Murine weight loss exhibits significant genetic variation during dietary restriction

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Murine weight loss exhibits significant genetic variation during dietary restriction (DR) for females eating 60% ad libitum (AL). We examined 5 cohorts across 81 different strains (22 strains tested twice) that included the LXS and LSXSS recombinant inbred strains, the LXS parental strains ILS and ISS, and the classical inbreds 129S6, A, BALB/c, C57BL/6, C3H, and DBA. Weight loss exhibited highly significant genetic variation, with DR body weights ranging from ~60 to ~85% of AL body weight. This variation was not explained by the strain differences in absolute food intake, feces calorie content, motor activity, or AL body fat. Heritability was 40–50%, and several provisional quantitative trait loci were mapped. This variation can be used to test whether weight loss correlates with the health benefits of DR, independently of the reduction in calories. The genetic variation also implies the existence of genes that would be novel therapeutic targets, distinct from genes affecting AL body weight or body fat, for enhancing (or mitigating) weight loss during food restriction.

Body weight; caloric restriction; dieting; energy restriction; food restriction; genetics; quantitative trait loci; weight control; weight modulation

WEIGHT LOSS is one of the major physiological hallmarks of dietary restriction (DR; also referred to as food restriction, caloric restriction, or energy restriction), affecting a large number of organs and potentially affecting many physiological processes. Not surprisingly, therefore, it has frequently been suggested that weight loss could be linked to one or more of DR’s health benefits (26). However, the relationship between weight loss and the extension of life span by DR appears to be a complex one (7, 12). On the one hand, weight loss would seem to imply a reduced risk for metabolic syndrome, obesity, diabetes, cardiovascular disease, and cancer (23), and there is suggestive evidence that weight loss partially mediates the life-extending effects of DR in rodents (6, 24). On the other hand, weight loss also reduces energy reserves, which could potentially impair functional capacity (strength, endurance, ability to maintain homeostasis) and resistance to aging-related illnesses, and there is also evidence to suggest that, under DR, the rodents with higher body weight or body fat tend to live the longest (4, 7, 25). To critically test the relationship between DR-induced weight loss and other responses to DR, DR health benefits, and DR-induced life extension, we are studying the genetic basis of DR across a large number of murine strains. In this study we asked, under carefully controlled conditions of food intake, whether weight loss exhibits significant genetic variation, and, if so, what genetic loci are most strongly associated with this variation.

MATERIALS AND METHODS

Strains and Husbandry

ILS, ISS, 129S6, A, BALB/c, C57BL/6, C3H, and DBA were studied along with Cohort 1 of the LSXSS recombinant inbreds (RIs) (20). A, BALB/c, C57BL/6, C3H, and DBA were among the eight progenitor strains leading to the LSXSS and LXS panels (16, 29) and were maintained at the Institute for Behavioral Genetics (IBG) for >40 generations; therefore, these should be considered IBG substrains. 129S6 was imported from Taconic Farms (Germantown, NY) and maintained at IBG for ~2 yr before its use in this study. The two LSXSS cohorts were inbred >40 generations (20, 21). In all cohorts, the mice were singly caged, female, closely age-matched, and started on DR at 50–60 days of age (Supplemental Tables S1 and S2; the online version of this article contains supplemental data) (20, 21).

Detailed information on the three LXS cohorts, including the number of mice (typically 4 per strain and diet), birth dates, ages at which DR was started, strain means for food intake, and number of premature deaths is provided in Supplemental Tables S1 and S2. These cohorts were studied under the same husbandry conditions used previously for LSXSS (20, 21). The strains in LXS Cohorts 1 and 2 were inbred 14–16 generations; the strains in Cohort 3 were inbred 20–23 generations.

The DR rations [60% ad libitum (AL)] were calculated separately for each strain and cohort and recalculated each week based on the prior week’s AL food intake (20, 21). LXS Cohort 3 was initially fed 80% AL for 2 wk before being switched to 60% AL. With the exception of the first 14–15 wk of DR in LSXSS Cohort 1, the DR mice were fed on a Monday-Wednesday-Friday feeding schedule as others have used (18). All protocols were approved by the University of Colorado’s Institutional Animal Care and Use Committee.

Body Weight Measurements

Body weights (BW)s were measured once each week in the afternoon, 0–4 h before feeding the DRs (except 5 strains in LXS Cohort 1 that were weighed on Tuesdays during the Monday-Wednesday-Friday feeding schedule, which caused their BW measures to increase slightly, as is evident in RESULTS, composite plots of LXS Cohort 1). In LXS Cohort 2, we conducted a 3-day trial in which AL BWs were measured every 4 h to test for significant circadian variation that might affect our quantitation of weight loss. We found that our measures of BW in the afternoon matched closely with the 24-h means for BW (within 1–4%). There was also no appreciable strain variation in the circadian pattern of AL BW (Supplemental Fig. S1).
For regressing the DR BWs on AL, we included ILS and ISS with the LSXSS RIs because these strains were studied at the same time as LSXSS Cohort 1. We included both LSXSS cohorts in the regression and simultaneously adjusted for a potential cohort effect using a general linear model univariate analysis. General linear model univariate analysis was also used to regress the DR BWs of the LXS on the AL BWs while simultaneously adjusting for cohort effects.

**Time Period Divisions**

To quantify and compare the strain variation across all five cohorts, we separated the data into three periods with respect to how long the mice were on DR. The strain means for BW for each time period were calculated by first averaging the weekly BW measures from each mouse and then averaging the means from the same strain and diet.

The start of the first time period was defined as when the DR mice began their long-term, stable-BW phase, estimated separately for each cohort by visual inspection. The end of this period was based on when we had stopped our study of LSXSS Cohort 1 (16 wk after DR). Because this time period in Cohort 1 covered 10 wk, we set the end point for the other cohorts to also cover 9–10 wk. When discussing multiple cohorts, we will refer to this period nominally as weeks 7–16 of DR. The second period will be referred to nominally as weeks 17–30 when we discuss multiple cohorts; it began where the first period left off and stopped at when we discuss multiple cohorts; it began where the first period left off and stopped.

**Other Measurements**

See Supplemental Materials and Methods for the other measurements and procedures conducted on these mice. These included measures of the ratio of feces to food (feces:food), home-cage activity, runwheel activity, body temperature, tail growth, and hair growth.

**Statistical Methods**

**Treatment of missing BWs.** Some weekly BW measurements were inadvertently missed (<2% of total measurements). In addition, the BWs in LXS Cohort 2 were only measured every other week from week 27 to week 43 of DR. Therefore, to optimize the basis for comparison among all strains and cohorts, we imputed estimated values for these missing measurements as follows (Supplemental Fig. S2, flowchart A). When no data from other mice of that strain and diet were available, we simply imputed the mean of that mouse’s two flanking BW measures. When data from other mice were available, we calculated the average change in BW for the other mice and added that to the previously recorded BW. We also calculated the average change in BW for the other mice from the time of the missing BW to the time of the next recorded BW; this change was subtracted from the next recorded BW. The mean of the two estimates was then imputed for the missing BW.

**Treatment of mortalities.** Deaths were typically preceded by a short period of marked weight loss (2 wk, SD = 2); in one case, there was a marked increase in BW (LXS68, which had multiple, small tumors and was overeating). Although similar changes occurred at times among mice that did not die, a sudden change in BW before death was used as a post hoc indication of poor health. The BWs associated with this time period were thus censored and treated as missing values to avoid the appearance of sudden, large shifts in the strain mean for BW (owing to the small sample sizes within strain and diet). These missing means were imputed by adding the mean change in DR BW for the other mice of that strain and diet to the dead mouse’s mean BW from the previous period.

**Treatment of baseline differences in BW between AL and DR mice.** Because the DR measures of weight loss are relative to the AL means for BW, a large baseline difference in mean BW between the AL and DR mice would confound the results. The AL and DR mice of each strain were thus matched for BW 1–2 wk before starting DR, so that the AL and DR means typically differed by <0.3 g, a negligible difference. For baseline differences ≥0.5 g (a conservatively low cutoff), we used a mathematical adjustment as follows (Supplemental Fig. S2, flowchart C). The mean BWs of the individual mice for the period of interest (e.g., weeks 7–16) were regressed on their baseline BWs. The AL- and DR-assigned mice were regressed separately to give regression equations that were used to predict 1) a DR strain mean for the AL mice for that period and 2) an AL strain mean for the DR mice for that period. Dividing the predicted DR mean for the ALs by the observed AL mean gave an estimate of percent AL BW during DR. A second, independent estimate was obtained by dividing the observed DR mean by the predicted AL mean for the DRs. The two estimates were then averaged (mean of the numerators divided by mean of the denominators). The two estimates were generally in close agreement with each other (within 5 percentage points); however, five of the predicted DR means were not biologically plausible (such as a negative BW or a BW of just 3 g), and the associated percent AL estimate was not averaged with the other estimate.

**Censored mice.** We censored two DR and four AL mice from LXS Cohort 3 (<2% of LXS total) because their BW means were >2 SD different (~3 g) from the next closest BW for that strain and diet, as assessed from the distribution of such differences across all strains. These mice also represented some of the early deaths and repeated exclusions of AL food intake for calculating the DR rations. [These exclusions were per the criterion described by Rikke et al. (20) of excluding food measures that were >4 g from the next closest mean BW for that strain. In this study, we discovered that such chronically high measures of food intake were often due to an uncorrected amount of food wastage, although actual food consumption was often high as well. To minimize such exclusions, we switched to a mouse-specific, rather than strain-specific, food-wasted measure for these mice.]

**Statistical software and settings.** All statistical analyses were conducted using SPSS for Windows 13.0 (analyses of variance, general linear model univariate analyses, linear regressions, curve estimations, Pearson correlations, and scatter plots) or Microsoft Excel (linear regressions and scatter plots for adjusting baseline BW differences between AL and DR).

The genetic mapping was conducted using Map Manager QTXb20, released April 2004, which also estimated the quantitative trait locus (QTL) effect sizes based on regression and maximum likelihood methods (15). To optimize the mapping power in the LXS, we removed redundant markers with ≤2 recombinations between them and used the regression mapping function with a final set of 266 randomly distributed microsatellite markers, which equates to an average marker spacing of just 5 cM. Details of the marker genotypes, density, distribution, fixation, and segregation have been previously described (29). We used the interval mapping function for the LSXSS because of the much lower density of markers (89 markers). The genotyping of this panel has also been previously described (16). Map Manager’s permutation function was used to assess statistical significance, with 10,000 permutations used for single markers and 5,000 permutations used for marker interactions. The map positions (cM) of the markers were assigned according to the Mouse Genome Informatics database (http://www.informatics.jax.org/).
RESULTS

Composite Characterization

Before analyzing the strain variation, we examined the composite DR weight loss response over time by plotting the AL and DR grand means for BW of each cohort (strains equally weighted; Fig. 1, A and B, and Fig. 2, A and B). As illustrated by LXS Cohort 3 and LSXSS Cohort 2a (Fig. 1, A and B), which were followed the longest (1 yr), BW after initiating DR was typified by 1–4 wk of acute weight loss, followed by 1–4 wk of no change, followed by 1–4 wk of weight gain. The BWs then appeared to be relatively stable after 6–8 wk of DR. The analysis of DR strain variation was thus limited to this stable BW period.

There were some minor perturbations in BW after weeks 6–8 of DR that were associated with other testing activities such as temperature measurement trials, hair plucking, and runwheel measurements (Figs. 1 and 2). Perturbations in DR BW also tended to correlate with the fluctuations in absolute food intake. For example, in LXS Cohort 3 (Fig. 1A), there was a suggestive correlation between the weekly BW measures and the weekly amount of food eaten starting at week 7 of DR ($R = 0.20, P = 0.1$, 1-tailed) that reached statistical significance after week 14 ($R = 0.35, P = 0.024$). Likewise, there was a highly significant correlation between BW and food intake during the stable BW phase starting at week 7 in LSXSS Cohort 2a (the other cohort studied 1 yr on DR; Fig. 1B, $P = 0.001$, 1-tailed, $R = 0.48$).

The composite characterization indicated that, during weeks 7–16 of DR, the LXS strains maintained their BWs at 77% of AL (SE = 1%), whereas the LSXSS mean was 72% of AL (SE = 1%), a small but highly significant difference ($P = 0.0002$, 2-tailed $t$-test). There was a tendency for these percent AL values to decrease slightly over time due to an increase in the AL BWs (Figs. 1 and 2).

Strain Variation

To analyze the strain variation in the weight loss response, we first examined the DR BWs of each strain over time as a percentage of their respective AL strain means. The strains representing the extreme differences are shown for several cohorts in Fig. 1, C and D, and Fig. 2, C and D. The lowest extremes typically had BWs that were just 60–65% of AL.
whereas the highest had an impressive ability to maintain 80–85% of AL BW. This range of ~20 percentage points was observed in each cohort. It was also evident that this variation 1) began almost immediately, 2) was maintained for the entire time the mice were on DR, and 3) was largely impervious to the perturbations in the composite BW means noted above.

To further explore the robustness of the strain variation, we examined in LSXSS Cohort I the effect of changing the meal frequency from seven times to three times per week (double rations on Mondays and Wednesdays and triple rations on Fridays). We found that the extremes still maintained their differences in weight loss after the shift in meal frequency and did so regardless of what day they were being weighed (Fig. 2, A and B).

**Regression vs. normalization.** The DR strain means for BW were significantly correlated with their AL means (Supplemental Fig. S3). For all three LXS cohorts combined, these correlations were 0.69, 0.64, and 0.68 for weeks 7–16, 17–30, and 31–50, respectively (P values < 10^-6). In the LSXSS panel, the correlations were higher, 0.92 and 0.87 for weeks 7–16 and 17–30, respectively. Therefore, to analyze only the effect of DR on BW, we normalized the DR strain means by their respective AL means and expressed these as percent AL. These values, as noted above, indicated strain variation ranging from lows of 60–65% to highs of 80–85% of AL BW (Fig. 3).

The strain variation relative to the variation within strain was highly significant for each cohort and each time period [all P values < 0.005, ANOVA; each DR mouse normalized with respect to the AL strain mean and transformed using square root and arcsine functions to avoid artifacts due to combining ratios (1)].

However, as was also evident in Fig. 3, E and F, normalizing the DR BWs in the LSXSS panel did not remove the correlation with the AL BWs. To completely remove the AL correlation in all cohorts, we regressed the DR strain means on the AL strain means for both the LSXSS and the LXS. Nevertheless, to maintain the utility of expressing weight loss on a scale that would be physiologically meaningful, we transformed the residuals into percent AL values by adding the DR grand mean to the DR strain means for both the LSXSS and the LXS. Neverthe-

**Correlations with potential confounds.** The strain variation in the weight loss response (adjusted %AL) was not explained by covariation with the absolute differences in food intake in either LXS or LSXSS (P values ≥ 0.18; Supplemental Table S3), which also means no correlation with the absolute differences in protein, fat, carbohydrate, vitamin, or mineral content. There was also no correlation with DR feces:food (regressed on AL), suggesting that strains maintaining higher BWs were not more coprophagic or more efficient at extracting nutrients (P values ≥ 0.35; Supplemental Table S3). DR also had no effect on the calorie content of the feces (20), and the strain variation in feces calorie content did not covary with weight loss (P values > 0.05; Supplemental Table S3).

The weight loss response also was not explained by covariation with measures of gross motor activity such as home-cage

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<table>
<thead>
<tr>
<th>A</th>
<th>LXS Cohort 1-Weighed Mondays (6 strains)</th>
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<tbody>
<tr>
<td>B</td>
<td>LXS Cohort 1-Weighed Tuesdays (5 strains)</td>
</tr>
<tr>
<td>C</td>
<td>LXS Cohort 1-Weighed Mondays (extremes)</td>
</tr>
<tr>
<td>D</td>
<td>LXS Cohort 1-Weighed Tuesdays (extremes)</td>
</tr>
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</table>

Fig. 2. Plots of AL BW, DR BW, and the strain variation in weight loss during DR for LXS Cohort I to show the effect of changing the DR feeding schedule. A and B: plots of the AL and DR composite means for BW (strains equally weighted) for the 6 strains weighed on Mondays (A) and the 5 strains weighed on Tuesdays (B). Top line represents the composite for AL BW; bottom line is the composite for DR BW. C and D: DR strain means for BW expressed as %AL for the strains at the high and low extremes of the strain distribution, corresponding to A and B, respectively. In C, top line with solid squares is strain LXS25, top line without squares is LXS30, and bottom line with open circles is LXS36. In D, the line with solid squares is LXS51, the line with open circles is LXS122. The sudden drop in %AL BW in C (and to a lesser extent in D) during weeks 16–17 and 21–22 coincides with BWs measured during, and 5 days after, our 1-wk temperature measurement trials (tt). M, Monday; W, Wednesday; F, Friday.
activity (P values > 0.5; Supplemental Table S3) or runwheel activity in LSXSS (R = 0.31, P = 0.25, n = 16; Dr weeks 7–16). For LXS, there also was no correlation with home-cage activity for weeks 7–16 (P = 0.16; Supplemental Table S3), but there was possibly a small, negative correlation for weeks 17–30 (R = 0.32, P = 0.02).

In the LSXSS strains, there was a small correlation (R = 0.39; Supplemental Table S3) with our previous measures of AL body fat (regressed on eviscerated BW; Ref. 20), which was not measured in LXS. This correlation reached a 0.04 level of significance for weeks 7–16 because of a strong correlation in Cohort 1 (R = 0.49, P = 0.01) but was not significant in replicate Cohort 2 (R = 0.21, P = 0.18) or for weeks 17–30 (Supplemental Table S3). Even if the correlation were valid for weeks 7–16, AL fat only explained 15% of the variance, and the strain variation in weight loss remained very highly significant after regressing on AL fat (P < 10⁻¹³) with no discernable effect on the range.

Heritability

Heritability is the relative contribution of genetic factors to phenotypic variation. For the LXS panel, the calculated heritability (h², narrow sense) of the weight loss response (adjusted %AL) during weeks 7–16 was 42% and highly significant [95% confidence interval (CI) of 29–53%, calculated as described in Ref. 21]. The heritabilities during weeks 17–30 and 31–50 were somewhat higher, at 53 and 54%, respectively (Supplemental Table S4; 95% CIs of 41–64%). By comparison, the heritability estimates for AL BW were 54, 59, and 48% for weeks 7–16, 17–30, and 31–50, respectively; therefore, weight loss, defined as being independent of AL BW, was still a trait that was nearly as heritable as BW itself. There was
a high degree of phenotypic (individual mice) and genetic (strain means) correlation across time periods ($R$ values of 0.7–0.9; Supplemental Table S4), suggesting that the same genetic loci were largely responsible for the heritability in all three time periods.

The heritability of weight loss in the LSXSS panel was quite similar, with values of 41, 54, and 58% for weeks 7–16, 17–30, and 31–50, respectively (Supplemental Table S4). The heritabilities for weeks 17–30 and 31–50 were thus again ~10 percentage points higher compared with weeks 7–16 and were again nearly as high as those for AL BW (68, 66, and 58%, respectively). The strain means for weight loss across time periods were again highly correlated ($R$ values of 0.8–0.9; Supplemental Table S4).

**QTL Mapping in the LXS**

When we mapped on the weight loss response (adjusted %AL) in the LXS panel for weeks 7–16 of DR (Fig. 4), two loci exceeded the threshold for being provisional QTLs, i.e., had genome-wide $P$ values < 0.05 by permutation testing (13, 15). These loci on chromosomes 6 [peak logarithm of the odds (LOD) at ~19 cM, D6Mit223] and 16 (peak LOD at ~22 cM, D16Mit103) did not reach the provisional threshold for weeks 17–30 (Fig. 4) but did have single-marker $P$ values of just 0.05 and 0.07, respectively. There were two loci, on chromosomes 4 (peak LOD at ~14 cM, D4Mit286) and 7 (peak LOD at ~18 cM, D7Mit270), that exceeded the provisional threshold for weeks 17–30. These loci had single-marker $P$ values for weeks 7–16 of just 0.008 and 0.02, respectively. When we averaged the strain means for both time periods (equally weighted), these loci on chromosomes 4 and 7 exceeded the provisional QTL threshold (LOD > 1.78).

**Expanded candidate pool.** The loci on chromosomes 4, 6, 7, and 16 together only explained ~54% of the genetic variance. In addition, the effect sizes are uncorrected for low statistical power and are thus likely to be overestimates (2, 3, 8), suggesting that there must be at least a similar number of additional QTLs. Therefore, to expand the pool of top candidates, we identified all loci with single-marker $P$ values < 0.05 (Table 1). All 10 of these loci together explained roughly 105% of the genetic variance (again, likely to be a large overestimate). On the basis of the overrepresentation of these loci with $P$ values < 0.05 (10 observed vs. 4 expected by chance, assuming 75 linkage groups of 20 cM each), the false discovery rate is roughly 0.4 (28), suggesting that approximately six of these candidates are likely to be true positives.

**Epistasis.** For weeks 7–16, we also identified a provisional epistatic interaction between loci on chromosomes 18 and X (peak LODs at 37 cM, D18Mit40, and 62 cM, DXMit197, respectively). This interaction was again provisional for weeks 17–30. For this time period, there were also two additional interactions that exceeded the provisional threshold (chromosomes 1 and 4, 14 and X). For weeks 7–16 and 17–30 combined, the only interaction that was provisional was that
Table 1. Suggestive QTLs affecting weight loss during DR*

<table>
<thead>
<tr>
<th>Marker</th>
<th>Chr No.</th>
<th>cM Peak</th>
<th>LOD</th>
<th>P Value†</th>
<th>Effect Size‡</th>
<th>Potential Confound§</th>
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LOD, logarithm of the odds of linkage; AL, ad libitum. *Weeks 7–16 and 17–30 of dietary restriction (DR) combined (unweighted average). †Single-marker P values. ‡Percent genetic variance explained. §Loci with single-marker P values < 0.05 for the potential confounds of absolute DR food intake, DR feces: DR calorimetry, DR home cage activity, and AL fat (LSXSS only).

between the loci on chromosomes 18 and X, with a genome-wide P value of just 0.08. The LOD scores and contributions associated with these loci acting individually were quite low (LODs <0.4).

**Mapping directly on DR BW.** For the mapping on weight loss, our construct (adjusted %AL) was defined to be independent of AL BW. To test whether this definition was handicapping our ability to detect weight loss QTLs, we tried mapping directly on DR BW. The heritability estimates increased substantially (0.69 and 0.77 for weeks 7–16 and 17–30, respectively), but the genetic mapping sensitivity seemed worse (weeks 7–16 and 17–30 combined by averaging). Only one locus surpassed the provisional QTL threshold, the same locus on chromosome 1 that was identified above as provisional (again with P = 0.002). The other loci with P values < 0.05 were largely loci that had lower P values for mapping on AL BW. There was a provisional epistatic interaction between loci on chromosomes 4 and 15, the same loci noted above for having suggestive individual effects when mapping on adjusted percent AL.

**Mapping on metabolic efficiency.** We also tried using the change in “metabolic efficiency” as our construct. Metabolic efficiency is the ratio of average daily food intake to BW (lower values representing greater efficiency); it exhibited a small, but significant, inverse correlation with AL BW (R values = −0.31 and −0.25 for weeks 7–16 and 17–30, respectively; P values < 0.05, 1-tailed). QTL mapping on the change in metabolic efficiency (DR regressed on AL) indicated two provisional QTLs on chromosomes 4 and 15 (weeks 7–16 and 17–30 combined by averaging). The locus on chromosome 4 (single-marker, P = 0.003) was again the same QTL identified above as being provisional using adjusted percent AL. The locus on chromosome 15 (single-marker, P = 0.002) was one of the additional loci identified by expanding the candidate pool for adjusted percent AL. The other loci with single-marker P values < 0.05 also tended to be the same as those identified using adjusted percent AL. One notable exception was a locus on chromosome 14 (peak LOD at D14Mit34) having a single-marker P value of 0.01. The suggestive epistatic interaction between loci on chromosomes 18 and X noted above for adjusted percent AL BW did not quite reach provisional status, nor did any other interactions. Overall, therefore, metabolic efficiency did not appear to have an advantage over mapping on our adjusted percent AL construct.

**QTL Mapping in the LSXSS**

Mapping on the weight loss response (adjusted percent AL) in the LSXSS panel (Fig. 4) identified a provisional locus on chromosome 1 for weeks 7–16 (peak LOD at ~43 cM, D1Mit46). However, this locus was not close to being provisional for weeks 17–30. Instead, there were provisional loci on chromosomes 4 and 9 (peak LOD 45–66 cM, D4Mit205–D4Mit54), and peak LOD ~71 cM, D9Mit18) that also had single-marker P values for weeks 7–16 of just 0.07 and 0.01, respectively. (The LSXSS set of markers typically requires a single-marker P value of ~0.01 to be provisional by permutation testing, which is 2× higher than that required in the LXS panel because of the many fewer markers being tested.) When we averaged the strain means for both time periods (equally weighted), only the locus on chromosome 9 was provisional, whereas the loci on chromosomes 1 and 4 had single-marker P values of 0.04 and 0.02, respectively. The locus on chromosome 4 is not the same locus mapped above in the LXS panel (cM position of 45–66 cM vs. ~14 cM in LXS).

The loci on chromosomes 1, 4, and 9 together explained ~70% of the genetic variance (both time periods combined), again suggesting that the pool of top candidate QTLs could be expanded. Therefore, we again identified all of the loci with single-marker P values < 0.05, which suggested three additional loci on chromosomes 8, 11, and 13 (Table 1). All six loci actually had P values ≤ 0.04 and together explained ~140% of the genetic variance (genetic effect sizes ranging from 17 to 33%, but again likely overestimates). Given that approximately three loci with P values ≤ 0.04 were expected by chance (~75 independent linkage groups × 0.04), the false discovery rate would be ~0.5, and the expected number of true positives would be approximately three.

**Epistasis.** There were provisional epistatic interactions between loci on chromosomes 2 and 4 for weeks 7–16 and between chromosomes 3 and 14 for weeks 17–30. However, neither interaction was provisional when the strain means were averaged. Instead, interactions between chromosomes 2 and 7, and 2 and 14, were provisional.

**DISCUSSION**

Our major observation is that, under carefully controlled conditions of food intake, mice exhibit a highly significant amount of genetic variation in their weight loss response to DR. For females eating 60% AL, this response produced BWs that ranged from lows of ~60% of AL BW to highs of ~85%. Heritability tended to be ~50% or 10 percentage points higher in both the LXS and LSXSS for weeks 17–30 and 31–50 of DR compared with weeks 7–16. It is not clear why heritability would increase; perhaps it reflects a greater loss of fat reserves...
relative to lean body mass. The genetic variation was not explained by correlations with the strain differences in absolute food intake, feces:food, feces calorimetry, gross motor activity, or AL body fat. We also found that the RI panels did a good job of capturing virtually all of the phenotypic variation that was present among five of the classical inbred strain progenitors of the panels and 129S6, which also represent some of the more diverse classical inbred strains. Such marked strain variation indicates that these RI strains can provide a useful test of whether the weight loss response to DR is genetically correlated with other DR responses, DR health benefits, or DR-induced life extension.

By definition, our construct for weight loss was distinct from mapping on AL BW or percent body fat, setting this study apart from many previous QTL studies (5). Therefore, the genes underlying this component, if identified, would likely represent novel therapeutic targets for enhancing or mitigating weight loss. Enhancements to weight loss would be especially beneficial given that obesity has reached epidemic proportions and weight loss is notoriously difficult to maintain (14, 17). That there is also a genetic component to weight loss in humans during food restriction has been reported by Hainer and colleagues (10, 11) in a short-term study of 14 obese twins.

Given that we did not find a clear overlap between our LXS and LSXSS mapping results, we used our LSXSS data set for weeks 7–16 to ask whether our results were at all reproducible by mapping separately on replicate Cohorts 1 and 2 (studied 2 yr apart). The heritability estimates were 40 and 45%, respectively, and the strain means were highly correlated, with $R = 0.74 (P < 0.0001)$, which is not much lower than the correlation between the AL strain means for BW of 0.89. There were two loci, on chromosomes 9 and 13, that had single-marker $P$ values $< 0.05$ in both cohorts analyzed separately. Therefore, two of six suggestive QTLs were reproducible even though both had genome-wide $P$ values $> 0.1$ for the cohorts combined. Interestingly, the locus on chromosome 13 also gave a single-marker $P < 0.05$ in the LXS; however, it is not clear that this represents the same QTL, as the marker allele associated with greater weight loss in the LXS was associated with reduced weight loss in the LSXSS, although there is precedent for QTL alleles to act with opposite effects in different backgrounds (22). The LSXSS would also be expected to have at least some QTLs that differ from the LXS because this panel was derived from noninbred parents and is known to have alleles that are not present in the LXS.

That we did not detect QTLs at a genome-wide 0.05 level of significance is not due to marker density or distribution, which are both excellent in the LXS panel (29). They are also adequate in the LSXSS panel (16), as we have previously mapped with this panel, and the very same mice, a significant QTL affecting the body temperature response to DR (21). This QTL achieved statistical significance because it explained 49% of the genetic variance. In this study, however, all of the QTLs were much smaller, and their validation would require a much larger panel of RIs. As previously noted (29), even the full LXS panel of 77 strains, although it is the largest murine RI panel currently available, would likely not detect QTLs at a genome-wide 0.05 level unless they explained $\geq 25\%$ of the genetic variance. This means that, even with 100% heritability or an infinite sample size per strain, the QTLs in this study would not have achieved statistical significance. Significance, however, can be achieved by using a second stage of confirmation as is commonly done after RI mapping (19). For example, confirmation could be obtained by developing congenic strains from ILS and ISS that target the suggestive QTLs (19), which can be prioritized based on the results of replicate studies and by identifying genetic correlates with other responses to DR. Such studies are in progress.

That there were no major QTLs affecting weight loss is perhaps not too surprising given that QTLs affecting AL BW also tend to be small, and similarly we did not detect significant QTLs for AL BW in this study. Nevertheless, we were able to identify for weight loss an epistatic interaction between loci on chromosomes 18 and X that had a genome-wide $P$ value of just 0.08. A provisional QTL on chromosome 4 in the LXS (peak LOD at $\sim 14$ cM, D4Mit286) also exhibited notable robustness in that this locus was also a provisional QTL when we mapped on DR BW directly and on metabolic efficiency. Two other individually acting loci, on chromosomes 7 and 9, also reached provisional QTL status in this study for weeks 7–16 and 17–30 combined (unweighted average).

There is also a possibility that using other types of weight loss constructs, such as mapping more directly on the loss of body fat and/or liver mass, would improve QTL detection sensitivity. Targeting fat loss might be especially useful considering that Sprague-Dawley rats restricted $\sim 40\%$ lose $\sim 70\%$ of their fat mass (accounting for 53% of the loss in BW) but only 40% or less of their other organ masses (27). Similarly, male Fischer 344 rats restricted 34% lose $\sim 75\%$ of their fat mass (accounting for just 34% of the loss in BW) but just 45% or less of their other organ masses (9).

On a final practical note, our results suggest caution when inferring the percentage level of food restriction based on weight loss in mice without recognizing the potential for marked strain or genotype variation. In particular, given that some strains are highly effective at maintaining their BW (such as 85% AL when restricted 40%), mild levels of food restriction (such as 15% or less) may not be readily detectable in terms of weight loss. Consequently, using only BW to infer that a given mutation or intervention that modestly extends murine lifespan does so without significantly reducing food intake is potentially misleading.

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