



Published in final edited form as:

Ann N Y Acad Sci. 2016 January ; 1363(1): 50–58. doi:10.1111/nyas.12958.

Molecular correlates of fat mass expansion in C57BL/6J mice after short-term exposure to dietary fat

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Abstract

Heterogeneity of obesity within a population of inbred mice fed an obesogenic high-fat diet (HFD) is associated with changes of gene expression in white adipose tissue (WAT). One gene in particular with large variations among mice, mesoderm-specific transcript (*Mest*), has been shown to be highly inducible after being fed a short-term HFD, and its expression in WAT before HFD feeding is predictive for susceptibility to the development of obesity. To gain further insight on the association of *Mest* with rapid changes in body composition, 96 individually housed C57BL/6J mice were fed an HFD for only 2 weeks, resulting in a 12-fold and 90-fold variation in *Mest* mRNA in visceral epididymal and subcutaneous inguinal WAT, respectively. WAT *Mest* mRNA was positively associated with interindividual variation of fat mass. Surprisingly, there was only a slight association of WAT *Mest* with food intake when normalized by body weight or lean mass. In addition, WAT *Mest* expression coincided highly with the expression of the transcription factor Kruppel-like factor 14 (*Klf14*), an imprinted gene that regulates lipid metabolism in WAT. Our data suggest that KLF14 transcriptional activity may partially mediate, or act in concert with, MEST as part of an epigenetic mechanism that promotes fat mass accumulation in mice fed an obesogenic diet.

Keywords

Mest; *Klf14*; adiposity; obesity; epigenetics

Introduction

Complex metabolic phenotypes, such as obesity and type 2 diabetes (T2D), have been shown to be highly variable among individuals within genetically identical populations of mice.^{1–3} Our studies have also demonstrated that variation of fat mass expansion in inbred mice fed an obesogenic diet is highly stable and distinctive among individual animals and suggests that an epigenetic etiology, possibly established via developmental programming, could be involved in these phenotypic differences between mice.^{3,4} Analyses of gene expression in subcutaneous white adipose tissue (WAT) of male C57BL/6J (B6) mice fed a

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Conflicts of interest

The authors declare no conflicts of interest.

high-fat diet (HFD) for 4 weeks identified a subset of genes that correlate highly with fat mass accretion, including imprinted genes and genes involved in the Wnt and Tgf- β signaling pathways.^{3,5} Mesoderm-specific transcript (*Mest*), a maternally imprinted gene belonging to the α/β hydrolase family, showed the largest variation (almost 80-fold) among mice, and its expression was highly and positively associated with secreted frizzled-related sequence protein 5 (*Sfrp5*) and bone morphogenetic protein 3 (*Bmp3*). In addition, mRNA expression of *Mest*, *Sfrp5*, and *Bmp3* in WAT biopsies of mice before exposure to an obesogenic diet was predictive for the future development of adiposity.³

Although evidence for a role of *Mest* in facilitating fat accumulation in cell-based *in vitro* models and in WAT^{3,5-8} is compelling, little is known about its catalytic function and how it is transcriptionally regulated. Evidence suggests that *Mest* is localized within the endoplasmic reticulum/Golgi apparatus of the adipocyte where it may act to facilitate fat storage in lipid droplets.^{5,6} We have also demonstrated that increased WAT *Sfrp5* expression is associated with enhanced adiposity in B6 mice after an HFD, which is consistent with findings on the role of these soluble inhibitors of Wnt signaling in the development of adiposity in mice.^{3,9} Recent evidence suggests that *Sfrp5* may act to stimulate adipocyte hypertrophy via inhibition of oxidative metabolism and/or act as an anti-inflammatory adipokine that regulates metabolic dysfunction and inflammation.¹⁰⁻¹² Bmps have been shown to be involved in a wide variety of morphogenetic processes during development.¹³⁻¹⁷ However, *Bmp3*, unlike other Bmps, antagonizes osteogenesis by activation of the Tgf- β /activin pathway.^{18,19} This antagonistic effect of *Bmp3* on osteogenesis is consistent with a morphogenetic role for *Bmp3* in adipogenesis.¹⁹⁻²¹

The recent identification of Kruppel-like factor 14 (*Klf14*), a maternally-expressed transcription factor, as a regulator of lipid metabolism and lipid signaling^{22,23} suggests that KLF14 may potentially play a role in mediating fat mass expansion in populations of genetically identical inbred mice. Subsequent analyses of global gene expression data of inguinal (ING) fat from male C57BL/6J mice fed an obesogenic diet for 4 weeks revealed significantly elevated *Klf14* expression (1.52-fold; $P = 0.0054$) in mice with high versus low weight gain (deposited in NCBI's Gene Expression Omnibus (GEO), with accession number GSE4692).³ In this study, a cohort of 96 male C57BL/6J mice were subjected to short-term (2 week) feedings of dietary fat to determine the effects on WAT *Mest* mRNA expression and the association of *Mest* with indices of fat mass expansion, caloric intake, and WAT gene expression. As expected, our results showed strong associations between adipose *Mest* mRNA with indices of adiposity, but, surprisingly, little association was observed for caloric intake during the 2-week dietary-fat feeding regimen. In addition, adipose *Mest* expression correlated very highly with *Klf14* expression in both subcutaneous and visceral WAT, suggesting that KLF14 transcriptional activity may at least partially mediate WAT MEST to promote fat mass accumulation in this model designed to investigate the epigenetic basis for the development of obesity.

Materials and methods

Animals and phenotyping

All *in vivo* experiments were carried out in age-matched male C57BL/6J (B6) mice ($n = 96$) purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were maintained in a temperature-controlled room (23 °C) with a 12-h light/12-h dark cycle. They were reared under conventional conditions and fed PicoLab Rodent Diet 20 (LabDiet, 13% kcal fat) until 8 weeks of age. Mice were singly housed at 7 weeks of age and then fed an HFD (Surwit Diet D12331, Research Diets; 58% kcal fat; 23.26 kJ/g) for 2 weeks starting at 8 weeks of age. Body composition was analyzed at 8 and 10 weeks of age with a nuclear magnetic resonance (NMR) minispec (Bruker), which uses the contrasting hydrogen density and/or hydrogen spin properties from fat and lean mass to estimate 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. Food intake was measured for all mice during the 2 weeks of HFD feeding. 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 the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Gene expression analyses

Epididymal (EPI) and ING WAT of mice were 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 RNase inhibitor (Life Technologies). RNA quantity and quality was determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). Quantitative reverse-transcription polymerase chain reaction (RT-PCR) was performed using total RNA, with specific primers and probes designed using Primer Express Software v3.0.1 (Life Technologies) as previously described⁵ except that a BioRad CFX Real-Time System was used for analyses. Gene expression data were normalized to cyclophilin B (*Ppib*).

Statistics

Statistical calculations were performed using GraphPad Prism 6 and Microsoft Excel 2010. Differences between two groups were calculated using a two-tailed unpaired parametric *t*-test with confidence level at 95%. Significance among multiple groups was calculated with a one-way ANOVA followed by Tukey's multiple comparison test with an alpha of 0.5. Pearson correlation coefficients (two-tailed; assuming Gaussian distribution) were calculated with a confidence interval of 95%. Data are presented as mean \pm SEM. A *P* value of < 0.05 was considered to be significant.

Results

Body weight and composition of male C57BL/6J mice fed HFD for 2 weeks

Frequency distribution profiles for body weight (BWT) of 96 male B6 mice (Fig. 1A) fed an HFD from 8 (Wk 0) to 10 weeks of age (Wk 2) showed an average weight gain of

approximately 3.7 g, with a range of 1.5–7.4 grams. Surprisingly, after feeding mice an HFD for only 2 weeks, a broad and somewhat bimodal distribution of BWTs became evident, with peaks at about 25 and 28 grams. Indices of adiposity as measured by the adiposity index (AI; fat mass/lean mass ratio; Fig. 1B) or fat mass (Fig. 1D) showed frequency distribution patterns that are consistent with the observed change in BWT and exhibit a modest bimodal distribution. The average increase in fat mass was 2.19 g/mouse, with a range of 0.2–5.5 g, whereas lean mass increased by an average of only 1.27 g/mouse, with a range of 0.1–2.7 g (Fig. 1C), indicating a significantly greater contribution of adiposity to changes in BWT during the 2-week obesogenic diet. Results from a regression analysis comparing fat mass ($R = 0.83$; $P = 1.8E-25$) and lean mass ($R = 0.46$; $P = 3.0E-06$) gain versus the change in BWT are consistent with the significance of the contribution of fat mass to overall BWT gain.

Association of *Mest* with BWT, body composition, and food intake

Data in Figure 2 show the level of *Mest* expression in both EPI and ING WAT of mice within the lowest and highest 10th percentile (10 mice per group) for BWT (Fig. 2A), AI (Fig. 2B), and the change of AI during the 2 weeks of feeding an obesogenic diet (Fig. 2C). Although differences in WAT *Mest* mRNA in mice can be observed 2–7 days after the initiation of an HFD,⁵ it was somewhat unexpected that changes in both BWT and adiposity indices occurring after feeding mice an obesogenic diet for only 2 weeks would show significant associations with *Mest* mRNA expression in both EPI and ING WAT.

A comparison of the lowest and highest 10th percentiles of *Mest* expression in both EPI and ING fat showed highly significant differences between subsets of mice, as indicated by a lack of overlap in gene expression values (Fig. 3A). Although the range for *Mest* expression in ING fat was large compared with EPI fat after 2 weeks of an HFD, the average for *Mest* expression among the cohort of 96 mice was not significantly different between the two WAT depots (EPI, 9.23 ± 0.26 AU/cyclo; ING 9.67 ± 0.62 AU/cyclo; $P = 0.52$). In addition, regression analysis showed a strong correlation ($R = 0.69$; $P = 1.2E-14$) between EPI and ING fat *Mest* expression in individual mice.

The analyses of the changes in BWT, fat mass, and lean mass in mice with the lowest and highest 10th percentiles of EPI and ING fat *Mest* expression after two weeks on an HFD showed highly significant increases in BWT (BWT; Fig. 3B) and fat mass (FM; Fig. 3D), but not lean mass (LM; Fig. 3C), in mice in the highest 10th percentile of *Mest* expression, regardless of fat depot. These results are consistent with the data in Figure 2 showing EPI and ING WAT *Mest* expression in mice with the lowest and highest 10th percentiles of BWT and adiposity. Surprisingly, although the association of WAT *Mest* with increased BWT and fat mass was highly significant, measurements of food intake, adjusted to either BWT (Fig. 3E) or fat-free mass (FFM; Fig. 3F) for each mouse, showed no association with WAT *Mest* expression.

Molecular correlates of *Mest* gene expression and adipose tissue expansion

Previous studies showed a strong association between adipose tissue *Mest* expression and genes associated with Tgf- β (i.e., *Bmp3*) and Wnt signaling (i.e., *Sfrp5* and Naked 1 (*Nkd1*))

in mice fed an HFD for 4 weeks.^{3,5} In the present study, expression of *Bmp3*, *Sfrp5*, and *Nkd1* were measured in EPI and ING fat of mice with the lowest and highest 10th percentiles of *Mest* expression in order to determine whether coexpression of these genes emerges after a short 2-week HFD feeding regimen. Data in Table 1 show significantly upregulated expression for *Bmp3*, *Sfrp5*, and *Nkd1* in the highest 10th percentile of *Mest* for both fat depots, consistent with previous observations following long-term exposure to obesogenic diets. Leptin (*Lep*) mRNA expression was also shown to be more highly expressed in both WAT depots of the mice with the highest 10th percentile of *Mest* expression, possibly reflecting the increased adiposity in these groups (Fig. 3D). Because of recent studies suggesting a role for *Klf14* as a master regulator of gene expression in WAT,²² and its possible link to obesity and type 2 diabetes,^{24,25} *Klf14* mRNA was measured in WAT of mice with the lowest and highest 10th percentiles *Mest* expression. Results in Table 1 show significantly higher *Klf14* expression in the highest-percentile groups for both WAT depots. Furthermore, regression analyses with the entire cohort of 96 mice showed a similar range of expression (*Mest*, 0.32–30.03 AU/cyclo; *Klf14*, 1.78–21.91 AU/cyclo) and an extremely strong correlation between *Mest* and *Klf14* (Fig. 4) in ING WAT ($R = 0.91$; $P = 1.4E-38$). Measurements of *Pparg* and *Cebpa*, genes involved in adipogenesis, were not significantly different in EPI WAT of mice with the lowest versus highest 10th percentile of *Mest* expression; and only *Cebpa* showed a modest, albeit significant, increase in expression in ING WAT of the highest-percentile mice (Table 1). However, analyses of the complete cohort of 96 mice showed no association of EPI or ING fat *Pparg* or *Cebpa* with fat mass accumulation (Table 2), whereas *Mest*, *Nkd1*, and *Lep* all showed significant associations with fat mass accumulation during the 2 weeks of HFD feeding, regardless of the WAT type used for gene expression analyses. Correlation analyses of genes previously identified as being associated with nongenetic variation of obesity in the cohort of 96 mice showed *Mest* to be most robustly associated with *Klf14* (Table 3) in both ING and EPI fat.³ Strong correlations of *Mest* with *Bmp3*, *Sfrp5*, *Nkd1* and *Lep* were also observed and are consistent with previous observations.³

Discussion

In previous studies aimed to ascertain epigenetic mechanisms underlying the development of obesity within an inbred, genetically identical population of mice, we identified several genes including the imprinted gene *Mest*, inhibitors of Wnt signaling (i.e., *Sfrp5* and *Nkd1*), and genes associated with Tgf β /activin signaling (i.e., *Bmp3*) that are associated with heterogeneity in fat accretion and storage. Because prior studies examined WAT gene expression in mice fed an HFD for at least 4 weeks, when WAT has already undergone robust development, the aim of the current study was to determine whether these same gene targets continue to emerge as correlative indicators of fat mass expansion after a short-term 2-week exposure of mice to an obesogenic diet. It was anticipated that short-term dietary studies will provide a greater likelihood for the identification of genes linked with epigenetic mechanism(s) involved in the regulation fat mass accumulation, rather than genes that show changes in expression as a result of the development of obesity.

Feeding mice an HFD for 2 weeks resulted in an approximate 3.7-g average increase in BWT, with fat mass accounting for more than 60% of the weight gained. In addition, a

bimodal pattern of BWT emerged that paralleled the pattern for indices of adiposity (Fig. 1). Although a similar distribution of BWT was also observed in a previous study of male B6 mice fed an HFD for 4 weeks,³ this was originally regarded as a possible artifact caused by the combination of two independent cohorts of mice for this dataset and thus was interpreted as a bell-shaped curve. The consistent replication of a bimodal distribution in BWT and corresponding indices of adiposity may be indicative of a metabolic difference in fat mass accumulation between these two subsets of mice. Since all mice in our study are genetically homogeneous, it is unlikely that genetic differences contribute to this BWT and adiposity pattern; however, it is possible that epigenetic variation could at least be partly responsible for the emergence of this phenotypic profile. A recent study determined that differences in intestinal microbial profiles in 129/SvEv mice acquired from different vendors resulted in mice that were susceptible or resistant to the development of obesity.²⁶ Subsequent analyses with 129/SvEv and C57BL/6J mice determined that environmental history, diet, and genetic background can strongly affect gut microbiota content.²⁶ Since the mice in our study are reared in the same environment and fed the same diet, the likelihood for significant differences in gut microbiota between individuals, at least initially, is relatively small. However, as mice begin to metabolically diverge when fed an obesogenic diet, it is possible that differences in bacterial taxa profiles in intestinal microbiota may begin to emerge in animals with low or high weight gain, which could exacerbate adipose inflammation and insulin resistance. Another potential nongenomic developmental variable that could mediate behavior and metabolic status of mice is the intrauterine position (IUP) of the fetus and resulting prenatal exposure to androgens.²⁷ An early study by Kinsley *et al.* suggested that the IUP of male and female fetuses between two male fetuses could establish a metabolic set point that promotes increased BWT and fat storage during postnatal growth.²⁸

We have shown in previous studies that WAT *Mest* gene expression can be rapidly, but variably, induced in mice fed an HFD for only 2–5 days.⁵ However, variability in the induction of WAT *Mest* gene expression among individual mice after this short duration of HFD feeding does not reflect differences in adiposity. Although it is unclear whether mice susceptible to rapid induction of WAT *Mest* are more susceptible to the development of dietary obesity, we have shown that *Mest* expression in WAT biopsies in mice prior to exposure to an obesogenic environment is predictive for the development of obesity as they age.³ A recent study by Voigt *et al.*²⁹ has also determined that WAT *Mest* expression is highly predictive for adipose tissue expansion after a 5-day HFD-feeding trial with C57BL/6J mice and can be used to test efficacy of anti-obesity treatments. In addition, this same study showed that *Mest* mRNA in whole blood correlated strongly with adiposity, leading to the possibility of developing a minimally invasive approach to identify mice that differ in their susceptibility to the development of obesity. After two weeks on an HFD, we determined WAT *Mest* to be highly associated with mice within the lowest and highest 10th percentiles for both BWT and measurements of adiposity (Fig. 2). When examined from the perspective of how WAT *Mest* expression affects phenotypic traits, analyses of changes in BWT, body composition, and food intake in mice with the lowest and highest 10th percentiles of EPI and ING WAT *Mest* mRNA showed a positive association with changes in BWT and fat mass, but not with lean mass or food intake (Fig. 3). This strong association of WAT *Mest* with indices of adiposity after a short 2-week dietary fat regimen is consistent

with past studies of mice fed an HFD for 4 weeks.³ Food intake during the 2-week dietary study normalized to either BWT or fat-free mass showed no association with mice in the lowest and highest 10th percentile WAT *Mest* expression groups (Fig. 3) and only marginal significance ($R = 0.25$; $P = 0.015$) to ING WAT *Mest* mRNA in a regression analysis of the entire cohort of 96 mice. These data can be interpreted to suggest that heterogeneity in the development of adiposity observed among individual mice is not caused by increased energy intake, but rather by differences in the utilization and compartmentalization of energy, metabolic efficiency, or capacity for fat storage in adipocytes.

Analyses of WAT gene expression within the lowest and highest 10th percentiles for EPI and ING WAT *Mest* expression (Table 1) show similar strong associations between *Mest*, *Bmp3*, *Nkd1*, and *Sfrp5* after HFD feeding for 2 weeks, as observed in studies with mice fed an HFD for 4 weeks.³ Both *Lep* and *Klf14*, previously shown to be upregulated in ING WAT in a global gene expression study (GEO accession number GSE4692) of mice with low and high weight gain when fed an HFD for 4 weeks,³ were also strongly associated with *Mest* in this 2-week dietary study. In addition, regression analyses of EPI and ING WAT gene expression in the entire cohort of 96 mice versus the change of fat mass during the 2 weeks of HFD feeding (Table 2) show strong associations for *Mest*, *Bmp3*, *Nkd1*, *Sfrp5*, *Lep*, and *Klf14*. WAT expression of the well-characterized adipogenic genes *Pparg* or *Cebpa* was not associated with indices of fat mass expansion, which is consistent with observations in previous studies.³

Our interest in the potential contribution of *Klf14* in the heterogeneous development of obesity within a population of genetically identical mice stems from recent studies suggesting that *Klf14*, an imprinted maternally expressed transcriptional regulator, is an important mediator of the regulation of lipid metabolism and signaling in adipose tissue.^{22,23,30} In addition, since *Mest* and *Klf14* are located in close proximity on the same genomic locus in mice (Chr 6; 30.73–30.96 mb) and humans (CHR 7; 130.49–130.73 mb), it is possible that a common mechanism (e.g., imprinting control region (ICR) could be involved in their regulation.³⁰ The observation of consistent patterns and range for gene expression for *Mest* and *Klf14* in ING WAT among mice was confirmed by regression analyses (Fig. 4), which showed an almost linear relationship ($R = 0.91$; $P < 10^{-37}$) between the two genes. Correlation matrices of gene expression in Table 3 showed that the strongest association for *Mest* in both fat depots was with *Klf14*. Although this association was not as strong in EPI WAT ($R = 0.70$; $P < 10^{-14}$) as in ING WAT, these data were consistent with a possible interaction and contribution of the imprinted genes *Klf14* and *Mest*, and genes previously shown to co-regulate with *Mest* (i.e., *Sfrp5*, *Nkd1*, and *Bmp3*),³ in an epigenetic model for the regulation of fat mass expansion in mice fed an obesogenic diet.

The identification of a strong association of *Mest* and *Klf14* in WAT with indices of adiposity in male C57BL/6J mice fed an HFD for only 2 weeks establishes an exciting opportunity for further study. Although loss of imprinting (LOI) of *Mest* has been shown to increase its expression in fat of interspecies hybrids and results in increased body weight,³¹ our studies and those of others have shown that variation in *Mest* mRNA in WAT does not show loss of imprinting, and *Mest* mRNA is derived exclusively from the paternal allele.^{32–34} Although it is possible that epigenetic modifications of distal enhancers and

regulatory elements could lead to variation in the expression of *Mest* mRNA from the paternal allele, it is equally plausible that the epigenetic regulation of a gene, or a transcription factor that regulates *Mest* transcription from the paternal allele, could lead to a similar result. On the basis of the data presented here, studies are now underway to investigate *Klf14*-mediated transcriptional regulation of *Mest*, as well as global changes in histone modifications in DNA from WAT with low and high *Mest* and *Klf14* expression. We anticipate that the results of this current study will begin to delineate a novel epigenetic mechanism for the regulation of fat mass accumulation in an obesogenic environment.

Acknowledgements

We would like to thank David C. Higgins for assistance in generating some of the data for this study. These studies were supported by the Molecular Phenotyping Core Facility (funded by NIGM 8P30-GM106391) at the Maine Medical Center Research Institute and by NIH RO1DK090361 (R.A.K).

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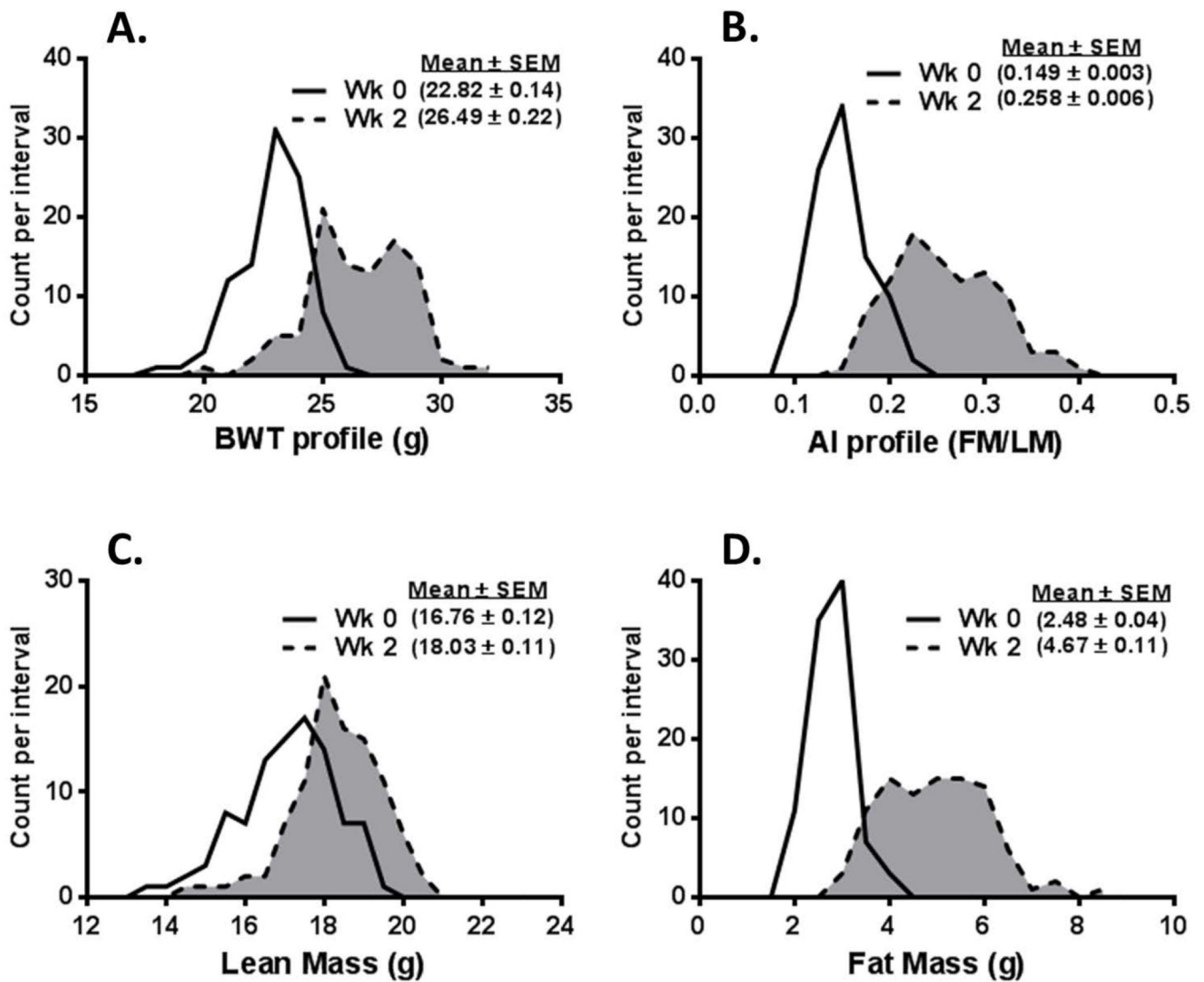


Figure 1.

Data represent the frequency distribution for (A) body weight (BWT), (B) adiposity index (AI) represented as fat mass (FM)/lean mass (LM), (C) LM, and (D) FM of 96 male C57BL/6J mice before (solid line, unshaded) and after (dashed line, shaded) being fed a high-fat diet for 2 weeks.

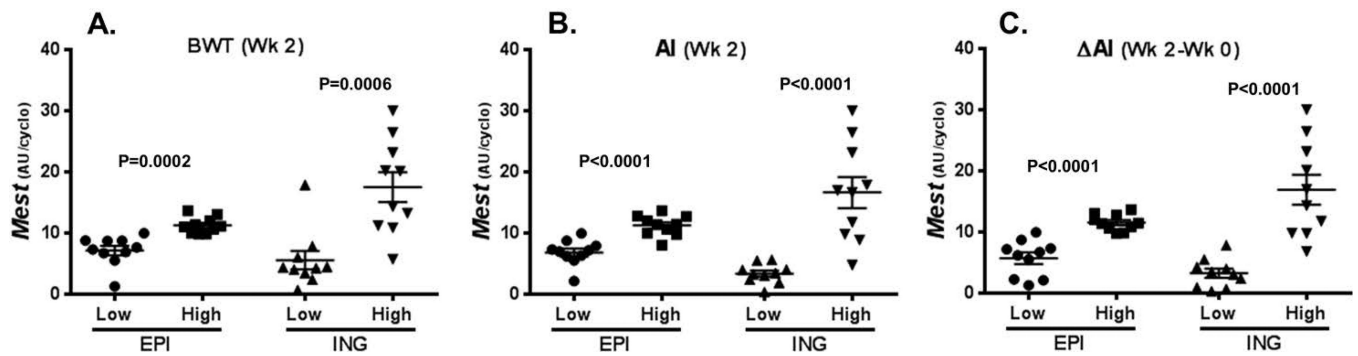


Figure 2.

The expression of *Mest* mRNA was measured in epididymal (EPI) and inguinal (ING) adipose tissue of mice within the lowest and highest 10th percentile (10 mice/group) for (A) body weight (BWT), (B) adiposity index (AI), and (C) change in adiposity index (Δ AI) after 2 weeks of feeding an obesogenic diet. Gene expression measured by TaqMan quantitative RT-PCR is represented as arbitrary units (AU) normalized to cyclophilin B. Significance between groups measured by two-tailed unpaired parametric *t*-tests is indicated on each figure.

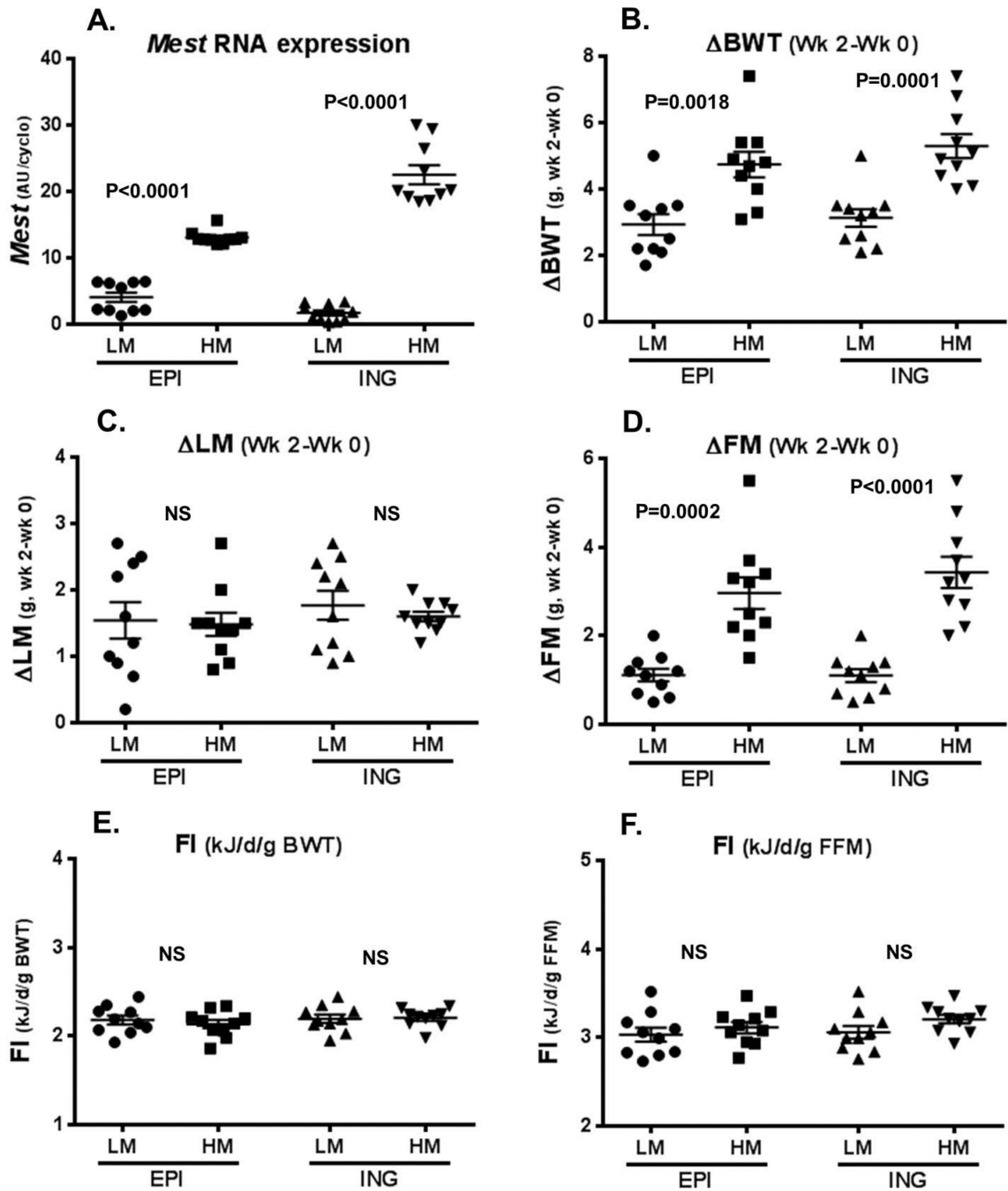


Figure 3. Data represent a comparison of phenotypes of mice within the lowest (low *Mest*; LM) and highest (high *Mest*; HM) 10th percentile groups (10 mice/group) for epididymal (EPI) and inguinal (ING) adipose tissue *Mest* mRNA after the 2 weeks of feeding an obesogenic diet. A represents *Mest* mRNA levels of the LM and HM cohorts in EPI and ING adipose tissue; and B–D show the change in body weight (BWT), lean mass (LM), and fat mass (FM) in the LM and HM mice. Panels (E–F) represent food intake in mice with LM and HM normalized to BWT or fat-free mass (FFM), respectively. Gene expression measured by

TaqMan quantitative RT-PCR is represented as arbitrary units (AU) normalized to cyclophilin B. Significance between groups measured by two-tailed unpaired parametric *t*-tests is indicated on each figure.

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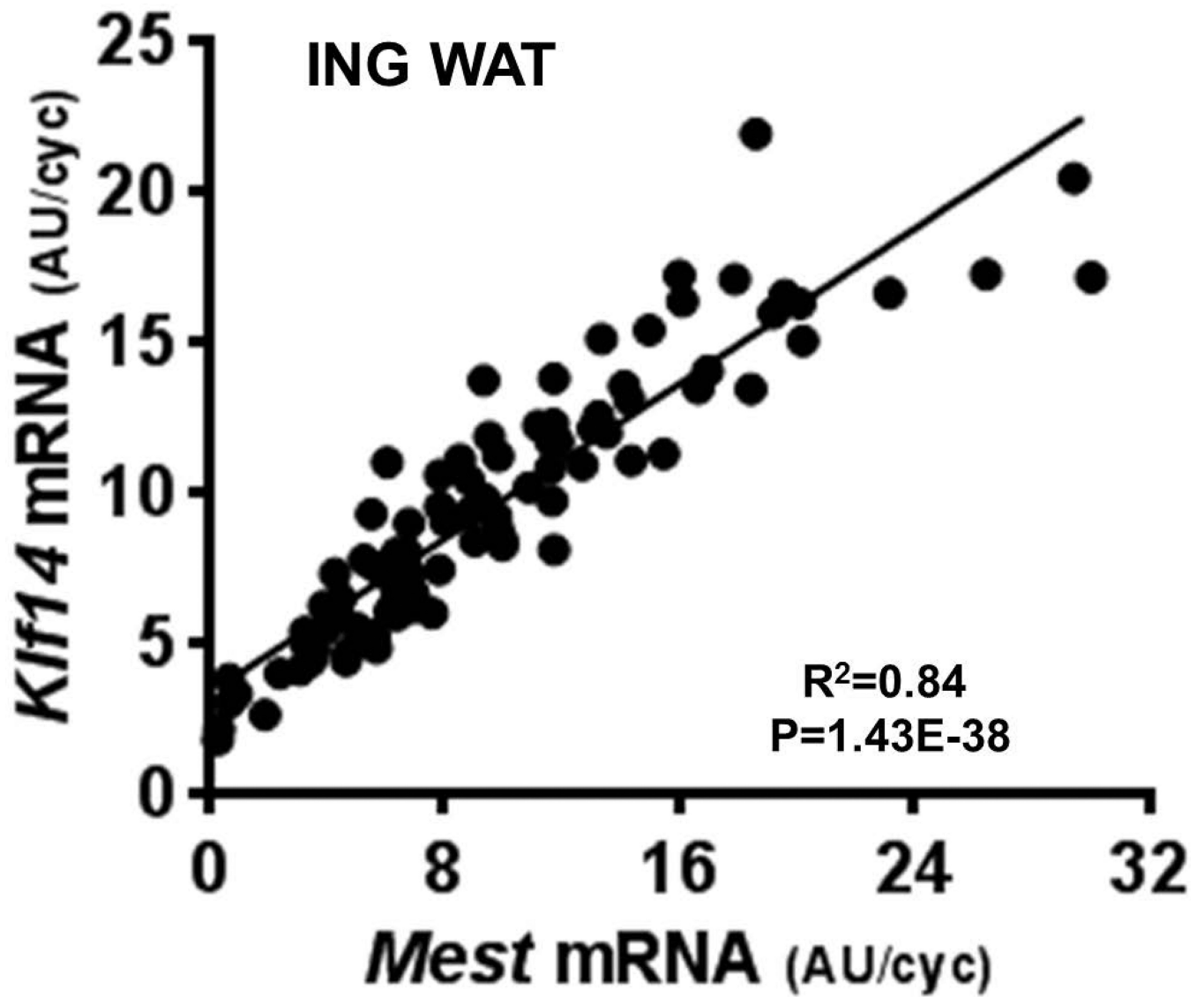


Figure 4. A highly significant correlation between inguinal (ING) fat mRNA expression for *Mest* and *Klf14* is represented in a scatterplot. Gene expression measured by TaqMan quantitative RT-PCR is represented as arbitrary units (AU) normalized to cyclophilin B.

Table 1
Analyses of gene expression in mice within the lowest and highest 10th percentile for adipose tissue *Mest* mRNA

Gene	EPI fat			ING fat		
	LM	HM	P value	LM	HM	P value
<i>Mest</i>	4.06 ± 0.71	13.06 ± 0.33	3.28E-08	1.70 ± 0.39	22.52 ± 1.42	6.40E-08
<i>Bmp3</i>	6.99 ± 0.64	14.44 ± 0.87	2.78E-06	5.99 ± 0.61	13.68 ± 0.69	1.31E-07
<i>Nkd1</i>	5.87 ± 0.28	9.90 ± 0.49	5.41E-06	5.14 ± 0.30	14.61 ± 1.16	1.31E-05
<i>Sfrp5</i>	3.02 ± 0.49	7.44 ± 1.27	0.0070	4.52 ± 0.52	17.47 ± 1.59	8.82E-06
<i>Lep</i>	6.00 ± 0.43	11.53 ± 0.74	1.52E-05	5.95 ± 0.85	15.68 ± 1.60	9.79E-05
<i>Klf14</i>	3.27 ± 0.52	10.14 ± 1.19	0.00019	3.50 ± 0.36	17.05 ± 0.78	7.38E-10
<i>Pparg</i>	10.52 ± 0.66	10.90 ± 0.53	0.67	8.47 ± 1.52	12.14 ± 0.95	0.058
<i>Cebpa</i>	9.92 ± 0.38	9.00 ± 0.27	0.064	7.63 ± 0.77	10.36 ± 0.33	0.0068

LM, lowest 10th percentile *Mest* mRNA expression; HM, highest 10th percentile *Mest* mRNA expression

Table 2

Correlation between adipose tissue gene expression and fat deposition in mice fed an obesogenic diet for 2 weeks

Gene	EPI GenExp vs. FM (wk 2–wk 0)		ING GenExp vs. FM (wk 2–wk 0)	
	R	P value	R	P value
<i>Mest</i>	0.57	1.9E-09	0.66	3.7E-13
<i>Bmp3</i>	0.39	7.2E-05	0.46	2.2E-06
<i>Nkd1</i>	0.68	3.1E-14	0.65	5.6E-13
<i>Sfrp5</i>	0.25	0.015	0.40	4.8E-05
<i>Lep</i>	0.68	2.3E-14	0.59	2.3E-10
<i>Klf14</i>	0.31	0.0022	0.50	1.6E-07
<i>Pparg</i>	0.01	0.91	0.07	0.51
<i>Cebpa</i>	0.01	0.90	0.05	0.63

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Table 3 Pearson (R) correlation matrix of ING (unshaded) and EPI (shaded) fat gene expression in mice fed an obesogenic diet for 2 weeks

Gene expression	<i>Mest</i>	<i>Bmp3</i>	<i>Sfrp5</i>	<i>Klf14</i>	<i>Nkx1</i>	<i>Lep</i>	<i>Pparg</i>	<i>Cebpa</i>
<i>Mest</i>		0.79	0.83	0.91	0.86	0.76	0.30	0.36
<i>Bmp3</i>	0.48		0.82	0.85	0.80	0.77	0.52	0.57
<i>Sfrp5</i>	0.41	0.62		0.88	0.70	0.67	0.30	0.37
<i>Klf14</i>	0.71	0.62	0.78		0.76	0.74	0.41	0.43
<i>Nkx1</i>	0.62	0.30	0.23	0.40		0.86	0.41	0.48
<i>Lep</i>	0.51	0.48	0.41	0.44	0.66		0.62	0.56
<i>Pparg</i>	-0.05	0.19	0.12	0.10	-0.20	0.20		0.81
<i>Cebpa</i>	-0.03	-0.17	-0.005	-0.08	0.07	0.01	0.02	