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Genetic factors contributing to obesity and body weight can act through mechanisms affecting muscle weight, fat weight, or both

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Genetic factors contributing to obesity and body weight can act through mechanisms affecting muscle weight, fat weight, or both. Obesity is a major health risk factor for humans in modern societies. Therefore, the prevention or at least reduction of obesity is strongly recommended. For targeted prevention or therapies, it is helpful to understand the factors that predispose some individuals toward excess adiposity. The development of predictive genetic tests could lead to individualized approaches to this complex, multifaceted health risk. Twin studies, family-based studies, and population-wide association studies in humans as well as animal models have shown that obesity has a genetic component. In twin studies, the heritability of the body mass index as an often-used measure for obesity in humans was estimated to range between 40% and 70% (1, 16), and environmental factors contribute ~26% of the phenotypic variance (30). However, for polygenic obesity, the most common form of obesity in humans and other species, only very few genes have been identified and verified as obesity genes. These include MC4R (13, 20), FTO (12), and INSIG2 (15), for which genetic variants were repeatedly linked with body weight variation in different human populations. Except for MC4R, all other genes had been identified first in genomewide association studies before they were examined for their biological role in the development of obesity. This shows how difficult it is to predict and identify genes that contribute to body weight regulation, regardless of the tremendous progress in understanding physiological, endocrine, and metabolic changes in fat, muscle, liver, brain, and many other cells, tissues, and organs as a result of malnutrition and in response to diet, behavior, and physical activity.

To better understand the genetic determinants for fat deposition and body weight regulation, many genomewide scans for quantitative trait loci (QTLs) have been performed in experimental crosses, in particular in mice, under defined genetic, feeding, and housing conditions. As a result, the obesity map that collects QTL data for body weight and all related subphenotypes from different mammalian species is almost saturated (34). Although body weight itself is easy to measure, the genetic factors regulating whether an individual remains rather lean or becomes obese are poorly understood. In laboratory animals, lean and fat weight as subphenotypes are easily assessable as weight of muscle and fat depots. The challenge consists in understanding how single genetic loci act directly or indirectly on different measurable traits that all are related to body weight and composition.

Recently, Li et al. (19) proposed the method of structural equation modeling (SEM) to infer and describe the simultaneous effects of QTLs on multiple phenotypes and interactions among phenotypes. SEM is a generalized multiple regression approach that is based on the covariance matrix of observed genetic variation at QTLs and affected phenotypes. The randomization mechanism of meiosis in cross populations provides a basis to infer the causal relationship of genetic variations to phenotypes. Under this framework, we can explore and distinguish the directed interactions between the phenotypes. Application of SEM to genetic systems can help us understand the directed interactions between the phenotypes.
how genetic factors work together in affecting phenotypes, as well as how these related phenotypes interact with one another. Detailed methods were described previously (19).

We used SEM to assess the relationship between muscle and fat depot weights and genetic factors controlling these traits in two different mouse populations, which had been used previously for QTL mapping of body composition traits (3, 4). The F2 intercross populations were derived from the initial crosses NMRI8 × DBA/2 and DU6i × DBA/2. NMRI8 and DU6i originate from different genetic base populations that had been selected for high body weight at the age of 6 and 8 wk under different selection pressures, respectively. DBA/2 mice were used as an unselected counterpart to the extremely growth-selected lines. Males of line NMRI8 differed from the unselected mouse line DBA/2 by 2.5-fold and DU6i males by 3.7-fold increase in body weight at 6 wk. QTLs for body weight as well as for fat pad weights and muscle weights have been mapped across the genome in both intercross populations. Interaction with sex and epistatic interactions between genetic loci have also been identified (3, 4, 8). Some QTLs were colocalized in small chromosomal regions, which had multiple effects on body weight and composition that might indicate pleiotropic effects (21). Both experiments were carried out under the same experimental conditions. Here we use the method of SEM to investigate the genetic architecture affecting body weight components in each of these crosses, in order to understand how genetic factors work together in regulating these complex traits. We demonstrate pleiotropic effects of some QTLs on both fat and muscle weight and provide evidence that significant fat loci in strains selected for body weight contribute to fat weight both directly and indirectly via an influence on lean weight.

METHODS

Mouse Populations

SEM was performed in the two intercross populations, NMRI8 × DBA/2 and DU6i × DBA/2. NMRI8 (Technical University Munich, Munich, Germany) and DU6i (FBN, Dummerstorf, Germany) are long-term high body weight-selected mouse lines, which are partially inbred. Both lines are extremely different in body composition from the control mouse line DBA/2 (DBA/2OlaHsd, Harlan Nederland, Horst, The Netherlands). The mouse strain NMRI8 was selected for high body weight at the age of 8 wk from the outbred strain NMRI. Selection was carried out in a population of eight breeding pairs, following the strategy of weight selection (32). Line DU6i is an inbred derive of line DU6 that was selected for high body weight at the age of 6 wk for 78 generations. Breeding was performed from a base cross of 8 strains, using sib selection with 60 breeding pairs (26).

The two crosses were generated by crossing one single male from the selection line with a single DBA/2 female. F1 males and females were crossed repeatedly to build up pedigrees with large F1 subfamilies. The intercross population NMRI8 × DBA/2 consisted of 275 complete data sets (142 males, 133 females) and the cross DU6i × DBA/2 of 411 data sets (233 males, 178 females). All animals were analyzed at the postpubertal age of 6 wk. Housing, feeding, and protocols for the phenotypic data collection as well as genotyped markers and the genetic maps have been described previously (3, 4). All experimental protocols were approved by the German Animal Welfare Authorities (approval no. VI 522 a/7221.3-TV-003/97).

In the present study we focus on fat weight and lean weight as subphenotypes of body weight and major components to evaluate adiposity. We used the gonadal fat pads, which were the biggest fat depots at 6 wk, as representative for fat weight and the weights of the quadriceps comprising musculus rectus femulus, musculus vastus intermedicus, musculus vastus lateralis, and musculus vastus medialis, which were the biggest muscle packages, as representative of lean weight. Pedigree data, phenotypes, genotypes, and marker maps were used as previously described in detail for the crosses NMRI8 × DBA/2 (3) and DU6i × DBA/2 (4). Because R/qtl software requires homozygosity of marker alleles in the parental lines, heterozygous markers had to be transformed. Under the assumption of homozygosity of QTL alleles contributing significantly to the selection responses after long-term selection for high body weight, the two alleles of the selected line were transferred into a unique allele representing the selection line. This was necessary for 1 of 92 markers in the NMRI8 parent (D18Mit152 on chromosome 18, 37 cm) and 3 of 92 markers in the DU6i parent (D3Mit77 on chromosome 3, 40 cm; D5Mit10 on chromosome 5, 54 cm; D8Mit245 on chromosome 8, 72 cm).

Combined Cross for QTL Analysis

Combining data from two or more crosses can increase the power and resolution for QTL mapping (17). In a single cross, the power of QTL analysis depends on the QTL effect size and the population size; the location of a QTL depends on the distribution of recombination events, as well as the number and distribution of genetic markers used. Studies in mice indicated that the distribution of recombinational “hot spots” varies between inbred strains and between crosses as well (24). Thus QTL analysis on combined crosses can provide a better QTL localization than the analysis on a single cross. The increased power of QTL analysis rests on the increased sample size. One of the strategies in application is the chromosome-specific combining of data from multiple crosses (17). Because we detected QTLs that overlap in regions on chromosome 7, which affected muscle and fat mass in our two crosses, we performed a series of statistical tests to seek evidence for multiple QTLs on the chromosome. The tests included a two-QTL additive model contrasting a single-QTL model, and we considered a three-QTL additive model contrasting a two-QTL additive model. In the combined cross analysis, cross was included as an additional additive covariate.

Development of a Structural Equation Model

The development of SEMs was performed according to the method described by Li et al. (19). The modeling comprised five steps: 1) identification of direct QTLs for fat and muscle weights, 2) identification of pleiotropic, epistatic, and sex-specific QTLs, 3) definition of an initial path model, 4) assessment of the model, and 5) refinement of the model.

Identification of direct QTLs. In subsequent steps, we performed one-dimensional genome scans to detect QTLs with main effects and pairwise genome scans to detect epistatic interactions. Sex and subfamily (F2 animals from the same pair of F1 parents) were included as fixed effects in the QTL analysis. The experiment-specific empirical threshold values of the test statistics were estimated with the permutation test proposed by Churchill and Doerge (9, 10). One thousand replicates were performed. Levels for genomewide highly significant ($P < 0.01$) and significant ($P < 0.05$) linkage were used. All detected main-effect and interacting QTLs were used to fit multiple regression models. We estimated the effect of a QTL or an interaction of the phenotypic F2 variance as reduction of adjusted (type III) sum of squares with and without the QTL or locus pair in the regression model. For the analysis of the summed net effects, we compared the reduction of residual sum of squares of the model with and without all detected QTLs on the chromosome. The tests included a two-QTL additive model contrasting a single-QTL model, and we considered a three-QTL additive model contrasting a two-QTL additive model. In the combined cross analysis, cross was included as an additional additive covariate.

Identification of pleiotropic, epistatic, and sex-specific QTLs. To assess the effect of lean weight on fat weight, we performed a second set of scans with muscle weight as an additive covariate. In a third set of genome scans, we included sex as an interacting covariate in the model to identify sex-specific QTL effects. The effects of muscle...
weight on fat weight QTLs and the sex effects were significant if the change of the peak logarithm of odds (LOD) values between the models was larger than 2 (ΔLOD > 2). On the basis of our previous simulation studies in an intercross population with a sample size of 200 mice, a threshold of 2 in ΔLOD is significant (P < 0.05) with 2 degrees of freedom (17). We also performed pairwise genome scans to detect epistatic interactions.

Definition of an initial path model. A covariance matrix of phenotypes and corresponding QTL genotypes is the foundation for structural modeling. An initial model can be formed based on the results from unconditional and conditional genome scans for QTLs and includes QTLs with significant (P < 0.05) effects on respective lean and fat mass, QTLs with pleiotropic effects on both traits, and interacting QTLs (19). QTL genotypes were represented by the genotypes of LOD score peaks based on the imputation algorithm of Sen and Churchill (27). Decomposition of QTL effects includes additive (a) and dominant (d) components for a single QTL and a*a, a*d, d*a, and d*d components for interacting QTLs (11).

Assessment of the model. On the basis of the initial model, the first step in model assessment is testing all possible relationships between the phenotypes included in the model. In the present study, we have only two phenotypes, fat weight and muscle weight, and three possibilities of their relationship must be tested in each model. The three possibilities are muscle weight affects lean weight, lean weight affects muscle weight, and muscle and fat weight affect each other.

We know that fat weight and muscle weight are affected by many genetic and environmental factors. Any other factors that cannot be explained by the QTLs detected in these two crosses are included in the residuals. If there is a common unmeasured factor affecting both muscle weight and fat weight significantly, we should detect a covariance between these two residuals. To compare these models, we use model selection statistics including Akaike information criterion (AIC) and Bayesian information criterion (BIC) (7, 19). These statistics are robust for model section and model fitting. The modeling was carried out with SAS using PROC CALIS (SAS, Cary, NC; Ref. 25).

Refinement of the model. Based on the initial model and model assessment, model refinement involves adding a new path to and/or removing a path from the model, as suggested by the Lagrange and Wald tests (see Ref. 14). On the basis of our strategy for initial model formation, it can take several iterations of model assessment and refinement before we reach a final model that meets the goodness-of-fit standards (19). Thus there is no issue of multiple-test correction. The final model structure is represented as a directed graph between measured variables.

RESULTS

Body Composition

In the parental strains NMR18 and DU6i, the gonadal fat pad weights in males were 4.2-fold and 7.5-fold increased compared with DBA/2 and the quadriceps muscles were 1.8 and 2.6 times heavier than in DBA/2 males, respectively. Gonadal fat pad weights and muscle weights are positively significantly (P < 0.01) correlated in the intercross populations NMR18 × DBA/2 and DU6i × DBA/2 (Fig. 1). Because single individuals can be lean without having fat depots but cannot be adipose without having supporting lean

Fig. 1. Relationship of fat weight to muscle weight for female and male mice in the crosses NMR18 × DBA/2 (A) and DU6i × DBA/2 (B). Dotted line indicates ratio standardization between fat weight and muscle weight if both grow constantly beginning from zero. Solid line is regression of fat weight on lean body weight.
weight, the regression standardization provides a more accurate adiposity index than the fat weight-to-lean weight ratio as an adiposity index (31). Significant \( P < 0.01 \) correlation coefficients between lean and fat weights are 0.31 and 0.34 for females and 0.38 and 0.31 for males in the crosses NMRI8 × DBA/2 and DU6i × DBA/2, respectively.

**Direct QTLs Identified in Unconditional QTL Scans**

In the cross NMRI8 × DBA/2, two highly significant \( P < 0.05 \) QTLs influencing fat weight were identified on chromosomes 7 and 14 in a genomewide scan based on a single-QTL model. A QTL for muscle weight was also localized to chromosome 14 (Fig. 2A).

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**Fig. 2.** Unconditioned and conditioned genomewide scans for fat and muscle weights in intercross population NMRI8 × DBA/2. Dashed horizontal line represents genomewide significance at \( P < 0.05 \), and dotted line gives the genomewide suggestive significance threshold at \( P < 0.1 \). Arrows indicate quantitative trait locus (QTL) positions that were significant and thus considered for structural equation modeling. A: from top to bottom, genome scan for fat weight, genome scan for fat weight with muscle weight as an additive covariate, difference in logarithm of odds (LOD) scores between genome scans for fat weight with and without muscle weight as additive covariate, genome scan for muscle weight. Sex was an additive covariate in all these scans. B: from top to bottom, genome scan for fat weight with sex as an additive covariate, genome scan for fat weight with sex as an interactive covariate, LOD between genome scans for fat weight with and without sex as an interactive covariate. C: from top to bottom, genome scan for muscle weight with sex as an additive covariate, genome scan for muscle weight with sex as an interactive covariate, LOD between genome scans for muscle weight with and without sex as an interactive covariate. In all tests, inclusion of muscle weight as an additive covariate and sex as an interactive covariate were significant at \( P < 0.05 \) if LOD was \( >2 \).
In the cross DU6i × DBA/2, highly significant ($P < 0.05$) QTLs for fat weight were mapped on chromosomes 7 and 12. QTLs for muscle weight were localized on chromosomes 3, 7, 11, 12, and 13 (Fig. 3A). In addition to our published data (3, 4), we found two muscle weight QTLs in the cross DU6i × DBA/2 on chromosome 3 and on chromosome 7. The QTL on chromosome 3 at 40 cM was detectable only after the unification of the two different DU6i parental alleles to one common DU6i allele at the marker D3Mtt77.

Because the QTLs on proximal chromosome 7 (0–30 cM) for fat weight partially overlap between these two crosses, as do the chromosome 7 QTLs for lean weight (Fig. 4), we consider combining these two crosses. Following the method of Li et al. (17), we recoded NMRI8 and DU6i alleles as a
“high” allele and DBA/2 alleles as a “low” allele. For the QTL on chromosome 7 affecting fat weight, the LOD score peak is at 6 cM in the NMRI8 × DBA/2 intercross and the peak is at 16 cM in the DU6i × DBA/2 intercross, suggesting that there might be two different QTLs in this region (0–30 cM). First, we compare a two-QTL additive model against a single-QTL model in this region, after accounting for the effect of different crosses. These two QTLs correspond to regions 0–10 and 10–30 cM on chromosome 7. The LOD scores differ by 0.65 by contrasting these two models, indicating that we do not have significant evidence to support two QTLs in this region (0–30 cM). A similar situation is observed for lean weight (the LOD scores differ by 0.95). We then consider the entire chromosome 7 and compare a three-QTL additive model against a two-QTL additive model. These three QTLs correspond to regions 0–10, 10–50, and 50–60 cM on chromosome 7. The LOD scores differ by 0.7 for fat weight, indicating again that we do not have significant evidence to support two QTLs on this chromosome after accounting for the genotypes in the region of 50–60 cM. Similar results have been observed for muscle weight. Both the two-QTL and three-QTL models indicate a strong LOD score peak at 16 cM affecting both fat weight and muscle weight (data not shown). Evidence from our model tests also indicates that the distal QTL on chromosome 7 has a significant effect on muscle weight only. To further test the locations of the QTLs on chromosome 7 for muscle weight with the combined data, we rescanned this chromosome, using a single-QTL model conditioning on the genotype at 16 cM, and detect a second peak at 56 cM. Similarly, conditioning on the genotype at 56 cM, we detect the QTL with a peak at 16 cM. Collectively, we conclude that the proximal QTL on chromosome 7 at 16 cM affects both fat weight and lean weight significantly in these two crosses; the distal QTL on this chromosome at 56 cM affects muscle weight significantly in the intercross DU6i × DBA/2. In addition, these data provide another example that combined cross analysis can offer better QTL localization compared with analysis on single crosses.

Pleiotropic, Sex, and Epistatic Effects Identified in Conditional QTL Scans on Lean Weight

Pleiotropic effects. Genomewide scans for fat weight with muscle weight included as an additive covariate identified QTLs that affect fat weight independently of an effect on lean weight. These conditional scans provided evidence for effects on fat weight on chromosome 13 at 6 cM and on chromosome 19 at 34 cM that increased significantly after adjustment for muscle weight in the cross NMRI8 × DBA/2. In addition, the peak LOD score values for the QTLs on chromosomes 7 and 14 were significantly reduced (ΔLOD > 2), suggesting pleiotropic effects of these QTLs on fat and lean weights (Fig. 2A).

In the cross DU6i × DBA/2, a QTL for fat weight on chromosome 1 at 74 cM increased significantly (ΔLOD > 2) and another QTL on chromosome 3 at 38 cM reached significance after muscle weight was included as an additive covariate. In contrast, the peak LOD values for the major fat QTLs on chromosomes 7 and 12 dropped from highly significant to suggestive levels (Fig. 3A).

Sex effects. The QTL for fat weight on chromosome 6 at 32 cM in cross NMRI8 × DBA/2 affected males and females differently (Fig. 2B). This was the only locus for which a significant sex-specific effect on either fat or muscle weight was detected (Fig. 2C). No significant sex-specific QTLs were detected in cross DU6i × DBA/2 (Fig. 3B and C).

Epistatic interaction effects. To identify epistatic interactions, we performed a locus pairs genome scan. In cross NMRI8 × DBA/2, we found a significant interaction between QTLs on chromosome 6 at 32 cM and chromosome 14 at 16 cM affecting fat weight. As expected, individuals with homozygous DBA/2 QTL alleles on each of chromosomes 6 and 14 are lean. The DBA/2 QTL allele of chromosome 6 (homozygous or heterozygous) protects against obesity also if the second locus is homozygous for the NMRI8 allele. The protective DBA/2 allele effect gets lost if individuals are homozygous for the DBA/2 QTL allele on chromosome 6 but het-
erozygous on chromosome 14. In individuals that are heterozygous on the QTL on chromosome 14, fat deposition is highest independently of the genotype for the second QTL on chromosome 6; this is considered a result of an overdominance effect of this locus (Fig. 5B).

In cross DU6i × DBA/2, a significant interaction for muscle weight was detected between loci on chromosome 11 at 48 cM and chromosome 12 at 18 cM. In both loci, the DU6i alleles have increasing effect on muscle weights and heterozygous individuals have intermediate phenotypes. However, double heterozygous mice have higher muscle weights then expected from the additive genetic model (Fig. 6).

Initial Path Model, Assessment of the Model, and Refinement

We formulated an initial structural model for each of the two crosses based on the results of the unconditional and conditional QTL scans. The initial model includes significant QTLs affecting fat weight or lean weight and QTLs affecting both traits pleiotropically, as well as interacting QTLs. For all QTLs, additive and dominant genetic effects were assumed.

On the basis of the initial models, we tested all three possible path models for the relationship between fat weight and muscle weight. The model selection statistics including AIC and BIC indicate that a muscle weight-to-fat weight relationship fits the data best (see Table 1).

After model assessment and refinement, the final models for intercross populations NMRI8 × DBA/2 and DU6i × DBA/2 were established (Fig. 7). These models show how QTLs work together in affecting fat and lean weight. Table 2 summarizes the predictors for lean and fat weight, the mode of inheritance, the effect sizes, and the test statistics for both crosses.

Fig. 5. Effect plots for QTLs identified in cross NMRI8 × DBA/2 for fat weight (A), single loci and epistatic interaction effects influencing fat weight (B), and muscle weight (C). x-Axis, the 3 genotypes. y-axis: effect size. In case of interaction between two loci (Chr6@32 × Chr14@16 in B), x-axis represents the first locus genotypes and key indicates the genotypes of the second locus. N, NMRI8 allele; D, DBA/2 allele.
The Final Models

In cross NMRI8 × DBA/2, the NMRI8 QTL alleles on chromosome 13 at 6 cM and chromosome 19 at 34 cM increase fat weight directly by additive genetic effects. The two highly significant fat QTLs on chromosome 7 at 16 cM and chromosome 14 at 16 cM have pleiotropic effects on both muscle and fat weight. We use the peak at 16 cM to represent proximal QTL on this chromosome as mentioned above. The NMRI8 chromosome 7 QTL allele at 16 cM contributes equally to increased fat weight and lean weight (standardized coefficients of 0.18 and 0.16, respectively).
while the NMR8 chromosome 14 QTL at 16 cM acts in a complex manner. The additive effect reduces muscle weight, while the dominance effect accounts for pleiotropic increasing effects on muscle weight and fat weight. The net effect on fat weight is weak. If the QTL on chromosome 14 at 16 cM interacts with the locus on chromosome 6 at 32 cM, only fat weight is significantly influenced. Here, the interaction of additive alleles leads to a reduction and the interaction between dominance and additive alleles to an increase of fat weight.

In cross DU6i × DBA/2, only the QTL on chromosome 3 at 28 cM directly affects fat weight. The highly significant fat QTLs on chromosome 7 at 16 cM and chromosome 12 at 18 cM have pleiotropic effects on fat and muscle weights. Dominant DU6i alleles cause increased muscle weight and fat weight for the chromosome 7 QTL at 16 cM. The chromosome 12 QTL at 18 cM on muscle weight is additive. Five additional QTLs contribute to higher muscle weight with the DU6i alleles. The QTLs on chromosome 1 at 74 cM, chromosome 3 at 48 cM, and chromosome 7 at 56 cM show additive genetic effects, and the QTLs on chromosome 11 at 48 and chromosome 13 at 60 show dominance effects. The interaction of the muscle QTL on chromosome 11 at 48 cM with the fat and muscle QTL on chromosome 12 at 18 cM contributes an additional increase on muscle weight. The variables contributing to the SEM in the DU6i × DBA/2 cross are summarized in Table 2.

As expected, the sex of an individual has a direct effect on both fat weight and muscle weight. The structural models show that sex can act directly on fat weight and on muscle weight. Sex has a larger direct effect on fat weight and also influences fat weight indirectly via its effect on muscle weight. In both pedigrees, males are heavier and have bigger muscles and larger gonadal fat depots than females.

The SEMs of both crosses clearly show that most QTL alleles derived from the selected mouse lines NMR8 and DU6i

![Table 1. Model selection statistics for model comparisons](image)

<table>
<thead>
<tr>
<th>Cross</th>
<th>AIC</th>
<th>BIC</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DU6i × DBA/2</td>
<td>M1 -9.3</td>
<td>M2 0.3</td>
<td>M3 -8.5</td>
<td>M4 -0.7</td>
<td>M1 -49.3</td>
<td>M2 -39.6</td>
</tr>
<tr>
<td>NMR1 × DBA/2</td>
<td>M1 -18.6</td>
<td>M2 -17.8</td>
<td>M3 -16.9</td>
<td>M4 -17.8</td>
<td>M1 -72.8</td>
<td>M2 -72.1</td>
</tr>
</tbody>
</table>

Based on the initial model, we tested the following relationships: muscle weight to fat weight (M1); fat weight to muscle weight (M2); bidirectional muscle weight to fat weight and fat weight to muscle weight (M3); no direct connection between muscle weight and fat weight, but a covariance between the residuals of muscle weight and fat weight (M4). AIC, Akaike information criterion; BIC, Bayesian information criterion.
have the effect of increasing both fat and muscle weight. In intercross population NMRI8 × DBA/2, five of six fat QTLs and one of three muscle QTL effects are positive. QTL effects account for 35.2% and 19.9% of the phenotypic F2 variance for fat weight and muscle weight, respectively. In the other cross, DU6i × DBA/2, one of four fat QTLs and all eight muscle QTLs have positive DU6i allele effects. QTL effects explain 14.8% and 7.8% of the phenotypic F2 variance for fat weight and muscle weight, respectively. The positive allele effects are the result of selection and explain the extreme high phenotype of the selected parental lines compared with the phenotype distribution within the intercross populations (Fig. 8). In the NMRI8 × DBA/2 cross, none of the F2 animals was more extreme than the average NMRI8 animal in respect to fat and muscle weight and only a single F2 individual had higher fat weight than the average DU6i animal. On the other hand, many F2 mice were leaner and had less muscle weight than the DBA/2 parental line.

**DISCUSSION**

SEM as applied to complex trait analyses provides a richer description of the relationships between phenotypic and genetic parameters than standard linear regression models. In the present study, SEMs were built for two intercross populations generated from initial crosses between a high body weight-
selected line, either NMRI8 or DU6i, and DBA/2, a standard-body weight mouse line. The large phenotypic differences between selected and unselected mice allowed for successful QTL mapping and SEM in pedigrees of 275 and 411 animals.

All QTL positions and direct QTL effects for fat and muscle weights reported in our earlier studies (3, 4) were reidentified in the present analysis. In addition, in this study we detected the interacting QTLs on chromosomes 6 (32 cM) and 14 (16 cM), which have a significant effect on fat weight in cross NMRI8 × DBA/2. Regarding cross DU6i × DBA/2, we identified in this analysis additional QTLs on chromosomes 3 and 7 for muscle weight. The resolution of two QTLs on chromosome 7 was possible only after combining the data of the two crosses.

The developed structural models give us further insight into the relationship between muscle and fat weight, sex, and genetic factors. The direction of effects between phenotypes indicates that muscle weight serves as an important mediator for the regulation of fat deposition in both crosses. This is consistent with previous reports (18, 19). How can we interpret this finding? Fat is deposited, generally or only in gonadal tissue, only if a minimum of muscles have developed. This is in line with the fact that an individual can be lean without having fat depots but not vice versa. On the other side, this is also consistent with the observation that individuals of many species more often become obese at later ages, when muscle growth slows down (28).

In this study, we have considered only models based on measured variables. We do not wish to imply that the weight of the quadriceps muscle has a direct causal effect on the size of the gonadal fat pad, even though this is what we have modeled. Instead we propose that the measured variables are acting as surrogates for other quantities that we might refer to as “size” and “fatness.” These quantities are difficult to define precisely and hence not possible to measure directly. It is possible to develop models that incorporate latent variables that are not directly measured. In a latent variable model, the causal effect would most likely arise between the latent factors “size” and “fatness.” However, because of statistical considerations of identifiability, a latent variable requires at least three measured variables to support the latent construct in the model. Thus we are limited to models that include only measured variables.

The structural models support the overall finding that fat weight and lean weight are differentially influenced in males and females. The male organism exerts larger enhancing effects on both fat and muscle weights. But beside these direct tissue-specific actions, the muscle again can act as a mediator of sex-specific effects to increase fat weight. Sex hormones have biologically diverse functions, targeting both reproductive tissue and nonreproductive tissues such as skeletal muscle. The effect on decreased muscle protein breakdown and increased muscle protein synthesis is used, for example, in androgen therapy to enhance skeletal muscle weight (28). One molecular mechanism for sex hormone-specific effects has recently been found in the triggering of estrogen-induced gene expression through estrogen receptor-targeted demethylation of histones and subsequent DNA conformational changes that are essential for estrogen-induced transcription (23).

Furthermore, the modeling of the relationship between phenotypic data together with QTL mapping data provides insights into a genetic system and allows us to distinguish between loci having effects on fat or muscle weight and pleiotropic loci affecting both fat and muscle weight. Our models provided evidence that loci showing highly significant effects on fat weight in a genomewide QTL screen without considering lean weight may contribute only indirectly to fat deposition. Indeed, each of the two highly significant fat QTLs in our populations (chromosomes 7 and 14 in NMRI8 × DBA/2; chromosomes 7 and 12 in DU6i × DBA/2) show pleiotropic effects on both fat and muscle weight, suggesting that the selection for high body weight could act on a modular structure that either constrains or facilitates multivariate evolution (22, 33). Thus the high-fat QTL peaks may be misleading in the further search for “obesity” genes. The gene or genes underlying the QTL effect could be regulators of energy distribution and storage rather than fat deposition-controlling genes. This finding sheds new light on the evaluation of QTL effects and our understanding of weight regulation as a complex trait. It clearly shows that the inclusion of correlated phenotypes in conditional QTL scans helps to pinpoint the biological function of genes responsible for an observed QTL effect. This is particularly important to consider in human genomewide linkage and association studies, in which usually body weight as a whole and the body mass index are the only measured phenotypes for population studies (e.g., Refs. 12, 15, 20). If magnetic resonance methods or computer tomography is used in human studies (29) to better characterize obesity by dissecting body weight into body composition subphenotypes, we strongly suggest also recording lean weight data and performing statistical analyses with and without inclusion of lean weight as a covariate to distinguish hidden QTLs with overall body size effects from those that primarily influence fat deposition.

We found that the number of overlapping QTLs between the two populations is small. Because the male parent came from the different high body weight-selected mouse lines and the DBA/2 parent was genetically identical, differences between the two SEMs must have their origin in selection line-specific QTL alleles. The overlap between the two models consists only in one QTL, which resides in the centromeric region of chromosome 7 and has been mapped to 12 cM in the NMRI8 × DBA/2 cross and to 16 cM in the other cross as most likely positions. These effects could result from one and the same gene, because the locus has pleiotropic effects on fat and muscle weights in both populations. But the mode of inheritance is additive in one cross and dominant in the other cross, indicating that different alleles might occur in the different selection lines. However, the possibility that these are two different genes cannot be excluded.

Because the overlap between the two models is surprisingly small, we may conclude that the genetic determinants for very similar selected phenotypic traits are very different. In cross NMRI8 × DBA/2 five QTLs and in cross DU6i × DBA/2 eight QTLs contributed significantly to fat and lean weight. Because the threshold for selection of QTLs was genomewide significance at \( P < 0.05 \), it can be ruled out that nonoverlapping QTLs of the model are false positives. Among the genetic loci, two and one loci in the different crosses showed exclusively effects on fat deposition. Two additional loci in every cross had pleiotropic effects on fat and muscle weight, with the
one locus discussed above likely acting in both crosses. Five loci contributed exclusively to muscle weight in cross DU6i/H11003 DBA/2. Additional analyses such as transcriptional profiling may be necessary to identify whether the same or different functional modules (33) are affected via the different genetic factors.

The differences in the SEMs mirror the selection process in the two selection lines. The two selection lines differ largely in the genetic diversity of the base populations of the selection experiments, which is the pool of genomic variants that potentially can respond to the selection procedure. During selection over many generations, these mouse lines have reached the selection limits, a state where no further increase in selection response can be seen from one generation to the next. At this stage we expect optimal allele combinations to realize the extreme performance of the reached selection response. For additive and dominance allele effects we expect homozygosity. However, overdominant QTL alleles and alleles under the influence of allelic interaction could favor heterozygosity even in a long-term selection line. Whether alleles are fixed or not depends not only on allele effects but also on the selection condition itself. The higher number of QTL alleles contributing to the high body weight phenotype in DU6i animals together with smaller effect size of DU6i QTL alleles compared with NMRI8 is the result of a long-term selection strategy in a large population with relatively low selection pressure (2, 6). The population size of only 8 breeding pairs in the NMRI8 line compared with 60 in the DU6i line combined with high selection pressure leads to fast fixation of favored high-effect alleles and is critical for the random loss of positive alleles as a consequence of genetic drift.

The occurrence of negative QTL alleles in the selection lines compared with DBA/2 indicates the complex pattern of inheritance and gene effects contributing to the selection response. Interestingly, among significant QTLs, negative QTL allele effects were only found for QTLs that exerted positive effects on either fat or muscle weight. Thus the negative effect might result from different polymorphisms in one gene or close linkage of genes having independent effects. Random fixation of genomic regions carrying negative gene effects can result from genetic drift or linkage to positive QTL alleles, in particular if the negative effect is hidden by recessive or interacting alleles. The fixation of unfavorable linked genes may happen more frequently if the selection pressure is high and fixation of alleles is realized within only a few generations. This prevents recombination and leads to long selection signatures in the genome.

Conclusions

SEM is a suitable method to infer the relationship between fat depot and muscle weights. From our models, we conclude that muscle mass is a mediator for fat deposition. Muscle mass is also important for sex effects on fat deposition. Sex can act either directly on fat mass or indirectly via the muscle as a mediator. The QTLs exert a complex pattern of actions including pleiotropic effects on both fat and muscle mass. Genes underlying fat QTLs could have their molecular function in biological processes that affect muscle mass. Furthermore, genetic components affecting similar traits can be very different between different selected populations. The conclusions drawn from our findings are based on studies in experimental animal populations and the statistical models applied. Although gonadal fat pads and quadriceps muscles are the biggest lean and fat depots in mice at 6 wk, it must be considered that these weights are inaccurate in respect to whole body lean and fat mass. Further experiments are necessary to generalize these results and conclusions.
Innovative Methodology

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