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

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CHAPTER 6

MICE SELECTIVELY BRED FOR HIGH VOLUNTARY RUNNING ARE RESISTANT TO PERINATAL PROGRAMMING OF WEIGHT GAIN BY A HIGH FAT DIET

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Summary

Feeding a high-fat/high sucrose (HF) diet during perinatal life programs offspring energy balance and may cause several health disturbing diseases associated with obesity at adulthood. However, a trait for high physical activity may balance and prevent these effects in the offspring. We hypothesized that selective breeding for high voluntary running in mice would protect offspring against these deleterious effects of perinatal HF feeding. Regression analysis, besides mean comparisons, was performed to investigate contribution of naturally occurring litter size in the effect of diet. At adulthood, HF diet caused proportional weight gain in male but not female offspring of the control line with elevated plasma insulin and low adiponectin levels. This was observed independent of the size of litter, but with a higher risk in small litter male offspring. Control female offspring from larger litter sizes developed elevated levels of glucose and cholesterol which might be the consequence of their increased food intake behavior. In contrast, male and female offspring of active lines became lean with elevated adiponectin levels and increased water intake, and particularly active female offspring developed lower levels of plasma leptin and insulin relative to controls. From these results, we conclude that male offspring are more susceptible to weight gain by the programming effects of HF diet. The trait for high voluntary running behavior in both genders reverses and/or defends against these effects and may provide a mechanism to prevent diet-induced weight gain.

1. Introduction

Locomotion enables animals to travel across the environment in the attempt to find partners, nutrients and fluids. In doing so, they need to combust extra fuels above the amount to maintain resting metabolic rate, in order to provide the energy for skeletal and cardiovascular muscular activity. The display of locomotor activity can vary tremendously between species, but also considerable individual differences can be observed within certain species (Scholz et al. 2008). In an extensive selective breeding program, Garland and colleagues assessed individual differences in running wheel activity of outbred mice, and created four replicate lines with high and four with normal locomotor activity (Swallow, Carter, and Garland, Jr. 1998). We and others have found that these high activity lines of mice have a number of metabolic and behavioral characteristics which may be viewed as adaptive to sustain increased physical activity levels in the natural environment (see Chapters 2, 3, and 4). From an energy balance point of view, these high activity mice are more resistant to develop diet-induced obesity (DIO) than control mice when subjected to feeding a high-fat (HF) diet at adulthood (Vaanholt et al. 2008). This is highly relevant in light of the view that dietary fat combined with a sedentary life-style is a frequent cause of human obesity in the modern industrialized societies (Dourmashkin et al. 2005; Lee and Korner 2008; Sanderson et al. 2008).

Besides studies dealing with regulation of energy balance at adulthood, also the perinatal origins of energy balance are becoming subject of intense investigation. Mechanism include maternal behavioral, hormonal and/or metabolic factors that can program fundamental regulatory systems in the fetal-newborn state, and this can subsequently influence the risk for attracting metabolic diseases in the offspring later in life (Levin 2006; Plagemann 2004; Plagemann 2005; Srinivasan and Patel 2008). One of the potential causal factors underlying the fetal origins of excess body weight at adulthood is early maternal overnutrition of dietary fat (Armitage, Taylor, and Poston 2005). Indeed, offspring from HF diet-induced obese female rats develop increased adiposity associated with cardiovascular and metabolic dysfunction indicative of the metabolic syndrome at adulthood (Samuelsson et al. 2008). Besides absorbed nutrients, also physical activity is an important determining factor in the energy balance equation (McMurray and Hackney 2005; Teske, Billington, and Kotz 2008). Physical activity is inversely related to the risk of attracting obesity (Patterson and Levin 2008; Tappy, Binnert, and Schneiter 2003), and it improves metabolic abnormalities even in the absence of weight loss in adults (Colberg 2007; Kruk 2007; Pearce 2008) as well in as children (Colberg 2007; Fogelholm 2008; Pearce 2008). In line with this view is the finding that already as little as 3 weeks of voluntary running wheel exercise at the juvenile stage is able to postpone obesity in diet-induced obese (DIO) male rats (Patterson, Dunn-Meynell, and Levin 2008). As mentioned earlier, mice selectively bred for voluntary high wheel running activity are shown to be resistant against HF DIO, even in the absence of running wheels (Vaanholt et al. 2008). Thus, while the trait for increased physical activity in mice is sufficient to maintain leanness by increasing metabolic rate and fat oxidation in the context of feeding a HF diet (see Chapter 3), it is unknown whether such

a trait also protects mice from developing disturbances in energy balance regulation when their mothers were fed a HF diet during pregnancy and lactation. Therefore, in the present study, selectively bred highly active mice and their randomly-bred controls were used to investigate the interactions between a physical activity trait and the effects of the peri-gestational HF diet feeding on several energy balance parameters in the offspring at adulthood, including body composition, food and water intake, and plasma levels of fuels and hormones. Since litter size is a major determinant of early growth as well (chapter 5 and (Johnson, Thomson, and Speakman 2001)), we investigated whether this factor also explained variations in the aforementioned energy balance parameters at adulthood of control and selected mice.

2. Materials and methods

2.1. Origin of animals and housing

Offspring of two selectively bred lines for high voluntary wheel running activity (line 7 and line 8) and of one randomly-bred control line (line 2) in generation 50 were used in these experiments. Parents of these mice were born to breeding couples within selected lines in their 48th generation, which were obtained from T. Garland Jr, Riverside, CA. For a detailed description of the selection procedure see (Swallow, Carter, and Garland, Jr. 1998). Selected lines were used for breeding without further selection at our facility in Haren. Mothers of offspring mice were fed either a standard low-fat (LF) lab chow diet (3.8 kcal/g; 58 % carbohydrate, 6 % fat, 22 % protein; Standard lab chow RMH-B 2181, HopeFarms BV, Woerden, NL) 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) ad libitum 3 weeks before pregnancy until day 16 of lactation. After this period, the HF was replaced by the standard chow diet to avoid that the developing pups ingested the HF. This was necessary since mice start to ingest solid food after day 16. At birth, litter characteristics were assessed and litter sizes were kept non-manipulated (see Chapter 4).

2.2. Offspring and conditions

At weaning, offspring gender was determined and 3-4 mice of similar sex and perigestational diet were housed in standard cages (Macrolon Type II, UNO Roestvaststaal BV, Zevenaar, NL) with wood shavings and EnviroDry® bedding and they had ad libitum assess to standard lab chow diet and water. The room temperature was 22±1 °C with a 12:12 light-dark cycle (lights on at 8 am).

At 5-6 weeks of age, mice were characterized for running wheel activity over a 6 day period in similar type cages with bedding material and food and water available ad libitum, and with a running wheel (diameter: 14cm, code 0131 Savic®, Kortrijk, BE) attached to the side of the cage. The wheel revolutions were assessed by a computer, which collected data on a minute to minute basis. Data on running wheel activity over day 5 and day 6 were averaged, in the same

way as in the selection protocol. Right after the running wheel measurements, body mass was assessed. From each litter, two male and two female mice were blindly assigned to be included in these experiments, while the rest was used for other purposes.

Offspring included in this study were housed in couples of similar sex and from the same litter, with ad libitum access to food and water with the above-mentioned light, temperature and cage conditions. When they reached 4 months of age, food and water intake in each cage was assessed on two consecutive days, and was averaged per day per animal. Following food and water intake measurements, all offspring were anaesthetized by CO2, their nose-anal length was determined, and they were decapitated. Trunk blood was collected in tubes with Trasylol- EDTA, which then were centrifuged at 2600 g for 15 min at 4°C. Plasma was collected and stored at -80°C for later analysis of insulin, adiponectin, and leptin (analyzed by radioimmuno assays of Linco Research, Nucli lab, The Netherlands), and for cholesterol (enzymatic kit from Roche/Hitachi) and for glucose (by ferrycyanide method of Hoffman). Whole carcasses were kept without heads for total analysis. This included weighing of wet and dry masses (after drying to constant weight at 103°C for 24 plus 1 hour) and followed by fat extraction with petroleum ether in a soxhlet apparatus for 8 complete cycles. Thereafter, bodies were dried again to obtain dry-lean masses and calculate total fat- and fat-free mass (FFM).

2.3. Statistical analysis

Results were analyzed with GLM Univariate Analysis to test line and diet effects and their interaction in the measured parameters. Secondly, a Linear Regression Forward method was applied, which allowed us to investigate the contribution of litter size in the measured parameters. P value less than 0.05 was considered significant for all tests.

3. Results

3.1. Offspring characteristics at the adolescent stage

3.1.1. Running wheel activity

Between 6 and 7 weeks of age, all offspring animals were tested for running wheel activity. In figure 1, averages of running wheel activity on the 5th and 6th day of the 6-day subjection period are shown. In both genders, running wheel activity was significantly influenced by line (♂: $F(2,191)=141.3$; $p<0.001$, ♀: $F(2,188)=36.03$; $p<0.001$), but not by diet. Post-hoc analysis revealed that line 8 offspring ran significantly more than those of line 2 in both genders (♂: 2.9 times more, ♀: 1.7 times more). Males of line 8 also ran significantly more (2.3x) than those of line 7, but this effect was not observed in females. Females of line 7, on the other hand, ran 1.7x more than those of line 2, an effect not seen in males.

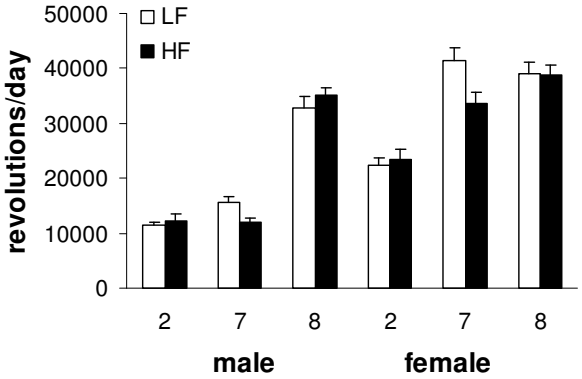

Figure 1. Average running wheel activity of male and female offspring of mothers perinatally fed LF and HF diet in line 2 (control line), line 7 and line 8 (selected lines) at 6-7 weeks of age.

3.1.2. Body weight

At the 7th week of age, body weight was significantly influenced by line in both genders (♂: $F(2,207)=93.5$; $p<0.001$; ♀: $F(2,204)=48.6$; $p<0.001$). Post-hoc analysis revealed increased body weight of line 2 males and females relative to those of line 7 and 8. Furthermore, body weight of line 7 female offspring was lower than that of line 8, an effect not observed in males. Body weight was also influenced by perigestational diet in males ($F(1,207)=51.7$; $p<0.001$), but not in females. Post-hoc analysis revealed that offspring of HF diet fed mothers had increased body weight relative to those of LF fed mothers. This effect appeared to interact with line ($F(2,207)=5.7$; $p<0.01$), meaning that line 2 and line 7, but not line 8 male offspring from HF fed mothers were found to be heavier than those of LF fed mothers (see table 1).

3.2. Offspring characteristics at the adult stage

3.2.1. Food and water consumption

At 4 months of age, food intake of offspring was significantly influenced by line ($F(2,80)=9.62$; $p<0.001$) in males, but not in females. Specifically, male offspring of line 8 consumed more food than those of line 2 and line 7. Perigestational HF diet had no effect on food intake (see Figure 2). In both genders, however, water intake was significantly influenced by line (♂: $F(2,79)=10.06$; $p<0.001$, ♀: $F(2,73)=19.85$; $p<0.001$) as well as diet (♂: $F(1,79)=7.57$; $p<0.01$, ♀: $F(1,73)=4.45$; $p<0.05$). Post-hoc analysis revealed increased water intake by line 7 and line 8 offspring relative to that of line 2 offspring, and offspring of HF-fed mothers consumed more water than that of LF-fed females irrespective of line (see Figure 3).

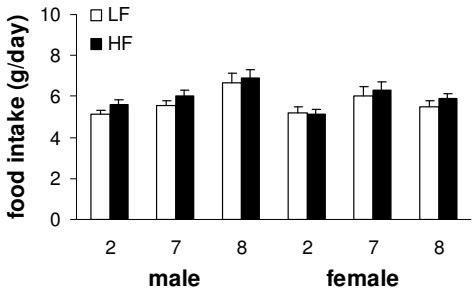

Figure 2. Food intake of male and female offspring of mothers perinatally fed LF and HF diet in line 2 (control line), line 7 and line 8 (selected lines) at 4 months of age.

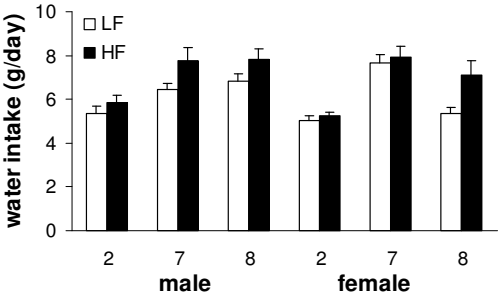

Figure 3. Water intake of male and female offspring of mothers perinatally fed LF and HF diet in line 2 (control line), line 7 and line 8 (selected lines) at 4 months of age.

3.2.2. Body weight and composition

At 4 months of age, offspring body weight was influenced by line in both genders (♂: $F(2,133)=23.28$; $p<0.001$, ♀: $F(2,130)=71.92$; $p<0.001$), and post-hoc analysis revealed that line 2 offspring was heavier than line 7 and line 8 offspring. Only in males, body weights were influenced by perigestational diet ($F(1,133)=13.41$; $p<0.001$), and increases were found in offspring of HF-fed mothers. Post-hoc analysis only revealed increased body weight in offspring from HF-fed mothers in line 2 (see Table 1).

Nose-anal length of offspring was influenced by line in both genders (♂: $F(2,130)=10.53$; $p<0.001$), ♀: $F(2,128)=37.81$; $p<0.001$). In line with the effects on body weight was the observation that line 2 offspring had higher nose-anal length than offspring of line 7 and line 8. Furthermore, nose-anal length was also influenced by perigestational diet ($F(1,130)=6.19$; $p<0.05$), but only in males; specifically male offspring of HF diet-fed mothers had higher nose-anal length than those of LF diet-fed mothers. Moreover, in males, an interaction between line

and diet ($F(2,130)=7.24$; $p<0.01$) was observed, and post-hoc analysis revealed that the nose-anal length of male offspring from HF diet fed mothers was increased only in line 2 (See table 1).

By absolute measures, drymass (♂: $F(2,132)=14.30$; $p<0.001$, ♀: $F(2,130)=65.31$; $p<0.001$), dry-lean mass (♂: $F(2,132)=22.11$; $p<0.001$, ♀: $F(2,130)=54.52$; $p<0.001$), body water content (♂: $F(2,132)=29.61$; $p<0.001$, ♀: $F(2,130)=47.47$; $p<0.001$) and body fat content (♂: $F(2,132)=12.70$; $p<0.001$, ♀: $F(2,130)=51.33$; $p<0.001$) were influenced by line in both genders. In general, these parameters were significantly increased in line 2 relative to lines 7 and/or 8 (see table 1). In addition, differences were found between lines 7 and 8 for dry mass (line 7 ♂ > line 8 ♂) and body water content (line 7 ♂ < line 8 ♂). Expressed as a proportion of body weight, %dry mass (♂: $F(2,128)=10.52$; $p<0.001$, ♀: $F(2,126)=46.51$; $p<0.001$), %dry-lean mass (♂: $F(2,128)=3.74$; $p<0.05$, ♀: $F(2,126)=20.37$; $p<0.001$), %body water (♂: $F(2,128)=10.13$; $p<0.001$, ♀: $F(2,126)=41.88$; $p<0.001$), and %body fat (♂: $F(2,128)=12.28$; $p<0.001$, ♀: $F(2,126)=45.15$; $p<0.001$) were all affected by line in both genders. Line 8 had the smallest %dry mass and %body fat and the highest %dry lean mass and %body water. In these respects, males of line 7 were similar to those of line 2, whereas females of line 7 were intermediate phenotype and significantly different from those of line 2 and line 8.

Perigestational diet influenced the absolute measures of dry mass ($F(1,132)=4.58$; $p<0.05$), dry-lean mass ($F(1,132)=9.68$; $p<0.01$), and body water content ($F(1,132)=17.16$; $p<0.001$) in males. In females, no effect of perigestational diet was observed. Post-hoc analysis revealed that above-mentioned parameters were increased in line 2 offspring of HF diet-fed mothers relative to those fed the LF diet. Body fat content was not influenced by diet in either gender when all lines were included in the analysis. However, when viewing line 2 males separately from lines 7 and 8, the HF diet condition significantly increased the level of body fat by 41%. When expressed proportionally to body weight, however, the perigestational diet effects were lost in male offspring, and was not observed in females either.

3.2.3. Metabolic fuel and hormone levels

Plasma insulin (♂: $F(2,131)=9.48$; $p<0.001$, ♀: $F(2,127)=11.64$; $p<0.001$), leptin (♂: $F(2,132)=8.74$; $p<0.001$, ♀: $F(2,130)=23.31$; $p<0.001$) and adiponectin levels (♂: $F(2,129)=6.44$; $p<0.01$, ♀: $F(2,129)=13.58$; $p<0.001$) were influenced by line in both genders. Post-hoc analysis revealed that offspring of line 2 had higher plasma levels of insulin and leptin, and lower plasma levels of adiponectin than those of line 7 and/or line 8 (see Table 2). Plasma glucose levels were also influenced by line ($F(2,127)=9.08$; $p<0.001$) but only in females; specifically, offspring of line 2 had higher levels of glucose than offspring of line 8. Furthermore, a line effect was revealed on plasma cholesterol levels ($F(1,129)=5.64$; $p<0.01$) in male, but not in female offspring; specifically, line 2 had lower cholesterol levels that those of line 7 and line 8. Perigestational diet had no effect on the above-mentioned fuel and hormone levels.

Table 1. Characteristics male and female offspring mice from line 2 (control line) and line 7 and line 8 (selected lines) mothers feeding either a LF diet or a HF diet during pregnancy and lactation.

	2		7		8	
	LF	**HF**	**LF**	**HF**	**LF**	**HF**
MALE						
Body mass						
7 weeks	30.5 ± 0.4	33.6 ± 0.4##	25.3 ± 0.5**	28.0 ± 0.4##	26.6 ± 0.4**	27.9 ± 0.3#
4 mnths	37.0 ± 0.6	40.7 ± 0.8##	34.0 ± 0.6**	35.9 ± 0.9	34.4 ± 0.7**	35.4 ± 0.7
Body composition						
Length	9.3 ± 0.1	9.9 ± 0.1##	9.1 ± 0.1	9.3 ± 0.1	9.2 ± 0.1	9.1 ± 0.1
Drymass	10.9 ± 0.3	12.8 ± 0.5##	10.3 ± 0.4	11.1 ± 0.9	9.6 ± 0.3**	9.4 ± 0.3
Drylean mass	8.0 ± 0.1	8.7 ± 0.2##	7.2 ± 0.1*	7.6 ± 0.2#	7.5 ± 0.1**	7.7 ± 0.2
Body water	21.2 ± 0.3	22.8 ± 0.4##	18.6 ± 0.3**	20.1 ± 0.4##	20.1 ± 0.4*	20.8 ± 0.4
Body fat	2.9 ± 0.2	4.1 ± 0.4	3.2 ± 0.3	3.4 ± 0.8	2.0 ± 0.2**	1.7 ± 0.2
% Dry mass	33.9 ± 0.5	35.8 ± 0.7	35.5 ± 0.8	34.9 ± 1.8	32.1 ± 0.5*	30.8 ± 0.6
% Dry-lean mass	25.0 ± 0.1	24.3 ± 0.3	24.8 ± 0.2	24.5 ± 0.3	25.5 ± 0.2	25.4 ± 0.3
% Body water	66.1 ± 0.5	64.2 ± 0.7	64.5 ± 0.8	65.1 ± 1.8	67.9 ± 0.5*	69.2 ± 0.6
% Body fat	8.9 ± 0.5	11.4 ± 0.8	10.7 ± 1.0	10.4 ± 2.0	6.7 ± 0.7*	5.4 ± 0.6
FEMALE						
Body mass						
7 weeks	25 ± 0.5	25.8 ± 0.6	19.6 ± 0.4**	21.6 ± 0.6	21.8 ± 0.4**	21.9 ± 0.3
4 mnths	34.2 ± 0.8	35 ± 1.5	26.7 ± 0.5**	27.6 ± 0.7	26.3 ± 0.5**	26.9 ± 0.6
Body composition						
Length	9.3 ± 0.1	9.4 ± 0.1	8.7 ± 0.1**	8.8 ± 0.1	8.6 ± 0.1**	8.5 ± 0.1
Drymass	11.1 ± 0.4	12.3 ± 1.0	7.8 ± 0.2**	7.6 ± 0.3	7.2 ± 0.3**	7.1 ± 0.2
Drylean mass	6.9 ± 0.1	6.8 ± 0.2	5.6 ± 0.1**	5.5 ± 0.1	5.6 ± 0.1**	5.7 ± 0.1
Body water	18.3 ± 0.4	18.0 ± 0.6	14.9 ± 0.3**	15.1 ± 0.3	15.2 ± 0.2**	15.5 ± 0.3
Body fat	4.2 ± 0.3	5.4 ± 0.9	2.2 ± 0.2**	2.1 ± 0.2	1.6 ± 0.2**	1.4± 0.1
% Dry mass	37.4 ± 0.6	39.8 ± 1.6	34.1 ± 0.5**	33.5 ± 0.6	31.9 ± 0.7**	31.1 ± 0.3
% Dry-lean mass	23.6 ± 0.3	22.8 ± 0.5	24.6 ± 0.1**	24.4 ± 0.3	25.1 ± 0.3**	25.2 ± 0.2
% Body water	62.6 ± 0.6	60.2 ± 1.6	65.9 ± 0.5**	66.5 ± 0.6	68.1 ± 0.7**	68.9 ± 0.3
% Body fat	13.8 ± 0.9	17.0 ± 2.0	9.5 ± 0.6**	9.0 ± 0.8	6.8 ± 0.9**	5.9 ± 0.4

Values given are means ± SEM. * denotes significant difference with line 2 (line effect) (*, $p<0.05$; **, $p<0.01$). # denotes significant difference with LF diet (diet effect) (#, $p<0.05$; ##, $p<0.01$).

4. Regression analysis

In chapter 4 it was observed that a number of growth and developmental pup characteristics were negatively correlated to litter size during lactation. An explanation for these correlations is that individual pups in relatively large litters received relatively less nutrients than those in

Table 2. Plasma hormone and fuel levels of 4 months old male and female offspring mice from line 2 (control line) and line 7 and line 8 (selected lines) mothers feeding either a LF diet or a HF diet during pregnancy and lactation.

	2		7		8	
	LF	**HF**	**LF**	**HF**	**LF**	**HF**
MALE						
Glucose (mM)	8.5 ± 0.3	8.1 ± 0.3	8.2 ± 0.4	7.5 ± 0.3	7.6 ± 0.3	7.8 ± 0.3
Insulin (ng/ml)	1.9 ± 0.2	2.7 ± 0.4	1.4 ± 0.2	1.6 ± 0.4	1.2 ± 0.2*	1.2 ± 0.2
Leptin (ng/ml)	1.8 ± 0.2	3.1 ± 0.5#	2.7 ± 0.4	3 ± 0.8	1.3 ± 0.3	1.1 ± 0.2
Adiponectin (µg/ml)	5 ± 0.4	4.8 ± 0.4	6.5 ± 0.5*	5.7 ± 0.6	6.9 ± 0.5**	5.9 ± 0.4
Cholesterol (mM)	2.6 ± 0.1	2.4 ± 0.1	3 ± 0.1*	3 ± 0.2	2.9 ± 0.2	3 ± 0.2
FEMALE						
Glucose (mM)	7.8 ± 0.3	8.2 ± 0.4	7.7± 0.2	7.0 ± 0.2#	6.6 ± 0.2**	7.1± 0.3
Insulin (ng/ml)	1.9 ± 0.3	2 ± 0.4	1 ± 0.1*	0.9 ± 0.2	0.9 ± 0.2*	0.8 ± 0.1
Leptin (ng/ml)	4 ± 0.5	4.5 ± 0.7	2.3 ± 0.3**	2.2 ± 0.4	1.3 ± 0.5**	1.1 ± 0.2
Adiponectin (µg/ml)	9.4 ± 0.8	9.5 ± 1.0	14.9 ± 1.4**	13.8 ± 1.2	16.8 ± 1.9**	15.7 ± 1.2
Cholesterol (mM)	1.9 ± 0.1	2 ± 0.2	2 ± 0.1	2.1 ± 0.1	1.8 ± 0.1	2 ± 0.1

Values given are means ± SEM. * denotes significant difference with line 2 (line effect) (*, $p<0.05$; **, $p<0.01$). # denotes significant difference with LF diet (diet effect) (#, $p<0.05$).

relatively small litters. Using multiple linear regression analysis, some of these "litter size-correlated pup characteristics" were line and/or diet specific. Here we investigated whether nutritional/energetic state parameters during adulthood were correlated to the size of the litter at peak lactation, and whether these correlations provided additional information above the statistical outcomes provided by mean comparisons.

4.1. Offspring characteristics at the adolescent stage

4.1.1. Running wheel activity

No overall correlation of running wheel activity with litter size was found, indicating that variation in litter size does not improve discrimination between groups.

4.1.2. Body weight

At 7 weeks of age, body weight of male and female offspring was negatively correlated to litter size, meaning that offspring mice reared in relatively large litters weighed less than those reared in relative small litters. Within this correlation, no overall effects of diet were observed, indicating that the effects of diet found by mean comparison are weakened when corrected for litter size. There was, however, an effect of diet in line 8 male offspring which appeared to interact with litter size (see Table 3), meaning that line 8 males born to HF diet fed mothers were smaller than line 2 males from HF diet fed mothers at large litter sizes, but not at small ones.

4.2. Offspring characteristics at the adult stage

4.2.1. Food and water consumption

Food intake was overall negatively correlated to litter size in female offspring, but no overall correlation was found in male offspring. Within this correlation, female offspring of HF diet fed mothers ate less than those of LF diet fed mothers. Since diet interacted positively with litter size in female offspring, this decreased food intake of female offspring of HF fed mothers occurred at small litter sizes, but not at large ones. Overall, water intake was not related to litter size.

4.2.2. Body weight and composition

Body weight at 4 months of age in males, but not in females, was negatively correlated with litter size. Within this correlation, line and diet effects were observed. First, male offspring from HF diet feeding mothers were heavier than those from LF feeding mothers at corresponding litter sizes. Secondly, line 7 and line 8 male offspring weighed less than those of line 2 mothers at corresponding litter sizes, irrespective of diet. Nose-anal length was negatively correlated with litter size at 4 months of age in both genders, with offspring from HF diet feeding mothers longer than those from LF feeding mothers at corresponding litter sizes, but this HF diet effect was only observed in males. Additional interactions showed that this correlation was significantly more negative in line 8 male and female offspring from HF diet feeding mothers relative to line 2 offspring, indicating that line 8 males and females were smaller than those of line 2, particularly when they were from large litters. In line 7 male and female offspring from LF feeding mothers, this correlation was less negative than found in line 2 offspring from LF feeding mothers irrespective of diet. None of the other body composition parameters were correlated with litter size.

4.2.3. Metabolic fuel and hormone levels

Plasma levels of adiponectin levels in males but not in females, were positively correlated with litter size. Within this correlation, plasma adiponectin levels were more negatively related to litter size in line 8 males than in line 2 males irrespective of diet. Furthermore, maternal HF diet feeding increased plasma adiponectin levels at each corresponding litter size in males irrespective of line. Plasma insulin levels in the male offspring were significantly correlated with litter size, but only in the HF diet condition, meaning that plasma insulin levels of male offspring from HF diet feeding mothers were only increased compared to those of LF feeding mothers at small litter sizes. In female offspring, on the other hand, plasma glucose and cholesterol levels were correlated with litter size only in the perigestational HF diet condition, meaning that plasma glucose and cholesterol levels were increased in female offspring from HF diet feeding mothers at large litter sizes, compared to female offspring from LF feeding mothers.

Table 3. Results of Regression Analysis, where B is the regression coefficient and p-level is the level of significance. Line and diet indicated as dummy variables, whereas litter size is the continuous independent variable. In the left column, Intercept represents the value of line 2 (reference group) crossing the y-axis and litter size represents the slope of the line representing the reference group (line 2). When line, diet and/or their interactions were found significant, the intercept of that particular group differed from line 2 (reference group). When an interaction with litter size was found significant, the slope of that particular group differed from line 2 (reference group).

	BW (7week)		BW (4 mon)		Body length		Drylean mass		% Drylean mass		Body water		Body fat		Insulin		Adiponectin		Cholesterol		Glucose		Food intake	
	B	p	B	p	B	p-level	B	p-level	B	p-level	B	p-level	B	p-level	B	p-level	B	p-level	B	p-level	B	p-level	B	p-level
MALES																								
Intercept	36.26	<0.001	40.99	<0.001	9.75	<0.001	8.68	<0.001			23.01	<0.001	3.06	<0.001	1.98	<0.001	2.76	0.009						
littersize	-0.59	<0.001	-0.39	0.002	-0.05	<0.001	-0.07	0.024			-0.19	0.045					0.23	0.030						
Diet			2.10	<0.001	0.50	<0.001	0.42	<0.001			1.17	<0.001	1.07	0.011	1.63	0.004	3.23	0.010						
Line 7	-5.5	<0.001	-5.90	0.003			-1.11	0.010			-3.25	0.006					1.70	0.001						
Line 8	-6.04	<0.001	-3.42	<0.001									-1.05	0.016	-0.81	0.004	5.07	<0.001						
Line 7 x Diet					-1.00	0.005																		
Line 8 x Diet	6.64	0.018																						
Line 7 x Litter size																								
Line 8 x Litter size							-0.06	<0.001									-0.31	0.033						
Diet x Litter size															-0.14	0.014	-0.36	<0.001						
Line 7 x Diet x Litter size																								
Line 8 x Diet x Litter size	-0.74	0.008			-0.05	<0.001							-0.12	0.028										
FEMALES																								
Intercept	30.17	<0.001			9.92	<0.001			20.12	<0.001									1.94	<0.001	7.74	<0.001	7.21	<0.001
littersize	-0.54	<0.001			-0.07	0.003																	-0.24	0.014
Diet									-0.35	0.049													-2.27	0.019
Line 7	-5.19	0.015			-1.18	<0.001			0.79	<0.001														
Line 8					-1.06	0.024			1.34	<0.001											-1.26	<0.001		
Line 7 x Diet																					-1.32	<0.001		
Line 8 x Diet																								
Line 7 x Litter size					0.07	0.024																		
Line 8 x Litter size																								
Diet x Litter size																			0.02	0.021	0.06	0.012	0.26	0.010
Line 7 x Diet x Litter size																								
Line 8 x Diet x Litter size					-0.10	0.032																		

5. Discussion

The aim of the present study was to investigate the effects of feeding a high-fat (HF) diet during pregnancy and lactation on offspring body weight gain and several associated parameters related to energy balance. This study was performed in a group of randomly bred control mice (line 2), and in two mouse lines that were from the same ancestral line as the controls, but were selectively bred for high voluntary wheel running behavior over 48 generations (Swallow, Carter, and Garland, Jr. 1998). We have previously observed that these highly active mice are hyperphagic but nonetheless resistant to diet-induced obesity (DIO), when fed a HF diet at adulthood. Control mice, on the other hand, were prone to develop DIO when subjected to a HF diet (Vaanholt et al. 2008). We hypothesized that the randomly bred female control mice, but not the highly active female mice, would program their offspring to exaggerated weight gain and related changes at adulthood when they would be fed a HF diet during pregnancy and lactation.

At 7 weeks of age, perigestational HF diet had an overall effect to increase body weight in male but not female offspring irrespective of line compared to the LF diet condition. This means that the trait for voluntary high-wheel running behavior did not protect the offspring against the stimulatory effect of perigestational HF feeding on body weight gain at the adolescent stage. Differences in running wheel activity between control and high activity lines were not influenced by the perinatal HF diet indicating that the HF diet did not program weight gain via behavioral inactivity. At the adult stage of 4 months of age, the stimulatory effect of perigestational HF feeding on body weight gain persisted in the highly active males irrespective of line. Viewing the data more closely, this effect of perigestational HF diet appeared to be skewed towards the control line, and was less pronounced in line 7 and even less so in line 8 males. Fluctuations in body weight can be the result of changes in fat-free mass, fat mass and/or body water. Analysis of these parameters revealed that particularly fat free mass and body water contributed to the increased body weights of the male offspring from HF diet fed line 2 mothers, and pointing out that these line 2 offspring from HF fed mothers were simply larger animals than the line 2 offspring from LF feeding mothers. This is reflected by the finding that also nose-anal lengths of line 2 offspring from HF feeding mothers were increased compared to the offspring from LF feeding mothers. Almost none of these parameters (except for the elevated water content and dry-lean mass content in the male line 7 offspring from HF fed mothers compared to the LF mothers) were significantly affected by the perigestational HF diet in the line 7 and 8 male offspring. Thus, it may be concluded that 1) male offspring is more susceptible to weight gain by perigestational HF diet than female offspring, and 2) programming effects of perigestational HF diet feeding are largely corrected in male offspring with a trait for increased voluntary wheel running behavior.

In previous studies, it was observed that feeding a HF diet during the perigestational period causes disproportional increases in adipose tissue (Lemonnier 1972; Samuelsson et al. 2008; Srinivasan et al. 2006). The finding in the present study that perigestational HF diet feeding did not cause an overall effect to increase fat mass is inconsistent with those previous

observations. This has two reasons. First, when only considering line 2 offspring, the perigestational diet caused a 41% increase in fat mass, which was not observed in line 7 and 8. Thus, the effect of perigestational HF feeding on adiposity was "missed" because of inclusion of all lines. Secondly, a strong disproportional increase in male line 2 offspring from perigestational HF fed mothers might have been ameliorated due the fact that the HF diet feeding mothers in the present study were switched back to the LF diet on day 16 of lactation, and the offspring were maintained on the LF diet as well. Most other studies investigating dietary programming effects on offspring energy balance left lactating mothers and progeny on the HF diet at least throughout weaning (see references above). The reason for the "early switch" was that we wanted to avoid direct effects of dietary fat on the offspring, and ended dietary treatment before pups have been observed to start ingesting solid foods (Kounig, Riester, and Markl 1988). Despite the absence of disproportional adiposity we found that plasma levels of insulin and adiponectin were increased in male offspring from HF feeding mothers relative to the LF feeding ones, irrespective of line. An elevation of plasma insulin has been shown previously to result from perigestational HF diet feeding (Parente, Aguila, and Mandarim-de-Lacerda 2008; Srinivasan et al. 2006), and together with the unchanged glucose levels this may be seen as an early sign of insulin resistance. An interesting phenomenon in light of the previous observations is that the perigestational HF diet feeding caused an increase in water intake, which was most pronounced in the high activity lines. In fact, the high activity lines had higher water intakes compared to the control line irrespective of diet. Since increased water intake stimulates cellular metabolism (Thornton, Even, and van Dijk 2009), it might be hypothesized that the high level of water intake is connected to the resistance of high activity lines to develop weight gain and potential metabolic disturbances induced by perigestational HF diet feeding.

Since litters of mice in the present study were not manipulated in size, it might be possible that naturally occurring differences in litter size among lines could have influenced body weights and associated parameters at adulthood. As mentioned in Chapter 5, sizes of litters the animals were born in were larger in lines 7 and 8 compared to line 2, and line 2 mothers feeding the HF diet tended to have even smaller litters than mothers feeding the LF diet. Although line 7 mothers had considerable pup loss during lactation, also litter size in this line tended to be lower in the HF condition than in the LF diet condition. In contrast, line 8 mothers feeding the HF diet tended to have even larger litters than in the condition of the LF diet. These differences between lines and diets might have contributed to differences in offspring energy balance parameters at adulthood. Indeed, it is known that extreme manipulation to very small (3-4 pups) or very large litters (18-20 pups) causes small litter offspring to become obese due to early overfeeding and large litter offspring to remain lean in the absence of overfeeding (Aubert, Suquet, and Lemonnier 1980; Plagemann et al. 1999). However, also relatively small differences of 2 pups less or more in the litter may contribute to differences in body weight parameters at adulthood (Epstein 1978). For example, observations in the study of Buckley et al. where litter size was reduced by perinatal HF diet from 15 to 12 probably contributed to the increased

adiposity of adult offspring from HF diet feeding mothers (Buckley et al. 2005). We therefore performed a Linear Regression Analysis including litter size, diet, line, as well as their interactions as independent factors and body weight and related parameters as dependent factor. We consistently observed in male, but also in female offspring that litter size contributed significantly in a negative direction to body weight at 7 weeks of age and only in male offspring at 4 months of age. Within this correlation in males, we observed that perigestational HF diet feeding contributed significantly to the increase in body weight at adulthood at each corresponding litter size, and line 7 and 8 males were smaller than line 2 offspring at each corresponding litter size. Furthermore, dry lean mass and body water content were also increased by perigestational HF feeding, and reduced in line 7 and 8 compared to line 2. These results are largely consistent with those obtained from the mean comparisons by GLM analysis.

The regression analysis between energy balance characteristics and litter size also revealed some novel effects that were not observed by mean comparisons in the GLM analysis. The level of body fat, for example, was not significantly increased by perigestational HF feeding in the overall mean comparison analysis, but was significantly elevated in the regression analysis by perigestational diet when ignoring line as a factor. Also line effects were found on the level of body fat, specifically line 8 male offspring had significantly less fat than line 2 males, irrespective of diet. Other effects found with regression analysis, which were not observed with mean comparisons related to differences in plasma hormone and fuel levels. Plasma levels of insulin in male offspring were negatively correlated to litter size, but only in the HF diet condition. The regression analysis revealed that differences between plasma insulin levels of HF and LF offspring was largest in small litters. Plasma adiponectin levels, on the other hand, were positively correlated with litter size in male offspring. Although plasma adiponectin levels within this correlation were increased somewhat at smaller litter sizes by perigestational HF diet feeding, these opposite correlations of insulin and adiponectin with litter size may have important consequences. Taking into account that the HF diet caused the largest weight gain in line 2 male offspring born in small litters, with the highest plasma insulin levels, but lowest plasma levels of adiponectin (despite some recovery as mentioned above), these animals were probably at highest risk to develop insulin resistance. The slight recovery of plasma adiponectin at small litter sizes by perigestational HF feeding might have protected them from developing type-II diabetes mellitus. Hence, adiponectin stimulates fat oxidation in skeletal muscle and contributes to glucose disposal and insulin action (Fruebis et al. 2001; Yamauchi et al. 2002), and reduces the risk of type-2 diabetes mellitus (Fruebis et al. 2001; Hara, Yamauchi, and Kadowaki 2005; Kadowaki et al. 2006). This is most relevant for offspring of line 2 since the high activity lines 7 and 8 have significantly elevated levels of plasma adiponectin. These latter effects are consistent with the findings in Chapter 4 and with previous work of Vaanholt et al. The final effects observed with regression analysis and not observed with mean comparisons related to plasma levels of glucose and cholesterol in female offspring, which were both positively correlated to litter size only in the HF diet condition. This means that the fuel levels were increased

independent of line in female offspring from HF feeding mothers at large litter sizes compared to offspring from LF feeding mothers. These effects may be related to food intake, since female offspring from HF feeding mothers ate more than those of LF feeding mothers at large litter sizes.

In summary, perigestation HF diet feeding caused weight gain in male offspring from randomly bred line 2 mothers, and this effect was largely absent in male offspring from the highly active line 7 and 8 mothers feeding the HF diet. Fat mass, fat-free mass as well as body water content appeared to contribute proportionally to the increased weight gain of the line 2 male offspring of HF fed mothers, and this effect was independent from litter size. Besides similarities between the high activity lines to resist effects of the perigestational diet, it was also clear that this resistance was strongest in line 8 offspring. It is likely that these differences results from the fact that the line 7 mothers lost an average 37% of pups in their litters during lactation, whereas line 8 mothers did not lose pups (see Chapter 5). The latter is remarkable since line 8 mothers had the largest litters among lines. It is therefore conceivable that individual pups in litters of line 8 mothers received less nutrition than individual pups in litters from line 7 mothers, and therefore remained the smallest and the lightest after lactation and at adulthood. Thus, even though line 7 offspring was relatively overnourished, the programming effects of perigestational dietary fat were probably offset by the genetic trait for increased wheel running behavior. Contributing mechanisms to this trait – besides increased voluntary activity and increased associated energy expenditure - could be related to the relatively high level of water intake, and a relatively high plasma level of adiponectin.

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