Genetic Loci Controlling Body Fat, Lipoprotein Metabolism, and Insulin Levels in a Multifactorial Mouse Model

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Abstract

We analyzed the inheritance of body fat, leptin levels, plasma lipoprotein levels, insulin levels, and related traits in an intercross between inbred mouse strains CAST/Ei and C57BL/6J. CAST/Ei mice are unusually lean, with only ~ 8% of body weight as fat, whereas C57BL/6J mice have ~ 18% body fat. Quantitative trait locus analysis using > 200 F2 mice revealed highly significant loci (lod scores > 4.3) on chromosomes 2 (three separate loci) and 9 that contribute to mouse fat-pad mass for mice on a high-fat diet. Some loci also influenced plasma lipoprotein levels and insulin levels either on chow or high-fat diets. Two loci for body fat and lipoprotein levels (on central and distal chromosome 2) coincided with a locus having strong effects on hepatic lipase activity, an activity associated with visceral obesity and lipoprotein levels in humans. A locus contributing to plasma leptin levels (lod score 5.3) but not obesity was identified on chromosome 4, near the leptin receptor gene. These data identify candidate regions and candidate genes for studies of human obesity and diabetes, and suggest obesity is highly complex in terms of the number of genetic factors involved. Finally, they support the existence of specific genetic interactions between body fat, insulin metabolism, and lipoprotein metabolism. (*J. Clin. Invest.* 1998. 101: 2485–2496.) Key words: genetics • obesity • mouse • insulin • lipoproteins

Introduction

Adipose energy stores are regulated by feedback signaling mechanisms that influence energy expenditure and food intake. Under conditions of nutritional abundance, a large fraction of the population accumulates excessive lipid stores, and such obesity is commonly associated with insulin resistance, type 2 diabetes, high blood pressure, dyslipidemias, heart disease, and stroke. Which individuals become obese is determined in large part by genetic factors (1). With the exception of certain rare syndromes, such as Prader–Willi and Bardet–Biedl Syndromes, human obesity is multifactorial. The number of major genes contributing to obesity, their identities, and the mechanisms contributing to diseases are largely unknown. Physiologic, biochemical, and genetic studies have provided a number of candidate genes and pathways. It is clear, for example, that the hypothalamus is a key brain region since a variety of lesions in this region can cause hypophagia or hyperphagia (2). Biochemical studies of fat cell gene expression have suggested that several transcription factors, including PPARγ, ADD1/SREBP1 and C/EBPs are important in adipogenesis (3). Studies of monogenic mutations resulting in massive obesity in mice have been particularly informative (4). The genes for five such mutations have now been identified by positional cloning or positional candidate gene approaches. The *ob* gene encodes leptin, a protein secreted by adipocytes in proportion to triglyceride stores, which interacts with a receptor in the hypothalamus, encoded by the *db* gene, to influence energy expenditure and food intake. One consequence of leptin action is decreased expression of the hypothalamic neuropeptide Y, which modulates energy expenditure and brown adipose tissue activity. The agouti mutation, resulting in ectopic expression of agouti protein, a pigmentation factor normally expressed in skin, appears to induce obesity by antagonism of the melanocortin-4 receptor, a G protein–coupled receptor that activates adenyl cyclase production in the brain (5). The physiologic systems perturbed by the other mouse obesity genes, "fat" and "tubby," are as yet unclear (3).

Attempts to identify genetic factors contributing to human obesity have not yet produced definitive results, although several potential linkage results with candidate gene regions have been reported. Thus, several studies have reported linkage of obesity parameters to the leptin gene region on human chromosome 7 (6–8) but extensive studies of the *ob* gene failed to identify any functionally significant mutations within the coding regions of the gene (9, 10). Recently, significant linkage was observed for circulating leptin levels and fat mass to a locus on human chromosome 2p21 (11). In collaborative studies with C. Bouchard and colleagues, our laboratory observed linkage of insulin levels and obesity parameters with a region near the human agouti gene on human chromosome 20 (12), although peak linkage was clearly distal to the agouti gene on the long arm of the chromosome (12). Several other potential linkages have been observed with various candidate genes, such as the β-3 adrenergic receptor (13), the glucocorticoid receptor (6), and a sodium–potassium ATPase (14), although these studies require confirmation. Such studies of genes predisposing to obesity in humans are complicated by the multigenic nature of obesity, important environmental influences, and genetic heterogeneity. Thus, for example, complete genome scans of large numbers of families have failed to reveal clear-cut linkage with measures of body fat, although some suggestive loci were identified (15).

An alternative to direct human studies is the analysis of genetic factors contributing to multigenic obesity in animal models, particularly rodents. Such studies greatly simplify the anal-

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yses, since large numbers of animals can be bred and studied under defined experimental conditions. A number of loci affecting body weight in mice have been identified by such strategies (see, for example, references 16–18). Once loci are mapped, they can be isolated on common genetic backgrounds as congenic strains, facilitating studies of their interactions and physiologic effects and simplifying identification by positional cloning. We previously reported the identification of four chromosomal loci contributing to multigenic obesity in a backcross between strains C57BL/6J (B6)[1] and SPRET/Ei. The four loci exhibited nonadditive interactions and had distinct effects on various fat depots and plasma lipoproteins. One locus, designated *Mob1*, on distal chromosome 7, exhibited striking effects on both hepatic lipase activity, HDL and LDL (17, 18). West and colleagues (19, 20) identified three loci contributing to dietary obesity in a cross between strains SWR/J and AKR/J. Interestingly, one of the loci coincided in location (proximal chromosome 15) with a locus identified in the B6 and SPRET/Ei cross (18). Loci on chromosomes 1 and 7 contributing to mouse obesity were identified in a cross between strains 129 and EL (21). Recently, we identified an obesity locus on distal chromosome 2 in a cross between NZB and SM (12), a locus we designated *Mob5.* We also found that the homologous human locus, on chromosome 20, was linked to obesity and insulin levels in a large set of French–Canadian families analyzed by the sib–pair method (12). The identities of the genes underlying multifactorial obesity are as yet unknown, although Fleury and colleagues provided suggestive evidence that the *Mob1* locus may result from variations in the gene for uncoupling protein-2 (22).

We now report the identification of four highly significant loci (lod scores exceeding 4.3) contributing to obesity parameters in a genetic cross between strains CAST/Ei (CAST) and B6. We have designated these multigenic obesity loci as *Mob5*, *Mob6*, *Mob7*, and *Mob8.* The loci *Mob5*, *Mob6,* and *Mob8* correspond to the locations of previously identified multigenic loci (12, 18–20, 23), and *Mob5*, *Mob6,* and *Mob7* control insulin levels. The finding of common loci in crosses involving widely divergent strains of mice suggests that multigenic obesity results from a limited set of major genes, at least 10, contributing to the control of body fat in mice. Moreover, these results suggest specific interactions between obesity, insulin levels, and lipoprotein metabolism and provide candidate regions in the search for loci controlling obesity in humans.

Methods

Mice. Parental mice were purchased from the Jackson Laboratories (Bar Harbor, ME) and all mice were housed under conditions meeting Association for Assessment and Accreditation of Laboratory Animal Care, International accreditation standards. CAST males were mated with B6 females and the resulting F1 progeny were intercrossed to produce F2 intercross progeny. The F2 mice were weaned at about 21 d old onto rodent chow containing 12% of calories as fat (Purina 5001); at about 4 mo old, they were switched to a high-fat, high-cholesterol diet for 8 wk. This diet was 75% chow supplemented with 7.5% cocoa butter (resulting in 30% of calories as fat) and also 2.5% dextrose, 1.625% each of sucrose and dextrin, 1.25% cholesterol, and 0.5% sodium cholate (Diet No. 90221; Harlan Teklad, Madison, WI). The high-fat diet had little impact on body weight (our unpublished observations) in the parental strains and was used to examine the genetic control of dietary responsiveness of plasma lipoprotein levels. The mice were given free access to food and a 12-h light–dark cycle was maintained throughout.

Quantitation of obesity and plasma lipids, glucose, leptin, and insulin. These were as previously described (17, 18). Briefly, mice fasted overnight before collection of blood and were killed $\sim$ 3 h into the diurnal phase of the light cycle. After the animals were weighed and measured for body length (anal-to-nasal distance), the kidneys, liver, and spleen were collected for DNA isolation and other analyses. Four fat pads, consisting of three intra-abdominal fat pads (retroperitoneal, mesenteric, and gonadal) and the femoral fat pad (a subcutaneous fat pad on the outer thigh) were dissected, weighed, and returned to the carcass. Body composition was based on the remaining carcass, which was dried and homogenized; an aliquot was extracted for lipid in a Soxhlet apparatus (Kimble Glass Co., Vineland, NJ). Carcass water and lipid were determined gravimetrically. We separated plasma lipids and lipoproteins and quantitated them using enzymatic procedures as previously described (24). Hepatic lipase activity was quantitated as previously described (18). We determined insulin and glucose levels using commercial kits as described (17). Leptin levels were determined using a commercial immunoassay (Linco, St. Louis, MO).

Linkage and data analyses. A complete linkage map for all chromosomes except the Y was constructed using microsatellite markers and restriction fragment length variants. PCR primers for microsatellite typing were purchased from Research Genetics (Huntsville, AL). Methods for PCR analyses were as described (18, 25). Linkage maps were constructed using the Map Manager v2.6.5 (26) and MAPMAKER/QTL (27) programs. Statistical comparisons of quantitative traits between groups as shown in the tables was by ANOVA. Analysis of variance and regression analyses were performed using the Statview 4.5 program for the Macintosh Computer (Abacus Concepts, Inc. Berkeley, CA). Lod scores for quantitative traits were calculated using MAPMAKER/QTL. The data were adjusted for the effects of age and sex by regression.

Results

Study design. Preliminary studies revealed large differences in body fat content and plasma lipoprotein levels between the strains CAST and B6 (see below). To identify genetic factors contributing to body fat and to test for possible interactions between body fat, insulin levels, and lipoprotein metabolism, we performed quantitative trait locus (QTL) analysis on an intercross between the CAST and B6 mice. Thus, F2 mice were typed for genetic markers spanning the genome and for parameters related to body fat. Chromosomal regions segregating with the traits were identified using analytic programs and loci exhibiting suggestive linkage (lod scores $\geq$ 3.0) or highly significant linkage (lod scores $\geq$ 4.3) are presented. The highly significant loci were then examined for possible effects on insulin and glucose levels and plasma lipoprotein metabolism.

Inheritance of body fat and plasma lipid levels in B6 $\times$ CAST intercross. Table I shows the characteristics of the inbred strains B6 and CAST with respect to body fat and lipoprotein levels. CAST mice are unusually lean, with $\sim$ 8% of body weight as fat, whereas strain B6 mice have $\sim$ 18% body fat (Fig. 1). We observed some significant differences in fat pad weights between males and females, but these were small compared with the differences between the two strains (Table I). The lipoprotein profiles of the two strains also differed, as CAST mice exhibited significantly lower HDL levels than B6 mice (Table I). To examine the inheritance of these traits, B6

1. *Abbreviations used in this paper:* B6, C57BL/6; CAST, CAST/Ei; QTL, quantitative trait locus.

Table I. Body Fat and Plasma Lipoprotein Characteristics of B6 and CAST Parental Mice and (B6 × CAST)F1 Mice

		CAST	C57BL/6J	(B × C)F1
Total cholesterol (mg/dl) (Chow)	Total	73±6	82±4	55±1.6*‡‡
HDL cholesterol (mg/dl) (Chow)	Total	34±4*	50±2	45±10
	Male	26±4*,***	53±3‡‡‡	47±1‖¶
	Female	39±4	46±3	42±3
Carcass weight (g)	Total	13.3±0.5*	22±1	19.6±0.4‖¶
	Male	14.0±0.3*‡‡‡	26±2¶¶	20.5±0.5‡¶‖‖
	Female	12±1*	18.9±0.6	18.1±0.3
Retroperitoneal fat pad (mg)	Total	11±4*	109±20	23±4*‡‡
	Male	12±8	140±150	29±6‡‡‡
	Female	10±5*	91±20	15±3‡
Mesenteric fat pad (mg)	Total	101±15*	218±30	154±8‖**
Subcutaneous fat pad (mg)	Total	102±21*	299±40	118±11*
	Male	121±27	240±90	130±16‡‡‡
	Female	78±32*	327±60	98±8*
Epididymal/parametrial fat pad (mg)	Total	113±36*	524±70	193±22*§§
Insulin (ng/ml) (High fat)	Total	1.0±0.2§	0.47±0.09	0.27±0.04*§§
Leptin (ng/ml) (High fat)	Total	2.2±0.3§	1.31±0.04	2.31±0.09*
Glucose (High fat)	Total	194±30	194±39	180±21
Hepatic lipase (chow) (nmoles FFA/h/μl)	Total	19.4±0.8	25±4	20.1±0.3

Data are shown±SEM. *$P < 0.0001$ vs. C57BL/6. ‡$P < 0.001$ vs. C57BL/6. §$P < 0.01$ vs. C57BL/6. ‖$P < 0.05$ vs. C57BL/6. ¶$P < 0.0001$ vs. CAST. **$P < 0.001$ vs. CAST. ‡‡$P < 0.01$ vs. CAST. §§$P < 0.05$ vs. CAST. ‖‖$P < 0.0001$ for sex difference. ¶¶$P < 0.001$ for sex difference. ***$P < 0.01$ for sex difference. ‡‡‡$P < 0.05$ for sex difference.

females were crossed with CAST males and the resulting F1 mice were intercrossed to generate > 200 F2 mice. (B6 × CAST)F1 mice exhibited body fat and lipoprotein levels that were approximately intermediate between the parental strains (Table I). For some traits, sex differences were observed and these reached statistical significance for carcass weight and HDL cholesterol (Table I). In general, however, the sex differences were small in comparison with the differences between the parental strains.

Fig. 1 shows the distributions of the traits in the F2 mice. The wide range of trait values, much larger than the variation observed in either of the parental strains, reflects primarily genetic influences. The traits all exhibited a continuum of values, suggesting multigenic inheritance. In some cases, the range of F2 values considerably exceeded the parental ranges, probably reflecting novel combinations of genetic influences due to independent assortment of parental alleles. Several of the traits exhibited significant correlations in the F2 mice (Table II).

Mapping of loci controlling body fat in B6 × CAST F2 mice. To map genes contributing to body fat, a dense linkage map spanning all chromosomes except the Y was constructed for ~ 200 of the F2 mice. We scored a total of 116 genetic markers, resulting in an average spacing of about 10 cM between markers. We constructed a linkage map of the markers

Table II. Correlation Matrices between Plasma Lipoprotein and Body Weight and Body Fat Values in (CAST × B6)F2 Animals

	TChol	HDL	HL act	Weight	Mes fat	Retro fat	Paramet fat	Sub fat	Percent lipid	Insulin	Glucose	Leptin
Total cholesterol		**0.82**	**0.72**	0.14	−0.05	−0.02	0.06	0.13	0.16	0.11	−0.16	−0.14
HDL cholesterol	**0.82**		**0.78**	0.20	−0.10	0.10	0.07	0.16	0.22	0.13	−0.19	−0.08
Hepatic lipase activity	**0.59**	**0.67**		0.30	−0.03	0.07	0.12	0.17	0.35	0.64	0.10	−0.41
Weight	0.24	0.30	0.40		**0.55**	0.31	0.39	0.28	**0.50**	0.19	0.20	−0.15
Mesenteric fat	0.20	0.26	0.27	**0.67**		**0.56**	**0.70**	0.32	**0.58**	0.47	−0.05	−0.17
Retroperitoneal fat	0.25	0.24	**0.55**	**0.60**	**0.81**		**0.80**	0.45	**0.85**	0.23	0.02	−0.04
Epididymal fat	0.35	0.38	0.51	**0.77**	**0.83**	**0.76**		0.40	**0.81**	0.17	0.21	−0.07
Subcutaneous fat	0.29	0.33	0.35	**0.72**	**0.71**	**0.74**	**0.76**		**0.48**	0.09	0.04	0.19
Lipid (*percent*)	0.29	0.37	**0.41**	**0.66**	**0.67**	**0.59**	**0.86**	**0.69**		0.17	0.10	−0.04
Insulin	**0.45**	**0.49**	**0.53**	0.37	0.35	0.26	0.42	0.35	**0.62**		0.02	−0.08
Glucose	0.13	0.09	0.20	0.22	0.04	0.02	−0.02	0.15	−0.10	−0.09		−0.09
Leptin	−0.17	−0.31	−0.59	−0.21	0.12	0.05	0.13	0.01	0.19	0.09	−0.09	

Correlation coefficient (ρ) involving plasma lipoproteins and body fat values. Values to the right of the diagonal are for females, values to the left of the diagonal are for males. Values in bold are significant at the $P \leq 0.0001$ level.

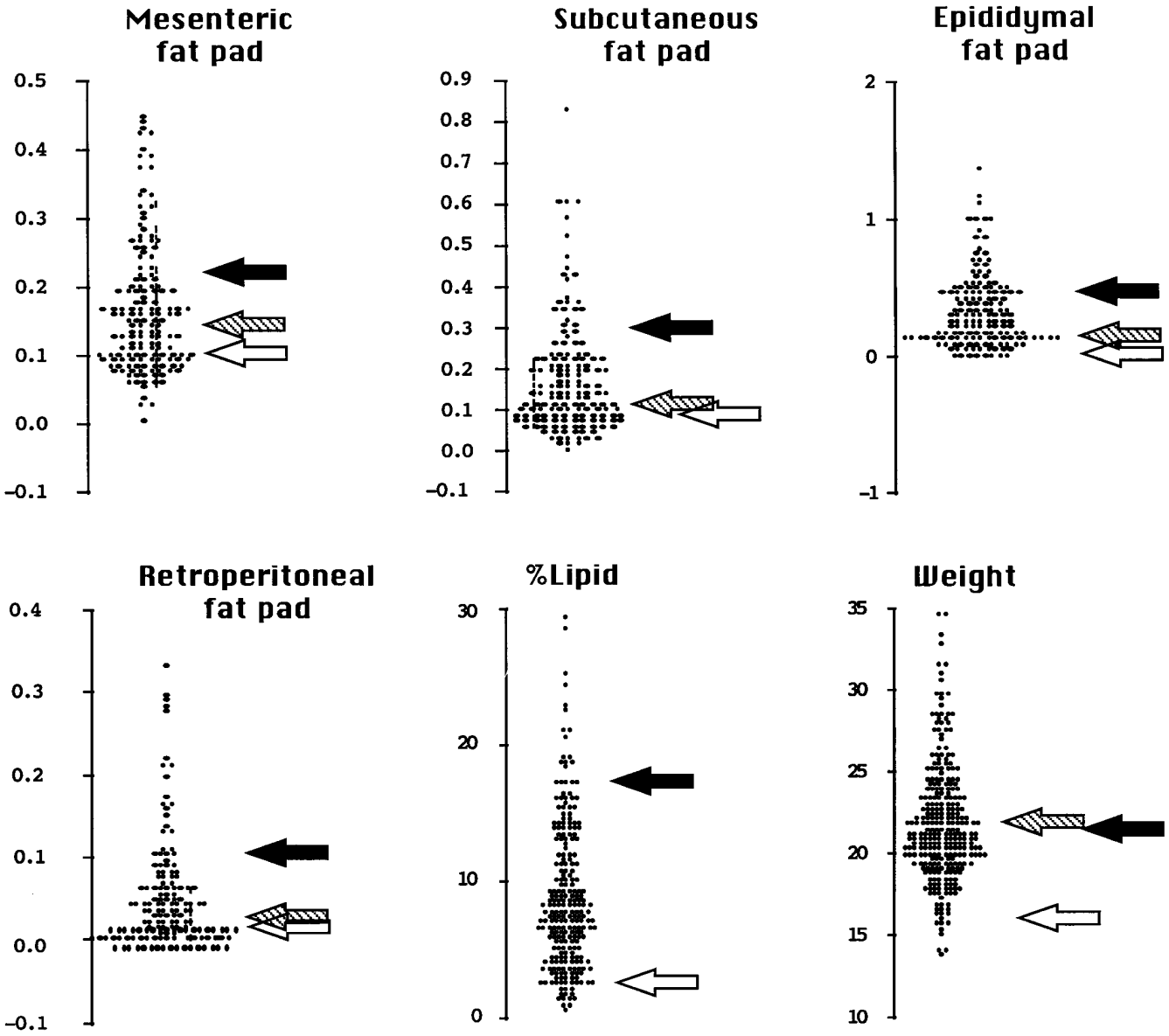

Figure 1. Frequency distribution of fat-pad weights, percent body fat and total body weight in (B6 × CAST)F2 mice. Each point represents an individual mouse. *Filled arrow*, average values for the B6 parental; *open arrow*, average values for CAST mice; *hatched arrow*, average values for (B6 × CAST)F1 mice.

using the Map Manager program (Fig. 2). The locations of the markers shown are consistent with published data. We performed QTL analysis for obesity-related traits, including the weights of four fat pads and total body fat. We examined the linkage of body fat measures to regions of the genome using the MAPMAKER/QTL program, which calculates the strength of associations between genotypes (markers) and phenotypes (traits) as the log base ten of the odds ratio (lod score). It has been estimated that in the mouse, in an F2 cross, an lod score of $\sim$ 4.3 is statistically significant (28). We obtained similar thresholds when we performed simulations with shuffled data using the Map Manager program (data not shown).

Four highly significant loci, with peak lod scores exceeding 4.3, were identified for body fat (Table III). We designate these loci as *Mob* loci consistent with our previous notation for genes contributing to multigenic obesity in mice. As discussed below, the distal chromosome 2 locus identified here appears identical with the locus previously observed in a cross between mouse strains NZB/BINJ and SM/J (12) that we designated *Mob5.* Therefore, we tentatively assign the same designation to the distal chromosome 2 locus observed in the present cross. We designate the central and proximal chromosome 2 loci as *Mob6* and *Mob7*, respectively and the chromosome 9 locus as *Mob8.* Finally, we identified an additional obesity locus exhibiting suggestive evidence of linkage (lod score exceeding 3.0) on chromosome 8 near D8Mit12.

To define the locations of the four significant loci more precisely, and to strengthen the evidence for linkage, additional genetic markers in the regions were typed in the 200 F2 mice and selected markers were typed in up to 100 additional F2 mice. Three of the observed highly significant loci are linked on chromosome 2, and the lod score plots for the loci

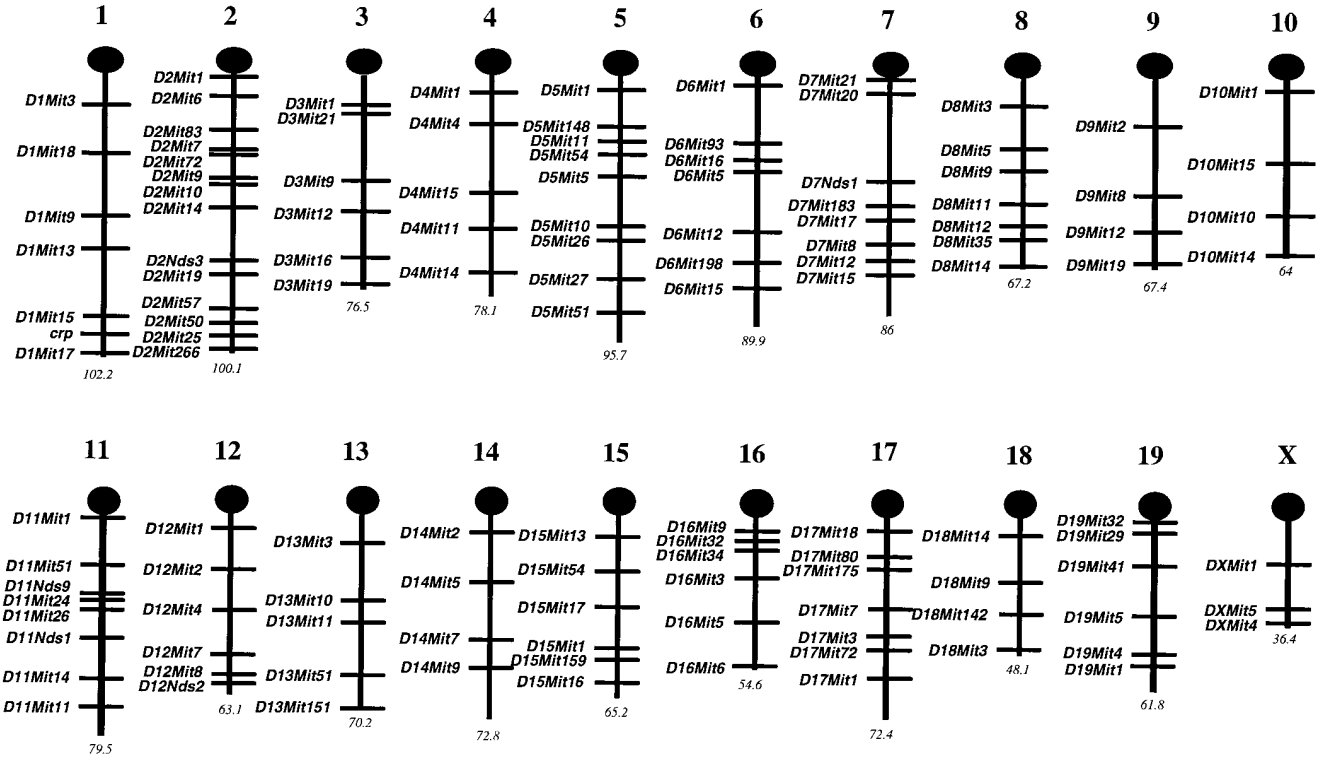

Figure 2. A linkage map constructed for (B6 × CAST)F2 mice. *Horizontal lines*, simple sequence repeat markers that were typed in ~ 200 (B6 × CAST)F2 mice. Chromosomes are drawn with the centromere (*knob*) at the top of each chromosome. The distance spanned by markers on each chromosome is given, in centimorgans, at the bottom of each chromosome representation.

overlap (Fig. 3). The lod scores at each of these loci improved as additional mice were typed after the initial genomic scan of 200 F2 mice. The evidence that these are distinct loci is based on their differential effects on measures of body fat and other traits as well as the shape and breadth of the lod score plots (Fig. 3). Thus, the proximal (*Mob7*) and central (*Mob6*) loci primarily influenced subcutaneous fat, a trait not significantly influenced by the distal locus (*Mob5*). By contrast, the distal locus impacted primarily on percent of body weight present as fat (percent body lipid), whereas the proximal and central loci affected percent body lipid only slightly. The proximal and central loci also appear to be distinct from each other based on their differential effects on traits such as hepatic lipase activity, HDL levels, and insulin levels (see below). The colocalization of QTL peaks for obesity and related traits is suggestive of a single gene within each QTL that impacts several related phenotypes. However, the regions identified by QTL mapping are necessarily broad, and much higher resolution approaches will be required to establish whether the genes that determine fat pad weights are the same or different from those responsible for other traits that colocalize on the chromosome (see discussion). Also, the possibility that there are only two, rather than three, loci on chromosome 2 cannot be excluded.

Table III. Significant and Suggestive Loci for Body Fat Measures

Trait	Chromosome	Peak marker	LOD score
Subcutaneous fat	2	D2Mit9	5.8
Retroperitoneal fat	2	D2Mit9	3.2
Subcutaneous fat	2	D2Mit14	7.4
Retroperitoneal fat	2	D2Mit14	4.0
Percent body fat	2	D2Mit50	5.8
Percent body fat	8	D8Mit12	3.2
Percent body fat	9	D9Mit8	4.7
Epididymal/parametrial fat	9	D9Mit8	3.8

The effects of CAST and B6 alleles at the obesity QTL are summarized in Table IV. In the case of *Mob5*, *Mob6*, and *Mob7*, the CAST alleles were associated with reduced body fat, with each B6 allele conferring a 1–2% increase in percent body lipid. In each case, the mice homozygous for CAST alleles were leanest, those homozygous for B6 alleles had the most fat and heterozygous mice were intermediate. Thus, the genes exhibit additive or codominant inheritance. The *Mob8* locus, however, exhibited the least body fat in mice homozygous for the B6 allele, indicating that, at that locus, the CAST alleles confer increased body fat. Also, the *Mob8* locus appears to exhibit dominant inheritance, as heterozygous mice and mice homozygous for the CAST allele exhibited similar percent body fat and subcutaneous fat.

Leptin levels in (B6 × CAST)F2 intercross mice are poorly correlated with body fat and are determined by a locus near the leptin receptor. As discussed in the introduction, plasma leptin levels in humans and a number of animal models tend to reflect triglyceride stores (29). By contrast, plasma leptin levels

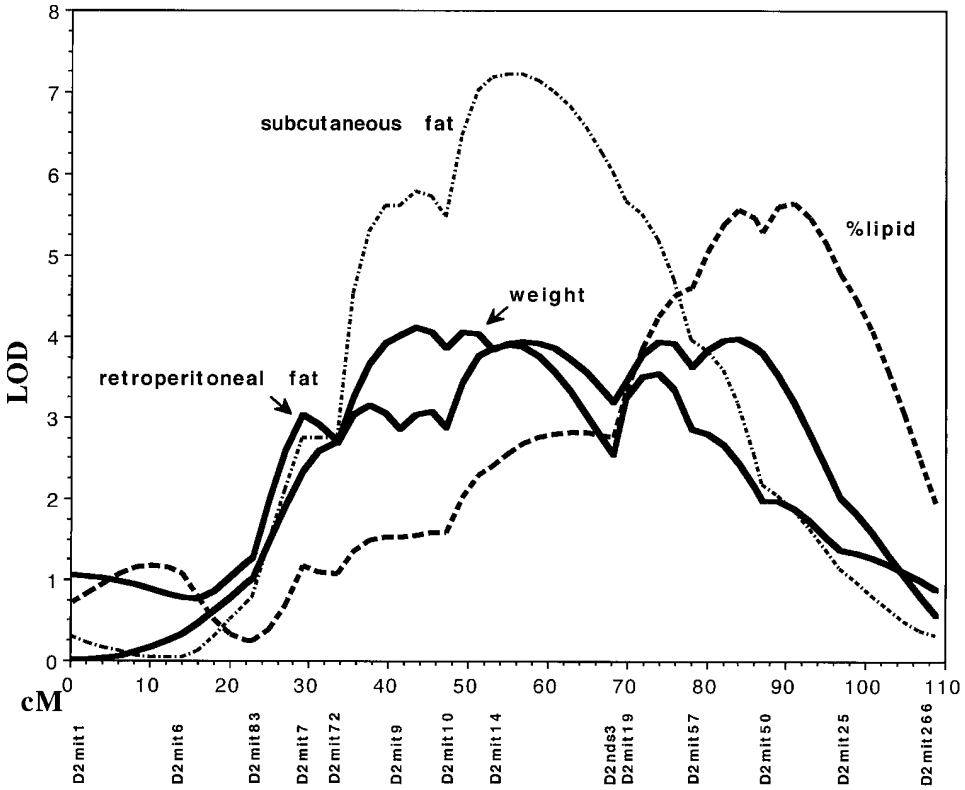

Figure 3. Lod scores on chromosome 2 for fat-pad weights (retroperitoneal and subcutaneous), percent of body weight as lipid (percent lipid), and total body weight. *Bottom*, markers scored in the F2 mice. The lod score, on the y-axis, was calculated at 2-cM intervals along chromosome 2 using the MAPMAKER/QTL program. As discussed in the text, chromosome 2 appears to contain three distinct obesity-related QTLs, a proximal QTL near D2Mit9 (*Mob7*), a central QTL near D2Mit14 (*Mob6*), and a distal QTL near D2Mit50 (*Mob5*).

of the F2 intercross mice (ranging from 2.3 to 17.1 ng/ml) were poorly correlated with measures of body fat (Table II). Interestingly, by far the strongest correlation of leptin levels was with hepatic lipase activity ($r = -0.41$ in females and -0.59 in males). None of the obesity loci noted above cosegregated with leptin levels but a highly significant QTL (lod 5.2) was observed on chromosome 4 near the marker *D4Mit15*. As described below, the 95% confidence limits of this locus include the leptin receptor gene (*db*). This locus exhibited a dominant inheritance pattern, since mice homozygous for the CAST allele at a marker near the QTL peak had leptin levels similar to heterozygous mice, while mice homozygous for the B6 allele had considerably lower leptin levels (Table IV).

Specific interactions between QTLs for body fat and parameters related to insulin resistance and type 2 diabetes. Obesity is the strongest risk factor for non–insulin-dependent diabetes mellitus (NIDDM), but the relationship between the two traits is unclear. Since strain B6 mice fed a high fat diet (without cholesterol) become hyperinsulinemic (30), we examined the relationship between insulin levels and the obesity loci. Highly suggestive linkage for insulin levels (ranging from 0.04 to 0.96 ng/ml in F2 animals) was observed with all three body fat loci on chromosome 2 (*Mob5*, *Mob6*, and *Mob7*) (Fig. 4) but not with the locus on chromosome 9 (Table IV). As expected, insulin levels were highest in mice inheriting chromosomal regions from B6 (BB) and lowest in mice inheriting the CAST chromosomal regions (CC), with intermediate levels being observed in heterozygous mice (CB) (Table IV). Interestingly, in F2 mice, insulin levels were most significantly correlated with with hepatic lipase activity ($r = 0.64$ in females and $r = 0.53$ in males). Insulin levels were also correlated with the size of various fat depots in both males and females and, in males, with HDL cholesterol levels ($r = 0.49$). Glucose levels, which ranged from 0 to 726 mg/dl in the F2 mice were poorly correlated with the traits examined in this cross (Table II) and we observed no significant or suggestive QTLs for plasma glucose levels.

Specific interactions between QTLs for body fat and plasma lipoprotein metabolism. Both human epidemiological studies and genetic studies in rodents have demonstrated metabolic interactions between body fat and plasma lipoprotein levels (31). To further examine the nature of such interactions, we tested for possible effects of the loci for body fat on plasma lipoprotein levels. Of the four highly significant loci identified in this cross, the loci *Mob5*, *Mob6*, and *Mob7* exhibited effects on HDL–cholesterol levels on both chow and high-fat diets (Fig. 5 and Table IV). Interestingly, the *Mob5* locus exhibited the highest lod score for HDL–cholesterol levels on a chow diet (peak lod score 3.5), whereas the *Mob6* and *Mob7* loci exhibited much stronger linkage with HDL levels on the high-fat diet (peak lod scores 4.3 and 5.7, respectively). Thus, while there were only small dietary effects on absolute HDL levels, it appears that different genes act to regulate HDL cholesterol levels on chow and high fat diets. Further, the *Mob5*, *Mob6*, and *Mob7* loci exhibited additive or codominant inheritance as observed for body fat. In contrast, the *Mob8* locus did not significantly influence HDL cholesterol levels. No significant QTLs for triglyceride levels or low density lipoprotein levels were observed at the four obesity loci (data not shown). We conclude that there are specific genetic interactions between plasma lipoprotein metabolism and body fat.

Coincidence of hepatic lipase QTL with those for body fat and plasma lipoprotein metabolism. Previous studies in hu-

Table IV. Effects of CAST (C) and B6 (B) Alleles on Body Fat and Related Traits at Chromosomes 2, 4, and 9 QTLs

Chromosome locus (symbol)	Trait	Peak Marker	CC	CB	BB
Distal chromosome 2 (*Mob-5*)	Leptin	D2Mit50	8.6±0.5	9.1±0.4	9.1±0.6
	Percent lipid		6.9±0.6‡¶	8.5±0.5	10.9±0.6*
	Subcutaneous fat		0.14±0.02‖‡‡	0.18±0.02	0.23±0.03
	Chow HDL		36±2*¶	44±1	45±2
	High-fat HDL		28±3*¶	42±3	49±6
	Insulin		0.23±0.01*¶	0.32±0.02	0.38±0.03‖
	Chow hepatic lipase		35±3§¶	44±3	54±0.4*
Central chromosome 2 (*Mob-6*)	Leptin	D2Mit14	8.8±0.4	9.4±0.4	8.4±0.7
	Percent lipid		7.8±0.5¶	8.3±0.5	10.5±0.7§
	Subcutaneous fat		0.13±0.01¶	0.15±0.02	0.28±0.03*
	Chow HDL		38±2‖¶	42±2	46±2§
	High-fat HDL		30±3§¶	40±3	50±5*
	Insulin		0.25±0.02‡¶	0.32±0.02	0.37±0.03
	Chow hepatic lipase		36±2‡**	46±3	52±4
Proximal chromosome 2 (*Mob-7*)	Leptin	D2Mit9	8.6±0.4‖	9.5±0.4	8.3±0.7
	Percent lipid		7.6±0.6¶	8.6±0.5	10.3±0.7‖
	Subcutaneous fat		0.12±0.01‖¶	0.17±0.02	0.28±0.04§
	Chow HDL		38±2‡¶	43±2	45±2
	High-fat HDL		29±3‡¶	42±3	48±5
	Insulin		0.24±0.01‡¶	0.31±0.02	0.40±0.04‖
	Chow hepatic lipase		36±2‡**	47±3	51±4
Chromosome 4	Leptin	D4Mit15	9.5±0.5¶	9.9±0.4	7.5±0.5*
	Percent lipid		9.6±0.7‡‡	8.9±0.5	7.7±0.6‖
	Subcutaneous fat		0.15±0.01‖§§	0.19±0.02	0.19±0.02
	Chow HDL		40±0.2‖	43±1	42±2
	High-fat HDL		43±4§§	44±4	33±3§
	Insulin		0.28±0.03‖	0.35±0.03	0.30±0.02
	Chow hepatic lipase		45±4	43.6±0.03	42±3.4
Chromosome 9 (*Mob-8*)	Leptin	D9Mit8	9.3±0.6	8.9±0.3	8.8±0.5
	Percent lipid		9.3±0.9**	9.7±0.5	6.3±0.5*
	Subcutaneous fat		0.22±0.04§§	0.20±0.02	0.13±0.02‡
	Chow HDL		45±2**	43±1	38±2‖
	High-fat HDL		50±6‡‡	42±3	30±4§
	Insulin		0.36±0.03**	0.33±0.02	0.25±0.03§
	Chow hepatic lipase		46±4§§	46±3	37±4‖

All measurements are for animals on high-fat diet unless marked as chow. Measurements are given as ±SEM. The units for these measurements are: Leptin (ng/ml), subcutaneous fat (g), HDL cholesterol (mg/dl), insulin (ng/ml), and hepatic lipase (nmoles FFA per hour μl). *$P < 0.0001$ vs. heterozygote. ‡$P < 0.001$ vs. heterozygote. §$P < 0.01$ vs. heterozygote. ‖$P < 0.05$ vs. heterozygote. ¶$P < 0.0001$ vs. BB alleles. **$P < 0.001$ vs. BB alleles. ‡‡$P < 0.01$ vs. BB alleles. §§$P < 0.05$ vs. BB alleles.

mans and mice have revealed that hepatic lipase activity is associated with both body fat and plasma HDL levels (32). Therefore, we tested whether any of the four obesity loci contributed to the levels of hepatic lipase activity. As shown in Fig. 5, the *Mob5* and *Mob6* loci exhibited linkage with hepatic lipase, with peak lod scores of 4.2 and 2.8, respectively. Neither of the other two loci exhibited significant or suggestive linkage with hepatic lipase activity. Hepatic lipase activity at the *Mob5* and *Mob6* loci exhibited codominant inheritance, with mice homozygous for CAST alleles exhibiting the lowest levels and those homozygous for the B6 allele exhibiting the highest levels (Table IV).

A locus for body size on chromosome 15. Body length, measured as anal to nasal distance, ranged from 7.8 to 10.6 cm in the F2 animals at time of sacrifice. A locus centered near the chromosome 15 marker *D15Mit54* gave a significant lod score of 4.3 for body length, and coincident with this was a QTL for body weight (lod score = 2.5). Although this locus did not exhibit significant linkage for percent body fat or body fat depots, it was of interest since QTLs for body fat have been observed in this region in two other studies (18, 19).

Discussion

We report here the identification of four loci contributing to body fat stores in a genetic cross between strains CAST and B6. We previously reported the identification of four loci contributing multigenic obesity in a cross between the SPRETUS and B6 strains, and we designated these loci *Mob1*, (chromosome 7), *Mob2* (chromosome 6), *Mob3* (chromosome 12), and *Mob4* (chromosome 15). The designation *Mob* refers to multigenic obesity (18). Subsequently, in a cross between strains

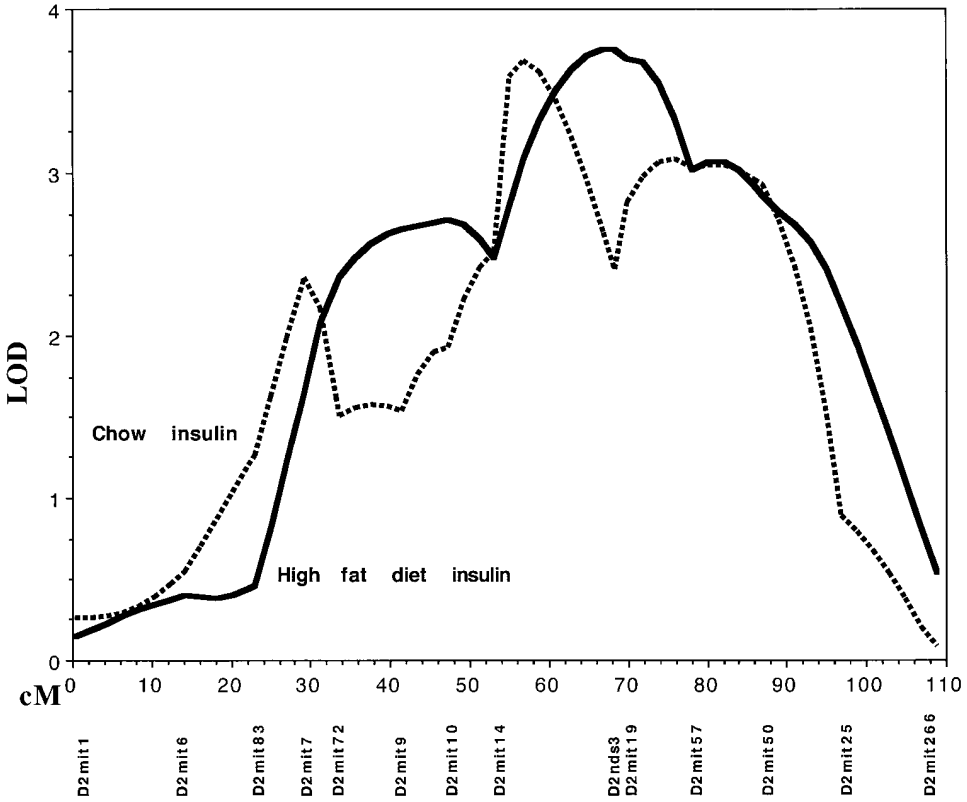

Figure 4. Lod scores on chromosome 2 for insulin levels on a chow diet and a high-fat diet. Suggestive lod scores for plasma insulin levels were observed at each of the three Chromosome 2 obesity loci presented in Fig. 3. The insulin levels were observed before and after placing the mice on a high fat diet for 5 wk and did not differ significantly.

NZB and SM, we identified an obesity locus on distal chromosome 2, which we referred to as *Mob5* (12). This latter locus coincided with the distal chromosome 2 locus observed in this study. In keeping with that nomenclature, we designate the present loci as *Mob5* (distal chromosome 2), *Mob6* (central chromosome 2), *Mob7* (proximal chromosome 2), and *Mob8* (chromosome 9). West and colleagues (19, 20) previously reported loci for diet-induced obesity in a cross between strains

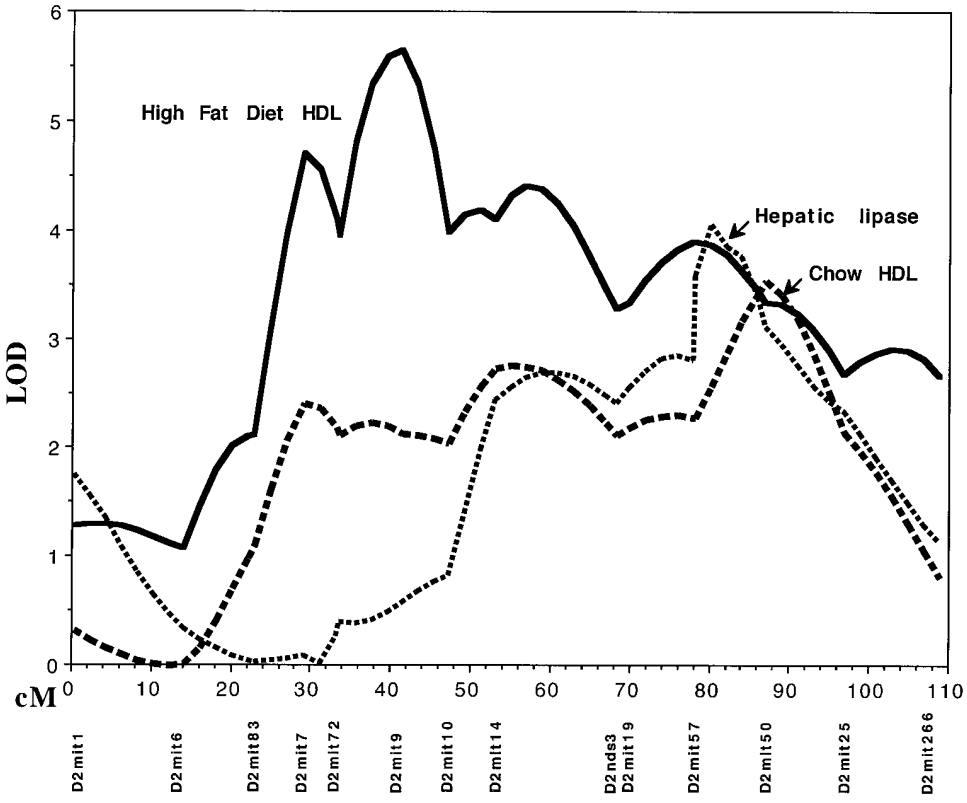

Figure 5. Chromosome 2 obesity loci contribute to variations in HDL levels and hepatic lipase activity levels. HDL levels were determined for mice maintained on a chow diet (chow HDL) or on a high-fat diet (high-fat diet HDL), and hepatic lipase activities were determined on a chow diet (hepatic lipase).

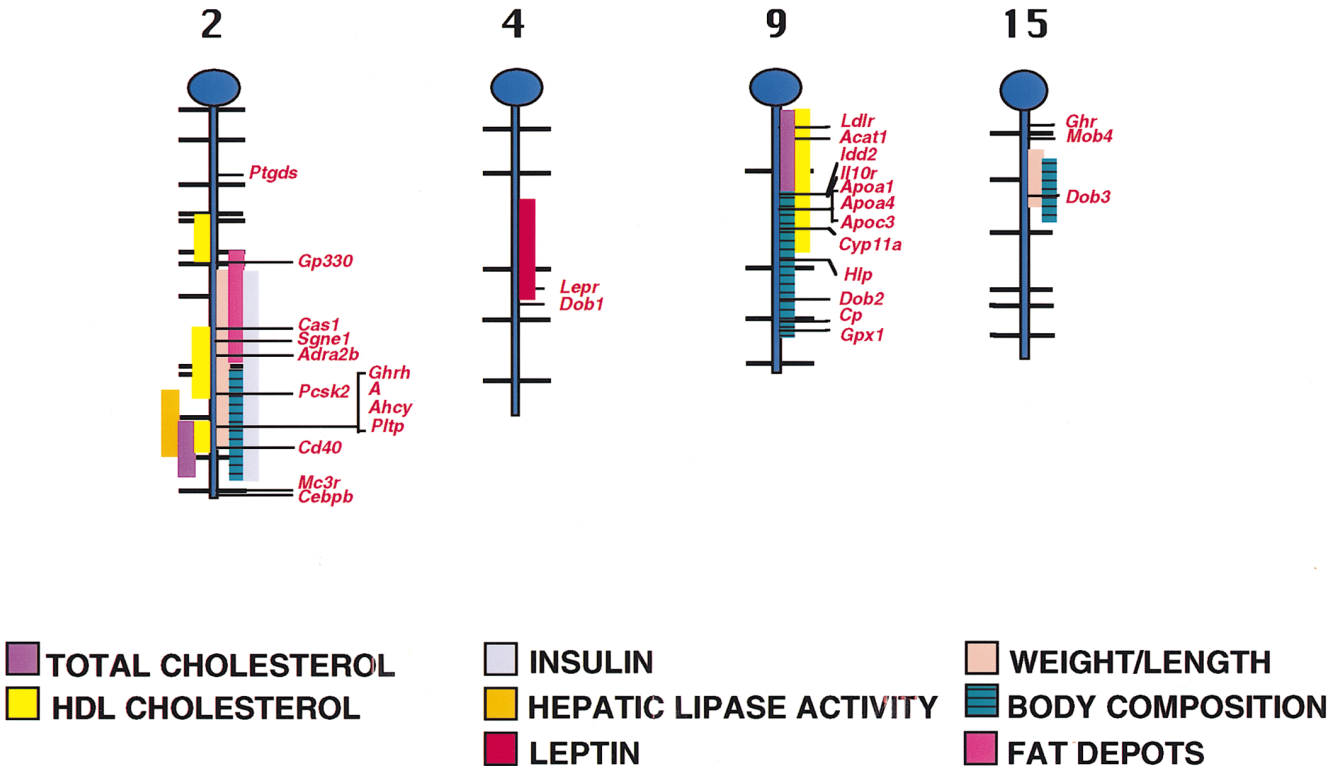

Figure 6. Summary of QTLs and positional candidate genes for (B6 × CAST)F2 mice. The locations (95% confidence intervals) for various QTLs are shown. *Bars*, the levels of total cholesterol, HDL cholesterol, insulin, hepatic lipase activity, leptin, either weight or length (weight/length), percent of body weight as fat (body composition), and weights of the fat depots examined. Gene symbols shown: chromosome 2: *Ptgds* (prostaglandin–endoperoxidase synthase), *Gp330* (glycoprotein-330), *Cas1* (catalase1), *Sgne1* (secretory granule neuroendocrine protein 1), *Adra2b* (adrenergic receptor, α-2b), *Pcsk2* (proprotein convertase subtilisin/kexin type 2), *Ghrh* (growth hormone–releasing hormone), *A* (agouti), *Ahcy* ([S]-adenosylhomocysteine hydrolase), *Pltp* (phospholipid transfer protein), *Cd40* (CD40 cytokine receptor), *Mc3r* (melanocortin 3 receptor), *Cebpb* (CCAAT/enhancer binding protein [C/EBP], β). Chromosome 4: *Lepr* (leptin receptor). Chromosome 9: *Ldlr* (LDL receptor), *Acat1* (acetyl-CoA acyltransferase), *Il10r* (IL-10 receptor), *Apoa1* (apolipoprotein-AI), *Apoa4* (apolipoprotein-AIV), *Apoc3* (apolipoprotein-CIII), *Cyp11a* (Cytochrome P450, 11a), *Hlp* (hepatic lipase), *Cp* (ceruloplasmin), *Gpx1* (glutathione peroxidase). Chromosome 15: *Ghr* (growth hormone receptor). In addition, the location of previously observed diabetes/obesity loci are noted: *A*, *Dob1*, *Dob2*, *Dob3*, *Idd2*, and *Mob4*.

AKR and SWR on mouse chromosome 9 that overlaps with the *Mob8* locus. Similarly, Taylor and Phillips (23) observed an obesity QTL in a cross between AKR/J and C57L/J that overlaps with *Mob6* on chromosome 2. These data clearly demonstrate that genetic factors determine the location of fat deposition and specific interactions between body fat, insulin metabolism, and lipoprotein metabolism, consistent with human studies (31–34). These data also provide information about candidate genes for molecular studies of body fat homeostasis. Fig. 6 summarizes the highly significant loci identified (95% confidence intervals). Within or near these regions are a number of candidate genes with possible roles in obesity or regulation of plasma lipoprotein metabolism. These include: (*a*) receptors for possible regulatory signals (*Adra2b* [adrenergic receptor, alpha 2b], *Mc3r* [melanocortin receptor], *Ghr* [growth hormone receptor], *Ghrh* [growth hormone–releasing hormone], *Lepr* [leptin receptor], *Il10r* [IL-10 receptor]); (*b*) transcriptional regulatory molecules (*Cebpb* [CCAAT/enhancer-binding protein-β {C/EBP}]); (*c*) proteins that play a role in lipid transport (*Pltp* [phospholipid transfer protein], *Apoa1* [apolipoprotein A-I], *Apoa4* [apolipoprotein A-IV], *Apoc3* [apolipoprotein CIII], *Hpl* [hepatic lipase], *Ldlr* [LDL receptor]); and (*d*) enzymes in lipid metabolism (*Acat1* [acetyl CoA acetyltransferase 1]). Several of these positional candidate genes are attractive but, with the exception of agouti, none have clearly established involvement with differences in body fat homeostasis. Finer mapping of the loci using congenic strains or other approaches (35) will allow exclusion of most of these candidates. Remaining candidates can then be tested using biochemical or transgenic studies. As discussed below, the loci overlap with several previously identified obesity-related QTLs.

The three separate loci that we propose on chromosome 2, *Mob5*, *Mob6*, and *Mob7*, are based on the shapes of the QTL peaks for fat-pad weights that appear to colocalize with QTL peaks for other obesity-related traits including hepatic lipase activity, HDL cholesterol, and insulin levels in mice fed different diets. In some cases, higher-resolution mapping may ultimately resolve the loci for fat pad weights from the loci for the obesity-related traits. While larger numbers of animals in the cross might eventually refine the QTL peaks, our approach to this problem is to generate congenic mice carrying the individ-

ual *Mob* loci. While *Mob* congenics will take several years to construct, they, and subcongenics derived from them will be much more powerful tools in studying the metabolism of obesity, specifically allowing us to identify whether fat-pad weights and the related traits are the separate effects of a single gene or are independently determined by nearby but separate genes. Also, it is possible that the observed QTLs are, in some sense, specific to the high-fat diet that was used in these experiments and that other diets might reveal other QTLs or obscure one or more of the loci presented here. However, obesity is the major focus of this study and, while we expect major changes in serum lipoprotein profiles over the course of an 8-wk high-fat diet, it seems likely that accumulated body weight is more reflective of a lifetime on chow diet than of the relatively short exposure to high fat.

Including the loci reported here, a total of over a dozen loci contributing to multigenic forms of obesity have now been identified in genetic studies in mice (Table V). The picture that emerges is one in which a large number of separate genes contribute to differences in body fat among inbred strains. At present, results have been reported for at least six separate genetic crosses with mice, some involving strains, such as CAST and SPRETUS, that widely diverge from most laboratory strains. Although additional loci are likely to be identified in future studies, the fact that several of the loci were observed in separate independent crosses (loci on chromosomes 2, 7, 9, and 15) suggests that most of the loci responsible for common variations of body fat among laboratory mice have now been identified. A similar level of genetic complexity might be expected in human populations. Clearly, these genetic loci represent only those with the largest impact on body weight and there are undoubtedly numerous additional loci that exert small effects on body fat but cannot be detected using the number of animals investigated in these and previous studies. There are also a number of mendelian mutations that dramatically influence body fat homeostasis in mice, including "agouti" on chromosome 2, "diabetes" on chromosome 4, "obese" on chromosome 6, "tubby" on chromosome 7, and "fat" on chromosome 8 (3). Some of these occur in chromosomal regions contributing to multigenic variations in body fat and thus may represent more dramatic mutations of the same underlying genes. Each of the genes underlying the above mendelian mutations has now been cloned and can be examined for possible involvement in multigenic obesity. Recently, a mutation of the prohormone convertase 1 gene, which underlies the mouse *fat* mutant, was discovered in an obese human subject (36). Several human syndromes feature obesity as a characteristic feature (37), but, in general, these do not appear to correspond to the mouse mendelian mutations; however, Prader–Willi syndrome, an obesity locus in humans, is syntenic to *Mob1*, the multigenic obesity locus on distal mouse chromosome 7 identified in a genetic cross between SPRET and B6 (18).

An interesting feature of the genetic control of body fat in mice is the specificity of the individual loci with respect to the location of fat deposition and effects on lipoprotein metabolism and insulin metabolism. In the present cross, one of the loci (*Mob5*) was observed to control total body fat whereas the others acted primarily on specific fat depots. Three of the loci (*Mob5, Mob6*, and *Mob7*) contributed to differences in insulin levels and lipoprotein metabolism, whereas *Mob8* had little or no effect on either (Table IV). These data are similar to those we previously reported in the BSB mouse model for multigenic obesity, where loci on chromosomes 6, 7, 12, and 15 exerted specific effects on the location of body fat stores and lipoprotein metabolism (18). They are also consistent with human obesity, as humans exhibit marked differences in the sites of fat accumulation (31–34, 38–41). Moreover, there are strong sex differences in fat metabolism whose mechanisms are unclear. Thus, in both mice and humans, females and males show significantly different sites and extent of fat deposition. In this study (Table II), insulin levels were significantly correlated in males with percent body lipids, total cholesterol, and HDL cholesterol, whereas in females, insulin levels failed to show significant correlation with any of the measures of obesity or fat metabolism.

In humans, low HDL levels are associated with obesity, especially abdominal fat, and insulin resistance (32). In contrast to humans, however, fat stores in mice are positively correlated with HDL levels. This presumably reflects a metabolic difference between the species. One potential explanation relates to cholesteryl ester transfer protein, which is not present in significant amounts in mice. Recent studies have shown that obese human subjects have elevated cholesteryl ester transfer protein compared with nonobese controls, providing an attractive possible mechanism for the reduction in HDL cholesterol in obesity (42). Other potential mechanisms linking obesity and lipoprotein metabolism may be related to lipoprotein lipase expression and hepatic lipase. Both are altered in obesity in humans, and our present studies have revealed coincident loci for body fat, hepatic lipase activity, and HDL cholesterol levels. The interaction of obesity with hepatic lipase expression appears to be specific, since it was not observed in all of the major loci detected. We previously observed coincident QTLs for hepatic lipase, body fat and HDL levels on distal mouse chromosome 7. Significant linkage with obesity was not observed at the chromosome 7 locus in the present cross, but, nevertheless, a suggestive QTL for hepatic lipase activity (lod score > 3) was observed in the region (data not shown).

Table V. Quantitative Trait Loci for Obesity-related Traits (Fat Pad Weights or Percent Body Weight as Fat) in Various Mouse Genetic Studies

Cross	Symbol	Chromosome	LOD	Reference
C57BL/6J × *M. spretus*	*Mob1*	7	4.2	18
	Mob2	6	4.8	
	Mob3	12	4.8	
	Mob4	15	3.4	
AKR/J × SWR/J	*Dob1*	4	4.5	20
	Dob2	9	4.8	19
	Dob3	15	3.9	19
129/SvJ × EL/Suz	*Obq1*	7	8.0	21
	Obq2	1	5.5	
AKR/J × C57L/J	*Obq3*	2	5.1	23
	Obq4	17	4.6	
NZB/BINJ × SM/J	*Mob5*	2	5.2	12
C57BL/6J × CAST/Ei	*Mob5*	2	5.7	This study
	Mob6	2	7.3	
	Mob7	2	5.7	
	Mob8	9	4.7	

Whether hepatic lipase expression mediates, in part, the effect of obesity on lipoprotein levels is unclear. Hepatic lipase activities in F2 animals ranged from 7.6 to 81 mU (nmoles free fatty acids per hour per microliter). These levels differed with genotypes at each of the three obesity loci influencing plasma lipoprotein levels (Table IV), but in contrast to expectations from studies with mice in which the hepatic lipase gene was inactivated by targeting (43), elevated hepatic lipase activities were associated with elevated HDL levels (Table IV). The very strong correlation between hepatic lipase levels and leptin levels ($p = -0.41$ in females and $r = -0.59$ in males) and between hepatic lipase levels and insulin levels ($r = 0.64$ in females and $r = 0.53$ in males) suggests that leptin or insulin may influence hepatic lipase expression. Leptin and insulin would appear to influence hepatic lipase expression independently, since leptin levels and insulin levels were not correlated ($r = -0.04$ in females and $r = 0.09$ in males).

In contrast to a number of previous studies (29), we did not observe a strong correlation between body lipid stores and circulating leptin levels. The major locus contributing to leptin levels was located on chromosome 4, and this locus was not significantly linked to body fat. Interestingly, however, the locus contained, within the 95% confidence interval, the gene for the leptin receptor (*db*). The leptin receptor gene product exhibits alternative splicing, and some of the resulting polypeptides are expressed in peripheral tissues or are secreted, whereas some lack a carboxyl-terminal domain required for signaling upon leptin binding. Thus, the various splice forms of the receptor may mediate leptin levels and leptin signaling by means of nonproductive interactions (44, 45). We are presently examining the leptin receptor gene in CAST and B6 mice to determine whether variations of the gene may underlie the QTL.

Because of the homology of human and rodent chromosomes (46), it is in some cases possible to predict the chromosomal locations of the human loci corresponding to mouse multigenic obesity loci. As yet, few of the corresponding human loci have been examined for genetic contributions to body fat. Recently, however, we examined a region of the long arm of human chromosome 20 which corresponds to the distal chromosome 2 locus for body fat presented here (*Mob5*) and observed significant linkage with measures of body fat and insulin levels (12). Also, a human locus on chromosome 20q (47) has recently been implicated in susceptibility to non–insulin-dependent diabetes and may correspond to one of the chromosome 2 *Mob* loci reported here. Thus, the loci in mice will provide a means of identifying and characterizing some, but probably not all, loci contributing to common forms of obesity and related phenotypes in humans. Murine QTLs are also proving useful in evaluating candidate genes for obesity, an outstanding example being the recent report describing a novel uncoupling protein (UCP-2) not restricted to brown adipose tissue, which maps near QTLs for body fat on mouse chromosome 7 (22). A number of large scans of human families for obesity-related and type 2 diabetes-related traits have been performed. Although some putative loci have been identified, most of these remain to be confirmed. It is interesting to speculate that if the complexity of these traits in human populations approaches that in mouse populations, with at least 10 and, most likely, considerably more major loci contributing to common variations in body fat, the dissection of obesity and type 2 diabetes in humans will be a very formidable task.

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