Nature and nurture effects of voluntary activity and nutrition on energy balance and nutrition

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CHAPTER 7

POSTNATAL VERSUS PRENATAL EFFECTS ON OFFSPRING ENERGY BALANCE REGULATION IN CONTROL MICE AND MICE SELECTIVELY BRED FOR HIGH VOLUNTARY WHEEL RUNNING

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Summary

Maternal behavior and nutritional factors have been shown to imprint neurobiological controls over energy balance in the offspring during postnatal life. This is particularly relevant in species that give birth to multiple pups at once, and are faced with nursing large litters where above-mentioned maternal factors can easily become limited for the individual kids. In these experiments, we investigated the effects of the postnatal influences in mice selectively bred for voluntary high running under low-fat (LF) and a high-fat (HF) feeding conditions. The activity selected lines generally give birth to relative large litters, but have mixed success in nursing their offspring, presumably due to the activity trait. Our aims were 1) to examine the effects of perigestational diet on maternal and offspring characteristics in equalized litter conditions, and 2) to investigate postnatal environment exchange between active and control lines by cross-fostering of pups. Firstly, we found that litter size at birth needs to be taken account in equalizing litter sizes. Manipulation with already two pups difference in the litter can result in contradictory effects, potentially due to a mismatch with expected number of pups the mothers prepared for. Secondly, control offspring fostered by active mothers resulted in smaller growth, lower glucose and insulin levels at adulthood of the fostered controls, meaning that the physical activity trait of mothers strongly influences the control offspring later in life. The opposite, in which selection line pups were fostered by control mothers did not have a major effect on adult energy balance regulation in these animals. Thus the prenatal, genetic make-up of activity selected offspring overpowered the relatively rich postnatal environment of control mothers, even when they were subjected to a HF diet. In conclusion, postnatal epigenetic effects seem to play only a limited role in the phenotype of mice selectively bred for high running wheel behavior.

1. Introduction

During the perinatal stage, metabolic, physiological and behavioral traits can be programmed which could affect maintenance of health and proneness for certain diseases later in life (Bouret 2009; Armitage et al. 2005; MohanKumar et al. 2007; McGowan, Meaney, and Szyf 2008; Barnes et al. 1966). It has been claimed for example that pregnant and lactating subjects eating high amounts of sugars and saturated fats adversely influence their offspring, with increased risk of developing obesity and related co-morbidities later in their lives. In our previous study in control mice, we consistently observed that feeding a 40% high-fat diet combined with refined sugars until day 16 of lactation, was able to increase weight gain and adiposity in the adult male offspring. These perinatal diet effects, however, were not observed in mice from the same ancestral line, but subsequently selectively bred for increased voluntary wheel running behavior over 50 generations. Programming effects of an unhealthy diet can apparently be offset by a trait for voluntary behavior. In that study, we also observed that the reproduction output of the selected and control animals differed, i.e., selected mice gave birth to on average larger litters than control mice. Large litters are generally comprised of relative small pups, while pups in relatively small litters tend to be larger. This could imply that individual pups from selected mothers are less well nourished than those of controls. It was also shown previously that energy intake of lactating mice becomes limited intrinsically, when they have to nurse too large litter, which additionally influences average pup growth and development (Johnson, Thomson, and Speakman 2001). Therefore, litter size could be a major postnatal contributing factor to programming of energy balance in the newborn and later life.

Perinatal under- and overnourishment has been studied by experimentally increasing and decreasing litter size (Schultze 1954). The quantity of nutrition in suckling animals indeed resulted in permanent effects on growth and metabolism as well as mental development. Overnutrition early in life as a result of relatively fewer pups in a litter is an important cause of obesity and related metabolic diseases in the adult mice (Aubert, Suquet, and Lemonnier 1980; Winick and Noble 1967; Plagemann 2006; Faust, Johnson, and Hirsch 1980), and may be a model for human disease as well. To study the contribution of litter size in the effects of perinatal HF diet feeding and the trait for increased physical activity in offspring body weight development and associated metabolic, hormonal, and behavioral parameters, we equalized litter sizes in control and high activity lines under the different diet conditions. We hypothesized that line differences would be maintained with respect to consequences of perinatal HF diet feeding on offspring.

Next we addressed whether perinatal diet/ line interactions on pup characteristics and subsequent body weight related factors at adulthood are due to genetic differences between lines, or caused by postnatal effects of mothers on pups. The technique of cross-fostering is generally used to distinguish between the influence of genes and the postnatal environment (Hager, Cheverud, and Wolf 2009). For this reason equalizing litter size was combined with cross-

fostering of pups a) between litters born to mothers of similar lines, or b) between litters of mothers born to different lines. These effects were investigated in the situation when mothers were either feeding a low fat (LF) carbohydrate rich diet, or when they were feeding a HF diet. When a parameter relevant to regulation of body weight is not altered by cross-fostering between lines, a genetic basis for this parameter inside the cross-fostered pup may be considered more persistent than the postnatal influence exerted via the mother.

2. Materials and methods

2.1. Animals and housing

Female mice 5-7 months of age were selectively bred for voluntary wheel-running behavior (the base population was the Hsd:ICR strain) in generation 49 and they were the siblings of the animals used in chapter 4 and 5. Their founders from generation 48 were obtained from T. Garland Jr, Riverside, CA, which were used for further breeding for 2 separate selection lines (line 7 and line 8) and one separate control line (line 2) at our facilities in Haren without further selection for wheel-running activity. For a detailed description of the selection procedure see (Swallow, Carter, and Garland, Jr. 1998). All female mice were individually housed in type C cages with wood shavings and EnviroDry® bedding with food and water *ad libitum*. The food was either a healthy fibered low-fat diet (LF) (3.8 kcal/g; 58 % carbohydrate, 6 % fat, 22 % protein; Standard lab chow RMH-B 2181, HopeFarms BV, Woerden, NL) for half of the animals or a 40 % fat diet, additionally containing fast sugars (HF) (4.7 kcal/g; 30 % carbohydrate, 45 % fat, 18 % protein; AB Animal Diets, Woerden, NL. Animals were housed in a room with a temperature of 22±1 °C on a 12:12 light-dark cycle with lights on at 8 am. Virgin female mice were paired with males from the same line, diet and generation. After 3 weeks of pairing, males were removed and totally 39 females gave birth and allowed to raise litters.

Handling of lactating mothers was exactly the same as it was described in Chapter 4 (see in details in Chapter 4). Briefly, maternal body mass and food intake were assessed daily throughout lactation. Feces produced over day 13-16 of lactation were collected to determined energy absorption. Growth Efficiency (GE) was calculated for body weight gain of mother and pups (i.e., expressed as mg body weight gain/ kJ food absorbed).

2.2. Equalizing of litters and cross-fostering

At the day of delivery, the size and the mass of the litter were assessed and the proportion of males and females in the litters were scored. Based on that outcome male as well as female pups were either A) not exchanged and left with their own mother, B) exchanged between mothers within the same line and diet, C) exchanged between the lines on the same diet, or D) exchanged within the same line on different diets. Exchanging of pups according to C) was never done between lines 7 and 8. Only when two or more litters were born within one day difference or

less, exchanging pups was allowed before the 3rd day of post-natal life, because after the 3rd day pup-mother bonding is does not occur anymore. In the process of exchange on the first post-natal day, the largest variations in litter size were first narrowed to 9-12, and manipulated to 10-11 on the second or third post-natal day. At day 8, all the litters were equalized to 10, if possible. Individual pups (recognized by sex and toe-clip) were weighed at day 8, 13, 16 and 21. Cages were cleaned at the time of weighing. During peak lactation, between day 13 and 16, feces were collected from bedding material for analysis of energy density. At day 16, HF diet was changed for chow (LF) diet, for the reason that offspring start to eat from food hoppers. At day 21, offspring were weaned and group-housed into 3-4 with their same sex littermates.

At 4 weeks of age, half of the own offspring that was raised by the mother that delivered them, were sacrificed for hormone and body composition analysis. At 6-8 weeks of age running wheel characterization was performed with body mass measurement. At 3 months of age, body mass of offspring was assessed again and they were individually housed for food and water intake measurements. At 4 months of age, they were sacrificed for body composition and hormone analysis (the procedures more in details in Chapter 5).

2.3. Statistical analysis

Data was analysed with General Linear Model Univariate Analysis with the exception of GLM Repeated Measures of maternal body weight and food intake over the course of lactation. Body masses of offspring during lactation were analyzed with ANOVA nested design where mothers were nested into line*diet interaction.

3. Results of mothers and litters at birth

3.1. Mother characteristics

3.1.1. Body mass during pregnancy and lactation

Before pregnancy the average body weights of line 2, 7, and 8 females on LF diet were respectively 30.9g; 24.0g; and 25.6g. On the HF diet these body weights were respectively 33.9g; 24.5g; and 27.8g. GLM Univariate Analysis revealed effect of line ($F(2,43)=14.11$; $p<0.001$), and post-hoc analysis revealed that line 7 and 8 females weighed less than line 2 females irrespective of diet. Over the course of pregnancy, body weight increases were found without effects of line (Figure 1).

During lactation (d1-d16), GLM Repeated measures revealed line effects ($F(2,34)=5.85$; $p<0.01$), where line 7 mothers had significantly lower body masses than line 2, and line 8 mothers appeared to have an intermediate body weight. Time interacted with diet ($F(15,525)=2.62$; $p<0.001$), meaning that HF fed mothers weighed significantly less over time (Figure 2).

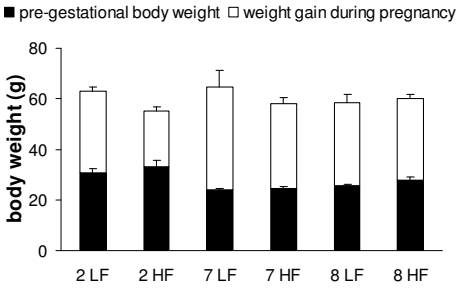

Figure 1. Pre-gestational body weight and weight gain during pregnancy in females fed LF diet (A) and HF diet (B) in line 2 (control line), line 7 and line 8 (selected lines).

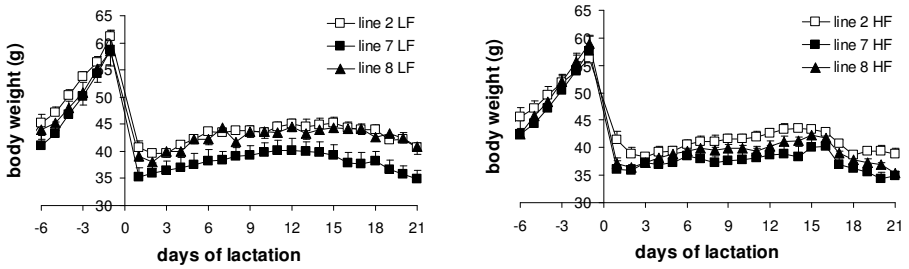

Figure 2. Body weight of females fed LF diet (A) and HF diet (B) in line 2 (control line), line 7 and line 8 (selected lines) during lactation.

3.1.2. Food intake during lactation

With repeated measures between day 1 and day 16 of lactation, a diet effect ($F(1,33)=5.67$; $p<0.05$), but not line effect was found on maternal energy intake (in kJ). Specifically, females feeding the HF diet increased their food intake compared to females eating the LF diet. There was an interaction between time and line ($F(28,476)=1.57$; $p<0.05$), time and diet ($F(14,476)=8.83$; $p<0.001$) and between time, line and diet $F(28,476)=1.79$; $p<0.01$), meaning that both line 7 and line 8 mothers increased their food intake when fed a HF diet, but not line 2. When the cumulative amount of food eaten during lactation was assessed from day 8 to day 16, an effect of line was revealed ($F(2,35)=3.70$; $p<0.05$), and post-hoc analysis showed that specifically line 8 females ate significantly more than line 7 females. Also an effect of diet was found ($F(1,35)=6.97$; $p<0.05$); i.e., mothers fed the HF diet ate more than those fed LF diet. (Figure 3).

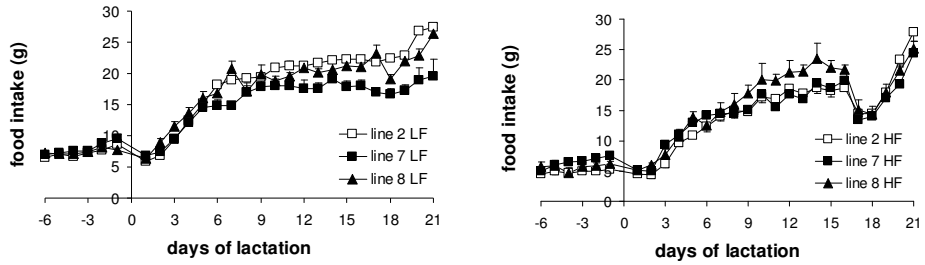

Figure 3. Food intake of females fed LF diet (A) and HF diet (B) in line 2 (control line), line 7 and line 8 (selected lines) during lactation.

3.2. Litter characteristics at birth

At birth, litter sizes and masses were assessed. We observed that line 8 had significantly larger litter sizes than line 2 ($F(2,38)=3.41$; $p<0.05$). Line 7 did not differ from either line (Table 1). Litter mass did not differ significantly between groups, however line 8 tended to have larger litter masses than line 2 ($F(2,38)=3.06$; $p=0.06$). Mean pup mass at birth did not differ between lines and diet conditions.

Table 1. Litter characteristics of mothers fed LF and HF diet during pregnancy and lactation in line 2 (control line), line 7 and line 8 (selected lines) at birth.

	2		7		8	
At birth	**LF**	**HF**	**LF**	**HF**	**LF**	**HF**
Litter size	11.8 ± 0.8	8.1 ± 1.0#	12.0 ± 1.3	11.5 ± 0.7	12.2 ± 1.2	13.3 ± 1.1
Litter mass	18.7 ± 0.8	13.6 ± 1.8#	19.6 ± 1.6	18.0 ± 1.7	19.3 ± 1.8	20.3 ± 1.0
Mean pup mass	1.6 ± 0.0	1.7 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	1.6 ± 0.0	1.5 ± 0.1

# denotes significance difference with LF diet ($p<0.05$).

Based on the outcome of the counted male and female pups, they were divided according to cross-fostering protocols A), B), C) mentioned earlier in the material and methods section. There were totally 3 litters in which adjusting the litter size to 10 was not possible, because of pup loss after day 3. These 3 litters were from one line 7 mother feeding HF diet (with 9 pups), from one line 8 mother feeding the LF diet (with 7 pups) and from one line 8 mother feeding HF diet (with 9 pups). Further details on the number of cross-fostered pups are provided in table 2 at pre-weaning. At post-weaning, the half of the own and all the cross-fostered offspring were used.

Table 2. Number of cross-fostered male and female pups raised by own, xf (condition B), line 2, line 7 and line 8 (condition C) mothers.

mothers	**own**	**xf**	**7**	**8**	**own**	**xf**	**2**	**own**	**xf**	**2**
	2LF offspring				**7LF offspring**			**8LF offspring**		
male	15	3	4	3	18	6	6	17	4	6
female	15	4	6	3	24	4	5	19	6	4
	2HF offspring				**7HF offspring**			**8HF offspring**		
male	8	4	3	3	13	3	3	25	4	7
female	9	4	4	5	12	4	5	15	4	3

4. Part A. Characteristics of own offspring

4.1. Body weight of own offspring

During the pre-weaning period, offspring characteristics were continuingly assessed at 8, 13, 16 and 21 days after birth. Using an ANOVA with a nested design, in which mother was a random factor and nested into line*diet interaction, males and females were analyzed separately. Mother attained a strong significant factor in each time point in the body mass differences of offspring during lactation in both genders. In general, maternal HF diet caused male and female offspring to be larger than those of LF diet fed mothers in each of the above-mentioned time points (d8: ♂: $F(1,92)=38.86$; $p<0.001$ and ♀: $F(1,89)=49.97$; $p<0.001$, d13: ♂: $F(1,92)=72.31$; $p<0.001$ and ♀: $F(1,89)=42.05$; $p<0.001$, d16: ♂: $F(1,92)=131.90$; $p<0.001$ and ♀: $F(1,89)=136.09$; $p<0.001$ and d21: ♂: $F(1,92)=57.03$; $p<0.001$ and ♀: $F(1,89)=61.47$; $p<0.001$) (see table 3). In males, line differences were revealed; i.e., at day 8 ($F(2,92)=8.27$; $p<0.001$) line 2 offspring were significantly heavier than that of line 7 and line 8. From day 13 onward (d13: $F(2,92)=13.27$; $p<0.001$, d16: $F(2,92)=17.07$; $p<0.001$, d21: $F(2,92)=30.27$; $p<0.001$), male offspring from different lines differed significantly from one another, with line 2 offspring being the heaviest and line 7 the lightest, and line 8 offspring of intermediate body weight. In the case of females, line effects were revealed only from day 16 onward (d16: $F(2,89)=17.46$; $p<0.001$, d21: $F(2,89)=22.92$; $p<0.001$), with line 2 offspring being the heaviest and line 7 the lightest with line 8 intermediate, similar to male offspring.

After weaning, body weights were assessed at 4 weeks, 6 weeks, at 3 months, and 4 months of age. At 4 weeks, maternal HF diet increased body mass in male ($F(1,39)=15.28$; $p<0.001$) offspring, but an effect in female offspring was lost. The HF diet effect was not attributed to the male offspring of line 2, but both line 7 and line 8 male offspring of HF feeding mothers were heavier than from LF feeding mothers (shown by interaction of line and diet). At 6 weeks of age, body mass was affected by line in both genders (♂: $F(2,52)=11.72$; $p<0.001$; ♀:

F(2,44)=12.06; p<0.001), but diet effects were lost. These effects were also observed at 3 months (line effects ♂: F(2,35)=4.70; p<0.05; ♀: F(2,41)=13.84; p<0.0001), and 4 months of age (line effects ♂: F(2,32)=6.71; p<0.01; ♀: F(2,36)=9.80; p<0.001). Specifically, line 2 offspring were heavier than those of line 7 and 8.

Table 3. Body weight of male and female offspring of mothers perinatally fed LF and HF diet during lactation in line 2 (control line), line 7 and line 8 (selected lines).

	2		**7**		**8**	
	LF	**HF**	**LF**	**HF**	**LF**	**HF**
MALE						
day 8	5.1 ± 0.1	5.8 ± 0.1	4.4 ± 0.1*	5.4 ± 0.2#	4.7 ± 0.2	5.2 ± 0.1
day 13	7.5 ± 0.2	9.2 ± 0.3#	6.3 ± 0.2*	8.2 ± 0.1##	7.0 ± 0.2	8.2 ± 0.2#
day 16	8.7 ± 0.2	10.9 ± 0.3##	7.1 ± 0.2**	9.7 ± 0.1##	8.0 ± 0.2	10.0 ± 0.2##
day 21	12.0 ± 0.3	14.3 ± 0.3#	9.1 ± 0.3*	11.6 ± 0.3##	11.2 ± 0.3	12.8 ± 0.4##
4 weeks	23.3 ± 0.5	23.3 ± 0.6	18.1 ± 1.0	22.1 ± 0.8#	17.0 ± 2.2	23.9 ± 0.6##
6 weeks	31.7 ± 1.0	32.3 ± 1.5	28.2 ± 0.6**	28.2 ± 0.6	30.4 ± 0.4	32.4 ± 0.8#
3 months	35.1 ± 0.7	35.9 ± 1.1	31.2 ± 0.8*	32.8 ± 1.5	34.1 ± 1.2	33.1 ± 0.9
4 months	40.6 ± 2.4	36.6 ± 0.9	33.9 ± 1.0*	33.7 ± 1.4	35.5 ± 0.9	34.9 ± 0.9
FEMALE						
day 8	4.8 ± 0.1	5.7 ± 0.2	4.2 ± 0.2*	5.4 ± 0.2	4.5 ± 0.1	5.0 ± 0.2
day 13	7.1 ± 0.1	9.5 ± 0.2##	6.2 ± 0.2	8.4 ± 0.1#	6.7 ± 0.1	8.1 ± 0.3#
day 16	8.3 ± 0.1	11.3 ± 0.2##	6.9 ± 0.2*	9.9 ± 0.2##	7.7 ± 0.2	10.2 ± 0.3##
day 21	11.3 ± 0.2	13.9 ± 0.4##	8.6 ± 0.4**	11.7 ± 0.2##	10.8 ± 0.3	12.8 ± 0.6
4 weeks	16.9 ± 1.5	15.4 ± 3.5	14.7 ± 1.1	17.3 ± 0.3	15.3 ± 0.7	18.4 ± 0.8#
6 weeks	25.3 ± 0.8	26.1 ± 1.5	20.5 ± 1.0**	22.1 ± 0.5	23.9 ± 0.8	26.3 ± 1.0
3 months	28.7 ± 1.0	28.5 ± 1.5	23.8 ± 0.5**	25.1 ± 0.4	26.2 ± 0.6*	28.2 ± 0.9
4 months	31.7 ± 1.2	31.6 ± 2.0	24.8 ± 1.3**	27.2 ± 0.4	27.5 ± 0.6**	29.6 ± 1.3

* 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).

4.2. Body composition of own offspring

Offspring cohorts of all groups were sacrificed and processed for body composition analysis at 4 weeks of age as well as at 4 months of age. At 4 weeks of age (see Table 4), body length was increased in male and female offspring by the maternal HF diet (♂: F(1,38)=6.56; p<0.05; ♀: F(1,34)=4.18; p=0.050), similar to the effects found on body weight, and this was attributed mostly to increases in line 7 and 8 offspring from HF diet fed mothers compared those from LF diet fed mothers. Furthermore, maternal HF diet increased dry mass (♂: F(1,38)=11.7; p<0.01; ♀: F(1,34)=5.10; p<0.05), dry-lean mass (♂: F(1,38)=9.3; p<0.01; ♀: F(1,34)=6,16; p<0.05) in both offspring genders, irrespective of line. Absolute levels of body fat (F(1,38)=11.1; p<0.01) as well as %body fat (F(1,38)=6.6; p<0.05) were increased only in male offspring from HF diet feeding mothers relative to offspring from LF diet feeding mothers, irrespective of line. Also

body water content ($F(1,38)=7.3$; $p<0.05$) was increased but %body water decreased in male offspring from HF diet feeding mothers relative to those from LF feeding mothers, and an interaction of line and diet revealed that effects of the peri-gestational diet on dry mass ($F(1,38)=3.2$; $p=0.052$) and body water content ($F(2,38)=3.5$; $p<0.05$) were mainly attributed to increased levels in lines 7 and 8, but not line 2 offspring. Female offspring of line 8 had increased dry mass ($F(2,34)=3.82$; $p<0.05$) and a tendency for increased dry-lean mass ($F(2,34)=3.28$; $p=0.052$) relative to line 2 female offspring.

With respect to the liver of 4 week old mice, male offspring of mothers on the HF diet had increased fresh liver weight ($F(1,38)=6.75$; $p<0.05$), liver dry mass ($F(1,38)=7.0$; $p<0.05$), liver dry-lean mass ($F(1,38)=7.6$; $p<0.01$) and liver water content ($F(1,38)=6.43$; $p<0.05$), effects not seen in female mice. Furthermore, compared to line 2 male offspring line 7 male offspring had livers with lower fresh weight ($F(2,38)=3.76$; $p<0.05$), dry mass ($F(2,38)=4.84$; $p<0.05$), dry-lean mass ($F(2,38)=4.06$; $p<0.05$) and water content ($F(2,38)=3.35$; $p<0.05$). The %liver dry lean mass ($F(2,34)=4.46$; $p<0.05$) was smaller in female offspring line 7 than that of line 2 offspring. On the other hand, liver fat content (♂: $F(2,38)=5.76$; $p<0.01$; ♀: $F(2,34)=4.97$; $p<0.05$) and %liver fat content (♂: $F(2,38)=3.55$; $p<0.05$, ♀: $F(2,34)=6.23$; $p<0.01$) was the higher in male and female offspring of line 8 relative to male offspring of line 7 and female offspring of line 2.

At 4 months of age (See Table 5), prior differences in body length caused by maternal HF diet were lost in both genders. The maternal HF diet increased %body water content in male offspring ($F(1,32)=6.30$; $p<0.05$), but none of the other body parameters in either gender was affected by maternal diet. In male offspring, maternal HF diet decreased dry-lean mass ($F(2,32)=3.73$; $p<0.05$) as well as total body water content ($F(2,32)=4.64$; $p<0.05$) in line 7 relative to line 2. An increased %body water content ($F(2,32)=4.16$; $p<0.05$) in line 8 male offspring was observed in male offspring from HF diet fed mothers relative to those of line 2 mothers. None of the other parameters reached significance. In female offspring, line effects revealed smaller dry mass ($F(2,36)=9.66$; $p<0.001$), fat mass ($F(2,36)=10.24$; $p<0.001$), %body water ($F(2,36)=6.04$; $p<0.01$), %dry-lean mass ($F(2,36)=3.70$; $p<0.05$) and %body fat ($F(2,36)=9.73$; $p<0.001$) in line 7 and line 8 relative to line 2 and in addition smaller dry-lean mass ($F(2,36)=8.22$; $p<0.01$) and body water ($F(2,36)=8.04$; $p<0.01$) in line 7 but not in line 8 relative to line 2.

Composition of the livers of 4 month old mice was not affected by diet. In general, effects of line revealed smaller liver fresh weight (♂: $F(2,37)=12.08$; $p<0.001$; ♀: $F(2,37)=6.04$; $p<0.01$), liver dry mass (♂: $F(2,37)=9.49$; $p<0.01$; ♀: $F(2,37)=6.96$; $p<0.01$), liver dry-lean mass (♂: $F(2,37)=7.25$; $p<0.01$; ♀: $F(2,37)=6.24$; $p<0.01$), liver water content (♂: $F(2,37)=12.60$; $p<0.001$; ♀: $F(2,37)=5.42$; $p<0.01$) and %liver water content (♂: $F(2,37)=3.4$; $p<0.05$; but not in ♀) mostly in line 7 male offspring relative to line 2 and line 8 male offspring. Liver fat content, %liver fat content and other % parameters were not affected by line or diet.

Table 4. Body composition of male and female offspring of mothers perinatally fed LF and HF diet in line 2 (control line), line 7 and line 8 (selected lines) at 4 weeks of age.

	2		7		8	
	LF	**HF**	**LF**	**HF**	**LF**	**HF**
MALE						
Body mass (g)	23.3 ± 0.5	23.3 ± 0.6	18.1 ± 1.0**	22.1 ± 0.8#	17.0 ± 2.2*	23.9 ± 0.6##
Length (cm)	7.9 ± 0.1	7.7 ± 0.1	7.3 ± 0.2	7.8 ± 0.2	7.1 ± 0.3	8.0 ± 0.1##
Drymass (g)	5.1 ± 0.1	5.1 ± 0.1	3.9 ± 0.3	4.9 ± 0.2#	3.6 ± 0.4	5.3 ± 0.2##
Dry-lean mass (g)	3.9 ± 0.1	3.8 ± 0.1	3.0 ± 0.2	3.7 ± 0.1#	2.8 ± 0.3	3.9 ± 0.2##
Water content (g)	12.4 ± 0.2	11.7 ± 0.4	9.4 ± 0.6	11.7 ± 0.4#	8.7 ± 1.0	12.0 ± 0.5##
Fat mass (g)	1.2 ± 0.1	1.3 ± 0.2	0.9 ± 0.1	1.2 ± 0.1	0.8 ± 0.1	1.4 ± 0.1##
%Dry-lean mass	22.2 ± 0.5	22.8 ± 0.4	22.4 ± 0.3	22.3 ± 0.2	22.8 ± 0.7	22.4 ± 0.2
%Water	71.1 ± 0.3	69.8 ± 0.9	70.8 ± 0.4	70.6 ± 0.5	71.1 ± 0.3	69.5 ± 0.5#
%Fat mass	6.7 ± 0.4	7.5 ± 1.2	6.7 ± 0.3	7.1 ± 0.6	6.1 ± 0.6	8.1 ± 0.5#
Liver fresh mass (g)	1.5 ± 0.0	1.5 ± 0.1	1.2 ± 0.1**	1.3 ± 0.1	1.1 ± 0.2*	1.5 ± 0.1#
Liver fat content (mg)	50.5 ± 3.4	47.4 ± 6.5	31.9 ± 2.4**	36.4 ± 4.8	37.7 ± 6.4	46.6 ± 2.2
%Liver fat content	3.4 ± 0.2	3.1 ± 0.3	2.8 ± 0.1*	2.7 ± 0.3	3.6 ± 0.5	3.2 ± 0.1
FEMALE						
Body mass (g)	16.9 ± 1.5	15.4 ± 3.5	14.7 ± 1.1	17.3 ± 0.3	15.3 ± 0.7	18.4 ± 0.8#
Length (cm)	7.4 ± 0.2	7.4 ± 0.3	6.9 ± 0.2	7.5 ± 0.1	7.1 ± 0.1	7.6 ± 0.1#
Drymass (g)	3.8 ± 0.3	3.4 ± 0.8	3.2 ± 0.3	3.8 ± 0.0	3.4 ± 0.1	4.9 ± 0.4##
Dry-lean mass (g)	2.9 ± 0.2	2.7 ± 0.4	2.4 ± 0.2	2.9 ± 0.0	2.5 ± 0.1	3.5 ± 0.3##
Water content (g)	8.6 ± 0.9	7.4 ± 1.7	7.4 ± 0.6	8.7 ± 0.2	7.4 ± 0.3	10.2 ± 1.0##
Fat mass (g)	0.9 ± 0.1	0.7 ± 0.3	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.4 ± 0.3#
%Dry-lean mass	24.1 ± 1.4	25.8 ± 1.6	22.6 ± 0.3	23.0 ± 0.3	23.4 ± 0.6	23.4 ± 0.8
%Water	69.3 ± 0.7	68.5 ± 0.4	69.9 ± 0.5	69.5 ± 0.5	68.8 ± 0.4	67.3 ± 1.5
%Fat mass	6.6 ± 1.0	5.7 ± 1.4	7.3 ± 0.5	7.5 ± 0.5	7.6 ± 0.8	9.2 ± 2.1
Liver fresh mass (g)	1.1 ± 0.1	0.9 ± 0.3	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.1	1.4 ± 0.1#
Liver fat content (mg)	27.3 ± 4.2	20.9 ± 10.9	27.4 ± 3.1	29.2 ± 2.2	35.1 ± 3.4	42.8 ± 7.3
%Liver fat content	2.4 ± 0.3	2.1 ± 0.6	2.8 ± 0.2	2.8 ± 0.2	3.4 ± 0.2	3.1 ± 0.2##

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

4.3. Plasma hormone and glucose levels of own offspring

Analysis of blood samples taken from 4 month old mice revealed that maternal HF diet tended to decrease insulin levels in both genders (♂: $F(1,40)=4.06$; $p=0.052$; ♀: $F(1,37)=3.83$; $p=0.058$) and glucose levels in female offspring ($F(1,37)=3.82$; $p=0.059$), see table 6. In fact, these effects were attributed by line 2 offspring from mothers fed the HF diet. None of the other assessed circulating parameters were affected by diet. Line effects revealed low levels of insulin (♂: $F(2,40)=5.26$; $p<0.05$; ♀: $F(2,39)=4.87$; $p<0.05$) and leptin (♂: $F(2,40)=5.90$; $p<0.01$; ♀: $F(2,39)=11.74$; $p<0.001$) in male offspring of line 8 and female offspring of line 7 and line 8 relative to those of line 2. Glucose levels were also lower in line 7 and line 8 female ($F(2,37)=4.01$; $p<0.05$), but not in male offspring. Adiponectin levels were significantly different among all the three lines ($F(2,39)=15.03$; $p<0.001$) with line 8 having the highest and line 2 having the lowest levels of adiponectin and line 7 being intermediate.

Table 5. Body composition of male and female offspring of mothers perinatally fed LF and HF diet in line 2 (control line), line 7 and line 8 (selected lines) at 4 months of age.

	2		7		8	
	LF	**HF**	**LF**	**HF**	**LF**	**HF**
MALE						
Body mass (g)	40.6 ± 2.3	36.6 ± 0.9	33.9 ± 1.0*	33.7 ± 1.4	35.5 ± 0.9	34.9 ± 0.9
Length (cm)	9.7 ± 0.3	9.5 ± 0.1	9.4 ± 0.1	9.2 ± 0.2	9.2 ± 0.2	9.2 ± 0.2
Drymass (g)	9.6 ± 0.6	8.9 ± 0.4	8.6 ± 0.2	8.6 ± 0.5	8.6 ± 0.5	7.8 ± 0.3
Dry-lean mass (g)	7.6 ± 0.3	7.4 ± 0.2	6.9 ± 0.3	6.7 ± 0.2	7.1 ± 0.2	6.8 ± 0.1
Water content (g)	19.3 ± 0.3	20.0 ± 0.4	18.0 ± 0.6*	18.2 ± 0.8	19.4 ± 0.4	19.3 ± 0.4
Fat mass (g)	2.1 ± 0.5	1.5 ± 0.3	1.6 ± 0.3	1.9 ± 0.4	1.6 ± 0.4	1.0 ± 0.2
%Dry-lean mass	26.1 ± 0.9	25.7 ± 0.2	26.0 ± 0.7	25.2 ± 0.4	25.2 ± 0.3	25.1 ± 0.2
%Water	66.8 ± 1.4	69.2 ± 0.7	67.7 ± 0.6	68.0 ± 1.0	68.0 ± 6.8	71.3 ± 0.5
%Fat mass	7.1 ± 1.6	5.1 ± 0.8	6.3 ± 1.1	6.8 ± 1.2	6.8 ± 1.4	3.6 ± 0.5
Liver fresh mass (g)	2.2 ± 0.1	2.1 ± 0.1	1.7 ± 0.1**	1.8 ± 0.1	2.1 ± 0.0	2.1 ± 0.1
Liver fat content (mg)	80.0 ± 9.4	66.5 ± 6.5	53.3 ± 3.2*	64.8 ± 8.1	60.8 ± 6.3	72.4 ± 10.0
%Liver fat content	3.6 ± 0.4	3.1 ± 0.3	3.2 ± 0.2	3.6 ± 0.5	2.9 ± 0.3	3.5 ± 0.5
FEMALE						
Body mass (g)	31.7 ± 1.2	31.6 ± 2.0	24.8 ± 1.3**	27.2 ± 0.4	27.5 ± 0.6**	29.6 ± 1.3
Length (cm)	9.2 ± 0.1	9.1 ± 0.2	8.8 ± 0.2	8.8 ± 0.1	8.9 ± 0.1*	8.9 ± 0.2
Drymass (g)	9.4 ± 0.6	8.9 ± 0.9	6.3 ± 0.4**	6.9 ± 0.3	6.6 ± 0.2**	7.3 ± 0.8
Dry-lean mass (g)	6.0 ± 0.2	5.7 ± 0.4	5.0 ± 0.2**	5.2 ± 0.0	5.4 ± 0.1*	5.7 ± 0.2
Water content (g)	15.7 ± 0.5	15.5 ± 0.8	13.3 ± 0.7*	14.3 ± 0.2	14.5 ± 0.3*	15.7 ± 0.4#
Fat mass (g)	3.5 ± 0.5	3.2 ± 0.6	1.3 ± 0.3**	1.7 ± 0.3	1.2 ± 0.1**	1.6 ± 0.7
%Dry-lean mass	23.7 ± 0.4	23.6 ± 0.5	25.6 ± 0.7*	24.7 ± 0.3	25.8 ± 0.3**	24.7 ± 0.6
%Water	62.7 ± 0.8	63.8 ± 1.3	68.0 ± 0.9**	67.4 ± 1.2	68.7 ± 0.7**	68.6 ± 1.7
%Fat mass	13.6 ± 1.1	12.7 ± 1.7	6.3 ± 1.2**	7.9 ± 1.4	5.6 ± 0.6**	6.7 ± 2.2
Liver fresh mass (g)	1.9 ± 0.1	1.7 ± 0.2	1.4 ± 0.1**	1.6 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
Liver fat content (mg)	107.6 ± 12.6	98.5 ± 5.6	71.0 ± 15.5	74.4 ± 5.6	76.7 ± 8.5	94.5 ± 15.7
%Liver fat content	5.7 ± 0.5	5.7 ± 0.5	4.8 ± 1.0	4.8 ± 0.3	4.6 ± 0.5	5.1 ± 0.6

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

4.4. Food and water intake of own offspring

Assessment and analysis of food intake by the offspring at 3 months of age revealed effects of diet in male offspring ($F(1,35)=6.12$; $p<0.05$), but no line effect was observed. Male offspring of HF diet feeding mothers ate significantly more than male offspring of LF diet feeding mothers, irrespective of line. In female offspring, a line effect ($F(2,41)=12.96$; $p<0.0001$), and a line*diet effect ($F(2,41)=5.35$; $p<0.01$) was observed on food intake. Specifically, female offspring of line 2 ate significantly less than line 7 and line 8 irrespective of diet, and female offspring of HF feeding mothers only had increased food intake in line 8. (see Figure 4).

In male offspring water intake revealed effects of diet ($F(1,35)=7.67$; $p<0.01$), but not of line. Specifically, male offspring of HF diet fed mothers drank more than offspring of LF diet fed mothers. In female offspring, however, water intake revealed a line effect ($F(2,41)=7.54$; $p<0.01$), but not a diet effect; with line 7 female offspring drinking more than 2 female offspring (see Figure 5).

Table 6. Hormonal and fuel levels in male and female offspring of mothers perinatally fed LF and HF diet in line 2 (control line), line 7 and line 8 (selected lines) at 4 months of age.

	2		7		8	
	LF	**HF**	**LF**	**HF**	**LF**	**HF**
MALE						
Glucose (mM)	9.4 ± 0.9	7.8 ± 0.5	8.5 ± 0.6	7.7 ± 1.0	6.9 ± 0.9	7.9 ± 0.6
Insulin (ng/ml)	1.7 ± 0.5	0.8 ± 0.3	1.2 ± 0.2	1.0 ± 0.2	0.6 ± 0.1	0.5 ± 0.1
Leptin (ng/ml)	2.5 ± 0.6	2.3 ± 0.5	3.0 ± 0.4	2.6 ± 0.6	1.8 ± 0.3	1.2 ± 0.2
Adiponectin µg/ml)	3.8 ± 0.6	4.5 ± 1.1	5.2 ± 0.4	4.7 ± 0.3	4.3 ± 0.6	4.3 ± 0.5
FEMALE						
Glucose (mM)	9.5 ± 0.8	7.5 ± 0.6	7.4 ± 0.4*	6.3 ± 0.5	7.1 ± 0.4**	7.4 ± 0.7
Insulin (ng/ml)	1.5 ± 0.4	0.7 ± 0.3	0.4 ± 0.2*	0.3 ± 0.1	0.6 ± 0.2*	0.3 ± 0.2
Leptin (ng/ml)	5.4 ± 1.0	4.3 ± 0.8	2.6 ± 0.6*	2.2 ± 0.3	1.6 ± 0.2**	1.9 ± 0.7
Adiponectin µg/ml)	8.3 ± 0.5	6.9 ± 1.0	9.6 ± 0.8	10.0 ± 0.8	11.2 ± 0.7**	12.7 ± 0.9

* denotes significant difference with line 2 (line effect) (*, $p<0.05$; **, $p<0.01$). No diet effect was observed.

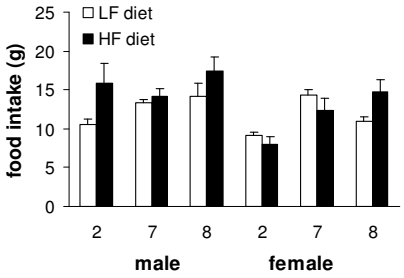

Figure 4. Food intake of male and female offspring of mothers perinatally fed LF and HF diet during lactation in line 2 (control line), line 7 and line 8 (selected lines) at 3 months of age.

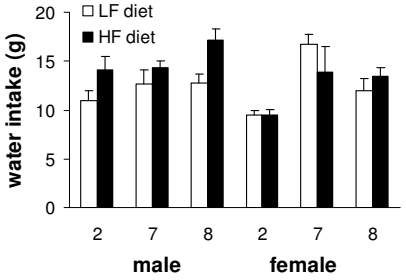

Figure 5. Water intake of male and female offspring of mothers perinatally fed LF and HF diet in line 2 (control line), line 7 and line 8 (selected lines) at 3 months of age.

4.5. Running wheel behavior of own offspring

Running wheel behavior was assessed according to the same methodology as used for selective breeding of lines. The average of the 5th and 6th day of total number of wheel revolutions was analyzed and since rodents are active in the dark phase, the dark phase of the 6th day was also examined specifically. In male offspring, running wheel behavior did not differ between lines and diet in either time period (see figure 6). In females, however, GLM revealed effect of line ($F(2,38)=4.20$; $p<0.05$), but not of diet. Specifically, line 8 offspring ran significantly more revolutions than line 2 offspring when the whole two days were examined. When only the dark phase was considered, both line 7 and 8 females ran significantly more than line 2 females ($F(2,38)=4.69$, $p<0.05$).

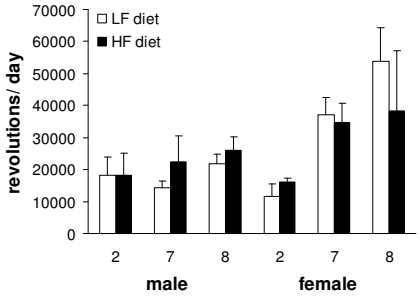

Figure 6. Average running wheel activity of male and female offspring of mothers perinatally fed LF and HF diet in line 2 (control line), line 7 and line 8 (selected lines) at 7 weeks of age.

4.6. Plus maze performance of own offspring

In male offspring, GLM Univariate Analysis revealed that maternal HF diet feeding increased the percentage of time spent in open arms ($F(1,48)=9.12$; $p<0.01$), the number of open arm entries ($F(1,48)=4.62$; $p<0.05$) and the percentage of open arm entries ($F(1,48)=10.42$; $p<0.01$). The percentage time spent in closed arms tended to be significant in the interaction of line and diet ($F(2,48)=3.14$; $p=0.053$). Closed arm entries were affected by line ($F(2,48)=3.28$; $p<0.05$), diet ($F(1,48)=7.09$; $p<0.05$), and by a line*diet interaction ($F(2,48)=4.25$; $p<0.05$). Specifically, offspring of HF diet fed mothers decreased their entries to closed arms, particularly in line 7, while offspring of line 8 was not affected. Percentage time spent in center area was affected by line ($F(2,48)=3.95$; $p<0.05$), diet ($F(1,48)=12.93$; $p<0.001$), and by a line*diet interaction ($F(2,48)=7.81$; $p<0.01$). Specifically, maternal HF diet feeding significantly decreased the time spent in the center, particularly strongly in line 7 offspring, while line 8 offspring were not affected. Total arm entries showed effect of line ($F(1,48)=3.64$; $p<0.05$); offspring of line 7 had increased total arm travels relative to offspring of line 2 (see Table 7).

In female offspring, GLM Univariate Analysis revealed that percentage of time spent in the center area was affected by line ($F(2,43)=7.10$; $p<0.01$), and by a line*diet interaction ($F(2,43)=7.01$; $p<0.01$). Specifically, offspring of line 2 spent significantly more time in the center relative to offspring of line 8, and this effect was amplified in line 2 offspring relative to lines 7 and 8 offspring from mother feeding the HF diet. While no effects were observed on time spent in closed or open arms, total arm entries in female offspring revealed effect of line ($F(2,43)=4.40$; $p<0.05$). Specifically, increased total arm entries of line 7 offspring relative to line 2 offspring were observed, with line 8 being intermediate. None of open arm or closed arm entries or percentage of open arm or closed arm showed difference among groups (see Table 7).

Table 7. Plus maze performance in male and female offspring of mothers perinatally fed LF and HF diet in line 2 (control line), line 7 and line 8 (selected lines) at adolescence age.

	2		7		8	
	LF	**HF**	**LF**	**HF**	**LF**	**HF**
MALE						
% Open time	13.6 ± 3.6	26.9 ± 12.9	4.2 ± 2.5*	16.7 ± 6.0#	8.6 ± 1.9	15.3 ± 1.8#
% Closed time	40.2 ± 3.6	49.4 ± 11.8	40.8 ± 4.6	51.3 ± 8.0	62.6 ± 3.9**	50.1 ± 4.0#
% Center time	46.2 ± 2.4	23.7 ± 3.0##	55 ± 3.9	32.1 ± 9.3#	28.9 ± 3.2**	34.7 ± 3.6
Open entries	4 ± 1.0	3.6 ± 1.3	2.3 ± 1.4	7.7 ± 2.1#	2.7 ± 0.6	4.3 ± 1.0
Closed entries	14.1 ± 1.5	9.2 ± 2.3	19.9 ± 1.9*	12.5 ± 1.7#	12.9 ± 1.1	14.3 ± 1.5
Total entries	18.1 ± 1.9	12.8 ± 1.5	22.2 ± 1.7	20.2 ± 3.7	15.6 ± 1.5	18.5 ± 1.8
% Open entries	21.4 ± 5.1	31.9 ± 12.4	9.9 ± 5.4	34.4 ± 4.1##	16 ± 2.6	22.6 ± 3.5
% Close entries	78.6 ± 5.1	68.1 ± 12.4	90.1 ± 5.4	65.6 ± 4.1##	84 ± 2.6	77.4 ± 3.5
FEMALE						
% Open time	8.5 ± 3.0	7.6 ± 4.4	10.2 ± 2.6	16.7 ± 6.9	13.6 ± 2.2	13.1 ± 2.8
% Closed time	51.4 ± 5.4	35 ± 5.2	45 ± 3.9	51.7 ± 5.8	53.8 ± 2.4	52.8 ± 3.4
% Center time	40.2 ± 3.8	57.4 ± 6.2#	44.8 ± 4.7	31.6 ± 1.5#	32.6 ± 2.4	34.2 ± 4.4
Open entries	3.4 ± 0.7	3.3 ± 1.6	5.2 ± 1.3	7.6 ± 2.2	4.7 ± 0.9	4.8 ± 0.7
Closed entries	15.3 ± 0.9	15.8 ± 2.8	18.2 ± 1.9	19.6 ± 2.1	15.8 ± 1.2	16.5 ± 1.3
Total entries	18.6 ± 1.3	19 ± 2.7	23.4 ± 2.9	27.1 ± 2.7	20.5 ± 1.2	21.3 ± 1.1
% Open entries	17.1 ± 3.1	16.7 ± 7.8	20.1 ± 3.9	26.3 ± 7.8	22.8 ± 3.7	23 ± 3.9
% Close entries	82.9 ± 3.1	83.3 ± 7.8	79.9 ± 3.9	73.7 ± 7.8	77.2 ± 3.7	77 ± 3.9

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

5. Discussion on perinatal nutritional effects on own offspring

In a previous study, we observed that control mice subjected to a diet with an high fat (HF) and refined sugar content during pregnancy and lactation give birth to pups with increase growth rates and subsequently increased body weights at adulthood (see Chapter 4 and 5). This effect was found in males only. These data are seemingly consistent with many other studies

investigating the effects of western type cafeteria diets during the perinatal stage on programming of energy balance in the offspring (Lemonnier 1972; Parente, Aguila, and Mandarim-de-Lacerda 2008; Samuelsson et al. 2008; Srinivasan et al. 2006). We additionally found that the perinatal effects of feeding a HF diet did not result in increased growth rates in mice from the same ancestral line, but subsequently selectively bred for increased voluntary wheel running behavior over 50 generations. Programming effects of an unhealthy diet can apparently be offset by a trait for voluntary physical activity, even in the absence of running wheels. In that particular study, however, we observed that the number of pups per litter at birth of the selected mothers was significantly higher in the high activity selected mice than of the control line. Moreover, the HF feeding control mothers, but not the selected mothers, gave birth to fewer pups per litter than the low fat (LF) diet feeding controls. These effects were largely repeated in the present study. Since litter size is inversely related to individual pup weight (Chapter 4, (Johnson, Thomson, and Speakman 2001)) during lactation, and thus may be an important factor determining pup weight gain and development and subsequent body weight at adulthood (Chapter 5), a permissive role of reduced litter size in the perinatal HF diet effects on offspring body weight homeostasis can not be ruled out.

The mechanism underlying this effect is probably related to the fact that nutrient procurement of the mother to each extra pup is not entirely met by increased maternal nutrient intake (Schultze 1954; Johnson, Thomson, and Speakman 2001), which renders individual pups in large litters smaller than those in relatively small litters. In the present study, we aimed at investigation the consequences of equalizing litters of all mothers to 10 pups, and hypothesized that diet and line effects would be maintained despite equalizing litter sizes. Hence, adjusting litter size to 10 pups would cause line 2 HF feeding mothers to nurse two pups extra per litter, whereas it would cause line 7 mothers to nurse on average 1.5 and line 8 to nurse on average 3 pups less per litter. There is supporting data pointing out that even a difference of 2 pups additionally or less in the litter could cause a significant difference in body weight gain of offspring after weaning (Epstein 1978), which could indeed explain the observed effects in the previous chapters.

Consistent with our hypothesis was the observation that equalizing litter sizes to 10 caused individual pup weights of HF feeding mothers during lactation to be larger than those of LF feeding mothers, and secondly, differences between lines during lactation (i.e., with line 2 pups being heavier than line 7 and 8 pups) were maintained. While the HF feeding line 2 mothers were the only group nursing more pups than they were prepared for, their food intake was markedly reduced on the HF diet and remained behind that of line 7 and 8 females feeding the HF diet. It seems unlikely that the mothers of the control line feeding the HF diet were limited intrinsically by maximized heat dissipation, and unable to increase their food intake to nurse the additional two pups in the litter above the ones they received at birth. The argument for this idea is that dietary fat has a lower specific dynamic action than carbohydrates (Donato 1987), and this would allow higher energy intake before running the risk over over-heating (Krol

and Speakman 2003). At this moment, we have no explanation for this phenomenon. Line 7 and line 8 mothers, on the other hand increased their food intake, despite the fact that they nursed a lower number of pups relative to the number they were prepared for at birth. This provided extra procurement of nutrients from the HF diet feeding mothers to their pups, and probably amplified the HF diet effect leading to transiently increased body weights of line 7 and 8 offspring after weaning; i.e., an effect not seen in the unmanipulated litter condition of chapter 4 and 5. Because ingestive behavior of line 2 mothers feeding the HF diet did not compensate the two additional pups they were not prepared for, differences in offspring weight gain of HF- and LF feeding line 2 mothers were lost at weaning.

The lack of HF diet effects in line 2 controls, and the emergence of HF diet effects in line 7 and 8 at weaning - both phenomena contrasting with the findings in the unmanipulated litter condition in Chapters 4 and 5 and against our initial hypothesis- were largely reflected by body composition at 4 weeks of age. Thus, male offspring from HF diet fed line 7 and line 8 mothers had increased body fat content, lean mass, and body water compared to line 7 and 8 male offspring from LF feeding mothers. In line 2, male offspring from HF feeding and LF feeding mothers did not differ in body composition, and these effects differ from the unmanipulated litter condition in Chapters 4 and 5, where only line 2 HF feeding male offspring was heavier and had more body fat content that line 2 male offspring from LF feeding mothers. In female offspring in the present study, body fat content and body mass even increased in line 8 offspring from HF diet feeding mothers relative to the HF diet feeding line 2 mothers. Taken together, these data indicate that litter size appears to be an important factor in HF diet-induced alterations in body weight homeostasis at the pre-pubertal stage. Since line 8 females prepared for the largest litters, and thus nursed relatively fewest pups per litter, it is plausible to find the largest weight gain of individual pups at equalized litters in this particular line.

In the offspring at 4 months of age, diet effects on adiposity and lean mass were generally lost, while line effects became stronger, with line 7 and 8 male and particularly female offspring being smaller and leaner than line 2 male and female offspring. The hormonal profile of these animals very well fit these differences, with plasma leptin and insulin levels particularly lower in line 7 and 8 female offspring relative to those in line 2 offspring. In addition, increases were observed in plasma adiponectin levels in the high activity female offspring, reflecting the findings we had in previous studies (Vaanholt et al. 2007). These data demonstrate that perinatal effects of dietary fat content are limited on the long term in the offspring, even if initial body weight gain is in the opposite direction due to equalizing litter sizes. The trait for increased voluntary physical activity apparently overpowers pre-pubertal increases in body adiposity. Interestingly, food and water intake were increased in the male offspring from HF feeding mothers irrespective of line. This points out that perinatal HF diet feeding increased turn-over of nutrients specifically in male offspring, and may be related to early imprinting on neural circuits in the hypothalamus regulating energy fluxes. There are indeed supporting studies showing that early overfeeding or underfeeding either by diet or litter size manipulation caused permanent

changes in hypothalamic hormonal controls on food intake at adulthood (Davidowa, Li, and Plagemann 2003; Plagemann et al. 1999; Bouret 2009; Bouret and Simerly 2006). Other behavioral effects of perinatal HF feeding were decreased anxiety-like behaviors in male (i.e., increased time spent in the open arms in the plus maze test), but to a lesser extent in female (i.e., increased time in the center of the plus maze test) offspring during the plus maze performance test. Besides these effects, there were line effects to indicate that the offspring from the high activity lines showed more anxiety-like behavior and running wheel activity (in females only) than the control offspring, which is in agreement with the results of previous chapters.

In summary, energy supply, both qualitatively (maternal diet) and quantitatively (determined by litter size manipulation) during lactation have huge impacts on offspring development. Feeding a HF diet caused female mice in the control line to deliver smaller litters, but those in the selection lines to deliver the same or even larger litters than when they were feeding the fed LF diet. Equalizing litter sizes caused control females on a HF diet to nurse two pups more than they delivered, while selected lines nursed 1.5-3 pups less than they delivered irrespective of diet. Thus, control mothers faced increased requirements from their litters despite HF diet feeding, and this permanently diminished perigestational HF diet effects on body characteristics including weight, length, and adiposity. Selected mothers, on the other hand, were feeding less pups than they prepared for, which resulted in perinatal HF diet-induced obesogenic effects at the adolescent stage, particularly in line 8. These effects, however, were transient and overpowered by the physical activity trait at adulthood leading to smaller and leaner mice in lines 7 and 8 relative to lines 2.

6. Part B. Characteristics of cross-fostered offspring

Litter characteristics at birth were already described in paragraph 3.2 and shown in table 1. In this part, growth and development will be described of pups that were either cross-fostered by mothers within the same line and diet (condition B), or cross-fostered between lines 2 and 7, or between lines 2 and 8 within the same diet condition (condition C). Cross-fostering of pups between mothers on different diets within the same line was also performed (condition D), but yielded too few cases and is not described further. Table 2 shows numbers of cross-fostered and non cross-fostered pups in the different conditions. To assess whether body weight and related parameters are determined 1) by a genetic basis for these parameters inside the cross-fostered pup, or 2) by the postnatal influence exerted via the mother ("environment"), characteristics of cross-fostered offspring between lines (condition C) were compared to non cross-fostered "own" offspring (condition A; described in the previous part of this chapter). Effect of cross-fostering per sé was investigated by comparing non cross-fostered "own" offspring (condition A) with cross-fostered offspring between mothers within the same lines (condition B). Differences between offspring were analyzed with GLM Univariate Analysis according to comparisons mentioned above. Males and females were included in the analysis to investigate interactions between "environment" and "gender". Gender effects alone were not discussed.

6.1. Body weight of cross-fostered offspring before weaning

Before weaning, body masses of offspring were assessed at day 8, 13, 16 and 21. Before weaning, cross-fostering of pups between mothers within the same line on either diet never caused body weights of pups to differ from the non cross-fostered pups (comparison of condition A versus B). This means that the procedure of fostering was a negligible factor in adolescent growth.

Line 2 offspring from LF feeding mothers (further mentioned as 2LF, 7LF, etc) fostered by 7LF mothers yielded significant body weight effects from day 8 onwards (day 8 ($F(3,55)=6.94$; $p<0.001$), day 13 ($F(3,52)=23.20$; $p<0.001$), day 16 ($F(3,52)=26.48$; $p<0.001$) and day 21 ($F(3,52)=31.44$; $p<0.001$)). Specifically, 2LF offspring weighed significantly less when they were fostered by 7LF mothers than when they were raised by their own mothers, and in fact weighed less than 7LF own pups. Line 2LF offspring by fostered by 8LF mothers did not change body weight. Line 7LF offspring fostered by 2LF mothers significantly affected body weight from day 16 onward (d16: $F(2,63)=4.10$; $p<0.05$ and d21: $F(2,63)=3.39$; $p<0.05$). Specifically, 7LF offspring were heavier when they were fostered by 2LF mothers, but they weighed significantly less than 2LF own offspring (d16 and d21: $p<0.001$). Body weight of 8LF offspring was not altered due to fostering by line 2LF mothers or by the interaction with gender at any age. See Figure 7 left column.

In the HF diet condition, body weight of line 2HF offspring fostered by 7HF mothers caused significant effects on body weight only at day 21 ($F(3,44)=2.89$; $p<0.05$). Specifically, 2HF offspring weighed less when they were fostered by 7HF mothers, and in fact had similar weights as 7HF own offspring. No effect of fostering of 2HF offspring by 8HF mothers was found. From day 13 onward, body weight of 7HF (d13: $F(2,39)=6.03$; $p<0.01$, d16: $F(2,39)=8.61$; $p<0.001$ and d21: $F(2,39)=7.49$; $p<0.01$) as well as 8HF offspring (d13: $F(2,57)=4.70$; $p<0.05$, d16: $F(2,56)=4.60$; $p<0.05$ and d21: $F(2,47)=3.96$; $p<0.05$) fostered by 2HF mothers yielded significant effects. Both 7HF and 8HF offspring were heavier when they were raised by 2HF mothers compared to when they were raised by their own mothers, and in fact had similar weights as 2HF own biological offspring. See Figure 8 left column.

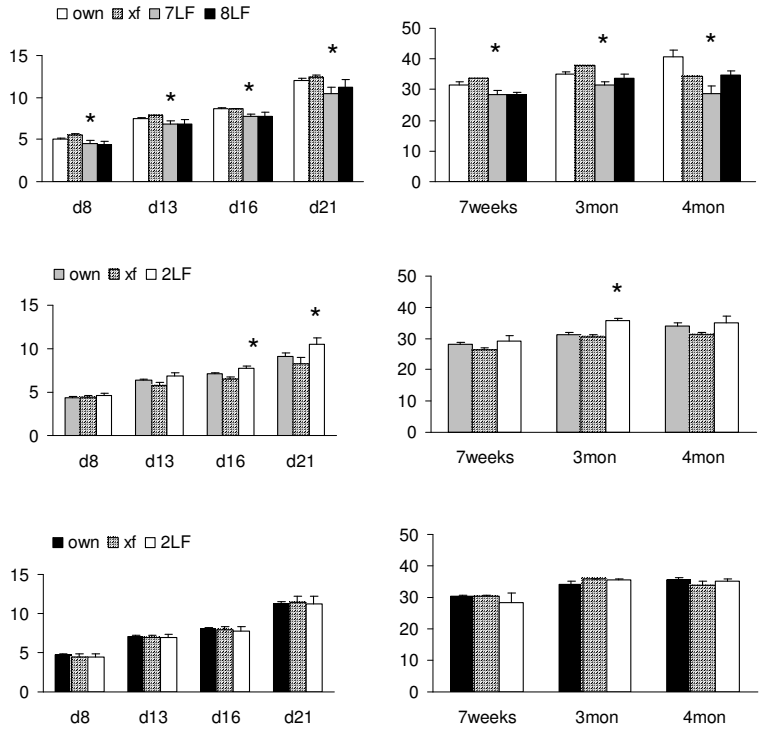

Figure 7. Offspring (males and females) body weight development described preweaning (left column) and postweaning (right column) when perinatally fed LF diet. Top raw shows 2LF offspring, middle raw shows 7LF offspring and bottom raw shows 8LF offspring raised by different mothers indicated in legend. * denotes significant difference between cross-fostered and own offspring ($p<0.05$).

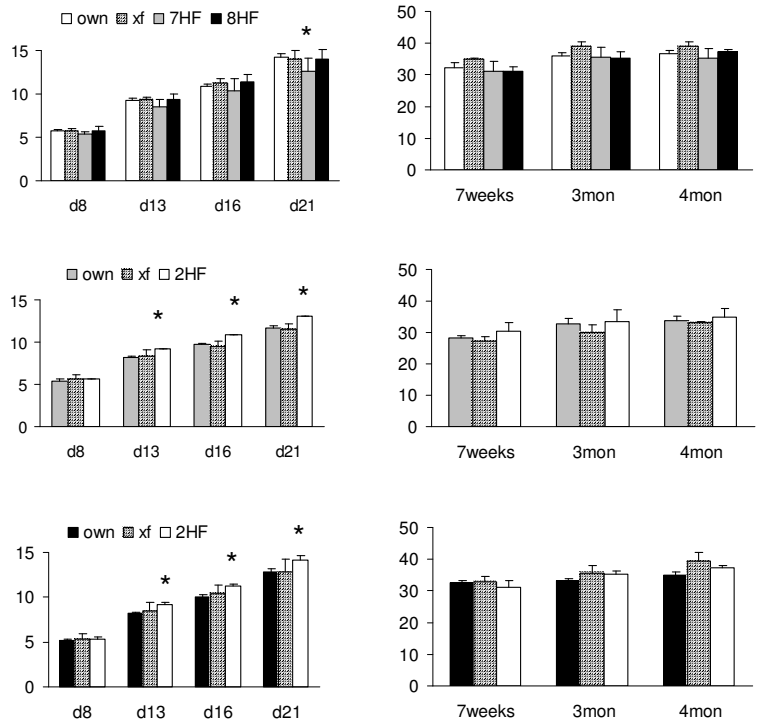

Figure 8. Offspring (males and females) body weight development described preweaning (left column) and postweaning (right column) when perinatally fed HF diet. Top raw shows 2HF offspring, middle raw shows 7HF offspring and bottom raw shows 8HF offspring raised by different mothers indicated in legend. * denotes significant difference between cross-fostered and own offspring ($p<0.05$).

6.2. Energy fluxes during peak lactation

During peak lactation (day 13-16), maternal food intake (g) was significantly influenced by line ($F(2,35)=3.38$; $p<0.05$) and by a line*diet interaction ($F(2,35)=3.49$; $p<0.05$); i.e., line 7 ate significantly less than line 8 mothers. When food intake was expressed as energy (kJ) effect of line ($F(2,35)=3.44$; $p<0.05$), diet ($F(1,35)=19.42$; $p<0.001$), and interactions of line and diet ($F(2,35)=3.41$; $p<0.05$) were revealed, and post-hoc analysis showed that mothers fed a HF diet ate more that those fed a LF diet, with the exception of line 2. Line 8 mothers ate more than line 7 mothers irrespective of diet (see Table 8).

The total amount of defecated waste during 3 days of peak lactation was significantly influenced by diet ($F(1,34)=12.33$; $p<0.01$), but not by line. Specifically, lactating females feeding HF diet defecated less than when they were feeding LF diet. The total energy content of the waste was also decreased by diet ($F(1,34)=5.99$; $p<0.05$). The waste energy content per unit

weight was significantly influenced by line ($F(2,34)=4.29$; $p<0.05$), diet ($F(1,34)=197.85$; $p<0.001$) and by a line*diet interaction ($F(2,34)=5.56$; $p<0.01$). Specifically, line 8 mothers had increased energy content in the feces per unit weight relative to line 2 mothers, and HF feeding also increased the energy content per unit weight.

Table 8. Energetic parameters during peak lactation (day 13-16) in females fed LF diet and HF diet in line 2 (control line), line 7 and line 8 (selected lines).

	2		7		8	
	LF	**HF**	**LF**	**HF**	**LF**	**HF**
Food intake (g)	66.5 ± 1.1	55.7 ± 2.3##	55.1 ± 3.1**	58.1 ± 5.4	62.8 ± 2.5	67.3 ± 4.1
Food intake (kJ)	1057.7 ± 18.0	1095.5 ± 45.1	875.6 ± 49.4**	1141.9 ± 106.4#	998.2 ± 39.7	1323.6 ± 80.6##
Feces						
total dry weight (g)	10.0 ± 1.8	6.0 ± 0.2#	6.3 ± 0.4	5.2 ± 0.3	8.4 ± 1.0	5.6 ± 0.3#
total energy content (kJ)	177.9 ± 33.1	115.9 ± 3.6	112.1 ± 7.5	106.1 ± 7.1	147.6 ± 17.6	116.5 ± 5.4
energy density (kJ/g)	17.7 ± 0.1	19.4 ± 0.3##	17.9 ± 0.2	20.2 ± 0.3##	17.6 ± 0.1	20.6 ± 0.2##
Absorbed energy (kJ)	879.8 ± 48.1	979.6 ± 43.1	763.5 ± 46.1	1035.9 ± 102.6#	850.7 ± 27.6	1207.2 ± 80.0##
Absorption efficiency (%)	82.9 ± 3.5	89.4 ± 0.4	87.1 ± 0.8	90.6 ± 0.6#	85.4 ± 1.4	91.1 ± 0.5##

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

The energy absorption of the mothers was subtracted from the total ingested energy minus the total measured energy of the feces. This absorbed energy was higher in mothers fed a HF diet ($F(1,34)=24.3$; $p<0.001$). Absorption efficiency was increased by HF diet ($F(1,34)=14.69$; $p<0.001$). This energy was used by the mother itself and by the pups. Therefore to calculate the growth efficiency, the body weight gain of the mother and the pups was counted from day 13 to day 16 and both divided with the absorbed energy of the mother (mg/kJ). Growth efficiency was significantly influenced by line ($F(2,33)=4.16$; $p<0.05$), diet ($F(1,33)=258.10$; $p<0.001$) and line*diet interaction ($F(2,33)=6.11$; $p<0.01$). Specifically, line 7 had smaller GE when fed a LF diet. HF diet significantly increased GE with the exception of line 8. (Figure 9).

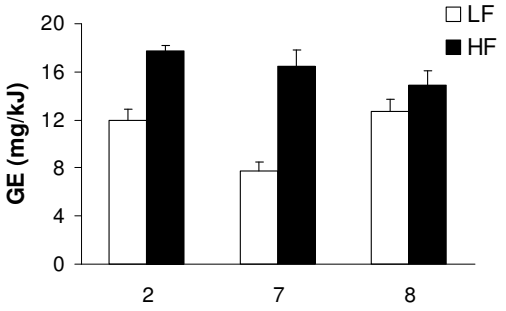

Figure 9. Growth efficiency of females fed LF and HF diet in line 2 (control line), line 7 and line 8 (selected lines) at peak lactation.

6.3. Body weight of cross-fostered offspring after weaning

Similar to the pre-weaning period, cross-fostering of pups between mothers in either line on either diet did not cause body weights of pups to differ from the non cross-fostered pups (comparison of condition A versus B).

Body weight of line 2LF offspring fostered by 7LF mothers yielded significant effects throughout the postweaning period to adulthood (at 6 weeks of age ($F(3,20)=7.30$; $p<0.005$), at 3 months of age ($F(3,20)=3.88$; $p<0.05$) and at 4 months of age ($F(3,23)=8.73$; $p<0.0005$)). See Figure 7 right column. Specifically, 2LF offspring weighed less when they were fostered by 7LF mothers compared to when they were raised by their own mothers, and in fact were indistinguishable from 7LF own offspring. Body weight of 7LF offspring fostered by 2LF mothers yielded interaction effect of environment and gender ($F(2,27)=4.49$; $p<0.05$) at 3 months of age but not at other time points. Thus at 3 months of age, 7LF male, but not 7LF female offspring were heavier when they were fostered by 2LF mothers and were indistinguishable from 2LF offspring. Body weight of 8LF offspring fostered by 2LF mothers was neither significantly by environment nor by the interaction with gender at any age. In the HF diet condition, neither body weights of 2HF, 7HF, nor of 8HF offspring were affected by cross-fostering. See Table 8 right column.

6.4. Body composition of cross-fostered offspring

Offspring body composition at 4 months of age is shown in Table 9. In the LF diet condition (Table 9A), 2LF offspring fostered by 7LF mothers yielded significant effects on body length ($F(3,23)=3.67$; $p<0.05$), dry-lean mass ($F(3,23)=4.28$; $p<0.05$), analogous to the effects on body weight. In none of the groups was body fat mass or %body fat significantly affected by cross-fostering. In stead, body length and dry-lean mass were smaller in 2LF offspring when they were fostered by 7LF, but not by 8LF mothers, and in fact indistinguishable from 7LF own offspring. When 7LF offspring were fostered by 2LF mothers, total body water content yielded an interaction effect of environment and gender ($F(2,28)=3.4$; $p<0.05$), but no other parameters were affected. Specifically, male offspring, but not female offspring, of 7LF mothers had increased body water content when they were fostered by 2LF mothers, and were indistinguishable from 2LF own biological offspring. Line 8LF offspring were not altered due to fostering by 2LF mothers. An effect of cross-fostering per sé was observed in 8LF offspring. Specifically, body water content ($p<0.001$) was increased in 8LF offspring by cross-fostering within lines.

In the HF diet condition (Table 9B), fostering of 7HF offspring by 2HF mothers caused significant effects on dry-lean mass ($F(2,17)=6.88$; $p<0.01$) and body water content ($F(2,17)=6.52$; $p<0.01$). Specifically, dry-lean mass and body water content were larger in 7HF offspring when they were fostered by 2HF mothers than when they were raised by their own mothers.

Table 9A. Body composition of mice selectively bred for high voluntary wheel running behavior (lines 7 and 8) and the control line raised by mothers they were born to ("own"), or when they were fostered by other mothers of the same line (xf), or by other mothers of other lines (xf2, xf7, or xf8). Males, females and LF- (A), HF diet (B) are shown in separate panels.

A	own	xf	7	8	own	xf	2	own	xf	2
	2LF offspring				7LF offspring			8LF offspring		
MALE										
BW	40.6 ± 2.3	34.4 ±	28.7 ± 2.6*	34.8 ± 1.2	33.9 ± 1.0	31.3 ± 0.5	35.2 ± 1.9	35.5 ± 0.9	33.9 ± 1.3	35.2 ± 0.6
Length	9.7 ± 0.3	9.3 ±	9.1 ± 0.2	9.1 ± 0.1	9.4 ± 0.1	9.5 ± 0.0	9.4 ± 0.3	9.2 ± 0.2	8.8 ± 0.3	9.4 ± 0.1
Drymass	9.6 ± 0.6	9 ±	7.7 ± 0.8	9.2 ± 1.0	8.6 ± 0.2	8.7 ± 0.4	9.5 ± 1.0	8.6 ± 0.5	8.9 ± 0.7	9.6 ± 0.3
Drylean	7.6 ± 0.3	8.3 ±	6.6 ± 0.4	7.3 ± 0.4	6.9 ± 0.3	6.7 ± 0.1	7.5 ± 0.4	7.1 ± 0.2	7.2 ± 0.4	7.5 ± 0.1
Water	19.3 ± 0.3	20.7 ±	16.3 ± 2.1	20.7 ± 0.6	18 ± 0.6	17.9 ± 0.4	20.7 ± 0.9*	19.4 ± 0.4	20 ± 0.7	20.8 ± 0.3*
Fat	2.1 ± 0.5	0.7 ±	1.2 ± 0.4	1.9 ± 0.6	1.6 ± 0.3	2 ± 0.3	2 ± 0.6	1.6 ± 0.4	1.6 ± 0.4	2.1 ± 0.3
Drylean%	26.1 ± 0.9	28.0 ±	24.9 ± 0.3	24.7 ± 0.2	26.0 ± 0.7	25.2 ± 0.4	27.9 ± 2.1	24.6 ± 0.3	25.0 ± 0.2	24.5 ± 0.5
Water%	66.8 ± 1.4	69.8 ±	68.8 ± 1.4	68.4 ± 0.7	67.7 ± 0.6	67.2 ± 0.8	67.6 ± 1.0	69.2 ± 1.2	69.4 ± 1.1	69.3 ± 2.0
Fat%	7.1 ± 1.6	2.2 ±	6.3 ± 1.6	6.8 ± 0.8	6.3 ± 1.1	7.6 ± 1.1	4.5 ± 1.2	6.2 ± 1.4	5.5 ± 1.1	6.2 ± 1.6
FEMALE										
BW	31.7 ± 1.2	33.8 ± 3.3	24.3 ± 2.3**	28.2 ± 0.6	24.8 ± 1.3	27.1 ± 0.8	25.8 ± 0.3	27.5 ± 0.6	28.7 ± 1.1	27.1 ± 1.4
Length	9.2 ± 0.1	9.5 ± 0.3	8.7 ± 0.2*	9 ± 0.2	8.8 ± 0.2	9.1 ± 0.3	8.7 ± 0.1	8.9 ± 0.1	8.7 ± 0.2	9 ± 0.2
Drymass	9.4 ± 0.6	9.8 ± 1.6	7.1 ± 0.8*	8 ± 0.5	6.3 ± 0.4	7.7 ± 0.6	6.8 ± 0.3	6.6 ± 0.2	8 ± 0.7	7.3 ± 0.5
Drylean	6 ± 0.2	6.2 ± 0.3	5.1 ± 0.4	5.9 ± 0.2	5 ± 0.2	5.4 ± 0.1	5.2 ± 0.1	5.4 ± 0.1	6 ± 0.1	5.8 ± 0.3
Water	15.7 ± 0.5	16.8 ± 0.8	14.4 ± 1.4	16.2 ± 0.2	13.3 ± 0.7	15.3 ± 0.3	14.6 ± 0.2	14.5 ± 0.3	16.4 ± 0.4*	16.5 ± 1.0*
Fat	3.5 ± 0.5	3.6 ± 1.3	2 ± 0.5	2.2 ± 0.3	1.3 ± 0.3	2.3 ± 0.5	1.6 ± 0.3	1.2 ± 0.1	2 ± 0.6	1.5 ± 0.2
Drylean%	23.7 ± 0.4	23.4 ± 0.9	24.4 ± 0.5	24.9 ± 0.3	25.6 ± 0.7	23.7 ± 0.3	24.2 ± 1.6	25.8 ± 0.3	24.8 ± 0.8	24.2 ± 0.3
Water%	62.7 ± 0.8	63.9 ± 2.8	68.2 ± 1.1	69.5 ± 0.6	68.0 ± 0.9	66.7 ± 1.6	67.1 ± 1.4	68.7 ± 0.7	67.3 ± 1.4	66.8 ± 1.2
Fat%	13.6 ± 1.1	12.7 ± 3.6	7.4 ± 1.6	7.7 ± 1.0	6.3 ± 1.2	9.6 ± 1.9	8.7 ± 1.5	5.6 ± 0.6	8.0 ± 2.1	9.0 ± 1.1

* denotes significant difference from the own (*, $p<0.05$; **, $p<0.01$).

Table 9B. Body composition of mice selectively bred for high voluntary wheel running behavior (lines 7 and 8) and the control line raised by mothers they were born to ("own"), or when they were fostered by other mothers of the same line (xf), or by other mothers of other lines (xf2, xf7, or xf8). Males, females and LF- (A), HF diet (B) are shown in separate panels.

B	own	xf	7	8	own	xf	2	own	xf	2
	2HF offspring				7HF offspring			8HF offspring		
MALE										
BW	36.6 ± 0.9	39.2 ± 1.4	35.4 ± 2.8	35.9 ± 0.7	33.7 ± 1.4	33.2 ± 0.4	34.9 ± 2.7	34.9 ± 0.9	39.4 ± 2.9	37.2 ± 0.7
Length	9.5 ± 0.1	9.6 ± 0.3	9.6 ± 0.2	9.3 ± 0.4	9.2 ± 0.2	9.5 ± 0.3	9.7 ± 0.3	9.2 ± 0.2	9.5 ± 0.0	9.7 ± 0.2
Drymass	8.9 ± 0.4	11.5 ± 0.3	9.5 ± 0.5	9.5 ± 0.5	8.6 ± 0.5	8.3 ± 0.4	9.5 ± 0.7	8 ± 0.3	10.8 ± 1.7	9.8 ± 0.6
Drylean	7.4 ± 0.2	8.5 ± 0.4	7.7 ± 0.5	7.6 ± 0.3	6.7 ± 0.2	7 ± 0.0	7.7 ± 0.5	6.9 ± 0.2	7.8 ± 0.4	7.9 ± 0.2
Water	20 ± 0.4	22.5 ± 1.2	20.8 ± 1.9	21.2 ± 0.3	18.2 ± 0.8	20.3 ± 0.1	21 ± 1.8	19.7 ± 0.5	23 ± 0.5	22.1 ± 0.5
Fat	1.5 ± 0.3	3.1 ± 0.4	1.8 ± 0.4	1.9 ± 0.3	1.9 ± 0.4	1.3 ± 0.4	1.8 ± 0.2	1 ± 0.1	3 ± 1.4	1.9 ± 0.5
Drylean%	25.7 ± 0.2	24.5 ± 0.3	25.3 ± 0.5	24.9 ± 0.2	25.2 ± 0.4	24.5 ± 0.2	25.4 ± 0.3	25.1 ± 0.2	23.3 ± 0.4	24.9 ± 0.3
Water%	69.2 ± 0.7	67.4 ± 0.5	68.9 ± 0.3	69.3 ± 1.4	68.0 ± 1.0	71.0 ± 1.2	68.4 ± 1.4	71.3 ± 0.5	68.2 ± 3.1	69.0 ± 1.0
Fat%	5.1 ± 0.8	8.1 ± 0.7	5.8 ± 0.3	5.8 ± 1.6	6.8 ± 1.2	4.6 ± 1.4	6.2 ± 1.4	3.6 ± 0.5	8.5 ± 3.5	6.1 ± 0.8
FEMALE										
BW	31.6 ± 2.0	28.6 ± 1.7	27.4 ± 3.1	31.8 ± 3.2	27.2 ± 0.4	26.8 ± 0.7	29 ± 1.0	29.6 ± 1.3	26.8 ± 0.7	28.1 ± 1.2*
Length	9.1 ± 0.2	9.1 ± 0.3	9 ± 0.4	9.2 ± 0.3	8.8 ± 0.1	8.9 ± 0.2	9.2 ± 0.2	8.9 ± 0.2	8.5 ± 0.1	9 ± 0.4
Drymass	8.9 ± 0.9	8.4 ± 0.3	8.4 ± 1.2	9.7 ± 1.4	6.9 ± 0.3	7.2 ± 0.2	7.9 ± 0.7	7.3 ± 0.8	6.7 ± 0.0	6.9 ± 0.4
Drylean	5.7 ± 0.4	5.9 ± 0.5	5.5 ± 0.5	6.2 ± 0.5	5.2 ± 0.0	5.8 ± 0.2	5.9 ± 0.3*	5.7 ± 0.2	5.9 ± 0.1	5.8 ± 0.2
Water	15.5 ± 0.8	16.2 ± 1.5	14.7 ± 1.6	17.3 ± 1.7	14.3 ± 0.2	15.5 ± 0.4	16.2 ± 0.5*	15.7 ± 0.4	15.7 ± 0.3	16.2 ± 0.8*
Fat	3.2 ± 0.6	2.5 ± 0.7	2.9 ± 0.7	3.5 ± 0.8	1.7 ± 0.3	1.4 ± 0.1	2 ± 0.4	1.6 ± 0.7	0.9 ± 0.1	1 ± 0.2
Drylean%	23.6 ± 0.5	23.8 ± 0.8	24.7 ± 0.3	25.2 ± 0.3	24.7 ± 0.3	25.4 ± 0.3	24.2 ± 0.8	24.7 ± 0.6	26.1 ± 0.2	23.2 ± 0.6
Water%	63.8 ± 1.3	65.6 ± 2.6	67.2 ± 1.8	70.3 ± 0.1	67.4 ± 1.2	68.2 ± 0.3	64.0 ± 1.4	68.6 ± 1.7	70.1 ± 0.5	64.2 ± 1.1
Fat%	12.7 ± 1.7	10.6 ± 3.3	8.1 ± 1.5	4.5 ± 0.5	7.9 ± 1.4	6.4 ± 0.3	11.8 ± 2.2	6.7 ± 2.2	3.8 ± 0.7	12.5 ± 1.6

* denotes significant difference from the own (*, $p<0.05$; **, $p<0.01$).

6.5. Hormone levels of cross-fostered offspring

In the LF condition, fostering of 2LF offspring by 8LF mothers caused significant effects on plasma levels of insulin ($F(3,30)=3.03$; $p<0.05$) and glucose ($F(3,30)=6.34$; $p<0.01$). See Table 10. Specifically, 2LF offspring had lower levels of insulin when they were fostered by 8LF mothers and in fact were indistinguishable from 8LF own offspring. Glucose levels were also decreased when 2LF offspring were fostered by 7LF and 8LF mothers, and were indistinguishable from to 8LF own offspring and even lower than in 7LF own offspring. No effects on plasma levels of adiponectin and leptin were observed by cross-fostering. Cross-fostering of pups between mothers in the same line caused an increased in insulin levels in 8HF offspring ($p<0.05$), but no other effects were observed.

6.6. Food and water intake of cross-fostered offspring

Food and water intake assessed at 3 months of age are shown in Table 11. No effect of cross-fostering within or between lines was found on food and water intake.

6.7. Running wheel behavior of cross-fostered offspring

Running wheel behavior was assessed according to the same methodology as used for selection of lines. Therefore the average of the 5th and 6th day of total number of wheel revolutions was analysed and since rodents are active in the dark phase, the dark phase of the 6th day was also examined specifically. No effect of cross-fostering was found in any line and diet condition. See Table 12.

6.8. Plus maze performance of cross-fostered offspring

Line 2LF offspring fostered by line 8LF mothers showed an interaction effects with gender on time spent in open arms ($F(2,25)=9.25$; $p<0.001$), %open arm entries ($F(2,25)=5.65$; $p<0.01$), closed arm entries ($F(2,25)=7.25$; $p<0.005$) and %closed arm entries ($F(2,25)=5.65$; $p<0.01$) (Table 13). Specifically, female offspring of 2LF fostered by 8LF mothers spent more time in open arms, had higher percentage open arm entries, less closed arm entries and lower percentage closed arm entries than 2LF own offspring. Line 7LF offspring fostered by 2LF mothers showed an interaction with gender on time spent in closed arms ($F(1,22)=5.05$; $p<0.05$) and time spent in the center ($F(1,22)=4.60$; $p<0.05$). Specifically, male offspring of 7LF spent more time and females spent less time in the closed arms. Male, but not female, line 7LF offspring spent less time in the center when they were fostered by 2LF mothers.

In the HF diet condition, 2HF offspring during plus maze test had only one male and one female mice in the group when they were cross-fostered to 8HF mothers, therefore, results were not shown. No effects were observed in 7HF and 8HF offspring.

Table 10. Plasma hormone and glucose levels of mice selectively bred for high voluntary wheel running behavior (lines 7 and 8) and the control line raised by mothers they were born to ("own"), or when they were fostered by other mothers of the same line (xf), or by other mothers of other lines (xf2, xf7, or xf8). LF-, HF diet are shown in separate panels.

	own	xf	7	8	own	xf	2	own	xf	2
	2LF offspring				7LF offspring			8LF offspring		
MALE										
glucose (mM)	9.4 ± 0.9	5.7 ±	6.4 ± 0.8	6.2 ± 0.8	8.5 ± 0.6	8 ± 0.4	8.9 ± 0.8	6.9 ± 0.9	9.2 ± 1.2	9 ± 0.3
insulin (ng/ml)	1.7 ± 0.5	1.2 ±	0.6 ± 0.1	0.9 ± 0.7	1.2 ± 0.2	0.8 ± 0.3	0.9 ± 0.5	0.6 ± 0.1	0.7 ± 0.3	1.5 ± 0.5
leptin (ng/ml)	2.5 ± 0.6	1.3 ±	1.3 ± 0.1	1.5 ± 0.2	3 ± 0.4	2.9 ± 0.6	2.4 ± 0.7	1.8 ± 0.3	1.8 ± 0.3	2.4 ± 0.6
adiponectin (µg/ml)	3.8 ± 0.6	2 ±	2.5 ± 0.2	2.7 ± 1.2	5.2 ± 0.4	6 ± 0.2	4 ± 0.6	4.3 ± 0.6	4.1 ± 0.6	5 ± 0.6
FEMALE										
glucose (mM)	9.5 ± 0.8	8 ± 0.8	6.2 ± 0.8*	6.9 ± 0.8	7.4 ± 0.4	6.6 ± 0.4	5.9 ± 0.5	7.1 ± 0.4	7.6 ± 0.7	6.2 ± 0.3
insulin (ng/ml)	1.5 ± 0.4	1.5 ± 0.6	0.7 ± 0.2	0.1 ± 0.1*	0.4 ± 0.2	0.6 ± 0.3	0.2 ± 0.1	0.6 ± 0.2	0.8 ± 0.3	0.2 ± 0.1
leptin (ng/ml)	5.4 ± 1.0	7.1 ± 2.0	2.5 ± 0.7	2.5 ± 1.0	2.6 ± 0.6	3.5 ± 0.9	1.6 ± 0.2	1.6 ± 0.2	2.4 ± 0.8	1.3 ± 0.5
adiponectin (µg/ml)	8.3 ± 0.5	9 ± 1.1	6.9 ± 0.7	8.5 ± 2.1	9.6 ± 0.8	8.5 ± 0.8	9.5 ± 2.0	11.2 ± 0.7	9.1 ± 1.8	9.5 ± 0.8
	2HF offspring				7HF offspring			8HF offspring		
MALE										
glucose (mM)	7.8 ± 0.5	7.6 ± 1.0	7.6 ± 1.3	9.5 ± 1.6	7.7 ± 1.0	7.1 ± 0.5	7.2 ± 0.0	7.9 ± 0.6	9.7 ± 1.8	9.2 ± 2.0
insulin (ng/ml)	0.8 ± 0.3	1.3 ± 0.2	1.3 ± 0.7	1 ± 0.3	1 ± 0.2	0.8 ± 0.1	1.4 ± 1.0	0.5 ± 0.1	1.2 ± 0.1*	0.6 ± 0.4
leptin (ng/ml)	2.3 ± 0.5	3.2 ± 0.7	2.5 ± 0.6	1.8 ± 0.5	2.6 ± 0.6	1.6 ± 0.0	1.3 ± 0.6	1.2 ± 0.2	2.2 ± 0.9	1.6 ± 0.6
adiponectin (µg/ml)	4.5 ± 1.1	3.5 ± 0.5	4 ± 0.9	3.6 ± 0.5	4.7 ± 0.3	5.4 ± 1.4	4.6 ± 0.7	4.3 ± 0.5	3.7 ± 0.7	4.7 ± 1.2
FEMALE										
glucose (mM)	7.5 ± 0.6	7.3 ± 0.9	7.5 ± 0.6	8.5 ± 0.7	7.1 ± 0.8	5.9 ± 0.9	8.3 ± 0.9	7.4 ± 0.7	7.5 ± 1.3	8.3 ± 0.2
insulin (ng/ml)	0.7 ± 0.3	0.8 ± 0.5	1.2 ± 0.2	1 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	0.9 ± 0.6	0.3 ± 0.2	0.9 ± 0.6	0.2 ± 0.2
leptin (ng/ml)	4.3 ± 0.8	3.1 ± 1.1	3.5 ± 0.9	4.2 ± 0.4	2.2 ± 0.3	1.7 ± 0.1	3.3 ± 1.2	1.9 ± 0.7	1.7 ± 0.2	1.3 ± 0.1
adiponectin (µg/ml)	6.9 ± 1.0	7.2 ± 0.5	10 ± 3.9	7.2 ± 1.2	10 ± 0.8	8.8 ± 0.7	10.8 ± 1.1	12.7 ± 0.9	9.1 ± 0.5	11.3 ± 1.4

* denotes significant difference from the own (*, $p<0.05$).

Table 11. Food and water intake of mice selectively bred for high voluntary wheel running behavior (lines 7 and 8) and the control line raised by mothers they were born to ("own"), or when they were fostered by other mothers of the same line (xf), or by other mothers of other lines (xf2, xf7, or xf8). LF-, HF diet are shown in separate panels.

	own	xf	7	8	own	xf	2	own	xf	2
	2LF offspring				7LF offspring			8LF offspring		
MALE										
Food intake (g)	10.5 ± 0.7	13.7 ±	11.7 ± 1.1	11.5 ± 0.2	13.4 ± 0.4	13.5 ± 0.3	12.2 ± 0.4	14.2 ± 1.7	12 ± 0.6	13.2 ± 0.4
Water intake (g)	11 ± 1.0	12.6 ±	10.8 ± 0.5	11.1 ± 0.3	12.7 ± 1.4	13.2 ± 0.5	13.1 ± 1.2	12.8 ± 0.9	8.7 ± 1.6	14.4 ± 2.9
FEMALE										
Food intake (g)	9.1 ± 0.4	10.83 ± 1.13	7.9 ± 0.6	8.9 ± 1.3	14.3 ± 0.7	12.8 ± 1.0	15 ± 1.3	11 ± 0.5	12.1 ± 1.3	11.1 ± 1.3
Water intake (g)	9.4 ± 0.5	8.3 ± 1.7	9.9 ± 0.7	10.3 ± 1.0	16.7 ± 1.0	14.4 ± 1.8	14.9 ± 0.8	12 ± 1.3	13.7 ± 0.6	11 ± 1.0
	2HF offspring				7HF offspring			8HF offspring		
MALE										
Food intake (g)	15.9 ± 2.6	11.4 ± 0.7	12 ± 0.9	9.8 ± 0.2	14.2 ± 1.0	16.4 ± 1.0	16 ± 1.0	17.4 ± 1.9	13.7 ± 0.5	12.6 ± 0.1
Water intake (g)	14.1 ± 1.3	11.1 ± 0.6	12.8 ± 1.2	5.5 ± 3.4	14.4 ± 0.7	17.4 ± 1.2	16.2 ± 0.5	17.2 ± 1.1	15.2 ± 1.2	13.3 ± 3.0
FEMALE										
Food intake (g)	8.1 ± 0.8	9.6 ± 2.1	13.1 ± 2.4	8.2 ± 1.9	12.4 ± 1.5	13.9 ± 0.6	14.5 ± 2.0	14.7 ± 1.6	14.3 ± 1.8	14.4 ± 0.6
Water intake (g)	9.5 ± 0.6	8.6 ± 1.3	9.8 ± 0.7	16.1 ± 6.3	13.9 ± 2.7	15.7 ± 1.6	21.1 ± 3.6	13.4 ± 1.0	13.4 ± 0.4	15.5 ± 0.8

No significant effect was found.

Table 12. Running wheel activity of mice selectively bred for high voluntary wheel running behavior (lines 7 and 8) and the control line raised by mothers they were born to ("own"), or when they were fostered by other mothers of the same line (xf), or by other mothers of other lines (xf2, xf7, or xf8). LF-, HF diet are shown in separate panels.

	own	xf	7	8	own	xf	2	own	xf	2
	2LF offspring				7LF offspring			8LF offspring		
MALE revolutions/day	18171 ± 5637	6403 ±	6662 ± 352	7924 ± 436	14443 ± 2031	27277 ± 1433	19279 ± 3322	21983 ± 2881	36462 ± 3798	22409 ± 4030
FEMALE revolutions/day	11603 ± 3935	20040 ± 2430	14342 ± 1753	14751 ± 3890	37103 ± 5363	48710 ± 28058	24736 ± 6451	53909 ± 10309	19623 ± 9161	31072 ± 5999
	2HF offspring				7HF offspring			8HF offspring		
MALE revolutions/day	18189 ± 7043	18766 ± 3494	7545 ± 1754	30448 ± 11447	22575 ± 7928	18088 ± 346	17716 ± 3723	25974 ± 4113	44616 ± 8627	16291 ± 7198
FEMALE revolutions/day	16253 ± 1323	17072 ± 10131	20784 ± 4907	17895 ± 3862	34784 ± 5928	28696 ± 4541	30822 ± 9677	38172 ± 18817	37292 ± 12674	39446 ± 8158

No significant effect was found.

Table 13. Plus maze performance behavior of mice selectively bred for high voluntary wheel running behavior (lines 7 and 8) and the control line raised by mothers they were born to ("own"), or when they were fostered by mothers of other lines (xf2, xf7, or xf8). Males, females and LF-, HF diet are shown in separate panels.

	own	7	8	own	2	own	2
	2LF offspring			7LF offspring		8LF offspring	
MALE							
%Topen	13.6 ± 3.6	6.4 ± 4.1	10.5 ± 5.9	4.2 ± 2.5	2.7 ± 1.5	8.6 ± 2.7	10.2 ± 5.1
%Tclosed	40.2 ±3.6	50.4 ± 3.9	53.8 ± 4.6	40.8 ± 4.6	61.7 ± 9.2*	62.6 ± 19.8	58.5 ± 8.2
%Tcenter	46.2 ± 2.4	43.2 ± 1.5	35.7 ± 4.6	55 ± 3.9	35.6 ± 8.0*	28.9 ± 9.1	31.3 ± 4.8
open entries	4 ± 1.0	3.7 ± 2.0	4 ± 2.6	2.3 ± 1.4	2 ± 1.4	2.7 ± 0.9	3.7 ± 1.6
closed entries	14.1 ± 1.5	11.7 ± 0.3	18.7 ± 4.3	19.9 ± 1.9	15.8 ± 2.6	12.9 ± 4.1	14.2 ± 1.1
total entries	18.1 ± 1.9	15.3 ± 2.3	22.7 ± 3.4	22.2 ± 1.7	17.8 ± 3.5	15.6 ± 4.9	17.8 ± 1.9
%open entries	21.4 ± 5.1	20.6 ± 10.9	18.3 ± 10.4	9.91 ± 5.4	8.9 ± 5.2	16 ± 5.1	18.3 ± 6.6
%close entries	78.6 ± 5.1	79.4 ± 10.9	81.8 ± 10.4	90.1 ± 5.4	91.1 ± 5.2	84 ± 26.6	81.7 ± 6.6
FEMALE							
%Topen	8.5 ± 3.0	12.2 ± 2.9	57.2 ± 19.2**	10.2 ± 2.6	12.4 ± 7.6	13.6 ± 2.2	12.8 ± 5.5
%Tclosed	51.4 ± 5.4	56 ± 5.4	15.3 ± 8.5**	45 ± 3.9	39.2 ± 7.6	53.8 ± 2.4	48 ± 5.9
%Tcenter	40.2 ± 3.8	31.8 ± 4.1	27.5 ± 11.0	44.8 ± 4.7	48.5 ± 2.8	32.6 ± 2.4	39.2 ± 4.3
open entries	3.4 ± 0.7	6.7 ± 1.9	6 ± 2.9	5.2 ± 1.3	4.3 ± 1.3	4.7 ± 0.9	2.3 ± 1.3
closed entries	15.3 ± 0.9	14.5 ± 1.3	6.3 ± 3.8**	18.2 ± 1.9	17.5 ± 3.9	15.8 ± 1.2	14.8 ± 1.1
total entries	18.6 ± 1.3	21.2 ± 2.4	12.3 ± 6.6	23.4 ± 2.9	21.8 ± 3.6	20.5 ± 1.2	17 ± 1.2
%open entries	17.1 ± 3.1	29.4 ± 6.3	65.3 ± 17.4**	20.1 ± 3.9	21.6 ± 8.2	22.8 ± 3.7	12.5 ± 6.5
%close entries	82.9 ± 3.1	70.6 ± 6.3	34.7 ± 17.4**	79.9 ± 3.9	78.4 ± 8.2	77.2 ± 3.7	87.5 ± 6.5
	2HF offspring			7HF offspring		8HF offspring	
MALE							
%Topen	26.9 ± 12.9	6.2 ± 1.9	29.3 ±	16.7 ± 6.0	8.5 ± 5.3	15.3 ± 1.8	11.6 ± 2.2
%Tclosed	49.4 ± 11.8	56.4 ± 8.8	6.6 ±	51.3 ± 8.0	58.6 ± 13.8	50.1 ± 4.0	62.7 ± 10.6
%Tcenter	23.7 ± 3.0	37.4 ± 9.1	64.1 ±	32.1 ± 9.3	32.9 ± 8.5	34.7 ± 3.6	25.7 ± 9.2
open entries	3.6 ± 1.3	4 ± 0.0	5 ±	7.7 ± 2.1	5 ± 2.5	4.3 ± 1.0	3.7 ± 1.5
closed entries	9.2 ± 2.3	12.7 ± 2.3	2 ±	12.5 ± 1.7	10 ± 2.5	14.3 ± 1.5	10.7 ± 3.2
total entries	12.8 ± 1.5	16.7 ± 2.3	7 ±	20.2 ± 3.7	15 ± 4.0	18.5 ± 1.8	14.3 ± 4.6
%open entries	31.9 ± 12.4	24.9 ± 3.4	71.4 ±	34.4 ± 4.1	32.1 ± 9.7	22.6 ± 3.5	23.5 ± 3.4
%close entries	68.1 ± 12.4	75.1 ± 3.4	28.6 ±	65.6 ± 4.1	67.9 ± 9.7	77.4 ± 3.5	76.5 ± 3.4
FEMALE							
%Topen	7.6 ± 4.4	25.5 ± 12.8	0.7 ±	16.7 ± 6.9	15.5 ± 8.0	13.1 ± 2.8	17.2 ± 3.0
%Tclosed	35 ± 5.2	42.9 ± 3.4	70.3 ±	51.7 ± 5.8	49.9 ± 8.1	52.8 ± 3.4	48.7 ± 5.1
%Tcenter	57.4 ± 6.2	31.6 ± 10.4	29 ±	31.6 ± 1.5	34.6 ± 0.1	34.2 ± 4.4	34.1 ± 3.7
open entries	3.3 ± 1.6	4 ± 2.3	1 ±	7.6 ± 2.2	6.7 ± 4.1	4.8 ± 0.7	5.3 ± 1.9
closed entries	15.8 ± 2.8	12.3 ± 2.6	18 ±	19.6 ± 2.1	18 ± 2.5	16.5 ± 1.3	15.7 ± 1.2
total entries	19 ± 2.7	16.3 ± 2.3	19 ±	27.1 ± 2.7	24.7 ± 4.7	21.3 ± 1.1	21 ± 3.0
%open entries	16.7 ± 7.8	24.4 ± 12.4	5.3 ±	26.3 ± 7.8	24.3 ± 12.4	23 ± 3.9	24.1 ± 4.9
%close entries	83.3 ± 7.8	75.6 ± 12.4	94.7 ±	73.7 ± 7.8	75.7 ± 12.4	77 ± 3.9	75.9 ± 4.9

* denotes significant difference from the own (*, $p<0.05$; **, $p<0.01$).

7. Discussion on perinatal nutritional effects of cross-fostered offspring

Mice selectively bred for increased running wheel behavior have several behavioral, physiological, and metabolic changes compared to non-selected mice, among which some may be viewed as co-adaptations to sustain endurance exercise (Girard et al. 2001; Gomes et al. 2009; Houle-Leroy et

al. 2003; Koteja et al. 1999; Vaanholt et al. 2007; Wong et al. 2009; Rezende et al. 2006a) and also (Chapter 2, 3, 4 of this thesis). We were interested to know to which extent the post-natal phase is critically important for the display of these phenomena. During the post-natal period, maternal influences are reported to cause programming effects in the developing offspring and subsequently cause long-lasting changes at adulthood (Armitage et al. 2005; Gallou-Kabani et al. 2007; Napoli and Palinski 2001; Samuelsson et al. 2008). For this reason, we performed an experiment in which we cross-fostered pups between mothers from different lines and between mothers from the same line. These comparisons were made when mothers were either feeding a low-fat (LF) carbohydrate-rich diet, or a high- fat (HF) diet enriched with refined sugars. Offspring growth during lactation and several parameters relevant to energy balance regulation after weaning were assessed in these cross-fostered mice, and were compared to the characteristics of the non cross-fostered offspring described in the first part of this chapter.

It was previously mentioned that growth rates of mice from the high activity lines 7 and 8 are lower than those of the control line 2 during the pre-weaning period as well as during adulthood. These effects were interpreted to indicate that selection for running wheel behavior causes a reduction in body size and mass which may be adaptive to sustain running wheel behavior (Rezende et al. 2006b). Cross-fostering of pups between line 2 and line 8 mothers feeding the LF diet (further referred to as 2LF and 8LF mothers) diet did not show alterations in growth during the pre-weaning stage compared to the line 2LF and 8LF pups raised by their own mothers. This indicates that differences between line 2 and 8 offspring are probably the result of differences in pre-natal (epi) genetic programming, and not by post-natal influences. In contrast, line 7LF offspring fostered by line 2LF mothers increased pup growth of line 7LF pups in the direction of 2LF own pups and became indistinguishable from cross-fostered 8LF pups. Vice versa, line 2LF offspring fostered by line 7LF mothers showed a markedly attenuated growth, even below the levels found in the line 7LF offspring. Therefore line 7 mothers provide a poor post-natal environment in the LF diet condition for offspring growth and development, and essentially similar effects are observed when mothers were feeding a HF diet. In the HF diet condition, both line 7 as well as line 8 offspring fostered by line 2HF mothers increased pup growth compared to condition when they were raised by their own mothers, thus presenting a richer environment for growth and development in line 2 mothers relative to mothers in the high activity lines, when the mothers are provided with an energy rich diet.

Effects of the poor pre-weaning maternal line 7 environment persistently reduced body weight of line 2 offspring in adulthood. In fact, the line 2 offspring at 4 months of age fostered by line 7 mothers were indistinguishable from line 7 own offspring in terms of dry-lean mass, fat mass, and body length. On the others hand, line 7LF offspring fostered by line 2LF mothers were only slightly and transiently heavier than line 7 offspring raised by their own LF mothers. These effects rely on the type of diet, because all cross-fostering effects are lost in adulthood when mothers are feeding a HF diet. Two conclusions can be drawn from these findings. First, the poor maternal line 7LF environment causes long-lasting physiological and morphological

changes that overrule pre-natal (epi)genetic make-up of line 2 offspring, and may potentially contribute to some extent to the phenotype of line 7 mice at adulthood as well. Secondly, the pre-natal (epi)genetic make-up of line 7 offspring cannot be overridden by the relatively rich post-natal environment provided by line 2 mothers, even when they are subjected to a HF diet. A poor environment may therefore have stronger effects on long-term programming of energy balance and growth than a rich environment. While this is consistent with the current conceptual framework of developmental metabolic programming founded on the idea of the thrifty phenotype and the pioneering work of Hales and Barker (Hales and Barker 1992), the directions of body weight changes in the current study are not consistent with this idea. According to the thrifty phenotype hypothesis, poor postnatal nutrition would be expected to cause catch-up growth (Gluckman and Hanson 2004; Gluckman, Hanson, and Pinal 2005), and in line with that we anticipated that line 2 offspring fostered by line 7 mothers would develop obesity and hyperglycemia at adulthood. In stead, they developed lower growth and lower growth efficiency (based on lower body weights combined with similar food intake), and lower levels of glucose and insulin at adulthood. The observed long-term fostering effects of line 7 mothers are not generalizable to selection for increased running wheel behavior per se, since cross-fostering of line 2 and line 8 pups by respectively line 8 and line 2 mothers did not yield aforementioned effects on adult body weights. In this case, differences in body weight and composition between these lines are apparently determined by prenatal gene-environment interactions. Neither did line 7 nor line 8 cross-fostering with line 2 result in changes in running wheel behavior, food intake nor water intake. The only effect of cross-fostering which we observed was that line 7 female offspring fostered by line 2 mothers became more anxious in the plus maze performance test, whereas line 2 female offspring fostered by line 8 mothers became less anxious. This indicates some postnatal effects of line 2 mothers to increase anxiety levels in their female offspring which might be offset in the high activity lines.

A remarkable observation in Chapter 5 was that line 7 mothers lost considerably more pups during lactation than mothers of other lines. Although litter characteristics at birth in Chapter 5 were very similar to those in this study, here we did not find pups missing during lactation. We suspect that these differences are the result of the cross-fostering procedure in the present study. Surviving the post-natal environment is not only dependent on maternal (line) and dietary (HF/LF) influences acting unidirectional on the offspring. For example, suckling (Schroeder et al. 2007; Fride, Bregman, and Kirkham 2005; Uvnas-Moberg 1996) and ultrasonic vocalization by pups (Shair 2007; Hofer et al. 1999) have been reported to markedly affect maternal behavior and physiology, and this in turn feeds back to pup growth and development. It may be speculated, that the pup losses of line 7 mothers that we have observed in Chapter 5 were "rescued" by line 2 pups fostered by line 7 mothers in the present study. A mechanism may be that line 2 pups suckled more vigorously than line 7 pups, and thus contributed to a higher milk production of line 7 mothers in the present study, benefiting both fostered and own offspring. Along these lines, also differences in growth efficiency (GE) during lactation found in

the present study and Chapter 5 may be explained. Thus, while line 7HF and 8HF mothers and offspring had the highest GEs in Chapter 5, here we observed that GE of line 2HF mothers was the highest. These effects may very well be explained by the contribution of growth efficiency of line 7HF and line 8HF offspring which were fostered by line 2 HF mothers. Further studies are needed to further explore these potential mechanisms, and whether they are relevant to behavioral and metabolic characteristics at adulthood.

References

Armitage, J. A., L. Lakasing, P. D. Taylor, A. A. Balachandran, R. I. Jensen, V. Dekou, N. Ashton, J. R. Nyengaard, and L. Poston. 2005. Developmental programming of aortic and renal structure in offspring of rats fed fat-rich diets in pregnancy. J. Physiol 565:171-184.

Aubert, R., J. P. Suquet, and D. Lemonnier. 1980. Long-term morphological and metabolic effects of early under- and over-nutrition in mice. J. Nutr. 110:649-661.

Barnes, R. H., S. R. Cunnold, R. R. Zimmermann, H. Simmons, R. B. MacLeod, and L. Krook. 1966. Influence of nutritional deprivations in early life on learning behavior of rats as measured by performance in a water maze. J. Nutr. 89:399-410.

Bouret, S. G. 2009. Early life origins of obesity: role of hypothalamic programming. J. Pediatr. Gastroenterol. Nutr. 48 Suppl 1:S31-S38.

Bouret, S. G. and R. B. Simerly. 2006. Developmental programming of hypothalamic feeding circuits. Clin. Genet. 70:295-301.

Davidowa, H., Y. Li, and A. Plagemann. 2003. Altered responses to orexigenic (AGRP, MCH) and anorexigenic (alpha-MSH, CART) neuropeptides of paraventricular hypothalamic neurons in early postnatally overfed rats. Eur. J. Neurosci. 18:613-621.

Donato, K. A. 1987. Efficiency and utilization of various energy sources for growth. Am. J. Clin. Nutr. 45:164-167.

Epstein, H. T. 1978. The effect of litter size on weight gain in mice. J. Nutr. 108:120-123.

Faust, I. M., P. R. Johnson, and J. Hirsch. 1980. Long-term effects of early nutritional experience on the development of obesity in the rat. J. Nutr. 110:2027-2034.

Fride, E., T. Bregman, and T. C. Kirkham. 2005. Endocannabinoids and food intake: newborn suckling and appetite regulation in adulthood. Exp. Biol. Med. (Maywood.) 230:225-234.

Gallou-Kabani, C., A. Vige, M. S. Gross, C. Boileau, J. P. Rabes, J. Fruchart-Najib, J. P. Jais, and C. Junien. 2007. Resistance to high-fat diet in the female progeny of obese mice fed a control diet during the periconceptual, gestation, and lactation periods. Am. J. Physiol Endocrinol. Metab 292:E1095-E1100.

Girard, I., M. W. McAleer, J. S. Rhodes, and T. Garland, Jr. 2001. Selection for high voluntary wheel-running increases speed and intermittency in house mice (Mus domesticus). J. Exp. Biol. 204:4311-4320.

Gluckman, P. D. and M. A. Hanson. 2004. Developmental origins of disease paradigm: a mechanistic and evolutionary perspective. Pediatr. Res. 56:311-317.

Gluckman, P. D., M. A. Hanson, and C. Pinal. 2005. The developmental origins of adult disease. Matern. Child Nutr. 1:130-141.

Gomes, F. R., E. L. Rezende, J. L. Malisch, S. K. Lee, D. A. Rivas, S. A. Kelly, C. Lytle, B. B. Yaspelkis, III, and T. Garland, Jr. 2009. Glycogen storage and muscle glucose transporters (GLUT-4) of mice selectively bred for high voluntary wheel running. J. Exp. Biol. 212:238-248.

Hager, R., J. M. Cheverud, and J. B. Wolf. 2009. Change in maternal environment induced by cross-fostering alters genetic and epigenetic effects on complex traits in mice. Proc. Biol. Sci.

Hales, C. N. and D. J. Barker. 1992. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 35:595-601.

Hofer, M. A., J. R. Masmela, S. A. Brunelli, and H. N. Shair. 1999. Behavioral mechanisms for active maternal potentiation of isolation calling in rat pups. Behav. Neurosci. 113:51-61.

Houle-Leroy, P., H. Guderley, J. G. Swallow, and T. Garland, Jr. 2003. Artificial selection for high activity favors mighty mini-muscles in house mice. Am. J. Physiol Regul. Integr. Comp Physiol 284:R433-R443.

Johnson, M. S., S. C. Thomson, and J. R. Speakman. 2001. Limits to sustained energy intake. I. Lactation in the laboratory mouse Mus musculus. J. Exp. Biol. 204:1925-1935.

Koteja, P., T. Garland, Jr., J. K. Sax, J. G. Swallow, and P. A. Carter. 1999. Behaviour of house mice artificially selected for high levels of voluntary wheel running. Anim Behav. 58:1307-1318.

Krol, E. and J. R. Speakman. 2003. Limits to sustained energy intake. VI. Energetics of lactation in laboratory mice at thermoneutrality. J. Exp. Biol. 206:4255-4266.

Lemonnier, D. 1972. Effect of age, sex, and sites on the cellularity of the adipose tissue in mice and rats rendered obese by a high-fat diet. J. Clin. Invest 51:2907-2915.

McGowan, P. O., M. J. Meaney, and M. Szyf. 2008. Diet and the epigenetic (re)programming of phenotypic differences in behavior. Brain Res. 1237:12-24.

MohanKumar, S. M., A. King, A. C. Shin, M. P. Sirivelu, P. S. MohanKumar, and G. D. Fink. 2007. Developmental programming of cardiovascular disorders: focus on hypertension. Rev. Endocr. Metab Disord. 8:115-125.

Napoli, C. and W. Palinski. 2001. Maternal hypercholesterolemia during pregnancy influences the later development of atherosclerosis: clinical and pathogenic implications. Eur. Heart J. 22:4-9.

Parente, L. B., M. B. Aguila, and C. A. Mandarim-de-Lacerda. 2008. Deleterious effects of high-fat diet on perinatal and postweaning periods in adult rat offspring. Clin. Nutr. 27:623-634.

Plagemann, A. 2006. Perinatal nutrition and hormone-dependent programming of food intake. Horm. Res. 65 Suppl 3:83-89.

Plagemann, A., T. Harder, A. Rake, T. Waas, K. Melchior, T. Ziska, W. Rohde, and G. Dorner. 1999. Observations on the orexigenic hypothalamic neuropeptide Y-system in neonatally overfed weanling rats. J. Neuroendocrinol. 11:541-546.

Rezende, E. L., F. R. Gomes, J. L. Malisch, M. A. Chappell, and T. Garland, Jr. 2006a. Maximal oxygen consumption in relation to subordinate traits in lines of house mice selectively bred for high voluntary wheel running. J. Appl. Physiol 101:477-485.

Rezende, E. L., S. A. Kelly, F. R. Gomes, M. A. Chappell, and T. Garland, Jr. 2006b. Effects of size, sex, and voluntary running speeds on costs of locomotion in lines of laboratory mice selectively bred for high wheel-running activity. Physiol Biochem. Zool. 79:83-99.

Samuelsson, A. M., P. A. Matthews, M. Argenton, M. R. Christie, J. M. McConnell, E. H. Jansen, A. H. Piersma, S. E. Ozanne, D. F. Twinn, C. Remacle, A. Rowlerson, L. Poston, and P. D. Taylor. 2008. Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. Hypertension 51:383-392.

Schroeder, M., Y. Lavi-Avnon, O. Zagoory-Sharon, T. H. Moran, and A. Weller. 2007. Preobesity in the infant OLETF rat: the role of suckling. Dev. Psychobiol. 49:685-691.

Schultze, M. O. 1954. Weight increments of suckling rats as affected by litter size and maternal diet. J. Nutr. 54:453-460.

Shair, H. N. 2007. Acquisition and expression of a socially mediated separation response. Behav. Brain Res. 182:180-192.

Srinivasan, M., S. D. Katewa, A. Palaniyappan, J. D. Pandya, and M. S. Patel. 2006. Maternal high-fat diet consumption results in fetal malprogramming predisposing to the onset of metabolic syndrome-like phenotype in adulthood. Am. J. Physiol Endocrinol. Metab 291:E792-E799.

Swallow, J. G., P. A. Carter, and T. Garland, Jr. 1998. Artificial selection for increased wheel-running behavior in house mice. Behav. Genet. 28:227-237.

Uvnas-Moberg, K. 1996. Neuroendocrinology of the mother-child interaction. Trends Endocrinol. Metab 7:126-131.

Vaanholt, L. M., P. Meerlo, T. Garland, Jr., G. H. Visser, and G. van Dijk. 2007. Plasma adiponectin is increased in mice selectively bred for high wheel-running activity, but not by wheel running per se. Horm. Metab Res. 39:377-383.

Winick, M. and A. Noble. 1967. Cellular response with increased feeding in neonatal rats. J. Nutr. 91:179-182.

Wong, L. E., T. Garland, Jr., S. L. Rowan, and R. T. Hepple. 2009. Anatomic capillarization is elevated in the medial gastrocnemius muscle of mighty mini mice. J. Appl. Physiol 106:1660-1667.