL) / 100\,. \quad (2)$$

MEO (kJ day^{-1}) was calculated from the difference between MEI and DEE of the individual females [for a full description of the method, see Król and Speakman (Król and Speakman, 2003b)].

Data analysis

All data were tested for normality using the Kolmogorov–Smirnov test in SPSS (version 18) and when necessary (for pre-pregnancy food intake and litter mass at birth) data were log-transformed to obtain a normal distribution. FI_{AS} was calculated as the mean food intake over the plateau in food intake (day 9–13 of lactation) (see Król and Speakman, 2003b) for mothers (F_0 generation) and daughters (F_1).

General linear models (GLMs) were used to test for differences between variables (e.g. body mass, litter size, litter mass) between mothers (F_0 generation) and daughters (F_1 generation). In these models, generation was added as fixed factor and family was added as a random factor to correct for family effects. Where variables are known to correlate with body mass (i.e. energy expenditure, food intake), this was added as a covariate to the models. Relationships between the various variables were tested using GLM with with generation added as a fixed factor to confirm that relationships between variables were similar in the two generations.

Parent–offspring regressions between traits measured in mothers and the same trait measured in their daughters was applied to assess heritability of the various traits. In this parent–offspring regression the slope of the fitted curve gives an indication of the proportion of the variation in, for example, FI_{AS} that can be attributed to genetic variation (Falconer, 1972). All tests were two-tailed and significance was set at $P \leq 0.05$.

RESULTS

FI_{AS}

Food intake increased during early lactation and then reached a plateau during late lactation (days 9–16; Fig. 1). After day 16, another increase in food intake was observed. At this point pups started eating food from the hoppers (L.M.V., R.E.S. and J.R.S., personal observations) and food intake thus no longer represented just the food intake of the mother. There were no differences in food intake between mothers and daughters (i.e. F_0 *versus* F_1 generation, repeated measures GLM, time: $F_{1,18}$=23.8.4, P<0.001, generation: $F_{1,52}$=0.47, P=0.49, time × generation: $F_{1,18}$=2.7, P=0.001; Fig. 1). The significant interaction between generation and time indicates that the two generations showed a different pattern in time, which is mainly reflected by an increased food intake in F_1 mice after day 16 of lactation when the pups had started eating the food (Fig. 1, *post hoc t*-tests).

FI_{AS} (food intake over day 9–13 of lactation) varied considerably between individuals: 17.7±1.5 g day^{-1} (mean ± s.d.), minimum 14.1 g day^{-1}, maximum 21.0 g day^{-1} (Table 1). No differences in the mean FI_{AS} between mothers from the F_0 or F_1 generation were observed (GLM with body mass as covariate, $F_{1,51}$=0.18, P=0.20, Table 1). In this model, family was a significant factor (F=4.2, P=0.001) indicating that FI_{AS} differed significantly between families.

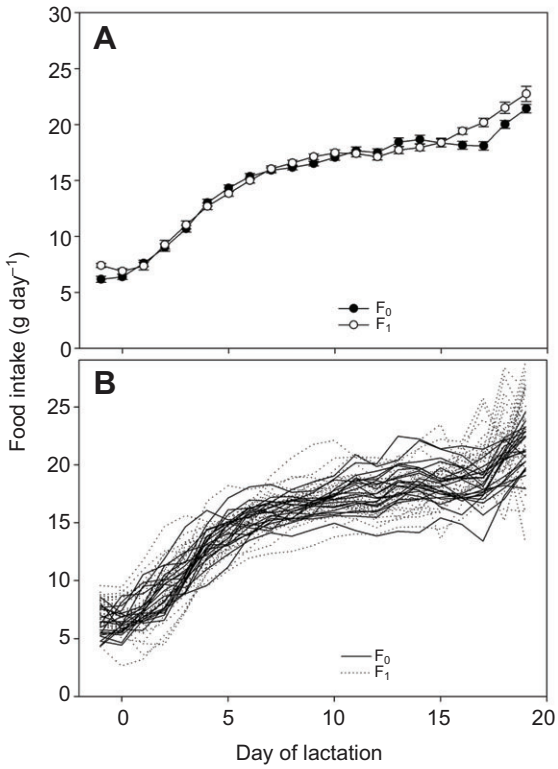

Fig. 1. (A) Relationship between mean food intake and day of lactation during the first breeding event in mothers (F_0, N=21) and their daughters (F_1, N=33). Day 0 is the day pups were born. Cross-fostering took place on day 2 of lactation and from this time onwards all mothers were raising 10 pups. (B) Relationship between food intake and day of lactation in individual mice from the F_0 and F_1 generation.

Reproductive performance

Litter size and litter mass at birth did not differ between the F_0 and F_1 generation, i.e. both groups gave birth to 12.2±0.5 pups that weighed 1.8±0.2 g on average (mass on day 1 of lactation, the day after birth, GLM, P>0.05 for all factors, Table 1). Litter mass at weaning was higher in offspring of the F_0 generation than in those of the F_1 generation, i.e. 144.5±8.7 and 135.8±11.2 g, respectively (day 21 of lactation, $F_{1,51}$=13.14, P=0.002, Table 1), but did not differ at peak lactation (i.e. day 16: F_0=93.0±6.5 g, F_1=91.9±7.1 g, $F_{1,51}$=1.5, P=0.2). Weaning mass of fostered pups (14.7±1.4 g) did not differ significantly from that of the biological offspring in the same litter (14.3±1.0 g), indicating that mothers allocated the same amount of energy to their own and fostered pups.

MEO was calculated by deducting DEE from the MEI and comprised ~50% of the animal's total energy intake (Table 1). Measurements of MEI and DEE did not differ between F_0 and F_1 generations (Table 1, GLM P>0.05). MEO did differ significantly between generations and a significant family effect on MEO was found (GLM: generation $F_{1,51}$=4.9, P=0.039, family F=2.3, P=0.044). Mean MEO was slightly reduced in mice from the F_1 generation compared with those from the F_0 generation (125.4±18.4 and 131.4±12.7 kJ day^{-1}, respectively).

Relationships between the variables measured and FI_{AS} and MEO were investigated using GLMs with generation (F_0 and F_1) added as fixed factor (Table 2, Fig. 2). Generation was never significant in these tests, indicating that any relationships observed

Table 1. Descriptive statistics

Variable	*N*	Mean ± s.e.m.	Minimum	Maximum
Body mass, pre-pregnancy (g)	54	32.1±0.4	25.0	38.2
Food intake, pre-pregnancy (kJ day^{-1})	50	91.7±1.2*	79.5	112.6
Litter size at birth (day 1)	54	12.2±0.4	4.0	18.0
Litter mass at birth (day 1, g)	54	22.0±0.6	8.2	29.8
Body mass (asymptote, day 9–13, g)	54	42.5±0.4	34.7	47.6
FI_{AS} (day 9–13, kJ day^{-1})	54	293.0±3.2	234.2	348.2
Litter mass (day 16, g)	54	92.3±0.9	76.5	113.2
Litter mass at weaning (day 21, g)	54	139.2±1.5*	109.4	170.7
MEI (kJ day^{-1})	54	258.1±2.9	199.7	310.3
DEE (kJ day^{-1})	52	130.6±2.0	89.8	171.7
MEO (kJ day^{-1})	52	127.2±2.3	91.6	167.1

FI_{AS}, asymptotic food intake; MEI, metabolisable energy intake; DEE, daily energy expenditure; MEO, milk energy output.
Values are shown for variables during the first reproductive event in F_0 (*N*=21) and F_1 (*N*=33) generation.
*Variable differed significantly (*P*<0.05) between F_0 and F_1 generation (mothers *versus* daughters) as determined by *t*-test or general linear model (GLM) with body mass as covariate. Four animals grinded their food pre-pregnancy and therefore no accurate measure of food intake was obtained. For two animals, the blood samples collected for doubly labelled water measurements could not be analysed and therefore sample size for DEE and MEO was reduced by 2.

between variables were similar in mice from the F_0 and F_1 generations. Pre-pregnancy body mass or food intake did not correlate with FI_{AS}, but body mass at the asymptote did (Table 2). FI_{AS} was significantly correlated with MEO (Table 2). Both FI_{AS} and MEO were positively correlated with litter mass at weaning, but did not significantly correlate with litter size or litter mass at birth (Table 2).

Fig. 3 shows that both DEE and MEI were significantly related to body mass, but only MEI was also positively related to litter mass at weaning, i.e. mothers with higher MEI weaned heavier litters. The amount of energy allocated to the pups (i.e. MEO=DEE–MEI) thus also increased with litter mass, i.e. females with higher MEO weaned larger pups (see Table 2).

No differences between biological daughters that stayed with their own mother or that were fostered were observed for any of the reproductive variables measured at peak lactation (i.e. pup mass, MEI, DEE or MEO) and data of both sets of daughters (i.e. the ones that were raised by their biological mothers and the ones that were raised by a foster mother) were therefore averaged in parent–offspring regression analysis.

Parent–offspring regressions

Parent–offspring regressions were applied to explore whether there was a relationship between variables measured in mothers and their daughters, which gives an indication of the heritability of these traits. There was a significant positive relationship between FI_{AS} of the mother and her biological daughters independent of who raised them (GLM: $F_{1,19}$=12.6, P=0.002; Fig. 2A, Table 3) and the slope of this relationship was 0.61±0.17. This relationship was independent of the body mass of the female (GLM with body mass included: $F_{1,19}$=9.8, P=0.006, body mass: $F_{1,19}$=6.5, P=0.021). No relationship between the FI_{AS} of mothers and foster daughters was found (GLM: $F_{1,13}$=0.1, P=0.74; Fig. 2B, Table 3).

Similarly, parent–offspring regression showed a significant positive relationship between MEO of mothers and biological daughters (Fig. 2C, Table 3, with a slope of 0.70±0.28), but no significant relationship was found between the MEO of mothers and foster daughters (Fig. 2D, Table 3).

Litter mass at weaning showed a positive relationship between the biological mother and her daughters (Fig. 2E, Table 3) and an inverse (not significant) relationship between litter mass at weaning of the mother and fostered daughters was observed (Fig. 2F, Table 3). Litter mass or size at birth showed a slightly positive, but not significant, relationship between mothers and biological daughters (litter mass: R^2=0.14, P=0.11, y=0.42x+12.1; litter size: R^2=0.02, P=0.52, y=0.16x+10.1) and no relationship between mothers and fostered daughters.

Table 2. Correlation with FI_{AS} and MEO

	FI_{AS}			MEO		
Covariate	*N*	R^2	*P*	*N*	R^2	P
Body mass, pre-pregnancy (g)	54	0.01	0.55	52	0.03	0.56
Food intake, pre-pregnancy (g day^{-1})	50	0.01	0.47	48	0.01	0.55
Litter size	54	0.07	0.07	52	0.09	0.06
Litter mass at birth (g)	54	0.07	0.07	52	0.03	0.41
Body mass (asymptote, g)	54	0.43	**<0.001**	52	0.10	0.04
Litter mass (day 16, g)	54	0.46	**<0.001**	52	0.23	**0.001**
Litter mass at weaning (g)	54	0.28	**<0.001**	52	0.26	**<0.001**
MEI (kJ day^{-1})	54	0.98	**<0.001**	52	0.53	**<0.001**
DEE (kJ day^{-1})	52	0.16	**0.004**	52	0.03	0.41
MEO (kJ day^{-1})	52	0.49	**<0.001**			

FI_{AS}, asymptotic food intake; MEO, milk energy output; MEI, metabolisable energy intake; DEE, daily energy expenditure.
General linear models (GLMs) with FI_{AS} or MEO as the dependent factor and various variables as covariate were performed. Generation (F_0 and F_1) was added to these models as fixed factor to confirm if any relationship between variables consistently occurred in both generations (total *N*=54). No significant effect of generation was found in any of the tests (*P*>0.1, results not shown). Reported are the R^2 and *P*-values for the covariate in the test. Significant results are in bold.

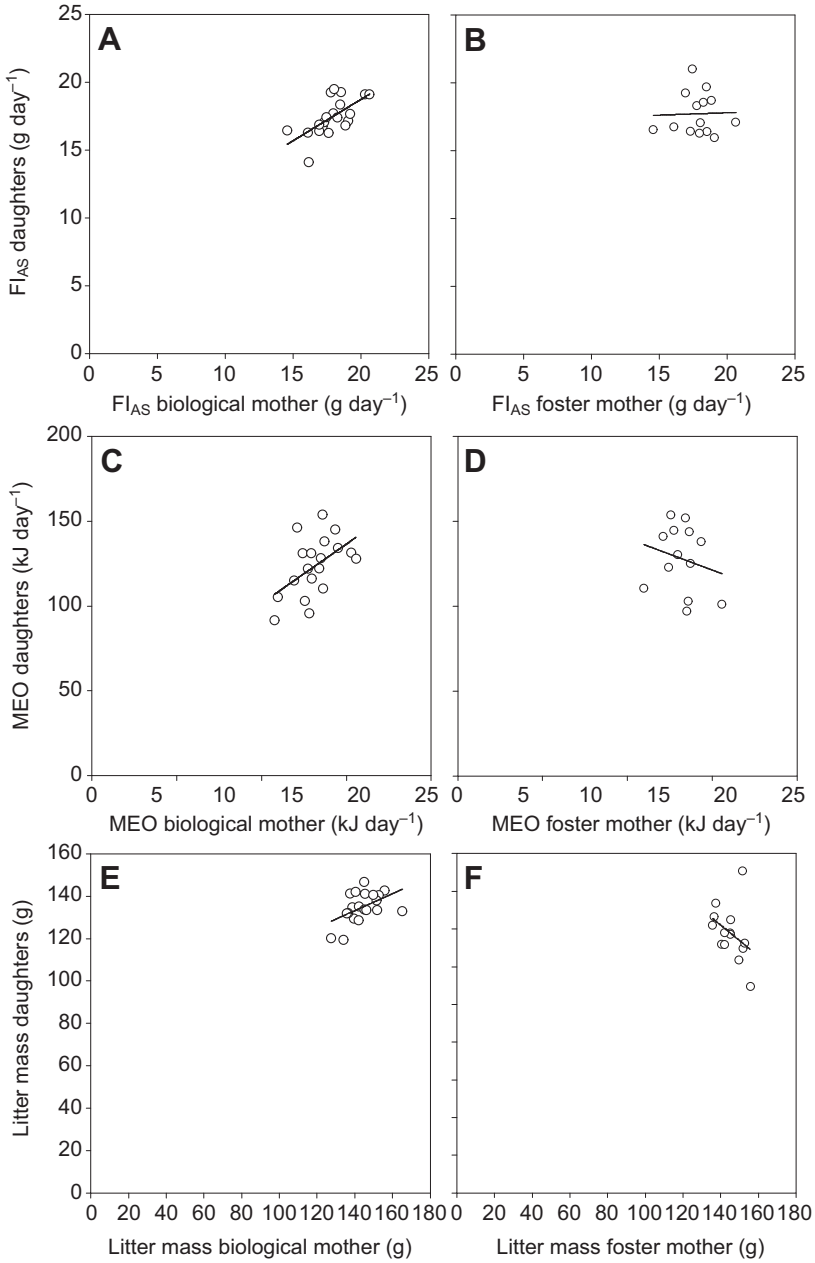

Fig. 2. Heritability of asymptotic food intake (FI_{AS}), milk energy output (MEO) and litter mass at weaning. FI_{AS} was calculated as the mean food intake over days 9–13 of lactation. (A,C,E) Parent–offspring regression between biological mothers and daughters for FI_{AS}, MEO and litter mass, respectively. (B,D,F) Parent–offspring regression between foster mothers and daughters for FI_{AS}, MEO and litter mass, respectively.

No significant relationship between mothers and biological or fostered daughters was found for DEE (parent–offspring regression for biological mother–daughter pairs: R^2=0.12, P=0.15 and foster mother–daughter pairs: R^2=0.001, P=0.95) or body mass at the asymptote (biological mother–daughter pairs: R^2=0.01, P=0.96 and foster mother–daughter pairs: R^2=0.27, P=0.06).

Adult food intake and body mass

Adult body mass (42.8±4.4 g) and food intake (3.8±0.4 g day^{-1}) measured at 6 months of age were similar in mice from F_0 and F_1 generations. Pearson correlations performed on data collected in mice from the F_1 generation indicated that body mass at weaning was not related to adult mass at 6 months of age (N=33, r=0.09, P=0.64). The FI_{AS} of mothers during peak lactation was positively related to body mass (N=54, r=0.58, P<0.001) and food intake (N=54, r=0.33, P=0.014) both at age 6 months.

Parent–offspring regression showed no significant relationships between adult body mass of mothers and the adult body masses of their biological or foster daughters (R^2=0.02, P=0.56, R^2=0.08, P=0.34, respectively) and neither was there a relationship between mothers and their biological or foster daughters for adult FI (R^2=0.07, P=0.25, slope=0.23±0.19, and R^2=0.14, P=0.19, slope=0.27±0.19, respectively).

DISCUSSION

Limits to SusEI are important because they affect all aspects of animal performance, including reproduction (Drent and Daan, 1980; Hammond and Diamond, 1997). Here, we showed that FI_{AS} (=SusEI) and MEO during lactation were related between mothers and biological daughters, and this reflected genetic factors rather than maternal effects during lactation – although maternal effects during pregnancy could not be separated from genetic effects using our protocols. Litter mass at weaning was positively related to both SusEI, as shown previously (Hammond et al., 1996; Johnson and Speakman, 2001; Zhao et al., 2010), and MEO (Król and Speakman, 2003b; Schubert et al., 2009), which is in agreement with previous

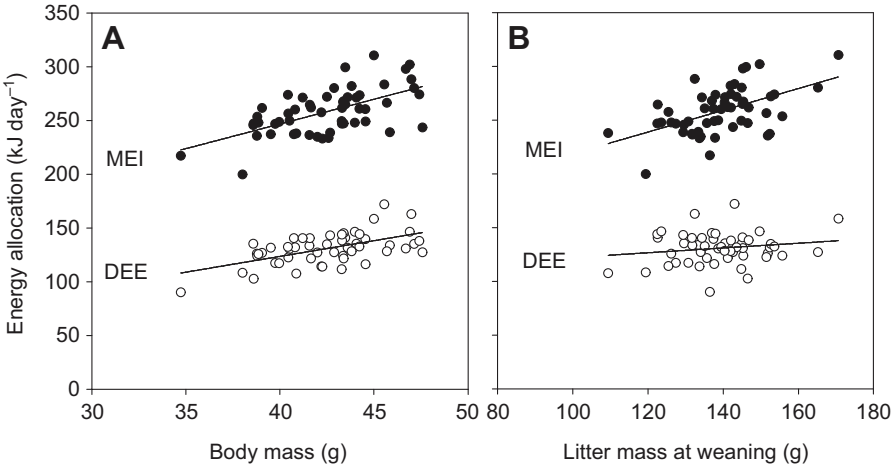

Fig. 3. Relationship between body mass (A) and litter mass at weaning (B) and MEI and DEE. MEO was calculated as the difference between MEI and DEE.

reports. As a consequence, the biological daughters of mothers with high reproductive output (i.e. higher litter mass at weaning) had a higher reproductive output than the biological daughters of mothers with low reproductive output – independent of the mother that actually raised them through lactation. Non-genetic maternal effects during the lactation period did not play a significant role in this effect as SusEI and MEO were not related between pups and their foster mothers and did not differ between daughters of the same mother that stayed with their biological mother or were fostered. Females with a higher SusEI thus raised larger pups and this may benefit their offspring later in life, as many studies have shown that low birth mass is associated with higher risk of cardiovascular diseases, hypertension, diabetes and metabolic syndrome as adults (Whincup et al., 2008), although a relationship between body size and longevity was not found in MF1 mice (Speakman et al., 2004b).

Dairy cows have been selected for high milk yields for many years and heritability of milk yield in dairy cows is estimated to lie between 0.04 and 0.67, but varies between different herds, depends on the generation of selection and is subject to sampling errors (Dechow and Norman, 2007; Lee, 1997; Schneider and Vanvleck, 1986; Vanvleck and Bradford, 1964; Veerkamp, 1998). In agreement with the present study, food intake and milk yield (or MEO) have been shown to be highly correlated (0.46–0.65 in dairy cows, 0.72 for MEO in the present study) and estimates for heritability of food intake during lactation and milk yield are generally similar (Veerkamp, 1998).

Our study indicates that SusEI and MEO are heritable and are not affected by epigenetic effects in lactation; we cannot, however, separate genetic effects from epigenetic effects that occur during pregnancy. A body of work (reviewed in Duah et al., 2013) suggests that variability in MEO may be pre-programmed during pregnancy by the number of gestated fetuses, and hence the expected number of offspring that the female anticipates she will have to support during lactation. It has, for instance, been shown in goats that the mass of mammary tissue correlated positively with placental mass and fetal number, and milk yield of mothers bearing twins or triplets was higher than that of mothers that carried singletons (Hayden et al., 1980). However, as shown in the companion paper (Duah et al., 2013), while growth of the mammary glands and associated structures may be initiated in gestation, and vary in relation to the number of placentas, the ultimate size and activity of the tissues depends primarily on factors during lactation. In our study the lactational burden was similar in all females, i.e. litter size was adjusted to 10 pups, and this may therefore suggest that the variability observed in SusEI and MEO was mainly driven by genetic factors.

Selection experiments for increased litter size have been performed in various strains of mice and heritability of litter size is estimated to be relatively low at 15% (Beniwal et al., 1992; Gutiérrez et al., 2006; Peripato et al., 2004). In the present study we showed a slight positive relationship (with a slope of 0.16) between litter size of mothers and their biological daughters, but this relationship did not reach significance. MF1 mice are known for their relatively large litter sizes and after many generations of selection, generally a selection plateau is observed (Beniwal et al., 1992; Eklund and Bradford, 1977), which may explain the absence

Table 3. Parent–offspring regression for FI_{AS}, MEO and litter mass at weaning

	N	Slope	Intercept	R^2	*P*
FI_{AS} (g day^{-1})					
Biological mother *versus* daughters	21	0.61±0.17	6.6±3.1	0.41	**0.002**
Foster mother *versus* daughters	14	0.04±0.31	17.1±5.6	0.009	0.740
MEO (kJ day^{-1})					
Biological mother *versus* daughters	21	0.70±0.28	31.7±37.0	0.27	**0.023**
Foster mother *versus* daughters	13	–0.37±0.52	177.3±68.6	0.05	0.487
Litter mass at weaning (g)					
Biological mother *versus* daughters	21	0.40±0.17	77.3±24.2	0.24	**0.028**
Foster mother *versus* daughters	14	–0.82±0.59	256.4±86.3	0.14	0.194

Results for general linear model (GLM) testing for a relationship between between asymptotic food intake (FI_{AS}), milk energy output (MEO) and litter mass of the biological mothers *versus* that of her daughters, and between foster mothers and daughters are shown.
Values for slope and intercept are means ± s.e.m. Significant results are in bold.

of a significant relationship for litter size. The selection plateau is generally attributed to a decrease in genetic variation in later generations, but this may, at least in part, also be attributed to selection limits on SusEI.

In contrast to studies that have selected mice for increased body mass (Bünger and Hill, 1999; Eisen, 1989) or food intake (Hastings et al., 1997; Sharp et al., 1984; Selman et al., 2001), no significant relationship between biological mothers and daughters was found for body mass or food intake. The slopes of the parent–offspring regressions were 0.10 and 0.23 for body mass and food intake, respectively, which are in line with heritabilities found in other studies, but the power to detect a significant relationship may have been too low.

Our results show that even when controlling for litter size, the individual variability in SusEI remained high and varied from 14.1 to 21 g day^{-1}. These results support the indication that pup demand during lactation is not the driving factor behind the variation in SusEI. SusEI could not be predicted from pre-pregnancy body mass or food intake, as shown previously (Speakman and Król, 2005a), and the variability in SusEI thus only became apparent when animals were pushed to their limits at peak lactation. SusEI was, however, positively related to subsequent food intake when the mice were 6 months old. Although this correlation was not very strong, this may indicate that mechanisms involved in the regulation of *ad libitum* food intake under non-limiting circumstances are also involved in the regulation of food intake when animals have to perform close to their limits (i.e. at peak lactation). However, this and previous studies (reviewed in Speakman and Król, 2005a) have shown that there was no relationship between SusEI and food intake prior to reproduction and thus changes that occur during reproduction may be important. Effects of reproduction on the alimentary tract (Hammond and Diamond, 1992; Speakman and McQueenie, 1996; Weiner, 1992) that subsequently affect food intake may explain the existence of a relationship between SusEI in reproduction and that in non-reproductive animals after, rather than before, reproduction.

For instance, the heat dissipation limit theory suggests that food intake at peak lactation may be limited by the capacity of individuals to dissipate body heat. Individuals with thinner pelages would have less external insulation and hence would be able to dissipate more heat and might be expected to have greater SusEI and MEO. During lactation, females may adaptively thin their pelages. Later, however, this thinner pelage would necessitate higher heat production and hence greater food intake under non-reproductive conditions. This may explain why a relationship is observed to subsequent non-reproductive intake, but no relationship is observed to pre-reproduction intake.

Alternatively, differences in neuroendocrine control could underlie variability in SusEI. Food intake is stimulated by a number of peripheral signals (e.g. leptin, insulin, ghrelin) that act in concert with several pathways in the brain (e.g. NPY, AgRP) to promote feeding behaviour (for reviews, see Chaptini and Peikin, 2008; Speakman and Król, 2005a). Endocrine systems cannot, however, be stimulated indefinitely because receptors become saturated. As suggested previously (Speakman and Król, 2005a), food intake during lactation may be stimulated by a combination of different signals that reach a point of maximal stimulation during the final part of lactation and therefore food intake cannot increase further and reaches a plateau (i.e. SusEI). The point at which animals reach this maximal stimulation may differ depending on, for instance, the number of receptors present and this may underlie individual differences in SusEI. Neuroendocrine control of food intake probably has an important role in regulating SusEI during lactation; however, the main signals involved remain to be elucidated (Speakman and Król, 2005a). For example, maximal stimulation of the leptin system occurs when leptin is absent, as in the *ob/ob* mouse, and although *ob/ob* mice have a much higher food intake than wild-types, they still only eat 8–10 g of food a day, which is much lower than food intake at peak lactation. Moreover, repletion of leptin had only a minor impact on food intake at peak lactation (Cui et al., 2011). Also, neuropeptide Y knockout mice have normal food intake during lactation (Hill and Levine, 2003). The physiological mechanisms that underlie the individual variability in SusEI thus remain obscure.

In conclusion, a large part of the variation in FI_{AS} at peak lactation could be attributed to genetic variation or maternal effects during pregnancy, whereas non-genetic maternal effects in lactation were negligible. MEO and litter mass at weaning were also heritable traits; biological daughters of mothers with high reproductive performance (i.e. that weaned heavier litters) had a higher performance than biological daughters of mothers with low reproductive performance.

LIST OF ABBREVIATIONS

DE	digestive efficiency
DEE	daily energy expenditure
DLW	doubly labelled water
F_{DM}	faeces, dry mass
F_{GE}	faeces, gross energy content
FI_{AS}	asymptotic food intake
FI_{DM}	food intake, dry mass
FI_{GE}	food intake, gross energy content
GLM	general linear model
MEI	metabolisable energy intake
MEO	milk energy output
SusEI	sustained energy intake
UEL	urinary energy loss

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AUTHOR CONTRIBUTIONS

L.M.V. was involved in the design, execution and interpretation of the work, and writing and revising of the article. R.E.S. was involved in the execution of the work and revising of the article. J.R.S. was involved in the design and interpretation of the work and in writing and revising of the article.

COMPETING INTERESTS

No competing interests declared.

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