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Weak Genetic Relationship Between Trabecular Bone Morphology and Obesity in Mice

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Abstract

Obesity, in addition to being associated with metabolic diseases, such as diabetes, has also been found to lower the risk of osteoporotic fractures. The relationship between obesity and bone trabecular structure is complex, involving responses to mechanical loading and the effects of adipocyte-derived hormones, both directly interacting with bone tissue and indirectly through central nervous system signaling. Here we examine the effects of sex, a high fat diet, and genetics on the trabecular density and structure of the lumbar and caudal vertebra and the proximal tibia along with body weight, fat pad weight, and serum leptin levels in a murine obesity model, the LGXSM Recombinant Inbred (RI) mouse strains. The sample included 481 mice from 16 RI strains. We found that vertebral trabecular density was higher in males while the females had higher tibial trabecular density. The high fat diet led to only slightly higher trabecular density in both sexes despite its extreme effects on obesity and serum leptin levels. Trait heritabilities are moderate to strong and genetic correlations among trabecular features are high. Most genetic variation contrasts strains with large numbers of thick, closely-spaced, highly interconnected, plate-like trabeculae with a high bone volume to total volume ratio against strains displaying small numbers of thin, widely-spaced, sparsely connected, rod-like trabeculae with a low bone volume to total volume ratio. Genetic correlations between trabecular and obesity-related traits were low and not statistically significant. We mapped trabecular properties to 20 genomic locations. Only one-quarter of these locations also had effects on obesity. In this population obesity has a relatively minor effect on trabecular bone morphology. Key Words: bone; trabecular morphology; obesity; quantitative trait loci; mice

Introduction

While the epidemic of obesity and overweight in the United States has contributed to a number of other serious health conditions, including diabetes and hypertension, heavier individuals are traditionally thought to have a lower risk of developing osteoporotic fractures than do those whose weight is at or below normal (1). It is generally thought that increased body weight acts on the skeleton to generate greater loading on bone, which

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results in an adaptive response leading to higher bone density. However, other factors such as the common pool of precursor stem cells producing adipocytes and osteoblasts, generalized inflammatory response (2) and common regulation through adipocyte-derived hormones may also play a role (3,4). Some have suggested that fat mass may have a stronger relationship with bone density than does lean mass (see 1), so that different body size components have differential effects on bone mass, a result not explained by the body-weight loading hypothesis.

Along with obesity, other common risk factors affecting osteoporosis include age, sex, and genetics. Relatively few studies have examined the complex way in which genetics, obesity, and gender act together to influence bone density. Studies in mouse models that have focused on the relationship between osteoporosis and obesity have often been conducted on specialized mutants, the homozygous obese (*ob/ob*) or diabetes (*db/db*) mice, or studied by directly injecting factors into the cerebral ventricles (5,6). While these studies are valuable, they take the organism out of a normal physiological range and limit their models' comparability to other mice and to humans. Even in these specialized mutants, the relationship between obesity and bone mass is complex with anabolic peripheral effects (7–9) and catabolic central effects (6). Furthermore, leptin administered to leptin-deficient human subjects results in an increase in vertebral bone density (10), while having no effect on bone in obese leptin-tolerant or leptin-resistant individuals (11). Even when considering only bone properties, the vast majority of murine genetic studies have focused on contrasts between C57BL/6J and either the DBA/2J or C3H/HeJ strains (12–19) limiting our knowledge of gene effects on bone to a small portion of mouse genetic diversity.

To address the complex interaction of genes and environment that contribute to bone mass and strength, we have studied the effects of high- and low-fat diets in a large series of recombinant inbred (RI) mice, taking several of the risk factors for osteoporosis into account. In contrast to models of osteoporosis and obesity using mice with specific mutations or mapping quantitative trait loci using C57BL/6J, we have based our research on descendents of the cross between homozygous LG/J and SM/J mice. The initial cross of these parents produced heterozygous offspring that were then inbred for over 25 generations to yield 16 distinct Recombinant Inbred (RI) strains, which have since been extensively studied and genotyped (20). Our experiments test for variation in bone traits in relation to obesogenic diet, sex, and genes.

Mouse models have consistently shown their usefulness as a proxy for human diseases because of the similarity between humans and mice in many areas of growth and development, their relatively short generation length, high reproductive rates, and relative ease with which skeletal traits can be observed. Additionally, inbred lines allow investigators to control the genetic variation in the population and to vary environmental influences, such as diet, in a controlled fashion. There are, however, also limitations to mouse models for osteoporosis research: mice do not load their skeletal elements in the same way humans do, mice do not have cortical remodeling through Haversian systems, and mice almost never develop spontaneous hip or vertebral fractures in contrast to older humans. Accordingly, mouse models are best utilized for studies of peak bone mass rather than bone loss; peak bone mass helps determine the amount of bone loss that can be tolerated later in life.

Research on the relationship between obesity and osteoporosis has included measures not only of body weight and fat deposition, but also of the hormone leptin, which regulates metabolism and energy intake. Leptin is produced by adipose tissue and its serum level tends to track the amount of fat. It binds to receptors on hypothalamic neurons to indicate satiety. When leptin levels are high, the increased stimulation of these neurons leads to an

escalation in metabolism coupled with a decrease in appetite. Morphological and mechanical properties of bone appear to be affected by leptin through a number of pathways. Individuals with larger body size tend to have greater bone mass (21), but it also appears that leptin signaling plays a more direct role in bone mass regulation through growth and remodeling (5,7,22,23). In particular, different bone types (cortical versus trabecular) and skeletal regions (appendicular versus axial) seem to be influenced in distinct ways by leptin signaling (7,23). Furthermore, feedback between bone and metabolism has been posited with the osteoblast-derived hormone, osteocalcin, regulating glucose metabolism and adiposity (24–27).

In part to test these seemingly disparate associations between obesity and bone mass, we turned to the LGXSM intercross mouse model. Using 16 recombinant inbred mouse strains, we collected data on trabecular bone from the proximal tibia, lumbar and caudal vertebrae, bones located in different regions and presumably subjected to different levels of biomechanical loading. We considered these skeletal properties in concert with a measure of obesity and serum leptin levels. Given the genetic structure and large size of our population, we can parse out genetic and environmental contributions to the phenotypic variance with the goal of clarifying the complex relationships between obesity, leptin, and trabecular bone properties.

We test several hypotheses in this study. We first examine the extent to which sex, obesity, and genes affect variation in the morphology of trabecular bone in the three skeletal elements, and whether these effects differ between elements. After defining the role of sex, obesity, and genes in determining variation in trabecular bone, we map genetic effects across the genome with special attention to locations where effects vary depending on sex and diet.

Materials and Methods

Sample

A total of 481 recombinant inbred (RI) mice representing 16 distinct strains from an initial LG/J x SM/J cross were used in this study (20). Individual strains were represented by between 7 and 45 mice, with an average of 28 mice per strain. The mice used to create the RI lines were derived from two parental strains selected for increased (LG/J) or decreased (SM/J) body size at 60 days of age that have been maintained by brother-sister mating over several decades. Male and female mice from each RI strain were randomly selected to be raised on high (42% kcal) or low (15% kcal) fat, near isocaloric, diets (see Table 1) after weaning at 3 weeks. Animals were euthanized by sodium pentobarbital overdose at 20 weeks of age after a 4 hour fast and animals were necropsied for carcass weight, and total fat pad weights (sum of the reproductive, inguinal, perirenal, and mesenteric fat depots) (28). We measured serum leptin levels from blood taken from the mice via cardiac puncture just prior to necropsy.

Trabecular Phenotypes

Using previous studies of trabecular properties as our guide, we harvested the right tibia, third lumbar and fourth caudal vertebrae from each mouse after all soft tissue had been fully removed by dermestid beetles. These elements were placed in an agarose gel solution and imaged by microCT (Scanco μ CT 40; energy = 70kVp, current 57 μ A, diameter 16.4 mm, integration time 200 ms, nominal resolution 16 μ m, 500 projections/180 degrees, 1024 samples/projection). Thirty cross-sectional slices of the proximal metaphysis of the tibia, and those of the entire vertebral bodies (generally between 100 – 150 slices per vertebra) were analyzed for trabecular bone properties using Scanco's 3D evaluation software. Manual contouring was used to separate trabecular from cortical bone, and a global

threshold (28% of full scale; Gaussian filtering, sigma 1.8, support 2) was used to segment bone from non-bone. Trabecular properties measured include bone volume/total volume (BV/TV), connectivity density (CD), structure model index (SMI), trabecular number (Tb.N), trabecular separation (Tb.Sp), and trabecular thickness (Tb.Th). These traits describe the relative volume (BV/TV), shape (SMI, Tb.Th), number (Tb.N), spatial density (Tb.Sp) and connectivity (CD) of trabeculae in the proximal tibia and vertebral bodies. In addition to analysis of these individual variables we also performed principal components analysis to determine combinations of variables that explained the most variation in trabecular morphology in our data set. Principal components analysis was performed separately for the lumbar and caudal vertebra and the proximal tibia.

Statistical Analysis

Most statistical analyses were carried out in SAS version 9.2 (SAS Institute, 2007) while principal components and quantitative genetic analyses were performed in SYSTAT version 12.0 (SYSTAT Software, Inc., 2007). We calculated broad-sense heritability and genetic correlation values using MANOVA pooled over the sexes and diets, providing an average heritability over the four sex-diet cohorts, but due to significant strain by sex interaction effects, we chose to separate males and females to model the effects of 512 single nucleotide polymorphisms (SNP) on the 19 autosomes and the X chromosome (see Supplementary Table 1). Gene, diet, and gene by diet interaction effects were assessed using univariate restricted maximum likelihood mixed linear models for each skeletal trait per element. Probabilities associated with the likelihood ratio tests were converted into log₁₀ of the odds ratio (LOD) scores and adjusted per chromosome for multiple comparisons (29). LOD scores that met or exceeded the chromosome-specific thresholds were circumscribed by a 1-LOD drop on either side of the peak LOD value. We compared regions of the chromosomes that were significantly associated with trabecular phenotypic variation in this sample of RI mice against previously published quantitative trait loci (QTL) for leptin, necropsy weight, and total fat pad weight (30) obtained from microsatellite data, as well as modeling these traits against the more dense SNP map. Shadow QTL, or regions of the genome where the phenotype and genotype appear to be significantly correlated only due to non-syntenic linkage disequilibrium with a distinct genomic region, were determined using standard linear regression for all significant QTL per trait (30).

Results

RI strain mean values for each sex by diet cohort are provided in Supplementary Table 2.

Principal Components Analysis

The first principal components of the trabecular traits in the lumbar and caudal vertebra and proximal tibia are presented in Table 2. The components are similar across all three bones, with vector correlations ranging from 0.89 between the caudal vertebra and the proximal tibia, to 0.99 between the lumbar and caudal vertebra. The first principal component (PC1) accounts for 75%, 69%, and 56% of the total variation in trabecular bone morphology in the lumbar and caudal vertebra and proximal tibia, respectively. We interpret the first principal component as a new synthetic measure that reflects trabecular structural density and will refer to this as “trabecular density”. Individuals with high positive values on the first principal component (i.e., high trabecular density) have many, closely spaced, thick, interconnected, plate-like trabeculae with higher trabeculae volume and are contrasted against individuals with low scores (i.e., low trabecular density) having relatively few, sparsely distributed, thin, poorly connected, rod-like trabeculae with lower trabeculae volume. While the dimensions of trabecular variation are basically the same for the three bones, the trabecular density scores are only moderately phenotypically intercorrelated, with

correlations of ~0.50 between each vertebral and the proximal tibial trabecular density, and a higher correlation of 0.77 for trabecular density across the two vertebra. Thus, there appear to be somewhat different ways to build a trabecular structure between the long bone and the vertebra.

Phenotypic correlations between trabecular parameters within and between bones ranged from non-significant to extreme, with most at least moderately strong (86 of 153 possible correlations had $\text{abs}(r) > 0.4$; Table 3). Phenotypic correlations between trabecular density and carcass weight, total fat depot weight, and leptin are all low ($r < 0.12$) and of only borderline statistical significance, indicating little association between body size/composition and trabecular bone morphology.

Sex and diet effects

All sex and diet effects reported here are pooled over the strains. When also pooled over the diets, males have higher trabecular density in the vertebra (+0.34 SD for lumbar and +0.70 SD for caudal, expressed as fractions of the Standard Deviations (SD) of trabecular density) and lower trabecular density in the proximal tibia (−0.53 SD) compared to females. Male lumbar vertebra have higher BV/TV (5%) and more (21%), highly connected (25% CD), thinner (−5%), closely spaced (−34%), rod-like (15% higher SMI) trabeculae than female vertebra. As with the lumbar vertebral body, male caudal vertebral bodies also had higher BV/TV (14%), and more (17%), highly connected (32%), closely-spaced (−25%) trabeculae than females. In contrast, in the proximal tibia males have less BV/TV (−19%) with less highly connected (−14%) and thinner (−12%) trabeculae. Thus, the sex difference reverses between the vertebrae and tibia.

For obesity traits carcass weight, total fat depot weight, and serum leptin levels are about 29%, 21%, and 50% higher in males than in females, respectively, on a low fat diet (28). These sex differences are not as apparent on the high fat diet because females respond more strongly than males to the dietary treatment. Sex differences in the high fat fed animals are reduced to 18%, 0%, and 7% for carcass weight, total fat depot weight and serum leptin levels, respectively.

When pooled across sexes, the high fat diet also had significant effects on bone volume and trabecular structure. Trabecular density is generally higher on the high fat diet than it is on the low fat diet (lumbar +0.32 SD; tibial +0.29 SD; caudal +0.12 SD). Animals fed a high fat diet had higher BV/TV (11%) and more highly-connected (10%), plate-like trabeculae in the proximal tibia. In the vertebrae, BV/TV was increased on a high fat diet (10% Lumbar, 2% Caudal), and a high fat diet led to more (8% L, 3% C), thicker (4% L, 1% C), less widely spaced (−13% L, −5% C), more connected (5% L, 5% C), and more plate-like trabeculae. There is a significant sex by diet interaction for vertebral trabecular density ($P = 4 \times 10^{-6}$ for lumbar and 1×10^{-4} for caudal). The high fat diet leads to higher vertebral trabecular density in females but has a relatively little effect in males.

There were massive increases in carcass weight (♀ 28%, ♂ 17%), total fat depot weight (♀ 100%, ♂ 63%), and serum leptin levels (♀ 120%, ♂ 56%) in animals fed a high fat diet (28). Note that the dietary differences are more extreme for females than for males because, as with trabecular bone, females respond more strongly to the high fat diet.

Quantitative Genetics

Genetic effects on trabecular density and its components are substantial. When pooled across sex and diet groups, trabecular density is relatively highly heritable in the vertebra ($H^2 = 0.68$ for lumbar and 0.58 for caudal) and moderately heritable in the proximal tibia

($H^2 = 0.37$). The individual components of trabecular density are moderately heritable, H^2 ranging from 0.20 to 0.70 (see Table 3).

There is substantial sex by strain interaction, indicating genetic variation in sexual dimorphism and that genes have different effects in males and females. However, the variances due to these interactions are much smaller than those due to strain as a main effect, statistically significant results ranging from 5% to 23%. Interaction effects for trabecular density are significant for both vertebra but not for the tibia. Strain by diet and strain by sex by diet interactions are rarely statistically significant for individual traits and fail to reach significance in multivariate tests so the genetic effect of mouse strain on trabecular density was similar for the low and high fat diet treatments.

Squared genetic correlations are the proportion of variance that two traits share due to genetic causes, determined in our study by comparing traits across RI strains and pooling across sex and diet groups. Genetic correlations between aspects of trabecular morphology are all extreme (see Table 3) with two sets of positively intra-correlated traits: (1) BV/TV, CD, Tb.N, and Tb.Th; and (2) SMI and Tb.Sp. Correlations across these two trait sets are significant but negative. All correlations are consistent with the major axis of variation in trabecular morphology being the contrast of high density (+BV/TV, +CD, +Tb.N, +Tb.Th, -SMI, -Tb.Sp) with low density (-BV/TV, -CD, -Tb.N, -Tb.Th, +SMI, +Tb.Sp) traits. Trabecular densities of the three bones are highly genetically intercorrelated, with an average genetic correlation of 0.88.

Aspects of obesity, including carcass weight ($H^2 = 0.46$), fat depot weights ($H^2 = 0.49$), and serum leptin levels ($H^2 = 0.43$) are significantly heritable (28) but are not significantly genetically correlated with trabecular density or any of its components (see Table 3). Genetic correlation estimates had to exceed 0.44, with the two traits sharing more than 20% of their genetic variance, to be considered statistically significant.

QTL Mapping

Table 4 presents a summary of the results for QTL mapping for trabecular traits. More detailed results, including individual trait LOD scores, QTL support interval limits, and effect sizes are provided in Supplementary Table 3. We found 20 distinct QTLs affecting trabecular morphology. Most of these QTL had effects restricted to a single sex, although 25% affected both sexes (*QTrab1.1*, *QTrab3.1*, *QTrab3.2*, *QTrab7.1*, *QTrab16.1*). There were only two female-specific QTLs against 13 male-specific QTLs. Most QTLs affected trabecular bone on both the low- and high-fat diets, only 20% being specific to the low fat diet (*QTrab2.1*, *QTrab9.1*, *QTrab10.1*, *QTrab14.1*) and none specific to a high fat diet. QTL support regions average 22 Mb with a standard deviation of 10.6 Mb. This likely corresponds to an average map distance of 13.25 cM (SD = 6.7 cM). Regions these sizes are likely to contain, on average, about 250 genes.

Trabeculae of different bones were often affected by different QTL. Six QTL affected only the lumbar vertebrae, five affected only the tibia, and three affected only the caudal vertebrae. The six remaining QTL affected multiple bones, four affecting all three, one affecting the lumbar and caudal vertebra, and one affecting the tibia and the caudal vertebra. Gene effect sizes are small, with absolute values centering on 1.0 SD between alleles. The maximum effect is 1.43 SD. At 12 loci the LG allele led to higher trabecular density with SM/J increasing trabecular density at eight loci.

First principal component scores reflecting overall lumbar, caudal or tibial trabecular density mapped to several locations, five affected lumbar trabecular density (*QTrab1.1*, *QTrab4.2*, *QTrab5.1*, *QTrab7.1*, *QTrab19.1*), five for caudal trabecular density (*QTrab4.2*, *QTrab5.1*,

QTrab6.1, *QTrab7.1*, *QTrab16.1*), and three for tibial trabecular density (*QTrab4.1*, *QTrab4.2*, *QTrab14.1*).

We mapped 11 locations affecting obesity, leptin, and body weight (Table 5; Supplementary Table 3). These QTL were nearly all sex-specific, with the exception of *QOb9.1* and *QOb15.2*. Of the remainder, 5 only affected males and 4 only affected females. Obesity QTLs were also more likely to be diet-specific with 5 only having effects on the high fat diet, one only on the low fat diet, and the remaining 5 affecting animals on both diets. There were 8 locations with effects on serum leptin levels, 6 affecting obesity, and 5 affecting carcass weight. Five loci affected multiple traits, 3 affecting carcass weight, obesity and leptin levels, one affecting just obesity and leptin levels, and one affected only carcass weight and obesity. At three loci the LG allele led to higher weights and/or leptin levels while at 6 loci the SM allele led to higher weights and/or leptin levels. The SM/J strain responds more strongly to the high fat diet than the LG/J strain (31). At two loci, effects switched sign depending on the diet, LG being heavier on the high fat diet and SM being heavier on the low fat diet.

When considering whether loci shown to have an effect on trabecular structure also affect obesity, we use a standard threshold of 5%, not correcting for multiple comparisons, as these loci are defined *a priori* based on their effects on trabecular structure. In effect, we asked: Of the QTLs that affect trabecular traits, which also affect obesity traits? This lowers the significance threshold for obesity effects at these trabecular bone QTLs to 1.30 LOD so that effects that were not significant at the chromosome-wide level, cited above, can be more easily detected. This provides a more realistic balance between Type I and Type 2 errors for the obesity traits, limiting the level of falsely accepting the null model of no effect. Only four of the 20 trabecular loci also had significant effects on obesity traits (*QTrab1.1*, *QTrab3.2*, *QTrab4.1*, *QTrab7.1*). At all four loci, the LG allele led to higher obesity, as it led to high trabecular mass for the corresponding trabecular QTLs. Unlike the trabecular QTLs, effects on obesity were restricted to a high fat diet with one locus (*QTrab1.1*) affecting only males, two (*QTrab3.2*, *QTrab4.1*) affecting only females, and one (*QTrab7.1*) affecting both sexes.

Discussion

We evaluated trabecular bone traits of 481 mice from 16 RI strains derived from the LGXSM intercross to look for phenotypic and genetic associations between obesity and trabecular bone. This genetic model is well suited for looking at obesity-bone associations because of the large difference in body size between the parent LG/J and SM/J strains (32), their major differences in response to a high fat diet (31,33,34), and our previous work demonstrating heritability of obesity-related traits (33–36). Our focus here was trabecular bone traits at three sites (tibia, caudal vertebra, lumbar vertebra), and their associations with obesity. Cortical traits will be considered in future reports.

Based on our results, we find that sex, diet, and genes all produce differences in trabecular bone morphology. However, these relationships are not simple. Males have higher trabecular density than females in the two vertebrae, while females have higher trabecular density in the proximal tibia. A study of trabecular morphology over time in the two sexes using BALB/cJ mice (37) also showed a site-sex interaction -- trabecular bone was denser in male caudal vertebra (18% higher in males) and in female proximal tibia (24% higher in females), as we found here with data pooled across strains and diets. In contrast, in C57BL/6J mice trabecular BV/TV was reported to be greater in males at both the distal femur and lumbar vertebra (38). In our study there was significant sex by strain interaction, indicating genetic variation in sex differences across the RI strains. For BV/TV male:female ratios

varied from 0.72 to 1.30 for lumbar vertebra, 0.92 to 1.37 for caudal vertebra, and 0.63 to 1.15 for the proximal tibia. The sex with greater BV/TV values changed from strain to strain so it may be inappropriate to generalize sex differences from studies of a single strain, such as BALB/cJ or C57BL/6J.

Dietary effects on trabecular properties were also documented with significant increases in trabecular density averaging 0.2 SD units and BV/TV showing a 2–11% increase in high-fat versus low fat diet. Females had a stronger trabecular density response to the high fat diet than males as indeed females respond more strongly than males in terms of obesity on the high fat diet (18). However, the magnitude of these differences in trabecular bone is quite small compared to differences in body size previously reported. Body mass is about 20 – 30% greater on the high fat diet, and total fat pad weights and serum leptin levels are about 50% higher in males and 100% higher in females on a high fat diet versus a low fat diet (28). Thus, the trabecular differences are only 10–20% of those realized for obesity. Clearly, the anabolic effects of a high fat diet, increasing trabecular bone density by about 10%, are minor relative to dietary effects on obesity and serum leptin levels.

Other studies of the effects of a high fat diet on bone have been performed using C57BL/6J mice (39–41). Effects of a high fat diet on body size and obesity are evident in each study and similar to our findings pooled over strains. However, the effects of the high fat diet on trabecular bone are divergent. In the studies on C57BL/6J the high fat diet led to less trabecular bone on the low fat diet, by 19% in the 4th lumbar vertebra (41), by 30% in the proximal tibia (39), and by 40% in the distal femur (40). Collectively, across the LGXSM RI strains an opposite effect was observed with a high fat diet leading to slightly more trabecular bone, 10% more in the lumbar vertebra and proximal tibia and 5% more in the caudal vertebra. These differences in trabecular response occur despite the similar effects of the high fat diet on obesity traits in the LGXSM mice and in the C57BL/6J studies (39,41). There are only a few significant diet by strain interactions in the RI strains indicating a general lack of genetic variation in the trabecular response to the high fat diet. Even so, changes in the amount of trabecular bone on a high fat diet varied across strains from increases of 1% to 37% for the lumbar vertebra, from a reduction of 7% to an increase of 23% for the caudal vertebra, and a reduction of 4% to an increase of 59% for the proximal tibia. Clearly, the effects of a high fat diet on trabecular bone are different in the LGXSM RI lines than they are in C57BL/6J. Generalizations from any one strain should be treated with caution as there is certainly a need for more strains to be included in these studies.

In contrast to the relatively minor effects of sex and diet, genetic variation in trabecular bone characteristics is of major importance in this population, typically being responsible for moderate to high proportions of the differences among individuals. Partial support for this conclusion comes from the moderate heritability levels. Additionally, the trabecular traits both within and between bones were highly genetically correlated with one another, suggesting a common genetic basis for their variation. We were able to map 20 genomic locations affecting trabecular bone properties. Thus, genetic variation in trabecular bone properties is due to many genes of relatively small effect (~1.0 SD). This genetic architecture has been observed for many of the complex traits studied in the LG/J by SM/J intercross, including body size growth (32,42,43), cranial and mandibular morphology (44–47), long bone length (48–50), obesity (30,33,35,36,51), diabetes (30,34), and serum lipid levels (30,52).

While the LG allele led to higher trabecular density at a majority of the loci, at 40% of the locations the SM/J allele led to higher trabecular density. This is somewhat surprising given that the parental strains were selected for greater (LG/J) or lesser (SM/J) body size at 60 days. For previously mapped body size-related traits, LG alleles produce larger size at nearly

all QTL with only a few *contra* loci where the SM allele leads to larger size (32,42–50). Presumably, these *contra* loci were fixed in the parent strain(s) due to genetic drift that accompanied the selection process. The ratio of LG:SM alleles increasing trabecular bone is much lower than for other traits mapped in this cross. Finding alleles increasing bone from both strains is common in other crosses, but these crosses (C57BL/6J with either C3H/HeJ or DBA/2J) only show minor differences in body size (1%–11%) or length (5%). The low observed genetic and phenotypic correlations between trabecular bone properties and obesity traits ($r \approx 0.14$), along with the distribution of LG and SM allelic effects indicate that trabecular bone morphology is largely independent of body size and obesity in this cross.

There have been many other murine QTL studies on trabecular bone and overall bone density (12–19, 53–58). Nearly all of these studies and 80% of the QTLs described in them are contrasts of C57BL/6J with either C3H/HeJ or DBA/2J. Of the 20 QTLs described here, nearly half (9 of 20) map to locations previously noted as bone QTLs, sometimes in as many as 7 different studies (*QTrab1.1*). The names and locations given to these QTLs are provided in Table 4. Even so, support regions in this (13.25 cM) and other F₂ intercross and RI strain studies are fairly large so it is difficult to say whether these effects, although mapping to common regions, are due to the same molecular variants or to different linked genes. It has not been uncommon for single QTL mapped in a F₂ intercross or RI strain set to divide into several loci with further accumulation of recombination (14–16,59–62). Two studies used one of the strains used here but there was only one result overlapping with our findings. There were 5 bone QTLs mapped in a cross of SM/J with NZB/B1NJ (54) none of which appear in the LGXSM RI lines, 4 between MRL/MpJ and CAST/Ei (58), one of which maps to *QTrab2.1*, and 12 between MRL/MpJ and SJL/J two of which map to *QTrab2.1* and *QTrab11.2* (63). MRL/MpJ is a close relative of LG/J sharing 75% of their genomes identical by descent (64) so we may expect some overlap in QTLs from these related crosses.

Unlike LG/J and SM/J, the strains most commonly used in mapping bone QTLs vary only modestly in size (C3H/B6 = 1.01 for body weight (BW) and 1.05 for body length (BL); DBA/B6 = 1.12 BW and 1.05 BL) (65) whereas LG/J weighs double SM/J and is 35% longer. Other crosses with large body size differences between parental strains include MRL/MpJ, LG/J's close relation, with CAST/EiJ (58) and SJL/J (63), SM/J with NZB/B1NJ (54) and C57BL/6J with CAST/EiJ (66). We posit that the LGXSM intercross is particularly well suited to examine interactions between bone traits and body size/obesity, providing a complementary approach to the growing literature on murine-derived bone QTLs.

Genetic correlations of obesity, leptin and body size with trabecular bone morphology are low and not statistically significant. This is in contrast to the very high genetic correlations observed among trabecular traits, and among the three aspects of obesity measured here. With a sample limited to 16 RI lines there is not enough power to detect minor genetic correlations ($r < 0.45$; $r^2 < 0.20$) so the lack of significance in this study should not be taken as evidence against any possible relationship, just evidence against a strong genetic relationship between obesity and trabecular density in this population. The QTL results also bear on this issue. Only 20% of the trabecular QTLs also had detectable effects on obesity. The influence of obesity on peak trabecular density is slight in this population relative to other biologically important factors affecting variation.

Also in human populations, studies of the relationship between obesity and bone mass have produced mixed results. Often the correlations, while statistically significant are low to moderate, in the range of 0.20 to 0.50, and can change depending on the age and sex of the subjects (3, 67–75). Correlations at this level may not have been detectable in our study.

Recent studies have found that obesity may actually decrease trabecular bone levels and increase fracture risk in humans (2,76–78).

Cao (2) points out that the relationship between obesity and bone is complex, involving many factors that may lead to varying effects of obesity on bone. Obesity may lead to increased bone due to increased mechanical loading but may also lead to decreased bone formation in the trade-off between adipogenesis and osteoblastogenesis in differentiation from a common set of precursor mesenchymal stem cells. The effects of leptin on bone can either be positive or negative depending on bone and compartment and whether effects are produced through the periphery or through the central nervous system. Obesity is associated with systemic inflammation and the increased secretion of proinflammatory cytokines which, in turn, can promote the production of osteoclasts and bone loss. This combination of anabolic and catabolic effects of obesity on bone may largely cancel out leaving a weak bone-obesity relationship like that described here. While some of these individual processes may be notable in isolation, as in a diet-induced obesity model or a single gene mutant model where all other genetic variation is eliminated, they may not rise to levels of great importance in segregating populations where their effects can cancel each other out or be swamped out by other genetic and environmental variants.

Conclusions

We have examined the effects of sex, diet, genes and obesity on trabecular bone properties in a genetic mouse model of dietary obesity, the LGXSM Recombinant Inbred mouse strains. We found increased trabecular density in male vertebra and decreased trabecular density in the male proximal tibia relative to females, but these effects were small. Likewise, the high fat diet led to uniform but small increases in trabecular bone in all three skeletal elements studied. Genetic effects on trabecular properties were substantial and highly intercorrelated, arguing for some common genetic basis underlying trabecular properties in the three regions examined. We mapped 20 trabecular quantitative trait loci for trabecular properties. Effects were relatively small (~1.0 standard deviation units) and about 25% affected multiple skeletal elements. In both the quantitative genetic and QTL analyses, the association between obesity and trabecular density was minimal, indicating that while traits within the trabecular and obesity sets are heritable and highly intercorrelated, the two sets of traits are largely independent, sharing only a small percentage (20%) of QTLs. Finally, we wish to further stress that there is great variability among mouse strains in the response of trabecular bone to genetic and environmental factors. This variability needs to be taken into account when attempting to generalize results based on a single strain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Reid IR. Fat and bone. *Arch Biochem Biophys*. 2010; 503:20–27. [PubMed: 20599663]
2. Smith BJ, Lerner MR, Bu SY, Lucas EA, Hanas JS, Lightfoot SA, Postier RG, Bronze MS, Brackett DJ. Systemic bone loss and induction of coronary vessel disease in a rat model of chronic inflammation. *Bone*. 2006; 38:378–386. [PubMed: 16256450]

3. Zhao L-J, Jiang H, Papasian CJ, Maulik D, Drees B, Hamilton J, Deng H-W. Correlation of obesity and osteoporosis: Effect of fat mass on the determination of osteoporosis. *J Bone Miner Res.* 2008; 23:17–29. [PubMed: 17784844]
4. Cao JJ. Effects of obesity on bone metabolism. *J Ortho Surg and Res.* 2011; 6:30.
5. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen J, Vinson C, Rueger JM, Karsenty G. Leptin inhibits bone formation through a hypothalamic relay: A central control of bone mass. *Cell.* 2000; 100:197–207. [PubMed: 10660043]
6. Karsenty G. Convergence between bone and energy homeostases: leptin regulation of bone mass. *Cell Metab.* 2006; 4:341–348. [PubMed: 17084709]
7. Hamrick MW, Pennington C, Newton D, Xie D, Isaacs C. Leptin deficiency produces contrasting phenotypes in bones of the limb and spine. *Bone.* 2004; 34:376–383. [PubMed: 15003785]
8. Williams GA, Callon KE, Watson M, Costa JL, Ding Y, Dickinson M, Wang Y, Naot D, Reid IR, Cornish J. Skeletal phenotype of the leptin receptor-deficient db/db Mouse. *J Bone Min Res.* 2011; 26:1698–1709.
9. Gordon CM. Leptin and the skeleton - where is the fat. *Metabolism Clinical and Experimental.* 2011; 60:1203–1206. [PubMed: 21742353]
10. Sienkiewicz E, Magkos F, Aronis KN, Brinkoetter M, Chamberland JP, Chou S, Arampatzi KM, Gao C, Koniaris A, Mantzoros CS. Long-term metreleptin treatment increases bone mineral density and content at the lumbar spine of lean hypoleptinemic women. *Metabolism Clinical and Experimental.* 2011; 60:1211–1221. [PubMed: 21741057]
11. Conroy R, Girotra M, Shane E, McMahon DJ, Pavlovich KH, Leibel RL, Rosenbaum M, Korner J. Leptin administration does not prevent the bone mineral metabolism changes induced by weight loss. *Metabolism Clinical and Experimental.* 2011; 60:1222–1226. [PubMed: 21489573]
12. Drake TA, Schadt E, Hannani K, Kabo JM, Krass K, Colinayo V, Greaser LE 3rd, Goldin J, Lusis AJ. Genetic loci determining bone density in mice with diet-induced atherosclerosis. *Physiol Genomics.* 2001; 5:205–215. [PubMed: 11328966]
13. Klein RF, Mitchell SR, Phillips TJ, Belknap JK, Orwoll ES. Quantitative trait loci affecting peak bone mineral density in mice. *J Bone Min Res.* 1998; 13:1648–1656.
14. Klein OF, Carlos AS, Vartanian KA, Chambers VK, Turner EJ, Phillips TJ, Belknap JK, Orwoll ES. Confirmation and fine mapping of chromosomal regions influencing peak bone mass in mice. *J Bone Miner Res.* 2001; 16:1953–1961. [PubMed: 11697791]
15. Beamer WG, Shultz KL, Donahue LR, Churchill GA, Sen S, Wergedal JR, Baylink DJ, Rosen CJ. Quantitative trait loci for femoral and lumbar vertebral bone mineral density in C57BL/6J and C3H/HeJ inbred strains of mice. *J Bone Miner Res.* 2001; 16:1195–1206. [PubMed: 11450694]
16. Beamer WG, Shultz KL, Ackert-Bicknell CL, Horton LG, Delahunty KM, Coombs HF 3rd, Donahue LR, Canalis E, Rosen CJ. Genetic dissection of mouse distal chromosome 1 reveals three linked BMD QTLs with sex-dependent regulation of bone phenotypes. *J Bone Miner Res.* 2007; 22:1187–1196. [PubMed: 17451375]
17. Bouxsein ML, Uchiyama T, Rosen CJ, Shultz KL, Donahue LR, Turner CH, Sen S, Churchill GA, Müller R, Beamer WG. Mapping quantitative trait loci for vertebral trabecular bone volume fraction and microarchitecture in mice. *J Bone Miner Res.* 2004; 19:587–599. [PubMed: 15005846]
18. Koller DL, Schrieffer J, Sun Q, Shultz KL, Donahue LR, Rosen CJ, Foroud T, Beamer WG, Turner CH. Genetic effects for femoral biomechanics, structure, and density in C57BL/6J and C3H/HeJ inbred mouse strains. *J Bone Miner Res.* 2003; 18:1758–1765. [PubMed: 14584885]
19. Kesavan C, Mohan S, Srivastava AK, Kapoor S, Wergedal JE, Yu H, Baylink DJ. Identification of genetic loci that regulate bone adaptive response to mechanical loading in C57BL/6J and C3H/HeJ mice intercross. *Bone.* 2006; 39:634–643. [PubMed: 16713414]
20. Hrbek T, Alves de Brito R, Wang B, Pletscher LS, Cheverud JM. Genetic characterization of a new set of recombinant inbred lines (LGXSM) formed from the intercross of SM/J and LG/J inbred mouse strains. *Mammalian Genome.* 2006; 17:417–429. [PubMed: 16688532]
21. Leonard MB, Shults J, Wilson BA, Tershakovec AM, Zemel BS. Obesity during childhood and adolescence augments bone mass and bone dimensions. *Am J Clin Nutr.* 2004; 80:514–523. [PubMed: 15277178]

22. Takeda S, Eleftheriou F, Levasseur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducy P, Karsenty G. Leptin regulates bone formation via the sympathetic nervous system. *Cell*. 2002; 111:305–317. [PubMed: 12419242]
23. Reid IR. Leptin deficiency--lessons in regional differences in the regulation of bone mass. *Bone*. 2004; 34:369–371. [PubMed: 15003783]
24. Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G. Endocrine regulation of energy metabolism by the skeleton. *Cell*. 2007; 130:456–469. [PubMed: 17693256]
25. Semenkovich CF, Teitelbaum SL. Bone weighs in on obesity. *Cell*. 2007; 130:409–411. [PubMed: 17693252]
26. Ferron M, Hinoi E, Karsenty G, Ducy P. Osteocalcin differentially regulates β cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. *PNAS*. 2008; 105:5266–5270. [PubMed: 18362359]
27. Confavreux CB, Levine RL, Karsenty G. A paradigm of integrative physiology, the crosstalk between bone and energy metabolisms. *Mol Cell Endocrinol*. 2009; 310:21–29. [PubMed: 19376193]
28. Cheverud JM, Ehrich TH, Kenney JP, Pletscher LS, Semenkovich CF. Genetic evidence for discordance between obesity and diabetes-related traits in the LGXSM recombinant inbred mouse strains. *Diabetes*. 2004a; 53:2700–2708. [PubMed: 15448104]
29. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity*. 2005; 95:221–227. [PubMed: 16077740]
30. Cheverud JM, Ehrich TH, Hrbek T, Kenney JP, Pletscher LS, Semenkovich CF. Quantitative trait loci for obesity and diabetes-related traits and their dietary responses to high fat feeding in the LGXSM recombinant inbred mouse strains. *Diabetes*. 2004b; 53:3328–3336. [PubMed: 15561968]
31. Ehrich T, Kenney J, Vaughn T, Pletscher LS, Cheverud J. Diet, obesity, and hyperglycemia in LG/J and SM/J Mice. *Obesity Research*. 2003; 11:1400–1410. [PubMed: 14627762]
32. Vaughn TT, Pletscher LS, Peripato A, King-Ellison K, Adams E, Erikson C, Cheverud JM. Mapping quantitative trait loci for murine growth—A closer look at genetic architecture. *Genetical Research, Camb*. 1999; 74:313–322.
33. Cheverud JM, Lawson HA, Fawcett GL, Wang B, Pletscher LS, Fox A, Maxwell TJ, Ehrich TH, Kenney-Hunt JP, Wolf JB, Semenkovich CF. Diet-dependent genetic and genomic imprinting effects on obesity in mice. *Obesity*. 2011; 19:160–170. [PubMed: 20539295]
34. Lawson HA, Lee A, Fawcett GL, Wang B, Pletscher LS, Maxwell TJ, Ehrich TH, Kenney-Hunt JP, Wolf JB, Semenkovich CF, Cheverud JM. The importance of context to the genetic architecture of diabetes-related traits is revealed in a genome-wide scan of a LG/J x SM/J murine model. *Mammalian Genome*. 2011; 22:197–208. [PubMed: 21210123]
35. Fawcett GL, Roseman CC, Jarvis JP, Wang B, Cheverud JM. Genetic architecture of adiposity and organ weight using combined generation QTL analysis. *Obesity*. 2008; 16:1861–1868. [PubMed: 18551125]
36. Fawcett GL, Jarvis JP, Roseman CC, Wang B, Wolf JB, Cheverud JM. Fine-mapping of adiposity quantitative trait loci in an F_{9/10} advanced intercross line. *Obesity*. 2010; 18:1383–1392. [PubMed: 19910941]
37. Willingham MD, Brodt MD, Lee KL, Stephens AL, Ye J, Silva MJ. Age-related changes in bone structure and strength in female and male BALB/c mice. *Calcif Tissue Int*. 2010; 86:470–483. [PubMed: 20405109]
38. Glatt V, Canalis E, Stadmeier L, Bouxsein ML. Age-related changes in trabecular architecture differ in female and male C57BL/6J mice. *J Bone Miner Res*. 2007; 22:1197–1207. [PubMed: 17488199]
39. Cao JJ, Gregoire BR, Gao H. High-fat diet decreases cancellous bone mass but has no effect on cortical bone mass in the tibia in mice. *Bone*. 2009; 44:1097–1104. [PubMed: 19264159]
40. Cao JJ, Sun L, Gao H. Diet-induced obesity alters bone remodeling leading to decreased femoral trabecular bone mass in mice. *Ann NY Acad Sci*. 2010; 1192:292–297. [PubMed: 20392249]
41. Patsch JM, Kiefer FW, Vargad P, Paila P, Rauner M, Stupphanna D, Reschf H, Moserg D, Zysset PK, Stulnig TM, Pietschmann. Increased bone resorption and impaired bone microarchitecture in

- short-term and extended high-fat diet-induced obesity. *Metabolism Clinical and Experimental*. 2011; 60:243–249. [PubMed: 20171704]
42. Cheverud J, Routman E, Duarte FM, van Swinderen B, Cothran K, Perel C. Quantitative trait loci for murine growth. *Genetics*. 1996; 142:1305–1319. [PubMed: 8846907]
 43. Wolf JB, Cheverud JM, Roseman CC, Hager R. Genome-wide analysis reveals a complex pattern of genomic imprinting in mice. *PLoS Genetics*. 2008; 4(6):e1000091. [PubMed: 18535661]
 44. Leamy L, Routman E, Cheverud J. Quantitative trait loci for early and late developing skull characters in mice: A test of the genetic independence model of morphological integration. *American Naturalist*. 1999; 153:201–214.
 45. Cheverud JM, Routman EJ, Irschick DK. Pleiotropic effects of individual gene loci on mandibular morphology. *Evolution*. 1997; 51:2004–2014.
 46. Ehrich T, Vaughn TT, Koreishi SF, Linsey RB, Pletscher LS, Cheverud JM. Pleiotropic effects on mandibular morphology I. Developmental morphological integration and differential dominance. *Journal of Experimental Zoology (Molecular and Developmental Evolution)*. 2003; 296B:58–79.
 47. Roseman CC, Kenney-Hunt JP, Cheverud JM. Phenotypic integration without modularity: Testing hypotheses about the distribution of pleiotropic quantitative trait loci in a continuous space. *Evolutionary Biology*. 2009; 36:282–291.
 48. Norgard EA, Roseman CC, Fawcett GL, Pavli ev M, Morgan CD, Pletscher LS, Wang B, Cheverud JM. Identification of quantitative trait loci affecting murine long bone length in a two-generation intercross of LG/J and SM/J mice. *Journal of Bone and Mineral Research*. 2008; 23:887–895. [PubMed: 18435578]
 49. Norgard EA, Jarvis JP, Roseman CC, Maxwell TJ, Kenney-Hunt JP, Samocha KE, Pletscher LS, Wang B, Fawcett GL, Leatherwood CJ, Wolf JB, Cheverud JM. Replication of long bone length QTL in the F₉–F₁₀ LG, SM advanced intercross. *Mammalian Genome*. 2009; 20:224–235. [PubMed: 19306044]
 50. Norgard EA, Lawson HA, Pletscher LS, Wang B, Brooks VR, Wolf JB, Cheverud JM. Complex factors and diet affect long bone length in the F₃₄ LG, SM advanced intercross. *Mammalian Genome*. 2011; 22:178–196. [PubMed: 21170743]
 51. Cheverud J, Vaughn T, Pletscher LS, Peripato A, Adams E, Erickson C, King-Ellison K. Genetic architecture of adiposity in the cross of Large (LG/J) and Small (SM/J) inbred mice. *Mammalian Genome*. 2001; 12:3–12. [PubMed: 11178736]
 52. Lawson HA, Zelle KM, Fawcett GL, Wang B, Pletscher LS, Maxwell TJ, Ehrich TH, Kenney-Hunt JP, Wolf JB, Semenkovich CF, Cheverud JM. Genetic, epigenetic, and gene-by-diet interaction effects underlie variation in serum lipids in a LG/J x SM/J murine model. *Journal of Lipid Research*. 2010; 51:2976–2984. [PubMed: 20601649]
 53. Benes H, Weinstein RS, Zheng W, Thaden JJ, Jilka RL, Manolagas SC, Shmookler Reis RJ. Chromosomal mapping of osteopenia-associated quantitative trait loci using closely related mouse strains. *J Bone Miner Res*. 2000; 15:626–633. [PubMed: 10780854]
 54. Ishimori N, Stylianou IM, Korstanje R, Marion MA, Li R, Donahue LR, Rosen CJ, Beamer WG, Paigen B, Churchill GA. Quantitative trait loci for BMD in an SM/J by NZB/BINJ intercross population and identification of *Trps1* as a probable candidate gene. *J Bone Miner Res*. 2008; 23:1529–1537. [PubMed: 18442308]
 55. Ishimori N, Li R, Walsh KA, Korstanje R, Rollins JA, Petkov P, Pletcher MT, Wiltshire T, Donahue LR, Rosen CJ, Beamer WG, Churchill GA, Paigen B. Quantitative trait loci that determine BMD in C57BL/6J and 129S1/SvImJ inbred mice. *J Bone Miner Res*. 2006; 21:105–112. [PubMed: 16355279]
 56. Reeves GM, McCreadie BR, Chen S, Galecki AT, Burke DT, Miller RA, Goldstein SA. Quantitative trait loci modulate vertebral morphology and mechanical properties in a population of 18-month-old genetically heterogeneous mice. *Bone*. 2007; 40:433–443. [PubMed: 17049325]
 57. Vitarius JA, Sehayek E, Breslow JL. Identification of quantitative trait loci affecting body composition in a mouse intercross. *Proc Natl Acad Sci USA*. 2006; 103:19860–19865. [PubMed: 17179051]

58. Yu H, Mohan S, Edderkaoui B, Masinde GL, Davidson HM, Wergedal JE, Beamer WG, Baylink DJ. Detecting novel bone density and bone size quantitative trait loci using a cross of MRL/MpJ and CAST/EiJ inbred mice. *Calcif Tissue Int.* 2007; 80:103–110. [PubMed: 17308992]
59. Edderkaoui B, Baylink DJ, Beamer WG, Shultz KL, Wergedal JE, Mohan S. Genetic regulation of femoral bone mineral density: complexity of sex effect in chromosome 1 revealed by congenic sublines of mice. *Bone.* 2007; 41:340–345. [PubMed: 17618849]
60. Shultz KL, Donahue LR, Bouxsein ML, Baylink DJ, Rosen CJ, Beamer WG. Congenic strains of mice for verification and genetic decomposition of quantitative trait loci for femoral bone mineral density. *J Bone Miner Res.* 2003; 18:175–185. [PubMed: 12568393]
61. Turner CH, Sun Q, Schrieffer J, Pitner N, Price R, Bouxsein ML, Rosen CJ, Donahue LR, Shultz KL, Beamer WG. Congenic mice reveal sex-specific genetic regulation of femoral structure and strength. *Calcif Tissue Int.* 2003; 73:297–303. [PubMed: 14667144]
62. Edderkaoui B, Baylink DJ, Beamer WG, Wergedal JE, Dunn NR, Shultz KL, Mohan SB. Multiple genetic loci from CAST/EiJ chromosome 1 affect vBMD either positively or negatively in a C57BL/6J background. *J Bone Miner Res.* 2006; 21:97–104. [PubMed: 16355278]
63. Masinde GL, Li X, Gu W, Wergedal J, Mohan S, Baylink DJ. Quantitative trait loci for bone density in mice: The genes determining total skeletal density and femur density show little overlap in F₂ mice. *Calcif Tissue Int.* 2002; 71:421–428. [PubMed: 12202954]
64. Murphy, ED.; Roths, JB. Autoimmunity and Lymphoproliferation: Induction by Mutant Gene *lpr* and Acceleration by a Male-Associated Factor in Strain BXSb. In: Rose, NR.; Bigazzi, PE.; Warner, NL., editors. *Genetic Control of Autoimmune Disease*. Elsevier; New York: 1979. p. 207-220.
65. Ackert-Bicknell, C.; Beamer, WG.; Rosen, CJ.; Sundberg, JP. Mouse Phenome Database. The Jackson Laboratory; Bar Harbor, Maine USA: 2012. Aging study: bone mineral density and body composition of 32 inbred strains of mice. web site (<http://phenome.jax.org>)
66. Beamer WG, Shultz KL, Churchill GA, Frankel WN, Baylink DJ, Rosen CJ, Donahue LR. Quantitative trait loci for bone density in C57BL/6J and CAST/EiJ inbred mice. *Mammalian Genome.* 1999; 10:1043–1049. [PubMed: 10556421]
67. Reid IR, Plank LD, Evans MC. Fat mass is an important determinant of whole body bone density in premenopausal women but not in men. *J Clin Endocrinol Metab.* 1992a; 75:779–782. [PubMed: 1517366]
68. Reid IR, Ames R, Evans MC, Sharpe S, Gamble G, France JT, Lim TM, Cundy TF. Determinants of total body and regional bone mineral density in normal postmenopausal women -- a key role for fat mass. *J Clin Endocrinol Metab.* 1992b; 75:45–51. [PubMed: 1619030]
69. Wardlaw GM. Putting body weight and osteoporosis into perspective. *Am J Clin Nutrition.* 1996; 63:433S–436S. [PubMed: 8615336]
70. Kirchengast S, Peterson B, Hauser G, Knogler W. Body composition characteristics are associated with the bone density of the proximal femur end in middle- and old-aged women and men. *Maturitas.* 2001; 39:133–145. [PubMed: 11514112]
71. Thomas T, Burguera B, Melton LJ III, Atkinson, O'Fallon WM, Riggs BL, Khosla S. Role of serum leptin, insulin, and estrogen levels as potential mediators of the relationship between fat mass and bone mineral density in men versus women. *Bone.* 2001; 29:114–120. [PubMed: 11502471]
72. Ho-Pham LT, Nguyen ND, Lai TQ, Nguyen TV. R Contributions of lean mass and fat mass to bone mineral density: a study in postmenopausal women. *BMC Musculoskeletal Disorders.* 2010; 11:59. [PubMed: 20346165]
73. Fu X, Ma X, Lu H, He W, Wang Z, Zhu S. Associations of fat mass and fat distribution with bone mineral density in pre- and postmenopausal Chinese women. *Osteoporos Int* (2011). 2011; 22:113–119.
74. Andreoli A, Bazzocchi A, Celi M, Lauro D, Sorge R, Tarantino U, Guglielmi G. Relationship between body composition, body mass index and bone mineral density in a large population of normal, osteopenic and osteoporotic women. *Radiological Medicine.* 2011; 116:1115–1123.

75. Bredella MA, Torriani M, Hosseini Ghomi R, Thomas BJ, Brick DJ, Gerweck AV, Harrington LM, Breggia A, Rosen CJ, Miller KK. Determinants of bone mineral density in obese premenopausal women. *Bone*. 2011; 48:748–754. [PubMed: 21195217]
76. Compston JE, Watts NB, Chapurlat R, Cooper C, Boonen S, Greenspan S, Pfeilschifter J, Silverman S, Díez-Pérez A, Lindsay R, Saag KG, Coen Netelenbos J, Gehlbach S, Hooven FH, Flahive J, Adachi JD, Rossini M, LaCroix AZ, Roux C, Sambrook PN, Siris ES. for the Glow Investigators. Obesity Is Not Protective against Fracture in Postmenopausal Women: GLOW. *Am J Med*. 2011; 124:1043–1050. [PubMed: 22017783]
77. Migliaccio S, Greco EA, Fornari R, Donini MLM, Lenzi A. Is obesity in women protective against osteoporosis? *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*. 2011; 4:273–282.
78. Yoo HJ, Park MS, Yang SJ, Kim TN, Lim KI, Kang HJ, Song W, Baik SH, Choi DS, Choi KM. The differential relationship between fat mass and bone mineral density by gender and menopausal status. *J Bone Miner Metab*. 2012; 30:47–53. [PubMed: 21644057]

Highlights

- Variation in trabecular bone morphology is due to sex, diet, obesity, and genes.
- Males have higher trabecular density in the vertebra and lower density in the tibia.
- High fat diet has minimal effects on bone.
- Bone morphology is heritable with 20 genomic locations identified.
- Genes affecting obesity and trabecular morphology are largely independent.

Table 1

Composition of high-fat (HF) and low-fat (LF) diets.

Dietary Components	HF	LF
Energy from fat (%)	42	15
Casein (g/kg)	195	197
Sugars (g/kg)	341	307
Corn starch (g/kg)	150	313
Cellulose (g/kg)	50	50
Corn oil (g/kg)	—	58
Hydrogenated coconut oil (g/kg)	—	7
Anhydrous milk fat (g/kg)	210	—
Cholesterol (g/kg)	1.5	—
Kilojoules per gram	18.95	16.99

Table 2

First principal component loadings for the Lumbar and Caudal Vertebra and Proximal Tibia

Trait	Lumbar	Caudal	Tibia
BV/TV	0.446	0.474	0.524
CD	0.386	0.241	0.454
SMI	-0.415	-0.432	-0.489
TbN	0.425	0.456	0.367
TbTh	0.374	0.360	0.380
TbSp	-0.398	-0.439	-0.036

Heritabilities are italicized on the diagonal ($SE = 0.0174$), genetic correlations are above the diagonal ($SE = 0.267$), and phenotypic correlations are below the diagonal ($SE = 0.046$). Significant results are in **boldface**.

Table 3

Trait	Lum BVTV	Lum CD	Lum SMI	Lum TbN	Lum TbTh	Lum TbSp	Tib BVTV	Tib CD	Tib SMI	Tib TbN	Tib TbTh	Tib TbSp	Cau BVTV	Cau CD	Cau SMI	Cau TbN	Cau TbTh	Cau TbSp	Leptin	Fat	Nec Wt
LumBVTV	<i>0.65</i>	<i>0.92</i>	<i>-0.99</i>	<i>0.97</i>	<i>0.98</i>	<i>-0.93</i>	<i>0.91</i>	<i>0.89</i>	<i>-0.85</i>	<i>0.69</i>	<i>0.61</i>	<i>-0.79</i>	<i>0.93</i>	<i>0.43</i>	<i>-0.92</i>	<i>0.89</i>	<i>0.92</i>	<i>-0.89</i>	<i>0.12</i>	<i>0.13</i>	<i>-0.15</i>
LumCD	<i>0.75</i>	<i>0.63</i>	<i>-0.88</i>	<i>0.90</i>	<i>0.90</i>	<i>-0.84</i>	<i>0.81</i>	<i>0.85</i>	<i>-0.72</i>	<i>0.66</i>	<i>0.39</i>	<i>-0.70</i>	<i>0.84</i>	<i>0.44</i>	<i>-0.82</i>	<i>0.77</i>	<i>0.85</i>	<i>-0.79</i>	<i>-0.10</i>	<i>0.06</i>	<i>-0.16</i>
LumSMI	<i>-0.95</i>	<i>-0.58</i>	<i>0.55</i>	<i>-0.93</i>	<i>-0.99</i>	<i>0.90</i>	<i>-0.90</i>	<i>-0.87</i>	<i>0.83</i>	<i>-0.66</i>	<i>-0.61</i>	<i>0.75</i>	<i>-0.93</i>	<i>-0.38</i>	<i>0.92</i>	<i>-0.87</i>	<i>-0.93</i>	<i>0.86</i>	<i>-0.15</i>	<i>-0.11</i>	<i>0.17</i>
LumTbN	<i>0.82</i>	<i>0.83</i>	<i>-0.64</i>	<i>0.69</i>	<i>0.91</i>	<i>-0.97</i>	<i>0.88</i>	<i>0.86</i>	<i>-0.83</i>	<i>0.70</i>	<i>0.58</i>	<i>-0.83</i>	<i>0.90</i>	<i>0.48</i>	<i>-0.86</i>	<i>0.90</i>	<i>0.86</i>	<i>-0.89</i>	<i>0.09</i>	<i>0.16</i>	<i>-0.10</i>
LumTbTh	<i>0.86</i>	<i>0.41</i>	<i>-0.89</i>	<i>0.53</i>	<i>0.48</i>	<i>-0.88</i>	<i>0.91</i>	<i>0.84</i>	<i>-0.88</i>	<i>0.62</i>	<i>0.69</i>	<i>-0.74</i>	<i>0.94</i>	<i>0.41</i>	<i>-0.93</i>	<i>0.88</i>	<i>0.94</i>	<i>-0.88</i>	<i>0.19</i>	<i>0.13</i>	<i>-0.13</i>
LumTbSp	<i>-0.74</i>	<i>-0.72</i>	<i>0.50</i>	<i>-0.92</i>	<i>-0.45</i>	<i>0.58</i>	<i>-0.85</i>	<i>-0.79</i>	<i>0.83</i>	<i>-0.59</i>	<i>-0.59</i>	<i>0.74</i>	<i>-0.87</i>	<i>-0.55</i>	<i>0.83</i>	<i>-0.88</i>	<i>-0.83</i>	<i>0.88</i>	<i>-0.20</i>	<i>-0.22</i>	<i>0.03</i>
TibBVTV	<i>0.61</i>	<i>0.34</i>	<i>-0.60</i>	<i>0.41</i>	<i>0.64</i>	<i>-0.36</i>	<i>0.42</i>	<i>0.90</i>	<i>-0.93</i>	<i>0.81</i>	<i>0.63</i>	<i>-0.77</i>	<i>0.94</i>	<i>0.53</i>	<i>-0.92</i>	<i>0.93</i>	<i>0.90</i>	<i>-0.91</i>	<i>0.15</i>	<i>0.24</i>	<i>0.00</i>
TibCD	<i>0.59</i>	<i>0.51</i>	<i>-0.54</i>	<i>0.48</i>	<i>0.49</i>	<i>-0.38</i>	<i>0.82</i>	<i>0.48</i>	<i>-0.73</i>	<i>0.94</i>	<i>0.27</i>	<i>-0.81</i>	<i>0.90</i>	<i>0.51</i>	<i>-0.88</i>	<i>0.90</i>	<i>0.82</i>	<i>-0.88</i>	<i>-0.03</i>	<i>0.10</i>	<i>-0.14</i>
TibSMI	<i>-0.45</i>	<i>-0.14</i>	<i>0.47</i>	<i>-0.22</i>	<i>-0.56</i>	<i>0.20</i>	<i>-0.84</i>	<i>-0.61</i>	<i>0.28</i>	<i>-0.64</i>	<i>-0.75</i>	<i>0.70</i>	<i>-0.87</i>	<i>-0.45</i>	<i>0.85</i>	<i>-0.85</i>	<i>-0.85</i>	<i>0.86</i>	<i>-0.18</i>	<i>-0.23</i>	<i>-0.08</i>
TibTbN	<i>0.31</i>	<i>0.28</i>	<i>-0.28</i>	<i>0.28</i>	<i>0.25</i>	<i>-0.17</i>	<i>0.53</i>	<i>0.63</i>	<i>-0.47</i>	<i>0.27</i>	<i>0.20</i>	<i>-0.91</i>	<i>0.69</i>	<i>0.29</i>	<i>-0.67</i>	<i>0.71</i>	<i>0.58</i>	<i>-0.70</i>	<i>-0.27</i>	<i>-0.11</i>	<i>-0.28</i>
TibTbTh	<i>0.26</i>	<i>-0.13</i>	<i>-0.34</i>	<i>-0.02</i>	<i>0.48</i>	<i>0.01</i>	<i>0.67</i>	<i>0.30</i>	<i>-0.70</i>	<i>0.19</i>	<i>0.23</i>	<i>-0.23</i>	<i>0.66</i>	<i>0.28</i>	<i>-0.66</i>	<i>0.65</i>	<i>0.68</i>	<i>-0.63</i>	<i>0.35</i>	<i>0.22</i>	<i>0.07</i>
TibTbSp	<i>-0.35</i>	<i>-0.32</i>	<i>0.30</i>	<i>-0.35</i>	<i>-0.27</i>	<i>0.26</i>	<i>-0.39</i>	<i>-0.45</i>	<i>0.35</i>	<i>-0.57</i>	<i>-0.09</i>	<i>0.20</i>	<i>-0.77</i>	<i>-0.34</i>	<i>0.74</i>	<i>-0.80</i>	<i>-0.66</i>	<i>0.76</i>	<i>0.10</i>	<i>-0.06</i>	<i>0.05</i>
CauBVTV	<i>0.76</i>	<i>0.66</i>	<i>-0.65</i>	<i>0.74</i>	<i>0.62</i>	<i>-0.64</i>	<i>0.47</i>	<i>0.47</i>	<i>-0.33</i>	<i>0.28</i>	<i>0.16</i>	<i>-0.32</i>	<i>0.59</i>	<i>0.28</i>	<i>-0.99</i>	<i>0.95</i>	<i>0.96</i>	<i>-0.92</i>	<i>0.35</i>	<i>0.33</i>	<i>0.09</i>
CauCD	<i>0.13</i>	<i>0.38</i>	<i>0.05</i>	<i>0.41</i>	<i>-0.10</i>	<i>-0.45</i>	<i>-0.06</i>	<i>0.06</i>	<i>0.19</i>	<i>-0.02</i>	<i>-0.33</i>	<i>-0.09</i>	<i>0.33</i>	<i>0.22</i>	<i>-0.17</i>	<i>0.48</i>	<i>0.48</i>	<i>-0.48</i>	<i>0.07</i>	<i>0.30</i>	<i>0.14</i>
CauSMI	<i>-0.71</i>	<i>-0.59</i>	<i>0.65</i>	<i>-0.64</i>	<i>-0.60</i>	<i>0.53</i>	<i>-0.45</i>	<i>-0.45</i>	<i>0.34</i>	<i>-0.28</i>	<i>-0.21</i>	<i>0.29</i>	<i>-0.93</i>	<i>-0.18</i>	<i>0.51</i>	<i>-0.91</i>	<i>-0.97</i>	<i>0.87</i>	<i>-0.34</i>	<i>-0.29</i>	<i>-0.06</i>
CauTbN	<i>0.62</i>	<i>0.64</i>	<i>-0.44</i>	<i>0.77</i>	<i>0.38</i>	<i>-0.71</i>	<i>0.32</i>	<i>0.39</i>	<i>-0.16</i>	<i>0.21</i>	<i>-0.05</i>	<i>-0.26</i>	<i>0.83</i>	<i>0.64</i>	<i>-0.70</i>	<i>0.57</i>	<i>0.83</i>	<i>-0.98</i>	<i>0.34</i>	<i>0.32</i>	<i>0.03</i>
CauTbTh	<i>0.74</i>	<i>0.51</i>	<i>-0.70</i>	<i>0.56</i>	<i>0.74</i>	<i>-0.45</i>	<i>0.53</i>	<i>0.45</i>	<i>-0.46</i>	<i>0.25</i>	<i>0.37</i>	<i>-0.26</i>	<i>0.83</i>	<i>-0.10</i>	<i>-0.83</i>	<i>0.49</i>	<i>0.48</i>	<i>-0.80</i>	<i>0.39</i>	<i>0.40</i>	<i>0.21</i>
CauTbSp	<i>-0.58</i>	<i>-0.59</i>	<i>0.42</i>	<i>-0.72</i>	<i>-0.36</i>	<i>0.71</i>	<i>-0.29</i>	<i>-0.35</i>	<i>0.14</i>	<i>-0.15</i>	<i>0.07</i>	<i>0.22</i>	<i>-0.74</i>	<i>-0.62</i>	<i>0.58</i>	<i>-0.93</i>	<i>-0.42</i>	<i>0.48</i>	<i>-0.35</i>	<i>-0.30</i>	<i>-0.04</i>
Leptin	<i>0.17</i>	<i>-0.03</i>	<i>-0.17</i>	<i>0.12</i>	<i>0.24</i>	<i>-0.16</i>	<i>0.12</i>	<i>-0.03</i>	<i>-0.12</i>	<i>-0.15</i>	<i>0.20</i>	<i>0.04</i>	<i>0.17</i>	<i>0.02</i>	<i>-0.14</i>	<i>0.13</i>	<i>0.21</i>	<i>-0.15</i>	<i>0.29</i>	0.83	0.73
Fat	<i>0.18</i>	<i>0.03</i>	<i>-0.18</i>	<i>0.12</i>	<i>0.26</i>	<i>-0.15</i>	<i>0.21</i>	<i>0.05</i>	<i>-0.22</i>	<i>-0.08</i>	<i>0.20</i>	<i>0.00</i>	<i>0.14</i>	<i>0.04</i>	<i>-0.10</i>	<i>0.09</i>	<i>0.20</i>	<i>-0.12</i>	0.76	0.24	0.92
NecWt	<i>0.16</i>	<i>0.16</i>	<i>-0.08</i>	<i>0.25</i>	<i>0.14</i>	<i>-0.26</i>	<i>0.07</i>	<i>-0.03</i>	<i>-0.06</i>	<i>-0.11</i>	<i>0.02</i>	<i>-0.02</i>	<i>0.20</i>	<i>0.25</i>	<i>-0.13</i>	<i>0.24</i>	<i>0.17</i>	<i>-0.26</i>	0.70	0.82	<i>0.24</i>

Summary of Trabecular QTLs

Table 4

QTL *	Pos	SEX**	Diet ⁺	TRAITs ⁺⁺	Pos allele [†]	SEX**	Diet ⁺	Obesity ⁺⁺	Pos allele [†]	QTL Names	Strain 1	Strain 2	Ref
<i>QTrab1.1</i>	145,049,833	B	B	Lum, LPC1	LG	M	HF	Lpt, Nwt	LG	<i>Bmd1a, Bmd5 Bvt2, Vnnmt1</i>	C57BL/6J	DBA, C3H, CAST, Balb	(13,15,17–19,56,62)
<i>QTrab2.1</i>	162,915,015	F	LF	Lum	LG					<i>Bmd6</i>	MRL C57BL/6J	CAST, SIL DBA, C3H	(12,13,15,58,63)
<i>QTrab3.1</i>	80,977,590	B	B	Cau, Lum, Tib	LG								
<i>QTrab3.2</i>	132,612,279	B	B	Lum	LG	F	HF	Nwt	LG				(12)
<i>QTrab4.1</i>	16,334,698	F	B	Tib, TPC1	LG	F	HF	Lpt, Fat, Nwt	LG				
<i>QTrab4.2</i>	113,292,708	M	B	Cau, Lum, Tib, CPC1, LPC1, TPC1	LG					<i>Bmd7, Bvt3 Vnnmt4</i>	C57BL/6J	C3H	(15,17,56)
<i>QTrab5.1</i>	3,974,781	M	B	Cau, Lum, CPC1, LPC1	LG								
<i>QTrab5.2</i>	134,365,541	M	B	Tib	SM								
<i>QTrab6.1</i>	145,879,903	M	B	Cau, CPC1	SM								
<i>QTrab7.1</i>	113,373,374	B	B	Cau, Lum, Tib	LG	B	HF	Lpt, Fat	LG	<i>Bmd9, Bvt4</i>	C57BL/6J	C3H, DBA,129	(13,15,17,55)
<i>QTrab8.1</i>	12,260,500	M	B	Cau	SM								
<i>QTrab8.2</i>	61,422,061	M	B	Cau, Tib	SM								
<i>QTrab9.1</i>	44,174,092	M	LF	Lum	LG								
<i>QTrab10.1</i>	15,783,280	M	LF	Lum	LG								
<i>QTrab11.1</i>	5,765,890	M	B	Tib	SM					<i>Vbt11</i>	C57BL/6J	C3H	(17)
<i>QTrab11.2</i>	80,618,774	M	B	Tib	SM					<i>Shmd4</i>	C57BL/6J SAMP6	DBA AKR, SAMP2	(13,53,63)
<i>QTrab13.1</i>	99,855,781	M	B	Tib	SM					<i>Fbiq6</i>	MRL C57BL/6J	SIL C3H, CASA	(18,57)
<i>QTrab14.1</i>	114,053,780	M	LF	Cau, Lum, Tib, TPC1	LG					<i>Vnnmt10</i>	DBA	C3H	(56)
<i>QTrab16.1</i>	87,960,594	B	B	Cau, CPC1	LG								
<i>QTrab19.1</i>	6,443,940	M	B	Lum, LPC1	SM								

* QTL names *Qtrab*X.Y where X is chromosome number and Y is the number of the QTL on the chromosome (Proximal to Distal)

** M = Male; F = Female; B = Both sexes

[†] LF = Low Fat Diet; HF = High Fat Diet; B = Both diets

⁺⁺ Lum = Lumbar vertebra; LPC1 = Lumbar first principal component; Cau = Caudal Vertebra; CPC1 = Caudal first principal component; Tib = Tibia; TPC1 = Tibial first principal component; Lpt = serum Leptin level; Nwt = Carcass weight; Fat = Sum of reproductive, renal, inguinal, and mesenteric fat depots

[†] LG = LG/J allele, SM = SM/J allele has a positive effect on amount of trabecular bone

Table 5

Obesity QTLs, abbreviations as in Table 4

QTL	Chr	Pos	SEX	Diet	TRAITS	Pos Allele *
<i>QOb1.1</i>	1	92,330,219	M	B	Lpt, Fat	LG
<i>QOb1.2</i>	1	118,834,067	M	B	Fat	LG
<i>QOb3.2</i>	3	3,793,662	M	HF	Nec	LG
<i>QOb8.1</i>	8	84,514,250	F	HF	Lpt, Nwt, Fat	SM
<i>QOb9.1</i>	9	118,451,530	B	HF	Lpt, Nwt, Fat	SM
<i>QOb11.1</i>	11	110,266,502	F	B	Lpt	LG(+)SM(-)
<i>QOb12.1</i>	12	13,452,538	M	B	Lpt	SM
<i>QOb13.1</i>	13	72,583,361	M	LF	Lpt	SM
<i>QOb15.1</i>	15	14,450,771	F	HF	Nwt, Fat	SM
<i>QOb15.2</i>	15	42,872,687	B	HF	Lpt, Nwt, Fat	SM
<i>QOb19.1</i>	19	28,235,010	F	B	Lpt	LG(+)SM(-)

* LG(+)SM(-) indicates that the effects switch between a low and high fat diet