

Genetic variation and correlation of dietary response in an advanced intercross mouse line produced from two divergent growth lines

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Summary

Levels of human obesity have increased over the past 20 years worldwide, primarily due to changes in diet and activity levels. Although environmental changes are clearly responsible for the increasing prevalence of obesity, individuals may show genetic variation in their response to an obesogenic environment. Here, we measure genetic variation in response to a high-fat diet in a mouse model, an F₁₆ Advanced Intercross Line derived from the cross of SM/J and LG/J inbred mouse strains. The experimental population was separated by sex and fed either a high-fat (42% of energy from fat) or low-fat (15% of energy from fat) diet. A number of phenotypic traits related to obesity and diabetes such as growth rate, glucose tolerance traits, organ weights and fat pad weights were collected and analysed in addition to serum levels of insulin, free fatty acids, cholesterol and triglycerides. Most traits are different between the sexes and between dietary treatments and for a few traits, including adult growth, fat pad weights, insulin and glucose tolerance, the dietary effect is stronger in one sex than the other. We find that fat pad weights, liver weight, serum insulin levels and adult growth rates are all phenotypically and genetically correlated with one another in both dietary treatments. Critically, these traits have relatively low genetic correlations across environments (average $r = 0.38$). Dietary responses are also genetically correlated across these traits. We found substantial genetic variation in dietary response and low cross environment genetic correlations for traits aligned with adiposity. Therefore, genetic effects for these traits are different depending on the environment an animal is exposed to.

1. Introduction

The genetics of human obesity has attracted much attention recently due to the increased prevalence of obesity worldwide. However, the recent dramatic increase in human obesity is not due to evolutionary genetic changes over the past generation (Yanovski & Yanovski, 1999), but rather to environmental changes in diet and activity. Even so, the response of an individual to these societal changes can vary by genotype. Some individuals react to a high-fat diet by becoming obese while other individuals remain relatively lean (Seidell, 1998).

Experimental populations derived from inbred mouse strains are an excellent model for the study of the genetic architecture of complex traits such as obesity, type 2 diabetes and dietary response (Fisler

& Warden, 1997). The use of previously characterized inbred lines as parental strains coupled with the precise environmental control that is possible with laboratory rodents makes them amenable to the rigorous statistical analysis necessary for the dissection of complex traits and their environmental interactions while minimizing other sources of variation. West *et al.* (1992, 1995) showed that an assortment of mouse strains varied in their response to a high-fat diet, indicating genetic variability in dietary response among strains. Studies by our group have demonstrated that the SM/J and LG/J inbred mouse strains differ in their response to increased levels of dietary fat with the SM/J strain demonstrating a greater response to a high-fat diet (Cheverud *et al.*, 1999; Ehrich *et al.*, 2003). Therefore, a population formed from the cross of these strains should display genetic variation in response to a high-fat diet. We have

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previously examined quantitative trait loci (QTL) for adiposity in an F₂ cross of LG/J and SM/J strains of mice (Cheverud *et al.*, 2001). To date this cross has yielded more QTL related to body size and adiposity than any other (Snyder *et al.*, 2004).

We have generated an Advanced Intercross Line (AIL) (Darvasi & Soller, 1995) from the cross of LG/J and SM/J inbred mouse strains. In the F₁₆ generation, half of each family was reared on a low-fat diet and the other half on a high-fat diet. We measured several phenotypes related to obesity and diabetes. This study design allows us to address questions of genetic variability in the traits themselves and in their dietary responses. A finding of significant family by environment interaction indicates the presence of genetic variation in response to a high-fat diet.

2. Methods

(i) Mouse population

The mouse population used in this study was derived from a cross of the SM/J ('Small') and LG/J ('Large') inbred lines. SM/J (MacArthur, 1944) and LG/J (Goodale, 1938) were originally derived from separate experiments selecting for small and large 60-day body size, respectively. The base population for the LG/J strain was composed of 'albinos' of no particular distinction obtained from a commercial breeder. Goodale (1938) reported that the base population was formed from the descendants of 5 males and 11 females. The base population for the experiment that produced the SM/J strain was formed by crossing seven strains in four separate crosses and then randomly interbreeding these hybrids. The strains used were 'dilute brown' (*dba*) and 'silver chocolate' (*sv ba*) from the Jackson Laboratories, 'black and tan' (*a'*), and pink-eyed, short-eared 'dilute brown' (*ps^e dba*) from the University of Michigan, and 'albino' (*c*), 'cinnamon spotted' (*bs*) and 'agouti' (*a*) from the University of Toronto. It is likely that the 'dilute brown' (*dba*) strain cited by MacArthur (1944) was related to the modern DBA/1J and DBA/2J strains held at the Jackson Laboratories. West *et al.* (1992) showed that the DBA/2J strain had a statistically significant but moderate response to a high-fat diet. SM/J is not especially closely related to DBA/2J as they differ at 37% of single-nucleotide polymorphisms (SNPs) genome-wide while random strain pairs differ at 40% of these SNPs on average (Pletcher *et al.*, 2004).

Original inbred mice for this experiment came from the Jackson Laboratories (Bar Harbor, Maine). The AIL was maintained by random mating, sib-matings excluded, from the F₂ intercross animals (Cheverud *et al.*, 2001) through the F₁₆ generation, with at least 65 mating pairs per generation resulting

Table 1. *Composition of diets*

	High-fat	Low-fat
Energy from fat	42%	15%
Casein (g/kg)	195	197
Sugars (g/kg)	341	307
Corn starch (g/kg)	150	313
Cellulose (g/kg)	50	30
Corn oil (g/kg)	–	58
Hydrogenated coconut oil (g/kg)	–	7
Anhydrous milkfat (g/kg)	210	–
Cholesterol (g/kg)	1.5	–
Total energy (kJ/g)	18.95	16.99

The diets used in this study were selected to be as similar as possible for nutrient composition with the exception of percentage of energy derived from fat.

in an effective population size over 250 (Darvasi & Soller, 1995). Eighty-two F₁₅ mating pairs were mated producing 1000 F₁₆ offspring in 148 litters and 82 sibships (families) of 12.2 pups per family. No standardization or adjustment was made for litter size.

Animals were weaned at 3 weeks of age, at which time each family was evenly divided by sex and diet, with no more than 5 animals per cage (Table 2). Maternal diet was PicoLab Rodent Chow 20 (#5053). The high-fat diet (Harlan Teklad catalog #TD88137) and low-fat diet (Research Diets catalog #D12284) compositions are shown in Table 1. All animals were fed *ad libitum*. The animal facility is maintained at a constant temperature of 21 °C, with 12-hour light and dark cycles.

All animals were weighed once per week on the day of their birth from 1 to 20 weeks. We divided growth into three consecutive periods to highlight three physiologically distinct growth phases. Three weeks was chosen for the first division because murine growth is controlled by different genetic and physiological systems before and after 3 weeks of age (Cheverud *et al.*, 1996; Cheverud, 2005a). Furthermore, skeletal growth is generally complete by 10 weeks of age so that growth after this time will be primarily due to increases in soft tissue weight. The pre-weaning growth period comprises the period from birth to weaning and was calculated as log₁₀(week 3/week 1). The post-weaning growth period consists of weeks 3 to 10 [log₁₀(week 10/week 3)], while the adult growth period consists of weeks 10 to 20 [log₁₀(week 20/week 10)].

(ii) Glucose tolerance

A subset of the animals received a glucose tolerance test at 10 and 20 weeks of age (Table 2). This subset includes 430 animals from 95 litters and 78 families. Animals were subjected to a 4-hour fast, at which

Table 2. Heritability (h^2) for each sex and diet cohort followed by the standard error of the heritability estimate in parentheses

	Family effect, by cohort			
	Females, low-fat diet	Females, high-fat diet	Males, low-fat diet	Males, high-fat diet
Glucose Tolerance				
<i>10-week test</i>				
Basal (mg/dl)	0.44 (0.25)	0.47 (0.27)	0.89 (0.37)	0.78 (0.34)
AUC (mg × min/dl)	0.65 (0.30)	0.06 (0.10)	0.88 (0.37)	0.47 (0.26)
Multivariate probability	<i>5.18 × 10⁻³</i>	<i>0.132</i>	<i>1.54 × 10⁻⁵</i>	<i>4.73 × 10⁻³</i>
<i>20-week test</i>				
Basal (mg/dl)	0.67 (0.31)	0.18 (0.17)	0.77 (0.35)	0.50 (0.27)
AUC (mg × min/dl)	0.38 (0.23)	0.95 (0.38)	1.13 (0.42)	0.05 (0.09)
Multivariate probability	<i>5.20 × 10⁻³</i>	<i>2.29 × 10⁻³</i>	<i>4.99 × 10⁻⁶</i>	<i>0.111</i>
Organ weights				
Heart (g)	0.52 (0.18)	0.20 (0.11)	0.08 (0.07)	0.24 (0.12)
Kidney (g)	0.33 (0.14)	0.22 (0.11)	0.29 (0.14)	0.12 (0.09)
Spleen (g)	-0.26 (NA)	-0.03 (NA)	-0.13 (NA)	0.37 (0.15)
Liver (g)	0.18 (0.11)	0.48 (0.18)	0.56 (0.19)	0.46 (0.17)
Multivariate probability	<i>0.0331</i>	<i>0.0142</i>	<i>6.23 × 10⁻³</i>	<i>5.01 × 10⁻⁴</i>
Fat pad weights				
Reproductive fat pad (g)	0.50 (0.18)	0.58 (0.19)	0.66 (0.20)	0.07 (0.07)
Renal fat pad (g)	0.48 (0.17)	0.50 (0.18)	0.57 (0.19)	0.63 (0.20)
Mesenteric fat pad (g)	0.36 (0.15)	0.23 (0.12)	-0.04 (NA)	0.48 (0.17)
Inguinal fat pad (g)	0.24 (0.12)	0.27 (0.13)	0.24 (0.12)	0.51 (0.18)
Multivariate probability	<i>4.13 × 10⁻⁴</i>	<i>7.10 × 10⁻⁸</i>	<i>2.97 × 10⁻⁷</i>	<i>8.58 × 10⁻⁶</i>
Serum levels				
Insulin (ng/ml)	0.34 (0.14)	0.37 (0.15)	0.30 (0.14)	0.001 (0.01)
Free fatty acids (mmol/l)	-0.08 (NA)	0.04 (0.05)	0.04 (0.05)	0.28 (0.13)
Cholesterol (mg/dl)	0.21 (0.11)	0.19 (0.11)	0.32 (0.14)	0.44 (0.17)
Triglycerides (mg/dl)	0.13 (0.09)	0.39 (0.16)	0.06 (0.06)	0.17 (0.10)
Multivariate probability	<i>0.0118</i>	<i>1.44 × 10⁻⁶</i>	<i>7.50 × 10⁻⁴</i>	<i>1.26 × 10⁻⁴</i>
Growth traits				
Pre-weaning (g/week)	0.36 (0.15)	0.59 (0.19)	0.41 (0.16)	0.89 (0.24)
Post-weaning (g/week)	0.36 (0.15)	0.67 (0.21)	0.73 (0.22)	0.76 (0.22)
Adult (g/week)	0.19 (0.11)	0.34 (0.15)	0.13 (0.09)	0.25 (0.13)
Multivariate probability	<i>4.72 × 10⁻⁵</i>	<i>1.86 × 10⁻¹¹</i>	<i>4.01 × 10⁻⁷</i>	<i>4.55 × 10⁻¹⁶</i>

Significant heritabilities are in boldface. Phenotypic traits are grouped by category, with multivariate probabilities for each multiple trait category indicated in italics.

time a basal glucose reading was obtained using a Glucometer Dex blood glucose meter (Bayer). Animals were then intraperitoneally injected with 0.01 ml of a 10% glucose solution for every gram of body weight. Additional glucose readings were obtained 15, 30, 60 and 120 minutes after the initial injection. The area under the curve (AUC) of the time-specific glucose levels plotted against time is a measure of an animal's ability to clear a fresh load of glucose from its bloodstream. This function is physiologically distinct from the fasting glucose level.

(iii) Necropsy

After 20 weeks of age, animals were again fasted for 4 hours, anaesthetized with sodium pentobarbital

and a terminal blood sample obtained via cardiac puncture. Blood plasma was separated through centrifugation and analysed for free fatty acids, cholesterol, triglycerides and insulin. Internal organs (liver, spleen, heart and kidneys) and fat pads (reproductive, renal, mesenteric and inguinal) were removed and weighed. The reproductive fat pad is adjacent to the epididymis in males and the uterine tubes in females and is typically the largest murine fat depot. The renal fat pads surround the kidneys and are enclosed in their own distinct fascial compartments. The mesenteric fat pad is contained within the dorsal mesentery of the gut between the stomach and rectum while the inguinal fat pad is a subcutaneous fat depot in the inguinal region.

(iv) *Multivariate analysis*

Phenotypes were analysed in four multivariate sets: glucose, necropsy, weekly weights and growth (see Table 2) using the following MANOVA in SYSTAT 10.0 (Wilkinson & Coward, 2000):

$$Y_{ijkl} = \mu + \text{Family}_i + \text{Sex}_j + \text{Diet}_k + \text{Family}_i \times \text{Sex}_j + \text{Family}_i \times \text{Diet}_k + \text{Sex}_j \times \text{Diet}_k + \text{Family}_i \times \text{Sex}_j \times \text{Diet}_k + e_{ijkl}. \quad (1)$$

In the above equation μ is the mean and Y_{ijkl} is the trait vector for the l th individual of family i and sex j , raised on diet k . Multivariate probabilities for each trait set were obtained with Wilk's Λ . These multivariate tests give the overall probability of no effect of the factor in question on each trait set as a whole, accounting for multiple traits. Sex and diet are treated as fixed effects while family and its interactions with other factors are treated as random effects. Parity, first or second litter, did not interact with the other factors so its effects were removed prior to analysis by subtracting the mean difference due to parity from phenotypic values of animals born in the second litter. A significant effect of family indicates significant genetic variation for the trait. A significant sex by family interaction indicates a genetic basis for variation in sexual dimorphism while a significant family by diet interaction indicates genetic variability in response to a high-fat diet. Finally, a significant three-way interaction (sex by diet by family) indicates genetic variation in sexual dimorphism of dietary response for the trait. The last two interaction effects are of primary interest here since they relate to genetic variation in response to a high-fat diet. If a multivariate trait set is statistically significant for an interaction factor, the set was analysed by individual cohorts comprising that factor. For example, if a trait set demonstrated a significant sex interaction, then males and females were analysed separately for that trait set. All trait sets were also analysed separately by sex and diet cohort according to the following MANOVA (Wilkinson & Coward, 2000):

$$Y_{ij} = \mu + \text{Family}_i + e_{ij}. \quad (2)$$

In the above equation, Y_{ij} is the trait set of the j th individual of family i .

(v) *Variance components*

Variance components for random effects are calculated as described by Sokhal & Rolf (1981):

$$\sigma_{family}^2 = \frac{MS_{family} - \sigma^2}{nab}, \quad (3)$$

$$\sigma_{sex \times family}^2 = \frac{MS_{sex \times family} - \sigma^2}{na}, \quad (4)$$

$$\sigma_{diet \times family}^2 = \frac{MS_{diet \times family} - \sigma^2}{nb}, \quad (5)$$

$$\sigma_{sex \times diet \times family}^2 = \frac{MS_{sex \times diet \times family} - \sigma^2}{n}. \quad (6)$$

In the above equations nab represents the number of pups per family, na represents the number of pups in each diet cohort per family, nb represents the number of pups of each sex per family and n represents the number of pups of each sex on each diet per family. Due to differences in sex ratios and litter sizes across families, the experimental population is not completely balanced by cohort. Therefore, a weighted average is calculated according to the method indicated by Sokhal & Rolf (1981) where a represents the total number of families and n is the number of pups in a sex–diet–family cohort:

$$n_{weighted} = \frac{1}{a-1} \left(\sum_i^a n_i - \frac{\sum_i^a n_i^2}{\sum_i^a n_i} \right). \quad (7)$$

Since the design used here is a full-sib design, the among-family variances (σ_{family}^2 , $\sigma_{sex \times family}^2$, $\sigma_{diet \times family}^2$, $\sigma_{sex \times diet \times family}^2$) are composed of one-half of the additive genetic variance, one-quarter of the dominance variance and various fractions of the epistatic variances (Falconer & Mackay, 1996) for each factor. In standard fashion, we assume a lack of dominance and epistatic variance and interpret twice the among-family variance as an estimate of the additive genetic variance for the trait itself (family), for sexual dimorphism in the trait (sex by family), for dietary response in the trait (diet by family) and for sexual dimorphism of dietary response (sex by diet by family) for the trait, respectively. Heritability estimates are given by the ration of the additive genetic variance to the sum of the between-family variance and the residual variance. Standard errors are calculated by the method described by Falconer & Mackay (1996). QTL analyses of this same intercross indicate that dominance is not very common, except for body weights and growth at early ages (Cheverud *et al.*, 1996; Vaughn *et al.*, 1999) and that epistasis is pervasive (Cheverud *et al.*, 2001; Cheverud, 2005b). The presence of dominance and epistasis may slightly inflate heritability estimates. Furthermore, no correction was made for maternal effects which will also contribute to between-family variance. This confounding factor may be important for early weights and growth but is minor at later ages and for necropsy traits (Kramer *et al.*, 1998).

Environmental variance (σ_E^2) is calculated as the difference between the total phenotypic variance and

the sum of the additive genetic variance components described above. The environmental variance also includes those portions of the non-heritable dominance and epistatic genetic effects not confounded with additive effects in a full-sib analysis (Falconer & Mackay, 1996). Heritability (h^2) is calculated as the additive genetic variance component divided by the sum of that variance component and the environmental variance.

(vi) Correlations

Phenotypic correlations are calculated using the Pearson correlation matrix feature of SYSTAT 10 (Wilkinson *et al.*, 2000). Genetic correlations are calculated by dividing genetic covariance between traits by the inverse of the square root of the product of the variances for those traits. Standard errors of genetic correlations are calculated using Robertson's approximation (Falconer & Mackay, 1996). Genetic covariances between traits were estimated as twice the among-family covariance obtained from the MANOVA using the multivariate version of equation 3. Because glucose-related traits have only been examined in a subset of the total population, correlations between glucose measurements and the remaining traits only utilize that subgroup having data for the glucose measurements.

In addition to genetic correlations between the traits themselves, we have calculated genetic correlations between dietary responses and sexual dimorphisms for traits with significant diet by family and sex by family interactions. Genetic correlations between dietary responses for various traits and between the sexes are measured using the family by diet and family by sex interaction variance/covariance matrices, respectively, obtained from the sums of squares and cross-products matrices for these factors using the multivariate versions of equations (4) and (5). Traits that have a common genetic basis for dietary response will have relatively high dietary response genetic correlation. Likewise, traits that share a genetic basis for variation in sexual dimorphism will have high genetic correlations for sexual dimorphism.

Genetic correlations between environments (Falconer & Mackay, 1996) are calculated by correlating the mean of a phenotype for a family on one diet with the mean of the same phenotype for the same family on the other diet. These means estimate diet-specific breeding values of the parents. This estimate introduces a slight bias towards lower correlations when compared with the method described by Lynch & Walsh (1998), but it is a mathematically more robust system, with no missing values. The slight bias is due to the inclusion of small amounts of the within-family variance/covariance into these

estimates of genetic correlations. A high positive genetic correlation between environments indicates that genes have very similar obesity-related effects on both diets while correlations near zero indicate that genes have independent effects on the two diets.

3. Results

(i) Sexual dimorphism and dietary effect

Phenotypic means and standard errors are listed by sex and diet cohort in the Appendix. Nearly all traits examined differ significantly by sex and diet. Females have lower basal glucose levels than males, and animals on a low-fat diet have lower basal glucose levels than animals on a high-fat diet. All cohorts had lower basal glucose levels at 10 weeks than at 20 weeks, but the age-related increase was slightly greater among animals on a high-fat diet. When overall response to glucose challenge is expressed as AUC, similar patterns emerge. As with basal glucose levels, females have lower AUCs than males and animals on a low-fat diet have lower AUCs than animals on a high-fat diet. Unlike basal glucose readings, however, both 10- and 20-week AUCs show a significant dietary effect on sexual dimorphism (sex by diet interaction), with males responding more to diet than females for this phenotype.

Heart, kidney and liver weights were all significantly lower for females than for males. Spleen weights did not differ significantly by sex. All organ weights show a significant diet effect: animals on a high-fat diet have heavier organs than animals on a low-fat diet. This is especially true of liver weight. At necropsy it was observed that animals fed a high-fat diet had noticeably more fat mottling their livers than did animals fed a low-fat diet. Liver weight also shows a significant sex by diet interaction with males responding more dramatically to the high-fat diet than females.

Fat pad weights generally follow same trends as organ weights: females are lighter than males, and animals fed a low-fat diet are lighter than animals fed a high-fat diet. The reproductive and renal fat pads both show significant sex by diet interactions. However, unlike organ weights, the weight of fat pads excised from female animals shows a proportionately greater increase in weight in response to a high-fat diet than the weight of fat pads excised from male animals.

Serum free fatty acids do not differ significantly by sex or by diet. Serum cholesterol differs significantly by sex as well as by diet: females have lower levels of serum cholesterol than males, and animals on a low-fat diet have lower levels of serum cholesterol than animals on a high-fat diet. Serum triglyceride levels do not differ significantly by sex or by diet, but

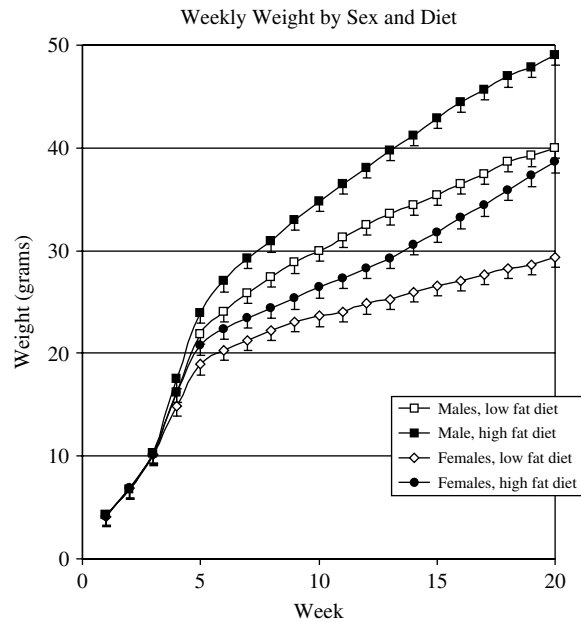


Fig. 1. Weekly weight plotted by sex and diet. The weekly weight in grams of each cohort of the experimental population is graphed. Bars indicate the lower standard error calculations. Open squares indicate males fed a low-fat diet, filled squares indicate males fed a high-fat diet, open diamonds indicate females fed a low-fat diet and filled diamonds indicate females fed a high-fat diet.

nevertheless do show a statistically significant sex by diet interaction: in males serum triglycerides are higher when the animal is fed a high-fat diet than when it is fed a low-fat diet, with the reverse in females. Serum insulin levels differ greatly depending on the sex and diet of the animal. Females have lower levels of serum insulin than males and animals fed a low-fat diet have much lower levels of serum insulin than animals fed a high-fat diet. The magnitude of the diet-based increase in serum insulin is much greater among males than females, accounting for the significant sex by diet interaction.

Growth curves for each sex by diet cohort are presented in Fig. 1. Weekly weights show a family by sex interaction effect that begins at week 4, peaks at week 8 and gradually diminishes. Consistent with earlier results (Cheverud *et al.*, 1996), pre-weaning growth does not differ significantly by sex. It does not differ by diet because this growth period precedes the dietary treatment. The post-weaning growth period differs significantly by both sex and diet: males grow more quickly than females, and animals grow more quickly on a high-fat diet. Relative growth rates during the adult growth period are similar: male animals grow more quickly than females fed the same diet, and animals fed a high-fat diet grow more quickly than animals fed a low-fat diet. However, the effect of a high-fat diet is much greater on females than on males, such that the rate of growth during this period

among female animals fed a high-fat diet is actually higher than the rate of growth of male animals fed a low-fat diet. This accounts for the observed significant sex by diet interaction. About 50% of the sex by diet interaction affecting adult growth is due to the greater weight of the reproductive fat pad in female animals fed a high-fat diet (see Appendix).

(ii) Genetic effects and correlations

Table 2 presents the genetic effect on the phenotypes examined, expressed as narrow sense heritability (h^2) for each sex and diet cohort. Multivariate probabilities are shown in italics below sets of related traits and indicate whether the traits considered, as a group, are statistically significantly heritable for the traits in question. Basal glucose and AUC are moderately or highly heritable at both 10 and 20 weeks in most cohorts. Organ weights, with the exception of spleen, have low to moderate heritability levels. Spleen weight is not heritable in this population. At the other extreme, liver weight shows moderate heritability. Fat pad weights are also significantly ($P < 0.001$) and moderately heritable. Most serum phenotypes have low heritabilities. Serum insulin and cholesterol, however, demonstrate low to moderate heritability. The multivariate probability for all cohorts is statistically significant for the serum traits as a whole in spite of the relatively low heritability of some traits. Pre- and post-weaning growth is moderately to highly heritable in all cohorts. In contrast, adult growth has low heritability in all cohorts. The multivariate probability for heritable variation in growth in each cohort is highly significant.

Phenotypic and genetic correlations are presented in Table 3, with the heritability of the trait pooled over diet and sex cohorts shown along the diagonal. Genetic correlations are slightly higher than their phenotypic counterparts due to statistical artefacts resulting from the diminished degrees of freedom used in estimating genetic correlations relative to phenotypic correlations (Cheverud, 1988). The 20-week AUC has been excluded from Table 3 because its very low genetic variance makes estimates of genetic correlation involving 20-week AUC unreliable.

Pre-weaning and post-weaning growth are not strongly correlated with any other phenotypes assayed in this study, with the exception of a moderate phenotypic correlation between post-weaning growth and liver weight. Adult growth, however, shows a moderate phenotypic correlation with reproductive and mesenteric fat pad weights, as well as a moderate genetic correlation with serum insulin levels.

Overall, the various fat pad weights are strongly correlated with each other as well as with liver weight, as indicated by the box enclosing these traits within Table 3. Insulin levels are also highly genetically

Table 3. Genetic (above diagonal) and phenotypic correlations (below diagonal) among obesity- and diabetes-related traits

	10-week basal	10-week AUC	20-week basal	Heart	Kidney	Liver	Reproductive	Renal	Mesenteric	Inguinal	Insulin	Cholesterol	Triglycerides	Free fatty acids	Pre-weaning	Post-weaning	Adult
10-week basal	0.55 (0.14)	-0.05 (0.20)	1.02 (NA)	0.43 (0.15)	0.09 (0.23)	0.36 (0.15)	0.30 (0.16)	0.34 (0.14)	0.19 (0.19)	0.30 (0.17)	0.94 (0.03)	0.17 (0.18)	-0.07 (0.23)	-0.06 (0.23)	-0.33 (0.13)	0.19 (0.14)	0.30 (0.17)
10-week AUC	0.18	0.39 (0.12)	0.16 (0.29)	0.69 (0.10)	0.87 (0.06)	0.37 (0.16)	0.65 (0.11)	0.81 (0.06)	0.74 (0.10)	0.39 (0.17)	0.68 (0.14)	0.46 (0.16)	1.26 (NA)	-0.30 (0.23)	0.06 (0.16)	0.03 (0.16)	-0.05 (0.21)
20-week basal	0.31	0.24	0.11 (0.06)	-0.06 (0.27)	0.36 (0.30)	1.31 (NA)	0.51 (0.20)	0.61 (0.15)	0.01 (0.30)	0.88 (0.06)	0.78 (0.14)	0.81 (0.09)	1.75 (NA)	-0.15 (0.34)	0.02 (0.23)	0.17 (0.21)	-1.05 (NA)
Heart	0.13	0.20	0.06	0.24 (0.06)	1.09 (NA)	0.90 (0.03)	0.78 (0.07)	0.62 (0.10)	0.44 (0.16)	0.45 (0.15)	0.58 (0.16)	0.35 (0.16)	0.08 (0.23)	0.10 (0.23)	0.07 (0.15)	0.15 (0.14)	0.04 (0.19)
Kidney	0.12	0.23	0.13	0.55	0.09 (0.04)	0.91 (0.04)	0.67 (0.13)	0.59 (0.13)	0.34 (0.22)	0.36 (0.21)	0.71 (0.16)	0.45 (0.19)	0.36 (0.26)	-0.20 (0.29)	-0.26 (0.18)	0.33 (0.17)	0.09 (0.25)
Liver	0.19	0.24	0.23	0.40	0.45	0.33 (0.07)	0.77 (0.07)	0.75 (0.06)	0.67 (0.10)	0.59 (0.11)	0.79 (0.09)	0.52 (0.12)	-0.12 (0.21)	-0.27 (0.20)	-0.03 (0.14)	0.11 (0.13)	0.20 (0.17)
Reproductive	0.05	0.06	0.16	0.26	0.21	0.54	0.24 (0.06)	0.82 (0.05)	0.80 (0.07)	0.71 (0.09)	0.99 (0.01)	0.03 (0.18)	-0.07 (0.23)	-0.15 (0.23)	0.29 (0.14)	-0.16 (0.14)	0.20 (0.19)
Renal	0.16	0.21	0.18	0.30	0.29	0.61	0.81	0.40 (0.08)	0.88 (0.04)	0.90 (0.03)	0.84 (0.06)	0.24 (0.15)	-0.18 (0.20)	-0.03 (0.21)	0.33 (0.12)	-0.09 (0.13)	0.19 (0.16)
Mesenteric	0.13	0.18	0.22	0.25	0.25	0.59	0.69	0.70	0.17 (0.05)	0.91 (0.03)	1.16 (NA)	0.14 (0.20)	-0.08 (0.25)	-0.31 (0.23)	0.36 (0.14)	-0.21 (0.15)	0.31 (0.19)
Inguinal	0.09	0.14	0.12	0.16	0.20	0.54	0.70	0.73	0.72	0.23 (0.06)	1.05 (NA)	0.23 (0.18)	-0.18 (0.23)	-0.29 (0.22)	0.26 (0.14)	-0.08 (0.15)	0.18 (0.19)
Insulin	0.09	0.00	0.08	0.23	0.21	0.50	0.34	0.42	0.40	0.35	0.07 (0.03)	0.22 (0.24)	0.05 (0.32)	0.02 (0.32)	-0.03 (0.21)	-0.26 (0.19)	0.57 (0.19)
Cholesterol	0.07	0.02	0.19	0.06	0.10	0.32	0.31	0.29	0.27	0.32	0.79	0.23 (0.06)	0.04 (0.24)	-0.03 (0.24)	-0.13 (0.15)	0.11 (0.15)	- 0.14 (0.19)
Triglycerides	0.04	0.06	0.13	0.02	0.09	0.04	0.01	-0.03	0.01	-0.01	-0.15	0.04	0.09 (0.04)	0.37 (0.26)	0.09 (0.19)	0.00 (0.19)	0.39 (0.21)
Free fatty acids	0.05	-0.08	-0.08	-0.06	-0.09	-0.03	-0.03	-0.06	0.02	-0.04	-0.57	-0.12	0.12	0.09 (0.04)	0.11 (0.19)	0.14 (0.19)	-0.05 (0.25)
Pre-weaning	-0.14	0.06	-0.03	0.20	0.25	0.24	0.33	0.33	0.28	0.29	0.12	0.04	0.03	-0.02	0.50 (0.09)	-0.36 (0.11)	0.30 (0.15)
Post-weaning	0.25	0.24	0.19	0.31	0.39	0.52	0.40	0.50	0.39	0.49	0.26	0.24	-0.02	-0.03	0.06	0.56 (0.09)	-0.46 (0.12)
Adult	0.04	0.10	0.24	0.22	0.20	0.39	0.50	0.46	0.52	0.44	0.27	0.21	-0.01	0.00	0.20	0.04	0.19 (0.06)

Genetic correlations are followed by their standard errors in parentheses. Genetic correlations greater than 0.50 are always significantly greater than zero. Standard errors of phenotypic correlations can be calculated as $\{\sqrt{(1-r^2)}/(428)\}$ for correlations involving glucose values and as $\{\sqrt{(1-r^2)}/(998)\}$ for other trait pairs. Phenotypic correlations greater than 0.0625 are significantly greater than zero. Adjectives ('reproductive', 'inguinal', etc.) indicate fat pads. Heritabilities pooled across sex and dietary cohorts are shown on the diagonal along with their standard errors. All heritabilities are statistically significant. Bold italics indicate significant moderately high (0.5 to 0.7) correlations, while boldface type indicates high (above 0.7) significant correlations. The box in the centre of the table indicates a suite of highly correlated traits. Correlation estimates greater than 1.0 should be interpreted as less than but approaching 1.0.

correlated with the liver and fat pad weights. Organ weights show moderate to high genetic correlations with fat pad weights, but low phenotypic correlations. Glucose-tolerance-related traits show moderate to high genetic correlations with serum insulin as well as organ and fat pad weights, but little phenotypic correlation with any other trait.

Inspection of the correlation matrix indicates that some estimates exceed the theoretical limit of 1.00, such as the nominal correlation of 1.31 between liver weight and 20-week basal glucose level. Estimates above the theoretical limit are common in quantitative genetic analyses using unbiased estimation methods (Hill & Thompson, 1978). Such estimates should be interpreted as indicating a relatively high correlation between the traits.

(iii) Genetic correlations of sexual dimorphism

Fat pad weights and adult growth demonstrate low to moderate heritability for sexual dimorphism (Table 4A), while most other traits have low heritabilities. Table 4A presents genetic correlations for sexual dimorphism among traits with significant sex by family interactions. Fat pad weights are very highly correlated with one another, and are moderately correlated with adult growth.

(iv) Inheritance and genetic correlations of dietary response

A similar set of obesity-related traits emerges when genetic variation in dietary response (diet by family interaction) is considered. Diet by family effects are moderately heritable for liver weight ($h^2=0.22$ (SE=0.12)), renal fat pad weight ($h^2=0.27$ (0.13)), reproductive fat pad weight ($h^2=0.22$ (0.12)) and adult growth ($h^2=0.20$ (0.11)). However, the heritability of dietary response is strongly sexually dimorphic for reproductive fat pad weight, renal fat pad weight and adult growth.

As shown in Table 4B, a subset of traits shows strong genetic correlations for dietary response, indicating that the same genes affect dietary response for liver weight and fat pad weights. As with Table 4A, only phenotypes demonstrating significant gene by diet interactions have been included. As can be seen by comparing Tables 4A and 4B with Table 3, traits that show strong genetic correlations for sexual dimorphism and dietary response also have strong phenotypic and genetic correlations between the traits proper.

The genetic correlations between environments are presented in Table 5. The upper and lower 95% confidence region boundaries are provided for each correlation. Most traits show moderate genetic correlations across diets, indicating that some but

Table 4. Genetic correlations for variation in sexual dimorphism and dietary response

<i>(A) Genetic correlations of sexual dimorphism</i>					
	Reproductive	Renal	Mesenteric	Inguinal	Adult
Reproductive	0.12 (0.06)				
Renal	0.80 (0.14)	0.10 (0.06)			
Mesenteric	0.91 (0.09)	0.98 (0.02)	0.03 (0.03)		
Inguinal	0.91 (0.09)	1.31 (NA)	0.97 (0.04)	0.03 (0.03)	
Adult	0.70 (0.15)	0.37 (0.27)	0.68 (0.22)	0.30 (0.37)	0.12 (0.04)
<i>(B) Genetic correlations of dietary responses</i>					
	Liver	Renal	Mesenteric	Adult	
Liver	0.22 (0.12)				
Renal	0.83 (0.12)	0.22 (0.12)			
Reproductive	0.98 (0.02)	1.11 (NA)	0.25 (0.13)		
Adult	0.28 (0.34)	0.12 (0.37)	0.23 (0.35)	0.20 (0.11)	

Table 4A shows the genetic correlations of sexual dimorphism for some traits with their standard errors. Heritability of sexual dimorphism along with standard errors is provided along the diagonal. Table 4B presents the genetic correlation of dietary responses along with standard errors. Heritability of dietary response is given along the diagonal. Correlation estimates greater than 1.0 should be interpreted as less than but approaching 1.0.

Table 5. Genetic correlations across environments are shown along with their 95% confidence intervals (CI) and the probability that the correlation is not different from zero

	Correlation	95% CI lower	95% CI upper	Probability
Glucose tolerance				
<i>10-week test</i>				
Basal	0.36	0.10	0.57	1.63×10^{-3}
AUC	0.25	-0.01	0.48	0.0303
<i>20-week test</i>				
Basal	0.39	0.13	0.59	1.47×10^{-3}
AUC	-0.01	-0.28	0.25	0.911
Organ weights				
Heart	0.50	0.27	0.67	2.95×10^{-6}
Kidney	0.31	0.05	0.53	5.47×10^{-3}
Spleen	0.36	0.10	0.57	1.12×10^{-3}
Liver	0.43	0.19	0.62	6.70×10^{-5}
Fat pad weights				
Reproductive fat pad	0.45	0.21	0.64	3.28×10^{-5}
Renal fat pad	0.50	0.27	0.67	2.73×10^{-6}
Mesenteric fat pad	0.35	0.09	0.56	1.64×10^{-3}
Inguinal fat pad	0.50	0.27	0.68	2.38×10^{-6}
Serum levels				
Free fatty acids	0.57	0.35	0.72	4.70×10^{-8}
Cholesterol	0.52	0.30	0.69	6.98×10^{-7}
Triglycerides	0.12	-0.15	0.37	0.304
Insulin	0.16	-0.11	0.41	0.148
Growth traits				
Pre-weaning	0.67	0.50	0.80	6.93×10^{-12}
Post-weaning	0.53	0.31	0.70	4.48×10^{-7}
Adult	0.25	-0.02	0.48	0.0270

not all genetic effects are the same on different diets. The correlation between diets of the pre-weaning growth phase is high ($r=0.67$) and not different from 1.0 (see Table 2) because the animals have not yet

experienced the dietary treatment. This level indicates the upper limit of genetic correlation across environments and can be used to informally normalize other values. The genetic correlation between environments

is moderate for post-weaning growth and low for adult growth. AUC levels at 10 and 20 weeks are only weakly correlated across environments. Serum triglycerides and insulin also show no correlation across diets, indicating again that different genes or gene effects are responsible for these phenotypes under the two diets tested.

4. Discussion

In this study we used quantitative genetic tools to examine the heritability and dietary response for a variety of obesity- and diabetes-related phenotypes on high- and low-fat diets. We found that most of the traits we examined are moderately to highly heritable, similar to the results of our earlier study of a subset of these traits in the F_3 generation of the AIL (Kramer *et al.*, 1998). When heritability levels for all traits are considered, variation in growth is the most subject to genetic influence, followed by fat pad weights. Although heritability levels for organ weights are not as high as for growth or fat pad weights, variation in liver weight demonstrates considerable genetic influence. Basal glucose levels and response to glucose challenge (AUC) as well as serum insulin levels are moderately heritable.

As can be seen in Table 3, a subset of obesity- and diabetes-related traits are very strongly genetically intercorrelated, including all four fat pads, liver weight and insulin level. Genetic variation in this suite of obesity-related traits is due primarily to a set of loci with common pleiotropic effects across the entire suite, leaving very little variation due to trait-specific effects on the various fat depots. Adult growth is also correlated with this obesity suite, albeit not as strongly, consistent with the view that much of adult growth is due to fat deposition in discrete fat depots, organs and muscle after 10 weeks of age. Basal glucose readings from 10 and 20 weeks are strongly genetically correlated with each other as well as with serum insulin. Somewhat lower, but still significant, genetic correlations were found between basal glucose levels at 20 weeks and the obesity suite and adult growth. While separate sets of genes may affect glucose levels and obesity traits, we would also expect some loci with pleiotropic effects across these traits.

A subset of traits in the obesity suite also demonstrates genetic variance in dietary response, including liver weight, reproductive fat pad weight in females, renal fat pad weight and adult growth in females. It is likely that much of the male/female difference in dietary response during the adult growth phase is due to differences in reproductive fat pad size: female animals show a significantly greater response to diet in reproductive fat pad size than do male animals.

This same subset of four traits – liver weight, reproductive fat pad weight, renal fat pad weight and, to a lesser degree, adult growth – demonstrate high levels of genetic correlation for their dietary responses. This indicates that genetic differences among individuals in levels of dietary response are due to a common set of diet-responsive genes.

The degree to which gene effects depend on diet is demonstrated by the remarkably low level of genetic correlation between environments. Fat pad weights, like organ weights, demonstrate moderate genetic correlations across environments. AUC levels and insulin, however, are very weakly correlated across environments, with neither trait showing statistically significant correlation across environments. From this study, we conclude that the genes influencing 20-week AUC and insulin on a low-fat diet are different from the genes or gene effects influencing these traits on a high-fat diet. This is not entirely surprising because serum insulin level is related to glucose tolerance. An early stage of diabetes is a condition with high insulin levels, normal fasting glucose levels and poor response to a glucose challenge. This is due to insulin resistance of peripheral tissues, so that more insulin and more time is needed to clear glucose from the bloodstream.

Serum triglycerides also diverge widely between the two diets measured in this study. With the exceptions of serum free fatty acids, serum cholesterol and pre-weaning growth, genetic correlations between the two diets for all traits examined show that less than 50% of the variance in these traits is common between the diets.

This study was large in scale compared with many quantitative genetic studies, including phenotypes on 78 families and 1000 individuals. This sample size results in heritabilities above 10% and genetic correlations above 0.50 being statistically significant when the whole population is considered. However, when the sample is subdivided into four cohorts with 250 animals per cohort, statistical power is somewhat compromised, so that heritability estimates need to be greater than about 20% to reach statistical significance. This is most apparent in the sometimes wide variation in heritability level among sex – diet cohorts for some traits.

We have shown that an F_{16} generation derived from a cross of SM/J and LG/J has substantial genetic variance for a variety of obesity- and diabetes-related phenotypes. Some of these traits, including liver weight, reproductive and renal fat pad weights, and adult growth rate, also show genetic variance in response to a high-fat diet. This indicates that these traits should be amenable to quantitative trait mapping in our experimental population. Future studies that examine and map dietary response loci in the LG/J by SM/J AIL will contribute to our

understanding of the biological bases of gene by diet interactions. Current efforts to understand the interaction of genotype and nutrition have produced a new field called nutrigenomics (Muller & Kersten,

2003). Further work in model systems and human populations (Ordovas *et al.*, 2002; Tai *et al.*, 2003) is needed to clarify the genetic architecture of environmental responsiveness.

Appendix. Sex- and diet-specific means and standard errors for obesity and diabetes-related traits in the F₁₆ generation of the LG/J by SM/J advanced intercross line

Significance of fixed effects for sex (S), diet (D), and Sex by Diet Interaction (SD) is indicated in the last column.

	Females, low-fat diet (n = 113)		Females, high-fat diet (n = 104)		Males, low-fat diet (n = 103)		Males, high-fat diet (n = 110)		Significance of fixed effects
	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error	
Glucose tolerance traits									
10-week test									
Basal (mg/dl)	92.89	2.74	103.96	2.69	110.58	3.16	130.76	3.75	S, D
AUC (mg × min/10 dl) ²	499.33	22.17	616.03	29.85	764.74	46.24	1174.44	60.18	S, D, SD
20-week test									
Basal (mg/dl)	106.16	2.62	129.62	2.63	132.59	3.64	157.20	4.12	S, D
AUC (mg × min/10 dl) ²	600.77	25.43	742.37	39.86	951.50	45.69	1626.36	84.60	S, D, SD
	Females, low-fat diet (n = 253)		Females, high-fat diet (n = 250)		Males, low-fat diet (n = 245)		Males, high-fat diet (n = 252)		Significance of fixed effects
	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error	
Organ weights									
Heart (g)	0.132	0.003	0.144	0.003	0.176	0.004	0.188	0.004	S, D
Kidney (g)	0.154	0.003	0.163	0.003	0.273	0.006	0.291	0.009	S, D
Spleen (g)	0.111	0.009	0.140	0.008	0.112	0.012	0.142	0.005	D
Liver (g)	1.265	0.034	2.097	0.065	1.530	0.031	2.659	0.079	S, D, SD
Fat pad weights									
Reproductive (g)	1.650	0.115	3.988	0.236	1.942	0.071	3.340	0.200	S, D, SD
Renal (g)	0.749	0.049	1.803	0.102	1.059	0.048	1.831	0.078	S, D, SD
Mesenteric (g)	0.707	0.039	1.254	0.069	1.087	0.058	1.549	0.050	S, D
Inguinal (g)	1.502	0.110	2.958	0.185	2.229	0.116	3.629	0.150	S, D
Serum levels									
Free fatty acids (mmol/l)	0.909	0.061	1.000	0.058	0.874	0.048	0.930	0.049	
Cholesterol (mg/dl)	118.503	3.684	174.158	5.960	130.608	3.628	192.169	5.784	S, D
Triglycerides (mg/dl)	101.932	5.723	87.588	5.067	87.317	4.101	103.106	6.424	SD
Insulin (ng/ml)	1.215	0.187	3.170	0.351	2.624	0.322	7.137	0.563	S, D, SD
Growth traits									
Pre-weaning [log10(week 3/week 1)]	0.765	0.010	0.764	0.010	0.767	0.013	0.771	0.010	
Post-weaning [log10(week 10/week 3)]	1.116	0.010	1.197	0.011	1.286	0.009	1.377	0.010	S, D
Adult [log10(week 20/week 10)]	0.672	0.033	1.023	0.027	0.962	0.021	1.125	0.021	S, D, SD
	Females, low-fat diet (n = 253)		Females, high-fat diet (n = 250)		Males, low-fat diet (n = 245)		Males, high-fat diet (n = 252)		Significance of fixed effects
	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error	
Weekly weights (g)									
Week 1	4.16	0.07	4.16	0.07	4.18	0.07	4.22	0.07	
Week 2	6.86	0.11	6.88	0.12	6.77	0.12	6.82	0.11	

Appendix (Cont.)

	Females, low-fat diet (n=253)		Females, high-fat diet (n=250)		Males, low-fat diet (n=245)		Males, high-fat diet (n=252)		Significance of fixed effects
	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error	
Week 3	10.12	0.17	10.12	0.17	10.25	0.19	10.28	0.17	
Week 4	14.89	0.23	16.21	0.25	16.17	0.29	17.54	0.26	S, D
Week 5	18.91	0.27	20.76	0.29	21.91	0.33	23.91	0.32	S, D
Week 6	20.27	0.26	22.35	0.32	24.05	0.35	27.02	0.36	S, D, SD
Week 7	21.28	0.30	23.48	0.36	25.84	0.36	29.21	0.43	S, D, SD
Week 8	22.23	0.31	24.37	0.40	27.40	0.38	30.87	0.53	S, D, SD
Week 9	23.05	0.35	25.33	0.44	28.81	0.41	33.01	0.53	S, D, SD
Week 10	23.63	0.37	26.42	0.48	29.98	0.45	34.76	0.58	S, D, SD
Week 11	24.06	0.36	27.30	0.51	31.28	0.47	36.48	0.62	S, D, SD
Week 12	24.83	0.41	28.29	0.52	32.52	0.50	38.03	0.62	S, D, SD
Week 13	25.28	0.40	29.26	0.56	33.53	0.52	39.73	0.65	S, D, SD
Week 14	26.01	0.43	30.55	0.61	34.42	0.55	41.19	0.69	S, D, SD
Week 15	26.57	0.44	31.76	0.63	35.40	0.57	42.87	0.69	S, D, SD
Week 16	27.07	0.47	33.17	0.66	36.47	0.57	44.48	0.70	S, D, SD
Week 17	27.70	0.49	34.38	0.73	37.50	0.59	45.69	0.70	S, D
Week 18	28.27	0.51	35.92	0.78	38.68	0.61	46.95	0.72	S, D
Week 19	28.63	0.54	37.27	0.82	39.21	0.62	47.88	0.71	S, D
Week 20	29.33	0.55	38.61	0.88	40.00	0.63	49.06	0.74	S, D

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