Maternal genotype affects adult offspring lipid, obesity, and diabetes phenotypes in LGXSM recombinant inbred strains[S]

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Abstract **Maternal effects on offspring phenotypes occur because mothers in many species provide an environment for their developing young. Although these factors are correctly "environmental" with respect to the offspring genome, their variance may have both a genetic and an environmental basis in the maternal generation. Here, reciprocal crosses between C57BL/6J and 10 LGXSM recombinant inbred (RI) strains were performed, and litters were divided at weaning into high-fat and low-fat dietary treatments. Differences between reciprocal litters were used to measure genetic maternal effects on offspring phenotypes. Nearly all traits, including weekly body weights and adult blood serum traits, show effects indicative of genetic variation in maternal effects across RI strains, allowing the quantitative trait loci involved to be mapped. Although much of the literature on maternal effects relates to early life traits, we detect strong and significant maternal effects on traits measured at adulthood (as much as 10% of the trait variance at 17 or more weeks after weaning). We also found an interaction affecting adult phenotype between the effects of maternal care between RI strain mothers and C57BL/6J mothers and a later environmental factor (dietary fat intake) for some age-specific weights.**—Jarvis, J. P., J. Kenney-Hunt, T. H. Ehrich, L. S. Pletscher, C. F. Semenkovich, and J. M. Cheverud. **Maternal genotype affects adult offspring lipid, obesity, and diabetes phenotypes in LGXSM recombinant inbred strains.** ***J. Lipid Res.*** **2005.** 46: **1692–1702.**

Supplementary key words maternal effects • quantitative trait loci • late-onset diseases • genotype-environment interaction

Maternal effects occur because mothers in many species provide an environment for their developing offspring. In mammals, this environment includes both the prenatal conditions of the uterus, which may vary in the availability of space and nutrition, and variable postnatal environmental components such as maternal care behaviors and milk quality and production. Because such factors are phenotypes attributable to individual mothers, they may be the products of both maternal genotype and environment. Variation in the magnitude of maternal effects among mothers may then be attributable to differences between maternal genotypes and/or differences in environments experienced by the mother. To the extent that they affect offspring phenotypes, maternal effects represent an indirect contribution of a mother's genotype and environment to trait expression in her offspring (1, 2). Thus, although maternal effects are properly "environmental" with respect to the offspring's genome, they may have a genetic basis and therefore an associated genetic variance.

In fact, indirect genetic effects of this sort have been repeatedly shown to account for a substantial proportion of the heritable phenotypic variance in many quantitative characters, particularly in neonatal and juvenile traits (3, 4). Because mortality during the early stages of life accounts for the majority of variation in total fitness in many species, maternal effects may play an exceedingly important role in natural selection during this selective window. For instance, >50% of the variation in human birth weight, a character with strong implications for infant survivorship, can be attributed to the effects of the maternal environment (5), and one natural study of a small-bodied mammal (*Tamiasciurus hudsonicus*) found that maternal effects for juvenile traits were 50% greater than direct genetic effects (6). As such, this phenomenon has become of particular interest to evolutionary biologists during the last decade (1, 7).

Other work has shown that maternal effects on offspring traits at later ages tend to be completely correlated with

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Abbreviations: AUC, area under the curve; QTL, quantitative trait loci; RI, recombinant inbred.

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[S] The online version of this article (available at http://www.jlr.org) contains an additional table.

maternal effects on preweaning traits, and genetic correlations between maternal effects and direct effects on offspring characters are usually strongly negative (4). This indicates that some individual genes with direct effects on offspring traits, when expressed in the offspring itself, have opposing indirect effects on those same traits through their contributions to the environment provided by the mother. For example, a gene when expressed in a young animal might lead directly to faster growth and early maturity. However, early maturity may lead to lower adult body size and consequent decreases in milk production when this same animal becomes a mother. This antagonistic pleiotropy has major consequences for the evolution of both maternal and offspring characteristics (1, 4, 8, 9). Furthermore, mounting evidence suggests that maternal effects may make important contributions to variation in quantitative traits beyond the crucial selective window of the neonatal and juvenile periods and into adulthood (10).

It has been suggested that prenatal and neonatal maternal effects can direct "permanent" physiological adaptations that, later, in interaction with the environment, produce common adult diseases such as obesity, hypertension, coronary artery disease, and non-insulin-dependent diabetes (5, 11, 12). This is the so-called "thrifty phenotype" hypothesis, in which animals exposed to lower levels of prenatal and neonatal nutrition become "thrifty" by being physiologically adjusted to make more efficient use of energy intake throughout life. When such individuals are later exposed to an enriched nutritive environment, their early-developed "thriftiness" results in obesity attributable to overefficient storage of nutrients, with consequences for a variety of late-onset human diseases. Thus, this phenomenon is the result of an interaction between early and later nutritive environments. The early mammalian environment is dominated by mother-offspring interactions, and as noted previously, variation in these can be attributable to both genetic and environmental variations.

The mouse is a particularly useful species in which to study the effects of maternal environment because of its life history characteristics, including extensive maternal care during the first 3 weeks of life, the large volume of genetic and phenotypic data collected on this species, and the existence of a variety of inbred strains, experimental crosses, and other material resources. These factors make mice amenable to statistical genetic analysis with a level of rigor not possible in natural systems. For instance, the LGXSM mouse system developed by Cheverud and colleagues (13) has proven quite useful in investigations of indirect genetic effects. Their study of maternal effects on a variety of traits included adult features in the F3 generation of an intercross between LG/J and SM/J inbred mouse strains. Data from cross-fostered families demonstrated significant age-specific maternal effects on the first 4 weeks of growth, but these effects disappeared completely by 10 weeks. Moreover, maternal effects on necropsy traits were low and not statistically significant. Wolf et al. (3) used the same intercross to map quantitative trait loci (QTLs) for maternal effects associated with the phenotype of early growth. This study did not consider adult traits but identified five direct effect QTLs and four maternal effect QTLs that contribute to variation in early growth (between weeks 1 and 2) and that also show extensive epistatic interactions.

In this study, we used the recombinant inbred (RI) strains derived from the LG/J and SM/J strains by Cheverud and colleagues (13) to examine a variety of diabetes- and obesity-related traits (**Table 1**) in an effort to *1*) explore the extent of the maternal effects on adult phenotypes associated with late-onset diseases in humans, *2*) examine the extent of the variation in these effects present in this set of RI lines, and *3*) directly test Barker's hypothesis (5) that maternity influences later physiological responses to environmental conditions, specifically dietary fat.

METHODS

Strains and crosses

The RI strains used in this study were derived from a cross between SM/J and LG/J lines selected for small and large body size at 60 days (14). Pups from each line effectively possess identical genotypes at all autosomal loci, as each line was maintained by brother-sister mating for >26 generations ($F > 0.986$) (15). C57BL/6J mice were obtained from Jackson Laboratories. After weaning, animals were housed in single-sex cages containing no more than five mice each and fed the diets described below ad libidum. The animal facility is maintained at a constant temperature of 21°C with 12 h light/dark cycles.

Animals from each of 10 RI strains were reciprocally crossed to C57BL/6J individuals (RI females × C57BL/6J males and C57BL/6J females × RI males). Other LGXSM strains were not included in this study because they failed to produce more offspring than were needed for their maintenance during the experimental period. Approximately four independent litters (~24 pups) were produced from each side of the reciprocal cross. This

TABLE 1. Measured phenotypes

Weight and Growth Periods	Necropsy Traits	Serum Data
Weekly weights from 1 to 20 weeks	Carcass weight	Response to a glucose challenge at 10 and 20 weeks
	Tail length	
	Organ weights	
Log-transformed ratios of the three growth periods:	Heart, kidney, spleen, and liver	Response at necropsy
Growth period 1 = 1–3 weeks	Fat pad weights	Insulin, cholesterol, free fatty acids, triglycerides, leptin
Growth period 2 = 3–10 weeks	Reproductive, renal, mesenteric, and inguinal	
Growth period 3 = 10–20 weeks	Total fat = sum of the four fat pads	

design resulted in genetically identical F1 offspring being born to genetically different mothers. Because differences between reciprocal crosses measure the genetic maternal effect on offspring phenotypes, this experimental design also allowed the calibration of maternal effects across strains to the C57BL/6J "standard" and so facilitated direct comparison among them (16). Experimental animals were not allowed to breed.

Weights and growth periods

Experimental offspring were weighed weekly on the day of their birth, beginning at 1 week and continuing through 20 weeks. Three growth periods of 1–3 weeks (preweaning; growth period 1), 3–10 weeks (postweaning; growth period 2), and 10–20 weeks (adult; growth period 3) were defined and represented by the $\log_{10}$(3 week weight/1 week weight), $\log_{10}$(10 week weight/3 week weight), and $\log_{10}$(20 week weight/10 week weight), respectively. This partition was implemented because growth during the first 3 weeks of life is mainly a function of maternal characters such as milk quality and production, making this period distinctly different from later stages of life. Moreover, skeletal growth is largely complete by 10 weeks of age; thus, increases in body weight after 10 weeks result mostly from soft tissue deposition rather than increasing skeletal size. The logarithmic transformations were applied to improve normality and eliminate correlations between group means and variances (17).

Glucose challenge

At 10 and 20 weeks, animals were fasted for 4 h, after which a basal glucose level was measured using the Glucometer Dex® blood glucose meter (Bayer Corp.). Animals then received 0.01 ml of a 10% glucose solution per gram of body weight via intraperitoneal injection, and blood glucose levels at 15, 30, 60, and 120 min after injection were recorded. These profiles were condensed into a single general measure of an individual's response to the challenge by calculating the area under the curve (AUC) specified by the five time-specific glucose values (0, 15, 30, 60, and 120 min) plotted against time.

Necropsy

After 20 weeks of age, animals were again fasted for 4 h and anesthetized with sodium pentobarbital. A terminal blood sample was collected by cardiac puncture. Blood plasma was separated through centrifugation and analyzed for levels of insulin, cholesterol, free fatty acids, triglycerides, and leptin. At necropsy, carcass weight and tail length were measured and internal organs (heart, spleen, liver, and kidneys) and fat depots (reproductive fat pad, renal fat pad, inguinal fat pad, and mesenteric fat pad) were removed and weighed (18–23).

Diet

To test Barker's (5) hypothesis of interaction between fetal and neonatal environment with adult environment as a source of chronic adult disease, the reciprocal litters described above were further divided into high-fat and low-fat dietary treatments from weaning at 3 weeks until the end of the experimental period at 20 weeks. Experimental animals in the high-fat group received 27% more of their kcal from fat than individuals on the low-fat diet (24) (**Table 2**). The percentage of calories from protein for the two treatments was equal. A standard rodent chow diet was used before weaning and throughout the life of the parental generation.

TABLE 2. High-fat and low-fat dietary treatment composition

Variable	High Fat	Low Fat
Calories 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	

Multivariate analysis

The data were analyzed using the following multi-way mixed-model ANOVA (17) with the offspring phenotypes (Table 1) as dependent variables and the main effect factors of sex (male vs. female), diet (high vs. low fat), maternity (born of an RI or a C57BL/6J mother), and strain (RI strain parent):

$$Y_{ijklm} = \mu + Sex_i + Diet_j + Maternity_k + Strain_l + Sex_i \times Diet_j + Sex_i \times Strain_l + Diet_j \times Strain_l + Maternity_k \times Diet_j + Maternity_k \times Sex_i + Maternity_k \times Strain_l + Diet_j \times Maternity_k \times Strain_l + e_{ijklm}$$

where μ is the trait mean and Y_{ijklm} is the trait vector for the m^{th} individual of sex i on diet j from mother k and strain l. The specific terms of interest for this study include $Strain_l$, which measures genetic variation attributable to the RI strain from which offspring were derived, $Maternity_k$, which represents differential genetic maternal effects between C57BL/6J mothers and RI mothers, $Maternity_k \times Diet_j$, which measures differential responses of strain and RI-mothered pups to the dietary treatment, $Maternity_k \times Sex_i$, which measures the effects of maternity on sexual dimorphism, $Maternity_k \times Strain_l$, which represents genetic variation in maternal effects across RI strains, and $Diet_j \times Maternity_k \times Strain_l$, which tests Barker's (5) hypothesis of an interaction between adult environment (Diet) and maternal environment ($Maternity_k \times Strain_l$).

Sex_i, $Diet_j$, $Maternity_k$, and their interactions were all treated as fixed effects and so are not used in the calculations of proportions of variance; rather, their effects are reported in SD units. SD was calculated by dividing the difference between group means by the residual SD for that trait. $Strain_l$ and its interactions were considered random effects. Proportions of variance attributable to these random effects were calculated as the percent phenotypic variance explained by the genetic factor [strain or its interactions; $V_G/(V_G + V_E)$], where V_G is the variance attributable to the specified random factor and V_E is the residual variance. A full accounting of the interpretations of each term included in the model is presented in **Table 3**. Analysis was carried out using the general linear model in Systat 10.2. In the event that a model term showed no significant effects, it was removed from the analysis.

RESULTS

Weekly weights and growth periods 1–3

The analysis of weekly weights and growth periods consisted of 500 F1 offspring individuals from 10 different sets of reciprocal C57BL/6J × RI strain crosses and showed a broad array of statistically significant genetic ($Strain_l$) and genetic maternal ($Maternity_k$, $Maternity_k \times Strain_l$) effects on offspring phenotypes.

There was highly significant variation ($3.38 \times 10^{-8} > P > 4.98 \times 10^{-27}$) between strains at all weeks and over all three growth periods as measured by the $Strain_l$ model term (**Fig. 1**). Strain accounted for slightly >10% of the phenotypic variance in size at week 1. The effect of strain

TABLE 3. Factors in the ANOVA design and their interpretations

Model Term	Interpretation
Sex_i	Sexual dimorphism
$Diet_j$	Effect of diet (high vs. low fat)
$Maternity_k$	Genetic maternal effects attributable to C57BL/6J versus RI strains
$Strain_l$	Genetic variation among strains
$Sex_i \times Diet_j$	Sexual dimorphism in response to diet
$Sex_i \times Strain_l$	Genetic variation in sexual dimorphism among strains
$Diet_j \times Strain_l$	Genetic variation in response to diet among strains
$Maternity_k \times Diet_j$	Genetic variation in response to diet between pups born of RI strain versus C57BL/6J mothers
$Maternity_k \times Sex_i$	Genetic variation in sexual dimorphism between pups born of RI strain versus C57BL/6J mothers
$Maternity_k \times Strain_l$	Genetic variation in maternal effects among strains
$Diet_j \times Maternity_k \times Strain_l$	Barker's hypothesis of persistent maternal effects attributable to early and late environment interactions

RI, recombinant inbred.

on size peaked at slightly <28% at week 5 and thereafter decreased exponentially to a low of just <13% at week 20. The growth periods also show stronger strain effects at earlier stages that decrease with time. Beginning at 22% for early growth (preweaning; growth period 1), the strain effect declined to 19% for the postweaning period (growth in overall body size; growth period 2) and decreased substantially to just <10% for the period of adult growth (primarily soft tissue deposition; growth period 3).

Maternity also had a significant effect on weekly weights and growth periods. The only nonsignificant value was for the third growth period (growth period 3; $P = 0.95$); all other weekly weights and growth periods were highly significant ($5.61 \times 10^{-13} > P > 4.33 \times 10^{-46}$ for weekly weights; 0.0174 and 5.27×10^{-13} for growth period 1 and growth period 2, respectively). Pups born of a strain mother were larger than pups born of a C57BL/6J mother for all weekly weights throughout the experimental period (**Fig. 2**). The differences between the two groups were >1.35 SD for each of the first 4 weeks, with a peak difference of nearly 1.5 SD at week 3. Thereafter, the effects gradually decreased with time to a fairly constant level of ~0.75 SD by week 12, which persisted to the end of the experimental period.

Data on the three growth periods, however, showed a markedly different pattern. Pups born of a strain mother grew more slowly in both the preweaning (growth period 1; −0.22 SD) and postweaning (growth period 2; −0.88 SD) periods. No significant difference was detected for growth during the adult growth period (growth period 3). Therefore, although animals with C57BL/6J mothers were born smaller and remained smaller throughout life, they grew at a faster rate than animals born to RI mothers.

Data on the $Maternity_k \times Diet_j$ interaction, which represents the differential response to the high-fat diet between pups born of the two different mothers, failed to meet multivariate significance, although they showed interesting trends (**Fig. 3**). Pups born of RI strain mothers appeared to react more strongly to the high-fat diet during the period between 7 and 11 weeks of age, spanning the two later growth periods defined in this analysis ($0.0243 > P > 0.00462$). The corresponding growth period data also showed no effect during the preweaning period, but the interaction between maternity and diet was positive for the postweaning growth period ($P = 0.0492$) and negative for the adult growth period ($P = 0.0192$), with values of 0.35 and −0.42 SD, respectively. This means that those born of RI strain mothers responded more strongly to the high-fat diet from 3 to 10 weeks but those born of C57BL/6J mothers responded more strongly between 10 and 20 weeks.

The $Maternity_k \times Sex_i$ interaction term represents variation in sexual dimorphism attributable to maternal genotype. These data reached multivariate significance but showed only significant effects on the first and third

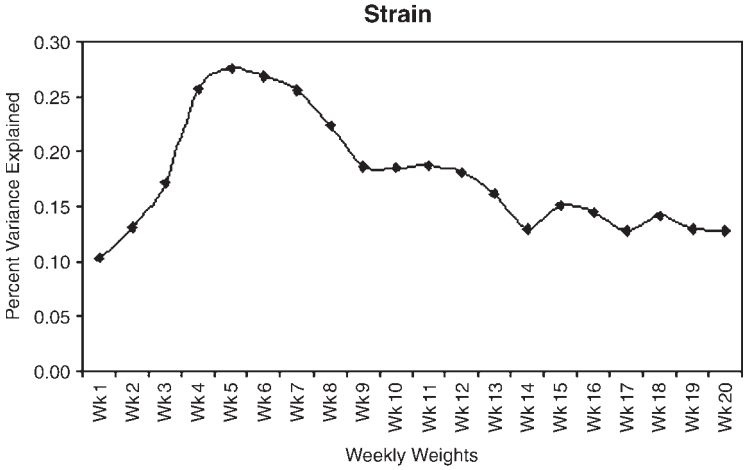

Fig. 1. Effect of genetic differences among recombinant inbred (RI) strains on weekly weights of F1 offspring of reciprocal crosses between 10 RI strains and C57BL/6J. Percentage variance explained was calculated as $V_G/(V_G + V_E)$, where V_G is the variance attributable to the specified random factor and V_E is the residual variance. All data points represent statistically significant effects ($1.47 \times 10^{-8} > P > 4.98 \times 10^{-27}$).

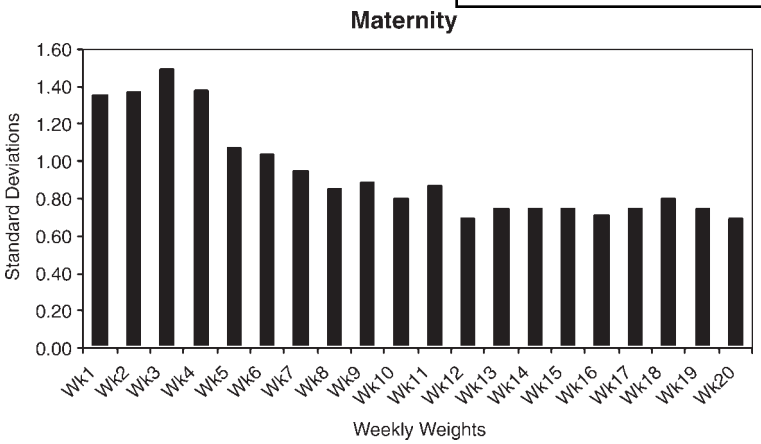

Fig. 2. Genetic maternal effects on weekly weights of F1 offspring of reciprocal crosses between 10 RI strains and C57BL/6J. Strain-mothered pups were consistently larger than C57BL/6J-mothered pups. Values are reported as differences between group means divided by the residual SD. All data points represent statistically significant effects ($5.61 \times 10^{-13} > P > 4.33 \times 10^{-46}$).

growth periods ($P = 0.00111$ for growth period 1 and $P = 0.00418$ for growth period 3). Before weaning, there was more sexual dimorphism (males − females) in pups born of RI strain mothers (0.60 SD). As adults, however, animals born of C57BL/6J mothers showed a greater difference between males and females (0.53 SD).

Results for the $\text{Maternity}_k \times \text{Strain}_l$ interaction term were highly significant for all weights ($9.20 \times 10^{-5} > P > 1.07 \times 10^{-17}$) and over all growth periods ($P = 0.00193$ for growth period 1, $P = 2.70 \times 10^{-10}$ for growth period 2, and $P = 2.08 \times 10^{-4}$ for growth period 3) (**Fig. 4**). They indicate substantial variation in maternal effects across this set of RI strains. The effects were ⩾25% over the first 5 weeks. Maternal effects peaked at 2 weeks at a value of ~33%. Between weeks 6 and 12, the effect leveled off to ~18%. From week 13 to week 20, there was a slight and gradual decline to ~11%. Data on growth periods showed a slightly different pattern. During the preweaning and adult growth periods, maternal effects were low to moderate, at 8% and 10%, respectively. However, during the postweaning growth period between weeks 3 and 10, maternal effects represented a substantial 22% of the variance. No significant values were found for the three-way interaction term $\text{Diet}_j \times \text{Maternity}_k \times \text{Strain}_l$.

Necropsy traits

The analysis of necropsy traits consisted of 487 F1 offspring individuals from 10 different sets of reciprocal C57BL/6J × RI strain crosses and showed a broad array of statistically significant genetic maternal effects on offspring phenotypes. Because the phenotype of total fat was the linear combination of the four fat pad weights, it was tested by itself in a separate ANOVA (n = 500).

Many necropsy traits showed significant variation across strains ($0.0189 > P > 2.17 \times 10^{-10}$), with the largest effect for tail length at 18%. All other traits showed effects ranging from 3% to 12% except heart weight, which was nonsignificant.

Maternity also played a significant role ($0.00976 > P > 1.20 \times 10^{-14}$) in necropsy trait values (**Fig. 5**), with only spleen weight nonsignificant. Generally, pups born of strain mothers had larger trait values, the only exception being tail length, for which C57BL/6J mothers produced pups with longer tails. Effect sizes ranged from 0.25 to 0.76 SD, with most clustered between 0.5 and 0.6 SD. The mean weight of the reproductive fat pad for strain-mothered pups was 2.2 g compared with 1.74 g for C57BL/6J-mothered pups ($P = 2.64 \times 10^{-8}$).

Although the $\text{Maternity}_k \times \text{Diet}_j$ term was removed from this analysis as no significant effects were detected, several effects were found for the $\text{Maternity}_k \times \text{Sex}_i$ interaction (**Fig. 6**). Interestingly, no significant effect of maternity on the degree of sexual dimorphism was detected for any organ weight. However, the fat depots showed varying levels of interaction. Differences between the sexes depending on maternity were nonsignificant for mesen-

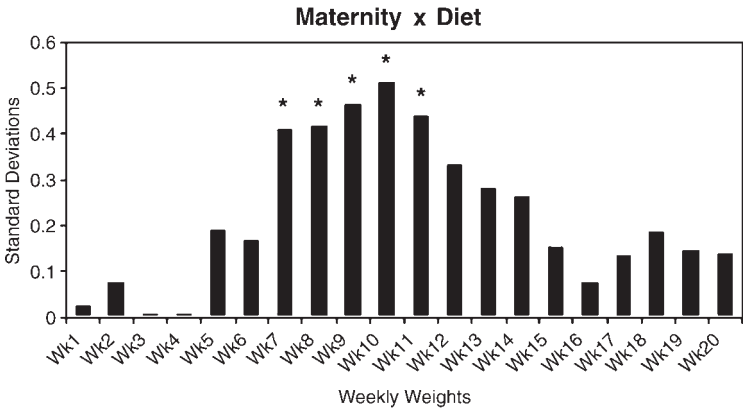

Fig. 3. Differential responses of RI- versus C57BL/6J-mothered pups to dietary fat. RI strain-mothered pups showed a greater response. Responses were calculated as differences between the high-fat and low-fat treatments. Values reported represent differences in this response between RI- and C57BL/6J-mothered pups. Significant values are indicated with asterisks ($0.0243 > P > 0.00462$).

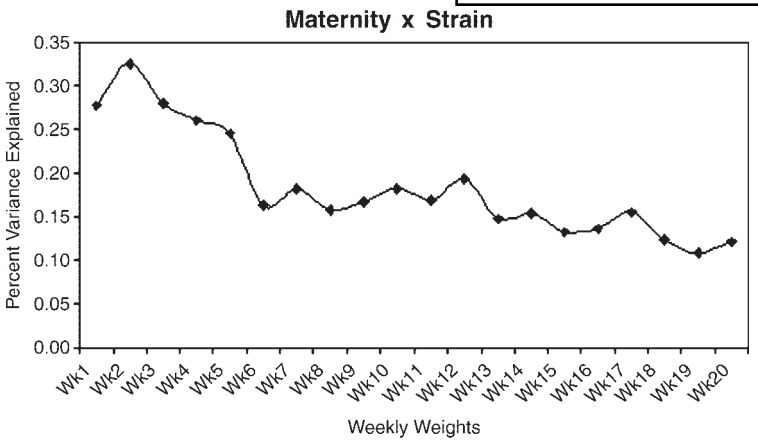

Fig. 4. Genetic maternal effects on the weekly weights of F1 offspring of reciprocal crosses between 10 RI strains and C57BL/6J. Percentage variance explained was calculated as $V_G/(V_G + V_E)$. All results were statistically significant ($9.20 \times 10^{-5} > P > 1.07 \times 10^{-17}$).

teric fat depot weight, but the reproductive ($P = 1.41 \times 10^{-5}$), renal ($P = 0.0202$), and inguinal ($P = 0.00201$) fat pads, as well as total fat pad weight ($P = 4.04 \times 10^{-4}$), all showed significant effects of maternity on sexual dimorphism. C57BL/6J-mothered pups showed greater dimorphism than RI strain-mothered pups. Effect sizes ranged from 0.43 SD for the renal fat pad to 0.82 SD for the reproductive fat pad.

Highly significant $\text{Maternity}_k \times \text{Strain}_l$ interactions were detected on carcass weight ($P = 5.69 \times 10^{-6}$), total fat pad weight ($P = 0.00496$), and tail length at necropsy as well as the weights of the kidney ($P = 0.00406$), liver ($P = 0.0290$), renal fat pad ($P = 3.98 \times 10^{-4}$), and mesenteric fat pad ($P = 1.08 \times 10^{-4}$) (**Fig. 7**). Effect sizes were generally moderate, with values ranging from 7% to 15%. Again, the fat depots showed varying degrees of interaction. In this case, genetic maternal effects among the RI strains explained 10% and 11% of the variation for the renal and mesenteric fat pads, respectively; however, no such effects were found for the reproductive and inguinal fat depots. Thus, genetic maternal effects contributed rather substantially to variation in fat deposition in some fat depots but not others. No significant values were found for any necropsy traits for the $\text{Diet}_j \times \text{Maternity}_k \times \text{Strain}_l$ interaction term.

Serum levels

The analysis of serum levels consisted of 487 F1 offspring from 10 different sets of reciprocal C57BL/6J × RI strain crosses and showed a broad array of statistically significant genetic maternal effects on offspring phenotypes. Because leptin levels were not obtained for some individuals, its analysis was carried out as a separate ANOVA to avoid the omission of information on the other serum traits (n = 351).

All blood serum traits, except for free fatty acid levels, showed significant variation ($0.00938 > P > 1.06 \times 10^{-39}$) across strains (**Fig. 8**). Effect sizes ranged widely from 3% for serum insulin to 39% for serum cholesterol. Both sets of 10 and 20 week glucose measures, basal glucose and AUC, showed larger effects at 10 weeks than at 20 weeks.

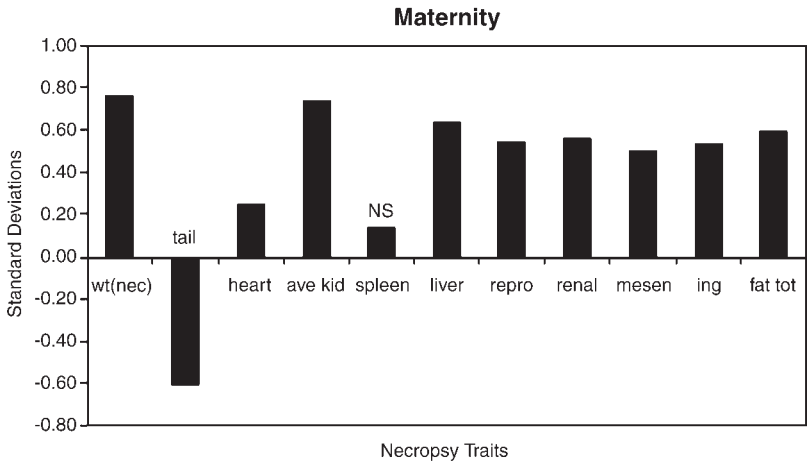

Fig. 5. Genetic maternal effects on necropsy traits measured after 20 weeks of age in F1 offspring of reciprocal crosses between 10 RI strains and C57BL/6J. Strain-mothered pups consistently displayed greater values for all necropsy traits except the length of the tail, for which C57Bl/6J-mothered pups displayed greater values (indicated by the "negative" effect on the tail). Values are reported as differences between group means (RI- vs. C57BL/6J-mothered pups) divided by the residual SD. Only the difference for the spleen was nonsignificant. ave kid, average kidney; ing, inguinal fat pad; mesen, mesenteric fat pad; renal, renal fat pad; repro, reproductive fat pad; fat tot, total fad pad; wt(nec), carcass weight at necropsy.

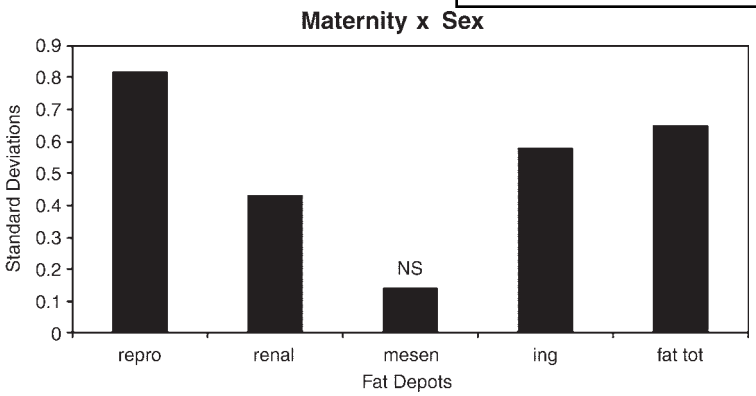

Fig. 6. Maternal effects on sexual dimorphism in fat deposition in F1 offspring of reciprocal crosses between 10 RI strains and C57BL/6J. C57BL/6J pups showed a greater difference between the sexes (male − female) in the weight of fat depots than did RI strain-mothered pups ($0.0202 > P > 1.41 \times 10^{-5}$). Only the effect on the mesenteric fat pad weight was nonsignificant.

The effect of maternity on blood serum traits was rather substantial (**Fig. 9**). AUC(10) ($P = 0.00110$), AUC(20) ($P = 0.0103$), insulin ($P = 0.00481$), cholesterol ($P = 0.0446$), basal glucose(10) ($P = 3.80 \times 10^{-4}$), and leptin ($P = 0.00393$) all showed significant effects. Effect sizes were >0.31 SD for AUC(10), basal glucose(10), and leptin and were 0.19, 0.2, and 0.25 SD for cholesterol, insulin, and AUC(20), respectively. The mean serum cholesterol level for strain-mothered pups was 129 mg/dl versus 123 mg/dl for C57BL/6J-mothered pups.

The $\text{Maternity}_k \times \text{Diet}_j$ interaction was removed from the blood serum analysis because no significant effects were detected. $\text{Maternity}_k \times \text{Sex}_i$ also failed multivariate significance testing but showed a notable effect on AUC(10) ($P = 0.0112$) of nearly 0.5 SD.

Significant $\text{Maternity}_k \times \text{Strain}_l$ interactions were detected for both glucose challenge variables [AUC(10) $P = 1.78 \times 10^{-11}$, AUC(20) $P = 1.71 \times 10^{-6}$] and their associated basal glucose values [basal glucose(10) $P = 1.64 \times 10^{-8}$, basal glucose(20) $P = 0.00799$) as well as for levels of insulin ($P = 0.00378$), cholesterol ($P = 3.59 \times 10^{-11}$), and triglycerides ($P = 0.0320$) at necropsy (**Fig. 10**). Effect sizes ranged from 4% phenotypic variance for triglyceride levels to 24% and 25% for cholesterol and AUC(10), respectively. AUC(20) and basal glucose at 10 weeks also showed effect sizes of 15% and 19%, respectively. Thus, genetic maternal effects are substantial for traits associated with adult-onset disease measured 17 or more weeks after mother-pup interactions ceased.

The only significant $\text{Diet}_j \times \text{Maternity}_k \times \text{Strain}_l$ interaction level in the entire analysis was for serum cholesterol ($P = 0.03$). However, none of the associated measures of multivariate significance were significant.

Differences in the number of individuals used in each of the analyses described above are the result of individuals removed by Systat 10.2 because of missing data. Subsequent analysis divided the complete population into high-fat and low-fat populations, and comparable models were applied to evaluate the same set of traits. Similar trends and patterns were observed, although many previously significant values became nonsignificant, most likely because of the reduced statistical power caused by the smaller sample sizes.

DISCUSSION

In many studies, the environmental variance component is intentionally minimized by experimental design

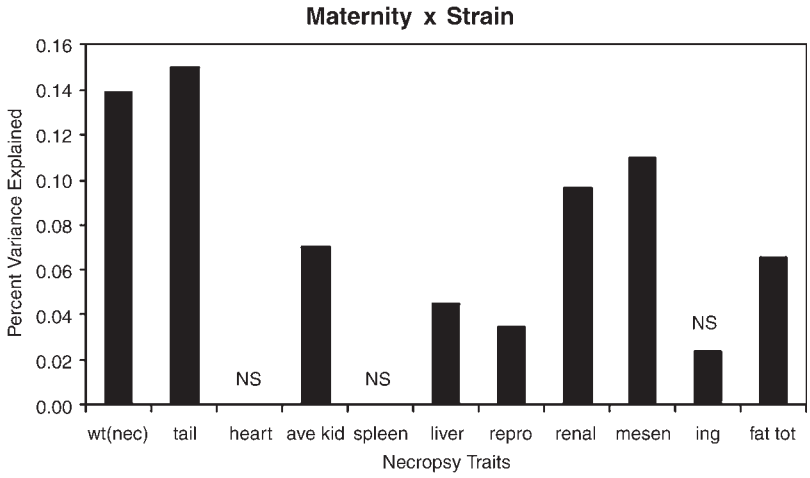

Fig. 7. Genetic maternal effects on necropsy traits of F1 offspring of reciprocal crosses between 10 RI strains and C57BL/6J. Percentage variance explained was calculated as $V_G/(V_G + V_E)$. Extensive differences existed among strains in the magnitude of maternal effects ($0.0290 > P > 1.59 \times 10^{-6}$). Effects on the weights of the heart, spleen, and inguinal fat pad were nonsignificant.

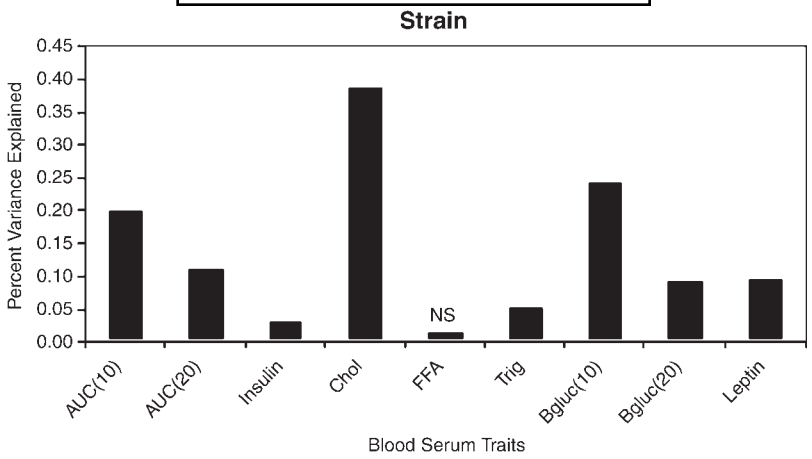

Fig. 8. Effects of genetic differences among RI strains on blood serum traits at necropsy of F1 offspring of reciprocal crosses between 10 RI strains and C57BL/6J. Percentage variance explained was calculated as $V_G/(V_G + V_E)$. Only the effect on free fatty acids was nonsignificant. All others represent significant effects ($0.00938 > P > 1.06 \times 10^{-39}$). AUC, area under the curve; Bgluc, basal glucose; Chol, cholesterol; FFA, free fatty acids; Trig, triglyceride.

(25). Maternal effects on offspring phenotypes, however, represent a component of this "environmental variation" that may have a genetic basis in the maternal generation. When this possibility is assessed, it is most commonly done through differences between reciprocal litters, as it was in this study. It should be noted that differences in reciprocal effects can arise from several sources: cytoplasmic inheritance, including mitochondria; the environment provided by the mother; genetic imprinting; and/or sex-linked effects (16). In this experiment, however, variation among strains in reciprocal litters was almost exclusively attributable to differences in the environment provided by the mother. Because imprinting was considered a form of indirect genetic effect, it was allowed to be confounded with other genetic maternal effects, all of which are environmental from the perspective of the offspring genome. Furthermore, cytoplasmic inheritance can be eliminated as a cause of variation in this study for two reasons. First, all standard inbred strains tested have identical mitochondrial genomes (15). Second, in the original cross from which these RI lines were derived, all mothers were LG/J; therefore, all descendants have passed on the same LG/J-based mtDNA to the entire RI strain set. This leaves sex-linked effects, which were statistically removed by the linear model. These sex-linked effects are confounded with the interaction effects of autosomes with the sex chromosomes in the $Sex_i \times Strain_l$ interaction. Significant $Sex_i \times Strain_l$ effects average 9.8% and ranged from 4% to 20% These effect magnitudes are approximately two-thirds the size of the genetic maternal effects ($Maternity_k \times Strain_l$; Figs. 4, 7, 10). As such, the effects identified in this study may be the result of some aspect of the environment provided by a particular mother for her offspring, genetic imprinting, or both.

Maternal effects on adult traits

Genetic maternal effects have been clearly demonstrated to play a role in offspring trait expression, especially in the neonatal and juvenile periods (1, 5, 10, 13). However, the first objective of this study was to explore the extent of maternal effects on adult traits, especially those thought to be associated with adult-onset diseases in humans, such as obesity and diabetes. Remarkably, although traits such as carcass and fat depot weights at necropsy, response to glu-

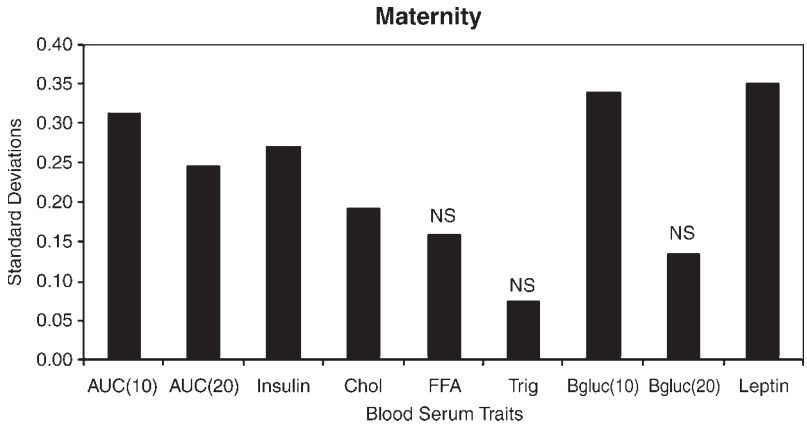

Fig. 9. Maternal effects on blood serum traits at necropsy in F1 offspring of reciprocal crosses between 10 RI strains and C57BL/6J. Strain-mothered pups consistently displayed greater values. Values are reported as differences between group means (RI- vs. C57BL/6J-mothered pups) divided by the residual SD. Differences for levels of free fatty acids, triglycerides, and the glucose challenge at 20 weeks were nonsignificant. All other values represent significant differences ($0.0446 > P > 3.80 \times 10^{-4}$).

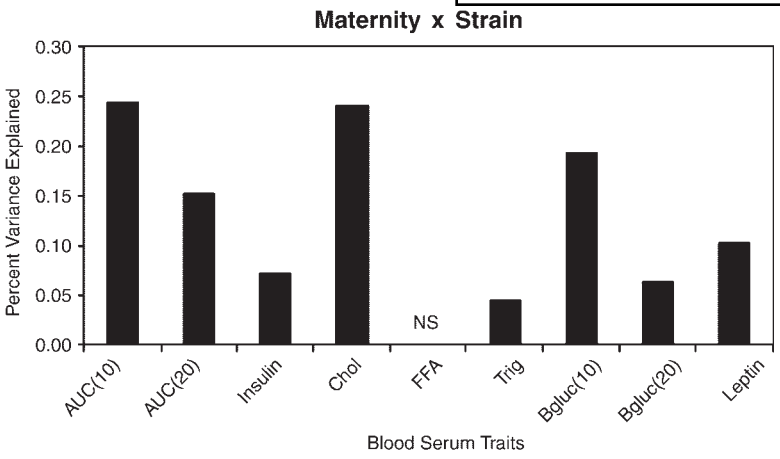

Fig. 10. Genetic maternal effects on blood serum traits at necropsy of F1 offspring of reciprocal crosses between 10 RI strains and C57BL/6J. Percentage variance explained was calculated as $V_G/(V_G + V_E)$. Extensive differences existed among strains in the magnitude of maternal effects ($0.0320 > P > 3.59 \times 10^{-11}$). Only the effect on free fatty acid levels was nonsignificant.

cose challenge at 10 and 20 weeks, and blood plasma levels of insulin, cholesterol, and leptin were measured on offspring as much as 17 weeks after direct mother-pup interactions ceased, highly significant maternal effects were still detected. In fact, although the percentage of the variance explained by the $\text{Maternity}_k \times \text{Strain}_l$ interaction term consistently decreased with age, it nevertheless explained >10% of the phenotypic variance for most of the period between 10 and 20 weeks. This is similar to the magnitude of the effects of RI strains themselves on these F1 animals (compare Figs. 1, 4). Even necropsy traits, which were the last features measured at >20 weeks of age, showed effects as large as 10–15%, for example on weight at necropsy, tail length, and mesenteric fat pad weight. Again, the effects attributable to genetic differences between mothers ($\text{Maternity}_k \times \text{Strain}_l$ interaction) are of the same magnitude of those for strain differences themselves. It should be noted that strain differences between these F1 animals were not as great as those among purebred strain animals (22) because each strain was mated to a common parent, C57BL/6J.

It is particularly interesting that the effects of any given factor or interaction on the four fat depots measured were not universal. For example, all four fat depots showed significant effects attributable to Maternity_k. However, only the renal and mesenteric fat pads showed significant genetic maternal effects represented by the $\text{Maternity}_k \times \text{Strain}_l$ term. Reproductive, renal, and inguinal fat pads, but not mesenteric fat pads, showed the effects of $\text{Maternity}_k \times \text{Sex}_i$. This indicates that localized fat deposition at these four depots may be modulated by independent maternal genetic factors.

As expected, maternal effects on weekly weights were strongest over the first 5 weeks. During this period ~18–25% of the variance in weight was explained by the $\text{Maternity}_k \times \text{Strain}_l$ term. The effect magnitude then decreased and oscillated around ~12% from week 6 to week 17, then declined to <10% for weeks 18, 19, and 20, but it was never <8%. Blood serum traits also showed remarkably strong maternal effects. Variance explained by the $\text{Maternity}_k \times \text{Strain}_l$ term for AUC(10) and AUC(20), which are measures of response to a glucose challenge at 10 and 20 weeks, were 12% and 10%, respectively. A staggering 14% of the variance in serum cholesterol at 20 weeks was explained by the $\text{Maternity}_k \times \text{Strain}_l$ model term. We find these diverse, sizable, and exceptionally long-lived effects rather surprising, because they indicate that the indirect genetic effects of the maternal genome remain a force shaping a remarkable variety of potentially medically relevant traits even at comparatively late stages of life.

The mechanisms by which maternity may affect adult phenotypes remain largely a matter of conjecture, because maternal effects are measured as a composite of any and all maternally derived factors contributing to offspring trait values. Identification and measurement of any one aspect of maternity that makes a contribution to trait variation is difficult and thus rare in the literature. One possibility is that offspring genotypes may be differentially sensitive to elements of the physical environment of the uterus or nest, such as space or temperature. A more exotic possibility, however, is that maternal factors directly modulate offspring gene expression patterns, which are then fixed even after direct contact between the mother and pup is lost. Support for this intriguing possibility is found in recent work by Weaver et al. (26), who described the regulatory effects of maternal behaviors in rats. They report that through undefined epigenetic processes, increased pup licking and grooming and arched-back nursing by rat mothers altered DNA methylation patterns at a glucocorticoid receptor gene promoter in the offspring hippocampus. The differences emerged during the first week of life, were reversed with cross-fostering, persisted into adulthood, and were associated with altered histone acetylation and transcription factor binding to the glucocorticoid receptor promoter. Such mechanisms may or may not be common, but we do find that a substantial portion of trait variance in medically relevant phenotypes measured in adult offspring is explained by the effect of the mother's genotype on prenatal and neonatal rearing environment.

Such findings have relevance for a number of fields and for a number of reasons. First, they provide further evidence of the potentially important evolutionary role of indirect genetic effects in general and maternal effects in particular. It is generally acknowledged that maternal effects can play a role in the dynamics of natural selection in early life traits, as the vast majority of the variation in total

offspring fitness occurs in this period of particularly high mortality in the natural world. But the evidence presented here that maternal effects can play a role in the variation of adult phenotypes suggests the possibility that indirect genetic effects may interact with selection at points throughout an organism's life cycle.

Second, these results represent a step toward refining practices used in the generation and maintenance of inbred strains for research, because maternal effects have often been shown to have negative pleiotropic interactions with traits important to the health of inbred strains; this consequence of inbreeding is well known in the agricultural literature. Thus, further investigation using this set of RI lines will contribute to future success in both the development of new lines and the maintenance of current stocks.

Finally, and most speculatively, such findings suggest that adult-onset diseases may have elements of causality and risk attributable to indirect genetic effects. As maternal effects can alter the genotype-phenotype relationship for medically relevant traits in mice, even in adults, maternal genotype may be an important factor to explicitly include in predictions of adult-onset disease susceptibility, recommendations of preventative measures, and potentially even the choice of treatment. This application, of course, requires that we have some indication of the maternal effect loci that contribute to the observed variation.

Mapping studies

QTL mapping is now a relatively common procedure used to help elucidate the genetic basis of complex traits by localizing genetic effects to particular physical regions of the genome (27). To do this, however, variation in the trait of interest is a necessary precursor. The second objective of this study was to examine the extent of variation in maternal effects present in this existing set of RI lines and determine whether or not they can be used to map the observed effects. Mapping would facilitate fine-scale analysis of the maternal effect loci involved.

As many of the traits showed highly significant effects of Maternity_k as well as $\text{Maternity}_k \times \text{Strain}_l$ interactions, this set of RI lines shows not only the presence of maternal effects but also substantial variation in their magnitude across strains. As such, these lines, and an associated set of advanced intercross lines derived from the same LG/J × SM/J cross, can be appropriately used to map these maternal effects to relatively small genetic regions (23, 28) in the parental generation. Thus, this set of tools represents a powerful system for investigating the architecture and physiological mechanism of maternal effects on offspring phenotypes.

The presence and persistence of maternal effects in this study also has implications for QTL studies in general. Although genetic maternal effects do not contribute to variation between animals in F2 intercross and single backcross studies because there is no genetic variation among the F1 mothers, environmental maternal effects can be substantial. Controlling for this environmental factor statistically can improve the power to detect QTLs (14). However, later generation intercrosses, derived from a F2 intercross, will segregate for genetic maternal effects (3). One means of addressing this issue is to cross-foster offspring to separate the contributions of genes inherited from the mother from her environmental effect on her offspring. However, this is limited to postnatal maternal effects. Alternatively, a two-generation research design, simultaneously analyzing both parental and offspring generations, can appropriately account for genetic maternal effects on trait variation. As seen in this study, substantial effects are missed if genetic maternal effects are ignored.

Barker's hypothesis: interaction between environments experienced early and late in life

The third objective of this study was to assess the recent suggestion that prenatal and neonatal maternal effects can direct "permanent" physiological adaptations that later, in interaction with the environment, produce common adult-onset diseases such as hypertension, coronary artery disease, and non-insulin-dependent diabetes (5, 11, 12). As this analysis found only one significant effect of $\text{Diet}_j \times \text{Maternity}_k \times \text{Strain}_l$ on an offspring phenotype (serum cholesterol level at the time of necropsy) and none of the multivariate measures was significant, we find no direct support for the Barker (5) hypothesis in terms of differences among the RI strains or in maternal differences between C57BL/6J and RI strain mothers.

However, there are at least two reasons why this test of the Barker hypothesis is incomplete, and these need to be noted. First, to ensure effective breeding and survivorship of pups, mothers were raised on a standard rodent chow diet ad libitum. A major component of the Barker hypothesis, however, is that the permanent physiological adaptations imposed by maternal influence on a developing offspring are adaptive to the nutritive environment in which the mother finds herself. Thus, without nutritionally stressing the mothers, this study and analysis fails to fully depict the conditions specified by the hypothesis. Second, the effects of individual dams could not be used to parse out the environmental maternal effects, because the data had inadequate representation of individuals attributable to the practical limitations of breeding. This effect would have been represented in the model by a Dam × Diet(Maternity × Strain) term and would also have directly addressed the Barker hypothesis. A final notable limitation of the model used here is that a Dam(Maternity × Strain) term, which would have addressed environmental variation in maternal effects, was also omitted because of the practical limitations of husbandry.

However, as we emphasized above, despite little evidence specifically supporting Barker's hypothesis of interaction between early and later life-stage environments, we do find extensive, persistent maternal effects on offspring phenotypes associated with diabetes and obesity.

Conclusions

Genetic maternal effects and other forms of indirect genetic effects occupy an increasingly important place in the development of our understanding of complex pheno-

types, especially in taxa with extensive maternal interactions, such as mammals. To date, these effects have been demonstrated to be relevant for a number of neonatal, juvenile, and, in this analysis, adult traits. In such cases, environmental variance, which is often not considered in evolutionary studies of natural populations, may be inflated by genetic maternal effects. From an evolutionary perspective, the presence of such effects complicates population-level analyses of selection and can lead to unexpected responses to selective pressures (1).

From a practical standpoint, maternal effects can be a confounding factor in the development and maintenance of inbred strains, both for research and in agriculture. A better understanding of their nature and their underlying genetic architecture will lead to better management of existing resources and to the production of new and more useful stocks in the future.

Finally, maternal effects on complex phenotypes may be particularly relevant for medical applications, because they can be an important and consistent source of phenotypic variation in traits associated with human diseases. Thus, taking into account the contribution of maternal genotype and environment to the environment provided to offspring by their mothers may prove an important stride toward a better understanding of complex phenotypes in general and in adult-onset diseases in particular.

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