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Variations in body weight, food intake and body composition after long-term high-fat diet feeding in C57BL/6J Mice

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Abstract

Objective—To investigate the variations in body weight, food intake and body composition of both male and female C57BL/6J mice during a diet-induced obesity (DIO) model with high-fat diet (HFD) feeding.

Design and Methods—Mice were individually housed and fed *ad libitum* either a low-fat diet (LFD, 10% calories from fat; n=15 male, n=15 female) or high-fat diet (HFD, 45% calories from fat; n=277 male, n=278 female) from 8 to 43 weeks of age. Body weight, food intake and body composition were routinely measured.

Results—Body weight was significantly increased with HFD (vs. LFD) in males from week 14 ($p=0.0221$) and in females from week 27 ($P=0.0076$). Fat mass and fat-free mass of all groups were significantly increased over time (all $p<0.0001$), with a large variation observed in fat mass. Baseline fat mass, fat-free mass and daily energy intake were significant predictors of future body weight for both sexes ($p<0.0001$). Baseline fat mass was a significant predictor of future body fat ($p<0.0001$).

Conclusions—Both males and females have large variations in fat mass, and this variability increases over time, while that of fat-free mass remains relatively stable. Sex differences exist in HFD responses and multivariate predicting models of body weight.

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Keywords

Variation; body weight; body composition; C57BL/6J

Introduction

With the increased prevalence of obesity, the use of mice as a model for diet-induced obesity (DIO) has increased dramatically. Although numerous mouse strains are susceptible to DIO, sensitivity varies greatly among strains. For instance, SWR/J and CAST/Ei tend to be DIO resistant, while the C57BL/6 strain is highly susceptible (1, 2, 3, 4). Thus, C57BL/6J mice are used frequently with HFD feeding in obesity research.

Many factors influence body weight in C57BL/6 mice, and some of the strongest and best-known factors are age, sex and diet (5). Previous studies on HFD-induced obesity with C57BL/6 mice have used relatively small sample sizes or short study durations, have reported results only for males or have used a very high-fat diet composition (around 60% calorie from fat) (5, 6, 7, 8). Increased sample size could help to understand the extent of variability, even within a reported susceptible strain. It has been suggested that male C57BL/6J mice have a large variation in stable obesity phenotypes after short-term HFD feeding despite their inbred and purportedly isogenic genetic status (9), but few studies have reported the long-term outcomes or the responses of female C57BL/6 mice to DIO (5, 6). Additionally, a diet with 60% of calories from fat is more effective than a diet with lower fat content (such as 45%) for inducing obesity in mice (10, 11); however, 60% kcal from fat is much higher than the normal human dietary fat intake, particularly when it comes largely from a single fat source (12). The NHANES dietary nutrients intake survey reported that 30–40% of energy intake for U.S. adult men and women is from fat (13). In addition to these factors, litter size and weaning weight have weak positive associations with adiposity in C57BL/6J mice (9).

In this paper, we examined the variations in body weight, food intake and body composition after long-term HFD feeding of both male and female C57BL/6J mice with a robust sample size.

Methods

Animals

Six-week-old C57BL/6J mice (n=277 males and n=278 females) were obtained from the Jackson Laboratory (Bar Harbor, ME) and group housed (n=5/cage) for one week with standard rodent chow (Teklad Global 16% Protein Rodent Diet; Harlan, Madison, WI). Mice were subsequently singly housed for one week and then fed a high-fat diet (D12451, 45% kcal fat and 20% protein; Research Diets, New Brunswick, NJ) starting from 8 weeks of age. A separate group of 30 mice of the same age and strain (n=15 males and n=15 females) were acquired one month earlier as a low-fat control group. After a one-week acclimation, these 30 mice were singly housed and subsequently fed a low-fat diet (LFD; D12450B, 10% kcal fat and 20% kcal protein, Research Diets) starting from 8 weeks of age.

All mice were housed in poly-carbonate, standard filter top mouse cages (31L × 20W × 15H cm^3) containing ~200 g of sterilized Beta Chip Bedding (NEPCO, Warrensburg, NY) and a paper tube for enrichment. The housing room was a specific pathogen free animal facility with sentinel monitoring. All cages were maintained on a ventilation rack system (Thoren Caging Systems, Hazleton, PA). Cages, with bedding and water (sterilized, in plastic bag with activator), were changed monthly, and the positions of cages on the rack were switched monthly to reduce the influence of environment (light intensity, noise, vibration, etc.). The animal room was maintained a 12:12 hour light-dark cycle beginning at 6 a.m., at an ambient temperature of 22±1°C and 50% humidity. All procedures were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) at the University of Alabama at Birmingham.

Measures

Body weight was measured weekly for the first month and every four weeks thereafter until 43 weeks of age (unless identified, all week numerals indicate mice age). Food was changed biweekly and measured every two of four weeks' interval. Food intake was obtained by subtracting remaining food, including any spilled food in cages, from a weighed aliquot for a period of two weeks. Energy intake was calculated on the basis of 4.73kcal/g for the HFD and 3.85kcal/g for the LFD (caloric values obtained from Research Diets, Inc.). Body composition (fat and fat-free mass) was determined *in vivo* using quantitative magnetic resonance (QMR) (EchoMRI™ 3-in-1 v2.1; Echo Medical Systems, Houston, TX) at 8, 23 and 43 weeks of age as described (14). Caloric content of the change in body mass was computed at 1.8 kcal/g for fat-free tissue and 8 kcal/g for fat tissue (15).

Moribund mice, including mice with ulcerative dermatitis or similar skin lesions (n=29) or those unable to eat/drink (one female), were euthanized in accordance with university and IACUC policies. One male mouse died spontaneously during the study. A total of 23 female and 8 male mice were dead or removed prior to 10 months of age; thus, 554 mice were included in the data analysis (255 HFD females, 269 HFD males, 15 LFD females and 15 LFD males).

Statistical analyses

All statistical analyses were performed using SAS (version 9.3; SAS Institute, Cary, NC). Student's t-test or repeated measures ANOVA with Tukey-Kramer adjustment for multiple comparisons was used where appropriate to determine significant differences in body weight, food intake and body composition over time. Repeated measures analysis of covariance (ANCOVA) was used to compare the changes in body weight among groups. For comparisons of body weight and increases in body weight, we employed cubic polynomial models. Time (weeks) is a continuous variable, with both squared and cubed terms, and the model includes main effects for diet and sex as well as their two-way interaction and their interactions with time. This permits a distinct cubic growth curve for each diet and sex combination and allows for comparisons at time points not common to both diets. Repeated measures are modeled with a spatial power covariance structure, since measurements were taken at unequal time intervals.

Non-parametric Mann-Whitney test was used to perform the body composition comparisons within each sex. Pearson correlation was used to test the inter-relationships among body weight, energy intake and body composition. Multivariate analysis of variance was used to predict, for each sex, body weight and fat mass with time, with body weights (or fat mass) as dependent variables and three baseline features considered as independent variables. The criterion for statistical significance was $p<0.05$ (2-tailed), using p-values adjusted for multiple comparisons.

Results

Body weight

Starting from baseline (8 weeks of age), both HFD and LFD mice significantly increased body weight over time (all $p<0.0001$) (Figure 1.A), with significant diet and sex effects. The average body weight increase from week 8 to week 43 was 20.53 g for HFD males and 12.55 g for HFD females, versus 10.5 g for LFD males and 5.62 g for LFD females. Within each sex, HFD male mice were significantly heavier than their LFD counterparts beginning at week 14 ($p=0.0221$), while HFD females showed statistical differences from LFD females at week 27 ($p=0.0076$).

As for the increases in body weight (Figure 1.B), a significant difference between HFD-fed males and females was first evident at week 10 ($p=0.0027$). Increases in body weight of HFD males remained significantly greater than LFD males and both female groups from week 14 onward ($p=0.0028$ between HFD males and LFD males at week 14; all others $p<0.0001$). In a manner analogous to the absolute body weight comparisons within the same sex, change in body weight of HFD males was significantly different from LFD males from week 14 ($p=0.0028$) and remained significantly different thereafter (all $p<0.0001$), while that of females occurred from week 23 ($p=0.0178$) and remained significantly different ($p=0.0009$ for week 27 and $p<0.0001$ for the remaining weeks). In contrast to the significant differences in absolute body weight between LFD males and HFD females (from week 8 to week 39), these two groups have a statistically similar increase in body weight for all weeks.

Energy intake

Within HFD, males had a significantly higher energy intake than females ($p<0.0001$ for all weeks); however, within LFD, there was no significant difference until week 43 ($p=0.0103$). Final measures of energy intake of HFD male and female mice were significantly higher than initial measures (both $p<0.0001$), but there was no significant difference for LFD mice ($p=0.9494$ for males and $p=1.0000$ for females). Significant differences in daily energy intake between HFD and LFD animals was first evidenced in males at week 27 ($p=0.0391$) but appeared only sporadically for females, at week 23 ($p=0.0091$) and again at week 43 ($p=0.0002$) (Figure 1.C).

Average daily energy intake across the study was significantly correlated with total body weight gain for all groups (Figures 2.A and C) and was similarly correlated with caloric content of the increased body mass (fat mass + fat-free mass) (Figures 2.B and D). The correlation between average daily energy intake and total weight gain was stronger in HFD

females (R=0.7268, p<0.0001) than in HFD males (R=0.3748, p<0.0001). Similarly, there were significant correlations between average daily energy intake and caloric content of the increased body mass (R=0.6989, p<0.0001 for HFD females; R=0.3377, p<0.0001 for HFD males).

Body composition

Both fat mass and fat-free mass significantly increased over time (all p<0.0001), except for the fat mass of LFD females (p=0.6597) (Figures 3.A and B). At baseline, there was no statistical difference between males and females in fat mass for either HFD (p=1.0000) or LFD (p=1.0000). However, the fat-free mass was significantly different between males and females for both HFD (p<0.0001) and LFD (p<0.0001). For weeks 23 and 43, males had greater fat-free mass than females, and HFD mice had greater fat mass than LFD mice. For the changes in body mass from week 8 to week 23, fat mass change was significantly higher in males than in females in both HFD (10.15g vs. 3.14g, p<0.0001) and LFD (4.36g vs. 0.98g, p=0.0066); there was no significant difference between males and females in fat-free mass change in either HFD (p=0.9030) or LFD (p=1.000 for Tukey-Kramer adjusted, unadjusted p=0.9734). For the increases in body mass from week 23 to week 43, fat mass increase was not significantly different between HFD males and females (p=0.9892) or between LFD males and females (p=0.1986); change in fat-free mass was significantly different between HFD males and females (1.95g vs. 1.26g, p<0.0001) but not significantly different between LFD males and females (p=0.8005).

Although the amount of fat-free mass significantly increased over time, the variation in fat-free mass remained relatively stable for each of the four groups. This is evidenced by the coefficient of variation, which ranges from a low of 0.042 for LFD males at week 43 to a high of 0.070 for HFD females at week 8. In contrast, variability in fat mass increased over time, with the lowest coefficient of variation 0.138 at week 8 and the highest 0.390 in week 43. This is illustrated in Figures 3.C to E. The distributions of fat mass and fat-free mass for all groups at week 8, 23 and 43 are shown in Figure 4.A to D. As shown in Figures 4.E and F, both males and females on HFD have significantly increased variance of fat mass over time (both p<0.0001), but the variance of fat-free mass did not significantly change over time except for HFD males from week 23 to week 43 (p<0.0001). For LFD mice, neither fat mass nor fat-free mass increased variability over time, with the exception of fat mass variance for LFD males, which increased significantly from week 8 to week 23 (p<0.0001).

Supplemental Figure 2 illustrates the relationship between gain in body weight and the gains in fat mass and fat-free mass from baseline (week 8) to the end of study (week 43). Fat mass gain (Δ fat mass) is highly correlated with body weight gain (Δ body weight) for HFD males (R=0.9692, p<0.0001), LFD males (R=0.9349, p<0.0001) and HFD females (R=0.9791, p<0.0001) but not for LFD females (R=0.3901, p=0.1506). Similarly but to a lesser extent, fat-free mass gain (Δ fat-free mass) was also significantly correlated with Δ body weight for HFD males (R=0.7456, p<0.0001), HFD females (R=0.5294, p<0.0001) and LFD females (R=0.6822, p=0.0051) but not for LFD males (R=0.5074, p=0.0535). In addition, there are significant correlations between absolute fat mass and fat-free mass for all four groups at most time points (Supplemental Table 1).

Multivariate analysis of variance on body weight (gain) prediction for HFD mice

Considering the large variability observed in final body weight (and fat mass) over the course of the study, baseline values for fat mass, fat-free mass and average daily energy intake were tested as predictors (or explanatory variables) of the DIO response. Multivariate analysis of variance for predictions of body weight (Table 1) indicates that these baseline measurements are significant for predicting future body weight, but the significance is sex-specific.

Of these three predictors for future body weight in HFD males, all of them were significant positive predictors for future weight throughout the study (all overall effect $p<0.0001$). For HFD females, baseline fat mass and fat-free mass were significant predictors for all time points (both overall effect $p<0.0001$); baseline energy intake was a significant predictor through week 35 ($p=0.0477$ at week 35).

For the prediction of fat mass (Table 2), baseline fat mass was a significant predictor for both sexes (both overall effect $p<0.0001$). Baseline fat-free was a significant predictor for HFD females for both of the two future measurements, but was only significant for the measurement at week 23 for HFD males. Baseline energy intake was a significant predictor only at week 23 for HFD males ($p<0.0001$) and at the same week for HFD females ($p=0.0054$).

Discussion

The data presented in this study are from the DIO phase of an ongoing longevity study using obesity-prone C57BL/B6J mice. The fat content of the diet used resembles the fat intake in the U.S. population (12, 13). While most of the published literature on DIO in C57BL/6 mice has focused on males only, we utilized both sexes. In agreement with other studies (6, 16), our results show that male C57BL/6J mice are susceptible to DIO, with females to a lesser extent and in a slower manner. Additionally, there are sex differences in the body weight variation and distribution, energy conversion to body weight and body composition changes, as well as body weight predictions with baseline features.

Male C57BL/6 mice have been a standard animal utilized for DIO research (1, 2, 3, 4), but few studies have reported the responses of female C57BL/6 to high-fat DIO, with limitations including small sample size or short study duration. Tortoriello *et al*. (17, 18) demonstrated that distinct female sex-specific resistance toward DIO exists in the C57BL/6J strain with 24% kcal fat diet for 20 weeks (n=20) and 35% fat diet for 24 weeks (n=10) and speculated that a prolonged feeding or higher-fat diet may be more effective. A later study (11) found higher body weight with higher dietary fat feeding, but it didn't support the concept of sex-specific resistance to obesity when a 24% body weight increase was observed with 45% kcal fat diet for 12 weeks (n=50) and a 39% increase in weight with a higher fat content diet (60%). We found that our HFD females (n=255) had an average 71% increase in body weight, compared to 93% in males (n=269), after 35 weeks of high-fat feeding. In the distribution of final body weight, males were skewed left (skewness=−0.284), while females were skewed right (skewness=0.659). This indicates a general tendency for a small proportion of females to greatly exceed their expected weight gain, while a small proportion

of males fall short of their expected weight gain. When male and female mice were fed with HFD and LFD, significant differences between the two diets were observed earlier in males than in females, which suggests males respond faster to high-fat diet than do females. The early difference in body weight between HFD and LFD in males also was observed by others (6, 19) with the same or similar diets. Estrogen level (20), sex-specific leptin resistance (4, 21) and differences in gross locomotor activity (16) may contribute to sex differences in response to DIO, but they were not tested in this study.

We found that both body weight and fat mass were highly variable among animals after long-term HFD feeding, which was consistent with results from other studies (8, 9, 11, 22). As mice grow larger, the variation in fat mass for both males and females becomes greater than the variation in fat-free mass, which suggests that fat-free mass is less influenced by dietary manipulation than fat mass. It would be important to determine when the body weight or fat mass variation increase occurs and what causes the variation in this genetically identical, inbred mouse strain. Koza *et al*. (9) indicated that variations in body weight occurred at an early age even with weeks of low-fat diet feeding, and this variation persisted with calorie restriction. We examined this phenomenon by separately analyzing the HFD animals that finished the study in either the top 10% or bottom 10% of all HFD animals of the same sex. Significant differences were found between them at baseline in both males ($p<0.0001$) and females ($p<0.0001$) (Supplemental Figures 1.A and B), which indicates that body weight variation already existed before receiving HFD. Additionally, even under HFD feeding, the body weight of the bottom 10% HFD mice (both males and females) was not significantly different from that of LFD mice for most of the time points.

It has been suggested (9) that food intake contributes to the long-term variation in adiposity among mice. This is supported by the significant correlation between average daily energy intake and total body weight gain (or caloric content of the increased body mass) in our data. Interestingly, the correlation between energy intake and body weight gain (or caloric content of the increased body mass) is stronger in HFD females than in HFD males. Furthermore, the average daily energy intake of the top 10% HFD animals was significantly higher than that of the bottom 10% across the study for males (all $p<0.0001$) and from week 14 for females ($p=0.0798$ at week 10, all other $p<0.0001$) (Supplemental Figures 1.C and D). This suggests that mice with lower initial body weight did not respond to HFD as well as those with greater body weight.

Given the variability in this DIO model, it would be useful and cost-effective if there were established parameters for future body weight or body fat prediction. Zhang *et al.* (8) investigated potential factors predicting non-genetic variability in body weight gain in 60 C57BL/6J mice and found that HFD-induced obesity was associated with baseline fat mass, fat-free mass and physical activity. They used similar-aged male C57BL/6J mice (Charles River UK) that received the same high-fat diet for 16 weeks and found that initial body fatness was the strongest predictor of the variability in weight gain independent of HFD feeding duration, and that baseline physical activity and fat-free mass were associated with late-stage body weight gain at 16 weeks of study. However, baseline food intake, resting metabolic rate and body temperature were not significant predictors at any of the time points. We also looked retrospectively at the prediction of body weight based on three

baseline features, including energy intake, body weight, fat mass and fat-free mass. We found that these features are significant predictors of future body weight for both sexes but baseline energy intake is only partially significant for females. Additionally, baseline fat mass is a significant predictor of future body fat for both sexes, while baseline fat-free mass is only significant for females throughout the study. These results suggest sex differences in growth and development.

In conclusion, male and female C57BL/6J mice have differences in growth patterns besides the fact that males have higher body weight, fat mass and fat-free mass. Both males and females have large variations in fat mass, and this variability increases over time, while the variation of fat-free mass remains relatively stable. The multivariate analysis of variance model indicates that baseline features such as fat mass, fat-free mass and energy intake could serve as predictors for future body weight estimate.. Researchers should take into consideration the variations and sex differences in this DIO-susceptible mouse strain. Studies to find the causes for the individual variations of HFD-induced obesity in the inbred mouse strain are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

DLS, DBA and TRN conceived the study and provided critical support on statistical analyses and data interpretation. YY and DLS helped data collection. YY and KDK performed statistical analyses. All authors were involved in writing the paper and had final approval of the submitted and published versions. This study was supported in part by NIH grants R01AG033682, P30DK056336, P60DK079626 and T32DK062710.

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What is already known about 19 this subject?

1. C57BL/6 mice are susceptible to dietary-induced obesity (DIO)
2. There is considerable variation in body weight of DIO mice
3. Some baseline features are associated with future weight gain

What does this study add?

1. Large variation in fat mass after long-term high-fat feeding in both sexes of C57BL/6J mice and variations in body weight occur before dietary intervention
2. Sex differences exist in the responses to high-fat diet feeding
3. Baseline fat mass, fat-free mass and energy intake can be used to predict future body weight

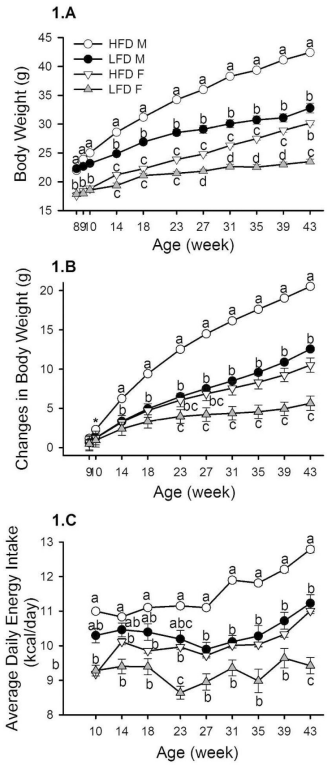

Figure 1.

Body weight. (A) Body weight changes with time. Male mice with high-fat diet (HFD M; 45% kcal fat; n=269), male mice with low-fat diet (LFD M; 10% kcal fat; n=15), female mice with high-fat diet (HFD F; n=255) and female mice with low-fat diet (LFD F; n = 15) at age 8–43 weeks. (B) Changes in body weight (body weight at each week subtract baseline body weight) for all groups. (C) Average daily energy intake change with time for all groups. Body weight and energy intake of week 18 for the HFD males and females were the average of week 17 and week 19 to make it comparable with LFD. * indicates significant differences between HFD females and HFD males at week 10 for figure 1.B. Different letters at each time point denote significant differences at $p<0.05$. Error bars represent SE.

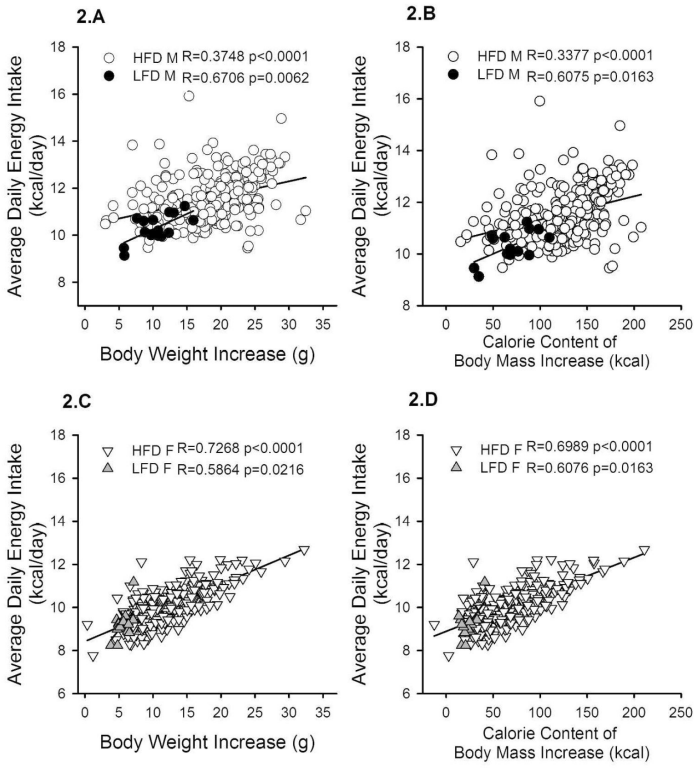

Figure 2.

Correlation of energy intake and total weight gain or caloric content of increased body mass. Average daily energy intake and total weight gain (A) and caloric content of increased body mass (B) for HFD males and LFD males; average daily energy intake and total weight gain (C) and caloric content of increased body mass (D) for HFD females and LFD females.

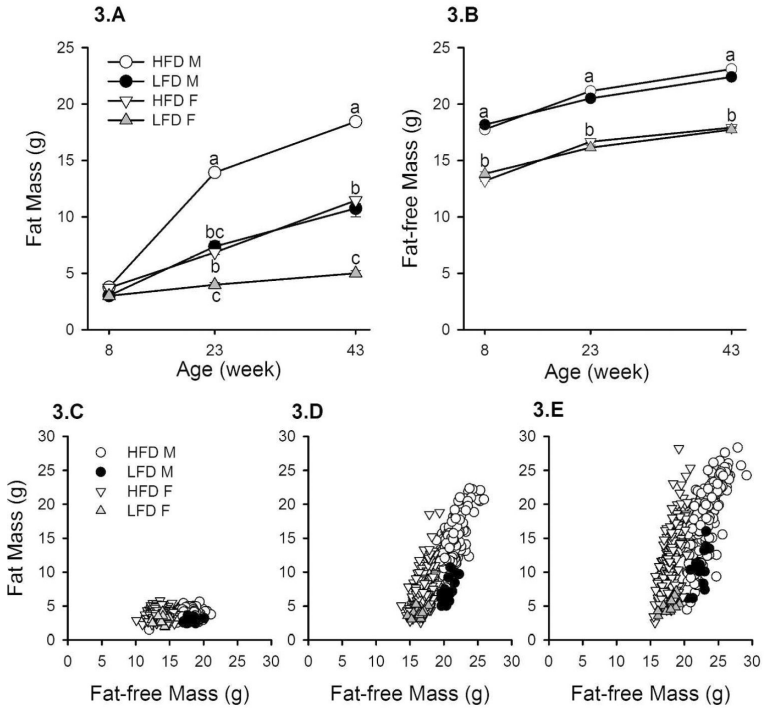

Figure 3.

Body composition changes. (A) Fat mass (mean with SE) for all groups; (B) fat-free mass (mean with SE) for all groups; body composition (fat mass vs. fat-free mass) scatter for all groups at week 8 (C), week 23 (D) and week 43 (E). Different letters denote significant differences at $p<0.05$.

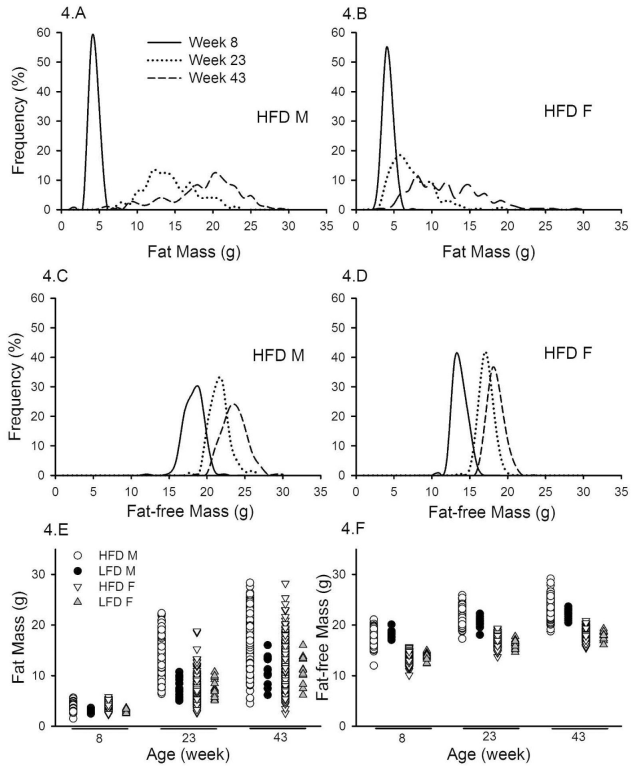

Figure 4.

Body composition distributions at week 8, week 23 and week 43. Histogram distribution (drawn with 30 bins, ranged from 0 to 30 g for fat mass and 10 to 30 gram for fat-free mass). (A) Fat mass of HFD males; (B) fat mass of HFD females; (C) fat-free mass of HFD males; (D) fat-free mass of HFD females. Individual scatter plot distributions of (E) fat mass for all groups and (F) fat-free mass for all groups.

Table 1

Multivariate analysis of variance predicting body weight using baseline features in HFD-fed mice*

			Overall Effect (Wilks' Lamda)	Weeks of Age						
				14	18	23	27	31	35	39	43
Male (n = 269)											
Models	R^2			0.4993	0.3760	0.2739	0.2279	0.1726	0.1357	0.1320	0.1097
	P			< 0.0001	< 0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Predictors (p-values and test statistics)	FM	P<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	0.0005	0.0010	<0.0001	0.0003
		WL=0.7878		(55.58)	(40.17)	(23.88)	(18.91)	(12.32)	(11.11)	(17.56)	(13.22)
	FFM	P<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	0.0005	0.0117	0.0173	0.0167
		WL=0.6991		(88.51)	(41.86)	(23.36)	(20.89)	(12.57)	(6.44)	(8.74)	(5.8)
	EI	P<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.0004	0.0075	0.0202
		WL=0.8466		(45.89)	(33.06)	(21.60)	(16.46)	(14.75)	(12.86)	(7.26)	(5.46)
Female (n = 255)											
Models	R^2			0.5091	0.4626	0.3207	0.2621	0.2233	0.1937	0.1684	0.1482
	P			< 0.0001	< 0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Predictors (p-values and test statistics)	FM	P<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0003	0.0005
		WL=0.6557		(105.54)	(87.29)	(30.25)	(22.47)	(20.15)	(16.26)	(13.46)	(12.63)
	FFM	P<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
		WL=0.7238		(84.32)	(79.81)	(60.19)	(49.31)	(37.5)	(32.23)	(26.76)	(21.88)
	EI	P<0.0001		<0.0001	<0.0001	0.0009	0.0191	0.0240	0.0477	n.s.	n.s.
		WL=0.8346		(43.33)	(26.05)	(11.32)	(5.57)	(5.16)	(3.96)		

*FM, baseline fat mass; FFM, baseline fat-free mass; EI, baseline average daily energy intake; HFD, high-fat diet; WL, Wilks' Lamda; n.s., not significant; #(#), p-value (F value); for weeks 14 through 43, the test statistic is a Type III F statistic; for the overall effect, the test statistic is Wilks' Lambda.

Table 2

Multivariate analysis of variance predicting fat mass using baseline features in HFD-fed mice*

		Male (n=269)			Female (n=255)		
		Overall Effects (Wilks' Lamda)	Weeks of Age		Overall Effects (Wilks' Lamda)	Weeks of Age	
			23	43		23	43
Models	R^2		0.2113	0.0706		0.2396	0.1169
	P		<0.0001	0.0002		<0.0001	<0.0001
Predictors	FM	P<0.0001	<0.0001	0.0004	P<0.0001	<0.0001	0.0008
		WL=0.9120	(24.52)	(12.76)	WL=0.8958	(28.62)	(11.52)
	FFM	p=0.0052	0.0019	n.s.	P<0.0001	<0.0001	0.0001
		WL=0.9610	(9.87)		WL=0.8819	(33.44)	(15.43)
	EI	p=0.0001	<0.0001	n.s.	p=0.0166	0.0054	n.s.
		WL=0.9332	(18.38)		WL=0.9677	(7.87)	

*FM, baseline fat mass; FFM, baseline fat-free mass; EI, baseline average daily energy intake; HFD, high-fat diet; WL, Wilks' Lamda; n.s., not significant; #(#), p-value (F value); for weeks 23 and 43, the test statistic is a Type III F statistic; for the overall effect, the test statistic is Wilks' Lambda.