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Adipose tissue *Mest* and *Sfrp5* are concomitant with variations of adiposity among inbred mouse strains fed a non-obesogenic diet

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Abstract

The expression of a subset of genes including mesoderm specific transcript (*Mest*), secreted frizzled-related protein 5 (*Sfrp5*) and bone morphogenetic protein 3 (*Bmp3*) in adipose tissue biopsies of C57BL/6J mice before exposure to an obesogenic diet were shown to be predictive for the development of obesity in mice after feeding a high fat diet for 8 weeks. This observation led to the supposition that adipose tissue expression of this subset of genes within inbred strains of mice could be associated with their susceptibility in the development of adiposity when fed a low fat diet. The analyses of male mice from 5 inbred strains showed average bodyweights ranging from 25.82–36.58 grams at 16 weeks of age. Bodyweight was highest for AKR/J and adiposity correlated highly with bodyweight for all strains. Analyses of epididymal fat gene expression showed *Mest, Sfrp5* and *Bmp3* to be highly concomitant with adiposity across all strains of mice. Naked 1 (*Nkd1*), a gene previously shown to be associated with variations of adiposity in mice fed a high fat diet, but not predictive for the development of adiposity, showed no correlation with adiposity. In addition, the expression of *Mest* and *Sfrp5* were tightly associated across the 5 mouse strains with the highest and lowest expression occurring in DBA/2J and C57BL/6J (B6) respectively suggesting a common mechanism for their regulation. Surprisingly, when independent cohorts for these 2 strains were fed high fat diet for 8 weeks, DBA/2J showed no further increase in *Sfrp5* expression whereas expression levels for B6 mice were induced almost 20-fold. Analyses of (B6 × DBA2/J) F1 mice fed a low fat diet for 8 weeks showed intermediate levels of adiposity and gene expression for *Sfrp5* and *Mest* suggesting a strong genetic basis for these differences.

Keywords

Mest; *Sfrp5*; *Bmp3*; adiposity; obesity; epigenetics; genetics; inbred strains; diet-induced obesity

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The authors declare that there are no conflicts of interest.

1. Introduction

Experiments using genetically homogeneous inbred mouse strains have demonstrated the emergence of large variations in obesity and diabetes phenotypes when fed a high fat diet. [1, 2]. In addition, phenotypic variability in traits such as adiposity and fat mass expansion within inbred populations of mice are highly stable over time suggesting that an epigenetic etiology, possibly involving developmental changes caused by pre- or postnatal programming events, is involved in phenotypic variation of diet-induced obesity. [2]. Previous studies using global gene expression analyses were able to identify genes that are expressed in adipose tissue that were positively associated with the development of adiposity in a population of inbred C57BL/6J (B6) male mice that included imprinted genes, as well as genes involved in Wnt and Tgf-β signaling pathways [2]. A subset of these genes, including the maternally imprinted mesoderm specific transcript (*Mest*), secreted-related frizzled protein 5 (*Sfrp5*) and bone morphogenetic protein 3 (*Bmp3*) were also shown to be highly predictive for future susceptibility to the development of adiposity in mice when expression was measured in fat biopsies prior to feeding mice an obesogenic diet [2].

There is large body of compelling evidence for a role of *Mest* in mediating fat accumulation in adipocytes and adipose tissue [2–6]; however, the mechanisms that regulate *Mest* and its catalytic function remain elusive. MEST is localized in the endoplasmic reticulum/Golgi apparatus of the adipocyte, a cellular component essential for lipid storage and metabolism where it may act to facilitate fat uptake in adipocytes and storage of fat in lipid droplets [6]. Although the association and role for *Sfrp5* in adipose tissue remains controversial, it has been demonstrated that addition of exogenous recombinant *Sfrp1* or *Sfrp2*, molecules with strong sequence homology with *Sfrp5* [7], to 3T3-L1 preadipocytes was shown to promote spontaneous differentiation into adipocytes [8]. Studies demonstrating increased adipose tissue *Sfrp5* expression associated with enhanced adiposity in B6 mice after a high fat diet are consistent for a role of these soluble inhibitors of Wnt signaling in the development of adiposity in mice [2]. *Sfrp5* may act to stimulate adipocyte hypertrophy via inhibition of oxidative metabolism [9]; and, in addition has been suggested to act as an anti-inflammatory adipokine that regulates metabolic dysfunction and inflammation [10, 11].

Bone morphogenetic proteins (Bmp's), members of the Tgf-β superfamily, were originally identified as peptides that induce bone and cartilage formation but have since been shown to be involved in a wide variety of morphogenetic processes during development [12–16]. The addition of recombinant *Bmp2* and *Bmp7*, or ectopic expression of *Bmp2* and *Bmp4*, is able to convert pluripotent mouse fibroblast cell line C3H10T1/2 into osteoblasts, chondroblasts or adipocytes [17–21]. *Bmp3*, unlike other Bmp's, antagonizes *Bmp2* signaling and osteogenesis via activation of the Tgf-β/activin pathway [22, 23]. Since evidence strongly indicates a reciprocal relationship in pathways leading to osteogenesis and adipogenesis, the antagonistic effect of *Bmp3* on osteogenesis is consistent with a morphogenetic role for *Bmp3* in adipogenesis [23–25].

Previous studies show *Mest, Sfrp5* and *Bmp3* in adipose tissue biopsies of C57BL/6J mice prior to feeding mice an obesogenic diet is highly predictive for future development of adiposity in an obesogenic environment; however, very little is known in regards to how

non-dietary fat-induced ‘genetic’ differences in the expression of these genes correspond to the development of adiposity among unique inbred mouse strains. In this study we evaluated body composition and adipose tissue expression of *Mest, Sfrp5* and *Bmp3* in 5 inbred mouse strains to determine the relationship of these molecular correlates with the development of adiposity.

2. Materials and Methods

2.1 Animals and phenotyping

All *in vivo* experiments were carried out with male A/J, C57BL/6J, AKR/J, DBA/2J, 129SVImJ and (B6 × DBA/2J)F1 mice purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were maintained in a temperature-controlled room (23°C) with a 12-h light/12-hr dark cycle. The mice were reared under conventional conditions and fed PicoLab Rodent Diet 20 (Lab Diet; 13% kcal fat) until 16 weeks of age. A subset of mice was fed high fat diet (D12331; 58 kcal% fat) for 8 weeks (8–16 weeks of age). Body composition (body fat, lean mass and free fluid) was analyzed by Minispec NMR (Bruker) which uses the contrasting hydrogen density and/or hydrogen spin properties from adipose tissue and muscle for estimating body composition. A quality control check of NMR parameters using a standard provided by the manufacturer was performed at the beginning of each day of testing. All animal experiments were approved by the Pennington Biomedical Research Center and Maine Medical Center Research Institute Institutional Animal Care and Use Committees and in accordance with National Institutes of Health guidelines for care and use of laboratory animals.

2.2 Gene expression analyses

Epididymal fat of mice was isolated from euthanized mice and quickly frozen in liquid nitrogen. RNA was extracted from tissue homogenized in TriReagent (Molecular Research Center, Inc.) and then purified using RNeasy Mini Kit and RNase-free DNAse (Qiagen). Isolated RNA was protected from RNAse contamination with SUPERase-In (Life Technologies). RNA quantity and quality was determined using Nanodrop 1000 spectrophotometer. Quantitative reverse transcription-PCR was performed using total RNA with specific primers and probes designed using Primer Express software v3.0.1 (Life Technologies) as previously described [6]. Gene expression data were normalized to cyclophilin b (*Ppib*).

2.3 Statistics

Statistical calculations were performed using GraphPad Prism software V.6 and Microsoft Excel 2010. Statistical differences between 2 groups were calculated using two-tailed unpaired parametric t-test with confidence level at 95%. Significance among multiple groups was calculated with ordinary one-way ANOVA followed by Tukey’s multiple comparisons test with an alpha of 0.5. Pearson correlation coefficients (Two-tailed; assuming Gaussian distribution) were calculated with a confidence interval of 95%. Data is presented as the mean ± SEM. $P<0.05$ was considered to be significant.

3. Results

3.1 Body weight and composition of inbred strains fed low fat (LFD) chow diet

Bodyweights (BWTs) of male mice from 5 unique strains (n=9–10 per strain) fed a LFD was measured at 8 and 16 weeks of age and body composition measured at 16 weeks of age. Data shown in Table 1 demonstrates a broad range of BWTs with differences ranging from ~7g (8 weeks) and ~10g (16 weeks) between strains with AKR/J being significantly larger than all other strains at both 8 and 16 weeks of age. BWT variation across all strains at 16 weeks corresponded strongly with lean mass ($R=0.972$; $P<10^{-30}$) as measured via NMR suggesting that strain-specific differences in longitudinal (lean mass) growth contributes highly to the diversity in BWT across inbred strains fed a LFD. The contribution of fat mass to overall bodyweight is lower but is a significant component of overall BWT in mice fed a LFD ($R=0.852$; $P<10^{-14}$) and shows a higher degree of variation between strains (~1.8-fold) compared to lean mass (~1.3-fold). Fat mass was highest in AKR/J and DBA/2J mice with both strains having >15% fat whereas B6 mice had less than 10% fat (Table 1). In addition, body composition profiles of DBA/2J and B6 mice (Table 1) show no difference in lean mass ($P=0.62$) whereas fat mass is significantly higher in DBA/2J mice ($P<0.0005$) indicating that adiposity is a principal component for differences in BWT between these two inbred strains. It is also important to note that at 8 weeks of age, DBA/2J had the lowest BWT of all strains (Table 1) but showed a ~32% increase in BWT during the subsequent 8 weeks suggesting that significant proportion of weight gain in this strain is likely due to increased fat mass. Epididymal (EPI) fat pad weights across all mouse strains were consistent with NMR-based measurements of fat mass ($R=0.963$; $P<10^{-27}$) providing confidence for the body composition measurements in Table 1.

3.2 Strain-dependent variation in gene expression

Gene expression for *Mest, Sfrp5, Bmp3* and *Nkd1* was measured in RNA isolated from EPI fat from each of the 5 strains as shown in Figure 1. The selection of EPI fat for these analyses was based on previous studies that analyzed the same subset of genes in EPI fat biopsies in 7 week old mice fed a LFD to determine whether pre-HFD levels of expression could predict susceptibility for the development of adiposity in C57BL/6J mice fed an obesogenic diet [2]. *Mest* expression was significantly higher in DBA/2J compared to all of the other mouse strains with almost a 4-fold increase compared to C57BL/6J (Fig. 1A). Importantly, EPI fat *Mest* expression in the LFD-fed mice in this study were consistent with levels detected in past experiments using cohorts of LFD-fed B6 mice as controls to determine effects of dietary fat on the induction of *Mest* [6]. Surprisingly, the strain-dependent pattern for EPI fat *Mest* expression was very similar to that of both *Sfrp5* (Fig. 1B) and *Bmp3* (Fig. 1C). EPI fat *Sfrp5* expression in DBA/2J was more than 20-fold higher than B6 mice and more that 2-fold higher than all of the other mouse strains. EPI fat *Nkd1* expression showed only a modest difference between A/J and B6 ($P=0.04$) but was essentially consistent among all strains. These data are consistent with previous studies showing the lack of association of *Nkd1* expression in EPI fat biopsies with susceptibility for the development of adiposity following an obesogenic diet [2].

3.3 Molecular correlates for strain-dependent variation of adiposity

Analyses of the correlation of EPI fat gene expression and phenotypic parameters across the entire cohort of mice from the 5 strains showed very strong positive associations between adiposity (% fat) vs *Sfrp5* (Fig. 2A) and *Mest* (Fig. 2B) with P values $<10^{-9}$ and 10^{-7} respectively. *Bmp3* was also significantly associated with adiposity (data not shown; R=0.644; $P<10^{-6}$). *Nkd1* showed no significant correlations with adiposity or any other phenotypic measurements. The strong correlation between *Mest* and *Sfrp5* across the entire cohort of mice (Fig. 1C; R=0.844; $P<10^{-13}$) or when plotted as the mean for each gene with respect to strain (Fig. 1D; R=0.984; P=0.002) suggesting the likelihood that a common mechanism is at least partially responsible for the regulation of these 2 genes. A correlation between *Bmp3* vs *Mest* ($P<10^{-5}$) and *Sfrp5* ($P<10^{-4}$) was also evident but was significantly reduced compared to that observed between *Mest* and *Sfrp5*.

3.4 Genetic diversity in the regulation of Sfrp5 and Mest between B6 and DBA/2J mice

Since past studies have shown that feeding mice an obesogenic diet for as short of a period as 1 week can induce the expression of adipose tissue *Sfrp5* and *Mest* in mice [2] we performed a study to determine whether the strikingly high expression of *Sfrp5* in EPI fat of chow-fed DBA/2J mice can be further induced by HFD. Two additional cohorts of DBA/2J (n=9) and B6 (n=9) male mice were placed onto a HFD at 8 weeks of age for 8 weeks (until 16 weeks of age). It was anticipated that EPI fat expression of *Sfrp5* and *Mest* would be robustly induced in B6 during this time [2, 6]. Physiological parameters of HFD-fed B6 and DBA/2J mice, as expected, showed highly significant increases in BWT (Fig. 3A) and adiposity (Fig. 3C and 3D) compared to the LFD-fed cohorts; however, lean mass was either not changed or only modestly increased by the HFD in B6 and DBA/2J mice respectively (Fig. 3B). EPI fat expression of *Mest* in HFD-fed B6 and DBA/2J mice shown in Figure 3E was more highly induced in B6 mice (>6-fold) compared with DBA/2J mice (~2.5-fold) when compared to LFD-fed mice but was still significantly higher in the HFD-fed DBA/2J mice compared to B6 (P=0.0006). In contrast, EPI fat *Sfrp5* expression was induced over 20-fold in HFD-fed B6 mice but remained unchanged in HFD-fed DBA/2J mice compared to their LFD-fed cohort. Interestingly, EPI fat *Sfrp5* mRNA in the HFD-fed B6 mice was not significantly different from either LFD or HFD-fed DBA/2J mice. To further test the genetic predisposition for the differences in EPI fat *Sfrp5* expression in B6 vs DBA/2J mice, an independent cohort of B6 and DBA/2J was compared with an F1 hybrid; (B6 × DBA/2J)F1 (B6D2F1), derived from the two parental strains of mice. The 3 cohorts of mice were fed a LFD until 16 weeks of age and physiological parameters were measured. Data shows significantly higher BWT (Fig. 4A) in the B6D2F1 compared to both parental strains and significantly (P<0.001) and modestly (P=0.058) increased lean mass (Fig. 4B) compared to DBA/2J and B6 respectively suggesting slightly increased longitudinal growth. Adiposity measured by fat mass (Fig. 4C) and %FAT (Fig. 4D) in the B6D2F1 mice showed an intermediate phenotype compared to the B6 and DBA/2J parental strains which was consistent with the expression of EPI fat *Mest* (Fig. 5A) and *Sfrp5* (Fig. 5B) among the 3 strains of mice. EPI fat *Bmp3* expression (Fig. 5C) did not show the same expression profile and was similarly expressed in both DBA/2J and B6D2F1 mice. In addition, the association of EPI fat *Sfrp5* with *Mest* (R^2=0.78; $P<10^{-7}$) across the 3 strains in this study was several orders of magnitude more significant than with *Bmp3* (R^2=0.45; P<0.001). These results

suggest that intermediate levels of EPI fat *Sfrp5* in B6D2F1 mice may be genetically regulated via a codominant or incompletely dominant manner which could lead to coordinated effects on the regulation of adipose tissue *Mest* expression and susceptibility for the development of adiposity.

4. Discussion/Conclusion

Our previous investigations were aimed at identifying epigenetic determinants of obesity utilizing the high degree of variability in adiposity phenotypes after B6 mice have been exposed to an obesogenic environment. The four genes whose expression was measured in this present study; *Mest, Sfrp5, Bmp3* and *Nkd1* all showed a highly variable expression with expression ranging almost 80-fold (*Mest*) in mice fed a HFD for 4 weeks [2]. In addition, the expression of all of these genes was significantly elevated in adipose tissue in HFD-fed mice compared to basal expression levels in mice maintained on a LFD. The effects of dietary fat on *Mest* was particularly striking with some mice exhibiting substantial increases of expression after feeding mice a HFD for only 2 days whereas other individuals showed no increase [6]. Of the four genes showing highly variable HFD-mediated expression three of these; *Mest*, *Sfrp5* and *Bmp3* was also predictive of future development of obesity based on their expression in EPI fat biopsies of B6 mice prior to feeding a HFD [2]. Because of the predictive relationship between *Mest, Sfrp5* and *Bmp3* in mice fed LFD with susceptibility for development of adiposity, we became interested in gaining further functional insight for these genes by determining how their ‘non-fat-induced’ expression in adipose tissue is associated with strain-specific variations in adiposity related phenotypes.

Data from 5 strains of mice fed a LFD for 16 weeks in Table 1 and Figures 1 and 2 showed remarkable consistency between the expression of EPI fat *Mest, Sfrp5* and *Bmp3* with indices of adiposity, as well as strong associations between EPI fat *Mest* and *Sfrp5* across the strains (Fig. 2D). A similar close association between adipose tissue *Mest* and *Sfrp5* was also observed for B6 mice after feeding a HFD for 4 weeks [2]. Although the consistent association of *Mest* with *Sfrp5* is not well understood in the context of fat mass gain in adult mice, it’s possible that *Sfrp5* inhibition of oxidative metabolism [9] could lead to physiological conditions that promote adipocyte hypertrophy and fat mass expansion. Interestingly however, other studies have shown that *Mest* is most highly expressed in early post-natal development when fat mass is rapidly expanding whereas *Sfrp5* expression in adipose tissue is expressed at very low levels during this time [6, 26].

One of the most intriguing outcomes of this present study is the high expression of *Sfrp5* in EPI fat in DBA/2J mice which ranged almost 20-fold higher than B6 (Fig. 1B and 3F). Interestingly, a second cohort of DBA/2J and B6 mice fed a HFD from 8 to 16 weeks of age showed no further increase in DBA/2J EPI fat *Sfrp5* expression compared with DBA/2J fed a LFD, whereas B6 mice showed a ~20-fold increase in EPI fat *Sfrp5* with levels equivalent to DBA/2J mice fed either LFD or HFD (Fig. 3F). Additional cohorts of B6, DBA/2J and a F1 hybrid generated from a cross between B6 and DBA/2J (B6D2F1) fed LFD until 16 weeks of age showed intermediate phenotypes for adiposity (Fig. 4C and 4D), *Sfrp5* and *Mest* (Fig. 5) in the B6D2F1 compared to the parental strains. The analyses of EPI fat *Bmp3* in B6, DBA/2J and B6D2F1 mice (Fig. 5) showed a reduced association of *Bmp3* with *Sfrp5*

compared with that of *Mest*. This observation was somewhat surprising since *Sfrp5* has been shown to modulate BMP signaling in the regulation of organogenesis [27] and retinal development [28] in zebrafish. However, although it is well recognized that significant cross-talk occurs between the Wnt and BMP/Smad signaling pathways [29, 30] and BMP and Wnt signaling are important mediators of adipogenesis [31], very little is known in regards to specific *Sfrp5*-mediated effects on BMP signaling in mammals. In a genetics context, the intermediate expression of EPI fat *Sfrp5* mRNA in the F1 progeny (B6D2F1) derived from B6 and DBA/2J suggests a codominant or incompletely dominant genetic contribution to this quantitative phenotype. Differentiating between codominance vs incomplete dominance with quantitative traits such as EPI fat *Sfrp5* mRNA expression is difficult since the complexity of the genetics regulating *Sfrp5* is not well defined. Since both B6 and DBA/2J do express *Sfrp5*, albeit at different levels when fed a HFD, it is more likely that the intermediate phenotype in the B6D2F1 is caused by codominant contribution of the two alleles [32]. Future quantitative analyses of EPI fat *Sfrp5* mRNA in backcross, intercross, and/or recombinant inbred lines (BXD) derived from the B6 and DBA/2J parental strains will provide significant insight into the genetic complexity in the regulation of adipose *Sfrp5*. A query of MGI (The Jackson Laboratory) revealed only a single nucleotide polymorphism (SNP) within the 3'UTR of *Sfrp5* (*Chr 19*) between B6 and DBA/2J. However, this same SNP also occurs in A/J mice which had levels of EPI fat *Sfrp5* level almost as low as B6 (Fig. 1B) reducing the potential for this polymorphism in having a significant impact on the regulation of *Sfrp5*. In conclusion, our studies show that adipose tissue expression of *Mest, Bmp3* and *Sfrp5* show positive strong association with the development of adiposity across multiple inbred strains of mice in the absence of a HFD. These results are consistent with previous studies of these same genes in the context of variations in adiposity phenotypes within a population of HFD-fed inbred B6 mice. In addition, we have identified a mouse model, DBA/2J, with constitutively high expression of EPI fat *Sfrp5* that is not further up-regulated via dietary fat. This model will be particularly useful for studies of the regulation of *Sfrp5* and its role in adipose tissue function, energetics, inflammation, and glucose homeostasis.

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Abbreviations

Mest	mesoderm specific transcript
Sfrp5	secreted related protein 5
Bmp3	bone morphogenetic protein 3
Nkd1	Naked 1

HFD	high fat diet; Surwit D12331
LFD	low fat diet; PicoLab 20
EPI	epididymal
SNP	single nucleotide polymorphism
Tgf-β	transforming growth factor beta
NMR	nuclear magnetic resonance

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Highlights

- Adipose *Mest, Sfrp5* and *Bmp3* are concomitant with strain-differences in adiposity.
- Strong correlation between *Mest* and *Sfrp5* suggests a common regulatory mechanism.
- DBA/2J mice express high adipose tissue *Sfrp5* compared to other mouse strains.
- DBA/2J, unlike B6 mice, shows no fat-inducible adipose *Sfrp5* expression.
- Characteristics of DBA/2J provide an excellent model to study SFRP5 function.

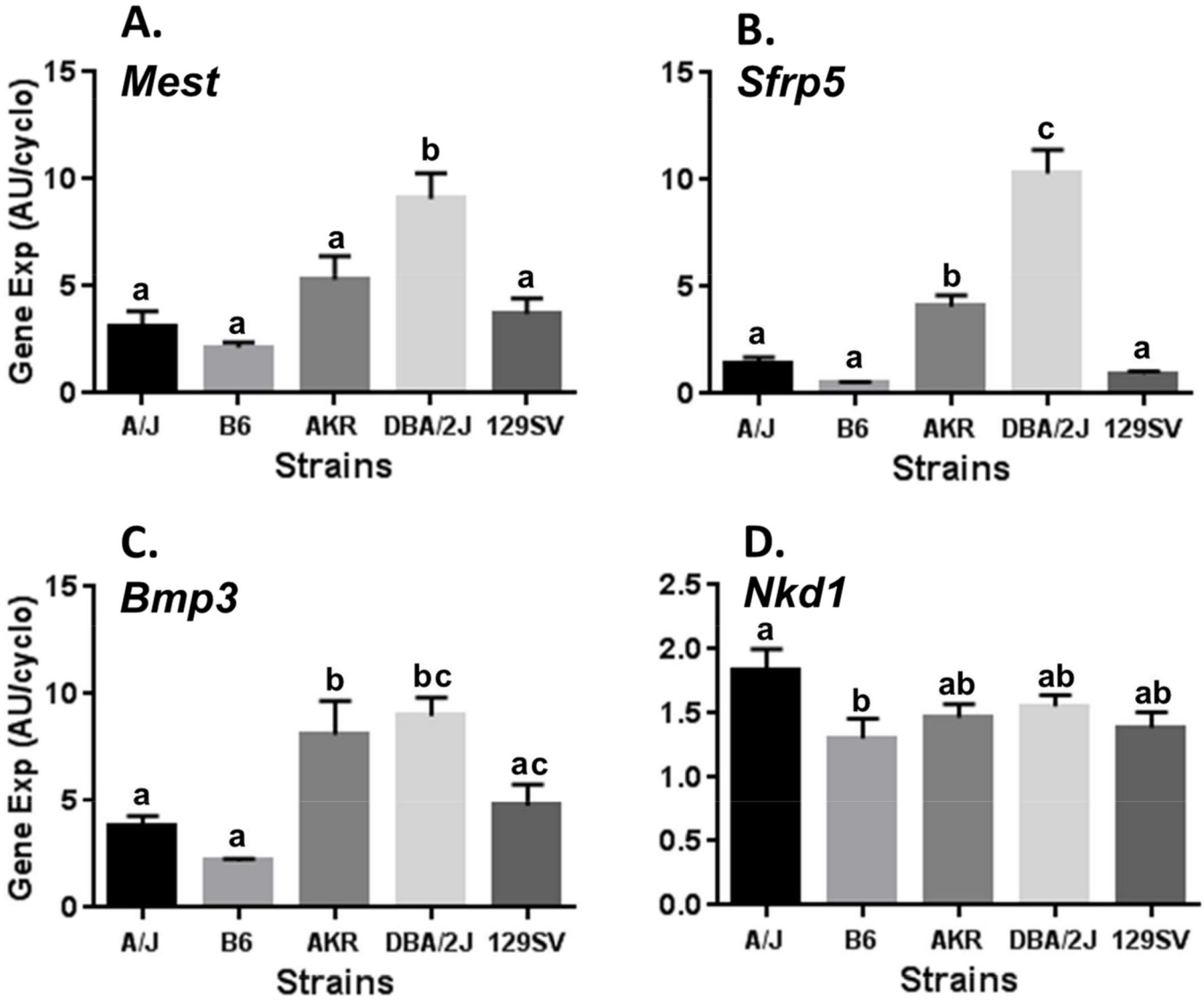

Figure 1.

Analyses of *Mest* (A), *Sfrp5* (B), *Bmp3* (C) and *Nkd1* (D) mRNA expression in epididymal fat (EPI) of 5 inbred mouse strains fed a low fat diet (LFD) until 16 weeks of age. Gene expression measured by TaqMan QRT-PCR is represented as arbitrary units (AU) normalized to cyclophilin b. Each strain represents the mean ± SEM of at least 9 mice. Datasets annotated with the same letter indicate no significant differences between groups. *Mest* expression (A) is significantly higher in DBA/2J compared with B6 ($P<0.0001$), A/J ($P=0.0002$), 129SV ($P=0.0007$) and AKR ($P=0.029$). *Sfrp5* expression (B) is significantly higher in DBA/2J compared with all strains ($P<0.0001$); and, AKR is higher than B6 ($P=0.0007$), 129SV ($P=0.004$) and A/J ($P=0.02$). *Bmp3* expression (C) is significantly higher in DBA/2J compared with B6 ($P=0.0003$), A/J ($P=0.006$), 129SV ($P=0.03$); and higher in AKR compared with B6 ($P=0.002$) and A/J ($P=0.03$). *Nkd1* expression (D) is significantly higher in A/J compared to B6 ($P=0.04$).

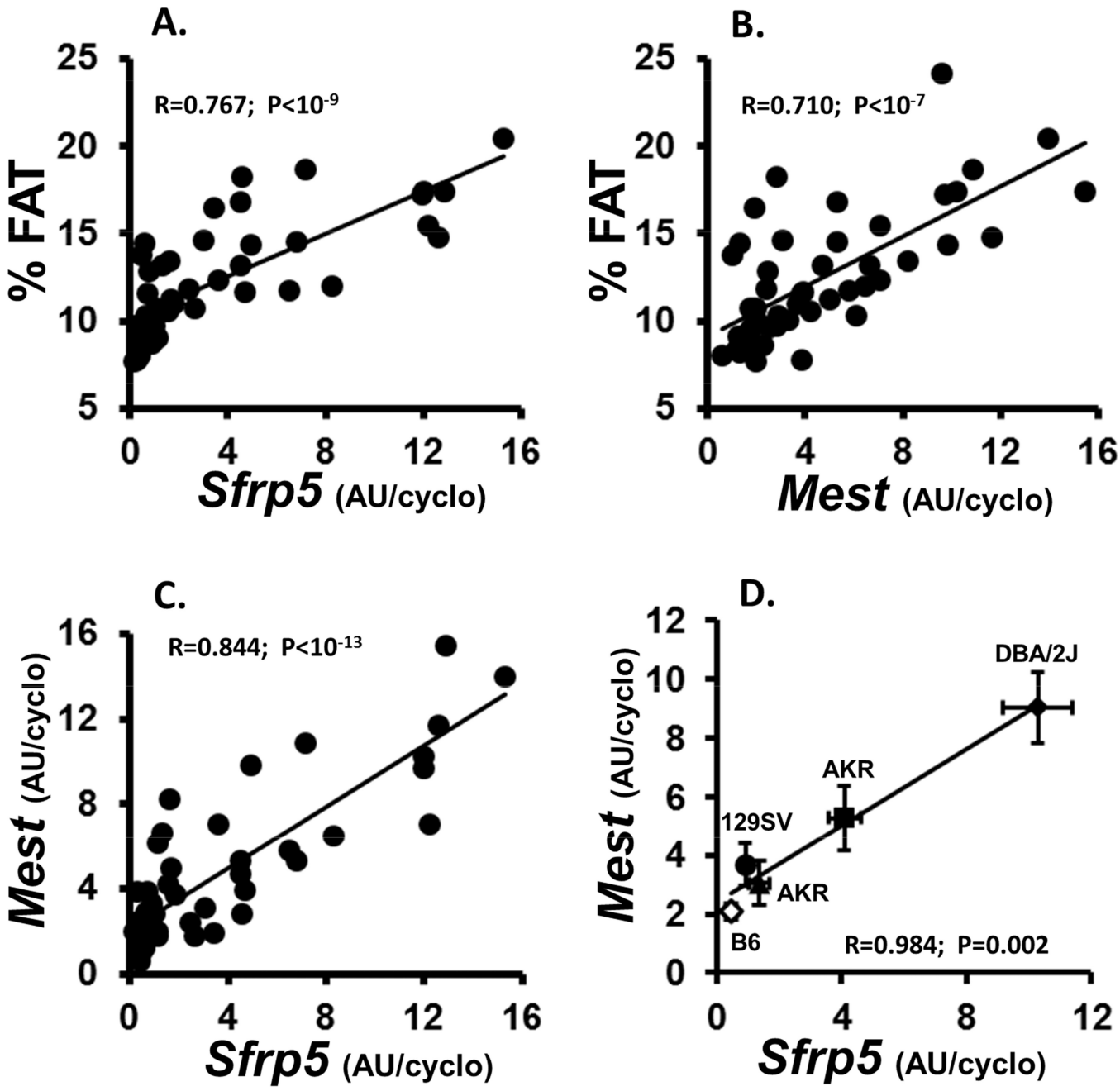

Figure 2.

Data represents the correlation between indices of adiposity (% FAT) with *Sfrp5* (A) and *Mest* (B) gene expression; and, the correlation between *Mest* and *Sfrp5* gene expression in all individuals from the 5 mouse strains (C) or by the mean ± SEM of each of the strains (D). Each strain represents the mean ± SEM of at least 9 mice. Body composition was measured via NMR. Gene expression measured by TaqMan QRT-PCR is represented as arbitrary units (AU) normalized to cyclophilin b.

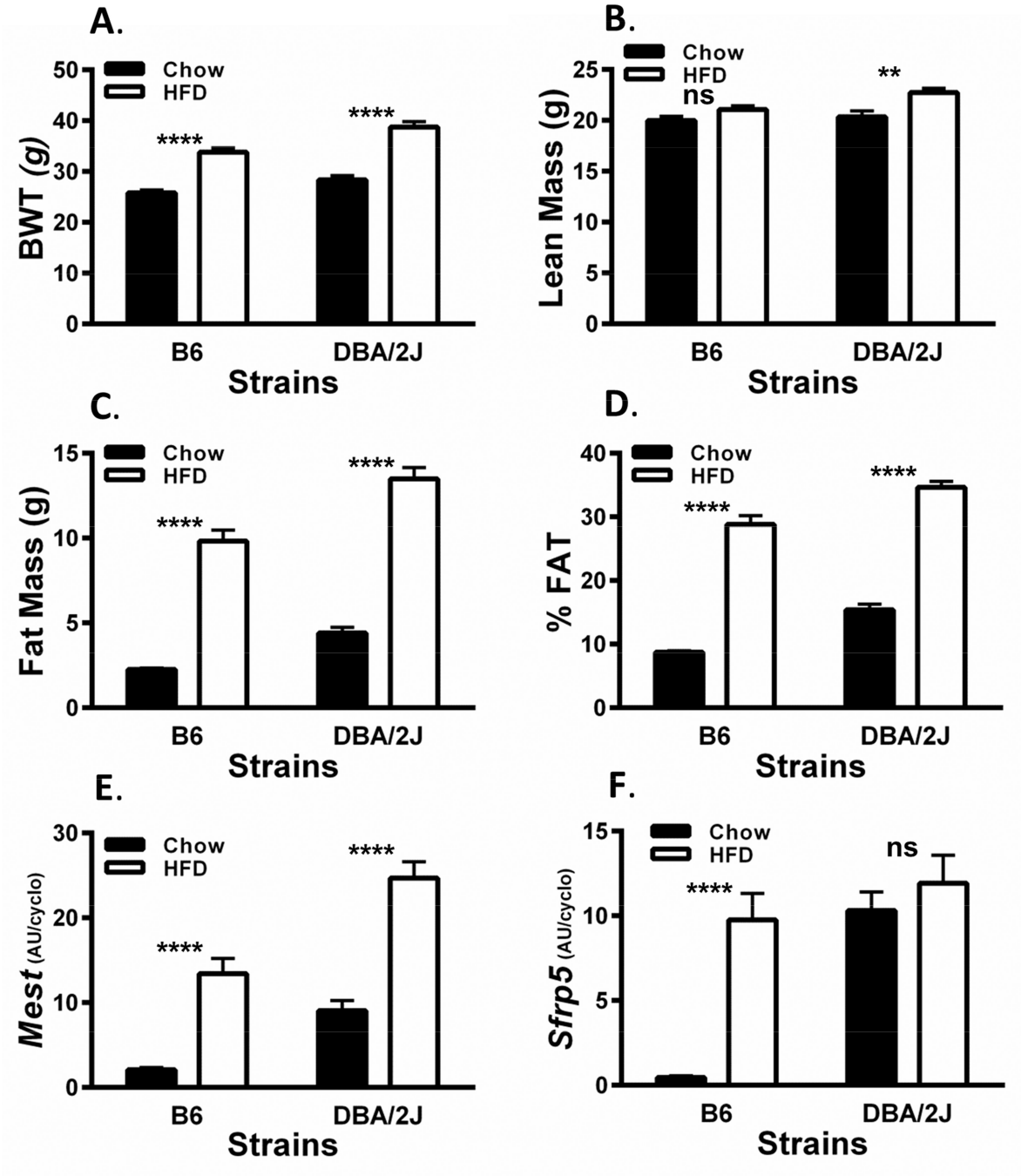

Figure 3.

Analyses of bodyweight (BWT; A), lean mass (B), fat mass (C), adiposity (%FAT; D); and, epididymal (EPI) fat *Mest* (E) and *Sfrp5* (F) mRNA expression in B6 and DBA/2J inbred mice fed a low fat diet (LFD) until 8 weeks of age and then fed either the LFD or a high fat diet (HFD; 58% kcal fat) for an additional 8 weeks until 16 weeks of age. Body composition was measured via NMR. Gene expression measured by TaqMan QRT-PCR is represented as arbitrary units (AU) normalized to cyclophilin b. Each group represents the mean ± SEM of

at least 9 mice. Datasets annotated with ns, ** and **** indicate no significant differences, $P<0.01$ and $P<0.0001$ between groups respectively.

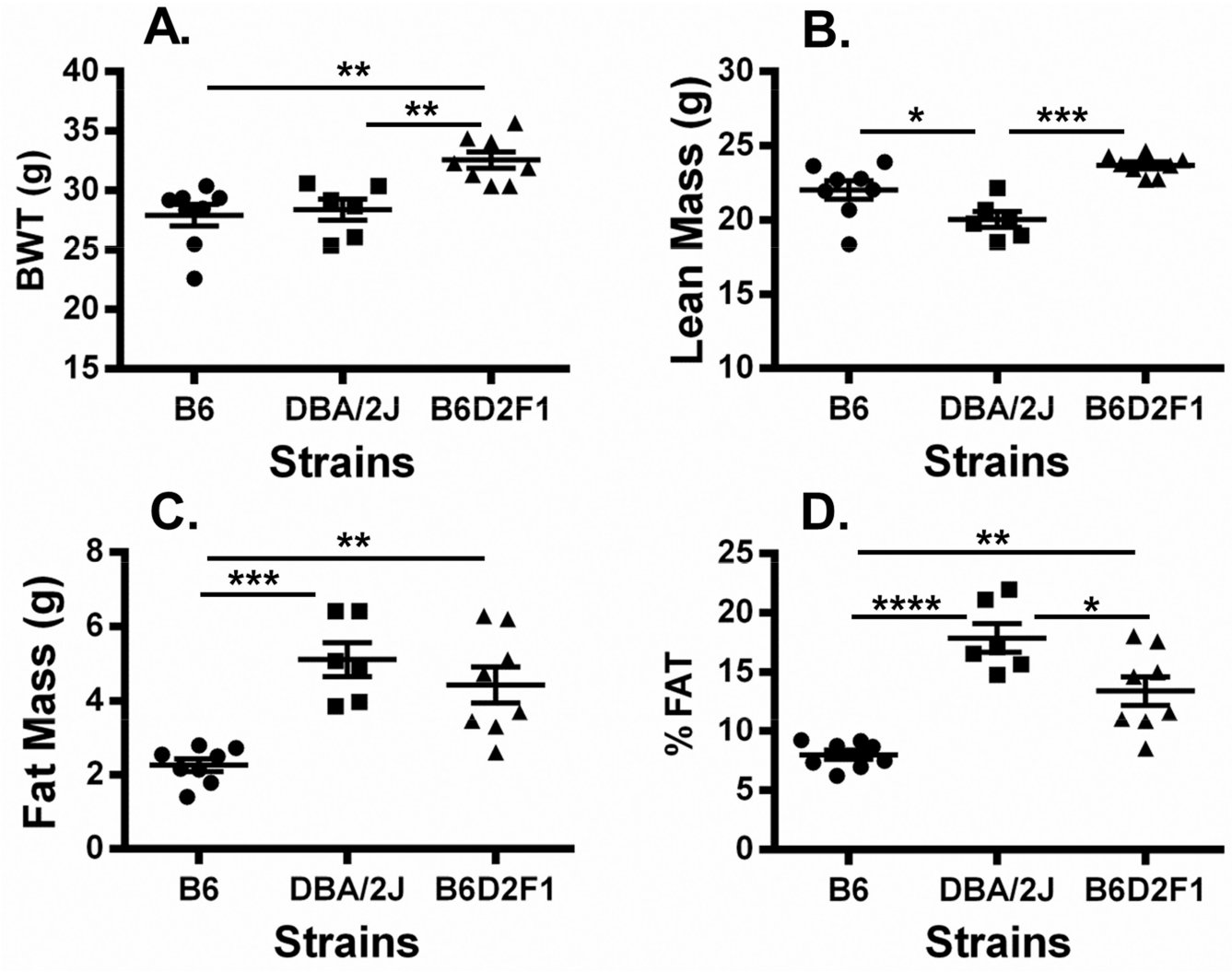

Figure 4.

Data shows a scatterplot of bodyweight (BWT; A), lean mass (B), fat mass (C) and adiposity (% FAT; B) in of B6, DBA/2J and (B6 × DBA/2J)F1 hybrid (B6D2F1) mice fed a low fat diet (LFD) until 16 weeks of age. Body composition was measured via NMR. Each group represents the mean ± SEM of 6–8 mice. Datasets annotated with 1, 2, 3 or 4 asterisks indicate significant differences of $P<0.05$, $P<0.01$, $P<0.001$ and $P<0.0001$ respectively.

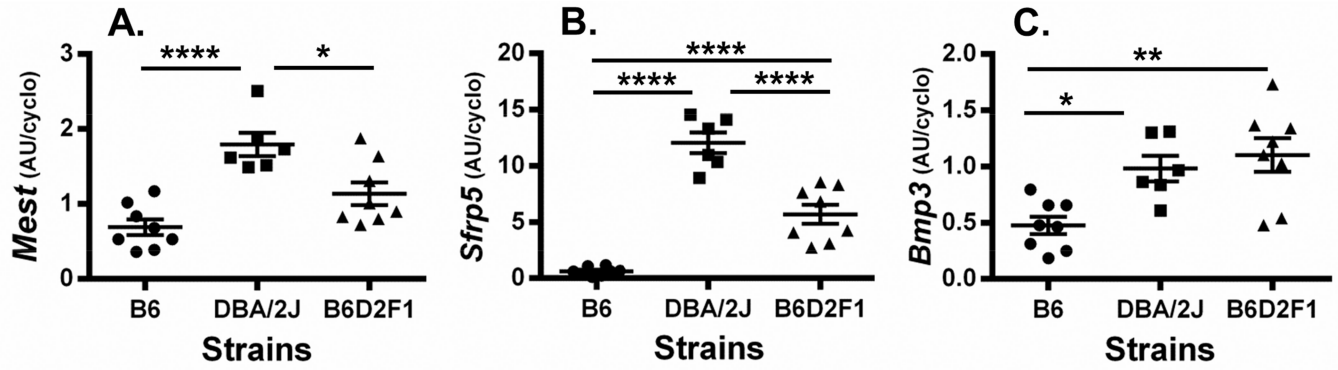

Figure 5.

Data shows a scatterplot of *Mest* (A), *Sfrp5* (B) and Bmp3 (C) mRNA expression in epididymal fat (EPI) of B6 (n=8), DBA/2J (n=6) and (B6 × DBA/2J)F1 (n=8) hybrid (B6D2F1) mice fed a low fat diet (LFD) until 16 weeks of age. Gene expression measured by TaqMan QRT-PCR is represented as arbitrary units (AU) normalized to cyclophilin b. Each group represents the mean ± SEM of 6–8 mice. Datasets annotated with 1, 2, and 4 asterisks indicate significant differences of $P<0.05$, $P<0.01$, and $P<0.0001$ respectively.

Table 1

Body weights and body composition of 5 inbred strains of mice fed standard chow diet for 8 weeks. Data in columns sharing the same superscript indicate no significant difference between strains.

		8 weeks	16 weeks			
Strain	**n**	**BWT (g)**	**BWT (g)**	**Lean Mass (g)**	**Fat Mass (g)**	**% Fat**
A/J	9	23.22 ± 0.33[a]	25.90 ± 0.40[a]	19.53 ± 0.26[a]	2.64 ± 0.15[a]	10.17 ± 0.46[ab]
C57BL/6J (B6)	10	21.66 ± 0.53[a]	25.82 ± 0.52[a]	19.98 ± 0.42[a]	2.26 ± 0.09[a]	8.72 ± 0.25[a]
AKR/J	10	28.66 ± 0.58[b]	36.58 ± 1.16[b]	25.76 ± 0.62[b]	5.83 ± 0.64[b]	15.66 ± 1.31[c]
DBA/2J	10	21.50 ± 0.73[a]	28.39 ± 0.86[a]	20.34 ± 0.59[a]	4.40 ± 0.34[c]	15.42 ± 0.87[c]
129SV/ImJ	10	22.14 ± 0.84[a]	27.42 ± 0.96[a]	20.46 ± 0.71[a]	3.28 ± 0.15[ac]	12.01 ± 0.55[b]