Genetic Determinants of Energy Expenditure and Insulin Resistance in Diet-Induced Obesity in Mice

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Diet-induced obesity is the primary determinant of the current epidemic of diabetes. We have explored the role of genetics in this phenomenon, using C57Bl/6 (B6), 129S6/SvEvTac (129), and intercross (B6 × 129)F2 mice on a low- or high-fat diet. Over an 18-week period, B6 and F2 mice gained more weight, had higher levels of insulin and leptin, and showed greater glucose intolerance than 129 mice, despite lower food intake. By contrast, metabolic rate and diet-induced thermogenesis were significantly higher in the 129 mice. Genome-wide scans identified several quantitative trait loci, including a quantitative trait locus that was linked with hyperinsulinemia/insulin resistance on chromosome 14 in a region similar to that seen in mice with genetically induced insulin resistance. Microarray analysis indicated significant changes in expression levels between B6 and 129 mice in the identified chromosomal area of Wnt5a and protein kinase Cδ (PKCδ). Thus, caloric efficiency, i.e., the "thrifty gene," is a dominant-acting genetic determinant of diet-induced obesity in mice and can be linked to a locus on chromosome 14, including genes linked to adipose development and insulin sensitivity. *Diabetes* 53:3274–3285, 2004

There is a worldwide epidemic of obesity and type 2 diabetes (1). This is being driven, at least in part, by the westernization of diet with high-fat and higher caloric intake (2). The impact of these changes in dietary pattern, however, varies dramatically depending on the genetic background of the population exposed, with higher rates of diabetes and obesity in Hispanic, black, and Native-American groups. Defining the background genes that modify this response is central to understanding the pathogenesis of these conditions.

Background genes play a major role in determining the phenotype of mice that are subjected to different diets (3,4), mice with naturally occurring mutations leading to obesity and diabetes (5,6), and mice that have been genetically altered by introduction of transgenes or knockout of endogenous genes (7,8). This complicates both the interpretation of genotype-phenotype relationships and the understanding of what is due to the primary genetic alteration and what is due to background genes or environmental modifiers (7,9).

Several studies have suggested that C57Bl/6 (B6) mice are "obesity prone" as well as "susceptible to insulin resistance and glucose intolerance," whereas other strains, such as C3H/He, 129/Sv, and A/J mice, are resistant to obesity and diabetes (3,4,10–13). We have previously shown that mice with a double-heterozygous deletion of the insulin receptor and insulin receptor substrate-1 (IRS-1) become markedly insulin resistant and hyperinsulinemic on the B6 background and that >90% of the mice develop diabetes by 6 months of age, whereas on the background of 129S6/SvEvTac (129), <2% of the double heterozygote mice become diabetic by 6 months, and the mice develop only a mild hyperinsulinemia (14). Using an intercross (B6 × 129)F2 generation, a genome-wide scan of the IR/IRS-1 double heterozygous mice revealed a locus with linkage to hyperinsulinemia on chromosome 14, a locus linked to hyperleptinemia on chromosome 7, and two loci with linkage to hyperglycemia on chromosomes 14 and 12, respectively (15). It is interesting that the donor strain in all four cases was B6. Recently, Colombo et al. (16) showed that mice with lipoatrophic diabetes have less insulin resistance in muscle and more insulin-resistant livers on a B6 background than on an FVB background, possibly as a result of differences in hepatic triglyceride clearance, although the exact loci contributing to this difference have not been mapped.

In the present study, we explored in detail how high-fat diet–induced insulin resistance affects the B6 and 129 strains, as well as an F2 intercross between these strains, and asked whether the genes that control diet-induced insulin resistance are the same as those that control genetic insulin resistance and are dominant-acting genes and whether these genes could be identified using a combination of genetics and genomics.

RESEARCH DESIGN AND METHODS

F2 mice were obtained by breeding wild-type C57Bl/6 (B6) mice with 129 wild-type mice (strain 129S6/SvEvTac) from Taconic (Germantown, NY) to create an F1 outcross generation. The F1 generation was then intercrossed in a random manner to obtain a total of 100 F2 male mice. Fifty intercross F2 mice, 8 B6, and 8 129 male mice were maintained on a low-fat diet that contained 14% of calories from fat, 25% from protein, and 61% from carbohydrates (Taconic), and an equivalent number of mice were fed a high-fat diet containing 55% fat, 21% protein, and 24% carbohydrates (Harlan Teklad, Madison, WI). All mice were kept on the low-fat diet from the time of weaning until the end of puberty (at the age of ~6 weeks), when the feeding study was initiated. The mice were maintained on the various diets for at least 18 weeks. Food intake was measured by placing the mice in individual cages and weighing the food every third day over 9 days. The mice were maintained on a 12-h light-dark cycle. All protocols for animal use were reviewed and

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IRS-1, insulin receptor substrate-1; LOD, logarithm of odds; LRS, likelihood ratio statistic; PKC, protein kinase C; QTL, quantitative trait locus.

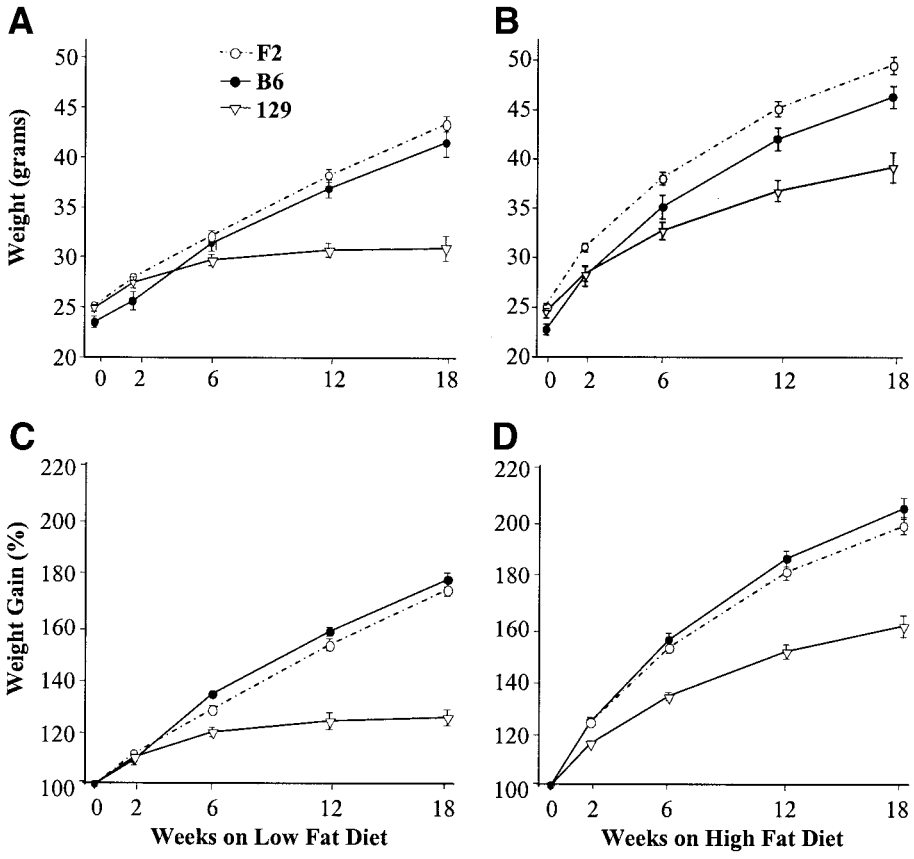

FIG. 1. Body weight and percentage weight gain of mice that were fed a low-fat and a high-fat diet. The weight of intercross F2 (○), B6 (●), and 129 (△) mice was measured during a period of 18 weeks on a low-fat (*A*) and high-fat (*B*) diet. Weight gain calculated as percentage of initial weight in B6, 129, and F2 mice on low-fat (*C*) and high-fat (*D*) diets. Results are expressed as mean ± SE. B6 (n = 8), 129 (n = 8), and F2 (n = 50) mice.

approved by the Animal Care Committee of the Joslin Diabetes Center and were in accordance with the National Institutes of Health guidelines.

Phenotype analysis. Weight, blood glucose, plasma insulin, and plasma leptin were measured at 0, 2, 6, 12, and 18 weeks. Fasted serum free fatty acids were measured at week 0 and week 18. Blood glucose values were determined from whole venous blood using an automatic glucose monitor (Glucometer Elite; Bayer, Mishawaka, IN). Insulin and leptin levels were measured in plasma samples by enzyme-linked immunosorbent assay using mouse insulin and mouse leptin as standards (Crystal Chem, Chicago, IL). Glucose tolerance was performed in the mice after the 18-week period (mice were ~7 months old) by intraperitoneal injection of 2 g glucose/kg body wt after an overnight fast. Insulin tolerance tests were performed in random-fed mice by injection of 1 unit insulin/kg body wt.

Indirect calorimetry and activity. Another set of B6 and 129 mice (n = 4 in each group) were maintained on low- or high-fat diet for ~6 weeks. The mice were placed individually in indirect calorimetry chambers (Oxymax OPTO-M3 system; Columbus Instruments, Columbus, OH). After 48 h to allow for adaptation to the metabolic chamber, O_2 consumption and CO_2 production were measured every 30 min for 48 h and activity was measured as beam break counts. During the first 24 h, the mice had free access to food and water, and during the second 24 h, the mice had access to water only.

Genome-wide scan in 100 F2 intercross mice. DNA was prepared from tail tips as previously described (15). The mice were genotyped using 114 polymorphic markers covering the 19 autosomal chromosomes and the X chromosome (Research Genetics, Huntington, AL) with a proposed average distance of 20 cM. Genotypes were scored using 4% agarose gels, and this was done blindly. Any ambiguous results were repeated in an independent analysis. Marker linkage maps were generated with our own data and the Celera database and compared with the public maps at the Mouse Genome Database. Reasonable agreement was found with these mouse databases, although the exact position of markers sometimes deviated slightly from the positions identified by genome-wide sequence analysis.

RNA isolation, cRNA preparation, array hybridization, and analysis. Skeletal muscle, liver, and epididymal fat pads were removed from B6 and 129 mice at the age of 6 months after the mice were maintained on a standard diet that contained 21% of calories from fat, 22% from protein, and 57% from carbohydrates. Total RNA was extracted and purified with RNeasy (Qiagen, Chatsworth, CA), and a total of 25 μg pooled from two to three mice was used for cRNA synthesis (17). A total of 15 μg of cRNA was hybridized on Affymetrix (Santa Clara, CA) murine chips U74Av.2 that contain 12,488 genes or expressed sequence tags with ~30–45% present on the chips. Three to four chips were used for each strain, and the data were normalized to 1,500 using the GeneChip software MAS 5.0. Genes in Table 2 were obtained using Celera Discovery System (Rockville, MD).

Statistical analysis. Data analysis was performed using MapMaker/EXP 3.0 and MapMaker/QTL 1.1 (18–20) as well as Map Manager version QTXb20 (21). MapMaker/QTL calculates the strength of the association between markers and traits as the log base 10 of the odds ratio (logarithm of odds [LOD] score). The permutation test (10,000 permutations) from Map Manager estimates an empirical genome-wide probability for a given likelihood ratio statistic (LRS). SPSS for Windows (version 11.5) was used for the other statistical analyses. $P < 0.05$ (two-tailed) was considered significant. Insulin levels in the F2 mice on both low- and high-fat diet deviated from a normal distribution and were log transformed for the quantitative trait locus (QTL) analysis.

RESULTS

Body weight gain. Parallel groups of ~6-week-old B6 (n = 8), 129 (n = 8), and intercross (B6 × 129)F2 (n = 50) male mice were placed on a low-fat (14%) or high-fat (55%) diet (see RESEARCH DESIGN AND METHODS) for an 18-week period. Although starting body weights in all three groups were almost identical, during the 18 weeks of study, the F2 and B6 mice gained ~18 g on the low-fat diet and 24 g on the high-fat diet, whereas the 129 mice gained only 6 g and 14.5 g on the two diets, respectively (Fig. 1). Indeed, the B6 and F2 mice gained more weight on a low-fat diet than the 129 mice gained even on the high-fat diet. The difference in weight gain between the low- and high-fat diets, however, was higher for the 129 strain (gained 35% more weight on the high-fat versus low-fat diet) than the B6 (gained 27% more weight on the high-fat versus low-fat diet). Thus, the B6 mouse gains more weight than the 129 independent of diet but is less sensitive to changes in dietary fat.

The variance is a measure of the spread of a distribution about its average value; thus, the larger the variance, the

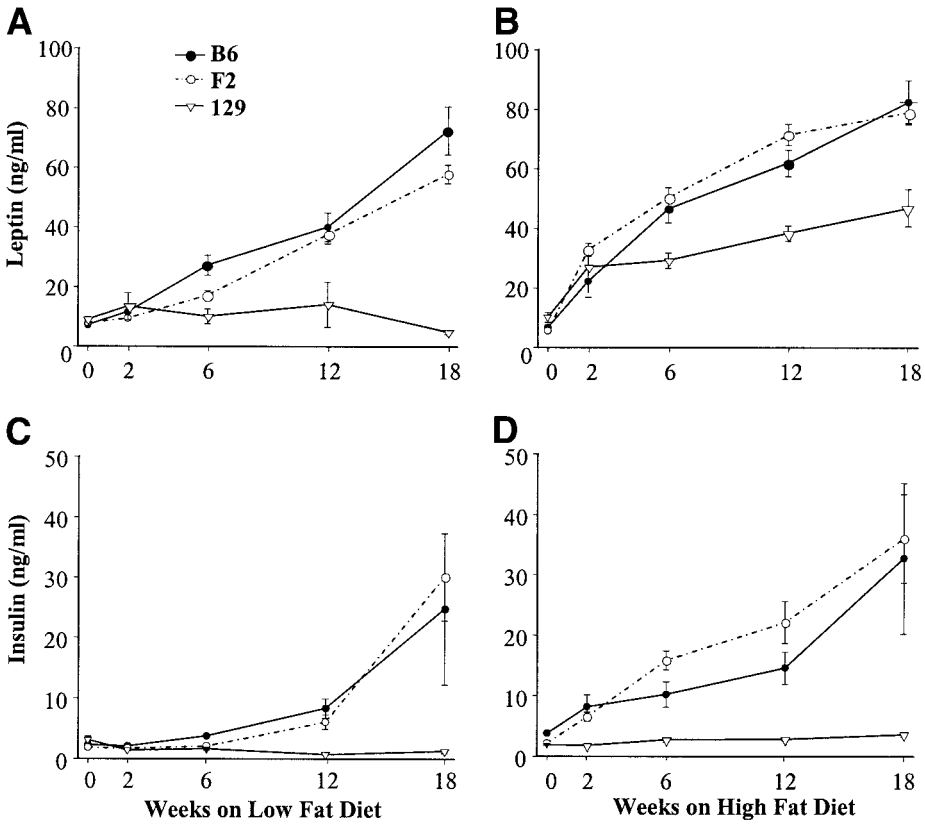

FIG. 2. Effect of diet on leptin and insulin levels. Random-fed plasma leptin levels of B6 (●), intercross F2 (○), and 129 (△) mice were measured over 18 weeks on a low-fat (*A*) and high-fat (*B*) diet, and insulin was measured in the same way (*C* and *D*). Results are expressed as mean ± SE. B6 (n = 8), 129 (n = 8) and intercross F2 (n = 50) mice.

more heterogeneous is the group. At week 18 on a low-fat diet, the variance of the percentage weight gain was 307, 55, and 36% for the F2, B6, and 129 mice, respectively, whereas on the high-fat diet, the variance was 534, 118, and 132% for the F2, B6, and 129 mice, respectively. The larger variance in the F2 mice confirms the effects of the mixed genetic background. The larger variance on the high-fat versus low-fat diet also suggests a larger degree of heterogeneity among mice on factors such as individual dietary intake, activity, etc.

The fact that the F2 mice show patterns of average weight gain almost identical to those of B6 suggests that B6 mice carry dominant genes in the control of body weight between these strains. In all cases, circulating leptin levels, which generally reflect adipose mass (22), paralleled the changes in body weight (Fig. 2*A* and *B*).

Effect of diet on glucose tolerance and insulin sensitivity. Both the F2 and the B6 mice showed an increase in insulin during the 18 weeks that was greater than that in the 129 mice, indicating higher levels of insulin resistance (Fig. 2*C* and *D*). This increase occurred faster when the mice were maintained on the high-fat diet. The variance of the insulin levels on low-fat diet at week 18 was 2,593, 1,251, and 0.4 ng/ml, and on high-fat diet it was 2,649, 1,242, and 1.3 ng/ml for the F2, B6, and 129 mice, respectively. Thus, the F2 mice show the effects of the mixed genetic background, and after 18 weeks, the spread of insulin was independent of the fat content of the diet. The random-fed plasma glucose levels on either diet were highest in the B6 mice (~150 mg/dl), slightly lower in the F2 mice (~140 mg/dl), and considerably lower in the 129 mice (~105 mg/dl), and these did not change during the 18 weeks. Glucose tolerance tests performed after 18 weeks on the test diets revealed impaired glucose tolerance in B6 mice on both low- and high-fat diets. In contrast, the 129 mice showed impaired glucose tolerance only on the high-fat diet (Fig. 3*A*). The response to exogenous insulin during the insulin tolerance was also reduced in B6 mice on either diet, indicating greater insulin resistance in this strain (Fig. 3*B*).

Indirect calorimetry and activity. The increased weight gain of the B6 strain could potentially be due to a higher level of food intake or decreased energy expenditure. To decide which factors were most important, we subjected B6 and 129 mice to indirect calorimetry after they were maintained on the various diets for ~6 weeks (age of ~12 weeks) and caloric intake was assessed. Despite the higher level of weight gain, B6 mice ate less than the 129 on the low-fat diet and a similar number of calories on the high-fat diet (Fig. 3*C*), indicating the higher caloric efficiency (i.e., grams of weight gained per gram of food intake) of the B6 strain (Fig. 3*D*). Consistent with this, the metabolic rate, measured as O_2 consumption, was 6% higher in the 129 mice than in the B6 mice during feeding on the low-fat diet, 5% higher during fasting on the high-fat diet ($P = 0.01$), and 14% higher ($P < 0.001$) during feeding on the high-fat diet (Fig. 4). In addition, metabolic rate showed significant increases in the 129 mice when changed from fasting to feeding (30% increase on low-fat diet and 10% increase on high-fat diet; Fig. 4*A*), whereas the B6 mice showed reduced thermic effect of food (Fig. 4). The higher metabolic rate of 129 mice was independent of activity. In fact, activity levels calculated by beam breaks over 24 h were ~50% lower ($P < 0.001$) in the 129 than in the B6 mice under all conditions (Fig. 5*A*). The respiratory quotient, an indicator of the relative rates of

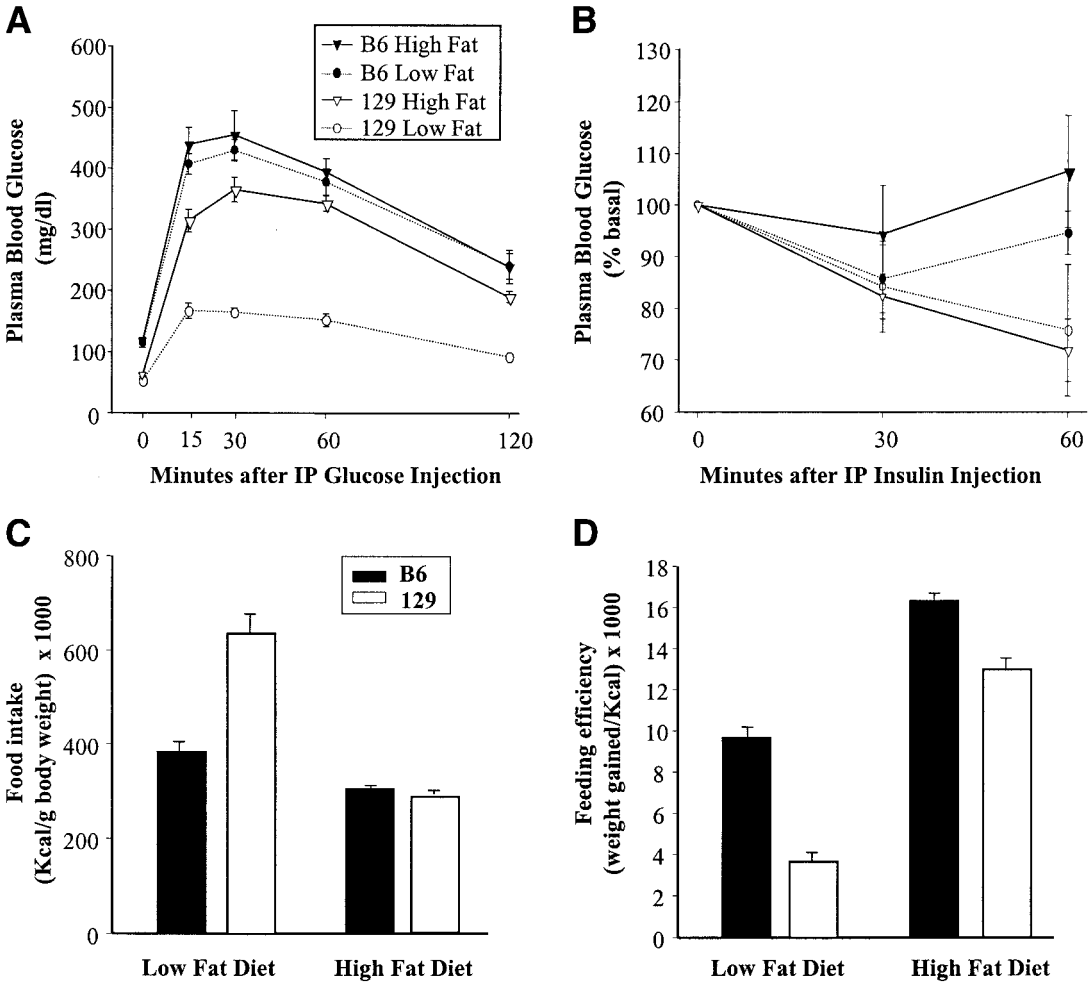

FIG. 3. Effect of diet on glucose and insulin tolerance. *A*: **Glucose tolerance tests were performed after an overnight fast in B6 and 129 mice on low- and high-fat diets, respectively. Animals received intraperitoneal injections of 2 g/kg body wt glucose. Blood glucose was recorded from tail-vein samples at 0, 15, 30, 60, and 120 min.** *B*: **Insulin tolerance tests were performed on random-fed B6 and 129 mice on low- and high-fat diets, respectively. Animals received intraperitoneal injections of 1 unit/kg body wt insulin. Blood glucose was measured at 0, 30, and 60 min. B6 on high-fat diet (▲), B6 on low-fat diet (●), 129 on high-fat diet (△), and 129 on low-fat diet (○). Data points are mean ± SE with eight mice in each group.** *C*: **Food intake determined as kcal/g body wt was measured in B6 (■) and 129 (□) on low- and high-fat diets. Feeding efficiency was calculated as weight gain/kcal of food intake in mice on both diets (*D*). Results are expressed as mean ± SE with four mice in each group.**

substrate oxidation (23), was significantly lower in B6 than in 129 mice during feeding on both a low-fat ($P = 0.027$) and high-fat ($P < 0.001$) diet and during fasting on the low-fat diet ($P < 0.001$), indicative of a higher percentage of fat oxidation in the B6 mouse, whereas the respiratory quotient was similar in both strains during fasting on the high-fat diet (Fig. 5*B*). When both B6 and 129 mice were shifted from fasting to feeding, there was a change in substrate oxidation from fat to carbohydrate, as expected (Fig. 5*B*).

Identification of QTLs in intercross (B6 × 129)F2 mice. F2 mice ($n = 100$) were genotyped using a total of 114 polymorphic markers covering the 19 autosomal chromosomes and the X chromosome. Linkage maps for weight, weight gain, glucose, insulin, and leptin at the five time points on either the low- or high-fat diet were constructed by using MapMaker/EXP; QTLs were calculated using MapMaker/QTL and MapManager (18,21); and permutations test were performed using MapManager (24). The QTLs with the highest LOD scores are described below and in Table 1 and depicted in Fig. 6.

A linkage map of chromosome 14, based on genotyping data of 15 markers in F2 mice, revealed a QTL for insulin levels at week 18 in mice on a low-fat diet with a peak at marker *D14Mit52* and a LOD score of 3.0, accounting for up to 25% of the variation of this trait (Fig. 6*A*, Table 1). The location of this QTL is virtually superimposable with the previously identified locus in the IR/IRS-1 double-heterozygous knockout mice (15). In the F2 generation, insulin levels of mice homozygous for the B6 allele at marker *D14Mit52* were 8.9-fold higher than the insulin levels of mice homozygous for the 129 allele ($P = 0.002$), and mice heterozygous at the marker were intermediate between the other two genotypes, suggesting a gene-dosage effect (Fig. 6*B*). A QTL with linkage to weight gain at week 18 was also found on chromosome 14 around marker *D14Mit192* with a LOD score of 3.0 (Fig. 6*A*, Table 1). Mice carrying two B6 alleles at marker *D14Mit192* gained 5.2 g (or 22.5%) more weight at week 18 than mice carrying two 129 alleles ($P = 0.007$). A third QTL on chromosome 14 at marker *D14Mit192* was found for glucose in mice that were maintained on a high-fat diet with a LOD score of 3.3 at week 12 (Table 1). For verifying the QTL analyses, tests with 10,000 permutations were

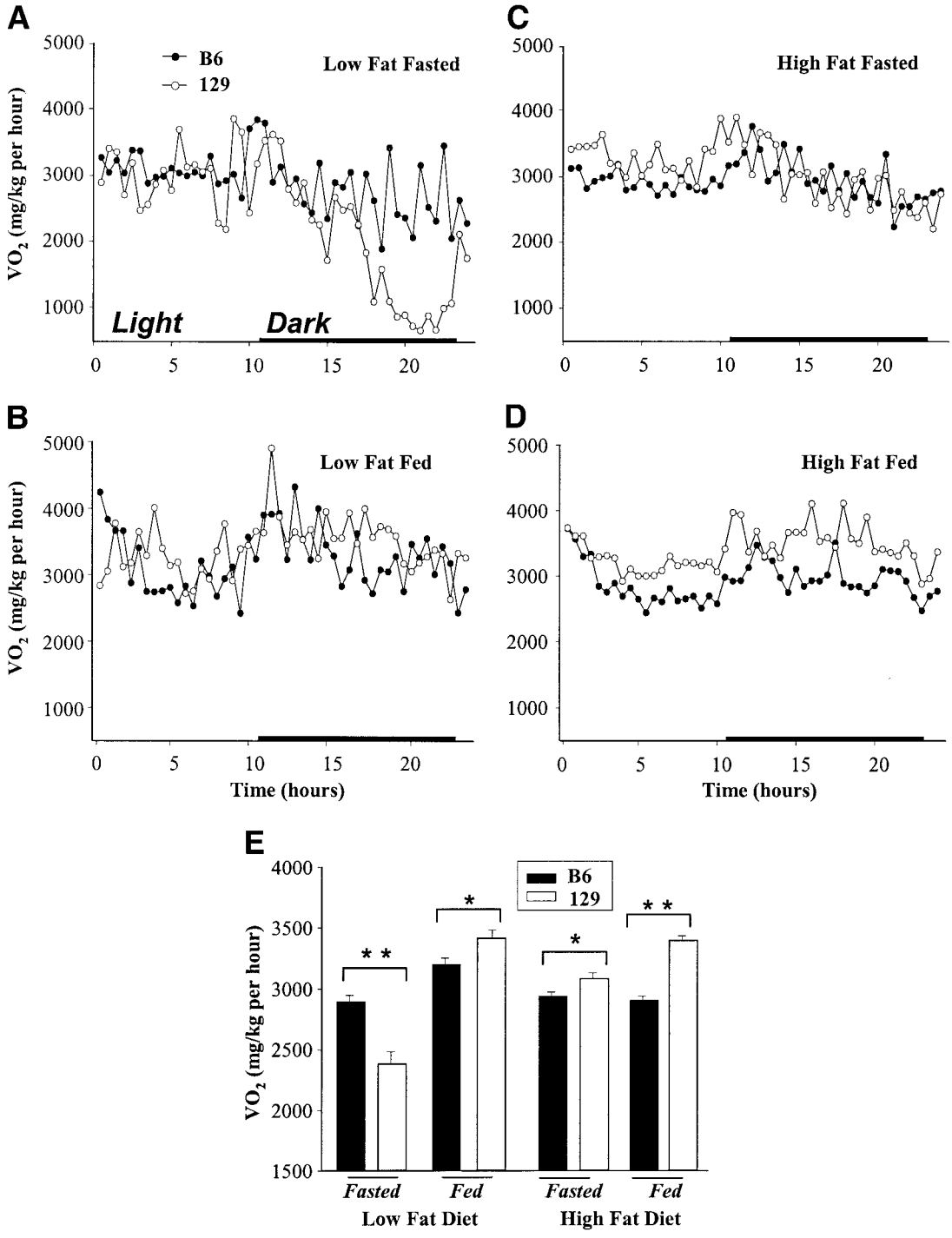

FIG. 4. Effect of diet on O_2 consumption. Oxygen consumption was measured by indirect calorimetry in B6 and 129 mice during 24 h of fasting (*A* and *C*) and 24 h of feeding (*B* and *D*) in mice that were maintained on either low-fat (*A* and *B*) or high-fat (*C* and *D*) diet. The thick black line on the abscissa denotes the dark period. *E*: The bars represent the calculated mean ± SE oxygen consumption during 24 h of feeding or fasting, respectively, in B6 (■) and 129 (□) mice. **P* = 0.01, ***P* < 0.001.

carried out using MapManager (21,24). The permutation test estimates an empirical genome-wide probability for a given LRS (21). The permutation tests confirmed that the identified QTL with linkage to insulin at *D14Mit52* as well as the QTLs at *D14Mit192* were significant (LRS score at *D14Mit52* for insulin = 11.3 with significance at 11.2, LRS score at *D14Mit192* for weight gain = 13.0 with significance at 11.4, and LRS score at *D14Mit192* for glucose = 14.8 with significance at 11.1).

Our previous results have suggested a locus on chromosome 12 (*D12Mit231*) with linkage to hyperglycemia and another on chromosome 7 linked to leptin levels (15). In the present study, there was a QTL on chromosome 12 (*D12Mit231*) with linkage to leptin at week 2 in mice that were fed a low-fat diet (peak LOD score of 3.2; Fig. 6*C*). Surprisingly, mice homozygous for the 129 allele exhibited 1.9-fold higher leptin levels than B6 homozygous mice (P = 0.005; Table 1), whereas the previously identified

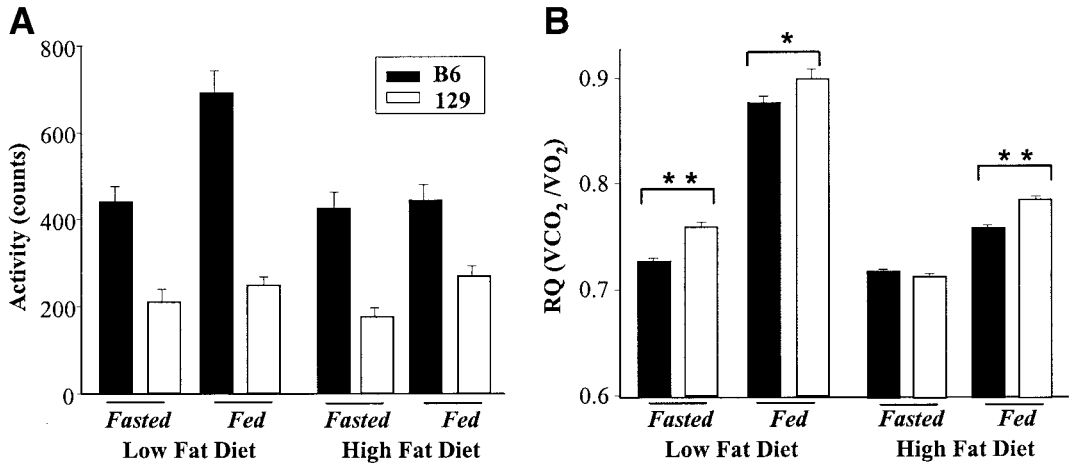

FIG. 5. Activity and respiratory quotient of fasted and fed B6 and 129 mice on low- and high-fat diets. *A*: Activity was counted as the number of times a beam was broken during fasting and feeding on low- and high-fat diets. The bars represent the mean activity during 24 h for B6 (■) and 129 (□) mice. *B*: Respiratory quotient was estimated during the 24 h of feeding or fasting, respectively. The bars represent the mean ± SE (n = 4). *P = 0.027, **P < 0.001.

locus on chromosome 7 linked with hyperleptinemia (15) was not statistically defined in these mice during diet-induced insulin resistance.

Five other QTLs were identified in the genome-wide scan (Table 1). These included a hyperleptinemia locus on chromosome 10 (Fig. 6*D*), and on chromosome 3, we identified two separate loci linked with hyperinsulinemia and hyperleptinemia in mice that were fed a low-fat diet for 12 weeks (Table 1, Fig. 6*E*). Insulin and leptin levels, respectively, were significantly higher in mice homozygous for the B6 alleles at these markers (P = 0.002). On chromosome 11, a locus was significantly associated with insulin after 6 weeks on high-fat diet (Fig. 6*F*), and finally on chromosome 15 (Fig. 6*G*), we identified a locus associated with fasting plasma blood glucose.

Expression analysis of genes located in identified chromosomal areas. To determine whether any of the annotated genes located in the chromosomal areas with QTLs for different phenotypes (Table 1) were differentially expressed between the B6 and 129 strains, we collected skeletal muscle, liver, and epididymal fat pads from 6-month-old mice that had been maintained on a standard diet containing 21.6% fat, extracted RNA, and performed Affymetrix microarray analysis. All of the genes located within 3 Mb of the marker with the highest LOD score (as shown in Table 1) that were present on the murine chip U74Av.2 are presented in Table 2 in the order of location on the chromosome. The interval of ±3 Mb was arbitrary but was chosen to focus on the differentially expressed genes closest to the LOD peak and probably represents approximately the 50% confidence interval for each QTL. Differential expression between B6 and 129 was observed of more genes than expected in the region of several of the chromosomal markers with significant LOD scores. For example, in the region within 3 Mb of *D14Mit52*, there were 23 annotated genes represented on the U72Av2 chip. Examining expression of these genes in liver, muscle, and fat demonstrated 11 differences in expression that were significant at the $P < 0.05$ or greater level. By random chance, one would have expected three or four significant differences at this level out of the 69 measured variables in the region. There were several interesting candidate genes among these 11, including the gene encoding PKCδ, which showed 22–57% upregulation in B6 mice in all examined tissues, with the largest change in adipose tissue reaching statistical significance. Wnt5a is also located under that peak and was expressed at lower levels in all tissues obtained from the B6 strain, with a significant decrease of 78% in skeletal muscle. Other potential candidate genes based on expression differences include nuclear receptor coactivator A; calcium channel, voltage-dependent, α2/δ subunit 3; and transketolase, all of which were lower in skeletal muscle of B6 mice as compared with 129. Inter-α trypsin inhibitor, heavy-chain 3, NIMA (never in mitosis gene a)-related expressed kinase 4, and stabilin were all lower in livers of B6 mice compared with 129, whereas transketolase was more highly expressed in this tissue in the B6 strain. The PHD (plant homeo domain) finger protein 7 was significantly higher in skeletal muscle of B6 mice.

A similar enrichment of differentially expressed genes was observed in the region of marker *D14Mit192*, where there were seven significant changes at the $P < 0.05$ level of 36 measured defined genes. These included a 2.6-fold higher level of expression of the tyrosine kinase substrate Dok2 in fat of B6 over 129 mice, as well as bone morphogenetic protein 1 and esterase 10. Significant differences in fat of 129 over B6 included erythrocyte protein band 4.9 and protein phosphatase 3, and the latter gene was also upregulated in skeletal muscle, whereas the expression of SH3-domain protein 4 was higher in muscle of B6 than 129. The large number of changes on chromosome 14 between these two strains of mice suggests the action of some global *cis*-acting regulator of gene expression, such as altered patterns of DNA methylation, chromatic binding, or chromatic structure in these regions between these two strains of mice.

DISCUSSION

The development of diabetes and the associated metabolic syndrome in response to either genetic or acquired defects in both rodents and humans is dramatically affected by background genes. In mice, this was first demonstrated by Coleman et al. (5) for the *ob/ob* and *db/db* traits and has

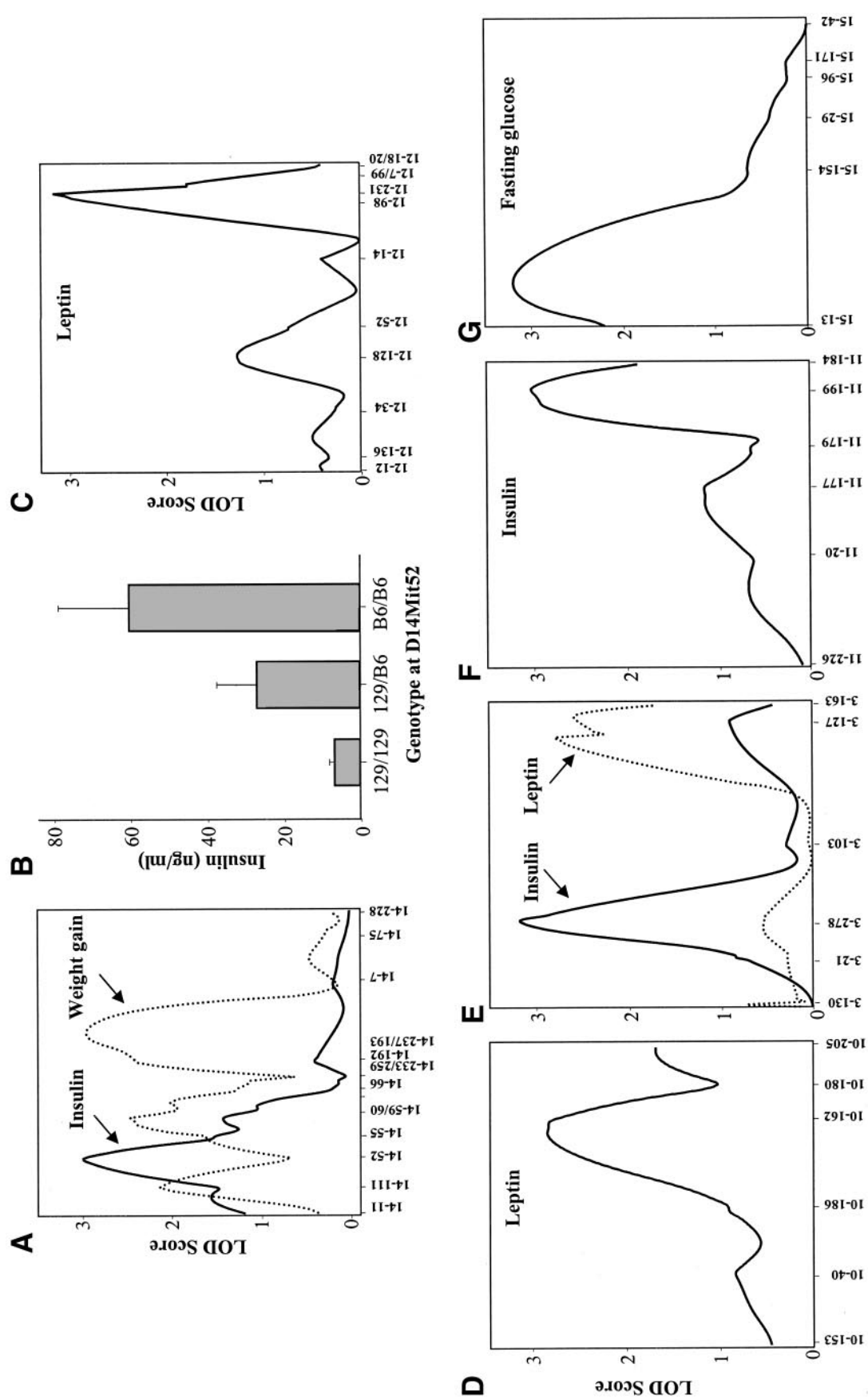

FIG. 6. QTL analyses of (B6 × 129)F2 mice on low- or high-fat diet. *A*: QTL analysis of genotyping data on chromosome 14 revealed a locus linked to insulin (solid line) at week 18 in intercross F2 mice at marker *D14Mit52* and a locus at marker *D14Mit192* linked with increased weight gain (broken line) on low-fat diet at week 18. *B*: Mice homozygous of the B6 allele around marker *D14Mit52* exhibited significantly higher insulin levels than mice homozygous of the 129 allele ($P = 0.002$). *C*: QTL linked with leptin in mice on low-fat diet at week 2 in the area of marker *D12Mit231*. *D*: QTL linked with leptin in mice on high-fat diet at marker *D10Mit162*. *E*: QTLs linked with hyperinsulinemia at *D3Mit278* and hyperleptinemia at *D3Mit127* in mice on low-fat diet at week 12. *F*: QTL linked with hyperinsulinemia in mice on high-fat diet at week 6 at marker *D11Mit199*. *G*: QTL linked with fasting hyperglycemia as measured after 19 weeks in mice on low-fat diet is located around marker *D15Mit13*.

TABLE 1
Results of QTL analysis of selected genotyping data

Chromosome	Marker	Trait, diet, duration	LOD score	Phenotypic variation (%)	Donor strain
3	*D3Mit278*	Hyperinsulinemia, low-fat diet, week 12	3.2*	29	B6
3	*D3Mit127*	Hyperleptinemia, low-fat diet, week 12	2.7*	78	B6
10	*D10Mit162*	Hyperleptinemia, high-fat diet, week 2	2.9*	28	129
11	*D11Mit199*	Hyperinsulinemia, high-fat diet, week 6	3.0*	30	B6
12	*D12Mit231*	Hyperleptinemia, low-fat diet, week 2	3.2*	25	129
14	*D14Mit52*	Hyperinsulinemia, low-fat diet, week 18	3.0*	25	B6
14	*D14Mit192*	Increased weight gain, low-fat diet, week 18	3.0*	52	B6
		Hyperglycemia, high-fat diet, week 12	3.3*	33	B6
15	*D15Mit13*	Fasting hyperglycemia, low-fat diet, week 19	3.3†	49	B6

Genotyping data were analyzed using MapMaker/QTL and MapManager. The indicated markers denote the marker closets to the peak. The LOD score values are according to MapMaker. *Significant LRS value and †suggestive significant LRS value according to MapManager. Phenotypic variation indicates the percentage of the phenotypic variation of the trait that can be explained by the locus.

been subsequently observed in several studies, including analysis of the impact of heterozygosity for insulin receptor and IRS-1 deletion in mice (14,25), the phenotype of genetically induced lipodystrophy (16), and diet-induced obesity (26). In the insulin receptor/IRS-1 double-heterozygous knockout, we have previously shown that the background genes of B6 mice cause severe hyperinsulinemia and diabetes, whereas background genes of the 129 strain protect against diabetes (14). In the present study, we focused further on this problem by determining what accounts for the differences in response to diet-induced obesity in mice by defining the genetic background loci that contribute to the differences in phenotypes between the B6 and 129 mice that are subjected to environmentally induced insulin resistance and obesity and comparing those with the modifiers of genetically induced insulin resistance.

We find that B6 mice are prone to becoming more obese on either high- or low-fat diets as they age compared with 129 mice. Although 129 mice are relatively lean, they are in fact highly susceptible to the content of fat in the diet, with a greater percentage increase in weight and greater deterioration in glucose tolerance than the B6 on a high- versus low-fat diet. Thus, mice of this strain are not obesity resistant, as previously described (4,13), but exhibit a complex interaction among genes, diet, age, and environment. In addition, we find that the higher body weight and tendency to glucose intolerance of the B6 mice are dominantly inherited when these two strains are crossed.

The most obvious potential explanation for differences in body weight would be differences in food intake. Surprising, however, is that 129 mice ate significantly more calories than B6 on the low-fat diet and an equal number of calories on the high-fat diet but still remained leaner than their B6 counterparts. Thus, the B6 mice showed higher feeding efficiency, i.e., greater weight gain per calorie consumed, as compared with 129 mice. This would represent an example of the "thrifty gene" phenotype and represent an advantage during times of food shortage (27).

In the absence of excessive food intake, low energy expenditure would be the major potential risk factor for body weight gain (28). Although B6 mice are actually more active than 129 mice, B6 mice have a lower metabolic rate than the 129 during feeding on both low- and high-fat diets and also during fasting on the high-fat diet. B6 mice also exhibit a smaller change in metabolism in response to a shift from a fasted to a fed state than 129 mice and a smaller change in response to the shift from a low- to a high-fat diet, i.e., reduced diet-induced thermogenesis. Whether the higher basal metabolic rate and higher diet-induced thermogenic response in 129 mice accounts entirely for the diminished weight is hard to predict. It is interesting that studies have shown that diet-induced thermogenesis is higher in lean humans compared with obese individuals (29). Furthermore, the higher metabolic rate in the 129 mouse in the basal state is independent of activity level, because these mice show approximately a 50% lower level of activity than the B6.

Besides a low metabolic rate, a low-fat versus carbohydrate oxidation ratio, i.e., a high respiratory quotient, has been suggested to be a risk factor for body weight gain (23,30,31). Contrary to this dogma, B6 mice exhibit a slightly lower respiratory quotient than the 129 mice.

An increase in body fat causes an increase in circulating leptin levels, which will normally decrease the energy intake and increase energy expenditure (32), yet in the B6 mice, the ratio of leptin to body weight and fat mass was much higher than that in the 129 mice. Thus, the B6 mice develop leptin resistance with age and obesity, mimicking humans with diet-induced obesity (15,33). It is interesting that leptin mRNA on high-fat diet is similar in B6 and 129 mice despite the differences in circulating leptin (manuscript in preparation), suggesting differences in either leptin translation efficiency or protein half-life between strains.

In parallel with the higher body weight, the intraperitoneal glucose tolerance testing showed greater hyperglycemia and little diet-induced change in the B6 mice, whereas 129 mice were glucose tolerant on the low-fat diet but intolerant on the high-fat diet. Likewise, the B6 mice exhibited insulin resistance during the insulin tolerance test on both the low- and high-fat diets. In contrast, 129 mice were relatively insulin sensitive even on a high-fat diet. The development of glucose intolerance in 129 mice on a high-fat diet is in contrast to our previous studies that have shown that 129 mice do not develop glucose intolerance when subjected to genetically induced insulin resistance as a result of heterozygosity of IR and IRS-1 (14). The B6 mice, conversely, become insulin resistant and hyperinsulinemic on either diet and also are more prone to

TABLE 2
RNA expression of genes located in the area of the QTLs determined in skeletal muscle, liver, and epididymal fat of B6 and 129 mice at 6 months maintained on standard diet (21.6% fat)

Marker	Gene	Probe set name	Skeletal muscle				Liver				Epididymal fat			
			B6	129	B6/129	*P*	B6	129	B6/129	*P*	B6	129	B6/129	*P*
D14Mit52	JNK1	104047_at	2113	2461	0.86	0.20	828	1097	0.75	0.28	1027	1404	0.73	0.059
	PKCδ	160698_s_at	2047	1675	1.22	0.09	2039	1673	1.22	0.58	**6405**	**4085**	**1.57**	**0.005**
	Wnt-5a	99390_at	**77**	**343**	**0.22**	**0.04**	37	92	0.40	0.47	279	576	0.48	0.074
	Ncoa4	97843_at	**1487**	**2021**	**0.74**	**0.035**	2634	2940	0.90	0.65	4264	4765	0.89	0.18
	Cacnaα2δ3	98300_at	**4**	**69**	**0.06**	**0.019**	111	250	0.44	0.19	79	67	1.18	0.73
	Tkt	101964_at	**2158**	**6665**	**0.32**	**0.008**	**10334**	**6403**	**1.61**	**0.032**	13026	14190	0.92	0.48
	Itih1	98014_at	63	34	1.85	0.39	9155	13811	0.66	0.30	44	39	1.13	0.77
	Itih3	100002_at	161	209	0.77	0.66	**26969**	**49473**	**0.55**	**0.014**	386	1153	0.33	0.31
	Itih4	98467_at	154	53	2.91	0.15	15128	22741	0.67	0.06	139	533	0.26	0.30
	Nek4	93210_g_at	272	97	2.80	0.17	**122**	**250**	**0.49**	**0.04**	169	266	0.64	0.17
	Stab1	96885_at	185	298	0.62	0.44	**439**	**925**	**0.47**	**0.048**	**1549**	**680**	**2.28**	**0.004**
	Nisch	94236_at	3924	4082	0.96	0.81	3495	4082	0.86	0.81	7759	6707	1.16	0.31
	Tncc	101063_at	11011	14369	0.77	0.12	36	41	0.88	0.78	35	66	0.53	0.11
	Phf7	162304_r_at	**311**	**73**	**4.26**	**0.026**	229	119	1.92	0.43	150	131	1.15	0.65
	Capn7	160718_at	1701	1843	0.92	0.71	964	802	1.20	0.21	1458	1827	0.80	0.21
	Sh3bp5	98766_at	643	580	1.11	0.25	446	405	1.10	0.77	458	575	0.80	0.35
	Btd	95469_at	1077	830	1.30	0.14	2323	2548	0.91	0.46	1446	1360	1.06	0.73
	Msmb	100715_at	66	42	1.57	0.52	26	61	0.43	0.45	20	27	0.74	0.59
	Parg	160949_at	364	353	1.03	0.96	323	305	1.06	0.87	632	653	0.97	0.92
	Slc18a3	101354_at	101	55	1.84	0.12	46	59	0.78	0.36	35	34	1.03	0.94
	Gdf10	160860_at	610	449	1.36	0.35	282	181	1.56	0.31	914	1043	0.88	0.62
	Anxa8	97529_at	428	122	3.51	0.29	115	112	1.03	0.97	917	710	1.29	0.23
	Ppyr1	101736_at	303	151	2.01	0.39	81	121	0.67	0.22	83	208	0.40	0.23
D14Mit192	**Ppp3** BMP 1	97989_at	**1466**	**2089**	**0.71**	**0.004**	175	161	1.09	0.86	**462**	**676**	**0.68**	**0.004**
	Bmp1	92701_at	901	495	1.82	0.12	1132	1047	1.08	0.81	**1440**	**816**	**1.76**	**0.031**
	Sh3d4	98848_at	**642**	**176**	**3.65**	**0.037**	138	284	0.49	0.33	187	214	0.87	0.79
	Sftpc	94900_at	707	436	1.62	0.31	222	205	1.08	0.90	267	350	0.76	0.38
	Hr	99875_at	3372	3150	1.07	0.88	768	655	1.17	0.65	2045	2397	0.85	0.74
	Epb4	103600_at	512	390	1.31	0.10	312	288	1.08	0.76	**1362**	**3792**	**0.36**	**0.005**
	Fgf17	98730_at	53	20	2.65	0.25	60	62	0.97	0.93	44	76	0.58	0.11
	Dok2	102334_at	765	687	1.11	0.69	662	505	1.31	0.27	**2483**	**967**	**2.57**	**0.000003**
	Gfra2	92448_s_at	1354	882	1.54	0.09	930	702	1.32	0.47	615	933	0.66	0.20
	Rb1	97948_at	232	651	0.36	0.09	118	101	1.17	0.81	893	1105	0.81	0.23
	Itm2b	101123_at	10824	9920	1.09	0.40	31886	25495	1.25	0.18	31553	35829	0.88	0.12
	Es10	92553_at	3744	3670	1.02	0.78	21599	17669	1.2	0.36	**12122**	**6424**	**1.89**	**0.003**
D12Mit231	Ptpn21	99970_at	2588	2770	0.93	0.42	1199	1028	1.17	0.19	819	1213	0.68	0.21
	Ches1	104664_at	324	165	1.96	0.28	170	143	1.19	0.74	148	252	0.59	0.54
	Psmc1	160152_at	4010	4453	0.90	0.52	2951	2707	1.09	0.57	**2480**	**2169**	**1.14**	**0.04**
	Cpsf2	104161_at	381	194	1.96	0.38	211	406	0.52	0.25	288	338	0.85	0.55
D3Mit278	Bche	94644_at	188	171	1.10	0.87	282	245	1.15	0.54	272	532	0.51	0.061
	Serpini1	99494_at	245	287	0.85	0.77	136	168	0.81	0.73	244	219	1.11	0.81
	Kpna4	100320_at	782	1264	0.62	0.41	139	186	0.75	0.57	871	1266	0.69	0.10
D3Mit127	Pdha2	99542_at	134	42	3.09	0.24	127	104	1.22	0.80	260	371	0.70	0.46
	Unc5h3	103742_at	498	94	5.30	0.34	188	258	0.73	0.55	**243**	**540**	**0.45**	**0.044**
	Gbp2	104597_at	554	581	0.95	0.84	323	106	3.05	0.19	4380	1995	2.20	0.21
	Gbp3	103202_at	445	461	0.97	0.88	358	357	1.00	0.98	1675	854	1.96	0.22
	BMPR1b	97725_at	125	298	0.42	0.31	61	120	0.51	0.80	100	186	0.54	0.053
	Lmo4	98122_at	1364	1308	1.04	0.80	586	605	0.97	0.80	4906	4343	1.13	0.68
	Hs2st1	102306_at	168	331	0.51	0.38	204	285	0.72	0.38	437	522	0.84	0.39
	Sh3glb1	98945_at	619	469	1.32	0.28	208	244	0.85	0.70	762	1090	0.70	0.08
	Cyr61	92777_at	789	484	1.63	0.10	366	281	1.30	0.53	739	586	1.26	0.38
	Clca2	103812_at	78	103	0.76	0.77	266	272	0.98	0.95	377	270	1.40	0.46
	Clca3	100623_at	162	178	0.91	0.86	345	206	1.67	0.10	186	273	0.68	0.23
	Bbcl10	94448_at	980	1155	0.85	0.35	846	623	1.36	0.07	1354	1123	1.21	0.21
D10Mit162	Myf 5	101162_at	56	95	0.59	0.25	15	21	0.71	0.73	20	26	0.77	0.76
	Myf 6	102265_at	**4487**	**7996**	**0.56**	**0.05**	276	390	0.71	0.49	173	170	1.02	0.97
	Syt1	93005_at	82	16	5.13	0.12	71	67	1.06	0.93	67	47	1.43	0.64
	Csrp2	93550_at	**181**	**351**	**0.52**	**0.009**	2138	2689	0.80	0.24	1107	1126	0.98	0.91
D11Mit199	Nme2	92625_at	33707	33024	1.02	0.54	20838	21971	0.67	0.93	18439	15031	0.82	0.17
	Tob1	99532_at	**8415**	**13977**	**0.6**	**0.02**	6687	4980	1.35	0.35	3698	4587	1.25	0.24
	Luc7a	160791_at	367	300	1.22	0.59	174	224	2.38	0.47	341	318	0.72	0.93
	Abcc3	103689_at	552	557	0.99	0.99	15375	12833	1.20	0.25	2545	1643	0.15	0.65
	Cacna1g	104460_at	57	52	1.08	0.86	37	48	0.77	0.37	42	47	0.25	1.15
	Chad	99913_at	2929	2489	1.17	0.40	1396	1404	0.98	1	633	706	1.12	0.58

Continued on following page

TABLE 2
Continued

Marker	Gene	Probe set name	Skeletal muscle				Liver				Epididymal fat			
			B6	129	B6/129	*P*	B6	129	B6/129	*P*	B6	129	B6/129	*P*
D15Mit13	**Dab2**	104633_at	595	507	1.17	0.72	393	477	0.83	0.57	**2134**	**1176**	**0.55**	**0.004**
	C9	104424_at	446	304	1.46	0.50	13868	22674	0.61	0.21	231	487	2.13	0.36

RNA levels are expressed in arbitrary units after global normalization. *P* was calculated using a Student's *t* test. All significant changes ($P < 0.05$) are indicated in bold. Abbc3, ATP-binding cassette, subfamily C (CFTR/MRP), member 3; Anxa, annexin; Bbcl, B-cell leukemia/lymphoma; Bche, butyrylcholinesterase; BMP, bone morphogenetic protein; Btd, biotinidase; C9, complement component 9; Cacnaα2δ3, calcium channel, voltage dependent, α2/δ subunit 3; Cacna1g, calcium channel, voltage dependent, T type, α1G subunit; Capn, calpain; Chad, chondroadherin; Ches, checkpoint suppressor; Clca, chloride channel calcium activated; Cpsf, cleavage and polyadnylation specific factor; Csrp, cystein-rich protein; Cyr, cystein-related protein; Dab2, disabled homolog 2; Dok2, downstream of tyrosine kinase 2; Epb4, erythrocyte protein band 4.9; Es, esterase; Fgf, fibroblast growth factor; Gbp, guanylate nucleotide binding protein; Gdf, growth differentiation factor 10; Gfra2, glial cell line–derived neurotrophic factor family receptor (α2); Hr, hairless protein; Itm, integral membrane protein; Hs2st1, heparan sulfate 2-O-sulfotransferase 1; Itih, inter-α trypsin inhibitor, heavy chain; JNK, c-Jun NH_2-terminal kinase; Kpna, karypherin α; Lmo, LIM domain only; Luc7a, cisplatin resistance-associated overexpressed protein; Mbtd1, malignant brain tumor–related domain containing 1; Msmb, β-microseminoprotein; Myf, myogenic factor; Ncoa, nuclear receptor coactivator; Nek4, NIMA (never in mitosis gene a)-related expressed kinase 4; Nisch, nischarin; Nme2, expressed in nonmetastatic cells 2, protein; Parg, poly(ADP-ribose) glycohydrolase; Phf, PHD finger protein; Psmc, protease (prosome macropain) 26S subunit ATPase; Ptpn21, protein tyrosine phosphatase nonreceptor type 21; Ppp3, protein phosphatase 3; Ppyr, pancreatic polypeptide receptor; Rb, retinoblastoma; Serpini, serine proteinase inhibitor, clade I; Sftpc, surfactant associated protein C; Sh3bp, SH3-domain binding protein; Sh3d, SH3-domain protein; Slc, solute carrier; Stab, stabilin; Syt, synaptotagmin; Tkt, transketolase; Tncc, troponin C; Tob1, transducer of ErbB-2.1; Unc5h3, unc5 homolog 3; Wnt5a, wingless-related MMTV integration site 5A.

become diabetic when they carry the double heterozygous deletion for IR and IRS-1 (14).

Characterization of the (B6 × 129)F2 intercross suggests that the differences between B6 and 129 mice are primarily determined by dominant-acting genes of the B6 mouse (24). In particular, we found that a locus on chromosome 14 between markers *D14Mit55* and *D14Mit52* previously identified as a modifier of genetically induced insulin resistance (15) also plays an important role in response to dietary insulin resistance.

Of the potential candidate genes in this area that are represented in the Affymetrix microarray, a disproportionate number of genes exhibited differential expression between B6 and 129 mice. These included several genes that are known to affect metabolism, such as PKCδ, Wnt-5a, and transketolase. PKCδ is one of several serine-threonine protein kinases that has been implicated in action, activity, and intracellular trafficking of the insulin receptor (34) and has been shown to be persistently activated in diabetes (35). Recent studies have also suggested an involvement of PKCδ in promotion of apoptosis (36), and it has been shown that increased glucose uptake promotes oxidative stress and PKCδ activation in adipocytes of B6 mice (37).

Wnt-5a is a member of a family of paracrine and autocrine factors that regulate cell growth and cell fate (38). Wnt signaling prevents preadipocytes from differentiating into adipocytes (38), and this could fit with the findings of the present study, showing that Wnt expression is upregulated in skeletal muscle, liver, and fat of the lean 129 strain as compared with the obese B6, in some cases by almost fivefold.

The expression level of transketolase was also significantly different between B6 and 129 mice; however, in this case, the expression was significantly higher in skeletal muscle of 129 versus B6, whereas it was significantly lower in liver of 129 versus B6, and the expression was similar in white fat. Transketolase is a ubiquitous enzyme involved in multiple metabolic pathways. Haploinsufficiency of transketolase results in reduced adipose tissue mass (39), whereas the deletion of PKCδ and Wnt-5a seems to affect β-cell proliferation and stimulate hematopoietic malignancies, respectively (40,41). Thus, future studies of the knockouts of each of these candidate genes may help to determine their roles in metabolism.

A separate QTL at marker *D14Mit192* was identified with linkage to both weight gain and hyperglycemia and inherited by the B6 strain. On the basis of differential expression, Dok2 (also known as p56[Dok2], FRIP, or Dok-R) is an interesting candidate gene located in the area around marker *D14Mit192*, which is highly upregulated in epididymal fat of B6 mice in comparison with 129. Dok2 is a RasGAP-binding protein suggested to have an important role downstream of growth factor receptors as a potential negative regulator of signal transduction (42). Additional studies will be needed to determine whether this gene or other genes revealed in Table 2 play a role in the development of hyperinsulinemia or insulin resistance. Furthermore, the list of genes under the peaks given in the Table 2 may potentially be the quantitative trait genes; however, several unidentified candidate genes may still be discovered in the areas.

A QTL with linkage to leptin at week 2 was detected around marker *D12Mit231* and is inherited from the 129 genome. The locus overlaps the hyperglycemia locus that was identified in the insulin receptor/IRS-1 double-heterozygous mice that interacts significantly with a locus on chromosome 7 in the control of leptin (15). In the New Zealand Obese mouse, this same marker (*D12Mit231*) has been shown to affect adiposity, BMI, and leptin (43) and is a diabesity locus when interacting with several other loci (44). Loci linked with hyperleptinemia were also discovered on chromosomes 3 and 10 in the present model. Because of the low number of markers in the area of the QTL on chromosome 10 as well as chromosome 15, there is some uncertainty about the significance of those QTLs.

Reed et al. (45) performed an analysis of an F2 intercross of C57BL/6ByJ and 129P3/J and identified QTLs for body weight, length, and adiposity on chromosome 2. We did not identify any significant QTLs on chromosome 2.

This discrepancy may be due to small differences of the inbred mouse substrains being used in the two studies (Jackson versus Taconic, 129Ps/J versus 129S6/SvEvTac, and C57Bl/6 versus C57BL/6ByJ), differences in dietary fat intake (4.4 vs. 9% fat) that were used to provoke the insulin-resistance phenotype, and differences in the age at the time of study or time course of study or other unidentified variables.

Combining the mapping data with the microarray expression profile provides an opportunity to establish a potential link between genotype and phenotype and thereby improve the chances of finding new diabetogenes and pathways. If the link between QTL and gene expression can be proved directly, then this would suggest a *cis*-acting gene, i.e., one in which a DNA variation in the gene itself (most likely the promoter) controls the expression level. As more data on the complete sequence of these candidate genes in both strains of mice become available, this hypothesis can be tested. However, a LOD score can also be due to a *trans*-acting effect, i.e., a gene at the defined locus acts on other genes to affect their transcription. It has previously been suggested that it is easier to detect effects that are caused by DNA variation acting on the gene itself (46), and this is also suggested by our data as a result of the high number of genes with differential expression at the QTLs (Table 2). For example, around marker *D14Mit192*, ~11 more genes than expected for a $P = 0.05$ were significantly differentially expressed in B6 versus 129 mice. Furthermore, at the other identified locus on chromosome 14 (*D14Mit52*), 23 annotated genes were present on the chip, and 11 of those genes were differentially expressed ($P < 0.05$) in muscle, fat, or liver of B6 as compared with 129, which is ~9 more genes than expected by random chance alone. Thus, the QTL and gene expression data support each other and suggest that genes located in the identified loci do play important roles in the outcome of a trait. At this point, however, we cannot determine whether this is the effect of only one or all three candidate genes at the QTL on chromosome 14 (at *D14Mit52*) or some other gene not yet mapped that is responsible for the hyperinsulinemia/insulin resistance phenotype. Moreover, these multiple changes at individual loci suggest that methylation, chromatin structure, or histone binding may be altered in one strain or the other and consequently affect the gene expression (47,48).

In conclusion, B6 mice are prone to becoming obese, hyperleptinemic, insulin resistant, and glucose intolerant independent of the fat content in food, whereas 129 mice exhibit more modest weight gain and glucose intolerance only on a high-fat diet. This is due to higher caloric efficiency and reduced diet-induced thermogenesis in the B6 mouse, two manifestations of the "thrifty gene." This "thrifty" phenotype with weight gain, hyperinsulinemia, and hyperleptinemia is dominantly inherited when these strains are bred. QTL and gene-array expression data suggest that some of the major genes that control hyperinsulinemia/insulin resistance both as a result of genetic modification and obesity are located on murine chromosome 14 in the region of marker *D14Mit52*.

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