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

Jónás, Izabella

CHAPTER 4

EFFECT OF DIET ON THE ENERGETIC AND BEHAVIOURAL CHARACTERISTICS OF MOUSE LINES SELECTED FOR HIGH ACTIVITY

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

Summary

Rodents subjected to forced physical activity are resistant to diet-induced obesity, but the procedure often involves emotional stress. For this reason, we studied interactions between voluntary activity and parameters related to energy balance and emotional stress in control mice, and in mice with a trait for increased wheel running behaviour. These latter animals show more voluntary behaviour even in the absence of running wheels. When subjected to a 40% high fat (HF) diet with added refined sugars, the activity selected lines were HF diet obesity resistant, with increased food intake, similar absorption rates and higher non-exercise thermogenesis (NEAT) relative to their randomly bred controls. While activity selected females had a higher anxiety level at baseline than control, this effect reversed on a HF diet, without concomitant changes in plasma levels of corticosterone. Since activity selected lines had a lower HF preference than controls in a two choice test of HF food vs. high-carbohydrate food, these data may be viewed such that the activity lines, and in particular the females improve their mood on a HF diet, without necessarily liking the HF diet more than control mice.

1. Introduction

In an attempt to unravel underpinnings of mammalian energy balance regulation and its derangements, numerous studies have been performed using rodents with traits for obesity proneness and resistance. Through experiments with these animals many candidate genes have been discovered involved in energy balance regulation (Brockmann and Bevova 2002). Selective breeding of rodents lines for diet-induced obesity refined these investigations by using diet as a default trigger, and allowed tracking the aetiology of obesity proneness-and resistance (Levin et al. 1997). It is currently believed that traits for obesity or resistance to it are not caused by single gene differences but is the result of multiple genes that interact at different levels. Indeed, in the seminal work of Singer and colleagues using chromosome substitution strains of mice, they identified that the trait for obesity involves genes almost on every chromosome. Secondly, they observed that high-fat diets differing in the level of refined sugars distort energy balance regulation probably via different mechanisms (Singer et al. 2004).

Another determinant of energy balance regulation is energy expenditure, but has been investigated not as extensively. In general, studies in rats and mice showed a decrease in body fat content in these animals when they had access to a running wheel even though their food intake increased (Tokuyama, Saito, and Okuda 1982; Looy and Eikelboom 1989; Bell, Spencer, and Sherriff 1997; Bell and McGill 1991). One approach to examine the effects of a high level of physical activity on energy balance regulation is to study this in animals that have been selectively bred for increased running wheel activity. Swallow et al consistently observed that selection lines ate more food compared to the controls (Swallow, Carter, and Garland, Jr. 1998b), but remained at a lower body weight during the entire period of the experiment compared to the controls. The activity-selected animals also showed lower fat accumulation which can explain the lower body weight (Swallow et al. 2001). A more recent study by Vaanholt et al showed that selected females, when fed a 60% high fat diet without refined sugars, had higher food intake than the controls but unlike the controls they showed remarkable resistance to weight gain on this high fat diet, even in the absence of running wheels. The activity-selected animals showed a higher mass corrected daily energy expenditure and had a higher resting metabolic rate compared to controls, and this was particularly evident in the females (Vaanholt et al. 2008). Thus, besides increased energy turn-over due to increases in spontaneous activity (Dauncey 1990), activity selected mice display increased metabolic rate independent of differences in activity levels. Together, these mechanisms contribute to resistance to high-fat diet-induced obesity.

As mentioned above, high fat diets differing in the level of refined sugars (i.e., sucrose) may cause distorted energy balance regulation via different mechanisms (Singer et al. 2004). For this reason, we aimed at reconciling the diet-obesity resistance of activity selected mice in the condition when these animals are feeding a high fat diet with an increased level of the refined sugar sucrose. Since it was shown in previous Chapters of this thesis and a number of published

reports (Girard et al. 2001; Swallow et al. 2001; Koteja et al. 1999; Gammie et al. 2003; Carter et al. 2000; Garland, Jr. et al. 1995; Vaanholt et al. 2007a; Belke and Garland, Jr. 2007; Malisch et al. 2009) that selective breeding for physical activity causes co-selection of several personality traits (including exploratory behavior, anxiety, stress sensitivity, and stereotypic behavior), we aimed at exploring these in control and activity selected mice in the face of feeding the high-fat high sucrose diet. The main hypothesis which we tested was that activity selected animals would be equally resistant to obesity on this high fat – high sugar diet as on the previously applied high fat alone diet, and that this obesity resistance would parallel outcomes of the behavioral tests.

2. Materials and Methods

2.1. Animals and housing

Thirty-six breeding pairs of Hsd:ICR mice (*Mus domesticus*) selected on high wheel-running activity (lines 7 and 8) for 50 generations and their random bred controls (lin 2) were obtained from T. Garland Jr, Riverside, CA (For a detailed description of the selection procedure see (Swallow, Carter, and Garland, Jr. 1998a). From these founder mice, separate breeding lines were continued at our facilities in Haren and further selected according to the same criteria. In the current experiments, 93 mice of the 52nd generation were used. From the age of 7 weeks onward, they were individually housed in standard cages (Macrolon Type II, UNO Roest Vast Staal B.V., Soest, NL) in the same room with an ambient temperature of 22±1°C. At the start of the experiment all animals had *ad libitum* food 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) or a 40 % fat diet, additionally containing sucrose (HFS) (4.7 kcal/g; 30 % carbohydrate, 45 % fat, 18 % protein; AB Animal Diets, Woerden, NL). and water; they were on a 12:12 light-dark cycle (lights on at 8:00). Wood shavings and EnviroDry® were used as bedding material.

2.2. Body mass and food intake

Four weeks before the start of the experiments, male and female mice of the different lines were assigned to cohorts either receiving a low-fat unrefined carbohydrate rich diet (LF), or a 40% high-fat diet with 25% added sucrose (HFS). Body mass and food intake of the LF and HFS feeding mice were measured weekly between 12:00 and 14:00 over the course of the entire diet manipulation. Intake was corrected for spillage.

2.3. Diet preference test

Diet preference of all animals was tested by allowing animals access to the LF and the HFS diet during a two-hour interval 1) in the evening at 20:00 at the beginning of their active phase or 2) at

8:00 in the morning at the beginning of their inactive phase. The active phase diet choice was done at the age of 12 weeks and the inactive phase diet choice was done at the age of 14 weeks. The food was provided in the lid of the cage, separated by the water bottle. Over the two-hour period, mice were allowed to eat from the food without the experimenter being in the room. Thereafter, the food was removed and intake was assessed, and animals were readjusted to their 'normal' diet.

2.4. Elevated plus maze

To determine the anxiety levels of mice, they were tested on an elevated plus maze over the period of week 11 to week 13. Plus maze tests separated from diet choice tests for more than two days. The maze consisted of two open arms and two closed arms facing each other, 40 cm above the floor. Each arm was 29 cm long and 5 cm wide. The closed arms had 15 cm high walls with a closed top. At the beginning of an experiment, the mice were placed at the center (5 x 5 cm) of the elevated plus maze after which the experimenter left the room. The animal was then videotaped during 5 minutes and the behavior on the plus maze was scored using Eline software. This way the time spent on each arm and the crossings from one arm to another was recorded. After finishing testing an animal the maze got cleaned with water and dried, before placing a new animal on the plus maze. The time spent on each of the different arms was analyzed, it represents the animal's anxiety level, the more time spent on the open arms the less anxious the mouse is and vice versa.

2.5. Stress test

At the age of 14-15 weeks a novel cage stress test was performed on all groups. At the start of the experiment, a blood sample was taken in their home cage by tail clip and 30-40µl of blood was collected in a heparinized tube. After 5 minutes, the animals were moved to a brightly lit room adjacent to their habituated room, and placed in a clean empty cage (identical to their home cage but without bedding). They were left in this cage for 15 minutes and then another blood sample was collected. After the experiment the blood was centrifuged and the blood plasma was collected and stored at -80°C. Later the amount of corticosterone in the plasma was measured by RIA (Linco Research, Nucli lab, The Netherlands).

2.6. Respirometry measurements

In week 21-23, animals were moved into respirometric chambers to determine oxygen consumption (VO_2, l/h) and carbon dioxide production (VCO_2, l/h) by indirect calorimetry for 24 hours. Eight animals could be measured simultaneously. Oxygen and carbon dioxide concentration of dried inlet and outlet air (drier: molecular sieve 3 Á, Merck) from each chamber was measured with a paramagnetic oxygen analyzer (Servomex Xentra 4100) and cardon dioxide by an infrared gas analyzer (Servomex 1440). The system recorded the differentials in oxygen and

carbon dioxide between dried reference air and dried air from the metabolic chambers. Flow rate of inlet air was measured with a mass-flow controller (Type 5850 Brooks). Ambient temperature in the chamber and cage, as well as activity (with passive infra-red detectors) were measured simultaneously. Samples were collected every 10 minutes for each animal and automatically stored on a computer. To reduce novel cage stress, the respirometric chambers (45x25x30 cm) were adapted to accommodate the home cage of the animal. Animals therefore did not need to be handled and stayed in their home cage during the entire measurements. Animals were measured at an ambient temperature of 22°C and food (standard chow or fat) and water were provided *ad libitum*.

Heat production (HP, kJ/h) was calculated using the following equation: HP= (16.18 x VO_2) + (5.02 x VCO_2) (Romijn and Lokhorst 1964). Resting metabolic rate (RMR, kJ/h) was defined as the lowest value of heat production calculated as the running mean over half an hour and was calculated for the first and second day in the respirometer separately. Maximal heat production was also calculated as the running mean over half an hour. In addition, the average heat production (daily energy expenditure: DEE, kJ/d), respiratory quotient (RQ= VCO_2/VO_2) and PIR (passive infrared) activity were calculated for both consecutive days.

2.7. Bom calorimeter measurements

Directly after respirometry, feces of animals were collected from the saw-dust bedding and were weighed. Then the caloric content of the feces was measured by bom calorimeter. First a known amount of benzoic acid (known energy content of 6320 cal/g) was combusted in the bom calorimeter. This was done three times and the average of those three measurements was used as the reference point for the samples of the feces. Then samples of the feces were combusted and the heat production was compared to the references to determine the energy content of the samples. With these data combined with the assessed food intake during the 24 hours in the respirometer, this enabled us to estimate metabolizable energy intake (MEI).

2.8. Data analysis

All of the data were analyzed using Statistica 7. The body mass and food intake data that were measured during 12 weeks were analyzed with repeated measures ANOVA. For the analysis of the other data general linear models were applied. To test the effect of diet within each line, the data from each line was analyzed separately with a general linear model. To test all subgroups a contrast test was performed.

3. Results

3.1. Body mass and food intake

Figure 1 shows changes in body weight of the mice over time when they were feeding a HFS or LF diet. Mice from the selection lines have a significantly lower body mass compared to the control animals (♂: $F(2,44)=25.53$; $p<0.001$ and ♀: $F(2,40)=39.95$; $p<0.001$). This is in agreement with the other studies that were done with the activity selected lines compared to the controls (Swallow et al. 2001; Vaanholt et al. 2008; Vaanholt et al. 2007b). Body weight gain over time was higher in line 2 ($F(11,44)=8.16$; $p<0.001$) and line 7 ($F(11,44)=2.69$; $p<0.001$) males on the HFS diet than on the LF diet. This pattern is not seen in the females.

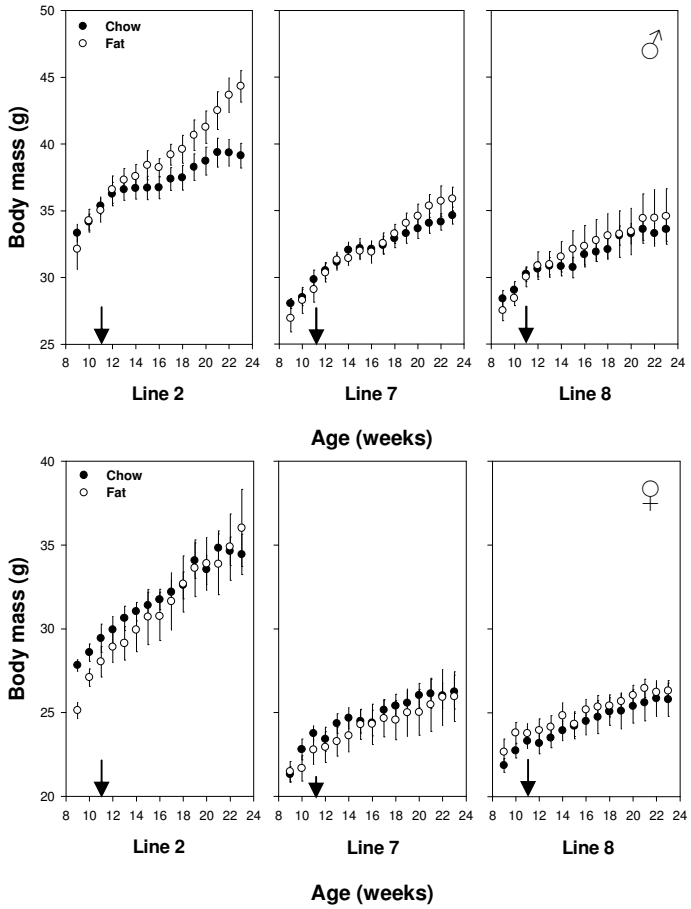

Figure 1. The average body mass over time for all groups in male (top panels) and female mice (bottom panels). The arrows depicts the age at which the HFS diet was provided.

The average daily food intake (per week) of the mice is shown in figure 2. In males food intake of line 8 ($p=0.004$), but not line 7 mice was generally increased above the levels found in line 2. In females, both line 7 and line 8 showed increased food intake above the levels observed in line 2 females ($F(2,40)=19.31$; $p<0.001$). Line 2 (♂: $F(1,44)=26.40$; $p<0.001$ and ♀: $F(1,40)=50.32$; $p<0.001$) and line 7 (♂: $F(1,44)=33.10$; $p<0.001$ and ♀: $F(1,40)=5.12$; $p<0.047$) males and females showed clearly reduced food intake on the HFS diet compared to respective groups on the LF diet, except for line 8.

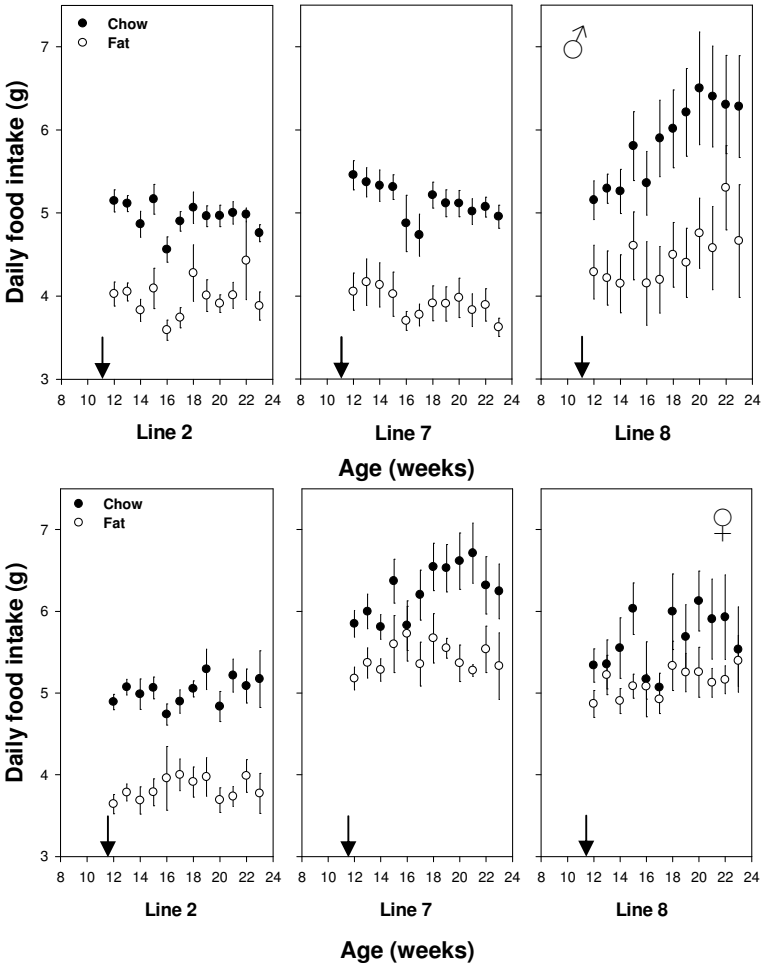

Figure 2. The average daily food intake over time for all the different groups in male (top panels) and female (bottom panels) mice. The arrows depicts the age at which the HFS diet was provided.

3.2. Absorption and growth efficiency

During the 24 hours in the respirometer, the amount of food eaten was assessed and the feces that were excreted were collected. The total calories of the food intake and the feces was determined (males only), and with this data the percentage of absorption of the food was calculated (figure 3). The high absorption rates of about 80-90% are comparable to data collected in other studies done with mice or rats (Carvajal et al. 2000; Chen et al. 2003; Lo et al. 2008; Santos, Coelho, and Coelho 2008). The animals that were on HFS diet show a significantly higher percentage of absorption compared to the chow fed animals ($F(1,37)=14.82$; $p<0.001$) irrespective of line.

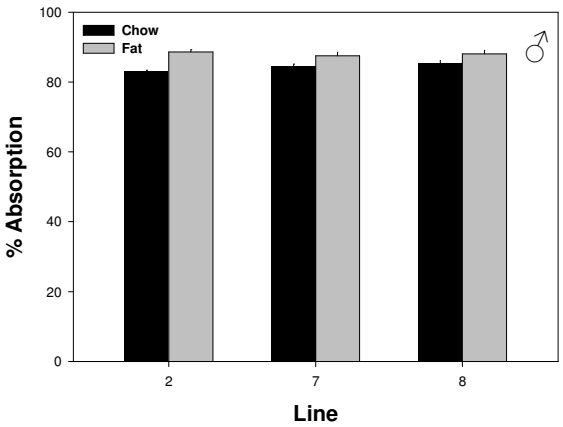

Figure 3. The percentage of food that was absorbed from the total food that the male mice consumed during their time in the respirometer.

Growth efficiency was calculated by dividing the weekly food intake by the weekly change in body mass. The HFS-fed animals had a significantly higher growth efficiency than the LF-fed animals (♂: $F(1,44)=26.30$; $p<0.001$ and ♀: $F(1,40)=11.12$; $p=0.002$). Line 2 males on the LF diet had a significantly higher growth efficiency compared to line 8 ($F(2,44)=4.64$; $p=0.025$) and for the females on the HFS diet the growth efficiency of line 2 was significantly higher than that of line 7 and 8 females ($F(2,40)=4.98$; $p=0.012$).

3.3. Respirometer measurements

From the data output of the respirometer the daily energy expenditure (DEE) could be determined, which is shown in figure 5. Overall females had a higher DEE when feeding the

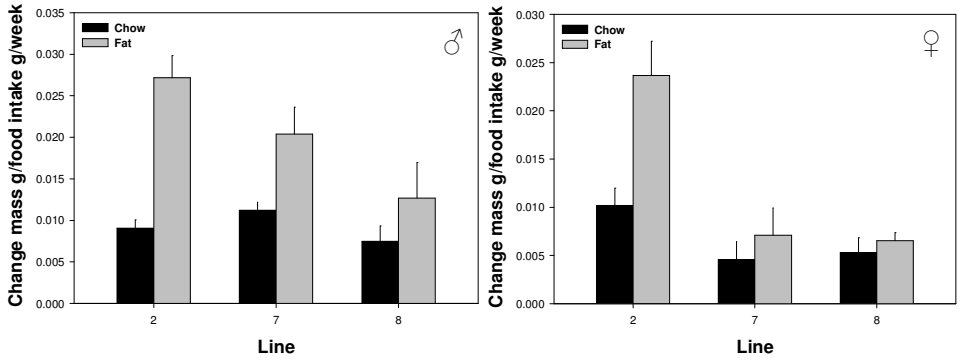

Figure 4. The average growth efficiency over a period of 12 weeks in male (left panel) and female (right panel) mice.

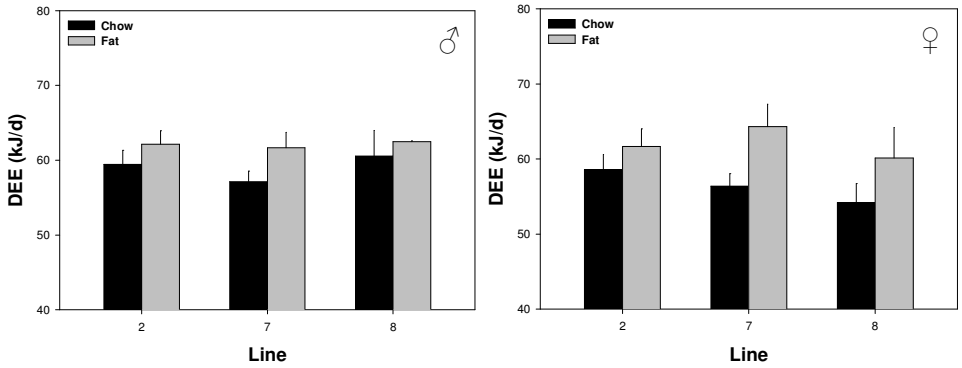

Figure 5. The daily energy expenditure (DEE) measured over 24 hours in the respirometer in male (left panel) and female (right panel) mice.

HFS diet than when they were feeding a LF diet ($F(2,39)=6.35$; $p=0.016$). In males a similar pattern can be seen but the difference was not significant.

Resting metabolic rate (RMR) is significantly influenced by body mass. Therefore, RMR is depicted as the lean mass-specific RMR in figure 6. The males show a significant line*diet interaction ($F(2,44)=3.52$; $p=0.040$) and line 2 animals appeared to have slightly lower RMR on the HFS diet compared to when they were feeding a LF diet. Lines 7 and 8 males, on the other hand, showed a slightly higher RMR when feeding the HFS diet (table 6). None of the groups, however, showed a significantly higher or lower RMR compared to the other groups in the post-hoc analysis, but there appears to be a trend of a higher RMR in the selection animals.

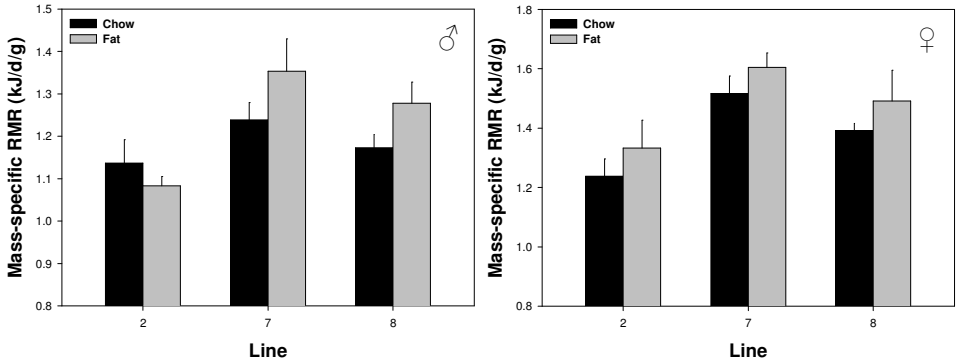

Figure 6. The resting metabolic rate calculated from the measurements in the respirometer in male (left panel) and female (right panel) mice.

By subtracting RMR (i.e., non corrected for body mass) from DEE in each animal, this reveals energy allotted to thermogenesis by spontaneous activity and diet; the so-called non-exercise activity thermogenesis (NEAT), and the averages of energy expenditure by RMR and NEAT are depicted in figure 7. The effects of line and diet on the RMR have already been discussed in the previous section. Line 8 males have a significantly higher NEAT compared to the line 2 males ($F(2,44)=4.36$; $p=0.020$), irrespective of diet. In the females, both line 7 and 8 show a significantly higher NEAT than the control line ($F(2,44)=3.65$; $p=0.036$), irrespective of diet.

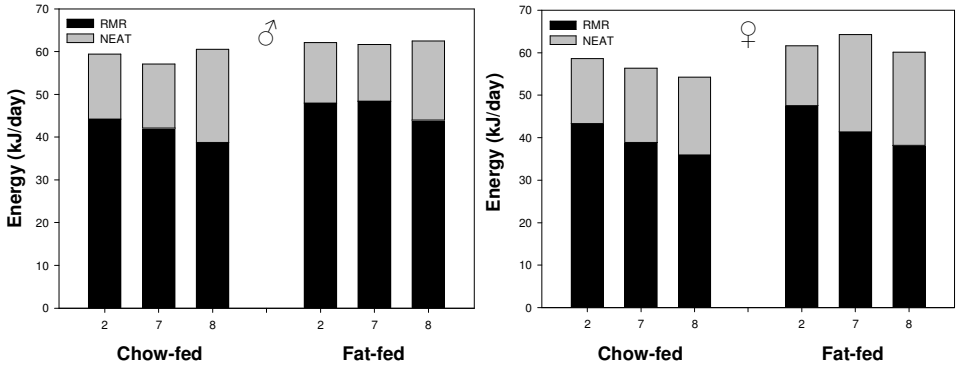

Figure 7. Energy spent during the time in the respirometer, consisting of the RMR and NEAT in male (left panel) and female (right panel) mice.

3.4. Diet choice

The results of the diet choice test are presented in figure 8. The two diet groups had markedly different effects on diet selection; i.e., mice feeding LF-fed diet as their regular food almost exclusively ate HFS-diet during when given the choice between HFS and LF food, at both the inactive and active phase. Mice feeding the HFS diet as their regular food ate both LF as well as HFS diet during the choice test. This pattern is most clearly seen for the test in the morning, when all lines of HFS-fed animals ate considerable amount of LF diet (♂: $F(1,43)=47.23$; $p<0.001$ and ♀: $F(1,40)=12.69$; $p<0.001$). In the males, lines 7 and 8 eat significantly more LF diet than the line 2 males during the inactive morning phase ($F(2,43)=6.10$; $p=0.005$). In the females, only line 8 mice select more LF diet compared to line 2 mice ($F(2,40)=5.66$; $p=0.035$), and this was predominantly seen in the active phase (see table 6).

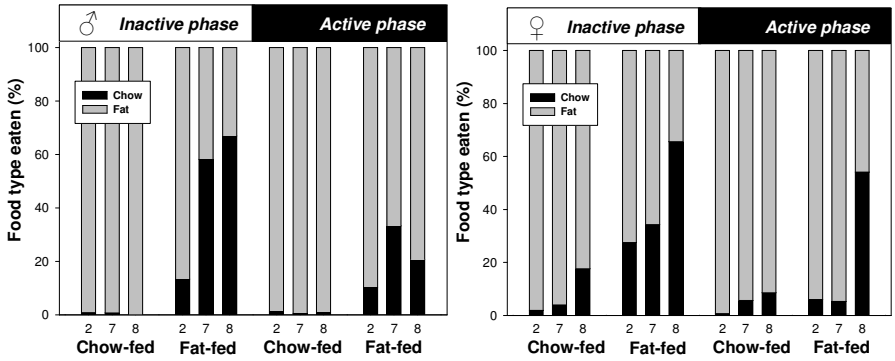

Figure 8. Diet choice test showing the food preference for both the inactive and active phase in male (left panel) and female (right panel) mice. Total intake is set at 100% for each animal, and the subsequent intake from HFS and LF diets are presented as grey and black bars.

3.5. Elevated plus maze

The results of the elevated plus maze test are shown in figure 9. Diet did not affect anxiety levels (expressed by time in open versus closed arm) in males. The females on the other hand, showed a significant line*diet interaction ($F(2,40)=5.76$; $p=0.007$). The three lines all showed a different response to the test. The LF-fed line 2 females spent significant less time in the closed arms $F(2,40)=6.45$; $p=0.004$) (indicating reduced levels of anxiety relative to selected lines), and line 8 females on the LF diet showed the lowest presence in the open arms ($F(2,40)=5.76$; $p=0.007$) (with line 7 as the intermediate). On the HFS diet, however, this picture in the females completely reversed. In males feeding the LF diet, a pattern with some similarity was found with the females on the LF diet, but these effects did not attain significance. On the HFS diet, males of the different showed exactly similar responses on the plus maze.

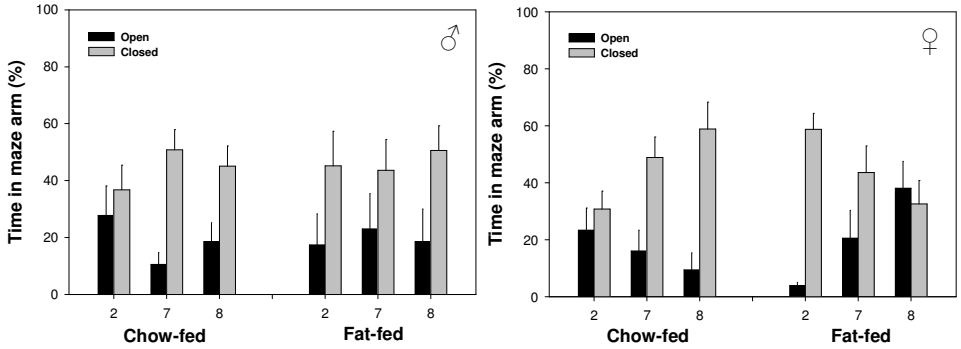

Figure 9. Elevated plus maze test showing the percentage of time spend on both the open arm and the closed arm in male (left panel) and female (right panel) mice.

3.6. Stress test

The results of the novel cage test on plasma corticosterone levels are shown in figure 10. At baseline, no differences were observed plasma corticosterone levels in males and females between lines and diets. During the stress, however, male mice showed a line effect ($F(2,41)=3.55$; $p=0.038$), and post-hoc analysis revealed that line 7 had lower plasma corticosterone levels than line 2 males irrespective of diet. Effects in the females pointed in the opposite direction (i.e., with plasma corticosterone levels higher in line 7 mice compared to line 2 mice), but this effect failed to reach significance.

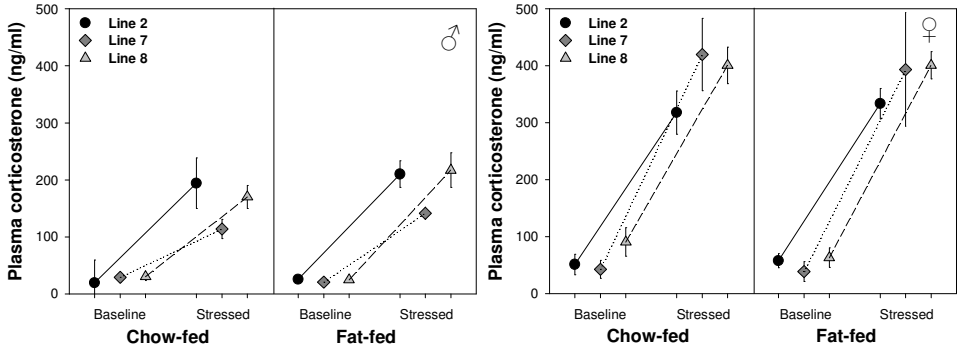

Figure 10. Stress test showing the amount of corticosterone measured in plasma for the baseline levels measured before the test and the stress levels measured after the test.

4. Discussion

The present study investigated the effects of feeding a diet consisting of 40% saturated fat and 25% refined sugars on energy balance regulation and an array of behaviors in control mice as well as in mice selectively bred for increased wheel running behavior. It was hypothesized that the high activity lines, and in particular the females would be resistant to the obesogenic actions of the HFS diet, and the control line would be obesity prone. Since selection for wheel running behavior has been shown to cause co-selection of various traits (i.e., which may be helpful to sustain endurance behavior), these co-adaptations may also be hypothesized to be resistant to change.

Consistent with our hypothesis and previous observations was the finding in the present study that the line 7 and line 8 activity-selected males and female mice did not respond with weight gain on the HFS diet compared to those feeding the LF diet. Mechanisms underlying this effect included increased mass specific-resting metabolic rate (RMR) and a higher level of non-exercise activity thermogenesis (NEAT) (Donahoo, Levine, and Melanson 2004). Although not assessed in the present study, the latter effect is obviously associated with increased levels of spontaneous activity in the activity selected animals which has been shown to be displayed by these animals even without running wheels (Rhodes et al. 2001). The effects on RMR were significantly increased in activity selected males compared to the control males (and with a strong trend in the females), but depended on diet. Thus, while RMR decreased slightly in the control males in the HFS condition relative to the LF condition, RMR increased in line 7 and line 8 males in the HFS condition. In contrast, the level of NEAT was significantly increased in the activity selected mice irrespective of diet in males, but in females this effect was strongest in the HFS condition. Together, this rendered the highly active mice less growth efficient than the control mice. Differences in food absorption (at least in the males) could not account for this effect since we did not find differences in the energy content of feces by bom calorimetry among lines.

The diet resistance in the line 7 and line 8 females is remarkable in light of the findings that they ingested between 15~20% more food than the control females did, and hardly responded with a reduction in food intake when subjected to the HFS diet. The latter would be a "normal" response when animals are subjected to high amounts of dietary fat (van Heek et al. 1997). Opposite to our previous observation was the finding in the present study that not the control females, but the control males were prone to weight gain on the HFS diet. We have no data on the body composition analysis, but since diet manipulations were started at 11 weeks of age, it is unlikely that the effects are not attributed to differences in adiposity. Furthermore, we do not know whether the increased weight gain is due to the differences in dietary fat percentage (i.e., 60% fat in our previous studies vs. 40% fat in the present) or the addition of the sucrose in the present study. Harris et al (Harris, Bowen, and Mitchell 2003) demonstrated previously that female, but not male mice retain leptin sensitivity on a 45% HF diet. Since blunting of leptin signalling is a major cause underlying weight gain in rodents (Ruffin et al. 2004), and might

underlie differences in resistance and proneness to diet-induced obesity (Levin and Dunn-Meynell 2002), this might very well explain differences in weight gain in the control and activity selected animals in the present study.

Despite resistance to weight gain in the selectively bred females, striking line differences were obtained in the female mice subjected to the elevated plus maze test. When feeding the LF diet, the line 2 females were the least anxious (indicated by the highest percentage of time present on the open arms) on the plus maze and the line 8 were most anxious. Line 7 LF feeding females showed an intermediate response. This confirms the findings of Chapter 2. On the HF diet, however, this pattern completely reversed, now the line 2 females were most anxious and line 8 ones the least. Thus, in control females, feeding the HFS diet increased anxiety levels whereas it reduced it in activity selected ones. Differential effects of dietary fat and sugars have been observed before on stress sensitivity and anxiety levels (Torres and Nowson 2007), and the direction of change may depend on the previous experience of animals (van Dijk and Buwalda 2008). One idea might be that the HFS diet enhances mood (Dallman et al. 2003) particularly in the highly activity females mice when they are not allowed to run in wheels, and through this mechanism could dampen anxiety and/or could replace diminished reward from abstinence of wheel running. In control females on the other hand, the HFS diet may have adverse effects on its own, which would be consistent with the study of Souza et al. (Souza et al. 2007).

If a diet is mood-enhancing, it would be of interest to test whether they also select more of it when given a choice of diets (la Fleur et al. 2007). We observed that the high-activity mice given a choice between the LF and HFS diet generally selected more of the LF diet than the line 2 mice did. The relative higher LF diet preference in the highly active mice was evident in males, and in females only in line 8. This differs slightly with the findings in Chapter 3, where particularly the line 7 females selected more the LF diet (although line 8 was not tested under those circumstances). Thus, if the HFS diet lightens up line 7 and line 8 animals (i.e., resulting in less anxiety in the plus maze test), this apparently does not correspond with the direct appreciation of the diet. While it is difficult to dissociate "wanting" and "liking" effects (Berridge, Robinson, and Aldridge 2009) of the diets in a two-hour diet choice test, one might speculate that the HFS diet gives less immediate reward in the high activity mice than the LF diet, but may enhance mood and dampens anxiety on the long-term. Another reason for selecting a certain diet could be that its macronutrient content is metabolically more appropriate (see Chapter 3). The present study does not confirm nor reject the latter possibility.

The differences in anxiety behavior and food selection were not reflected by differences in plasma corticosterone responses in the novel cage test in the present study. Line 7 males appeared to have significantly lower levels of plasma corticosterone than line 2 males irrespective of diet, but no differences were observed in the females between lines and diets. Thus, although Girard et al. found evidence for suppressed stress sensitivity in the selection mice compared to the controls (Malisch et al. 2008; Girard and Garland, Jr. 2002) - an effect which would be in line

with reports showing stress relief as a result of physical activity (Salmon 2001) - we did not unequivocally confirm these ideas in the present study.

In summary, activity selected mice were resistant to weight gain when chronically subjected to a HFS diet, and changes in RMR and NEAT contributed to this effect. Male control mice, on the other hand, increased body weight on this HFS diet. The resistance to weight gain in the activity selected mice did not parallel resistance to the effects of the HFS diet on affective traits. On the contrary, the levels of anxiety and appreciation of the diet were different between control and selected mice in the LF condition, but moreover differentially regulated in the HFS diet condition. Furthermore, if animals in the present study would have been housed in cages with access to running wheels, it is quite possible that anxiety levels in the LF diet condition would have been lower in the activity selected animals (Salmon 2001). In the present study, the highly active mice seem to recover by feeding a HFS instead, without necessarily appreciating this diet more than control mice do. As such, physical activity and nutrition may be regarded as default systems that determine mental and physical health.

References

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