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ORIGINAL ARTICLE

Metabolic and behavioral responses to high-fat feeding in mice selectively bred for high wheel-running activity

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Objective: Increased dietary fat intake is a precipitating factor for the development of obesity and associated metabolic disturbances. Physically active individuals generally have a reduced risk of developing these unhealthy states, but the underlying mechanisms are poorly understood. In the present study, we investigated the effects of feeding a high-fat diet (HFD) on obesity development and fuel homeostasis in male and female mice with a trait for increased physical activity and in their controls.
Methods: Male and female mice selectively bred for a high level of wheel running behavior over 30 generations and nonselected controls (background strain Hsd:ICR) were maintained on a standard lab chow high-carbohydrate diet (HCD) or on an HFD (60% fat). Food intake, body weight, indirect calorimetry parameters, spontaneous locomotor activity and several hormones relevant to metabolism and energy balance were measured.
Results: On HFD, mice reduced food intake and increased body fat mass and plasma leptin levels, with the notable exception of the selected females, which increased their ingested calories without any effects on body mass or plasma leptin levels. In addition, they had an elevated daily energy expenditure (DEE), increased spontaneous cage activity (~700% relative to controls) and higher resting metabolic rate (RMR) on the HFD compared with feeding the HCD. The selected males also had a higher DEE compared with controls, but no interaction with diet was observed. On HCD, adiponectin levels were higher in selected male, but not female, mice relative to controls. A marked increase in the level of plasma adiponectin was observed on the HFD in selected females, an effect of diet that was not observed in selected males.
Conclusion: Genetically based high locomotor activity renders female, but not male, mice resistant to HFD-induced obesity by alterations in behavioral, endocrine and metabolic traits that facilitate fat utilization rather than limiting HFD intake.
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Introduction

Obesity is becoming an increasingly prevalent health problem among individuals in affluent societies, because it is often associated with metabolic derangements such as impaired glucose tolerance, insulin resistance, high blood pressure, dyslipidemia and abdominal obesity. When these metabolic abnormalities are displayed in concert (often referred to as the 'metabolic syndrome'), they have a high risk of developing into life-threatening conditions such as cardiovascular disease and diabetes mellitus type 2 (for review, see Moller and Kaufman[1]). Consensus exists that increased dietary fat intake in combination with a sedentary lifestyle are precipitating factors (World Health Organization),[2] but the underlying endocrine and metabolic mechanisms are poorly understood.

A major part of the current knowledge on the etiology of obesity and the metabolic syndrome has come from studies on rodents subjected to a high-fat diet (HFD).[3–7] In particular, the use of selectively bred and genetically

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engineered rodents that display diet-induced obesity (DIO) or obesity resistance has provided considerable insight about underlying mechanisms (for reviews, see Caroll *et al*.[8] and Tschop and Heiman[9]) Reports on whether and how physical activity could be involved in preventing dietary fat-induced obesity and associated metabolic derangements are less abundant. In part, this is attributable to the methodological problems that can arise when animals are 'forced' to be active. For example, forced treadmill running, water immersion and so on.[10,11] are perceived as psychological stress,[12] which may complicate interpretation of results. A preferred method is the use of running wheels in which rodents can run voluntarily. Through this approach, Bell *et al*.[13,] for example, previously reported that spontaneous wheel running behavior was able to treat DIO. In addition, Patterson *et al*.[14] observed that 3 weeks of wheel running behavior at the juvenile stage delays DIO in selectively bred DIO-prone rats, probably by favorably altering hypothalamic pathways controlling energy balance.

In this study, we took an alternative approach by investigating the synergistic effects of dietary fat intake and spontaneous home cage activity (i.e., without running wheels) on energy balance in mice that were selectively bred for high voluntary wheel running relative to control lines (for selection procedure, see[15]). Mice selectively bred for increased wheel-running activity are leaner than control line animals and have relatively low circulating leptin levels but high adiponectin levels.[16–18] Even without the presence of a running wheel, these genetically selected animals display increased locomotor activity,[19] and therefore may represent a relevant model for the study of increased spontaneous activity in the development of HFD-induced obesity and metabolic derangements. Recently, we observed that males from this breeding line have higher plasma adiponectin levels in the adult and aged state when they were eating a regular high-carbohydrate diet (HCD) and this difference was found in animals with or without access to running wheels.[15] Given that adiponectin is known to facilitate triglyceride breakdown,[20–22] it might be hypothesized that these animals are resistant to HFD-induced obesity—over and above the effects of increased energy expenditure caused by elevated spontaneous activity. Thus, in this study, female and male mice from a line bred for high spontaneous wheel-running activity and from a nonselected control line were exposed to an HFD with two main objectives: (1) to test the hypothesis that a trait for high spontaneous physical activity can cause resistance to obesity and other indicators of the metabolic syndrome (2) and to investigate the potential behavioral, endocrine and metabolic mechanisms underlying this resistance.

Materials and methods

Animals and housing

Eighty breeding pairs of mice (*Mus domesticus*) that had been selectively bred for high wheel-running activity for 30 generations (S mice) and their nonselected controls (C mice) were obtained from T Garland Jr and colleagues. (for a detailed description of the selection procedure see[15]). From these founder mice, the eight separate breeding lines (four selected and four control) were continued at our facilities in Haren, NL, USA without further selection for wheel running. Animals in this study were fourth generation offspring from one control line and one selected line (lab designated lines 2 and 7, respectively).

At 5 months of age, a total of forty-eight mice (25 male and 23 female) were individually housed in standard cages (Macrolon type 2, UNO Roest Vast Staal BV, Soest, NL, USA) in a room with an ambient temperature of $22 \pm 1\,^{\circ}C$ and 12:12 light–dark cycle (lights on at 8:00, GMT + 1). Animals were fed *ad libitum* on a HCD (standard lab chow for Rat-Mouse-Hamster; RMH-B 2181), Hope Farms BV, Woerden, NL, USA. Wood shavings and EnviroDry® (BMI, Helmond, Netherlands) were used as bedding material.

Food intake and body mass were measured daily by weighing food hoppers corrected for spillage found in the cage. After 4 weeks, half of the mice were put on an HFD. Sixty percent of the energy content of the HFD came from fat, compared to only 14% in the HCD (Table 1). Importantly, the total energy content per gram of food is similar for both diets.

Respirometry

After 11–13 weeks on their respective diets, animals underwent metabolic measurements. Mice were housed in respirometric chambers to determine oxygen consumption (VO_2, $l\,h^{-1}$) and carbon dioxide production (VCO_2, $l\,h^{-1}$) for 48 h. To reduce novel cage stress, the respirometric chambers ($45 \times 25 \times 30$ cm) were adapted to accommodate the home cage of the animal. They, therefore, did not need to be handled and stayed in their home cages during the entire measurement. Animals were measured at an ambient

Table 1 Composition of the diets

	High-carbohydrate diet		*High-fat diet*	
	Content ($g\,kg^{-1}$)	*Energy* (%)	*Content* ($g\,kg^{-1}$)	*Energy* (%)
Protein	**228**	**23**	**200**	**20.4**
RMH-B lab chow	228		69	
Added casein			131	
Fat (saturated fat)	**55 (0.7)**	**14**	**260 (94.3)**	**60.2**
RMH-B lab chow	55		17	
Added corn oil			74	
Added beef tallow			169	
Carbohydrates	**625**	**63**	**190**	**19.4**
RMH-B lab chow polysaccharides	600		182	
Simple sugar	25		8	
Energy content	**16.1 kJ g^{-1}**		**16.3 kJ g^{-1}**	

temperature of 22 °C, and food (HCD or HFD) and water were provided *ad libitum*.

In this setup, eight animals could be measured simultaneously. Oxygen and carbon dioxide concentration of dried inlet and outlet air (drier: molecular sieve 3 Å, 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 ($601\,h^{-1}$) was measured with a mass-flow controller (Type 5850 Brooks). Ambient temperature in the chamber and cage, as well as activity (with a passive infrared (PIR) detector on the lid of the cage, Optex Wonderex FX-35) were measured simultaneously. Data were collected for each mouse over a 1-min interval every 10 min and automatically stored on a computer.

Heat production (HP; $kJ\,h^{-1}$) was calculated using the following equation: $HP = (16.18 \times VO_2) + (5.02 \times VCO_2)$.[23] Resting metabolic rate (RMR, $kJ\,h^{-1}$) was defined as the lowest value of HP calculated as the running mean over half an hour (i.e., three consecutive measurements) when animals were inactive (as determined by PIR activity), and was calculated for the first and second day in the respirometer separately. Maximal HP was also calculated as the running mean over half an hour. In addition, the average HP (daily energy expenditure: DEE, $kJ\,d^{-1}$), respiratory quotient ($RQ = VCO_2/VO_2$) and activity were calculated for both consecutive days.

Body composition and metabolic hormones

Following indirect calorimetry, mice were anesthesized (in the middle of the light phase) by brief CO_2 exposure and decapited. Trunk blood was collected in tubes with 10 µl ethylenediaminetetraaceticacid (EDTA) solution (9g/100ml water) as anticoagulant and organs and specific fat pads (i.e., retroperitoneal fat, gonadal fat and subcutaneous fat) were dissected out and weighed to the nearest 0.0001 g. All tissues were stored at −20 °C until further analyses. Blood samples were centrifuged at 2600 *g* for 15 min at 4 °C, plasma was then collected and stored at −80 °C for later analysis with RIA kits from Linco Research, Inc., St Charles, MI, USA (#RL-83K for leptin, #MADP-60HK for adiponectin, #RL-13K for insulin) and from Immunotech, Prague, Czech Republic (#IM1447-IM3286 for T4, and #IM1699-IM3287 for T3). Intra- and interassay coefficients of variation of reference plasma analyzed in duplicate of aforementioned hormones ranged between 6.5–11.3 and 10.3–16.8%, respectively. Dry and dry lean organ masses were determined by drying organs to constant mass at 103 °C (ISO 6496-1983(E)) followed by fat extraction with petroleum ether (Boom BV, Meppel, NL, USA) in a soxhlet apparatus.

Data analysis

Because males and females are known to differ with respect to activity levels, the hormonal regulation of metabolism and many other characteristics data for males and females were analyzed seperately. To test for effects of diet (HCD vs HFD) and line (selection vs control), general linear models in SPSS (version 12) were used. Line, diet and line × diet were fixed factors. Where appropriate, covariates (e.g., body mass) were used in the models. *Post hoc* independent *t*-tests were used to compare lines and diets. For several traits, repeated measures analysis of variance (ANOVA) was applied. Line, time (repeated factor) and line × time were used as fixed factors in these models. The significance level was set at $P<0.05$ and all tests were two-tailed.

Results

Changes in food intake and body mass

In the 4 weeks before the diet manipulation, while all mice were still on HCD, some differences in body mass and food intake were apparent between control and selected mice (data not shown, but see day 0 in Figure 1). The selected females, but not males, had a significantly lower body mass compared with control females ($P<0.01$), and both selected males and females had higher food intake compared with their respective controls. ($P<0.01$). Figure 1 shows daily changes in body mass and food intake of all mice from the start of the diet manipulation. In female mice, the HFD significantly influenced body mass, and there was a significant interaction between line and diet indicating that control and selected mice responded differently to the HFD. As seen in Figure 1, female control mice increased body mass on the HFD, whereas the body mass of selected females did not change. Food intake was not significantly affected by diet in females overall. In males on HFD, body mass significantly increased and food intake significantly decreased to a similar extent in control and selected mice.

Closer inspection of the day-by-day changes in body mass of individual animals, and the associated food intakes, revealed differences in food efficiency (FE) between control and selected females. With the energy content of the food being similar to the HCD and HFD, FE can be expressed as the mean amount of food ($g\,d^{-1}$) needed to maintain constant body mass (i.e., when energy expenditure equals energy intake; Figure 2) To maintain a stable body mass, control females ate less food ($g\,d^{-1}$) on the HFD than on the HCD. They thus obtained more energy from the HFD and had a greater FE on the HFD. The selected females, on the other hand, had slightly higher food intake on the HFD than on HCD and FE was thus not enhanced on the HFD. As in control females, both lines of males were able to maintain body mass on the HFD with less food than they needed on standard chow, thus, indicating the increased FE on the HFD relative to HCD (Figure 2).

Energy expenditure

After 11–13 weeks on the diets, all animals were put in respirometric chambers for 48 h to determine RMR, DEE,

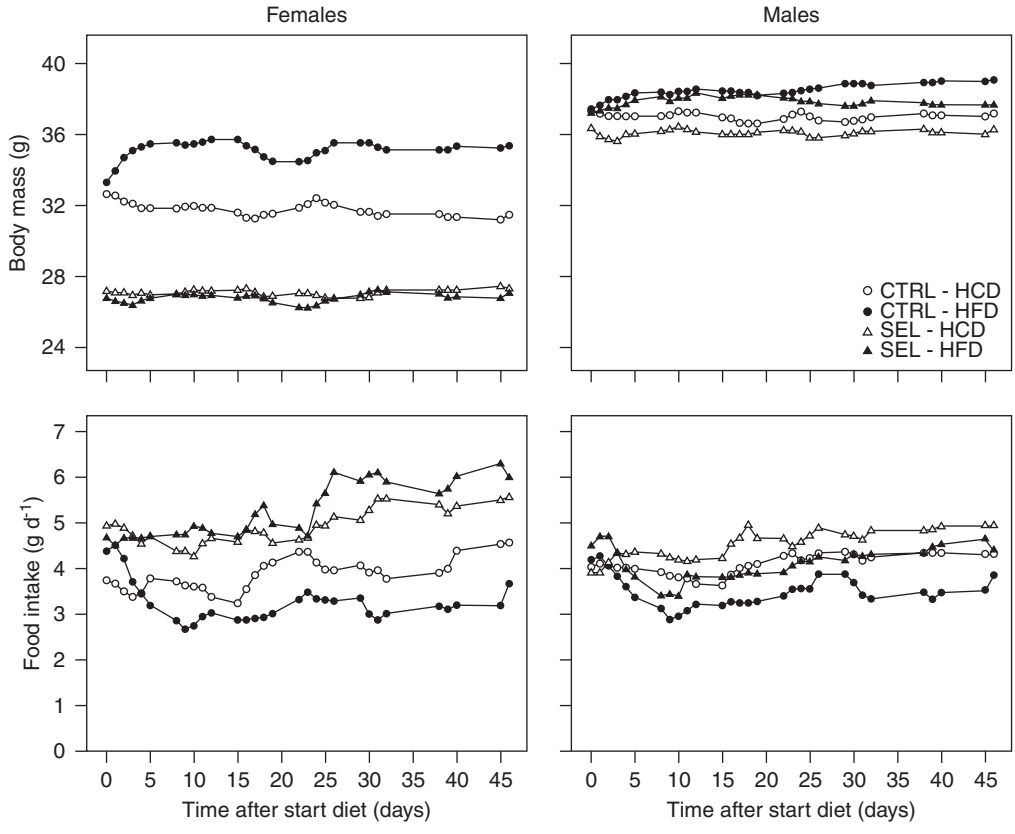

Figure 1 Body mass (top graphs) and food intake (bottom graphs) in male (right) and female (left) control (circles) or selected mice (triangles) in response to diet manipulation. Data are shown for the first 6 weeks after the manipulation. At day 0, animals were put on a high-fat diet (HFD; black symbol) or remained on standard high-carbohydrate chow diet (HCD; open symbols). Repeated measures analysis of variance (ANOVA) was performed to compare body mass and food intake of mice in the week before and after being put on the HFD for 4 weeks. In female mice, the HFD significantly influenced body mass ($F_{1,10} = 11.4$, $P < 0.01$) and there was a significant interaction between line and diet ($F_{1,10} = 7.5$, $P < 0.05$). Food intake (corrected for body mass) was not significantly affected by diet in females. In males, the HFD significantly affected body mass ($F_{1,11} = 10.2$, $P < 0.01$) and food intake ($F_{1,11} = 17.1$, $P < 0.01$), but there was no line x diet interaction for either variable.

spontaneous locomotor activity and RQ (see Tables 2 and 3). Data from day 2 were used in the analysis, because this day did not include novelty effects of first exposure to the respirometry cage. In females, there were no effects of line or diet on RMR. However, when body mass was added to the model as a covariate, selected females had a significantly higher RMR than control females. DEE was also significantly increased in selected females compared with controls (with or without body mass as a covariate). In both lines, mean DEE increased on the HFD, but this effect was stronger in the selected,females, as shown by a significant interaction effect between line and diet. Results for maximal HP were similar to those for DEE, with higher estimated HP in selected females compared with controls, and the highest levels in selected females on the HFD. In males, there was no effect of line on RMR, but HFD significantly increased RMR in both control and selected males. DEE was increased in selected males compared with controls and the HFD increased DEE in control as well as selected males. As in females, maximal HP was higher in selected males compared with controls, but in males, there was no interaction effect with diet.

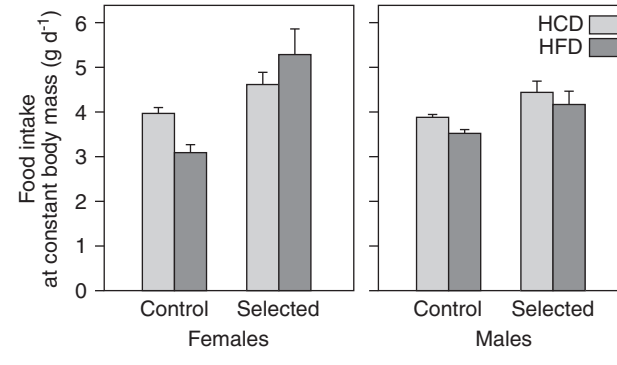

Figure 2 Food efficiency in control and selected mice on high-fat diet (HFD; dark gray) and standard high-carbohydrate chow diet (HCD; light gray). Food efficiency is expressed as the mean amount of food ($g\,d^{-1}$) needed to maintain constant body mass (weight change between −0.25 and 0.25 g on consecutive days) and was calculated over the first 6 weeks on the diet. Data were analyzed by two-way analysis of variance (ANOVA), with line, diet and line × diet as fixed factors. In addition to an effect of line in both sexes (females: $F_{3,19} = 14.5$, $P < 0.01$; males: $F_{3,19} = 8.8$, $P < 0.01$), there was a significant line × diet interaction in females ($F_{3,19} = 4.4$, $P < 0.05$) and a significant effect of diet in males ($F_{3,21} = 6.6$, $P < 0.05$).

Table 2 Metabolic rates of mice from a line selected for high wheel-running activity and their nonselected controls after 11–13 weeks on high-carbohydrate diet (HCD) or high-fat diet (HFD)

	Control		*Selected*	
	HCD	*HFD*	*HCD*	*HFD*
Females				
Body mass (g)	31.4 (1.8)	35.1 (1.4)	26.8 (0.9)	28.0 (0.7)
RMR (kJ d^{-1})	35.8 (4.1)	38.6 (2.2)	36.4 (1.7)	40.2 (1.4)
DEE (kJ d^{-1})	47.8 (4.6)	50.9 (2.5)	59.0 (3.5)	70.5 (3.2)
Maximum HP (kJ d^{-1})	74.3 (9.7)	72.8 (5.1)	84.1 (6.7)	113.8 (6.3)
RQ	0.93 (0.01)	0.76 (0.03)	0.87 (0.01)	0.76 (0.02)
Activity (number per day)	1249 (254)	1047 (155)	5845 (1705)	7643 (1211)
Males				
Body mass (g)	37.2 (1.3)	39.9 (1.1)	36.4 (1.6)	39.2 (2.3)
RMR (kJ d^{-1})	38.7 (1.7)	41.9 (1.7)	38.2 (2.0)	46.0 (0.6)
DEE (kJ d^{-1})	51.1 (2.3)	54.1 (1.6)	59.9 (7.1)	67.6 (3.3)
Maximum HP (kJ d^{-1})	72.5 (5.5)	73.5 (1.6)	86.1 (7.4)	103.9 (10.4)
RQ	0.90 (0.01)	0.75 (0.01)	0.88 (0.01)	0.75 (0.01)
Activity (number per day)	784 (238)	1109 (147)	1115 (151)	3453 (1681)

Abbreviations: DEE, daily energy expenditure; RMR, resting metabolic rate; RQ, respiratory quotient. Values were highly repeatable on the 2 consecutive days in the respirometer (Pearson correlation: RMR: $r=0.91$, $P<0.001$; DEE: $r=0.90$, $P<0.001$) and the table shows values measured on day 2 of measurements (24 h period). Mean and (s.e.m.) are given for body mass (average of masses measured at entering and leaving the respirometric cage), metabolic rates, RQ and activity. RMR is the lowest value of metabolic rate at rest (inactive) measured in this period (running means of 30 min.). The other values represent the mean over the whole 24 h period. $N=6$ per group, with the exception of control females on a HCD ($n=4$), control males on a HCD ($n=5$) and control males on a HFD ($n=7$).

Table 3 Respirometry, results of two-way ANOVA

Trait	*Model*		*Line*		*Diet*		*Line × diet*			*Covariate*
	N	*d.f.*	F	P	F	P	F	P		P
Females										
RMR (kJ d^{-1})	22	3.21	0.2	0.684	1.6	0.224	0.0	0.853		
	22	4.21	9.5	**0.007**	0.0	0.899	1.0	0.322	Mass	**0.001**
DEE (kJ d^{-1})	22	3.21	9.0	**0.008**	3.6	0.074	3.2	0.091		
	22	4.21	33.9	**<0.001**	0.8	0.372	10.3	**0.005**	Mass	**0.001**
Maximum HP (kJ d^{-1})	22	3.21	12.6	**0.002**	3.9	0.065	4.8	**0.042**		
	22	4.21	40.8	**<0.001**	1.0	0.330	14.1	**0.002**	Mass	**0.001**
RQ	22	4.21	11.5	**0.003**	195.4	**<0.001**	7.3	**0.015**		
Activity (number per day)	22	4.21	16.2	**0.001**	0.3	0.574	0.5	0.482		
Males										
RMR (kJ d^{-1})	24	3.23	1.3	0.373	11.4	**0.003**	1.0	0.173		
	24	4.23	2.1	0.161	7.1	**0.015**	2.3	0.143	Mass	**0.036**
DEE (kJ d^{-1})	24	3.23	10.4	**0.004**	4.9	**0.039**	2.3	0.144		
	24	4.23	9.8	**0.006**	6.4	**0.020**	2.4	0.137	Mass	0.233
Maximum HP (kJ d^{-1})	24	3.23	10.2	**0.005**	1.9	0.186	1.5	0.236		
	24	4.23	9.5	**0.006**	2.6	0.120	1.5	0.234	Mass	0.340
RQ	24	3.23	0.4	0.553	180.7	**<0.001**	1.2	0.278		
Activity (number per day)	24	3.23	2.4	0.136	2.4	0.137	1.4	0.255		

Abbreviations: ANOVA, analysis of variance; DEE, daily energy expenditure; HP, heat production; RMR, resting metabolic rate; RQ, respiratory quotient. Data recorded on day 2 of the respirometric measurement were analyzed with a two-way ANOVA with line (selected vs control line), diet (standard chow vs HFD) and line × diet (LxD) as fixed factors for females and males separately. Body mass is known to have a strong influence on metabolic rate and was added into the model as a covariate for these traits. Significant effects are shown in bold ($P<0.05$).

The selected mice were far more active than controls in the cage respirometers (Tables 2 and 3), which is in agreement with their higher DEE. These differences attained statistical significance in females, but not in males. When fed the HFD, control males and females did not show major differences in spontaneous activity compared with controls feeding the HCD. In contrast, selected males and females fed the HFD further increased spontaneous activity to ~300 and 700%, respectively, relative to controls (Table 2). These effects, however, did not attain statistical significance because of a relatively large degree of variation.

An RQ around 1 indicates that mice mainly utilize glucose as a metabolic substrate, whereas at RQ values around 0.7, mice are mainly utilizing fat. As expected, RQ was lower in all mice on the HFD (Tables 2 and 3). In females, but not in males, there was a significant effect of line and an interaction

between line and diet. *Post hoc* analyses revealed that the selected females on chow had lower RQ values than controls on the HCD (*t*-test, $P<0.05$), whereas RQ values did not differ between control and selected females on the HFD. RQ was similar in control and selected males on both diets.

Body composition

Following indirect calorimetry, seperate fat pads were collected and weighed to determine the distribution of fat deposition throughout the body (Tables 4 and 5). In control but not in selected females, the amount of fat increased in all fat pads (but not in organs) when put on the HFD. On the HFD, control females increased their total fat content by ~40%, whereas total fat content only increased by 5% in selected females. The distribution of fat (percentage of total fat) over the different fat pads was similar in control and selected females on standard chow. On an HFD, however, control but not selected females increased their relative parauterine fat pad weight of the total fat content from ~2% on the standard diet to 14% on the HFD. The other fat pads remained similar in controls.

In both control and selected males, there was an increase of ~25% in the total amount of fat in the separate fat pads (except for organ fat; see Tables 4 and 5) on the HFD. The distribution of fat over the different depots in the body was similar in both lines when they were on the HCD. Interestingly, the selected males on the HFD stored more fat in the intraperitoneal and epididymal compartment compared with controls (Tables 4 and 5).

Table 4 Fat content in mice selected for high wheel-running activity and their nonselected controls after 17–18 weeks on a high-carbohydrate diet (HCD) or a high-fat diet (HFD)

Mass (g)	*Control*		*Selected*	
	HCD	*HFD*	*HCD*	*HFD*
Females				
Body mass	33.9 (2.0)	43.6 (2.8)	27.3 (2.8)	29.2 (1.1)
Dry lean mass	7.2 (0.4)	7.4 (0.3)	6.3 (0.3)	6.2 (0.2)
Total fat	21.3 (1.3)	29.4 (2.2)	16.6 (1.7)	17.5 (0.5)
Parauterine fat	0.42 (0.12)	4.03 (1.19)	0.13 (0.08)	0.21 (0.05)
Retroperitoneal fat	0.10 (0.02)	0.48 (0.12)	0.04 (0.02)	0.09 (0.01)
Subcutaneous fat	3.4 (0.2)	6.0 (0.7)	2.5 (0.6)	2.5 (0.2)
Intramuscular fat	12.9 (0.8)	14.6 (0.6)	10.1 (0.9)	10.9 (0.3)
Organ fat	4.5 (0.3)	4.3 (0.3)	3.9 (0.2)	3.7 (0.1)
Males				
Body mass	39.4 (1.8)	43.5 (1.5)	37.1 (1.9)	43.0 (3.2)
Dry lean mass	8.2 (0.2)	8.3 (0.2)	7.7 (0.3)	7.9 (0.2)
Total fat	28.0 (1.7)	33.9 (1.9)	25.7 (2.0)	33.5 (3.5)
Epididymal fat	0.71 (0.11)	1.23 (0.21)	0.66 (0.19)	1.85 (0.40)
Retroperitoneal fat	0.22 (0.06)	0.45 (0.14)	0.24 (0.08)	0.76 (0.20)
Subcutaneous fat	4.6 (0.5)	6.4 (0.7)	3.5 (0.5)	6.2 (1.1)
Intramuscular fat	17.7 (1.0)	21.0 (0.8)	16.5 (1.2)	20.0 (1.9)
Organ fat	4.8 (0.2)	4.8 (0.2)	4.8 (0.1)	4.7 (0.2)

Mean and s.e.m. are given in the table. Values given for the separate fat pads include the water content. Gonadal (parauterine and epididymal) and retroperitoneal fat were dissected out and weighed. Mass of intramuscular fat was the fat content of the musculoskeletal system measured by fat extraction in a soxhlet apparatus. Organ fat was the sum of the fat contents measured for heart, liver, kidney, stomach, intestines, lung and spleen. $N=6$ per group, with the exception of control and selected females on an HCD ($n=4$ and $n=5$ respectively), control males on an HCD ($n=5$) and control males on an HFD ($n=7$).

Metabolic hormones

Plasma samples collected at decapitation were analyzed for the concentration of several metabolic hormones and the results are shown in Figure 3 (for adiponectin, insulin and leptin) and Figure 4 (for T3 and T4). No differences in hormone levels were observed between control and selected mice on the HCD, with the exception that adiponectin levels were significantly increased in selected males compared with controls on both diets. On the HFD, plasma adiponectin levels increased in selected females, an effect not observed in control females or males. Insulin levels were increased in control females on the HFD and this effect was not found in selected females. In both females and males, a significant effect of diet on leptin levels was observed *Post hoc* tests showed that in control mice, but not in selected mice, leptin was significantly increased in mice on the HFD compared with those fed HCD. In addition to the effect of diet in females, a significant effect of line and a significant interaction between line and diet was shown. This indicates that the control females increased their leptin levels more than the selected females did on the HFD, which is also apparent in Figure 3. Leptin highly correlated with fat content, and when total fat content was added to the model as a covariate, no significant effects of line, diet or line x diet remained.

Figure 4 shows plasma levels of T3 and T4. Plasma T3 levels were similar in control and selected females on both diets. In selected males on HCD, T3 levels were decreased compared with control males on the HCD. On the HFD, plasma T3 levels increased significantly in selected males compared with selected males on HCD (*Post hoc* t-test, $P<0.05$). T3 levels were similar on both diets in control males. On the HCD, plasma T4 levels were decreased in selected males and females compared with controls, but this effect did not reach statistical significance ($P=0.08$). On the HFD, T4 levels increased in selected mice up to a similar level as that of controls. This effect was significant in selected males (*Post hoc t*-test; $P=0.05$), but not in females. Both male and female control mice had similar levels of T4 on both diets.

Discussion

A high fat content in the diet increases weight gain and the risk of developing symptoms of the 'metabolic syndrome' in a number of species, including human beings[2,24] and various rodents.[3–6] This study investigated whether and how such an effect may be influenced by physical activity. For this

Table 5 Body composition, results of two-way ANOVA

Trait	*Model*		*Line*		*Diet*		*Line × diet*			*Covariate*
	N	*d.f.*	F	P	F	P	F	P		P
Females										
Body mass	21	3.20	20.2	**<0.001**	6.1	**0.024**	2.7	0.117		
Dry lean mass	21	3.20	11.3	**0.004**	0.1	0.737	0.2	0.640		
Total fat	21	4.20	8.4	**0.01**	8.3	**0.011**	4.9	**0.042**	Drylean	**0.026**
Parauterine fat	21	4.20	6.7	**0.020**	6.8	**0.019**	6.3	**0.023**	Drylean	0.561
Retroperitoneal fat	21	4.20	3.8	0.069	7.4	**0.015**	4.5	**0.048**	Drylean	0.663
Subcutaneous fat	21	4.20	5.8	**0.028**	5.8	**0.029**	5.2	**0.036**	Drylean	0.189
Intramuscular fat	21	4.20	8.3	**0.011**	9.3	**0.008**	0.1	0.711	Drylean	**<0.001**
Organ fat	21	4.20	0.1	0.749	2.4	0.134	0.4	0.528	Drylean	**<0.001**
Males										
Body mass	24	3.23	0.4	0.535	5.1	**0.036**	0.2	0.662		
Dry lean mass	24	3.23	3.8	0.064	0.5	0.469	0.0	0.851		
Total fat	24	4.23	1.4	0.239	10.1	**0.004**	0.1	0.725	Drylean	**<0.001**
Epididymal fat	24	4.23	6.3	**0.021**	11.5	**0.003**	2.1	0.165	Drylean	**0.005**
Retroperitoneal fat	24	4.23	4.3	**0.050**	6.6	**0.019**	1.0	0.312	Drylean	**0.037**
Subcutaneous fat	24	4.23	0.3	0.611	9.2	**0.007**	0.3	0.602	Drylean	**0.002**
Intramuscular fat	24	4.23	0.8	0.377	9.7	**0.006**	0.1	0.872	Drylean	**<0.001**
Organ fat	24	4.23	0.7	0.416	0.6	0.453	0.1	0.881	Drylean	**0.03**

Abbreviation: ANOVA, analysis of variance. Data on body composition were analyzed with a two-way ANOVA with line (selected vs control), diet and line × diet as fixed factors for females and males separately. Dry lean mass was added as a covariate when testing for differences in fat mass, and *P*-values are given in the table. Significant effects are shown in bold ($P \leqslant 0.05$).

purpose, we used a line of mice that had experienced long-term selective breeding for high spontaneous wheel-runnning activity and compared it with a nonselected control line. Even in the absence of running wheels, animals from the activity-selected line have been reported to display increased spontaneous activity.[19,25] In this study, we observed that the differences in spontaneous activity (recorded by infrared detection) between control and selected mice were most pronounced in females. When animals were given an HFD, spontaneous activity in the control line stayed the same, but activity in selected mice increased further to levels that were ~700% (females) and ~300% (males) higher than observed in respective controls subjected to an HFD. Consistent with the observation that the selected females fed HFD drastically increased their activity, these animals were almost entirely resistant to HFD-induced obesity. In contrast, control females accumulated ~40% body fat following HFD feeding relative to those fed on the HCD. In selected and control males on the HFD, there were comparable increases in total body fat content of ~25%. Interestingly, the distribution of fat storage in the selected mice occurred more in the visceral compartment than in control mice. This unexpected result seems counterintuitive at first, given that the increased physical activity is generally negatively associated with risk factors for the development of the metabolic syndrome in humans[26,27] and rodents.[28] However, because the visceral depot might overlap with the center of gravity of four-legged animals, we can speculate that fat storage in this area, rather than other areas, may be relatively advantageous to facilitate sustained endurance running behavior.

Consistent with the generally higher level of spontaneous activity in selected mice, we found that the selected females had increased intake of the HCD relative to control mice, particularly when controlling for differences in body mass. No differences in HCD intake were observed between control and selected males. When switched to an HFD, control females and both lines of males showed a considerable decrease in food intake. Food intake usually decreases when animals are switched to an HFD, and they apparently require less calories as fat to maintain energy balance compared with calories from carbohydrates.[6,29] In the control males and females, and to a lesser extent also observed in the selected males, the decrease in intake of HFD was not sufficient to prevent the gain of body fat mass. The selected females, however, showed an entirely different response to the HFD: their food intake actually slightly increased, whereas body mass remained constant (see Figure 1). This means that FE on the HFD was reduced in the selected females. FE is, among other factors, determined by physical activity as well as RMR. We, therefore, performed indirect calorimetry combined with infrared locomotion detection, which allowed us to dissociate RMR from DEE. Consistent with our expectation was the finding that the selected mice had increased DEE compared with control mice when correcting for body mass. Again, this difference was most pronounced in females, which also showed the largest differences in overall spontaneous activity. In addition, the selected females, but not the selected males had a higher RMR than control animals. Thus, both RMR and spontaneous activity-related metabolic rate contributed to the overall increased DEE in selected females relative to controls. Spontaneous activity-related metabolic

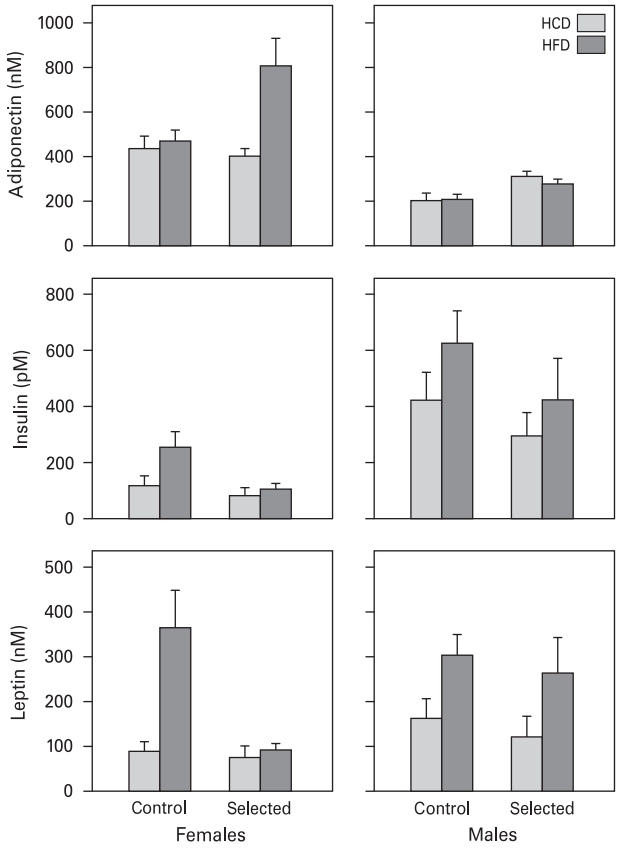

Figure 3 Plasma adiponectin, insulin and leptin levels of control and selected, male (right graph) and female (left graph) mice after 17–18 weeks on high-fat diet (HFD; dark gray) or standard high-carbohydrate chow diet (HCD; light gray). Values are means plus one standard error. Two-way ANOVAs were performed to analyze data. In females, diet significantly affected adiponectin levels (line; $F_{1,16}=3.0$, $P>0.1$, diet; $F_{2,16}=6.4$, $P<0.05$, line × diet; $F_{2,16}=4.2$, $P=0.057$), an effect mainly driven by the strong increase in adiponectin levels on the HFD in selected females. In males, line, but not diet significantly affected adiponectin levels (line; $F_{1,21}=7.2$, $P<0.001$, diet; $F_{2,21}=0.3$ $P>0.1$, line × diet; $F_{2,21}=0.6$, $P>0.1$). Leptin levels were significantly affected by line ($F_{1,16}=6.7$, $P=0.02$), diet ($F_{2,16}=6.9$, $P<0.05$) and line × diet ($F_{2,16}=5.3$, $P<0.05$) in females. Leptin levels did not differ between control and selected males, but a significant effect of diet was observed in both lines ($F_{2,21}=8.0$, $P=0.01$). Leptin highly correlates with fat content ($P<0.001$), and when total fat content was added to the models as a covariate, no significant effects remained. Plasma insulin levels increased in control females on HFD, an effect not observed in selected females (line; $F_{1,16}=4.8$, $P<0.05$, diet; $F_{2,16}=3.9$, $P=0.065$, line × diet; $F_{2,16}=1.6$, $P>0.1$). In males, no significant effects on insulin levels were found.

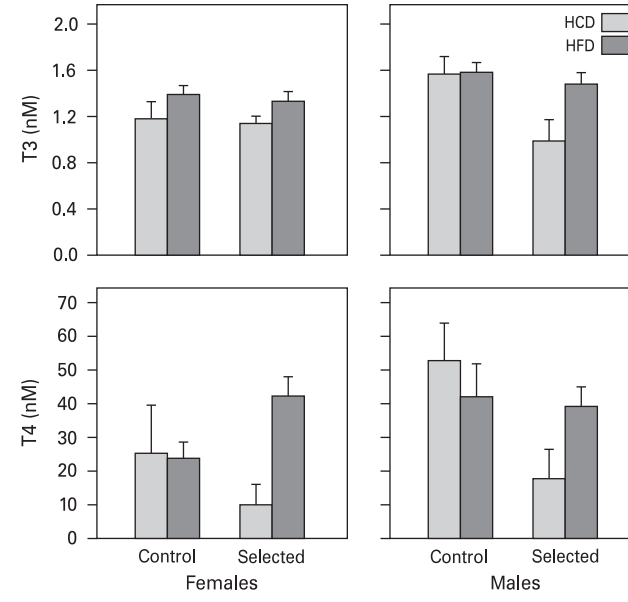

Figure 4 Plasma T3 and T4 levels of control and selected, male (right graph) and female (left graph) mice on high-fat diet (HFD; dark gray) and standard high-carbohydrate chow diet (HCD; light gray). Values are means plus one standard error. Two-way ANOVA showed no significant effects of line, diet or line × diet on T3 and T4 levels in females. In males, there was a significant effect of line ($F_{1,19}=6.8$, $P=0.017$) in T3 levels, but the effects of diet ($F_{1,19}=4.1$, $P=0.057$) and line x diet ($F_{1,19}=3.5$, $P=0.07$) did not reach significance. No significant effects were found on T4 levels (line; $F_{1,16}=3.9$, $P=0.067$ and diet; $F_{1,16}=0.3$, $P=0.58$, line x diet; $F_{1,16}=2.8$, $P=0.112$).

rate has previously been defined as 'NEAT' (nonexercise activity thermogenesis),[30] and has been implicated as an important factor in determining susceptibility or resistance to weight gain in humans as well laboratory rodents subjected to an HFD.[31,32]

Despite these increases in metabolism, control and selected males still increased body mass to a similar extent on the HFD. In control females, RMR and DEE were not increased on the HFD, which might explain the extra mass gain in control females compared with males. In conclusion, an increased RMR in combination with increased spontaneous activity-related metabolic rate in selected females resulted in the maintenance of constant body mass on an HFD in these mice. Apparently, the selected females cope with the dietary fat in an entirely different manner than control mice do. Given that the selected females already oxidized more fat, as was shown by a decreased RQ in these animals compared with control females on the HCD, this means that they have a higher capacity to utilize fat in their bodies. These outcomes are somewhat comparable to a recent study in rats selectively bred for high and low aerobic capacity, which showed that the latter have impaired transcription factors for mitochondrial biogenesis and the amount of oxidative enzymes in skeletal muscle.[33] Interestingly, a study of the selected and control lines of mice (fed standard chow) at 23–24 months of age suggests an environment-dependent difference in RQ for basal (i.e., fasted) metabolic rate in males but not in females.[34]

As has been observed in many other studies,[3,6,7] exposure to an HFD caused an increase in body fat content and an associated increase in plasma leptin levels in all animals of this study, with the expection of selected females. Previously, we found that the selectively bred mice had higher basal plasma adiponectin levels when feeding on the standard

HCD,[18] and this was replicated in this study. Whereas the selected females had no elevated levels of adiponectin compared with controls when feeding the HCD, they did have markedly augmented adiponectin levels when subjected to the HFD. This augmentation of adiponectin levels upon feeding the HFD was not observed in males of either line, nor in the control females. Adiponectin plays an important role in stimulating oxidation of lipids in liver and muscle.[21,22,35–37] It may therefore be argued that this mechanism underlies the differences in fuel oxidation in selected mice relative to that in control mice, and particularly in the condition of HFD given to the selected females. Besides increased adiponectin levels, responses in thyroid hormones may have contributed somewhat to the increased metabolic rate, but only in selected male mice on the HFD. Positive effects of physical activity on adiponectin levels have occasionally been reported in the human literature,[38] but the causal mechanism underlying this effect is unknown. It might be speculated that differences in central neural circuits that regulate behavioral arousal and spontaneous activity underlie changes in neuroendocrine activity/autonomic balance in peripheral tissues including adipose tissue.[39,40] In turn, these effects could lead to specific differences in synthesis and release of adipokines. As powerful stimulatory effects of estrogen replacement on circulating adiponectin levels have been observed in male gonadectomized pgtt-null mice,[41] a permissive role of gonadal hormones in the differential adipokine profile observed in selected male and female mice in the present study can be anticipated.

In conclusion, female mice from a breeding line artificially selected for high physical activity were fully resistant to HFD-induced obesity. Increases in spontaneous activity-related metabolic rate—by others refered to as NEAT—and RMR are probably important factors in these effects. Increased RMR (particularly at the expense of fat) can be related to a selective upregulation of circulating adiponectin levels. The selected males already had higher plasma adiponectin levels under conditions of HCD feeding, but this effect was not further augmented when feeding an HFD. The fact that these animals developed increased adiposity (particularly in the visceral depots) and hyperleptinemia may be a consequence of the failure to further augment circulating adiponectin levels. Thus, it may be argued that the selective breeding for high running-wheel activity leads to a correlated response of hyperactivity in regular cages (without wheels) and hyperadiponectemia during HFD feeding,[18,42] which underlie resistance to HFD-induced obesity in females, but not in males. Sex-specific effects of dietary fat are well-known in many polygenetic mouse models of DIO, and probably relate to differential profiles of gonadal hormones.[43] Future studies will focus on the central neural networks that potentially regulate behavioral arousal and adipokine profiles, and their potential interactions with gonadal hormones in control of fuel homeostasis and energy balance.

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