



## Factors influencing individual variability in high fat diet-induced weight gain in out-bred MF1 mice



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### HIGHLIGHTS

- Individual variability in high fat diet-induced weight loss was studied in MF1 mice.
- Pre-existing differences and changes in compensation were investigated.
- Fat free mass and sex predicted around 12% of the variability in body mass.
- Food intake during the 1st week of high fat feeding predicted 20% of the variability.
- Mice that gained more weight on high fat diet lost more when dietary restricted.

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### ABSTRACT

Easy access to high-energy palatable foods has been suggested to have contributed to the world-wide obesity epidemic. However, within these ‘obesogenic’ environments many people manage to remain lean. Mice also show variability in their weight gain responses to high-fat diet (HFD) feeding and their weight loss responses to calorically restricted (CR) feeding. In this study we investigated which factors contribute to determining susceptibility to HFD-induced obesity in mice, and whether the responses in weight gain on HFD are correlated with the responses to CR. One-hundred twenty four mice were exposed to 30% CR for 28 days followed by a 14 day recovery period, and subsequent exposure to 60% HFD for 28 days. Responses in various metabolic factors were measured before and after each exposure (body mass; BM, body composition, food intake; FI, resting metabolic rate; RMR, physical activity, body temperature and glucose tolerance; GT).

Weight changes on HFD ranged from  $-1$  to 26%, equivalent to  $-0.2$  g to 10.5 g in absolute mass. Multiple regression models showed that fat free mass (FFM) of the mice before exposure to HFD predicted 12% of the variability in weight gain on HFD ( $p < 0.001$ ). Also, FI during the first week of HFD feeding predicted 20% of the variability in BM and fat mass (FM) gain 4 weeks later. These data may point to a role for the reward system in driving individual differences in FI and weight gain. Weight gain on the HFD was significantly negatively correlated to weight loss on CR, indicating that animals that are poor at defending against weight gain on HFD, were also poor at defending against CR-induced weight loss. Changes in FM and FFM in response to HFD or CR were not correlated however.

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### 1. Introduction

The ‘obesogenic’ environment of modern society, with its high abundance of palatable energy-dense foods, has led to a gradual increase in the number of people that suffer from obesity and related diseases [12,23]. However, the response amongst people exposed to such ‘obesogenic’ environments is highly variable; some people are susceptible to weight gain while others remain lean [3]. Studying this individual

variability in responses could reveal processes of individual weight regulation and establish the biological factors that make people either susceptible or resistant to weight gain, which is crucial to increase our understanding of the aetiology of obesity. For example, in male Sprague Dawley rats on pure macronutrient or high fat diets, measures of weight gain, energy intake or fat preference are shown to vary considerably in direct proportion to ultimate body fat gain ([28,39,49], also see [55] in mice).

Substantial individual variability in weight loss is also observed in response to caloric restriction (CR) (e.g., humans [1,5], mice [47]). For instance, weight loss ranged from 1 to 36% in mice that had exposed to 30% CR for 4 weeks [47]. In this previous study, ~70% of the variation

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in weight loss could be attributed to individual variability in baseline food intake (FI), activity and resting metabolic rate (RMR) and changes in activity in response to CR. It is currently unclear if weight changes in response to high fat feeding (HFD) and CR are linked; i.e., are individuals that are poor at defending against weight gain also poor at defending against weight loss? Or does an individual that defends itself well against weight loss, defend itself poorly against weight gain? The dual intervention point model [33,42] suggests that body mass (BM) is regulated by an upper and lower intervention point above and below which physiological mechanisms are activated to maintain BM in the preferred range [42]. It is hypothesised that the upper and lower intervention points are potentially set by the risk of predation and starvation respectively, and it is assumed that they are independent. This model would thus predict no correlation between responses to over and under-nutrition. Alternatively, the general model of intake regulation [8], which hypothesises that energy intake is regulated by compensated factors (e.g., stomach content, hunger) and uncompensated factors (e.g., time of day, social factors), may predict a negative relationship between weight loss and gain. This model assumes that BM is maintained at a constant level until changes occur to a compensated or uncompensated factor, in response to which a new level of BM is reached and maintained. The magnitude of the change in BM depends on the magnitude of the individual response to the altered factor and individuals that respond strongly to changes in factors may be expected to be susceptible to both HFD and CR, whereas other individuals may be resistant to both.

Animal models have been developed that include HFD-induced obesity-prone and -resistant animals (rats [28,30,31,35] and mice [20]) and these animals have been shown to differ in their energy intake, glucose tolerance (GT), expression of (an)orexigenic neuropeptides and responses to CR [4,20,31,37]. These responses are generally observed after obesity has developed, and it is thus unclear whether they are the cause of differences in weight gain between animals fed HFDs, or a consequence of variable responses to the HFD. In addition, within these groups of animals (obesity resistant vs. obesity prone) individual variability in responses to HFD remains. Whether the extent to which individual variability prior to exposure of animals to HFD predisposes or protects individuals from weight gain has not been extensively studied in animal models (e.g., [3,28,55]). Zhang et al. [55] showed that pre-existing differences in baseline physical activity levels, lean BM and body fatness were all predictors of weight gain in C57Bl/6 mice when exposed to a HFD, but that baseline FI, body temperature (Tb) and lean BM were not. Some of the variation in these traits at baseline could be traced back to nutritional history during development.

The aim of this study was to investigate whether variation in pre-existing traits relevant to energy balance and body composition could predict variability in weight gain in response to HFD feeding. In addition, we aimed to establish whether individual responses in weight gain and weight loss are linked. We used an outbred mouse strain (i.e., MF1) to study these effects. MF1 mice vary considerably in their responses to CR and HFD and they develop age-related obesity on standard low-fat laboratory diets (10% kcal from fat); i.e., fat content is ~30% of BM at 6 months of age, compared to 10% in adult mice at 10 weeks of age [16]. These mice therefore provide a suitable model to investigate variability in responses to CR and HFD induced weight changes (see also [47]). Baseline FI, fat mass (FM), fat free mass (FFM), RMR, general physical activity, Tb and GT were selected as potential predictors. These predictors were selected because previous studies have suggested that individual variability in the tendency to gain weight is associated with high FI, low RMR and low activity [36, 44,54,55] and that there is an inverse relationship between Tb and obesity [29,38,45]. In addition to pre-existing variation in these traits, changes that occur in traits in response to high fat feeding may also contribute to the variability in HF diet-induced weight gain. Therefore we also studied whether variability in changes in RMR, Tb and/or activity in response to HFD feeding could predict HFD-induced weight gain. To

elucidate whether preference for the HFD (due to the palatability of the food) [2,10,11] contributed to weight gain, FI during the first week of HF feeding was also included as a predictor in these models, and food preference tests were performed.

## 2. Methods & procedures

### 2.1. Animals and housing

Male and female outbred MF1 mice were obtained from Harlan Ltd. UK at 4 weeks of age (parental generation,  $n = 46$ ) or bred in house (first generation of offspring, F1,  $n = 78$ ). Mice were maintained in a temperature controlled room ( $21 \pm 1$  °C) under a 12:12-h light–dark cycle, with lights on at 5:00 h and a “dawn/dusk” period of 20 min at either end of the light period. After a breeding event at 10 weeks of age all mice (males and females) were individually housed in standard cages containing shredded paper and a red dome-shaped house for enrichment. Out of the females used in this study ( $n = 65$ ) 53 gave birth and weaned successful litters and the others ( $n = 12$ ) were unsuccessful. Animals had ad libitum access to food (D12450B, 10% kcal fat,  $18.36 \text{ kJ g}^{-1}$ , Research Diets, New Brunswick, USA) and water. All mice ( $n = 124$ ) were implanted intraperitoneally with temperature transmitters (PDT-4000 E-Mitter, Mini Mitter Company Inc., USA) under general anaesthesia (mixture of isoflurane and oxygen). Males were implanted at 14 weeks of age and females at 17–18 weeks of age at least 10 days after their litters had been weaned. Mice were allowed at least 12 days to recover from the surgery before the start of the experiment. All procedures concerning animal care and treatment were approved by the ethical committee for the use of experimental animals of the University of Aberdeen, and licensed by the UK Home Office.

### 2.2. Experimental procedure

Baseline measurements (BL) started at the age of 19–20 weeks and were taken over a period of 4 weeks (days  $-28$  to  $-1$ ). During this time mice had ad libitum access to food (D12450B) and water. FI of all mice was then restricted to 70% of their individual BL FI (calculated in grammes over the last week of BL) for a period of 28 days (caloric restriction, CR; days 0–28). Food rations were weighed and delivered daily between 16:00 and 17:00. After the CR phase animals received ad libitum food for a period of 2 weeks to recover from CR (RC, days 29–42). Mice then received ad libitum high fat diet (D12492, 60% kcal from fat, Research Diets, New Brunswick, USA) for a period of 4 weeks (HF, days 43–70).

BM and FI were measured each day between 16:00 and 17:00 (1 h before lights off) throughout the experimental periods; i.e., BL, CR, RC and HF phases. Data on BM, FI, RMR, body composition, physical activity and Tb from the BL and CR phase of this experiment have been published previously [47] in a paper investigating predictors of individual variability in diet-induced weight loss.

### 2.3. Food preference test

A food preference test was performed on day  $-27$  of the BL period. Animals were given a choice of 4 diets over a 24 h period: 1.) high carbohydrate (HC) diet (CRM (P), 66:22:12% kcal from carbohydrates:protein:fat (C:P:F),  $18.4 \text{ kJ g}^{-1}$ , Special Diets Services, BP Nutrition, Witham, UK), 2.) medium fat (MF) diet (D12451, 35:20:45% kcal C:P:F,  $20.2 \text{ kJ g}^{-1}$ , Research Diets), 3.) high fat (HF) diet (D12492, 20:20:60% kcal C:P:F,  $23.9 \text{ kJ g}^{-1}$ , Research Diets, New Brunswick, USA) or 4.) custom-made high protein (HP) diet (DX04080301, 30:60:10% kcal C:P:F,  $21.1 \text{ kJ g}^{-1}$ , Research Diets). Animals were offered  $10.0 \pm 0.1 \text{ g}$  of each diet in small petri-dishes that were randomly distributed over the cage floor and the amount of food left from each diet after 24 h was measured to calculate how much food was consumed from each diet.

#### 2.4. Body temperature (T<sub>b</sub>) and general activity

Mice in their home cages were placed onto transponder energisers (ER-4000 Receiver, Mini-Mitter Company Inc., USA) allowing us to non-invasively monitor body temperature and general activity throughout the protocol. The VitalView™ Data Acquisition System (Mini-Mitter Company, Inc., USA) was used to collect the data in 1 minute intervals (for a detailed description, see [18]).

#### 2.5. Resting metabolic rate (RMR)

RMR was determined in all animals during BL (day  $-19 \pm 2$ ), CR (day  $16 \pm 2$ ) and HF (day  $58 \pm 2$ ) phase. All measurements took place during the light phase between 7:00 and 16:00. RMR (O<sub>2</sub> consumption and CO<sub>2</sub> production) was measured in an open-flow respiratory system [43]. In short, fresh air was pumped (Charles Austin Pumps) through a sealed Perspex chamber within an incubator (INL-401N-010, Gallenkamp) set at 30 °C. This temperature was chosen because it lies in the thermo-neutral zone for these mice (Speakman, 2014; [41]). Mass-flow controllers (MKS Instruments UK Ltd, Cheshire, UK) provided 500–700 ml O<sub>2</sub> min<sup>-1</sup> which was monitored using an Alexander Wright DM3A flow meter. Air leaving the animal chamber was dried using silica gel and 150 ml min<sup>-1</sup> was passed through a gas analyser (Servomex Xentra). CO<sub>2</sub> was not absorbed prior to gas analysis as this maximizes the accuracy of energy expenditure measures [27]. Gas concentrations were measured continuously, and averaged values were stored every 30 s for 180 min. RMR was quantified as the oxygen consumption over the lowest 20 consecutive values (10 min interval) and corrected for ambient temperature and pressure, using the appropriate equation [19]. Respiratory Quotient (RQ) was calculated by dividing CO<sub>2</sub> production by O<sub>2</sub> consumption and the data (in ml O<sub>2</sub> min<sup>-1</sup>) were converted to energy equivalents using the Weir equation [51]. Mean BM was calculated from mass before and after each run. RMR represents the energy expenditure of an animal at rest, and therefore measurements were repeated if the readings indicated that animals did not rest during the period of measurement. This mainly occurred in animals that were fed the HFD. When an animal did not rest during either measurement it was removed from final analysis (n = 10).

#### 2.6. Body composition

Fat mass (FM) and fat free mass (FFM) of mice was determined four times during the experimental protocol using dual energy X-ray absorptiometry (DXA; PIXImus2 Series Densitometers with software version 1.46.007, GE Medical Systems Ultrasound and BMD, Bedford, UK); day  $-8$  (BL), day 28 (CR), day 42 (RC) and day 70 (HF). Mice were anaesthetised using a face mask which provided a mixture of isoflurane and oxygen for the duration of the scan (~3 min). The software enabled a region of interest to be created to exclude the head with the mask from analysis [15]. Data were corrected with a calibration formula specific to our machine that has been generated by the linear regression of fat content determined by DXA analysis, with the fat content as determined by soxhlet chemical extraction (see [25] for detailed description of the procedure).

#### 2.7. Glucose tolerance tests

Glucose tolerance was determined in all animals during BL (day  $-13 \pm 1$ ), CR (day  $22 \pm 1$ ) and HF (day  $64 \pm 1$ ). Food was taken away and mice were placed in a clean cage with sawdust and their red house at 17:00 h the day before the test. Approximately 15 h later, fasted glucose levels were determined by collecting a drop of blood from the tail onto the Ultra OneTouch system (LifeScan, USA). Mice were then injected with a 20% glucose solution (0.1 ml per 10 g BM) and glucose concentration in the blood (tail tip) was determined after 15, 30, 60 and 120 min. Mice were re-fed after the test. Area under the

curve (AUC) for the response in glucose concentrations (at 0, 15, 30, 60 and 120 min.) was calculated using Prism 7. At BL gorging behaviour of all animals was assessed by measuring the amount of food consumed in the 5 h after the GTT test (i.e., after a 15 h fast).

#### 2.8. Data analysis

Data were tested for normality using the Kolmogorov–Smirnov test and when necessary data were log-transformed to obtain a normal distribution (i.e., FM, activity, glucose tolerance and RMR). All statistical tests were performed in SPSS version 18.0.

Repeated measures (RM) general linear models (GLM) were applied to explore differences between sexes and the effect of HF. In these models sex was added as fixed factor and diet (RC vs. HF) as repeated factor. For variables known to correlate with BM, BM was added to the model as a time-variant covariate (using MIXED models in SPSS).

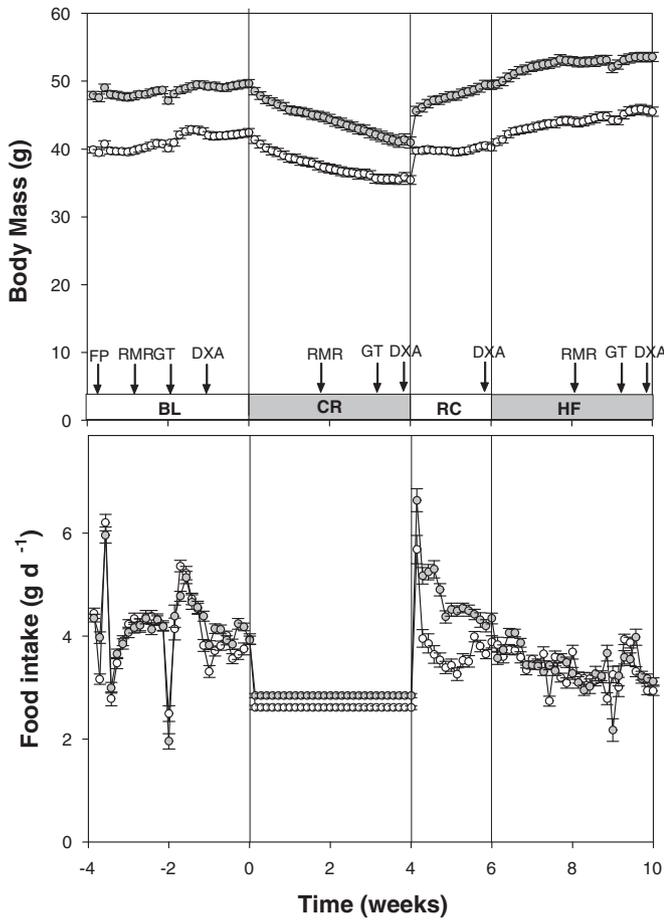
Stepwise linear regressions were performed with changes in BM or FM as the dependent variable and several independent variables (i.e., BL variables like FM, RMR, general activity and T<sub>b</sub>) to establish a model that predicted weight gain on HFD. Changes in BM and FM were expressed as the residuals from regressions between mass at BL and CR, or as absolute change in grammes (mass at CR – mass at BL). Regression models were performed including individuals of both sexes (where sex was included as a predictor variable) and for each sex separately when significant sex effects were found. Predictors were only included in the final model when they significantly contributed to the model, i.e.,  $p < 0.05$ . Data were investigated for unusual outliers (standardised residual  $> 2$ ) and influential data (leverage  $> (2k + 2)/n$ , where  $k$  is the number of predictors in the model and  $n$  is the number of observations). Some statistical outliers were identified, but we had no reason to assume that they did not represent true biological variation, and therefore they were not removed before final analysis. Several tests were included to confirm that our regression models met the assumptions for linear regressions (i.e., linearity of predictors, normal distribution of standardised residuals, homoscedasticity and absence of multicollinearity). These tests showed that the assumptions of linear regression were not violated in our models; i.e., there were no nonlinear effects of predictors, residuals were normally distributed ( $p > 0.05$ ) and homoscedastic (i.e., variance of the residuals was homogeneous across levels of the predicted values), and no multicollinearity was observed (variance inflation factor was  $< 2$  for predictors used in our models).

Simple linear regression (for relationships with BM) or Pearson correlations were applied to test for correlations between variables. Values are expressed as means  $\pm$  standard deviation (SD) unless stated otherwise. All tests were two-tailed and significance was set at  $p \leq 0.05$ .

### 3. Results

#### 3.1. Variability in weight gain and baseline variables

In both male and female mice, BM had stabilised at the end of the BL, decreased steadily during the CR phase and returned to the level of BL during RC (Fig. 1). A gradual increase in BM was observed over the period of HF feeding in both male and female mice (Fig. 1). In males, there were no significant differences between BM ( $49.6 \pm 4.0$  and  $49.5 \pm 5.0$  respectively), FM ( $15.0 \pm 3.4$  and  $15.1 \pm 4.6$ ) or FFM ( $34.6 \pm 2.9$  and  $34.3 \pm 2.8$ ; paired t-tests:  $p > 0.1$ ) at the end of BL and RC respectively (determined by DXA). In females, however, BM was slightly lower at the end of RC compared to BL ( $42.6 \pm 4.3$  vs.  $40.5 \pm 4.0$  for BL and RC respectively,  $p < 0.05$ ); FFM was slightly higher ( $29.7 \pm 2.4$  vs.  $30.5 \pm 2.8$ ,  $p < 0.05$ ), whereas FM was lower in females at RC compared to BL ( $12.9 \pm 3.7$  vs.  $10.0 \pm 3.4$ ,  $p < 0.05$ ). Residual weight loss was significantly higher in males than in females (t-test,  $p = 0.031$ ), but residual weight gain did not differ.



**Fig. 1.** Daily changes in body mass and food intake throughout the experimental procedure in male and female mice. Arrows indicate timing of procedures during the baseline (BL), caloric restriction (CR), recovery period (RC) and high fat feeding (HF) periods. FP = food preference test, RMR = resting metabolic rate, GT = glucose tolerance tests and DXA is dual X-ray absorptiometry. Open circles represent females and closed (grey) circles represent males.

The amount of BM gained on the 60% HFD was calculated from the change in BM between the ends of the RC and HF phase (calculated as the difference in mean BM over the last week of each phase) and varied considerably in both male and female mice (Fig. 2A). BM changes ranged from -1 to 26%, equivalent to -0.2 g to 10.5 g in absolute mass. Mice made adjustments to their body composition as they gained mass. There was a significant negative correlation between changes in

FM and changes in FFM in both male and female mice (Fig. 2B, Linear regression, females:  $R^2 = 0.47$ ,  $p < 0.001$ , males:  $R^2 = 0.57$ ,  $p < 0.001$ ); i.e., animals that showed the largest increases in FM showed the largest decreases in FFM (see also Table 1). Overall, FFM was significantly reduced by approximately 5% on the HFD in both males and females (RM GLM: diet:  $F_{1,122} = 68.9$ ,  $p < 0.001$ , sex \* diet:  $F_{1,122} = 0.7$ ,  $p = 0.41$ ), whereas FM significantly increased (diet:  $F_{1,122} = 43.2$ ,  $p < 0.001$ , see Table 2). FM increased significantly more in females (63%) than in males (39%) as indicated by a significant interaction effect between sex and diet (Table 1,  $F_{1,122} = 10.8$ ,  $p = 0.001$ ).

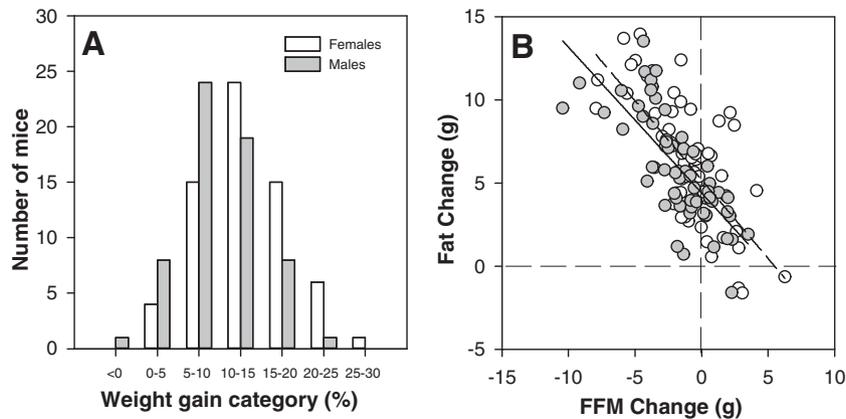
**3.1.1. Food preference test**

Food preference was tested in all animals at the start of the experiment. Animals had a choice of 4 diets (HC, MF, HF and HP) over a period of 24 h and the gross energy content of the amount eaten from each diet was calculated. During the food preference test the females' food consumption consisted of  $21.8 \pm 10.8\%$  of HC,  $29.1 \pm 22.3\%$  of MF,  $45.0 \pm 22.1\%$  of HF and  $3.5 \pm 4.8\%$  of HP diet. Females thus showed the strongest preference for the HF diet and consumed significantly more energy of this diet than any of the other diets (Paired t-tests comparing intake of each diet,  $p < 0.001$ ). Males' FI consisted of  $32.8 \pm 18.8\%$  of HC,  $37.3 \pm 24.7\%$  of MF,  $27.6 \pm 21.7\%$  of HF and  $2.3 \pm 3.7\%$  of HP diet. Males thus showed the greatest preference for the MF diet, but the intake of MF diet differed significantly only from the HP diet ( $p < 0.001$ ); indicating that males showed a similar preference for MF, HF and HC diets. T-tests comparing intake of each diet between the sexes, showed that females ate significantly less of the HC diet and more of the HFD than males ( $p < 0.001$ ), but no significant differences in intake of HP or MF diets were observed ( $p = 0.12$  and  $p = 0.08$ , respectively).

There was a variability in the preference for each diet as indicated by the large SDs, and it may be that animals that showed a greater preference for HFD during BL would consume more of this diet when exposed to it during the HF feeding phase. We therefore compared the food preference at BL to FI, and weight (or fat) gain on the HFD using ANOVA (FP of all animals was scored for each animal: 1 = animal consumed most of HC diet, 2 = MF, 3 = HF) and Pearson correlations (% HF eaten vs. FI and changes in mass). No significant relationships between food preference, and mean FI, FI consumed during the first week of HFD, or weight (fat) gain on HFD were observed.

**3.1.2. Predicting weight gain with baseline variables**

All variables measured under baseline conditions varied considerably between individuals (Table 1). For instance, FM at RC ranged from 4.4 to 20.9 g in females and 7.8 to 24.1 g in males, and activity ranged from 4.8 to 23.5 and 5.4 to  $14.0 \times 10^3$  counts  $\text{day}^{-1}$  in females and males respectively. To establish whether pre-existing differences



**Fig. 2.** Variability in high fat diet-induced weight changes in body mass and fat mass. A: Distribution of body mass gain in male and female mice, B: relationship between changes in fat free mass (FFM) and fat mass as determined by DXA. Regression lines for females are shown as a dashed line and for males as a solid line.

**Table 1**  
Descriptive statistics.

Baseline/recovery	Sex	n	Mean	SD	Min	Max	
Body mass (g)	F	65	40.0	3.9	32.0	49.5	
	M	59	48.7	4.9	38.0	59.7	
Fat free mass (g)	F	65	30.5	2.8	23.1	35.9	
	M	58	34.3	2.8	27.5	39.5	
Fat mass (g)	F	65	10.0	3.4	4.4	20.9	
	M	58	15.1	4.6	7.8	24.1	
Food intake (kJ day <sup>-1</sup> )	F	65	63.5	9.9	39.8	84.2	
	M	59	75.8	8.4	53.3	103.2	
Activity (*10 <sup>3</sup> counts day <sup>-1</sup> )	F	63	11.7	3.5	4.8	23.5	
	M	57	9.4	2.0	5.4	13.9	
Body temperature (°C)	F	63	37.1	0.3	36.6	37.7	
	M	57	36.5	0.5	34.6	37.7	
Resting metabolic rate (kJ day <sup>-1</sup> )	F	65	33.7	6.8	21.7	52.0	
	M	59	35.5	6.6	23.2	50.1	
Respiratory quotient (RQ)	F	65	0.86	0.08	0.70	0.99	
	M	59	0.86	0.07	0.71	0.97	
Glucose tolerance (AUC)	F	65	831	199	430	1315	
	M	59	1011	308	309	1590	
High fat diet	Sex	n	Mean	SD	Min	Max	Change
Body mass (g)	F	65	45.4 <sup>a,b</sup>	5.4	33.1	56.5	14
	M	59	53.5	5.1	41.8	67.6	10
Fat free mass (g)	F	65	29.4 <sup>a,b</sup>	3.4	19.5	37.4	-4
	M	59	32.7	3.2	26.8	38.9	-5
Fat mass (g)	F	65	16.2 <sup>a,b,c</sup>	4.9	7.1	28.1	63
	M	59	21.0	5.2	10.4	34.9	39
Food intake (kJ day <sup>-1</sup> )	F	65	75.0	10.7	60.7	100.2	18
	M	59	76.5	11.4	53.8	110.2	1
Activity (*10 <sup>3</sup> counts day <sup>-1</sup> )	F	63	11.4 <sup>a,c</sup>	4.3	5.6	36.8	-2
	M	56	8.1	1.7	5.1	13.2	-14
Body temperature (°C)	F	63	37.0 <sup>a,b,c</sup>	0.3	36.3	37.7	-0.4
	M	56	36.5	0.5	34.7	37.8	-0.1
Resting metabolic rate (kJ day <sup>-1</sup> )	F	59	34.9	6.0	20.1	46.4	4
	M	55	34.6	6.7	22.9	55.0	-3
Respiratory quotient (RQ)	F	59	0.85	0.06	0.75	0.98	-1
	M	55	0.85	0.05	0.73	0.96	-1
Glucose tolerance (AUC)	F	65	724 <sup>a,b</sup>	216	321	1260	-13
	M	59	994	302	416	2067	-2

Mean, SD, min and maximum values for various variables under baseline conditions (RC) and after exposure to high fat diet (HF) in male (M) and female (F) mice. To explore the difference between the sexes and effect of diet, repeated measures ANOVA was applied with sex as fixed factor and diet (RC vs. HF) as repeated factor (i.e., for body mass, body temperature and glucose tolerance). For variables known to correlate with body mass (i.e., food intake, resting metabolic rate (RMR), fat mass and fat free mass and activity) body mass was added to the model as a time-variant covariate (MIXED model in SPSS). The 'change' column shows the difference between variables at RC (or BL in the case of RMR, RQ and glucose tolerance) and HF in %. Data on activity and body temperature for 5 animals are missing due to failure of the implanted Mini-Mitter, and data on RMR and RQ at HF are missing for 10 animals because these animals did not rest during the measurement.

<sup>a</sup> Indicates that there was a significant effect of sex.

<sup>b</sup> Indicates a significant effect of diet.

<sup>c</sup> Indicates that a significant sex \* diet interaction was observed in the RM ANOVA ( $p < 0.05$ ).

(i.e., variability in BL/RC variables) between mice could predict subsequent changes in BM and FM on HFD stepwise linear regression was applied (for detailed methods see paragraph on 'Data analysis'). Sex, FM (g), FFM (g), FI (kJ day<sup>-1</sup>), activity (\*10<sup>3</sup> N counts day<sup>-1</sup>), body temperature (°C), RMR (kJ day<sup>-1</sup>) and glucose tolerance (area under the curve) were added as independent variables and residual BM or FM was added as dependent variables. Variables measured at RC were used except for RMR and GTT where data was only available for BL. All variables measured at BL and RC were significantly correlated (Pearson correlation,  $p < 0.01$ ). In the final models only significant ( $p < 0.05$ ) variables were entered.

Sex and FFM together predicted ~10% of the variation in BM changes (Table 2A, Fig. 3A), indicating that weight gain was greater in those with higher FFM over RC and that differences existed between the sexes. Repeating the analysis for both sexes separately did not yield a significant model in males, whereas in females FFM and FM were significant

**Table 2**

Regression analyses predicting individual variability in weight gain induced by feeding a HFD in male and female MF1 mice.

		Body mass	Fat mass
<b>A. Prediction of weight gain after 4 weeks of HFD feeding using baseline traits</b>			
Models	R <sup>2</sup>	0.12	0.11
	Adj. R <sup>2</sup>	0.10	0.10
Predictors	n	124	124
	p	0.001	0.001
	Sex	-0.359**	-0.264*
	FFM	0.370**	0.473**
<b>B. Prediction of weight gain after 4 weeks of HFD feeding using compensatory changes in traits in response to HFD feeding</b>			
Models	R <sup>2</sup>	0.20	0.10
	Adj. R <sup>2</sup>	0.19	0.10
Predictors	n	125	125
	p	0.001	0.001
	Sex	-0.809*	NS
	FI Wk1	0.0426**	0.326**

A: Stepwise linear regression with change in body mass (BM) or fat mass (FM) – expressed as residuals from the regression between mass at baseline (RC) and mass after high fat diet (HF) – as dependent and baseline variables as predictors, i.e., FM, fat free mass (FFM), food intake (FI), physical activity (PA), body temperature (Tb), resting metabolic rate (RMR) and glucose tolerance.

B: Stepwise linear regression with residual BM or FM as dependent and compensatory changes to traits in response to HF as predictors, i.e., FI Wk1 (FI during first week on HF), residual PA, residual Tb and residual RMR.

Analyses were performed for all animals together (with sex included as predictor). Where sex was a significant predictor analysis was repeated for females only and males only (see text). Standardised coefficients are shown for variables that significantly contributed to the final model and their significance is indicated by \*\* $p < 0.001$  and \* $p < 0.05$ . The sign before the coefficients (+ or -) indicates the direction of the relationship with changes in BM or FM. NS indicates variables that did not significantly predict weight changes, and that were not included in final models. Please note that for RMR and GT data collected at BL was used in the models as they were not measured during the RC phase.

predictors of weight gain ( $R^2 = 0.22$ , adj.  $R^2 = 0.19$ ,  $p = 0.001$ , with beta = 0.402, 0.274, respectively).

Sex and FFM together predicted ~14% of the changes in FM (Table 2A, Fig. 3B). In models where effects of BL/RC variables on FM gain were tested for males and females separately, FFM alone came out as a significant predictor and predicted 13% of the variability in fat gain in both sexes. Fat gain was greater when FFM was higher. None of the other predictors, e.g., FI, RMR, glucose tolerance, activity or Tb significantly contributed to any of the models.

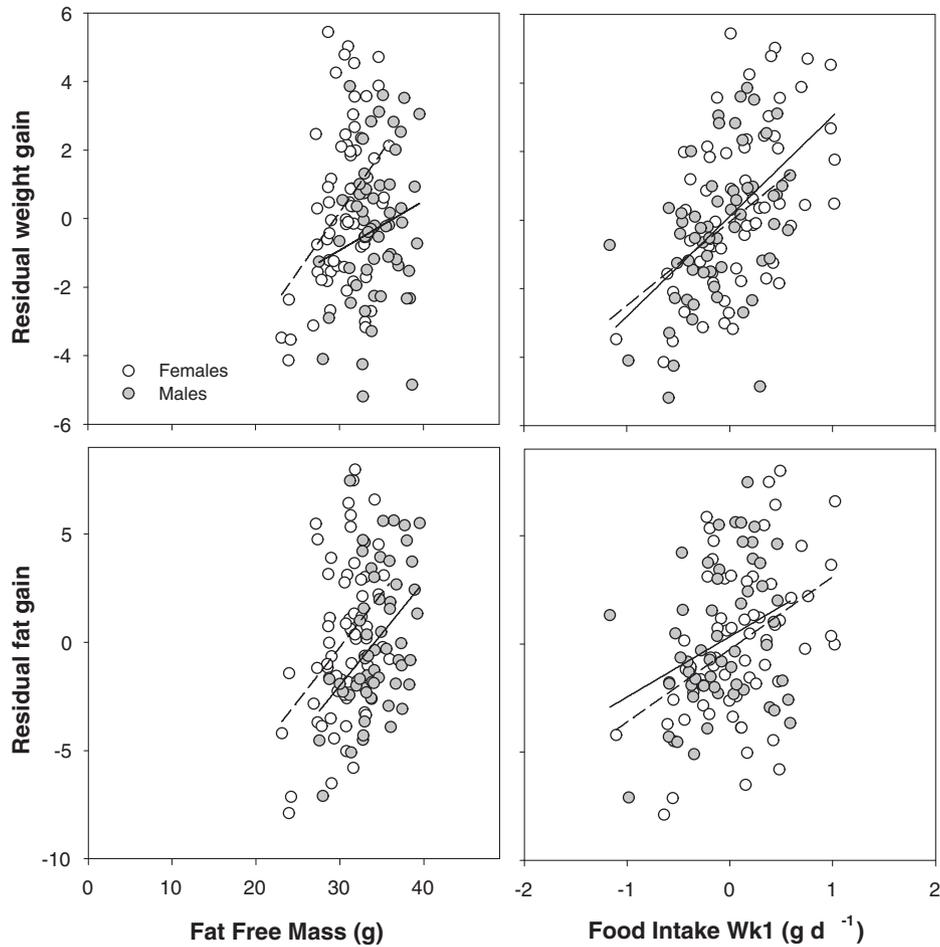
### 3.2. Behavioural and physiological changes in response to HF diet

#### 3.2.1. Food intake

Absolute FI decreased significantly on HFD compared to RC; i.e., from 3.7 to 3.3 g day<sup>-1</sup> in females and 4.4 to 3.4 g day<sup>-1</sup> in males (RM ANOVA with BM as covariate: diet:  $F_{1,122} = 191.5$ ,  $p < 0.001$ ). Due to the higher energy content of the HFD, gross energy intake (kJ day<sup>-1</sup>) however slightly, but not significantly, increased on the HFD in both sexes: 63.5 to 75.0 kJ day<sup>-1</sup> in females and 75.8 to 76.5 in males (RM ANOVA with BM as covariate: diet:  $F_{1,122} = 1.9$ ,  $p = 0.17$ , sex:  $F_{1,122} = 0.2$ ,  $p = 0.88$ , sex \* diet:  $F_{1,122} = 24.2$ ,  $p < 0.001$ ).

#### 3.2.2. General activity & body temperature

General activity significantly decreased on the HFD in both sexes and was lower in males than in females (Table 1, RM GLM with sex and diet as fixed factors, diet:  $F_{1,117} = 15.9$ ,  $p < 0.001$ ; sex:  $F_{1,117} = 38.2$ ,  $p < 0.001$ ). Mice are nocturnal and about 66% of activity took place during the dark phase. To explore whether effects of diet occurred during the active or inactive phase, activity during the dark and light phases was calculated (data not shown). A significant decrease in activity was observed during the dark phase when mice were feeding HFD compared to RC, but no effect of diet was observed during the light phase (RM GLM; diet:  $F_{1,117} = 18.8$ ,  $p < 0.001$  and  $F_{1,117} = 0.1$ ,  $p = 0.94$  for dark and light phase respectively).



**Fig. 3.** Predictors of high fat diet-induced body mass (BM) and fat mass (FM) gain in male and female mice (expressed as residuals). A: Relationship between residual BM gain and fat free mass (FFM) at baseline. B: Relationship between residual FM and FFM at baseline. C: Relationship between residual BM and residual food intake. D: Relationship between residual FM and residual FI. Regression lines for females are shown as a dashed line and for males as a solid line.

Activity is known to correlate with BM [46] and when BM was added to the models as a time-dependent covariate, the diet effect on activity was no longer significant (see Fig. 4,  $F_{1,117} = 1.9$ ,  $p = 0.17$  and  $F_{1,117} = 2.8$ ,  $p = 0.098$  for total activity or activity during the dark phase respectively); i.e., differences in activity between animals at RC and HFD could be fully explained by differences in BM, whereby larger fatter mice showed less general activity.

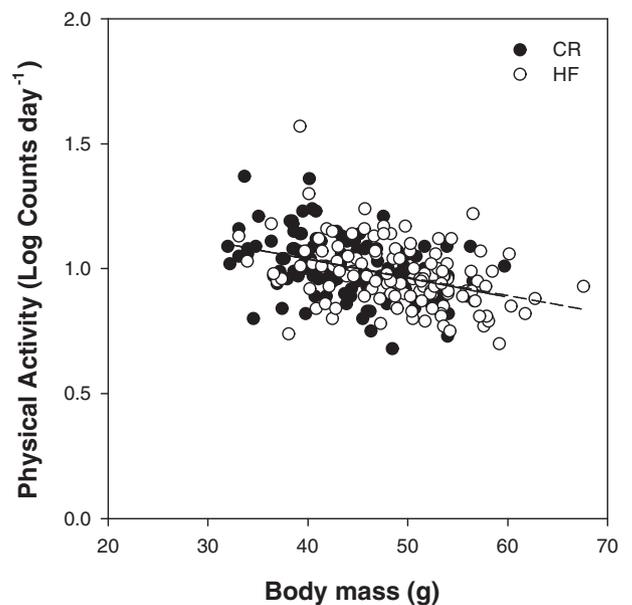
Tb was higher in females than in males (Table 1, RM GLM;  $F_{1,117} = 68.8$ ,  $p < 0.001$ ) and decreased significantly when animals were fed HFD compared to standard chow (RC;  $F_{1,117} = 12.9$ ,  $p = 0.001$ ).

### 3.2.3. Resting metabolic rate

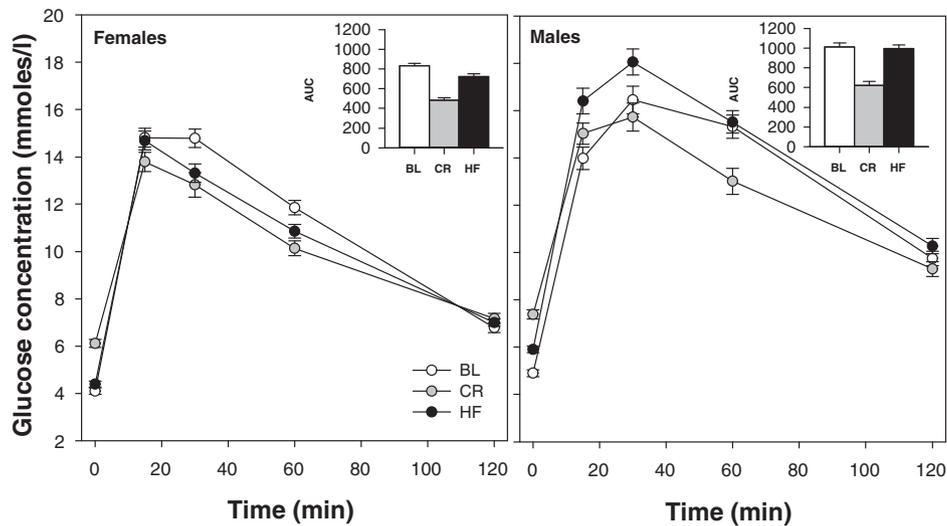
HF feeding did not significantly affect RMR (RM GLM with BM as time-variant covariate, diet:  $F_{1,185} = 2.9$ ,  $p = 0.09$ , sex:  $F_{1,123} = 2.9$ ,  $p = 0.09$ , sex \* diet:  $F_{1,120} = 1.2$ ,  $p = 0.26$ ); i.e., no differences were observed in RMR between BL and HF phase or between males and females (see Table 1). RQ also did not differ between the diets or sexes (diet:  $F_{1,112} = 0.8$ ,  $p = 0.36$ , sex:  $F_{1,112} = 1.0$ ,  $p = 0.32$ , sex \* diet:  $F_{1,112} = 0.5$ ,  $p = 0.5$ ).

### 3.2.4. Glucose tolerance

RM GLM were performed to test for differences in GT at BL or HF period (Table 1, Fig. 5). A significant effect of time of sampling ( $F_{1,244} = 218.6$ ,  $p < 0.001$ ) and sex ( $F_{1,244} = 79.2$ ,  $p < 0.001$ ) was observed. Diet was not significant ( $F_{1,244} = 1.8$ ,  $p = 0.177$ ), but an interaction between diet and time was found ( $F_{1,244} = 7.6$ ,  $p = 0.006$ ), indicating that



**Fig. 4.** Relationship between body mass and physical activity during baseline (RC, open symbols, solid regression line) and high fat diet feeding (HF, closed symbols, dashed regression line).



**Fig. 5.** Glucose tolerance test performed during baseline (BL), caloric restriction (CR) and high fat (HF) diet feeding in female (left graph) and male mice (right graph). The insert in each graph shows the area under the curve.

the pattern in time differed. Post-hoc tests, comparing glucose concentration at various time points between BL and HF, showed that mice on HF had higher levels of basal glucose, 0 min (i.e., basal levels,  $p < 0.001$ ) and 15 min after glucose injection ( $p = 0.009$ ), but did not differ from BL at any other time point.

GT significantly changed during the diet challenges (comparing area under the curve (AUC) for all three diets, BL, CR and HF, RM GLM, diet:  $F_{1,123} = 5.4$ ,  $p = 0.021$ , Fig. 5), and was greater in females than in males (sex:  $F_{1,123} = 32.3$ ,  $p < 0.001$ ). Compared to BL, GT (expressed as AUC) was improved on CR and HF (post-hoc tests,  $p < 0.001$  and  $p = 0.015$ ).

### 3.2.5. Predicting weight gain from behavioural and physiological changes on HF diet

Several behavioural and physiological changes were observed when animals were exposed to the HF compared to standard chow (RC) and to establish whether FI during the first week on HF, and changes in activity, Tb, RMR or GT (i.e., residuals of relationship between variable at BL/RC and HF) could predict changes in BM and FM on HF, stepwise linear regression was applied (for detailed methods see paragraph on 'Data analysis').

In models including both sexes, FI during week 1 of HF, and sex significantly predicted approximately 20% of the variability in BM changes on HF (Table 2B, Fig. 3C). These results indicated that there were differences between the sexes, however in prediction models with females or males separately, FI alone was a significant predictor and predicted ~22% and 15% of the variability in weight gain in females and males respectively (Fig. 3C).

Approximately 10% of the variation in FM changes could be predicted by FI on HF alone in both sexes (i.e., sex was not a significant predictor in the model, Table 2B, Fig. 3D). Residual activity, Tb, RMR or GT were not significant predictors in any of these models.

### 3.3. Relationship between changes in mass on CR and HF diet?

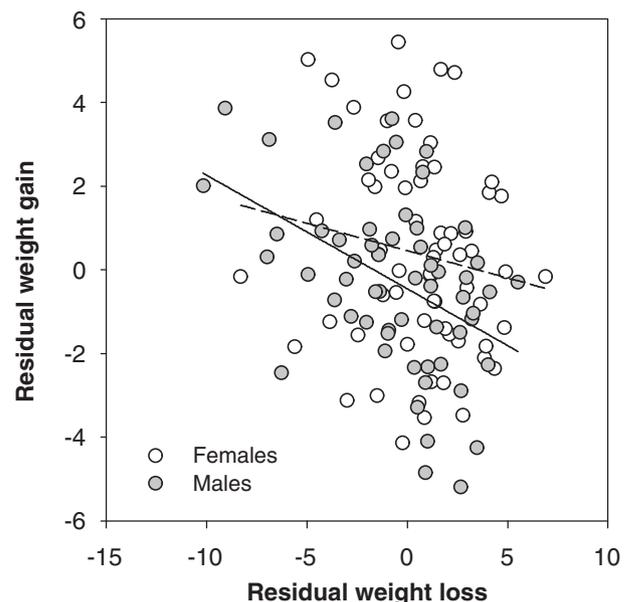
Relationships between changes in BM or body composition (FM and FFM) in response to CR and HF were explored (with changes expressed as residuals) using GLM with sex as a fixed factor.

No significant relationships were found between diet-induced changes in FM or FFM by CR vs. HF (GLM: FM,  $F_{1,123} = 0.05$ ,  $p = 0.83$ , sex =  $F_{1,123} = 0.01$ ,  $p = 0.97$ , FFM,  $F_{1,123} = 1.3$ ,  $p = 0.27$ , sex =  $F_{1,123} = 0.6$ ,  $p = 0.45$ ). Residual weight change did correlate significantly between CR and HF and there was a significant sex effect (GLM:  $F_{1,123} = 10.8$ ,  $p = 0.001$ , sex =  $F_{1,123} = 5.4$ ,  $p = 0.021$ , Fig. 6). Gorging

behaviour at BL (measured as the amount of food consumed within the first 5 h after an overnight fast; i.e., after GTT test) was significantly correlated with residual weight gain ( $r = 0.21$ ,  $p = 0.018$ ), but not weight loss ( $r = -0.15$ ,  $p = 0.10$ ) and post-restriction hyperphagia (measured as the amount of food consumed within the first 4 days after release from restriction; RC phase) was significantly correlated to residual weight loss ( $r = -0.69$ ,  $p < 0.001$ ), but not weight gain ( $r = 0.01$ ,  $p = 0.90$ ).

## 4. Discussion

As expected, HFD feeding resulted in increases in BM and FM, and large inter-individual differences were found in HFD-induced weight gain. Most animals increased their FM and reduced FFM on the diet.



**Fig. 6.** Relationship between changes in body mass in response to caloric restriction (CR) and high-fat feeding (HF) in male and female mice. There was a significant negative relationship between body mass changes in response to CR and HF in males ( $R^2 = 0.19$ ,  $p < 0.001$ , solid regression line), but this relationship did not reach statistical significance in females ( $R^2 = 0.02$ ,  $p = 0.22$ , dashed regression line). Body mass changes are expressed as residuals from the relationship with BL/RC.

Previous studies have indicated that obesity prone and resistant individuals differ with respect to their energy intake, GT, expression of (an)-orexigenic neuropeptides and responses to CR [4,20,31,37]. However, it is not always clear whether these differences occurred in response to HFD feeding or were already present before the obesity phenotype became apparent. Here, we investigated whether individual variability in HFD-induced weight gain could be explained by individual differences in metabolic factors that existed prior to HFD feeding (BL or RC) and/or differences in behavioural and physiological responses to HFD feeding between individuals.

Variability in FFM significantly predicted variability in BM and FM changes in response to HF feeding, although differences in the strength of this relationship were observed between the sexes (i.e., in males analysis for BM gain did not yield a significant model, but the other models run for each sex separately yielded similar results). Approximately 12% of the variation in BM and 11% of the variation in FM could be predicted by sex and FFM; animals that had a higher FFM before the diet intervention showed greater HFD-induced weight gain. These results agree with a previous study in inbred male C57BL/6 mice that found FFM to be a significant predictor of HFD-induced weight gain [55]. In that study, FM and physical activity were also shown to significantly contribute to the models, which is in agreement with a study using genetically obese *ob/ob* mice, which displayed reduced activity levels before the onset of obesity at the time when they were the same BM as their lean littermates [7]. In the current study physical activity measured before the onset of obesity, did not significantly contribute to the HFD-induced weight gain, which is in agreement with other studies, e.g., Simoncic et al. [40] observed that polygenic fat mice showed the same physical activity levels as their lean mice before they were put on a HFD. Also, in humans, studies have indicated that increasing adiposity is not related to previous levels of energy expended on activity [24,34]. Furthermore, it has been suggested that in human populations energy expended on physical activity has not declined over the same period that world-wide obesity has increased dramatically [52].

In agreement with previous studies in mice, we found no support for the hypothesis that individuals with lower RMR are more susceptible to HFD-induced weight gain [16,26,55]. There is also similar evidence from humans, i.e., Weinsier et al. [50] found that RMR was not correlated to 4-year weight changes in post-obese or non-obese women. In addition, although human studies have shown slight increases in RMR in response to HFD feeding, inter-individual changes in RMR did not account for the variability in fat gain [32,36]. Nevertheless, studies in Pima Indians that are obesity-prone showed a low RMR was a risk factor for weight gain in this population [44].

GT at BL did not predict HFD-induced BM or FM gain in our population of mice. These results are in agreement with studies in diet-induced obesity prone and resistant rats [30] and mice [20]. On chow-diet obesity prone rats had 44% more carcass fat than resistant rats, but energy intakes and oral fasted GT results were comparable between the groups [30]. Similarly, basal glucose levels were similar in obesity prone and resistant mice [20]. Obesity-prone mice on HFD did show some degree of glucose intolerance compared to obesity-resistant mice [20]. Obesity prone rats do, however, show increased basal levels of glucose and insulin in fed state [49]. These results indicate that the response to HFD differs between these animals with respect to GT, but differences in GT do not drive the differences in the response to HFD feeding.

High preference for HFD in a 24 h food preference test during BL, did not predict subsequent weight/fat gain on a HFD, which is in agreement with a study in rats that showed no differences in BM after 4 weeks on HFD between animals previously classified as high-fat preferring or low-fat preferring [6]. However, fat-preferring rats have been shown to have increased BM gain when given continuous access to HC, HP and HF diets [39]. In the current study, the amount of HFD consumed during the first week of exposure to HFD – which may indicate a preference for the HFD when it is offered on its own – did significantly predict individual variability in weight gain (20%). Reanalysis of data obtained

in inbred C57BL/6 mice also showed that 23% of the variability in weight gain could be attributed to the increase in FI during the first week of HFD feeding relative to baseline intake [55]. Hedonic control of FI, which is sensitive to palatability and rewarding properties of food, is an important factor regulating FI and may override homeostatic regulation of BM, even in the presence of satiety signals (for recent reviews see: [2, 14,23]). Our results may indicate that variations in reward-driven FI play a role in driving individual differences in weight gain, as animals with relatively high intake of the HFD during the first week of HF feeding showed greater weight gain. This agrees with studies in humans that have shown that habitual high-fat consumption is associated with obesity, and probably constitutes a risk factor for weight gain [3]. This also agrees with rodent studies showing that obesity-prone and resistant mice differ with respect to various components of the brains' dopaminergic system [9,21,22], as do CR-resistant vs. prone mice [48]. The dopaminergic system is involved in regulating goal-directed behaviour and the rewarding aspects of food, and administration of dopamine receptor (D2) agonists or antagonists to obesity prone and resistant mice, respectively, have indicated that the divergent metabolic phenotypes seen in response to HFD feeding can partly be reversed [9]. Alternatively, the relationship between weight gain and FI may be explained by individual differences in the requirements of certain micro- or macronutrients, i.e., individuals may vary in their requirements for certain nutrients that are low in the HFD, and compensate by over-consuming the diet. For example, there is evidence that intake of proteins is prioritized over non-protein intake – the protein leverage hypothesis – and this may drive differences in total energy intake according to the balance of macronutrients in the diet [13]. The diets used here differed in the content of carbohydrates and fat, but protein content was the same (20% kcal from proteins in both). Further research would be required to establish if individual differences in the requirements of macronutrients were involved in driving differences in FI and weight gain on the HFD.

Very few studies have investigated whether responses to over and under nutrition are linked [31]. Here we investigated whether there was a correlation between HFD-induced weight gain and CR-induced weight loss. Although, we did show that animals that gained more weight on HFD, also lost more when they were exposed to CR, no relationship was found between FM or FFM gain and loss. In addition, factors that have been shown to predict CR-induced weight loss in these mice (i.e., activity, FI and RMR [47]) did not predict HFD-induced weight gain. These results thus indicate that the underlying factors regulating diet-induced weight loss and HFD-induced weight gain are, at least partly, different. Studies comparing obesity-prone and resistant rats have shown them to respond differently to CR; i.e., obesity-prone rats primarily lowered their FM, whereas obesity-resistant rats altered their FFM on CR, but the rate and overall amount of weight loss did not differ [31]. To our knowledge, individual responses to CR and HFD feeding within the same individuals have not directly been compared in humans, but information is available on weight regain after a diet intervention. A recent study showed that weight regain was inversely related to diet-induced weight loss in both men and woman [53]; i.e., individuals who experienced larger reductions in BMI during a diet intervention were found to experience lower weight regain during the 6-month follow-up period. Interestingly, although sex differences were observed in response to the diet intervention – with men losing more weight than women (as has been shown in other studies, e.g., [17] and we found in this study) – no sex effects were found on weight regain. This again suggests that distinct molecular mechanisms may coordinate positive versus negative changes in BM [53]. Our results may agree with the existence of a dual intervention point model of BM regulation that hypothesises that the presence of a lower and upper intervention point, that both act independently to regulate BM [42]. If the upper and lower intervention points are wide BM would be allowed to fluctuate more than when they are closer together. It may thus be that the individuals in the current study that showed

both large diet-induced weight loss and large HFD-induced weight gain had a wide range of intervention points, allowing BM to drop lower on CR and increase more on HFD. Individuals that showed small changes in weight on either diet may have had narrow intervention points and thus only minor changes in BM in response to both HFD and CR. The upper and lower intervention points are thought to be set by risk of predation and starvation respectively [42,54] that act independently to control BM. Although a wide or narrow range of intervention points may explain the pattern observed in our dataset, it is hard to imagine how perception of starvation and predation risks would be linked. The general regulation of food intake model [8] may explain the data better, assuming that the level at which BM is regulated is more malleable in some individuals resulting in greater changes in BM in response to both HFD and CR, and less malleable in others resulting in small changes in BM in response to both diet manipulations.

In conclusion, ~12% of the variability in HFD-induced weight gain could be predicted by FFM at BL. A larger portion of the variability could be predicted by FI during the first week of exposure to HFD. These results are consistent with a role for the hedonic system in causing susceptibility to obesity in an environment with easy access to palatable foods.

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