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Relationship between obesity phenotypes and genetic determinants in a mouse model for juvenile obesity

Gudrun A. Brockmann,1 Nadine Schäfer,1 Claudia Hesse,1 Sebastian Heise,1 Christina Neuschl,1 Asja Wagener,1 Gary A. Churchill,2 and Renhua Li3

1Breeding Biology and Molecular Genetics, Department for Crop and Animal Sciences, Humboldt-Universität zu Berlin, Berlin, Germany; and 2Computational and Systems Biology Group, The Jackson Laboratory, Bar Harbor, Maine

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Obesity in humans has different faces spanning a range from obviously healthy to severely sick people. In addition, men seem to be more prone to some obesity-related diseases, diabetes for example, than women at the same amount of bodily fat. As such, the physiological and endocrine changes associated with increased fat mass differ greatly between people. The causes for different obese types are different genetic predisposition for obesity or leanness and various environmental aspects such as eating behavior and lifestyle, as well as their interaction. The ratio of fat to lean mass and the interaction between these tissues seem to influence the type of obesity as a risk factor for later onset of severe diseases. High lean mass is considered as protective against obesity and associated diseases. Molecules that are secreted from the adipose tissues and the muscle transfer information for the regulation of body mass or metabolism (47). While adipokines have been known since the discovery of leptin, the first hormone released from the muscle, irisin, has been identified recently (45).

Leptin and adiponectin are the most abundant adipokines primarily secreted by the adipocytes (44). Leptin is a satiety hormone that signals energy insufficiency or excess to the brain (13). Serum leptin concentrations correlate well with the amount of adipose tissue (14). Adiponectin has insulin-sensitizing effects and exerts its action via AMPK in suppressing gluconeogenesis and enhancing lipid oxidation in liver and muscle (21). In obesity, the production and secretion of adipokines are altered. Hyperleptinemia is a sign of low or lost sensitivity of the body to endogenous leptin production (32). In contrast to leptin, adiponectin levels are decreased in obese individuals (1) and contribute to impaired insulin sensitivity in the obese state. In addition, bone mineral density is negatively affected by obesity, which is a concern not only in obese adults, but in particular in obese children and postmenopausal women (16, 17).

The causality between different obesity phenotypes is challenging to infer from populations that differ in their lifestyle and are heterogeneous in their genetic backgrounds. Experimental crosses, where the genomes of the parents are randomly distributed and phenotypes can be recorded under controlled environment, however, are well suited to studies of the causal relationships between diverse genetic loci and phenotypes. The causality from genetic variations to phenotypes is intuitive, while the derived causal relationships between phenotypes depend on comprehensive model comparisons and stringent statistical tests. Structural equation modeling (SEM) is such an approach to model the network (27). This method was used, for example, to describe the relationship between lean and fat mass (7) in two mouse models for high body weight and to discover expressed genes that buffer the development of high cholesterol levels in mice on a high-fat diet (28). Recently, we have generated an intercross population between the Berlin Fat Mouse Inbred strain 860/Hber (BFMI) and the lean control mouse strain C57BL/6CrInN (B6N). The BFMI strain is a mouse model for juvenile obesity that is characterized by high body fat mass but little increase of body lean mass (48). On a standard maintenance diet, the body fat...
content is ~25% at 10 wk (29). The BFMI strain harbors a natural major gene defect resulting in early onset of obesity (33). Like many obese humans, BFMI mice show hyperleptinemia accompanied by hypoadiponectinemia (18). As these mice develop the described phenotype as a result of a major gene defect, they are a unique model for investigating genetic and pathophysiological mechanisms underlying these phenotypes.

We used an intercross population in which 351 mice were genotyped and phenotyped to model the causal relationship between the measured traits lean and fat mass, serum leptin and adipokine concentrations, as well as bone mineral density and quantitative trait loci (QTLs) affecting these traits. In contrast to previous findings in other crosses, we provide evidence for a directed relationship of fat mass to lean mass, which furthermore affects bone mineral density. This has helped generate a new hypothesis for further experimental tests.

**MATERIAL AND METHODS**

**Mouse Population**

We used 351 animals (186 females, 165 males) with full phenotype and genotype information of an F2 intercross of an initial cross between the BFMI860/Hber and the lean mouse strain B6N (Institutes of Health, Charles River Laboratories, Sulzfeld, Germany). The F2 population size was realized by repeated matings (one to five times) of 22 pairs of F1 animals, building 22 subfamilies. Recently, the same cross has been used for mapping QTL for fat mass and lean mass (33). The BFMI strain (Humboldt-Universität zu Berlin, Berlin, Germany) was generated from a genetically highly heterogeneous outbred population, the Berlin Fat Mouse (BFM), which has been repeatedly selected first for low protein content and afterward for high body weight before they were inbred as described in detail previously (48). All selection decisions were performed on mice fed a standard breeding diet.

The animals in this study were treated in accordance with and all experimental protocols were approved by the German Animal Welfare Authorities (approval no. G0152/04, T0149/04, O0145/04). After being weaned at 21 days, F2 mice were fed a standard maintenance diet (Ssniff diet V1534-0; Castrop-Rauxel, Germany) containing 19.0% crude protein, 3.3% crude fat, 4.9% crude fiber, 6.4% crude ash, 54.1% nitrogen-free extract (thereof 36.5% starch and 47.2% sugar), vitamins, trace elements, amino acids, and minerals (12.8 MJ/kg metabolizable energy; thereof 9% energy from fat, 58% from carbohydrates, and 33% from proteins). The fat was derived from soy oil (50–60%) and wheat and barley (40–50%). All mice had ad libitum access to food and water.

**Measured Phenotypes**

**Total body lean and fat mass.** All F2 animals were phenotyped at 10 wk of age. Total fat mass and total lean mass were determined in nonanesthetized mice by quantitative magnetic resonance interference analysis using the EchoMRI whole body magnetic resonance analyzer (Echo Medical Systems, Houston, TX) (42, 46). Each magnetic resonance measurement per animal was repeated two or three times, and the mean was used for further analyses. Total fat mass represented the sum of all fat in the body. Total lean mass included mainly muscle and inner organs. Skeletal muscle mass accounted for the largest portion of total lean mass.
Serum leptin and adiponectin. At 10 wk, after a fasting period of 2 h, mice were anesthetized under isoflurane and killed. During decapitation, blood was collected. Serum aliquots for the measurement of leptin and adiponectin were determined by enzyme-linked immunosorbent assay kits (Mediagnost, Reutlingen, Germany, and R and D Systems, Wiesbaden, Germany, respectively). For leptin, samples were diluted 1/10 vol/vol with dilution buffer. For adiponectin, samples were diluted 1/5,000 vol/vol or 1/8,000 vol/vol with Reagent Diluent. According to the manufacturer, intra-assay coefficients of variation were 4.4 and 5.8%, and interassay coefficients of variation were 4.7 and 6.0% with sensitivities of 0.01 and 0.003 ng/ml for mouse leptin and adiponectin, respectively.

Bone mineral density. The carcasses were stored at −20°C until the measurement of bone mineral density. Carcasses were thawed, and subsequently total bone mineral density was measured by dual-energy X-ray absorptiometry with the Lunar PIXIImus Densitometer (GE Medical Systems, Madison, WI).

SEM

The foundation for SEM in controlled population genetics rests on the randomization mechanism of the meiosis process. The causal relationship of QTL to phenotypes is intuitive, while the inference of causality between phenotypes needs stringent model comparisons. According to a procedure suggested by Li et al. (27), we first identified main-effect QTLs affecting the measureable variables lean mass, fat mass, serum leptin and adiponectin, respectively. The lower curves depict the scans for the same traits but conditioned for a pleiotropic phenotype. For these scans, the model includes the conditional phenotype as an additional covariate. The conditional scans are: FM|LM (A), LM|FM (B), and LM|FSM (C), adiponectin|LM+FM (D), and BMD|LM+leptin (E).

Fig. 3. Genome scans for QTLs affecting FM (A), LM (B), the serum concentrations of the adipokines leptin (C) and adiponectin (D), and BMD (E). The upper curves show the logarithm of the odds (LOD) score curves pertaining to the standard 1 QTL scan with sex and dam as fixed covariates. The lower curves depict the scans for the same traits but conditioned for a pleiotropic phenotype. For these scans, the model includes the conditional phenotype as an additional covariate. The conditional scans are: FM|LM (A), LM|FM (B), adiponectin|LM+FM (D), and BMD|LM+leptin (E).

Fig. 4. Genotype effect plots for males (A) and females (B) of the QTL for FM on Chr 4 at 38 Mb/2 (Q4@38), Chr X at 54 Mb/2 (QX@54) and interaction between them (A, B), and of QTL for total BMD on Chr 14 at 26 Mb/2, Chr 18 at 14 Mb/2 and interaction between them (C). F is the allele of B6N; B is the allele of B6N.
adiponectin concentrations, and bone mineral density and then checked them for pleiotropic, epistatic, and sex-specific effects in the F2 population. Using this information, we defined an initial path model, followed by rounds of model assessment and refinement.

**QTL analysis.** Linkage analyses for fat and lean mass, serum leptin, and adiponectin concentrations, and bone mineral density were performed with R/qtl software (9). Raw data were rank Z transformed (24) to obtain normal distribution for subsequent analyses. We performed a genome-wide search for main effect QTLs and another scan for pair-wise interaction between these QTLs to detect epistatic effects. The models included sex and dam (subfamily) as fixed effects. Significance thresholds for the main effect QTL scan were estimated based on 1,000 permutations (10). A logarithm of the odds (LOD) score threshold of 3.4 was considered as genome-wide significant (P < 0.05). Genome-wide suggestive LOD thresholds were taken from the chromosome-wise P values of 0.63. For the QTL interaction scan, we applied the LOD scores thresholds of LOD > 8 for the full model (including two single QTLs and their interaction effects) and LOD > 4 for the interaction model (contrasting the full model with the additive model) (8). Finally, we fit multiple regression models using all significant and suggestive main-effect QTLs and epistatic interactions. The genetic positions are given in Mb/2.

**Pleiotropic and sex effects.** To assess the altered QTL effect on a phenotype conditional on another related phenotype, we performed a set of scans with lean mass or fat mass as additive covariates. In another set of genome scans, we included sex as an interactive covariate in the model to identify sex-specific QTL effects. The effects were significant if the change of the peak LOD scores between the two models with vs. without covariates was > 2 (ΔLOD| > 2) (25).

**Definition of an initial path model.** The initial model was formed based on those QTLs with significant altered effects in the model comparisons mentioned above, as well as the interacting QTLs (27). QTLs were represented by the chromosomal position of LOD score peaks based on imputed genotypes (40).

**Assessment of the model.** On the basis of the initial model, we need to perform comprehensive model comparisons. The crucial step is to solve the fat mass-lean mass relationship. We considered four QTL models for comparisons: 1) fat mass | lean mass (fat mass conditional on lean mass); 2) lean mass; 3) lean mass | fat mass (lean mass conditional on fat mass), and 4) fat mass. Likelihoods of models 1 and 2 against models 3 and 4 will help infer the causality between the two phenotypes.

To compare the different models, we used model selection statistics including Bayesian information criterion (BIC) (38) carried out with the R/nlme package (35) and SAS using PROC CALIS (SAS, Cary, NC) (36).

The table gives data for the regression model fit for fat mass (FM) and lean mass (LM). The table shows the definition of an initial path model, the assessment of the model, and the models for comparisons: 1) fat mass | lean mass (fat mass conditional on lean mass); 2) lean mass; 3) lean mass | fat mass (lean mass conditional on fat mass), and 4) fat mass. Likelihoods of models 1 and 2 against models 3 and 4 will help infer the causality between the two phenotypes.

**Table 1. Regression models resolving the causality between fat and lean mass**

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Refinement of the model. The model refinement involved adding a new path to and/or removing a path from the model, as suggested by the Lagrange and Wald tests (19). Model assessment and refinement took several iterations before a final model was reached that met the goodness-of-fit standards (27). Thus, there was no issue of multiple-test correction. The final model structure is represented as a directed graph that accurately describes the relationships of the genetic variations and obesity-related phenotypes.

RESULTS

Phenotypic Variation and Correlations in the F2 Population

As a result of random segregation of alternative QTL alleles influencing fat deposition in the cross between the obese BFMI and the lean B6N strains, the variation of fat mass (2.79 ± 2.20 g) is high among the animals of the F2 population. In accordance with the high deviation in fat mass, serum concentrations of leptin, which is mainly secreted from adipocytes, were also highly deviant (1.81 ± 3.09 ng/ml). The standard deviation was lower, but still high for adiponectin, the second adipokine measured in the F2 population (5.30 ± 1.62 ng/ml). Low standard deviations were found for lean mass (22.73 ± 4.00 g) and total bone mineral density (0.053 ± 0.004 g/cm²). Testing differences between the 186 females and 165 males, we found 1.36 (P < 0.0001) and 1.06 (P < 0.0001) times higher lean mass and bone mineral density in males, respectively. Serum adiponectin concentrations were 1.32-fold (P < 0.0001) higher in females than in males (Fig. 1).

The correlation-based heat map indicates three phenotype modules: module 1 represented by fat mass and serum leptin concentrations, module 2 by serum adiponectin concentrations, and module 3 by lean mass and bone mineral density (Fig. 2).

QTL Detection for Measured Phenotypes

A genome-wide QTL scan (Fig. 3) for fat mass and serum leptin concentration, which are the two traits in the fat mass module of the correlation-based heat map, detected a highly significant pleotropic QTL on chromosome (Chr) 3 at 18 Mb/2 (LOD > 29.6 for fat, 95% confidence interval = 17–20 Mb/2). An additional significant QTL for fat mass was detected on Chr 6 at 12 Mb/2 (LOD = 5.4) and a suggestive QTL on Chr 5 at 36 Mb/2 (LOD = 2.5). A pair-wise genome scan for fat mass provided evidence for epistatic interaction between two loci on Chr 4 at 38 and Chr X at 54 Mb/2 (Fig. 4). The main effect QTL scan for serum adiponectin concentration detected a QTL on Chr 4 at 22 Mb/2 (LOD = 3.7). The genome scans for the two phenotypes in the lean mass module detected QTLs on Chr 4 for lean mass at 38 Mb/2 (LOD = 8.1) and for bone mineral density at 58 Mb/2 (LOD = 18.5). This suggested that the Chr 4 QTL affects body size by explaining a relatively large proportion of the phenotypic variation. The QTL peak positions on Chr 4 for the two traits shifted a little, but the 95% confidence interval of the QTL is the same (32–62 Mb/2). The genome-wide scan led to the mapping of additional QTLs for lean mass on Chr 5 at 36 Mb/2 (LOD = 3.5), Chr 6 at 13 Mb/2 (LOD = 5.5), and Chr 9 at 58 Mb/2 (LOD = 3.4), and QTLs for bone mineral density on Chr 9 at 16 Mb/2 (LOD = 5.9) and Chr 14 at 32 Mb/2 (LOD = 4.8). Lean mass and bone mineral density were also affected by epistatic interaction between the QTLs on Chr 14 at 40 Mb/2 and Chr 16 at 48 Mb/2 and between Chr 14 at 26 Mb/2 and Chr 18 at 14 Mb/2, respectively. We did not detect significant interactions for other traits. When we fitted multiple regression models for every trait using the significant single QTLs and QTL interaction effects identified for each trait, only two of the interactions, Chr 4 at 32 Mb/2 and Chr X at 54 Mb/2 for fat mass and Chr 14 at 26 Mb/2 and Chr 18 at 14 Mb/2 for bone mineral density, remained significant (Fig. 4). The direct genetic effects of the BFMI QTL alleles were positive for most traits. Exceptions were the QTL on Chr 9 at 16 Mb/2 where the BFMI allele reduced lean mass and bone mineral density, and the QTL on Chr 4 at 38 Mb/2 that led to the reduction of adiponectin (data not shown). The epistatic interaction effects were also negative for the BFMI alleles.

We tested potential pleiotropic effects of chromosomal regions where QTLs were detected for different traits. The scan for fat mass conditioned for lean mass did not affect the main QTL on Chr 3 at 18 Mb/2 but removed the significant QTL effect on Chr 6 (Fig. 3A). The scan for lean mass conditioned on fat mass did not change the QTLs on Chr 4 and Chr 9 but
removed the QTLs on chromosomes 5 and 6 (Fig. 3B). Effects of fat mass on serum leptin concentrations were evident in the scan for serum leptin concentration conditioned on fat mass where the LOD value for the Chr 3 leptin QTL significantly declined, and QTLs on chromosomes 9 and 14 disappeared (Fig. 3C). Surprisingly, the fat mass did not affect QTLs for adiponectin in the scan conditioned only for fat mass (not shown), but, if the scan was conditioned for fat and lean mass, the main QTL on Chr 4 dropped to the suggestive level ($P = 0.63$) of significance (Fig. 3D). A significant reduction of QTL effects was also evident for the main QTL effect of Chr 4 at 58 Mb/2 on bone mineral density, if the model to detect QTLs was conditioned for lean mass and serum leptin concentration (Fig. 3E). Furthermore, results from the genome scan for lean mass conditional on fat mass indicate that the Chr 4 QTL does not change, but the two QTL on chromosomes 5 and 6 dropped significantly (Fig. 3B). This suggests that the causality from fat mass to lean mass may be in these two QTLs.

Treat the causal path from fat mass to lean mass as a backbone, we developed the model following the same method by adding one more trait at a time to resolve the relationships between fat mass and serum leptin concentration, fat mass and serum adiponectin concentration, serum leptin concentration, and bone mineral density. In the process of model refinement, several new connections were found. We arrived at the final model that precisely characterizes the complex relationships of multiple loci and multiple traits (Fig. 5, Table 2). The model fitting $\chi^2$ is 34.1 with 30 degrees of freedom. The maximum likelihood based goodness-of-fit $P$ value is 0.28 ($P > 0.05$). These statistics indicate that this model fits the data well. In addition, the $t$-test results show that standardized path coefficients given in Fig. 5 are all significant (Table 3).

### Table 2. Best-fitting regression models for structural equation analysis

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The chromosomal positions of QTLs in Mb/2 from the centromere are given in the 1st column. QTLs are designated with Q<<chromosome number>>@<<Mb/2>>. BIC (38): significance thresholds are labeled as $P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. 

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The SEM model indicates causality from fat mass to serum leptin concentration and a major QTL on Chr 3 has a pleiotropic effect on these two traits. In addition, fat mass as well as lean mass have causal effects on adiponectin. There is also a causal relationship from lean mass to bone mineral density. The QTLs on Chr 4 at 38 Mb/2 and Chr 9 at 16 Mb/2, as well as sex, have significant effects on both lean mass and bone mineral density. A QTL on Chr 4 affects the serum adiponectin concentration, although the relationship between the locus at 38 Mb/2 affecting lean mass and bone mineral density and the locus at 22 Mb/2 affecting adiponectin could not be solved. Causality was also found from serum concentrations of both leptin and adiponectin to bone mineral density. There is no common QTL affecting either leptin and bone mineral density or adiponectin and bone mineral density. Bone mineral density is furthermore specifically influenced by genetic determinants on chromosomes 14 and 18 and interaction between them. The final SEM also depicts the interaction between the QTL on Chr 4 at 38 Mb/2 and the QTL on Chr X at 54 Mb/2 affecting fat mass. Sex has a direct effect on all traits, except on fat mass.

The path coefficients (Fig. 5) provided evidence for positive relationships of fat mass to lean mass and serum leptin and adiponectin concentration. There was no causal relationship directly from fat mass to bone mineral density. Bone mineral density was causally affected from all other traits; the effects were positive from lean mass and serum adiponectin concentration and negative from serum leptin concentration. Besides the positive association of fat mass to adiponectin, lean mass was negatively associated with serum adiponectin concentration.

**DISCUSSION**

Mammal body composition comprises two major parts: lean mass and fat mass. Their balance is the foundation of human health, whereas an imbalance between lean mass and fat mass promotes metabolic disturbances (22). Both are affected by the interplay of multiple genetic and environmental factors. Evidence from genome-wide association studies and functional validation has indicated that several genes, such as FTO and MC4R, have a consistent effect on obesity in men and mice (20, 41, 49). But mechanisms underlying the interaction between the muscle and adipose tissues remain unknown.

Previous studies have indicated that lean mass has a causative effect on adiposity in inbred mouse strains that were randomly inbred or selected for high body mass (7, 27). In the BFMI model that has been selected for juvenile obesity, however, the causality is switched from fat mass to lean mass. In addition, linkage studies and statistical modeling, based on the BFMI × B6N intercross, have indicated that QTLs on chromosomes 4, 5, and 6 affect both lean mass and fat mass (33). These lines of evidence indicate that the muscle and adipose tissues interact with one another and the interaction is modulated by genetic variations that are shaped by human and natural selections.

In the present study, we identified QTLs for serum leptin and adiponectin concentrations and for bone mineral density in addition to QTLs that had been identified for fat mass and lean mass before (33). A comprehensive data analysis using SEM suggests causal relationships between the measured body composition traits, adipokines, and bone mineral density and their connection to genetic determinants.

The body fat content in the 10 wk old animals of the F2 population is determined by genetic factors, mainly by the BFMI QTL allele on Chr 3, and to a lesser extent by the QTLs on chromosomes 5 and 6, which are counterbalanced by an epistatic interaction effect of QTLs on chromosomes 4 and X. While fat mass is causally affected neither by any of the other measured traits nor by sex, higher fat mass is associated with higher adipokine secretion into blood and higher lean mass in 10 wk old mice of the BFMI × B6N cross. That sex had a direct effect on all traits except fat mass seems to be surprising but is in line with findings in the intercross between BFMI and B6N mice, where males and females differed not in total fat mass but in the fat distribution patterns (33).

In most obese subjects, hyperleptinemia is associated with hypoadiponectinemia (2,11,15). Nevertheless, the relationship between total fat mass and serum adiponectin levels suggested by the final SEM in this study likely contributes to a delayed decline of serum adiponectin levels in BFMI mice and the ability to maintain a high glucose clearance capability at 10 wk, while at later ages (20 wk) impaired insulin sensitivity is evident (18, 37). Adiponectin has been shown to delay insulin resistance of the muscle associated with obesity (50). The upregulation of adiponectin expression in expanding adipocytes of the BFMI mouse model for juvenile obesity suggests coregulation with leptin as a normal cellular response to suppress potential malfunctions of the adipose tissue and to maintain insulin sensitivity. While high fat mass has the
favorable effect of increasing serum adiponectin level, high lean mass, inherited in males through the BFMI allele of the QTL on Chr 4, has the opposite effect of lowering the serum concentration of adiponectin. Higher muscle weight and unfavorable genetic predisposition are responsible for the resulting low net serum adiponectin concentration that is observed in most humans with manifested obesity and that contributes to impaired insulin signaling.

The model supports the directed relationship of fat mass to lean mass, but not vice versa. The direction of effect is likely caused by ectopic fat deposition in muscle tissue, which is positively correlated with adipose tissue mass (12). While the administration of adiponectin reduces intramuscular triglycerides in mice (3), heavier muscles as result of ectopic intramyocardial fat storage, in turn, likely lead to negative feedback effects on serum adiponectin concentration. Higher intracellular fat content in the muscle is associated with reduced gluconeogenesis and higher beta oxidation of fatty acids leading to insulin resistance, which impairs the function of the muscle for glucose clearance (6, 34). In addition, fatty acid oxidation produces metabolites, like acyl carnitines, that have been proven to negatively contribute to insulin signaling (23). These acyl carnitines are secreted into the circulation where they act as carriers of metabolic information (30). Furthermore, the muscle releases hormones like irisin that signal the metabolic state of the muscle to other organs (45). Such signals may also affect the adiponectin transcription and adiponectin concentration in serum.

While obesity has long been known to be a risk factor for fractures, high muscle mass is protective (16). In our BFMI mouse model, lean mass has the highest effect on bone mineral density, which is consistent with our previous report in other mouse strains (26). A high positive correlation between lean mass and bone mineral density in humans has been reported repeatedly (31, 39). Surprisingly, not fat mass itself but the adipokines leptin and adiponectin are causally associated with bone mineral density. A meta-analysis in humans showed that high serum concentrations of leptin and low concentrations of adiponectin are positively associated with bone mineral density in most studies; however, some examined groups provide evidence for opposite effects (5). Experiments in mice and humans have proposed leptin- as well as insulin-mediated signal transduction affecting bone cell metabolism and differentiation (43). Since the relationship between adipokines and bone mineral density, as well as between lean mass and bone mineral density, might not be direct but mediated by factors that were not measured or are still unknown, further experiments are necessary to test the indicated causality. While in this study, obesity-induced increased leptin and reduced adiponectin production in adipose tissues have been associated with low bone mineral density, and most genetic effects of BFMI are negative, the BFMI alleles of the QTLs on chromosomes 4 and 14 have positive effects on bone mineral density. The QTLs for bone mineral density identified in the cross BFMI × B6N are key genetic determinants that have been mapped in other mouse crosses and are associated with bone mineral density in humans (4). Males have a higher risk for low bone mineral density than females.

Analysis of the covariance among phenotypes in a structured intercross population and the segregation of gene variants contributing to the phenotypic variation provided statistical evidence for directional relationships between different phenotypes and genotypes. In the BFMI model, juvenile obesity affects lean mass and impairs bone mineral density via adipokines secreted from the white adipose tissues. In addition, direct gene and epistatic interaction effects counterbalance the action of endocrine regulators. These findings suggest an important influence of the genetic background on the consequences of obesity for the function of other organs like muscle and bones.

The causal relationships identified in this study by exploiting the covariance between traits can generate causal hypotheses. However, the inferred relationships between genetic and measured physiological parameters that were detected in this study solely by statistical means may lead to spurious effects since important components of the biological system might not have been measured or detected. Therefore, the biological meaning of the derived hypotheses has to be judged by experiments in which the genetic components are physically dissected or animals are physiologically challenged.

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Current addresses: Renhua Li, Windber Research Inst., Windber, PA 15963; N. Schäfer, Inst. for Research in Operative Medicine, Faculty of Health, Dept. of Medicine, Witten/Herdecke Univ., 51109 Köln, Germany.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


Segal NA, Torner JC, Mann Y, Curtis JR, Felson DT, Nevitt MC. Muscle mass is more strongly related to hip bone mineral density than is