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Identification of Quantitative Trait Loci Influencing Traits Related to Energy Balance in Selection and Inbred Lines of Mice

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ABSTRACT

Energy balance is a complex trait with relevance to the study of human obesity and maintenance energy requirements of livestock. The objective of this study was to identify, using unique mouse models, quantitative trait loci (QTL) influencing traits that contribute to variation in energy balance. Two F_2 resource populations were created from lines of mice differing in heat loss measured by direct calorimetry as an indicator of energy expenditure. The HB F_2 resource population originated from a cross between a noninbred line selected for high heat loss and an inbred line with low heat loss. Evidence for significant QTL influencing heat loss was found on chromosomes 1, 2, 3, and 7. Significant QTL influencing body weight and percentage gonadal fat, brown fat, liver, and heart were also identified. The LH F_2 resource population originated from noninbred lines of mice that had undergone divergent selection for heat loss. Chromosomes 1 and 3 were evaluated. The QTL for heat loss identified on chromosome 1 in the HB population was confirmed in the LH population, although the effect was smaller. The presence of a QTL influencing 6-wk weight was also confirmed. Suggestive evidence for additional QTL influencing heat loss, percentage subcutaneous fat, and percentage heart was found for chromosome 1.

ENERGY balance, or the difference between energy intake and energy expenditure, is a complex trait with important implications for human health and livestock production. Energy imbalance resulting in weight gain, and potentially leading to obesity, results when energy intake is greater than energy expenditure. The identification of low resting metabolic rate or total energy expenditure as risk factors for weight gain in certain human populations (Ravussin *et al.* 1988; Roberts *et al.* 1988; Griffiths *et al.* 1990) has led to the hypothesis that low energy expenditure provides a mechanism by which people susceptible to obesity can make excess energy available for weight gain (Saltzman and Roberts 1995). In contrast to the situation in humans in which low energy expenditure may be undesirable because of its potential contribution to unwanted weight gain and obesity, low energy expenditure and thus low maintenance energy requirements in livestock may be desirable to minimize the cost of feed required to maintain mature animals.

The genetic regulation of energy expenditure has been studied in mice through divergent selection for heat loss, which is measured using individual-animal direct calorimeters (Nielsen *et al.* 1997a). Because energy expenditure must equal energy intake at maintenance, and because energy is expended primarily as heat, heat loss was measured in mature mice as an indicator of energy expenditure and maintenance energy requirements. After 15 generations of selection, the difference in heat loss between lines selected in the high (MH) and low (ML) directions was 53% relative to the control line mean. The MH mice consumed significantly more feed, were leaner, more active, and had larger litters compared to ML mice (Moody *et al.* 1997; Nielsen *et al.* 1997a,b; Mousel 1998). These results demonstrate that the regulation of heat loss has a significant genetic component, and that selection for heat loss successfully changed energy expenditure as well as other traits related to energy balance. In particular, decreased fatness of MH relative to ML mice despite increased feed intake makes them a unique model with which to study correlations among fat deposition, feed intake, and energy expenditure (see Pomp and Nielsen 1999).

Although several studies have identified quantitative trait loci (QTL) contributing to energy imbalance measured as adiposity (see Pomp 1997), few have focused on component characteristics of energy balance, such as energy intake or energy expenditure. Because of the divergence in heat loss generated through selection, the MH and ML lines provide a valuable genetic resource with which to pursue such QTL. However, because the selection lines are outbred lines that originated from a common base population, they are likely to share common alleles at many molecular markers, which would make standard methods of QTL analysis for inbred lines inappropriate (Lander and Botstein 1989; Zeng 1993). Many inbred lines with variable phe-

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notypes are readily available, so that a resource population created from a cross between a selection and an inbred line with significantly different heat loss would allow QTL to be identified by using fully informative markers and statistical methods developed for inbred lines.

In a previous study, Moody *et al.* (1997) compared traits related to energy balance among three inbred lines and the heat loss selection lines of mice. The greatest difference in heat loss between an inbred and selection line was found between MH and the inbred line C57BL/6J (BL). Average heat loss and feed intake of BL mice were similar to ML, but their body composition was similar to MH. Thus, even though BL and ML lines have similarly low heat loss, this phenotype may arise from different mechanisms. In the ML line, the mechanism leading to low heat loss may also facilitate deposition of excess energy as fat, whereas low heat loss in the BL line may not result in increased fat deposition. Thus, it was hypothesized that some QTL alleles responsible for low heat loss would be shared between BL and ML, while others influencing both heat loss and fat deposition would be unique within each of the two lines. The present study has identified QTL that influence heat loss and other phenotypes related to energy balance in two resource populations. A complete genome scan was conducted in a cross between MH and BL. Regions harboring QTL with the greatest effects were then evaluated in a cross between MH and ML.

MATERIALS AND METHODS

Resource populations: Two different resource populations were created to utilize the resources available from diverse selection and inbred lines of mice. Detailed descriptions of the MH and ML lines divergently selected for heat loss have been presented elsewhere (Nielsen *et al.* 1997a,b). Briefly, selection was initiated from a composite base population created from four outbred strains of mice including NIH and ICR acquired from Harlan Sprague Dawley and CF-1 and CFW acquired from Charles River (Jones *et al.* 1992). Selection was based on measurement of heat loss (kcal/kg$^{0.75}$/day) in 9- to 11-wk males using individual-animal direct calorimeters. Following 16 generations of selection, the lines were maintained by random mating with care taken to minimize inbreeding. The cumulative realized selection differentials for generation 15 were 136.9 and −106.6 kcal/kg$^{0.75}$/day for MH and ML selection, respectively. Realized heritability for heat loss was 0.28 based on divergence of MH and ML. The first resource population (HB; $n = 560$) was created from a cross between the MH selection line and the BL inbred line. Average heat loss and food intake were similar for BL and ML and significantly less than for MH (Moody *et al.* 1997). A second F_2 intercross population (LH; $n = 560$) was created from a cross between the MH and ML lines. (See Table 1 for phenotypic means of BL, MH, and ML lines.) All MH and ML mice used in this study were sampled from the first of three replicated selection lines.

Each resource population was produced across four time periods separated by 4 wk. Matings in periods 1 and 2 were repeated in periods 3 and 4 such that each mating produced ~16 full-sibs in two time periods. BL males ($n = 5$) and MH females ($n = 15$) were mated to produce F_1 progeny, and male ($n = 22$) and female ($n = 36$) F_1 progeny were crossed to produce the HB resource population ($n = 560$). Similarly, males ($n = 12$) and females ($n = 12$) representing the MH and ML lines produced an F_1 generation, and F_1 males ($n = 22$) and females ($n = 37$) were crossed to produce the LH resource population ($n = 560$). All litters were standardized to eight pups at birth when possible. Pups were weaned at 3 wk and housed 3–4 per cage with *ad libitum* access to feed (Teklad 8604 Rodent Chow) and water until energy balance phenotypic measurement began at 10–12 wk. All mice were housed in stainless steel cages with wood-chip bedding and maintained at 22°, 35–50% relative humidity, and a light:dark cycle of 12:12 hr beginning at 0700 hr.

Measurement of phenotypes: Body weights were measured at 3, 6, and 10 wk. Direct calorimeters were used to measure heat loss at 10–12 wk of age as previously described (Nielsen *et al.* 1997a). Mice were placed into 1 of 10 individual-animal calorimetry units (Thermonetics Corporation, San Diego, CA) with a 3.5-g pellet of food at ~1630 hr. After a 30-min acclimation period, heat loss was measured every minute for a continuous 15-hr period. The average heat loss was adjusted for metabolic body weight and expressed on a 24-hr basis (kcal/kg$^{0.75}$/day). Data were discarded for mice that failed to consume at least 2.5 g of the food pellet because of a reduction in heat loss ($n = 16$ and 11 mice in the HB and LH populations, respectively). Following heat loss measurement, mice were housed in individual cages with hanging wire feeders. Food intake was measured by difference over a 14-day period, adjusted for metabolic body size, and expressed on a 24-hr basis (g/kg$^{0.75}$/day). Data were discarded for mice with significant food spillage (15 and 2 mice in the HB and LH populations, respectively). Otherwise, spillage was minimal and assumed to be randomly distributed. Body weights were measured and mice were killed 1 to 3 days after the food intake measurement period. Subcutaneous, gonadal (epididymal or perimetrial pads of males and females, respectively), and brown adipose pads, livers, and hearts were dissected and weighed. All adipose and organ weights were expressed as a percentage of body weight. The percentages of combined subcutaneous and gonadal fat pads were also evaluated as an indicator of total body fatness. All phenotypes were also measured on mice ($n = 20$ males and 20 females) representing each parental line (ML, MH, and BL) and F_1 generation (MH × ML [$n = 70$] and MH × BL [$n = 40$]).

Genetic Analysis

Genotyping of HB population: Standard methods were used to extract DNA from tail clips. Fully informative markers were identified by screening microsatellite markers (MapPairs; Research Genetics, Huntsville, AL) in grandparents of the HB population to identify markers with different alleles in the MH compared to BL line. Genotypes were determined by standard PCR and agarose gel electrophoresis protocols. Genotypes were scored as B, M, or H representing BL allele homozygotes, MH allele homozygotes, or heterozygotes, respectively. Genotypes were scored twice and discrepancies between the two scores were rectified.

Genotyping of the HB population was completed in three phases. In phase 1, mice with the highest and lowest heat loss within each full-sib family were selected after adjusting for effects of sex and period. Additional mice were selected until a total of 46 for each criterion (high or low heat loss) was identified with approximately equal representation of each time period and sex. This selected group was genotyped for a total of 62 markers representing each chromosome at 20-

to 40-cM spacing. In phase 2, the complete HB population was genotyped for 19 markers identified in phase 1 as having potential linkage to QTL influencing heat loss (see *Statistical Analysis* section). Phase 3 involved genotyping the complete HB population for additional markers located on chromosomes harboring markers identified in phase 2 as having significant effects on heat loss (see *Statistical Analysis* section).

Genotyping of LH population: Genotypes were determined by standard PCR using an infrared fluorescent dye-labeled forward primer followed by electrophoresis and analysis on the Li-Cor (Lincoln, NE) Model 4200 IR^2 system. Two reactions using primers with different labels (IRD700 and IRD800) were combined after PCR. Gels were analyzed using Gene ImagIR analysis software (Li-Cor) to determine allele sizes of each individual. The LH population was evaluated for chromosomes 1 and 3, which contained QTL having the largest effects on heat loss in the HB population. Markers on these chromosomes were screened in MH and ML grandparents of the LH population and selected if more than one allele was found. A total of eight and six markers on chromosomes 1 and 3, respectively, were genotyped in all MH and ML grandparents, F_1 parents, and LH F_2 mice.

Statistical Analysis

Description of phenotypes: The means and standard deviations of traits were determined for the HB and LH populations, as well as for mice representing each parental and F_1 line. Phenotypic differences among MH, ML, BL, and both F_1 populations were evaluated using the generalized least-squares procedure of SAS (1988) with fixed effects of line, sex, and line-by-sex interaction. Significant line effects were further evaluated by defining contrasts to test differences between MH and ML, MH and BL, and between each F_1 population and the average of its two parental lines.

HB population: Genetic distances between markers were determined using the Mapmaker 3.0 (Lincoln *et al.* 1992) analysis program with distances reported in Haldane centimorgan units.

Analysis of phase 1 and phase 2 genotypes: In phase 1, genotypic frequencies of selectively genotyped mice were evaluated using a chi-square analysis to test for equal allelic frequencies between high and low selected groups, which is expected under the null hypothesis of no linked QTL. Markers with a chi-square test statistic >2.71 ($P < 0.1$; 1 d.f.) were identified for further evaluation by whole-population genotyping. In phase 2, the effect of marker genotype on heat loss phenotype was evaluated by analysis of variance using the PROC MIXED procedure of SAS (1988). Fixed effects included time period, sex, marker genotype, and genotype-by-sex interaction, with sire-dam included as a random effect to account for effects that may be attributed to the genetic background of a specific sire and dam combination. Eight chromosomes with markers having nominally significant ($P < 0.05$) effects on heat loss were identified for evaluation by interval analysis in phase 3.

Interval analysis of phase 3 genotypes: Interval analysis (Lander and Botstein 1989) was used to better define the location and effects of QTL influencing all traits for the eight chromosomes identified in phase 2. Given the assumption that the MH and BL lines were fixed for alternate QTL alleles (Q and q) and the effects of QQ, Qq, and qq are a, d, and $-a$, respectively (Falconer and Mackay 1996), the expected value of an F_2 individual may be described in terms of a and d,

$$y_i = m + c_{ai}a + c_{di}d + e_i, \quad (1)$$

where m is a constant determined by fixed effects; c_{ai} is the coefficient for the additive component for individual i at the given location, defined as Prob(QQ) − Prob(qq); c_{di} is the coefficient for the dominance component for individual i at the given location, defined as Prob(Qq); and e_i is the residual error. Equation 1 may be expanded to account for fixed effects and effects of conditioning markers located on other chromosomes. The inclusion of conditioning markers has been recommended as a way to account for effects of background genes and other QTL (Zeng 1993). The expanded equation may be described in matrix notation as

$$\mathbf{y} = \mathbf{X}\,\mathbf{b} + \mathbf{C}\,\mathbf{m} + \mathbf{s}\mathbf{a} + \mathbf{t}\mathbf{d} + \mathbf{e}, \quad (2)$$

where **y** is a vector of phenotypes; **b** is a vector of fixed effects; **X** is an incidence matrix relating fixed effects to individuals; **m** is a vector of effects attributed to conditioning markers; **C** is a matrix of indicators that relate effects of genotyped conditioning markers to *y*; **e** represents the residual error; and **s** and **t** are vectors of c_{ai} and c_{di}, the coefficients of a and d, respectively, from Equation 1.

Analysis programs were developed to calculate the c_{ai} and c_{di} coefficients at 2-cM intervals on the basis of genotypes and locations of flanking markers (see Haley and Knott 1992), and the maximum-likelihood estimate for the residual variance for Equation 2 was obtained. The difference between the resulting −2 log-likelihoods of the full model (Equation 2, including a and d) and the reduced model (ignoring a and d) provided a likelihood-ratio test statistic (LR) that was converted to a LOD score (LOD = LR/4.601) for each 2-cM interval.

Fixed effects of sex, time period, and sire-dam were included for all traits. Litter size was also included as a fixed effect for 3- and 6-wk weights and percentage liver. Sex was accounted for as a fixed effect because no line-by-sex interaction was found when phenotypes of the MH, ML, and BL lines were compared for these traits (Moody *et al.* 1997) and because no marker by sex interaction was found for heat loss in the analysis of variance completed for phase 2. Conditioning markers were selected for each trait in a two-step process using the backward selection option of the PROC REG procedure of SAS (1988). In the first step, models including all markers on a single chromosome were evaluated. Markers remained in the model if their effect approached significance ($P < 0.1$). In the second step, markers identified from all chromosomes in the first step were included in a single model. Conditioning markers included in the QTL analysis were those that remained in the final regression model ($P < 0.05$). Conditioning markers were omitted from analyses of chromosomes on which they were located.

Effects of QTL: Effects of QTL are presented as the additional percentage of residual phenotypic variance explained by the QTL and the a and d effects of each QTL. The percentage variance was calculated as [(residual variance of reduced model − residual variance of full model)/residual variance of reduced model] × 100. Solutions and standard errors for a and d were obtained using option 4 of the MTDFREML programs (Boldman *et al.* 1995) with c_{ai} and c_{di} at the QTL location determined by the peak LOD score included in the model as covariates along with the same fixed effects and conditioning markers used in the interval analysis. Standard errors were calculated using the REML estimate of residual variance.

Confidence regions: One-LOD confidence regions are presented for significant QTL as the chromosomal region with LOD scores greater than or equal to one less than the peak LOD score at the QTL position.

Significance thresholds: Permutation testing described by Churchill and Doerge (1994) was used to determine empirically derived significance thresholds specific to the HB data. Permuted heat loss data were analyzed 1000 times with fixed

TABLE 1

Sex-adjusted means and phenotypic standard deviations for parental (BL, MH, and ML) and F_1 (BL/MH and ML/MH) populations for heat loss (kcal/kg$^{0.75}$/day), food intake (g food/kg$^{0.75}$/day), percentage subcutaneous, gonadal and brown fat pads, percentage liver and heart, and 10-wk weight

Population		HLOSS	INT	SUB	GON	FAT	BAT	LIV	HRT	WT10
BL	Mean[a]	111.2***	63.1***	0.548	0.510	1.057	0.355	5.437	0.563	24.6***
(n = 41)	SD	9.6	5.9	0.082	0.119	0.177	0.030	0.509	0.051	1.8
BL/MH	Mean[b]	131.3***	69.5	0.559	0.576	1.135	0.405*	5.831***	0.546	29.7***
(n = 39)	SD	8.1	6.8	0.138	0.180	0.300	0.120	0.345	0.045	3.1
MH	Mean	182.8	74.7	0.508	0.533	1.041	0.371	5.265	0.563	30.2
(n = 39)	SD	18.0	5.1	0.114	0.213	0.301	0.081	0.408	0.044	2.7
ML/MH	Mean[c]	144.5*	70.1	0.695***	0.880***	1.574***	0.370*	5.014**	0.489***	35.1***
(n = 77)	SD	17.4	6.3	0.218	0.319	0.490	0.088	0.387	0.052	3.6
ML	Mean[d]	118.6***	64.4***	0.686***	0.761***	1.447***	0.434**	5.146	0.489***	31.2
(n = 40)	SD	16.5	5.0	0.161	0.319	0.439	0.089	0.428	0.035	3.4

SD, standard deviation; HLOSS, heat loss; INT, food intake; SUB, GON, FAT, BAT, LIV, HRT, percentages subcutaneous fat, gonadal fat, subcutaneous + gonadal fat, brown fat, liver, and heart; WT10, 10-wk weight. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

[a] Differences between BL and MH.

[b] Differences between BL/MH and the average of BL and MH.

[c] Differences between ML/MH and the average of ML and MH.

[d] Differences between ML and MH.

effects and conditioning marker genotypes permuted along with associated phenotypes such that these effects were accounted for with each permutation. The maximum LOD for each chromosome, as well as the maximum across all chromosomes, was identified for each permuted analysis. The experiment-wise significance threshold level was defined as the 50th highest LOD among the 1000 maxima identified across all chromosomes, corresponding to an experiment-wise type 1 error rate of 0.05. Suggestive linkage threshold values were defined for each chromosome as the 50th highest LOD among the 1000 maxima for the respective chromosome.

LH population: Linkage maps for chromosomes 1 and 3 were constructed using Cri-map (Green and Crooks 1990) with results reported in Haldane centimorgan units. Interval analysis in the LH population was completed as described for the HB population, except that c_{ai} and c_{di} were calculated for 1-cM intervals conditional on genotypes in the three-generation pedigree for all markers in a linkage group (Haley *et al.* 1994). This method allows all available information to be used to calculate the probability of a QTL genotype at a given location. Fixed effects were the same as for the HB population, but no conditioning markers were included in the analysis of the LH population.

Because only specific regions of chromosomes 1 and 3 were evaluated in the LH population to test for the presence of significant QTL identified from the HB population, the problem of multiple testing was reduced. Significance of QTL in these specific regions (20–30 cM) was determined by $P < 0.01$ as suggested by Lander and Kruglyak (1995). However, because no previous evidence for QTL in other regions of these chromosomes exists, identification of QTL in these regions was handled as if they were evaluated as part of a complete genome scan. Permutation testing was carried out as described for the HB population to determine suggestive linkage threshold levels. Because only two chromosomes were genotyped, permutation testing could not be used to determine genome-wide significant threshold levels.

RESULTS

Description of phenotypes: The mean and standard deviation for each trait in the original BL, MH, and ML lines, as well as in the F_1 crosses, are shown in Table 1. The mean of the MH line was significantly greater than that of BL and ML for heat loss and food intake. Means of traits related to adiposity were greater in ML than in MH but similar in BL and MH. Body weight of MH was significantly greater than that of BL but similar to that of ML. Heterosis for heat loss was observed as the mean of both F_1 populations was less than the average of their two parental lines. The BL/MH F_1 mean was similar to the average of BL and MH for the remaining traits, except for brown adipose, liver, and 10-wk weights where the F_1 mean exceeded the mean of either of its parental lines. In contrast, mean of the ML/MH F_1 was different from the average of ML and MH for all traits except food intake. The ML/MH F_1 mice were significantly larger and had greater subcutaneous, gonadal, and combined fat than either of their parental lines, but their brown adipose, liver, and heart weights were less than the average of MH and ML. A large degree of phenotypic variation was generated in both F_2 populations for all traits evaluated (Table 2).

HB population: All markers genotyped in the HB population and their chromosomal locations are listed in Table 3. In general, positions of markers determined from the HB population agreed with those found in the Mouse Genome Database. However, three markers (*D1Mit234*, *D9Mit243*, and *D10Mit44*) were unlinked to their respective chromosomes in the HB population and were omitted from further analyses.

Phase 1 and 2 analyses: Markers with preliminary evidence of QTL influencing heat loss on the basis of selective genotyping are indicated in Table 3. Nineteen markers representing 11 chromosomes were identified as having potential linkage with QTL ($P < 0.1$) and were genotyped in the complete population. Of these,

TABLE 2

Male and female means adjusted for replicate and means and standard deviations adjusted for replicate and sex for pooled data are presented for the HB and LH F_2 resource populations for heat loss (kcal/kg$^{0.75}$/day), food intake (g food/kg$^{0.75}$/day), percentage subcutaneous, gonadal and brown fat pads, percentage liver and heart, and body weights (grams) at 3, 6, and 10 wk

Population		HLOSS	INT	SUB	GON	FAT	BAT	LIV	HRT	WT3	WT6	WT10
HB	Males	143.3	69.4	0.537	0.738	1.275	0.378	5.941	0.573	12.9	27.4	32.6
$n = 560$	Females	149.4	74.5	0.621	0.626	1.247	0.366	5.841	0.604	12.5	21.7	24.1
	Pooled	146.1	72.0	0.579	0.682	1.261	0.372	5.892	0.588	12.7	24.6	28.4
	SD	16.9	6.6	0.152	0.266	0.390	0.076	0.508	0.068	1.5	2.1	2.7
LH	Males	139.2	64.5	0.511	0.672	1.183	0.386	5.427	0.529	13.0	28.2	33.7
$n = 560$	Females	149.4	67.7	0.670	0.677	1.348	0.393	5.184	0.551	12.5	23.2	26.6
	Pooled	144.3	66.1	0.591	0.675	1.266	0.389	5.305	0.540	12.8	25.7	30.1
	SD	17.6	5.4	0.172	0.294	0.444	0.076	0.458	0.052	1.6	2.6	3.2

SD, standard deviation; HLOSS, heat loss; INT, food intake; SUB, GON, FAT, BAT, LIV, HRT, percentages subcutaneous fat, gonadal fat, subcutaneous + gonadal fat, brown fat, liver, and heart; WT3, WT6, WT10, 3-, 6-, and 10-wk weights. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

markers on chromosomes 5, 6, and 9 failed to influence heat loss on the basis of analysis of variance ($P > 0.05$) and were not included in subsequent interval analyses (Table 3).

Interval analysis: The experiment-wise 5% LOD threshold value based on permutation analysis across eight chromosomes was 3.28, which was defined as a threshold for declaring significant evidence for linkage of QTL. This value was essentially identical for the three traits subjected to permutation analysis (heat loss, subcutaneous fat, and 10-wk weight). Suggestive evidence for QTL was defined as the chromosome-wise 5% LOD threshold value. Because of constraints on computational time, the highest threshold value among the three traits on a particular chromosome was used as the threshold level for all traits on that chromosome. Suggestive threshold values ranged from 2.05 (chromosome 11) to 2.39 (chromosome 3). There was little variation in threshold values across the three traits within a chromosome.

TABLE 3

Microsatellite markers and their chromosomal positions (centimorgans, in Haldane units) in the HB population

Marker	cM	Marker	cM	Marker	cM	Marker	cM
D1Mit67[a]	9.0	*D4Mit235*[a,b,**]	1.9	*D8Mit94*[a]	13.0	*D15Mit11*[a]	10.4
D1Mit156[c]	46.2	*D4Mit164*	37.4	*D8Mit236*	36.0	*D15Mit5*	31.9
D1Mit303	48.1	*D4Mit175*	56.5	*D8Mit242*	40.6	*D15Mit156*	49.3
D1Mit30[b]	78.1	*D4Mit189*[b,**]	103.7	*D9Mit328*[a,b]	23.0	*D15Mit15*	81.9
D1Mit361[c]	122.5	*D5Mit352*[a]	20.0	*D9Mit302*	39.8	*D16Mit131*[a]	4.3
D1Mit407[b,***]	122.9	*D5Mit254*	29.5	*D10Mit175*[a]	41.8	*D16Mit4*	29.8
D1Mit209[c]	132.1	*D5Mit188*[b]	54.8	*D10Mit233*	62.2	*D16Mit152*	51.5
D1Mit155[c]	135.7	*D5Mit371*	65.2	*D11Mit140*[a]	28.0	*D17Mit21*[a]	9.8
D2Mit241[a]	30.0	*D6Mit183*[a]	26.5	*D11Mit284*[b,*]	49.6	*D17Mit88*[b]	26.9
D2Mit56[b,*]	42.3	*D6Mit188*[b]	35.1	*D11Mit333*[b,*]	67.9	*D17Mit142*[b,*]	46.5
D2Mit48[c]	63.1	*D6Mit254*	62.6	*D11Mit48*[c]	80.9	*D18Mit94*[a]	17.0
D2Mit423[b,***]	84.5	*D7Mit270*[a]	18.0	*D12Mit46*[a]	17.0	*D18Mit53*	23.2
D2Mit148[b]	118.0	*D7Mit62*[b,***]	40.7	*D12Mit14*	40.2	*D18Mit186*	43.9
D3Mit305[a,b]	11.2	*D7Mit301*[c]	42.8	*D12NDS2*[b,**]	81.2	*D19Mit28*[a]	9.8
D3Mit227[c]	23.5	*D7Mit222*[c]	51.3	*D13Mit256*[a]	40.0	*D19Mit19*	46.8
D3Mit49[b,***]	41.2	*D7Mit105*[b,***]	65.7	*D13Mit151*	78.0	*D19Mit105*	66.7
D3Mit102[c]	47.6			*D14Mit207*[a]	5.5		
D3Mit193[c]	71.4			*D14Mit158*	43.6		
D3Mit17[c]	75.1			*D14Mit228*	61.7		
D3Mit293	80.6						

Significant effects of marker genotype on phenotype are indicated. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.
[a] Positions of the first marker on each chromosome are from the Mouse Genome Database.
[b] Markers with preliminary evidence of QTL based on selective genotyping.
[c] Additional markers genotyped for interval analysis.

TABLE 4

Estimated locations and effects of significant and suggestive QTL for heat loss in the HB population

Symbol[a]	Chrom	cM	LOD	Var (%)[b]	Additive[c]	Dominance[d]
Hlq1	1	127	5.62	4.7	4.4 ± 1.0	2.3 ± 1.5
Hlq2	2	71	3.73	3.1	3.9 ± 1.0	1.5 ± 1.6
Hlq3	3	35	3.76	3.1	4.1 ± 1.0	−0.6 ± 1.6
Hlq4	3	47	4.70	3.9	4.0 ± 0.9	−1.3 ± 1.3
	4	12	2.92	2.4	3.9 ± 1.1	−1.0 ± 1.8
	4	77	3.27	2.7	3.8 ± 1.2	4.1 ± 2.4
Hlq5	7	61	4.06	3.4	4.2 ± 1.0	0.1 ± 1.5
	11	68	2.44	2.0	2.1 ± 0.9	−2.8 ± 1.3
	17	39	2.83	2.4	3.5 ± 1.1	2.7 ± 1.7

[a] Gene symbols are assigned to QTL exceeding the significant linkage threshold of 3.28.
[b] Percentage of additional residual variance explained by the QTL.
[c] Additive effect of replacing one BL allele with one MH allele (kcal/kg$^{0.75}$/day).
[d] Dominance deviation of a heterozygous QTL genotype from the mean of the two homozygotes (kcal/kg$^{0.75}$/day).

Locations of peak LOD scores and effects of QTL at these locations for all traits are presented in Tables 4, 5, and 6. Nine QTL influencing heat loss were identified including five (*Hlq1*, *Hlq2*, *Hlq3*, *Hlq4*, and *Hlq5*) that exceeded the significant linkage threshold level (Figure 1). Although two peaks are present on chromosome 3 (Figure 1), the confidence intervals for *Hlq3* and *Hlq4* overlapped, indicating they may represent a single QTL. The QTL that exceeded the significant threshold level each accounted for 3.1 to 4.7% of residual variance while those exceeding the suggestive level accounted for 2.0 to 2.7%. Together, these nine QTL explained

TABLE 5

Estimated locations and effects of QTL for percentage subcutaneous, gonadal, subcutaneous + gonadal, and brown fat in the HB population

Symbol[a]	Chrom.	cM	LOD	Var (%)[b]	Additive[c]	Dominance[d]
SUB						
	1	60	2.82	2.3	0.032 ± 0.010	0.023 ± 0.017
	4	53	2.75	2.2	−0.026 ± 0.009	−0.021 ± 0.014
	4	83	3.20	2.6	−0.035 ± 0.011	−0.038 ± 0.022
	7	34	3.27	2.7	−0.032 ± 0.009	0.022 ± 0.015
	12	52	2.79	2.3	0.033 ± 0.010	0.020 ± 0.018
GON						
Fatq1	1	60	7.95	5.9	0.086 ± 0.017	0.076 ± 0.030
	7	30	2.30	1.9	−0.031 ± 0.016	0.067 ± 0.027
	11	72	2.61	2.1	0.051 ± 0.016	0.034 ± 0.027
	12	56	2.79	2.3	0.060 ± 0.018	0.037 ± 0.038
FAT						
Fatq1	1	62	7.36	5.4	0.116 ± 0.025	0.121 ± 0.044
	7	32	2.91	2.4	−0.065 ± 0.024	0.086 ± 0.040
	7	59	2.48	2.0	−0.066 ± 0.024	0.067 ± 0.036
	11	62	2.60	2.1	0.068 ± 0.023	0.058 ± 0.038
	12	54	3.18	2.6	0.091 ± 0.026	0.060 ± 0.048
BAT						
Batq1	1	102	3.96	3.3	0.024 ± 0.006	−0.001 ± 0.011
Batq2	3	55	3.46	2.8	0.019 ± 0.005	−0.000 ± 0.008
	11	68	2.23	1.8	0.012 ± 0.004	−0.007 ± 0.006
	17	20	2.40	2.0	−0.015 ± 0.005	−0.014 ± 0.008

SUB, percentage subcutaneous; GON, gonadal; FAT, subcutaneous + gonadal; BAT, brown.
[a] Gene symbols are only assigned to QTL exceeding the significant linkage threshold of 3.28.
[b] Percentage of additional residual variance explained by the QTL.
[c] Additive effect of replacing one BL allele with one MH allele (percentage).
[d] Dominance deviation of a heterozygous QTL genotype from the mean of the two homozygotes (percentage).

TABLE 6

Estimated locations and effects of QTL for 3-, 6- and 10-wk weight and percentage liver and heart in the HB population

Symbol[a]	Chrom.	cM	LOD	Var (%)[b]	Additive[c]	Dominance[d]
WT3						
Wt3q1	1	72	5.13	4.1	0.37 ± 0.08	0.19 ± 0.14
Wt3q2	1	108	10.09	8.0	0.61 ± 0.10	0.20 ± 0.17
	7	32	2.39	1.9	0.01 ± 0.08	0.44 ± 0.14
	11	70	2.40	2.0	0.26 ± 0.08	−0.02 ± 0.13
Wt3q3	17	14	6.28	5.0	−0.40 ± 0.09	0.35 ± 0.14
WT6						
Wt6q1	1	27	4.02	3.3	0.57 ± 0.15	0.43 ± 0.26
Wt6q2	1	108	3.98	3.2	0.60 ± 0.15	−0.25 ± 0.27
Wt6q3	11	36	4.55	3.7	0.55 ± 0.13	0.31 ± 0.21
WT10						
Wt10q1	1	25	4.27	3.3	0.77 ± 0.19	0.19 ± 0.33
	3	11	2.43	2.0	−0.39 ± 0.14	0.36 ± 0.21
Wt10q2	3	61	4.76	3.8	−0.69 ± 0.17	−0.61 ± 0.27
	4	26	2.61	2.1	0.58 ± 0.18	−0.26 ± 0.30
Wt10q3	11	32	3.63	2.9	0.61 ± 0.16	0.28 ± 0.24
	12	60	3.27	2.7	0.69 ± 0.19	−0.27 ± 0.35
LIV						
	3	43	2.81	2.3	0.038 ± 0.029	0.130 ± 0.041
	4	89	2.52	2.1	0.112 ± 0.035	−0.039 ± 0.064
Livq1	7	66	3.70	3.0	0.110 ± 0.028	0.012 ± 0.039
Livq2	11	38	5.68	4.6	0.134 ± 0.030	−0.090 ± 0.049
Livq3	12	46	6.43	5.2	−0.156 ± 0.030	−0.029 ± 0.049
HRT						
Hrtq1	1	45	5.22	4.2	−0.020 ± 0.004	−0.005 ± 0.006
	7	51	3.06	2.5	0.009 ± 0.004	−0.017 ± 0.006

WT3, 3-wk weight; WT6, 6-wk weight; WT10, 10-wk weight; LIV, percentage liver; HRT, percentage heart.
[a] Gene symbols are only assigned to QTL exceeding the significant linkage threshold of 3.28.
[b] Percentage of additional residual variance explained by the QTL.
[c] Additive effect of replacing one BL allele with one MH allele (grams or percentage).
[d] Dominance deviation of a heterozygous QTL genotype from the mean of the two homozygotes (grams or percentage).

27.7% of residual variance for heat loss, after accounting for variation due to time period, sex, sire-dam, and conditioning markers. With the exception of the suggestive QTL region on chromosome 4, the effects of these QTL were primarily additive, ranging from 2.1 to 4.4 kcal/kg$^{0.75}$/day. The MH allele resulted in increased heat loss for all QTL.

Four QTL influencing brown adipose tissue weight were identified (Table 5), and each of them were within confidence intervals identified for heat loss QTL (Figure 1). The brown adipose QTL accounted for 1.8 to 3.3% of the residual variance and had additive effects ranging from 0.012 to 0.024%. The MH allele resulted in increased brown adipose for *Batq1*, *Batq2*, and for the suggestive QTL on chromosome 11, but the BL allele caused increased brown adipose for the suggestive QTL located on chromosome 17.

Significant evidence was found for one QTL influencing gonadal and combined fat (*Fatq1*) on chromosome 1 (Figure 2), while suggestive evidence (Table 5) was found for QTL on chromosomes 1 and 4 (subcutaneous), 7 and 12 (all three fat traits), and 11 (gonadal and combined). The MH allele resulted in increased fatness for QTL on chromosomes 1, 11, and 12, but decreased fatness for QTL on chromosomes 4 and 7. *Fatq1* on chromosome 1 explained 5.4 and 5.9% of the residual variance of combined and gonadal fat, respectively, while the remaining QTL each accounted for 2.0 to 2.7%.

Body weight QTL (Figure 3) were identified on chromosomes 1 (3, 6, and 10 wk), 3 (10 wk), 11 (6 and 10 wk), and 17 (3 wk). Suggestive evidence was also found for additional QTL on these chromosomes, as well as on chromosomes 7 and 4 (Table 6). The QTL with the largest effect was on chromosome 1, where *Wt3q1* accounted for 8% of the residual variance or 0.6 g of body weight at 3 wk. The remaining QTL explained from 1.9 to 5.0% of the residual variance (Table 5). Although most of these QTL demonstrated additive gene action with the MH allele causing increased body weight, the MH allele resulted in decreased body weight at *Wt3q3* and *Wt10q1*, and significant dominance effects

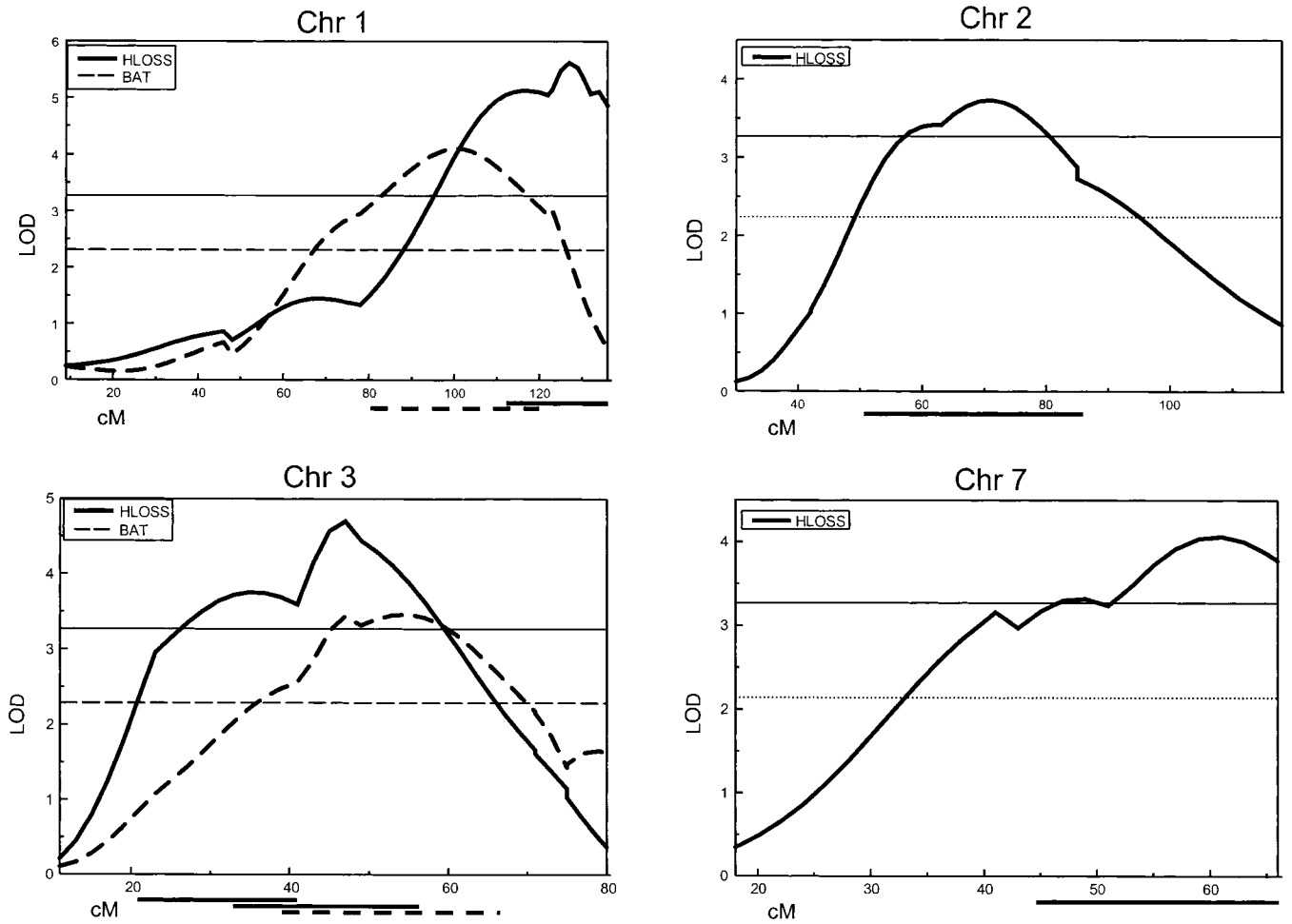

Figure 1.—Significant QTL influencing heat loss (HLOSS) and percentage brown fat (BAT) identified by interval analysis in the HB population are shown for chromosomes 1, 2, 3, and 7. Significant and suggestive threshold levels are shown as horizontal solid and dotted lines, respectively, for each chromosome. One-LOD confidence intervals are shown as lines below the *x*-axis for each QTL.

were identified for *Wt3q3*, *Wt6q1*, *Wt10q2* and for the suggestive QTL influencing 3-wk weight on chromosome 7.

Seven QTL that influence liver and heart weights were found, each accounting for 2.1 to 5.2% of residual variance. *Livq1* is located in a similar region of chromosome 7 as *Hlq5* (Table 6). Both additive and dominance effects were observed, and the MH allele resulted in both increases and decreases in liver and heart depending on the QTL. Interval analysis for food intake failed to identify QTL surpassing either the significant or suggestive threshold levels on the eight chromosomes evaluated.

LH population: Locations of the 14 markers genotyped in the LH population are shown in Table 7. The markers genotyped and total length of chromosome 1 for the HB and LH populations were different (135 and 103 cM, respectively), making it difficult to identify the expected location of significant QTL from the HB population. However, the confidence interval for *Hlq1* spanned ~20 cM on the distal end of chromosome 1 in the HB population. Therefore, the most distal 20 cM of chromosome 1 in the LH population was considered as the region where *Hlq1* would reside. Interval analysis in this region revealed a peak LOD score of 1.45 at 90 cM, which corresponds to $P < 0.01$ for a single position test. Following the guidelines proposed by Lander and Krugylak (1995), this is sufficient evidence to confirm the presence of *Hlq1* in the LH population. However, the effect of *Hlq1* was much smaller in LH than in HB, explaining only 1.2% of the residual variance with an additive effect of 2.01 kcal / $kg^{0.75}$ / day. Likewise, the confidence interval for the location of *Wt6q1* in the HB population included the proximal end of chromosome 1 from 9 through 40 cM. The peak LOD score at the proximal end of chromosome 1 (36.9 cM) in the LH population was 3.44, corresponding to $P < 0.0001$. Thus, *Wt6q1* was also confirmed in the LH population. Other QTL with significant evidence for linkage on chromosomes 1 (*Fatq1*, *Batq1*, *Wt3q1*, *Wt3q2*, *Wt10q1*, and *Hrtq1*) and 3 (*Hlq3*, *Hlq4*, *Batq2*, and *Wt10q2*) in HB were not confirmed in the LH population.

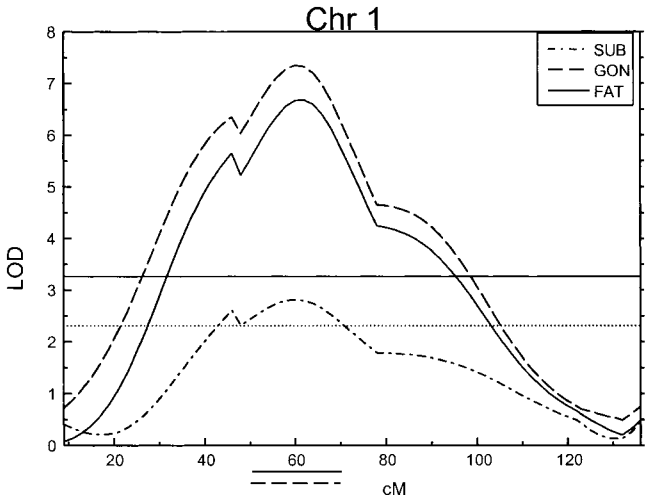

Figure 2.—Significant QTL influencing percentage subcutaneous (SUB), gonadal (GON), and subcutaneous + gonadal (FAT) fat pad weights identified by interval analysis in the HB population are shown for chromosome 1. Significant and suggestive threshold levels are shown as horizontal solid and dotted lines, respectively. One-LOD confidence intervals are shown as lines below the *x*-axis for each QTL.

Complete interval analyses of chromosome 1 revealed suggestive evidence (LOD $>$ 2.13) for three additional QTL on chromosome 1 (Figure 4). These QTL influencing heat loss, subcutaneous fat, and heart weights accounted for 2.1 to 2.5% of residual variance (Table 8). The QTL influencing subcutaneous fat in the LH population was located in the same region of chromosome 1 where suggestive evidence for a QTL was found in the HB population. No QTL influencing any of the measured traits were found on chromosome 3 in the LH population.

DISCUSSION

The development of the HB resource population from a cross between MH and an inbred line enabled fully informative markers to be identified and used for genotyping, which increased the power to detect QTL and simplified the genotyping process. Crossing MH with ML provided a second population in which to confirm QTL and provided a model with which to study the relationship between QTL influencing heat loss and fat deposition. Loci identified in both resource populations may represent QTL where similar alleles producing low heat loss were contributed by the ML and BL lines. Alternatively, a QTL effect observed in both resource populations may result from a high heat loss QTL allele originating from the MH line in both resource populations. Evidence for such unique MH alleles may be provided by the asymmetric response to selection observed in the development of MH and ML (Nielsen *et al.* 1997a). Additional heat loss QTL unique to each resource population were also expected, and it was hypothesized that these QTL may contribute to the similar heat loss but different fatness observed in ML and BL. Loci influencing heat loss in HB but not LH may represent genes that influence heat loss independently of fat deposition, while QTL unique to LH may have pleiotropic effects on heat loss and fatness. A similar model has been proposed by Rice *et al.* (1996) to describe the shared genetic regulation of resting metabolic rate, fat free mass, and fat mass in humans.

Evidence for QTL influencing heat loss was found on seven of the eight chromosomes evaluated by interval analysis. Of the five QTL exceeding the significance threshold level established by permutation testing, two (*Hlq1* and *Hlq4*) also exceeded the significance threshold (LOD $>$ 4.3) suggested by Lander and Kruglyak (1995). The presence of *Hlq1* but not *Hlq4* was confirmed in the LH population. Confirmation of *Hlq1* may indicate the presence of similar alleles responsible for low heat loss in both ML and BL, with a larger effect on heat loss resulting from the BL allele. The lack of confirmation of *Hlq4* may indicate that the allele responsible for the observed variation in heat loss originated from BL and that alleles at this locus did not differ between ML and MH. Alternatively, different effects observed at *Hlq1* and *Hlq4* in the two resource populations may result from the interaction of these QTL with different genetic backgrounds.

Suggestive evidence was found for QTL influencing subcutaneous fat and heat loss proximal to *Hlq1* in the LH population. Because these regions were linked and the presence of the ML allele resulted in decreased heat loss and increased fatness, these regions may contribute to the negative correlation between heat loss and subcutaneous fat found in the LH population (data not shown). The region of chromosome 1 influencing subcutaneous fat in the LH population corresponds to the region containing *Fatq1* in the HB population, which also had a suggestive effect on subcutaneous fat. Thus, even though the effect of *Fatq1* was not confirmed in the LH population, suggestive evidence of a QTL influencing subcutaneous fat was found in both resource populations in a similar chromosomal region. In the HB population, the MH allele resulted in increased fatness with greater effects observed for gonadal and combined fat compared to subcutaneous fat. In the LH population, the ML allele did not cause additional increases in gonadal or combined fat, but did have a suggestive effect of increased subcutaneous fat. Thus, this region may represent a locus responsible for differences in fat regulation between BL and the selection lines. The suggestive effect on subcutaneous fat observed in LH may be due to different alleles contributed by MH and ML or it may be caused by pleiotropic effects of heat loss QTL linked to a QTL influencing fatness.

Previous studies have identified QTL influencing adiposity using several different types of resource populations (see Pomp 1997). One QTL has been reported on

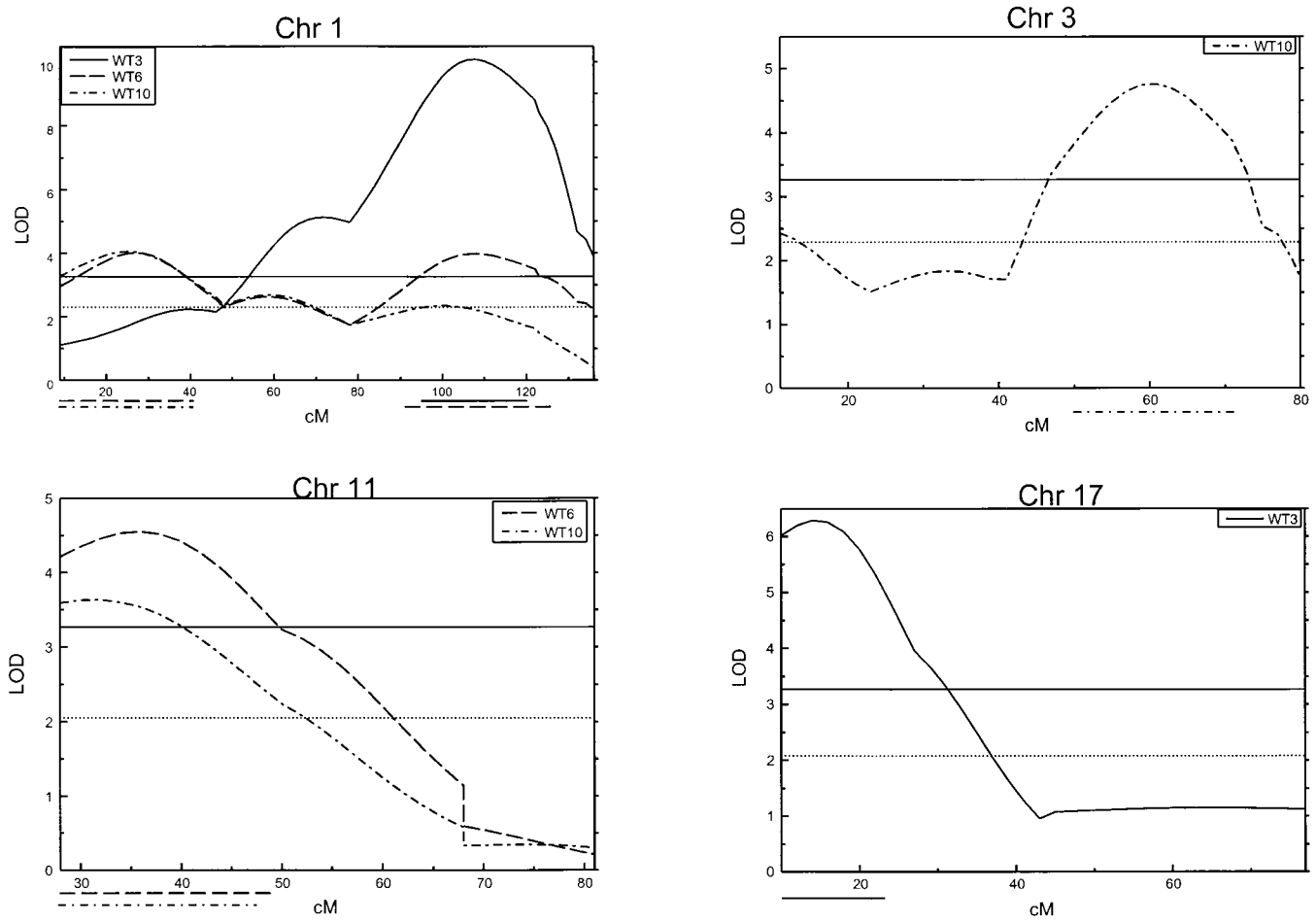

Figure 3.—Significant QTL influencing body weight measured at 3 (WT3), 6 (WT6), and 10 (WT10) wk identified by interval analysis in the HB population are shown for chromosomes 1, 3, 11, and 17. Significant and suggestive threshold levels are shown as horizontal solid and dotted lines, respectively, for each chromosome. One-LOD confidence intervals are shown as lines below the *x*-axis for each QTL.

TABLE 7

Microsatellite markers genotyped in the LH resource population, chromosomal positions estimated from the LH population (centimorgans, in Haldane units), and the number of alleles observed at each marker are presented

Marker	cM	No. alleles
D1Mit282	36.9[a]	5
D1Mit216	40.4	4
D1Mit444	54.3	4
D1Mit267	74.0	2
D1Mit110	83.4	3
D1Mit354	89.1	3
D1Mit359	91.4	3
D1Mit17	103.3	2
D3Mit167	16.5[a]	3
D3Mit22	38.3	3
D3Mit97	44.8	3
D3Mit10	50.8	4
D3Mit316	60.3	2
D3Mit127	74.6	3

[a] Position taken from the Mouse Genome Database.

chromosome 1 (*Obq2*; Taylor and Phillips 1996), but it appears to be distal to *Fatq1*. Several QTL influencing adiposity have been reported in other regions (see Pomp 1997). Regions containing *Dob1* on chromosome 4 (West *et al.* 1995), *Dob4* (West *et al.* 1995) and *Obq1* (Taylor and Phillips 1996) on chromosome 7, and *Mob3* (Warden *et al.* 1995) on chromosome 12 correspond to regions with suggestive evidence for QTL influencing fatness in the HB population.

An interesting result of this study was the identification of two significant and two suggestive QTL influencing percentage weight of brown adipose tissue. Each of these regions was closely linked to regions containing QTL for heat loss, suggesting a potential pleiotropic effect of these loci. Although the MH allele was associated with increased brown adipose for all QTL except chromosome 17, the effect of brown adipose differences on heat loss and energy expenditure is unclear. Increased brown adipose weight may indicate a proliferation of tissue to facilitate greater heat loss and energy expenditure. However, increased weight of brown adipose depots due to accumulation of lipid stores resulting

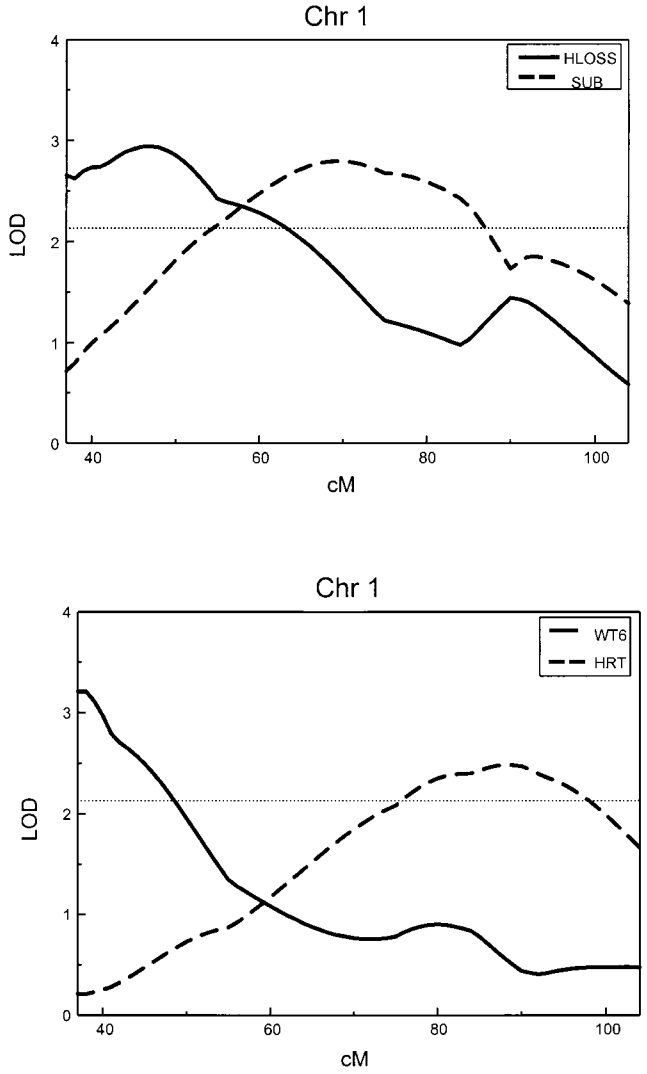

Figure 4.—Suggestive QTL influencing heat loss (HLOSS), percentage subcutaneous fat (SUB) and heart (HRT), and body weight measured at 6 wk (WT6) identified by interval analysis in the LH population are shown for chromosome 1. The suggestive threshold level is shown as a horizontal dotted line.

from reduced brown adipose tissue activity has also been observed (Enerback *et al.* 1997; Thomas and Palmiter 1997). Increases observed in ML relative to MH support the latter hypothesis, but the presence of QTL causing both increased heat loss and increased brown adipose supports the former. Further investigation of brown adipose composition and activity is needed to understand the impact of these QTL on the regulation of energy balance.

The chromosomal regions containing QTL identified in this study are relatively large and contain many known and unknown genes. Although the specific genes responsible for these QTL effects cannot yet be identified, the presence of intriguing candidate genes within these QTL regions deserves mention. Genes encoding the β-subunit of thyroid stimulating hormone (*Tshb*) and the neuropeptide Y receptor Y2 (*Npy2r*) are located in the region of chromosome 3 containing *Hlq4* and *Batq2* (Naylor *et al.* 1986; Nakamura *et al.* 1996). Both of these genes are involved in the regulation of several metabolic processes and have been implicated in the specific regulation of brown adipose activity (Himms-Hagen 1989; Cassard-Doulcier *et al.* 1994; see Woods *et al.* 1998). Genes encoding uncoupling proteins 2 and 3 are located on chromosome 7 within the region containing *Hlq5* (Boss *et al.* 1997; Fleury *et al.* 1997; Vidal-Puig *et al.* 1997). Uncoupling proteins facilitate the dissipation of energy as heat and have been actively investigated as candidate genes for obesity-related phenotypes (Bouchard *et al.* 1997).

Two heat loss QTL regions are homologous to regions in other species that harbor QTL for traits involved in energy balance. Norman *et al.* (1998) reported results of a genome scan for QTL influencing obesity and energy metabolism in Pima Indians. Evidence for a QTL influencing the ratio of carbohydrate oxidation to fat oxidation (24RQ) was found on human chromosome 1p22-p12, which is homologous to the region of mouse chromosome 3 containing *Hlq4*. Evidence for a QTL influencing percentage body fat was found on human chromosome 11 in the same study, but this region does

TABLE 8

Estimated locations and effects of QTL influencing heat loss, percentage subcutaneous fat and heart, and 6-wk body weight on chromosome 1 in the LH population

Trait (QTL)	cM	LOD	Var (%)[a]	Additive[b]	Dominance[c]
HLOSS	47	2.94	2.5	4.11 ± 1.17	0.89 ± 1.81
HLOSS *(Hlq1)*	90	1.44	1.2	2.53 ± 1.15	2.01 ± 1.78
SUB	69	2.80	2.3	−0.036 ± 0.012	0.034 ± 0.022
WT6 *(Wt6q1)*	37	3.44	2.8	0.51 ± 0.14	−0.28 ± 0.21
HRT	88	2.49	2.1	0.010 ± 0.003	0.004 ± 0.005

HLOSS, heat loss; SUB, percentage subcutaneous; HRT, heart; WT6, 6-wk body weight.
[a] Percentage of additional residual variance explained by the QTL.
[b] Additive effect of replacing one BL allele with one MH allele.
[c] Dominance deviation of a heterozygous QTL genotype from the mean of the two homozygotes.

not appear to be homologous to the mouse region harboring *Hlq5*. In pigs, Andersson *et al.* (1994) identified a QTL for fatness on porcine chromosome 4 in a region with homology to *Hlq1*.

In summary, several QTL influencing heat loss and other components of energy balance were identified. As more is learned about the genetic regulation of specific component characteristics that define energy balance, more factors explaining variation in this complex polygenic trait will be identified. Ultimately, it will be possible to study these individual factors and specific interactions among them to gain a more thorough understanding of the regulation of energy balance. This knowledge will be critical to the continued development of new methods to enable increased efficiency of livestock production and improved diagnosis and treatment of human obesity.

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