Genet. Res., Camb. (1997), **70**, *pp.* 225–235. *With 5 figures.* Printed in the United Kingdom © 1997 Cambridge University Press

Variability in metabolic rate, feed intake and fatness among selection and inbred lines of mice

D. E. MOODY, D. POMP* AND M. K. NIELSEN
Department of Animal Science, University of Nebraska, Lincoln, NE 68583-0908, USA

(*Received 8 April 1997 and in revised form 8 August 1997*)

Summary

Mouse populations differing in metabolic rate have been developed through selection for high (MH) and low (ML) heat loss (HLOSS), along with randomly selected controls (MC). Objectives of this study were to (a) compare MH, ML and MC lines for HLOSS and correlated traits of feed intake, body composition and organ weights; (b) compare three widely used inbred mouse lines with MH, ML and MC for the same traits; and (c) investigate potential genotype by diet interaction resulting from feeding diets differing in fat percentage. Heat loss (kcal/day) of MH and ML mice differed by 37% of the mean and remained significant (33%) when HLOSS was expressed on a fat-free mass basis. MH mice consumed more energy than ML with a greater difference in mice fed high-fat compared with standard diets (27% vs 13·9%). Despite greater energy consumption, MH mice were leaner than ML with a difference in total body fat percentage of 40%. The greatest difference in HLOSS between selection and inbred lines was between MH and C57BL/6J (BL), which differed by 26·3%. MH and BL mice also differed in energy intake (15·5%). Body composition of BL mice was similar to MH when fed a standard diet, but similar to ML when fed a high-fat diet. Crosses between MH and ML or between MH and BL would be useful to investigate the genetic regulation of, and identify quantitative trait loci influencing HLOSS, energy intake and body composition. Feeding of a high-fat diet may allow diet-specific loci influencing body composition to be identified in MH and BL lines.

1. Introduction

Obesity is a complex, multigenic trait that results from a long-term positive energy imbalance where energy intake exceeds energy expenditure. Reduced rates of total energy expenditure and resting metabolic rate have been identified as risk factors for subsequent weight gain in a human population prone to obesity (Ravussin *et al.*, 1988). Studies involving children of obese parents have also reported significant correlations between resting metabolic rate at early ages and subsequent weight gain (Roberts *et al.*, 1988; Griffiths *et al.*, 1990), and a low resting metabolic rate was identified in formerly obese patients who had undergone significant stable weight loss (Buscemi *et al.*, 1996). Thus, it has been suggested that reduced energy expenditure provides a mechanism by which individuals who are susceptible to obesity can make excess energy available for weight gain (Saltzman & Roberts, 1995). Identification of specific genes involved in the regulation of energy expenditure may be useful in the diagnosis, prevention and treatment of obesity.

Populations of mice that differ in heat loss (HLOSS) by nearly 50% of the mean have been developed through long-term selective breeding for HLOSS measured in 9- to 11-week-old males by direct calorimetry (Nielsen *et al.*, 1997*a*). Significant differences in HLOSS, as well as feed intake and body composition, have been described for these lines (Nielsen *et al.*, 1997*a*, *b*). However, only males were evaluated in these studies, and an indirect measurement of body composition was used. It has been estimated that 32 million adult women and 26 million adult men are overweight in the United States, indicating that women are more likely to be obese than men (Kuczmarski *et al.*, 1994). Thus, it will be useful to consider both males and females of the HLOSS selection lines to understand how they compare with the sex-related differences in obesity found in human populations. In addition, HLOSS has

* Corresponding author. Tel: +1 (402) 472-6416. Fax: +1 (402) 472-6362. e-mail: dpomp@unlinfo2.unl.edu.

previously been evaluated at a single age (9 to 11 weeks) to estimate energy requirements at maintenance (Nielsen *et al.*, 1997*a*). It will also be of interest to evaluate differences in HLOSS at later ages to determine whether divergent HLOSS is maintained in more mature animals when fat deposition accelerates.

The MH and ML selection lines provide a unique model in which to genetically dissect HLOSS and how it affects body composition. Because of differences in phenotypes between MH and ML mice, an F_2 population created from a cross between them would be useful for the identification of quantitative trait loci (QTL) influencing HLOSS and correlated traits. Identification of inbred line(s) that differ in HLOSS compared with MH and (or) ML would be useful for the development of additional crosses for QTL detection that could offer the advantage of increased marker informativeness and provide an independent population in which to compare QTL effects in different genetic backgrounds.

Finally, the environment in which a trait is measured is critical when evaluating the genetic regulation of a trait. Diet, as well as other environmental effects, is known to contribute to human obesity (Bouchard, 1994). In mice, high-fat diets have been shown to lead to obesity, increased weight gain per unit of feed consumed, and changes in plasma total cholesterol and triacylglyceride values (West *et al.*, 1992; Kirk *et al.*, 1995). However, responsiveness to high-fat diets varied among inbred mouse lines, indicating significant line by diet interaction effects (West *et al.*, 1992; Kirk *et al.*, 1995). Evaluation of the effect of differing dietary fat levels on HLOSS and body composition may reveal additional line by diet interactions important to understanding the relationships among diet, energy intake and expenditure, and obesity.

The first objective of the present study was to further characterize differences among MH, ML and MC (control) selection lines by describing differences in HLOSS in both males and females at two ages, by measuring additional traits potentially involved in energy utilization, and by directly evaluating total body fat percentage. Secondly, phenotypes of selection lines were compared with those of widely used laboratory inbred lines to extend the characterization of metabolic traits to a wider array of genotypes. Finally, comparisons among all lines were evaluated in the presence of a standard diet as well as a high-fat diet so that potential line by diet interaction effects could be identified.

2. Methods

(i) *Lines of mice*

Mice from lines that had undergone long-term selection for high (MH) and low (ML) HLOSS and unselected controls (MC) (Nielsen *et al.*, 1997*a*), as well as mice representing three widely used laboratory inbred lines (C57BL/6J, BL; DBA/2J, DB; and SWR/J, SW) were evaluated. Mice from the HLOSS selection lines represented generation 16 of the first of three independent replicate lines. Detailed descriptions of the selection lines have been published elsewhere (Nielsen *et al.*, 1997*a*, *b*). Briefly, selection was initiated from a composite base population created from four outbred stocks of mice (Jones *et al.*, 1992). Selection was based on measurement of HLOSS (kcal/kg$^{0.75}$/day) in 9- to 11-week males using individual-animal direct calorimeters. After 15 generations of selection, cumulative realized selection differentials in replicate 1 were 136·9 and −106·6 kcal/kg$^{0.75}$/day for MH and ML selection, respectively, and realized heritability for HLOSS was 0·28 based on the divergence of MH and ML selection. Retired breeders from inbred lines were purchased from Jackson Laboratories (Bar Harbor, ME) and mated to produce mice used in this study. Eighteen males (housed 3 per cage) and 16 females (housed 4 per cage) represented each line, except that only 17 BL males, 14 DB females and 15 SW females were available.

(ii) *Mouse care and maintenance*

All mice were weaned at 3 weeks of age and provided access to feed (Teklad 8604 Rodent Chow) and water *ad libitum*. At 4 weeks, mice were randomly assigned to either high-fat (38·2% fat, 40·9% carbohydrate, 20·9% protein; HIGH) or standard (12·7% fat, 66·4% carbohydrate, 20·9% protein; STN) synthetic diets (Research Diets, New Brunswick, NJ). The HIGH and STN diets contained 4·6 and 3·9 kcal/g metabolizable energy, respectively, and mice had access to the respective diets *ad libitum*. Diets were initially provided in powdered form but were replaced by pellets when mice were between 8 and 9 weeks of age. All mice were housed in stainless steel cages with wood-chip bedding and maintained at 22 °C, 35–50% relative humidity, and a light:dark cycle of 12 h:12 h beginning at 0700 hours.

(iii) *Data collection*

Body weights were measured every 2 weeks between 4 and 14 weeks of age. Feed intake was determined every 2 weeks on a cage basis. Average daily intake (g/day) was converted to metabolizable energy (kcal/day) for each diet and is presented for early (4–8 weeks) and late (8–12 weeks) growth, and weight maintenance (12–14 weeks) periods. Because of large differences in body weights among the selection and inbred lines, intake is also expressed relative to average metabolic body weight (kg$^{0.75}$) of the cage for each 2-week period. Diets were coloured to identify feed

https://doi.org/10.1017/S0016672397003017 Published online by Cambridge University Press

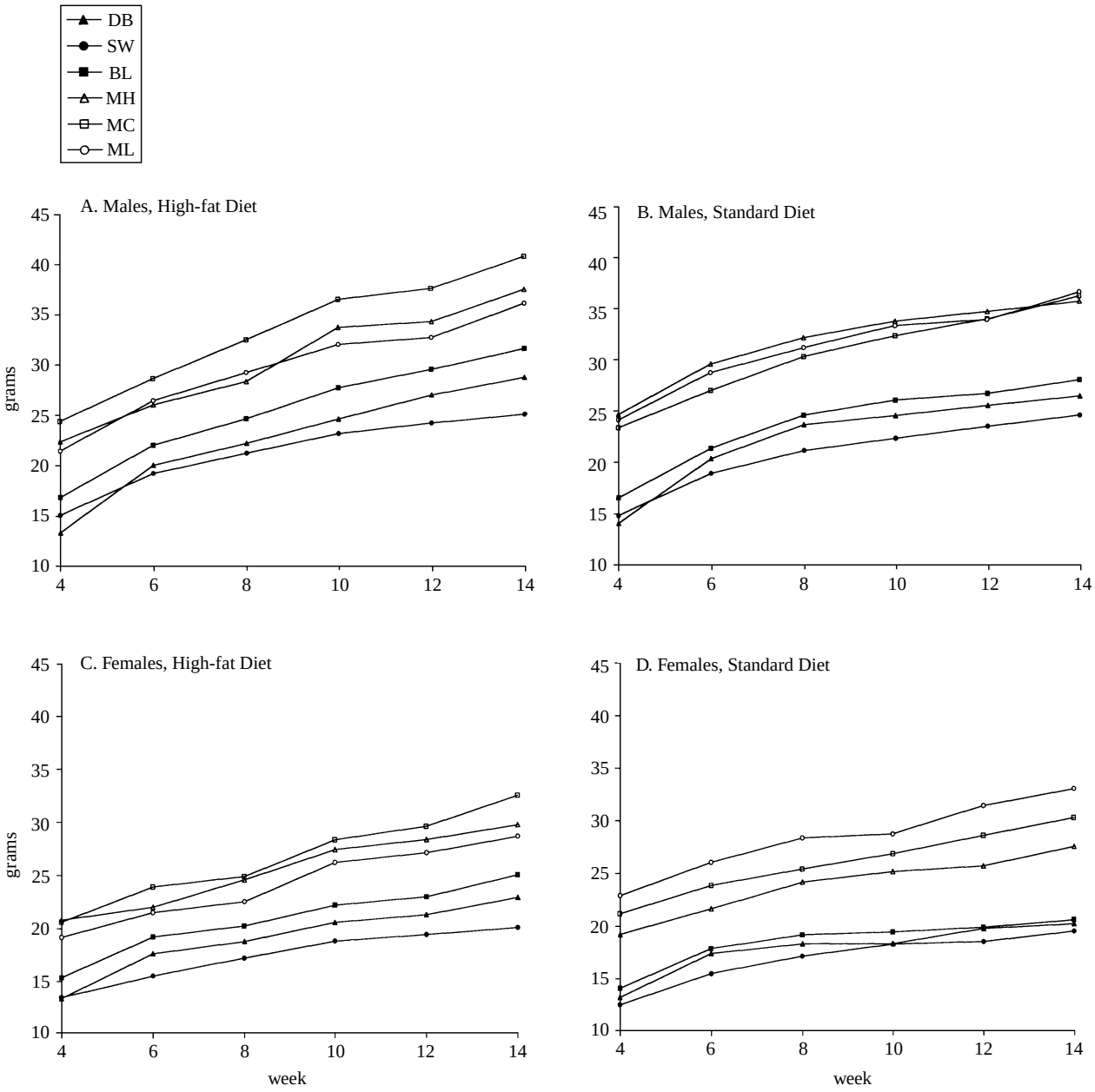

Fig. 1. Body weight (g) of male mice fed high-fat (38·2% fat) and standard (12·7% fat) diets (*A* and *B*, respectively), and female mice fed high-fat and standard diets (*C* and *D*, respectively), are shown for inbred lines DBA/2J (DB), SWR/J (SW) and C57BL/6J (BL), and high (MH), low (ML) and control (MC) heat loss selection lines.

spillage, which was found to be minimal and assumed to be randomly distributed.

Heat loss was measured at two ages – 9 to 11 weeks and 14 to 16 weeks – in individual-animal direct calorimeters (model 0601-S, gradient-layer Seebeck envelope; Thermonetics, San Diego, CA). Mice were placed in calorimeters with a 3·5 g pellet of feed at approximately 1630 hours. Average HLOSS (kcal/day) was recorded over a 15 h period after allowing mice to adapt to the calorimeters for 30 min. To account for variability in HLOSS associated with differences in body size and composition, average HLOSS measured from 14 to 16 weeks was expressed relative to fat-free mass (kcal/kg FFM/day). Because fat-free mass was not measured at 10 weeks, HLOSS measured from 9 to 11 weeks was expressed relative to metabolic body weight (kcal/kg$^{0·75}$/day) to account for variability in body size and surface area. Data were discarded from mice that did not eat during HLOSS measurement.

Mice were weighed and killed by cervical dislocation 1–4 days following their second HLOSS measurement. Livers, spleens and hearts were dissected and weighed. Livers and hearts were returned to the carcasses, while spleens were stored for future DNA extraction. Liver, spleen and heart weights were expressed as a percentage of carcass weight. Stomach contents were removed, weighed and discarded. Carcasses were placed in bags for chemical body composition analysis. Lipid weight was determined as the difference between

https://doi.org/10.1017/S0016672397003017 Published online by Cambridge University Press

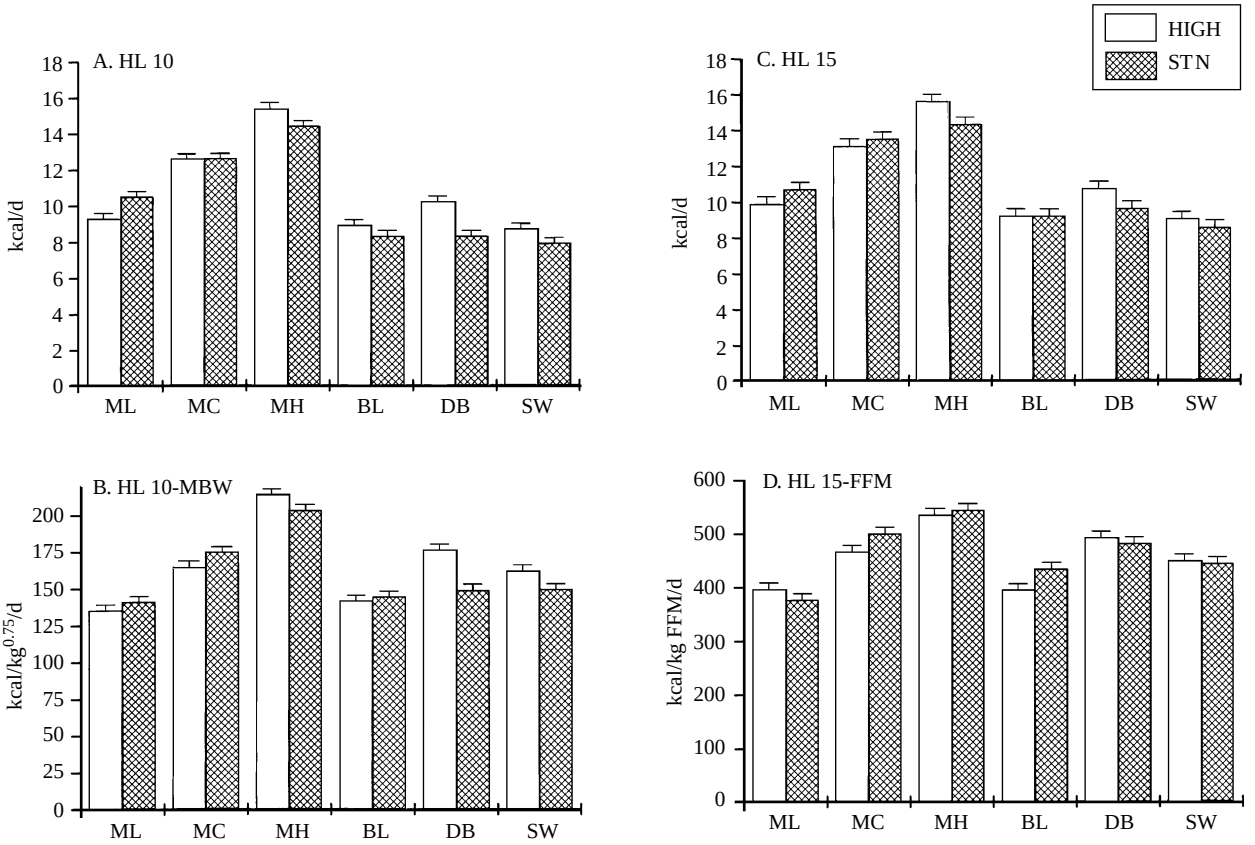

Fig. 2. Least-squares means for low (ML), control (MC) and high (MH) heat loss selection lines, and for C57BL/6J (BL), DBA/2J (DB), and SWR/J (SW) inbred lines, are shown for daily heat loss measured between 9 and 11 weeks (HL10) and between 14 and 16 weeks (HL15; *A* and *C*, respectively) for mice fed high-fat (38·2% fat; HIGH) and standard (12·7% fat; STN) diets. Least-squares means for HL10 adjusted for metabolic body weight (HL10-MBW) and HL15 adjusted for fat-free mass (HL15-FFM) are also presented (*B* and *D*, respectively).

dried carcass weights before and after a 96-h ether extraction, and was expressed as a percentage of the empty-stomach carcass weight to determine total body fat percentage. Fat-free mass (FFM) was calculated as carcass weight minus lipid weight. The ratio of FFM to carcass weight at the time of dissection was assumed to be equal to the ratio of FFM to carcass weight at the time of the second HLOSS measurement. The FFM used to adjust HLOSS measured from 14 to 16 weeks was calculated as the ratio of FFM to carcass weight at the time of dissection multiplied by body weight at the time of HLOSS measurement. The HLOSS measured from 14 to 16 weeks was then expressed as kcal/kg FFM/day.

(iv) *Statistical analysis*

Data were analysed as two separate data sets. The first data set (analysis 1) included data from MH, ML and MC lines, while the second data set (analysis 2) included data from all six lines evaluated. Both data sets were analysed using the generalized least-squares procedure of SAS (1988) with fixed effects of line, diet, sex, and all interactions. Least-squares means were generated for each line and diet combination in analysis 2. Significant line effects were further evaluated by defining contrasts to test specific differences between lines. For analysis 1, these contrasts were defined to test the difference between MH and ML selection lines (MH–ML), and the asymmetry of response, defined as the difference between MC and the average of MH and ML (ASYM). For analysis 2, contrasts were defined to test for differences between each inbred line and the MH and ML selection lines (BL–MH, DB–MH, SW–MH, BL–ML, DB–ML and SW–ML). When a significant line by diet interaction effect was observed, each contrast in analysis 1 and 2 was evaluated separately for mice fed HIGH and STN diets.

3. Results

Male and female body weights from 4 to 14 weeks are shown in Fig. 1. In general, selection line mice (MH, MC and ML) were heavier than inbred mice (BL, DB and SW), and mice fed HIGH diet continued to gain weight over a longer time period and were heavier compared with mice fed STN diet. Least-squares means of all line by diet combinations are presented for traits related to heat loss, fat percentage, organ size and energy intake in Figs. 2, 3, 4 and 5, respectively.

https://doi.org/10.1017/S0016672397003017 Published online by Cambridge University Press

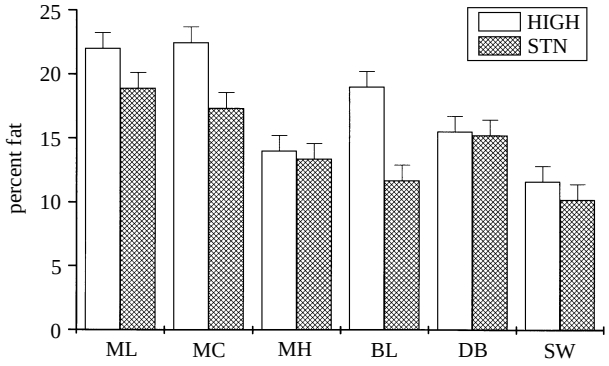

Fig. 3. Least-squares means for low (ML), control (MC) and high (MH) heat loss selection lines, and for C57BL/6J (BL), DBA/2J (DB), and SWR/J (SW) inbred lines, are shown for percentage body fat for mice fed high-fat (38·2% fat; HIGH) and standard (12·7% fat; STN) diets.

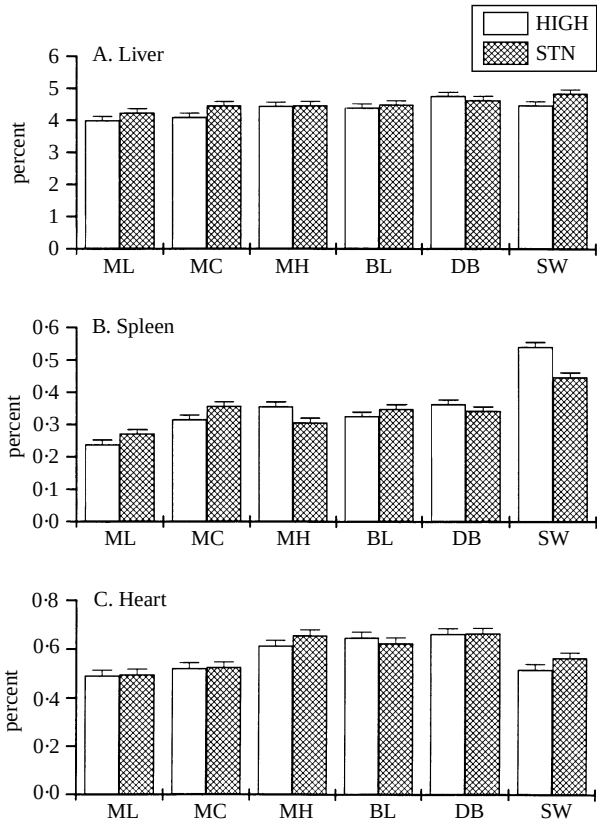

Fig. 4. Least-squares means for low (ML), control (MC) and high (MH) heat loss selection lines, and for C57BL/6J (BL), DBA/2J (DB), and SWR/J (SW) inbred lines, are shown for liver, spleen and heart expressed as a percentage of body weight (*A*, *B* and *C*, respectively) for mice fed high-fat (38·2% fat; HIGH) and standard (12·7% fat; STN) diets.

(i) *Analysis 1: Comparisons of selection lines*

The effect of line on HLOSS was significant for measurements at both age ranges, and remained significant when adjusted for metabolic body weight and FFM (Table 1). Differences between MH and ML were highly significant for all traits related to HLOSS, ranging from 33% to 41% of the mean (Table 2; Fig. 2). The line by diet interaction effect was significant for all measurements of HLOSS. The difference in HLOSS between MH and ML was greater for mice fed HIGH compared with STN diet for unadjusted HLOSS and HLOSS adjusted for metabolic body weight, but the difference was greater for mice fed STN diet when HLOSS was adjusted for FFM. The effect of sex on HLOSS traits was significant, with females having greater adjusted HLOSS than males (6–15% of the mean), but no line by sex interaction was found.

Fat percentage was significantly different among the selection lines as MH mice had 40% less fat than ML (Tables 1 and 2; Fig. 3). Mice fed HIGH diet had a greater fat percentage than mice fed STN diet (Fig. 3), but the fat percentage of males and females was similar.

The effect of line on organ weights expressed as a percentage of total body weight was significant (Table 1), with MH mice having significantly larger livers and hearts than ML mice (Table 2; Fig. 4). A significant line by diet interaction was found for spleens; when fed HIGH diet, MH mice had spleens larger than those of ML mice, but the difference was not significant for MH and ML mice fed STN diet.

All measurements of energy intake differed among the selection lines (Table 3), with MH mice consuming more energy than ML (Table 4; Fig. 5). For unadjusted intake measurements, significant line by diet interactions were observed for intake measured over all time periods. During early growth, differences in unadjusted intake between MH and ML were significant for mice fed both HIGH and STN diets. During late growth and maintenance periods, the difference in energy intake between MH and ML fed HIGH diet was 35% and 36% of the mean, respectively, but differences between MH and ML were not significant for STN diet. For intake measurements adjusted for metabolic body weight, a significant line by diet interaction was observed only for the late growth period. The difference in adjusted energy intake between MH and ML ranged from 15% to 21% of the mean for all time periods.

(ii) *Analysis 2: Comparisons of selection and inbred lines*

The effect of line was significant for all HLOSS traits (Table 1). The HLOSS of MH was significantly greater than that of each inbred line (Table 2; Fig. 2), with the greatest difference observed between MH and BL (37% of the mean for HLOSS adjusted for metabolic body weight, and 26% of the mean for HLOSS adjusted for FFM). Adjusted HLOSS of ML was significantly less than that of DB and SW (Table

https://doi.org/10.1017/S0016672397003017 Published online by Cambridge University Press

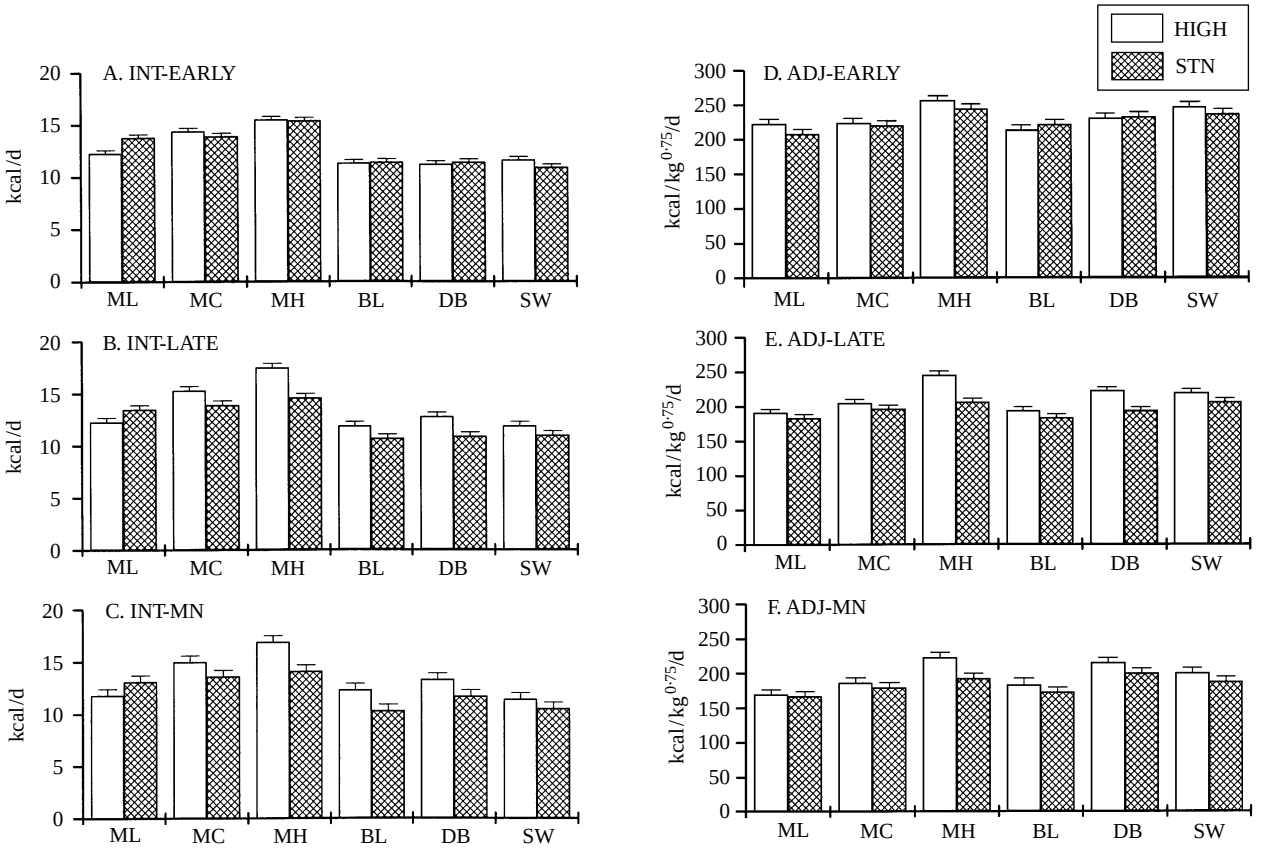

Fig. 5. Least-squares means for low (ML), control (MC) and high (MH) heat loss selection lines, and for C57BL/6J (BL), DBA/2J (DB) and SWR/J (SW) inbred lines, are shown for average cage energy intake measured between 4 and 8 weeks (INT-EARLY), 8 and 12 weeks (INT-LATE) and 12 and 14 weeks (INT-MN; *A*, *B* and *C*, respectively) for mice fed high-fat (38·2% fat; HIGH) and standard (12·7% fat; STN) diets. Least-squares means for average cage energy intake adjusted for metabolic body weight (ADJ-EARLY, ADJ-LATE and ADJ-MN) are also presented (*D*, *E* and *F*, respectively).

2). The HLOSS of BL was similar to that of ML when measured at 10 weeks and adjusted for metabolic body weight, but was greater than that of ML when measured at 15 weeks and adjusted for FFM. The effect of sex was significant for all HLOSS traits, with greater adjusted HLOSS observed in females compared with males (data not shown).

Line, diet, and line by diet interaction effects were significant for fat percentage. The fat percentage of MH was similar to that of DB and SW for both diets. Fat percentage of MH and BL was similar when fed STN diet, but BL had a greater fat percentage than MH when fed HIGH diet. The ML mice had a greater fat percentage than DB and SW for both diets, although the difference between ML and DB was greater for HIGH compared with STN diet (Table 2). The fat percentage of BL was similar to that of ML when fed HIGH diet, but was significantly lower when fed STN diet.

Organ weights expressed as a percentage of body weight differed among the lines (Table 1). In general, the relative size of organs of the inbred lines was similar to that of the MH line, but greater than that of the ML line (Table 2). However, spleens of SW mice were significantly larger than spleens of either selection line, and their hearts were smaller than MH hearts.

Energy intake differed significantly among the lines for all time periods measured (Table 3). For adjusted energy intake measurements, mice fed HIGH diet consumed more energy compared with mice fed STN diet for all time periods (Table 3; Fig. 5), but a significant line by diet interaction effect was observed for intake measured during early and late growth periods. Adjusted energy intake of MH mice was consistently greater than that of BL mice for all time periods. The MH mice consumed more energy than DB mice when fed HIGH diet during early and late growth, but adjusted energy intake of MH and DB mice did not differ when fed STN diet, or during the weight maintenance period. Adjusted energy intake of MH and SW mice was similar, except for mice fed HIGH diet during late growth. Adjusted energy intake of ML was similar to that of BL, but significantly less than that of SW. The DB mice has had greater intake than ML for both diets during early growth and weight maintenance periods, and when fed HIGH diet during late growth period (Table 4).

4. Discussion

Selection for high and low HLOSS created large differences in HLOSS as well as in energy intake and

https://doi.org/10.1017/S0016672397003017 Published online by Cambridge University Press

Table 1. *Mean squares resulting from analysis of variance of heat loss measured from 9 to 11 weeks* (*kcal/day; HL10*), *14 to 16 weeks* (*kcal/day; HL15*), *9 to 11 weeks adjusted for metabolic body weight* (*kcal/kg$^{0.75}$/day; HL10-MBW*) *and 14 to 16 weeks adjusted for fat-free mass* (*kcal/kg FFM/day; HL15-FFM*); *total body fat percentage* (%, *FAT*); *and liver, spleen and heart weights expressed as a percentage of body weight* (*LIVER*, *SPLEEN*, *HEART*)

Source	d.f.	HL10	HL15	HL10-MBW	HL15-FFM	FAT	LIVER	SPLEEN	HEART
Analysis 1[a]									
Line	2	192·03***	183·20***	38918·87***	189009·31***	473·95***	0·9024*	0·0669***	0·1677***
Diet	1	0·06	0·24	57·05	1199·67	220·31**	1·0159*	0·0017	0·0348
Line × Diet	2	9·51*	10·40*	967·24*	5905·54*	42·78	0·2530	0·0213**	0·0077
Sex	1	36·51***	14·34*	2550·60**	134496·46***	17·35	6·5305***	0·1164***	0·0036
Line × Sex	2	2·80	6·33	187·51	8139·61*	2·42	0·2600	0·0031	0·0000
Diet × Sex	1	1·52	17·71**	109·46	21725·10**	102·15	0·5769	0·0002	0·0037
Line × Diet × Sex	1	9·61	1·86	542·39	1189·10	26·98	0·3149	0·0081	0·0013
Error	89	2·11	2·40	224·79	1859·04	30·80	0·2082	0·0036	0·0097
Analysis 2[b]									
Line	5	212·41***	183·81***	20628·51***	96163·82***	444·02***	1·5340***	0·1462***	0·1958***
Diet	1	15·91**	5·24	1315·59*	2512·10	434·79***	1·2139*	0·0261	0·0060
Line × Diet	5	7·71***	5·75*	1464·79***	4539·14	62·18*	0·3153	0·0094***	0·0229
Sex	1	64·91***	36·36***	3199·18***	159099·22***	13·01	1·8170**	0·0276***	0·3652
Line × Sex	5	1·59	2·95	178·48	5862·38*	21·89	1·1193***	0·0132**	0·0094
Diet × Sex	1	0·14	9·01*	38·45	25981·56***	90·41	0·2807	0·0130	0·0000
Line × Diet × Sex	1	4·46*	2·54	283·47	919·39	29·86	0·2020	0·0087	0·0048
Error	173	1·62	2·01	211·60	2257·08	23·81	0·2260	0·0085	0·0029

[a] Analysis 1 included data from lines of mice selected for high and low heat loss, and unselected controls.
[b] Analysis 2 included data from the three selection lines as well as from three inbred lines of mice.
*$P < ·05$; **$P < ·01$; ***$P < ·001$.

body composition. Differences among three replicates of the MH, ML and MC selection lines have been described previously (Nielsen *et al*., 1997*a*, *b*), but only for males fed a standard laboratory diet and at a single age. The present study allows comparisons to be made for both males and females at two distinct time points and for diets differing in fat levels, and considers additional traits related to energy expenditure.

A difference between MH and ML males of 38·8% of the mean of HLOSS adjusted for metabolic body weight was observed, which is less than the 51% difference previously reported for replicate 1 (Nielsen *et al*., 1997*a*). This discrepancy may be due in part to sampling variance, as 18 males per line were measured in the present study, compared with 72–80 per line in the previous study. Different nutrient composition of diets may also contribute to the different results; the fat content of the STN synthetic diet used in the present study was greater than that of the rodent chow fed in the previous study (12·7% fat vs 4·0% fat). Heat loss expressed per unit of metabolic body weight or FFM tended to be greater in females than males, but the relative differences between MH and ML males and females were similar. Differences between MH and ML lines were also similar for HLOSS measured at 10 and 15 weeks. Clearly, selection for HLOSS, which was based on measurement of 9- to 11-week-old males only (Nielsen *et al*., 1997*a*), has been successful in changing energy expenditure in both males and females of the MH and ML lines at 9–11 weeks, and at older ages.

Because energy intake must equal energy expenditure to achieve energy balance, divergence of energy intake was expected to closely resemble divergence of HLOSS. The 15–21% difference in adjusted energy intake is similar to that previously reported (Nielsen *et al*., 1997*b*). Although these differences are significant, the divergence is roughly half as great as the divergence for HLOSS. This may be due in part to changes resulting from selection for factors other than maintenance energy requirements, such as physical activity and response to stress, that are unique to individual animal measurement in calorimeters and measured as a part of HLOSS. Alternatively, this discrepancy may be caused by measuring HLOSS at night when mice are most active, while energy intake is measured on the basis of 24-h days, including daytime when consumption is reduced.

Selection for HLOSS resulted in correlated changes in body composition. A significant difference in predicted total body fat percentage between MH and ML males of 5·5% was previously reported based on equations using measurement of electrical conductivity in live males at 12 weeks of age (Nielsen *et al*., 1997*b*). The present study identified much greater differences in total body fat percentage between MH and ML lines (40%) based on direct chemical analysis of body composition at 16 weeks. The methods and prediction equations described by Nielsen *et al*.

https://doi.org/10.1017/S0016672397003017 Published online by Cambridge University Press

Table 2. *Contrast of means of heat loss measured from 9 to 11 weeks (kcal/day; HL10), 14 to 16 weeks (kcal/day; HL15), 9 to 11 weeks adjusted for metabolic body weight (kcal/kg$^{0·75}$/day; HL10-MBW) and 14 to 16 weeks adjusted for fat-free mass (kcal/kg FFM/day; HL15-FFM); total body fat percentage (%; FAT); and liver, spleen and heart weights expressed as a percentage of body weight (LIVER, SPLEEN, HEART). Contrasts for high-fat (38·2%; high) and standard (12·7%; stn) diets are presented separately if a significant line by diet interaction was found from analysis of variance*

Contrast[a]	HL10	HL15	HL10-MBW	HL15-FFM	FAT	LIVER	SPLEEN	HEART
Analysis 1[b]								
MH–ML	2·42***	4·72***	70·07***	152·29***	−6·77***	0·3294**	0·0750***	0·1413***
ASYM	−0·14	1·52*	−6·76	40·06*	5·64*	−0·0064	0·0861***	−0·0227
MH–ML, high	3·24***	5·78***	78·21***	137·18***			0·1165***	
MH–ML, stn	1·60***	3·65***	61·94***	167·40***			0·0335	
ASYM, high	1·11	0·84	−19·03*	0·79			0·0370	
ASYM, stn	−1·40	2·20*	5·51	79·33**			0·1353***	
Analysis 2[c]								
BL–MH	−6·20***	−5·68***	−65·43***	−125·49***	1·66	−0·0114	0·0060	0·0012
DB–MH	−5·64***	−4·79***	−46·06***	−53·16***	1·66	0·2421*	0·0223	0·0283
SW–MH	−6·50***	−6·11***	−52·79***	−93·85***	−2.78	0·2075	0·1639***	−0·0950***
BL–ML	−1·26***	−0·97**	4·64	26·80*	−5·11***	0·3180**	0·0810***	0·1424***
DB–ML	−0·69*	−0·07	24·01***	66·13***	−5·10***	0·5715***	0·0973***	0·1695***
SW–ML	−1·55***	−1·39***	17·28***	58·44***	−9·56***	0·5369***	0·2389***	0·0462*
BL–MH, high	−6·49***	−6·41***	−72·08***		5·00**		−0·0298	
BL–MH, stn	−5·92***	−4·96***	−58·78***		−1·69		0·0418*	
DB–MH, high	−5·23***	−4·90***	−37·75***		1·48		0·0071	
DB–MH, stn	−6·05***	−4·68***	−54·37***		1·84		0·0375	
SW–MH, high	−6·57***	−6·47***	−51·97***		−2·40		0·1855***	
SW–MH, stn	−6·42***	−5·74***	−53·62***		−3·19		0·1422***	
BL–ML, high	−0·45	−0·63	6·13		−3·01		0·0868***	
BL–ML, stn	−2·07***	−1·31*	3·16		−7·21***		0·0752***	
DB–ML, high	0·81	0·88	40·46***		−6·53***		0·1235***	
DB–ML, stn	−2·20***	−1·02*	7·57		−3·68*		0·0710***	
SW–ML, high	−0·53	−0·69	26·25***		−10·41***		0·3020***	
SW–ML, stn	−2·57***	−2·09***	8·32		−8·71***		0·1757***	

[a] Contrasts for analysis 1 test differences between MH and ML selection lines (MH–ML), and differences between MC and the average of MH and ML (ASYM). Contrasts for analysis 2 test differences between each inbred line and MH and ML selection lines (BL–MH, DB–MH, SW–MH, BL–ML, DB–ML and SW–ML).

[b] Analysis 1 included data from lines of mice selected for high and low heat loss, and unselected controls.

[c] Analysis 2 included data from the three selection lines as well as from three inbred lines of mice.

*$P < ·05$; **$P < ·01$; ***$P < ·001$.

Table 3. *Mean squares resulting from analysis of variance of energy intake measured from 4 to 8 weeks (kcal/day; INT-EARLY), 8 to 12 weeks (kcal/day; INT-LATE) and 12 to 14 weeks (kcal/day; INT-MN), and energy intake adjusted for metabolic body weight measured from 4 to 8 weeks ($kcal/kg^{0·75}/day$; ADJ-EARLY), 8 to 12 weeks ($kcal/kg^{0·75}/day$; ADJ-LATE) and 12 to 14 weeks ($kcal/kg^{0·75}/day$; ADJ-MN) as a cage average*

Source	d.f.	INT-EARLY	INT-LATE	INT-MN	ADJ-EARLY	ADJ-LATE	ADJ-MN
Analysis 1[a]							
Line	2	14·06***	23·57***	23·25**	3195·74***	3631·78***	3704·72***
Diet	1	0·66	7·18*	6·75	758·16	2489·90*	1285·30*
Line × Diet	2	2·86*	10·82**	10·72*	78·34	787·75*	520·49
Sex	1	22·86***	28·85***	46·39***	399·81	450·75	28·26
Line × Sex	2	0·45	3·95	0·63	375·10	524·73	10·16
Diet × Sex	1	0·00	1·01	1·47	241·36	15·64	2·16
Line × Diet × Sex	1	3·10*	1·24	1·73	224·95	10·22	14·33
Error	18	0·56	1·14	2·32	276·72	185·47	282·55
Analysis 2[b]							
Line	5	30·27***	35·31***	28·56***	2427·31***	2092·80***	2378·24***
Diet	1	0·16	20·28***	20·52**	76·12***	4646·85***	2299·08**
Line × Diet	5	1·45*	4·69**	4·67*	146·89*	411·54*	213·43
Sex	1	43·78***	52·63***	54·80***	59·52	409·88	190·77
Line × Sex	5	0·30	1·63	1·17	232·98	236·81	117·70
Diet × Sex	1	0·00	0·03	0·10	24·00	108·87	369·83
Line × Diet × Sex	1	1·69	0·95	1·53	214·45	53·40	72·97
Error	36	0·53	0·91	1·62	125·01	129·12	249·84

[a] Analysis 1 included data from lines of mice selected for high and low heat loss, and unselected controls.
[b] Analysis 2 included data from the three selection lines as well as from three inbred lines of mice.
$*P < ·05$; $**P < ·01$; $***P < ·001$.

(1997*b*) were also used to predict fat percentage at earlier ages in the present study. These methods predicted similar trends in body composition (data not shown), but failed to identify the large magnitude of divergence that was found using direct measurement. The data in this study indicate that the correlated change in body composition in response to long-term selection for HLOSS is highly significant, providing support for the hypothesis that reduced energy expenditure contributes to obesity (Saltzman & Roberts, 1995). The change in body composition observed in MH and ML mice is of particular interest because few or no correlated changes in body weight occurred. Because a high genetic correlation exists between fat percentage and body weight (Eisen, 1987), the MH and ML populations provide a unique resource in which to study genetic regulation of weight-independent body composition.

Because of the significant difference in body composition between MH and ML lines, and because body composition can explain a significant amount of total energy expenditure (Ravussin *et al*., 1986), HLOSS was adjusted for FFM. The divergence in HLOSS between MH and ML mice decreased as a result of adjusting for body composition, but remained highly significant. Thus, even though MH mice may be expected to have greater HLOSS because of a lower percentage of body fat (Ravussin *et al*., 1986), the difference in HLOSS observed between MH and ML cannot be attributed solely to differences in FFM.

Organ weights adjusted for body weight were considered as an additional source of HLOSS variation. The larger livers and hearts of MH compared with ML mice were probably needed to accommodate the increased energy intake and expenditure of the MH line. This finding is consistent with results of Konarzewski and Diamond (1995), who reported that strains of mice with high resting metabolic rates also tended to have large organs.

The MH and ML lines described here and elsewhere (Nielsen *et al*., 1997*a*, *b*) offer a unique model for future investigation of the regulation of energy utilization and fat deposition. Of particular interest is the weight-independent change in body composition between MH and ML lines resulting from selection for HLOSS. This correlated response supports previous reports that identified energy expenditure as a risk factor for obesity in certain human populations (Ravussin *et al*., 1988; Roberts *et al*., 1988; Griffiths *et al*., 1990) and suggest that energy expenditure is an important regulator of body composition. Investigation to identify differences between MH and ML lines at the molecular level could identify genes or mechanisms involved in the regulation of HLOSS, and these factors could provide insight into the relationship between energy expenditure and obesity.

Several studies have employed genetic markers to identify chromosomal regions harbouring QTL contributing to differences in traits relating to obesity in mice (see Pomp, 1997). The MH and ML lines are clearly divergent for HLOSS and would produce an F_2 population with the phenotypic variation necessary

https://doi.org/10.1017/S0016672397003017 Published online by Cambridge University Press

Table 4. *Contrast of means of energy intake measured from 4 to 8 weeks (kcal/day; INT-EARLY), 8 to 12 weeks (kcal/day; INT-LATE) and 12 to 14 weeks (kcal/day; INT-MN), and energy intake adjusted for metabolic body weight measured from 4 to 8 weeks ($kcal/kg^{0·75}/day$; ADJ-EARLY), 8 to 12 weeks ($kcal/kg^{0·75}/day$; ADJ-LATE) and 12 to 14 weeks ($kcal/kg^{0·75}/day$; ADJ-MN) as a cage average. Contrasts for high-fat (38·2%; high) and standard (12·7%; stn) diets are presented separately if a significant line by diet interaction was found from analysis of variance*

Contrast[a]	INT-EARLY	INT-LATE	INT-MN	ADJ-EARLY	ADJ-LATE	ADJ-MN
Analysis 1[b]						
MH–ML	2·42***	3·13***	3·09***	34·45***	38·30***	38·85***
ASYM	−0·14	0·27	0·63	−20·86	−11·78	−10·19
MH–ML, high	3·24***	5·23***	5·17***		54·26***	
MH–ML, low	1·60**	1·03	1·01		22·35*	
ASYM, high	1·11	0·78	1·30		−26·65	
ASYM, low	−1·40	−0·24	−0·04		3·09	
Analysis 2[c]						
BL–MH	−4·14***	−4·73***	−4·21***	−32·78***	−37·32***	−29·87***
DB–MH	−4·12***	−4·19***	−3·00***	−18·76***	−17·90**	−0·09
SW–MH	−4·21***	−4·58***	−4·60***	−9·01	−13·09*	−13·55
BL–ML	−1·73***	−1·60***	−1·16	10·50*	0·98	8·98
DB–ML	−1·71***	−1·60*	0·09	24·52***	20·41***	38·76***
SW–ML	−1·79***	−1·45**	−1·51*	34·27***	25·21***	25·29***
BL–MH, high	−4·25***	−5·55***	−4·64***	−42·76***	−51·94***	
BL–MH, low	−4·04***	−3·91***	−3·78***	−22·80**	−22·71**	
DB–MH, high	−4·28***	−4·69***	−3·62***	−25·64**	−23·28**	
DB–MH, low	−3·96***	−3·70***	−2·38**	−11·88	−14·51	
SW–MH, high	−3·95***	−5·59***	−5·54***	−9·96	−26·35***	
SW–MH, low	−4·46***	−3·56***	−3·66***	−8·05	0·17	
BL–ML, high	−1·01*	−0·32	0·53	7·99	2·32	
BL–ML, low	−2·44***	−2·88***	−2·76**	13·01	−0·36	
DB–ML, high	−1·05*	0·54	1·55	25·11**	30·97***	
DB–ML, low	−2·36***	−2·67***	−1·37	23·93**	9·84	
SW–ML, high	−0·71	−0·35	−0·37	40·79***	27·90***	
SW–ML, low	−2·86***	−2·54***	−2·64**	27·76***	22·52**	

[a] Contrasts for analysis 1 test differences between MH and ML selection lines (MH–ML), and differences between MC and the average of MH and ML (ASYM). Contrasts for analysis 2 test differences between each inbred line and MH and ML selection lines (BL–MH, DB–MH, SW–MH, BL–ML, DB–ML and SW–ML).
[b] Analysis 1 included data from lines of mice selected for high and low heat loss, and unselected controls.
[c] Analysis 2 included data from the three selection lines as well as from three inbred lines of mice.
$*P < ·05$; $**P < ·01$; $***P < ·001$.

to detect QTL. However, the power of QTL detection in such a cross may be low due to shared marker alleles inherited from their common base population. One strategy to improve the power of QTL detection would be to identify an existing inbred line with HLOSS different from either MH or ML. A cross between MH or ML and an inbred line would be useful because markers could be identified that would be fully informative, despite heterogeneity within the selection lines. The greatest difference in HLOSS between selection and inbred lines evaluated in this study was observed between MH and BL. Therefore, a cross between MH and BL is likely to be useful for the identification of QTL influencing HLOSS, and would also be useful for evaluating QTL effects in a genetic background different from MH and ML.

The evaluation of genotype by environment interactions is important in understanding the complex and multifactorial nature of obesity. Significant interaction between genotype and dietary environment has been described where males representing six inbred mouse strains experienced a significant increase in carcass lipid content when fed a high-fat diet, while three other strains were resistant to the high-fat diet (West *et al.*, 1992). In the present study, the most notable effect of the HIGH diet was an increase in body fat percentage in BL mice, similar to that previously reported (West *et al.*, 1992). When fed STN diet, MH and BL mice had different adjusted HLOSS and energy intake but a similar fat percentage. However, adjusted HLOSS, energy intake and fat percentage of MH and BL mice were significantly different when animals were fed HIGH diet. Thus, it is possible that different subsets of QTL influencing fat deposition and HLOSS could be identified from a cross between MH and BL depending on the diet that is fed.

In contrast, MH and ML lines differed significantly

https://doi.org/10.1017/S0016672397003017 Published online by Cambridge University Press

for adjusted HLOSS, energy intake and fat percentage when fed either HIGH or STN diet. Thus, it is conceivable that a different mechanism is responsible for low HLOSS in ML compared with BL, allowing ML to store energy as fat regardless of diet. Rice *et al*. (1996) estimated that significant portions of shared variance between resting metabolic rate (RMR) and FFM, and between RMR and fat mass in humans are due to genetics. A model was proposed where three gene systems regulate RMR and body composition. Two of these systems, G1 and G3, have pleiotropic effects influencing both RMR and FFM or RMR and fat mass, respectively, while G2 regulates RMR independently of body composition. Crosses of MH with BL and ML may be useful to identify QTL belonging to these different gene systems. For example, QTL specific to a MH/ML cross that influence HLOSS and FAT may belong to the G1 or G3 system, while QTL specific to a MH/BL cross which influence only HLOSS may belong to G2.

Published as paper no. 11830, Journal Series, Agricultural Research Division, University of Nebraska. The authors thank Tong Zou, Jeryl Hauptman and Ruth Diedricksen for their assistance with data collection and mouse care.

References

Bouchard, C. (1994). *The Genetics of Obesity* Boca Raton, FL: CRC Press.

Buscemi, S., Caimi, G. & Verga, S. (1996). Resting metabolic rate and postabsorptive substrate oxidation in morbidly obese subjects before and after massive weight loss. *International Journal of Obesity* **20**, 41–46.

Eisen, E. J. (1987). Selection for components related to body composition in mice: direct responses. *Theoretical and Applied Genetics* **74**, 793–801.

Griffiths, M., Payne, P. R., Stunkard, A. J., Rivers, J. P. W. & Cox, M. (1990). Metabolic rate and physical development in children at risk of obesity. *The Lancet* **336**, 76–78.

Jones, L. D., Nielsen, M. K. & Britton, R. A. (1992). Genetic variation in liver mass, body mass, and liver: body mass in mice. *Journal of Animal Science* **70**, 2999–3006.

Kirk, E. A., Moe, G. L., Caldwell, M. T., Lernmark, J. A., Wilson, D. L. & LeBoeuf, R. C. (1995). Hyper- and hyporesponsiveness to dietary fat and cholesterol among inbred mice: searching for level and variability genes. *Journal of Lipid Research* **36**, 1522–1532.

Konarzewski, M. & Diamond, J. (1995). Evolution of basal metabolic rate and organ masses in laboratory mice. *Evolution* **49**, 1239–1248.

Kuczmarski, R. J., Johnson, C. L., Flegal, K. M. & Campbell, S. M. (1994). Increasing prevalence of overweight among US adults. *Journal of the American Medical Association* **272**, 205–211.

Nielsen, M. K., Jones, L. D., Freking, B. A. & DeShazer, J. A. (1997*a*). Divergent selection for heat loss in mice. I. Selection applied and direct response through fifteen generations. *Journal of Animal Science* **75**, 1461–1468.

Nielsen, M. K., Freking, B. A., Jones, L. D., Nelson, S. M., Vorderstrasse, T. L. & Hussey, B. A. (1997*b*). Divergent selection for heat loss in mice. II. Correlated responses in feed intake, body mass, body composition and number born through fifteen generations. *Journal of Animal Science* **75**, 1469–1476.

Pomp, D. (1997). Genetic dissection of obesity in polygenic animal models. *Behavior Genetics* **27**, 285–305.

Ravussin, E., Lillioja, S., Anderson, T. E., Christin, L. & Bogardus, C. (1986). Determinants of 24-hour energy expenditure in man. *Journal of Clinical Investigation* **78**, 1568–1578.

Ravussin, E., Lillioja, S., Knowler, W. C., Christin, L., Freymond, D., Abbott, W. G. H., Boyce, V., Howard, B. V. & Bogardus, C. (1988). Reduced rate of energy expenditure as a risk factor for body-weight gain. *New England Journal of Medicine* **318**, 467–472.

Rice, T., Tremblay, A., Deriaz, O., Perusse, L., Rao, D. C. & Bouchard, C. (1996). Genetic pleiotropy for resting metabolic rate with fat-free mass and fat mass: the Quebec family study. *Obesity Research* **4**, 125–131.

Roberts, S. B., Savage, J., Coward, W. A., Chew, B. & Lucas, A. (1988). Energy expenditure and intake in infants born to lean and overweight mothers. *New England Journal of Medicine* **318**, 461–466.

Saltzman, E. & Roberts, S. B. (1995). The role of energy expenditure in energy regulation: findings from a decade of research. *Nutrition Reviews* **53**: 209–220.

SAS. (1988). *SAS/STAT User's Guide*, release 6·03. Cary, NC: SAS Institute.

West, D. B., Boozer, C. N., Moody, D. L. & Atkinson, R. L. (1992). Dietary obesity in nine inbred mouse strains. *American Journal of Physiology* **262**, R1025–R1032.