*Brief Genetics Report*

# Identification of Major Quantitative Trait Loci Controlling Body Weight Variation in *ob/ob* Mice

**Jonathan P. Stoehr,[1] Jessica E. Byers,[2] Susanne M. Clee,[1] Hong Lan,[1] Igor V. Boronenkov,[1] Kathryn L. Schueler,[1] Brian S. Yandell,[3] and Alan D. Attie[1]**

**The adipocyte hormone leptin constitutes an important component of the regulation of energy homeostasis; leptin-deficient animals, such as obese mice, are strikingly overweight. The seemingly uninhibited weight gain in obese mice belies the fact that control of energy homeostasis remains under precise, heritably modifiable control. Herein, we report large, heritable differences in body weight and food intake between BTBR-*ob/ob* and B6-*ob/ob* mice. We have identified two loci, called modifier of obese (*Moo1* and *Moo2*), that explain the majority of the heritable variance in (BTBR × B6) $F_2$-*ob/ob* mice. Using interval-specific congenic mouse lines, we mapped *Moo1* to an 8-Mb segment of chromosome 2 and demonstrated that *Moo1* exerts its effects primarily by regulating total fat mass. Although null alleles of leptin are rare, the majority of overweight adults are leptin resistant, suggesting that leptin-independent pathways, such as those studied here, are important regulators of energy homeostasis. Thus, the identification of these loci may provide important new insights into the pathogenesis of human obesity. *Diabetes* 53:245–249, 2004**

Genetic factors help determine an individual's predisposition to obesity (1). Single-gene obesity syndromes notwithstanding, the major genetic determinants of human obesity are unknown. Understanding these determinants will greatly enhance our ability to diagnose and treat obese individuals. The cloning of the mouse obese gene (2) led to the discovery of pathways by which the brain senses nutritional status (3), allowing for precise control of food intake and energy expenditure, and maintenance of an internal body composition set point (4). The product of the obese gene, the 16-kDa hormone leptin, constitutes an afferent signal proportional to triglyceride stores within white adipose tissue. Leptin-deficient *ob/ob* mice exist in a state of perceived starvation, constitutively consuming food and suppressing energy expenditure (5).

**The BTBR genetic background potentiates the obesity syndrome of obese mice.** We have previously shown that two background genomes, BTBR (6) and B6, modify the type 2 diabetes syndrome in obese mice (7). Since BTBR-*ob/ob* mice are severely diabetic and markedly glycosuric, we expected to observe substantial secondary weight loss. Surprisingly, BTBR-*ob/ob* mice gained significantly more weight than B6-*ob/ob* controls despite having similar weaning weights (Fig. 1*A* and *B*).

At least part of this weight gain difference can be explained by food intake. Over a 20-day period, BTBR-*ob/ob* mice consumed significantly more food than B6-*ob/ob* controls (Fig. 1*D*). This increased food intake can be directly associated with weight gain during this period, as feed efficiency was not significantly different (Fig. 1*G*).

**Identification of obesity-modifier loci in $F_2$ mice.** We assembled 350 $F_2$-*ob/ob* mice derived from BTBR and B6. Sewall-Wright analysis (Fig. 1*C*) indicates that 30% of the $F_2$ population variance in body mass is heritable. We genotyped the $F_2$ mice at an autosome-wide panel of microsatellites and detected quantitative trait loci (QTLs) as described previously (7).

We detected two major body mass QTLs (Fig. 2). One locus, on chromosome 2, exhibits highly significant linkage to body mass (logarithm of odds [LOD] = 9.48) (Fig. 2*B*). At the peak marker, *D2Mit9*, each *BTBR* allele semidominantly increases 10-week body mass by 3.4 g (~7% of the total mass of the B6-*ob/ob* mouse). This locus, designated modifier of obese 1 *(Moo1*), explains 14.1% of the total $F_2$ population variance.

Another highly significant linkage, *Moo2*, resides on chromosome 13 around *D13Mit66* (Fig. 2*C*). The $Moo2^{B6}$ allele is dominant to *BTBR*, adding ~2.7 g to 10-week body mass, explaining ~12.5% of the $F_2$ population variance. Multivariate regression analysis of *Moo1* and *Moo2* revealed no significant nonlinear interactions (data not shown). Although *Moo2* cannot explain any of the parental strain difference reported here, it supports other studies showing B6 to bear alleles promoting increased feeding efficiency (8), adiposity, and insulin resistance (9,10).

Two other loci were discovered on chromosomes 5 and 17 (*Moo3* and *Moo4*) (Fig. 2*A*). Both loci exhibited suggestive linkage with interval mapping but showed significant linkage using multiple interval mapping (*Moo3*

From the [1]Department of Biochemistry, University of Wisconsin-Madison, Madison, Wisconsin; the [2]Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, Wisconsin; and the [3]Departments of Statistics and Horticulture, University of Wisconsin-Madison, Madison, Wisconsin.

Address correspondence and reprint requests to Alan D. Attie., Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Dr., Madison, WI 53706. E-mail: attie@biochem.wisc.edu.

Received for publication 7 May 2003 and accepted in revised form 13 October 2003.

ISC, interval-specific congenic; LOD, logarithm of odds; Moo, modifier of obese; QTL, quantitative trait locus.

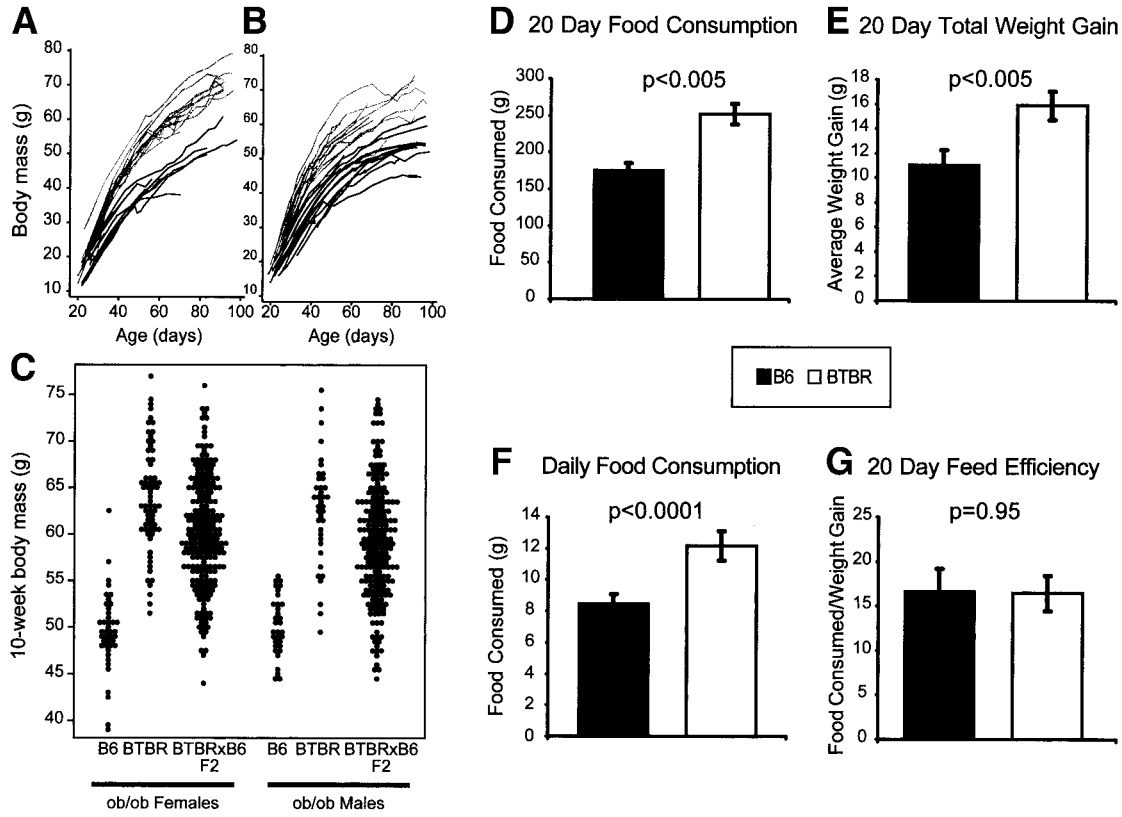


**FIG. 1. Growth curves for B6-*ob/ob* (black lines) and BTBR-*ob/ob* (gray) females (*A*) and males (*B*). The sex-adjusted strain difference is significant, $P < 0.01$, by sigmoidal mixed-model regression. *C*: Sewall-Wright analysis of 10-week body mass. Thirty percent of the variance in body mass is attributable to heritable factors. *D–G*: Twenty-day food intake study in B6-*ob/ob* (■, $n = 4$) and BTBR-*ob/ob* (□, $n = 4$) mice. *D*: Total food consumption. *E*: Total weight gain. *F*: Average daily food intake. *G*: Feed efficiency (food intake/weight gain). Error bars indicate SE.**

peak LOD = 3.60, D5mit136; *Moo4* peak LOD = 2.49, D17Mit109).

**Fine mapping of Moo1 using interval-specific congenic mouse lines.** Although the $F_2$ data establishes the existence of QTLs, it does not provide the positional resolution to support cloning without a candidate gene. We therefore refined the position of Moo1 using interval-specific congenic (ISC) mouse lines (11). We introgressed segments of the Moo1 support interval from B6 into BTBR. We defined the support interval as the 10-cM interval surrounding the LOD peak. Three congenic mouse lines are shown (Fig. 3*E*), named Moo1-A through -C.

Congenic lines Moo1-A and Moo1-B have inherited the centromeric half of the *Moo1* support interval from B6. The two distal recombinational break points arose independently in different founder mice. Each line's proximal recombination lies at least 19 cM (34 Mb) centromeric to the *Moo1* LOD peak. Longitudinal analysis of 28 growth curves from line Moo1-A (Fig. 3*A*) by sigmoidal regression shows no association between weight gain and genotype (difference in mean asymptotic body mass = $b_3 = -0.31 \pm 1.71$ g, $P = 0.82$). Similarly, 27 growth curves from line Moo1-B showed no association with genotype ($b_3 = -0.21 \pm 2.7$ g, $P = 0.94$) (Fig. 3*B*). Thus, ISC lines Moo1-A and -B detect no evidence of *Moo1* in the region of chromosome 2 centromeric to (and including) *D2Mit61*.

Line Moo1-C has retained an ~8-Mb segment of the support interval from B6. Inheritance of this region significantly alters expected weight gain among mice from this line (Fig. 3*C*). Analysis of 24 growth curves from line Moo1-C showed strong association between genotype and body mass ($b_3 = -8.70 \pm 1.72$ g, $P < 0.0001$), but no sex dimorphism ($b_2 = 0.4 \pm 1.8$ g, $P = 0.82$). Moo1-C thus establishes the existence of an obesity-modifier locus in an ~8-Mb interval between *D2Mit56* and *D2Mit159* (Fig. 3*E*). Of course, it remains a formal possibility that another modifier locus exists somewhere else in the *Moo1* QTL. We need to be mindful of the fact that other leptin modifier QTLs may exist elsewhere in our *Moo1* support interval not covered by the introgressed segment in the congenic mice.

Body composition analysis was performed on a randomly selected, representative subset of 15-week-old obese mice from Moo1-C. Nearly 90% of the observed difference in total carcass mass can be accounted for by differences in ether-extractable (lipid) mass ($-7.1 \pm 2.4$ g per *B6* allele) (Fig. 3*E*). No significant differences between genotypes were observed in moisture content or fat-free dry mass, indicating that *Moo1* regulates body mass by controlling accretion of lipid.

The *Moo1* position established by Moo1-C corresponds to a region associated with subcutaneous fat and diet-induced HDL levels in (B6 × CAST/Ei) $F_2$ mice (12), and with obesity in (AKR × C57BL6/J) $F_2$ mice (13). *Moo1* is distinct from an adiposity QTL in (NZB/B1NJ × SM/J) $F_2$ mice (14). Other linkages to body weight or adiposity on chromosome 2 are broad enough to potentially include *Moo1* (15,16). Together, the number of linkages on chromosome 2 suggests that a common locus may explain some or all findings and thus be of particular importance.

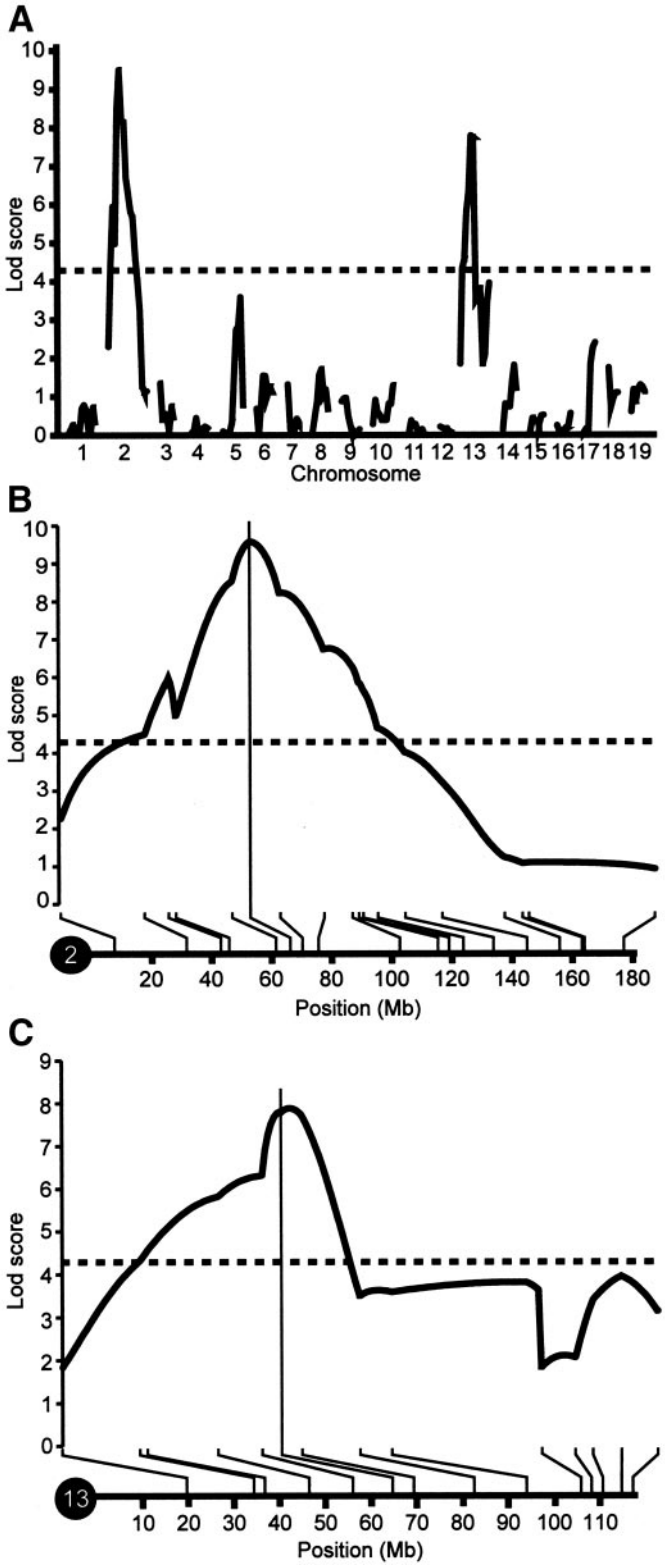


**FIG. 2. *A*: Whole-genome interval mapping of body mass in F2-*ob/ob* mice. The experiment-wise $P = 0.05$ threshold is shown. *B* and *C*: Detailed mapping results for *Moo1* and *Moo2*. The *x*-axis is scaled by empirical genetic distance but referenced to physical position per the February 2002 freeze of the Mouse Genome Browser (23).**

Such a locus would be syntenic with human 2q24-32 in a region containing <50 genes.

Since obesity and type 2 diabetes are so closely interrelated, it is tempting to hypothesize that *Moo1* and *Moo2* influence body weight by altering diabetes susceptibility or, conversely, that toggling the genotype at *Moo1* or *Moo2* could produce enough weight loss to ameliorate the risk of type 2 diabetes elicited by leptin deficiency. However, neither locus associates with fasting plasma glucose or insulin levels, despite the presence of an insulin locus, *t2dm3*, 30 cM telomeric to *Moo1* (7). Despite their strength, the obesity-regulating effects of *Moo1* and *Moo2* are insufficient to overcome the effects of *ob* on diabetes susceptibility.

The obesity-regulating effects of *Moo1–2* are silent in $Lep^{+/+}$ mice housed in standard conditions (data not shown). How then can our findings be extended to humans, in whom leptin deficiency is rare? Leptin has been shown to play a predominant role in regulating the physiological response to hunger and weight loss, but more recent data show that leptin's ability to regulate hypothalamic proopiomelanocortin and CART mRNA levels diminishes when plasma leptin concentrations exceed the physiological range (17), even though proopiomelanocortin mRNA levels decrease in overfed individuals (18). These findings suggest that at higher concentration ranges, leptin may not be the primary mediator of the satiety response in obese or overfed individuals or in high-fat fed mice. The majority of obese humans are hyperleptinemic (19), indicating that regulation of energy homeostasis in the obese state occurs downstream, or independent of leptin. We targeted mediators of such pathways by eliminating the leptin arm directly. The genes thus identified may help elucidate these leptin-independent pathways and therefore be of particular relevance to human obesity.

## RESEARCH DESIGN AND METHODS

BTBR, B6, and B6-*ob/+* mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred at the University of Wisconsin. Mice were weaned at 3 weeks of age onto a 6% fat diet (Purina 5008). Mice had ad libitum access to food and water, except for fasts (0730–1130) before blood draws and killing (by $CO_2$ asphyxiation). The facilities and research protocols were approved by the University of Wisconsin Institutional Animal Care and Use Committee. The lineage and characteristics of the BTBR strain have been reviewed by Ranheim et al. (6).

**Initial QTL detection.** Detection and mapping of QTLs was performed as previously described (7). Briefly, genotypes of 350 $F_2$ mice at 99 markers were assembled using MAPMAKER/EXP (20). Interval mapping with MAPMAKER/QTL and multiple-interval mapping with QTL Cartographer (21) were used to detect segregating loci. Other analyses, including multiple-trait interval mapping (21), composite interval mapping (21), and stepwise regression analyses, gave results consistent with those stated herein.

**Analysis of ISC mouse lines.** A support interval for each locus was defined as the 10-cM region surrounding the LOD peak. BTBR × B6 $F_2$ mice bearing recombinations in a support interval founded each ISC line. We used a marker-assisted backcrossing program (22) to introgress each recombination into BTBR. We referenced the mouse genome from the Draft Sequence Browser (23) and Celera (24) to map the recombinations and search for new microsatellites.

Each ISC line was scored as "retaining" or "not retaining" the *B6* allele of the QTL by comparing growth curves of obese backcross mice within each line, by genotype, i.e., comparing mice heterozygous for the congenic insert with their sibs who did not inherit the insert. No historical controls or cohorts were employed. Longitudinal body mass data for each mouse were fitted to a sigmoidal growth model:

$$\text{mass} = \frac{b_1 + b_2 \cdot \text{male} + b_3 \cdot \#B6\text{alleles} + u_1}{1 + \left(\frac{age}{b_4}\right)^{b_5}} + \varepsilon,$$

where $b_1$, $b_4$, and $b_5$ are curve-fitting parameters, $b_2$ quantifies the sex dimorphism, $b_3$ measures the effect of substituting the *B6* allele for *BTBR*, and

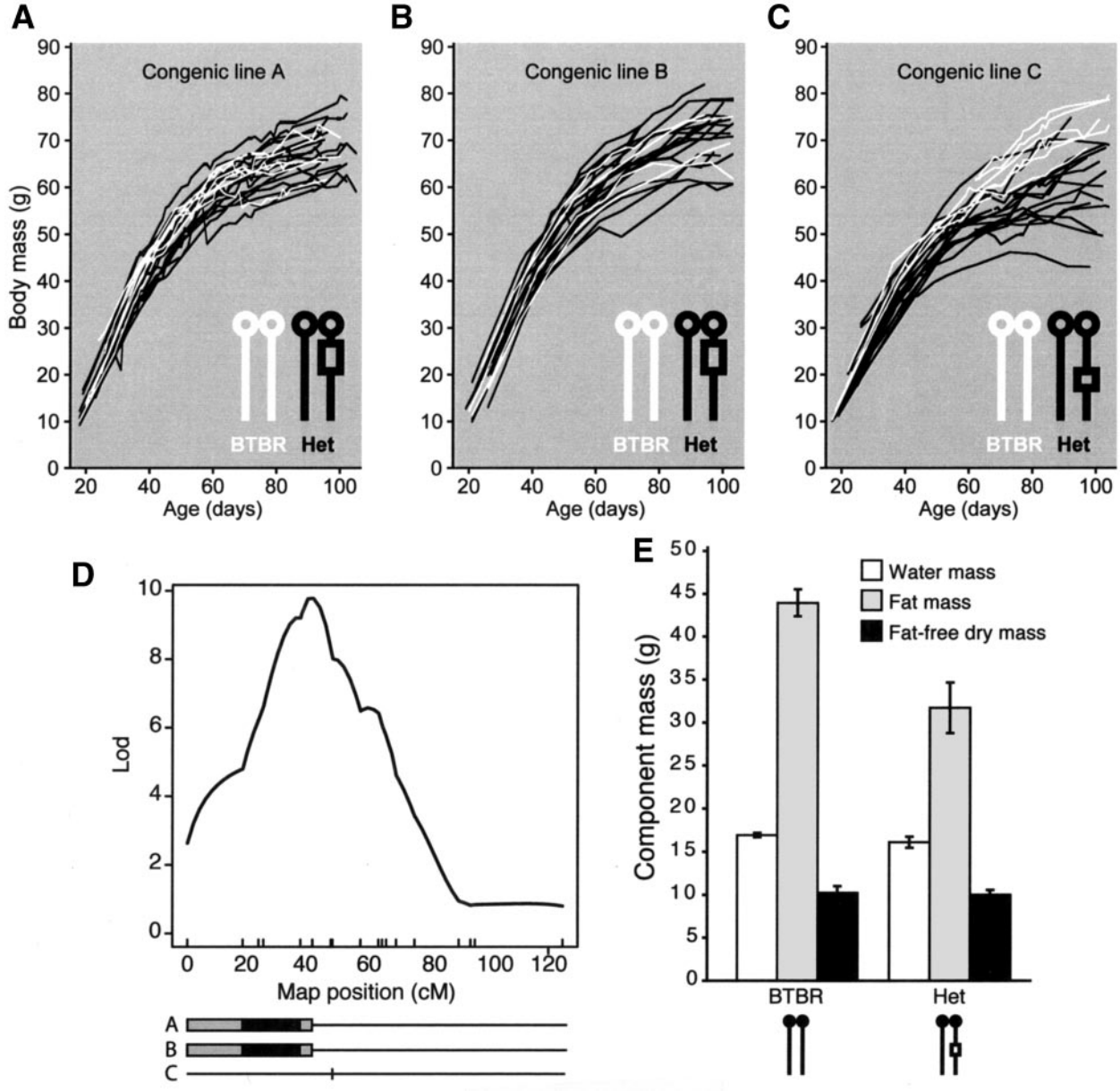


**FIG. 3. *A–C*: Phenotyping results for the three *Moo1* ISC lines. White traces are growth curves for mice that did not inherit any congenic chromosomes and are homozygous *BTBR* throughout. Black traces are growth curves from the sibs that inherited one congenic chromosome; heterozygous within the introgressed region, but *BTBR* elsewhere. Genotype does not associate with body mass for line Moo1-A or -B ($P > 0.05$) but significantly associates with body mass within line Moo1-C ($P < 0.01$). *D*: Dissection of *Moo1* by ISC mouse lines. The ruler indicates the genetic map distance (in cM) on chromosome 2 (23). Three ISC lines are shown as bars; thin line indicates no retention of *B6* alleles, and black indicates retention of *B6* alleles. Gray indicates the presence of a recombination somewhere between adjacent markers. *E*: Body composition analysis of 15 randomly selected obese mice from Moo1-C. Means ± SE of each carcass component is shown.**

$u_1$ measures random effects. The model was fitted using PROC NLMIXED (25).

**Food intake.** From ages 40 to 60 days, *ob/ob* mice were housed individually. Food was weighed and replenished daily. Body weights were recorded at the beginning, end, and on every 3rd day during the study. Data were compared by Student's $t$ test.

**Body composition.** At 15 weeks of age, fasted mice were killed by asphyxiation. The brain was removed for future study, the gastrointestinal tract was emptied, and the carcass was stored at −20°C. Carcasses were autoclaved for 7 h at 250°C. Water lost during autoclaving was replaced to within 0.01 g of the original mass, and the carcass was homogenized in a blender. Triplicate aliquots were quantitatively lyophilized, transferred to filter paper envelopes (Whatman #1), and extracted for 20 h with ethyl ether in a refluxing soxhlet apparatus. Each measured component of the carcass was regressed on sex and genotype using PROC GLM (25).

## ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grant DK-58037, Xenon Genetics, and an American Diabetes Association Mentor–based Fellowship. J.E.B was supported by National Institutes of Health Predoctoral Training Grant DK-07665-11.

We thank the NHLBI Mammalian Genotyping Service at the Marshfield Clinic (Marshfield, WI) for their genotyping efforts. We also acknowledge K. Albright, M. Pariza, W. Dove, and M. Rabaglia for their support and assistance.

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