Pleiotropy and Genotype by Diet Interaction in a Baboon Model for Atherosclerosis

A Multivariate Quantitative Genetic Analysis of HDL Subfractions in Two Dietary Environments

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Abstract—We investigated dietary effects on pleiotropic relationships among 3 HDL cholesterol (C) subfractions (HDL$_{1}$-C, HDL$_{2}$-C, and HDL$_{3}$-C; levels quantified by gradient gel electrophoresis) for 942 pedigreed baboons (Papio hamadryas) who were fed a basal (Chow) diet and a high cholesterol, saturated fat (HCSF) challenge diet. Using multivariate maximum likelihood methods we estimated heritabilities for all 6 traits, genetic and environmental correlations ($r_G$ and $r_E$) between them, and the additive genetic variance of each subfraction’s response to the diets. On the Chow diet, genetic correlations between the 3 subfractions were significant, and we observed complete pleiotropy between HDL$_{1}$-C and HDL$_{3}$-C ($r_G$ = 0.81). On the HCSF diet, only the genetic correlation between HDL$_{1}$-C and HDL$_{3}$-C ($r_G$ = 0.61) was significant. Genetic correlations between individual subfractions on the Chow and HCSF diets did not differ significantly from 1.0, indicating that the same additive genes influenced each subfraction’s levels regardless of diet. However, the additive genetic variance of response to the diets was highly significant for HDL$_{1}$-C and HDL$_{2}$-C, but not for HDL$_{3}$-C. Similar sets of genes influence variation in the 3 HDL subfractions on the Chow diet, and the same set influences variation in each subfraction on the HCSF diet. However, the expression of genes influencing HDL$_{1}$-C and HDL$_{2}$-C is altered by the HCSF diet, disrupting the pleiotropy observed between the 3 subfractions on the Chow diet. (Arterioscler Thromb Vasc Biol. 1999;19:1134-1141.)

Key Words: atherosclerosis risk factors ■ lipemic response ■ dietary challenge ■ animal models ■ statistical genetics ■ gradient gel electrophoresis

HDL particles are heterogeneous and comprise several distinct subfractions that differ in their composition of apolipoproteins and lipids. Although an inverse association between circulating concentrations of total HDL cholesterol (HDL-C) and risk of cardiovascular disease (CVD) in humans has gained wide acceptance during the past 2 to 3 decades, HDL-C subfractions also have been implicated as CVD risk factors. CVD risk is reported to decrease when HDL$_{2b}$-C is increased relative to HDL$_{3a}$-C and HDL$_{3b}$-C. Compared with total HDL-C, the HDL$_{2b}$-C and HDL$_{3b}$-C subfractions are better predictors of atherosclerotic plaque at specific sites, and HDL$_{2b}$-C is suggested to provide specific protective effects at early stages of atherosclerosis.

Associations between HDL-C subfractions and other CVD risk factors also have been reported. Reduced HDL$_{2b}$-C levels are correlated with a predominance of large LDL particles and increased CVD risk and with increased fibrogen levels in nonsmokers without CVD or infection. Hypertriglyceridemia, hyperinsulinemia, and high hepatic lipase activity are reported to be associated independently with decreased HDL$_{2b}$-C levels in men with or without coronary artery disease and non–insulin-dependent diabetes mellitus.

Several studies report associations of circulating levels of HDL-C and its subfractions with dietary intake of fat or cholesterol in humans. One study of 28 healthy subjects reports a negative correlation between the magnitude of postprandial triglyceridemic response to a standard oral fat meal and both total HDL-C and HDL$_{2b}$-C. Increased total HDL-C and HDL$_{2b}$-C levels are reported in response to postprandial lipemia in both carriers and controls in a study of the effects of the apolipoprotein apoA1Milano polymorphism.

In 56 normocholesterolemic and hypercholesterolemic subjects, Clifton et al observed increases in total HDL-C levels and, in a high-response subgroup, a 10% increase in the proportion of HDL$_{2b}$-C to total HDL-C in response to a high cholesterol dietary challenge. Katan and Beynen reported a positive correlation between responsiveness to dietary cho-
HDL-C and its subfractions respond to variation in fat and cholesterol in the diet, but only a limited number of studies have addressed the genetics of this response. Although a study of monozygotic twins reports a significant intrapair resemblance in HDL-C response to a 22-day overfeeding challenge, nearly all other published studies focusing on HDL-C report attempts to detect associations between variation in lipemic response and mutations in specific candidate genes. Mata et al observed no differences in HDL-C levels when individuals of different apoA4 genotypes are challenged with high fat, high cholesterol diets. However, one study reports that the apoE 4/3 genotype is associated with a greater response of HDL-C to a high fat, high cholesterol diet than other apoE genotypes.

There is substantial interspecies variability in responsiveness to dietary lipids and cholesterol. As is the case for studies of human subjects, nonhuman animal research has focused predominantly on the \( \beta \)-lipoproteins and pre-\( \beta \)-lipoproteins and associated components, ie, LDL-C, VLDL-C, apoB, apoE, and triglycerides, some of which have been posited as major factors in cholesterol transport. Only a limited number of groups have investigated the genetics of diet-induced changes in circulating HDL-C levels. In a laboratory marsupial (Monodelphis domestica), Rainwater and Vandenberg observed increased HDL-C levels in response to a cholesterol- and fat-enriched diet in animals that could be classified as both high and low responders on the basis of their VLDL+LDL-C responses to that same diet. In inbred mice the \( \text{Ath-}J \) locus influences HDL-C response to dietary challenge and also influences atherosclerosis in the aortic arch. Although these results suggest important insights into likely genetic influences on lipemic response in the studied species, salient differences in lipoprotein metabolism of these animal models may temper direct extrapolation to humans.

Their genetic proximity and metabolic similarities to humans increase the likelihood that studies using Old World monkeys to investigate genetic influences on lipoprotein metabolism and lipemic response will offer insights relevant to human physiology. Responses to dietary challenges have been reported for a number of nonhuman primate species, but those of baboons have proven to be more similar to humans in terms of cholesterol, lipoprotein, and lipid metabolism than those of other related species such as rhesus (Macaca mulatta), cynomolgus (Macaca fascicularis), or African green monkeys (Cercopithecus aethiops). Within these pedigrees there were 14 249 relative pairs. Kinship coefficients \( \Phi \) for these relative pairs were calculated by means of the Stevens-Boyce algorithm. Within these pedigrees there were 2015 relative pairs with \( \Phi = 0.25 \) (first-degree relatives), 21 with \( \Phi = 0.1875 \), 83 with \( \Phi = 0.15625 \), 10 142 with \( \Phi = 0.1250 \), 1955 with \( \Phi = 0.0625 \), and 33 with \( \Phi = 0.03125 \).

Baboons were maintained on a monkey chow diet low in cholesterol (0.03 mg/kcal) and fat (4% of calories, derived from vegetable oils; referred to below as “Chow” diet). Animals were fasted overnight and blood samples were drawn from the femoral vein after immobilization with ketamine (10 mg/kg body weight). The same animals were then fed a diet enriched in cholesterol (1.7 mg/kcal) and saturated fat (40% of total calories, derived from lard; HCSF diet). After eating this diet for 7 weeks, fasted animals were bled for the HCSF diet sample. All protocols were reviewed and approved by the institutional Animal Care and Use Committee.

Serum was isolated by low-speed centrifugation and was stored at \(-80^\circ\text{C as individual aliquots in plastic tubing segments. This method of storage guarantees that each sample is subjected to a single freeze-thaw cycle before analysis and is protected from oxidation and desiccation.}\n
**Measurement of Cholesterol Concentration in HDL Subfractions**

HDL-C concentrations were measured enzymatically in the serum supernatant after precipitation of apoB-containing lipoproteins by use of heparin-Mn\(^{4-42}\). HDL particles were resolved on the basis of size by use of gradient gel electrophoresis in nondenaturing gradient gels (PAA 2 to 16, Pharmacia). Distributions of cholesterol were visualized by pre-staining with Sudan black B, \(33,43\) which has been shown to be a reliable indicator of lipoprotein cholesterol concentrations. \(34-47\) Gels were subjected to densitometry at 610 nm with a Cliniscan Densitometer (Helena Laboratories). HDL absorbance profiles were then cut into 3 fractions on the basis of consistent features of a lyophilized baboon serum standard that was run on each gel. The fractions (and their approximate size intervals) were HDL\(_1\) (8.2 to 10.2 nm), HDL\(_2\) (10.2 to 14.2 nm), and HDL\(_3\) (14.2 to 19.3 nm). Absorbances within each fraction were summed and expressed as a fraction of total HDL absorbance and then multiplied by the HDL-C value to obtain cholesterol concen-
trations within each HDL subfraction (detailed description and validation are presented elsewhere\textsuperscript{43}). We have previously reported the multivariate coefficient of variation for these measures to be 8.2%.\textsuperscript{33}

### Statistical Genetic Analysis

We used the computer package PEDSYS\textsuperscript{48} for pedigree and phenotype data management and preparation. Statistical genetic analyses were conducted using maximum likelihood methods to compute the likelihoods of genetic models on data from pedigrees.\textsuperscript{49}

In accordance with established quantitative genetic theory,\textsuperscript{50} we partitioned the total phenotypic variance in the traits (\(\sigma^2\)) into \(\sigma^2_G\), the variance due to the additive effects of genes, and \(\sigma^2_E\), the variance due to nongenetic (or environmental) effects. We estimated heritability (\(h^2\)), the proportion of the phenotypic variance due to the additive effects of genes, for each of the subfractions as \(\sigma^2_G/\sigma^2\).

We modeled the multivariate phenotype of an individual as a linear function of the measurements on the individual's traits, the means of these traits in the population, and the covariates and their regression coefficients, plus the additive genetic values and unmeasured nongenetic deviations.\textsuperscript{51–55} We further partitioned the phenotypic variance-covariance matrix into the additive genetic and environmental variance-covariance matrices, given the relationships (kinship coefficients) observed in the pedigree. From these matrices, we estimated the additive genetic correlation, \(r_{\text{PE}}\), and the environmental correlation, \(r_{\text{EE}}\), between trait pairs. Respectively, these correlations are estimates of the additive effects of shared genes (ie, pleiotropy) and shared unmeasured, nonhereditary (often referred to as “random environmental”) factors on the variance in a trait. Because the contributions of the genetic and environmental components of the phenotypic variance-covariance matrix are additive,\textsuperscript{56} we used the maximum likelihood estimates of these 2 correlations to obtain the total phenotypic correlation, \(r_{\text{PE}}\) between 2 traits as

\[
(1)\quad r_{\text{PE}} = \sqrt{h_1^2 + h_2^2 r_{\text{PE}}} + \sqrt{1 - h_1^2 - 1 - h_2^2 r_{\text{PE}}}.
\]

We conducted a hexavariate quantitative genetic analysis of the 3 HDL subfractions (HDL\textsubscript{C}, HDL\textsubscript{L-C}, and HDL\textsubscript{L-C}) measured in 2 diets using the simultaneous orthogonalization methods of Blangero and Konigsberg\textsuperscript{57} implemented in our modified version of PAP v3.0.\textsuperscript{59} With this approach, we obtained simultaneous maximum likelihood estimates of the phenotypic means (\(\mu\)), phenotypic standard deviations (\(\sigma\)), heritabilities (\(h^2\)), and the effects of sex, age-by-sex, age\textsuperscript{1}-by-sex, nursery status (a dichotomous trait: baboons reared by mothers, “0”), and percent Yellow baboon admixture (method of calculation is reported elsewhere\textsuperscript{56}) for all 6 traits, as well as the genetic and environmental correlations between them. Before analysis, we performed a log (in) transformation on the HDL-C subfraction data to reduce skewness and to mitigate effects of scale on parameter estimation. No other prior adjustments to the data were made.

Although the hexavariate model applied in this study is a polygenic one, it is possible, in fact likely, that individual loci contribute some proportion of the shared variation between the phenotypes in the different dietary environments. The maximum likelihood methods used rely on the assumption of multivariate normality as a “working model,” but are robust to deviations from multivariate normality in the underlying distribution.\textsuperscript{53} Consequently, valid maximum likelihood estimates for the parameters of the genetic model can be obtained even if major loci, not modeled in this analysis, are involved.\textsuperscript{57}

We assessed the significance of each of the estimated parameters (excluding means, \(\mu\), and standard deviations, \(\sigma\)) by likelihood ratio tests, wherein \(-2\times\ln\) likelihood of a restricted model, in which a parameter value is fixed at 0, is compared with the similarly calculated value for the more general hexavariate model, in which all parameter values are estimated. The likelihood ratio test statistic, \(A_{\text{LRT}}\), (where \(i\) indicates degrees of freedom), is distributed approximately as a \(\chi^2\) variable with degrees of freedom equal to the difference in the number of parameters in the 2 models being compared.\textsuperscript{58} A hypothesis of pleiotropy is supported when an additive genetic correlation is found by likelihood ratio test to be significantly different from zero. Complete pleiotropy would indicate that the same gene or set of genes was influencing variation in HDL-C subfraction levels to the same degree in both diets. As a test for complete pleiotropy, we compared the likelihood of an unrestricted model in which the genetic correlation is estimated with that of a restricted model in which it is fixed at 1.0.

### Detecting Genotype by Diet Interactions

We have implemented an analytical approach that enables us to quantify the proportion of the variance in response to these 2 dietary environments that is attributable to the additive effects of genes. We consider the 2 dietary regimens as different environments. Genotype by environment interaction occurs when there is a significant genetic component to the variance in response to the environment.\textsuperscript{60} The additive genetic variance in an HDL-C subfraction’s response is a function of both that subfraction’s additive genetic variance in the 2 diets and the additive genetic correlation between levels of that subfraction in the 2 diets. An absence of additive genetic variance in response to the diet implies that there is no additive polygenotype by diet interaction. This occurs when the proportions of the variances due to the additive effects of genes are equal in both diets (when \(\sigma^2_G=\sigma^2_E\)) and when the proportion of the correlation between 1 subfraction in the 2 diets that is due to the additive effects of shared genes is complete (ie, positive pleiotropy, \(r_{\text{PE}}=1\)).\textsuperscript{60} These 2 conditions serve as null hypotheses. Although the rejection of either is indicative of polygenotype by diet interaction, the more salient of the two is the test of equal genetic variances for the same subfraction in 2 diets.

To test the first of these hypotheses for each of the \(i\) subfractions, we estimated the additive genetic variance of the response to the 2 diets as

\[
(2)\quad \sigma^2_G = h_i^2, \quad \sigma^2_E
\]

where \(\sigma^2_G\) is the maximum likelihood estimate of the 2 residual phenotypic standard deviations (one from each of the \(i=2\) environments), and \(h_i^2\) is the heritability estimate.

First-order Taylor series approximations of the standard errors of these estimates were obtained from the variance-covariance matrix of the genetic correlations with respect to the genetic variances. A Wald test with 1 degree of freedom\textsuperscript{61} was used to determine the significance of the genetic variance of the response for each HDL-C subfraction to the 2 diets.

### Results

Univariate descriptive statistics for the raw measures of the 3 subfractions measured in samples obtained from the 2 diets are presented for each sex in Table 1. On both diets, mean concentrations of both HDL\textsubscript{L-C} and HDL\textsubscript{L-C} are slightly lower in females than in males whereas the opposite is true for HDL\textsubscript{L-C} subfraction. In both sexes, the HCSF diet is associated with higher mean and median serum levels for all 3 HDL-C subfractions. Although the data in this table are from related individuals and tests of significance that do not account for this fact would be inappropriate, the magnitudes of the observed differences are suggestive of greater lipemic responses for the HDL\textsubscript{L-C} and HDL\textsubscript{L-C} subfractions than for HDL\textsubscript{L-C}.

Maximum likelihood estimates of \(h_i^2\) as well as of the phenotypic means, standard deviations, covariate effects, and their standard errors are found in Table 2 (note that because the 95% confidence intervals about the maximum likelihood parameter estimates are asymmetrical, the common practice of adding \(\pm 2\) times the standard error to the estimate will not reliably indicate significance). Likelihood ratio tests disclosed significant additive genetic effects, ie, significant \(h_i^2\) estimates, on all 3 HDL-C subfractions measured in both
TABLE 1. Descriptive Statistical Summary for Serum Concentrations (mg/dL) of Three HDL-C Subfractions in Chow and HCSF Dietary Environments in 944 Pedigreed Baboons (581 females, 363 males) by Sex

<table>
<thead>
<tr>
<th></th>
<th>Chow Diet</th>
<th>HCSF Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HDL1-C</td>
<td>HDL2-C</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7.62</td>
<td>0.20</td>
</tr>
<tr>
<td>Median</td>
<td>4.60</td>
<td>0.12</td>
</tr>
<tr>
<td>SD</td>
<td>9.04</td>
<td>0.23</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Maximum</td>
<td>80.60</td>
<td>2.08</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.42</td>
<td>0.24</td>
</tr>
<tr>
<td>Median</td>
<td>6.10</td>
<td>0.16</td>
</tr>
<tr>
<td>SD</td>
<td>10.05</td>
<td>0.26</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.20</td>
<td>0.01</td>
</tr>
<tr>
<td>Maximum</td>
<td>68.00</td>
<td>1.76</td>
</tr>
</tbody>
</table>

Table 2 presents maximum likelihood estimates of the additive genetic and environmental correlations and their standard errors plus the phenotypic correlations calculated using equation 1. In data from animals on the Chow diet, we observed significant genetic correlations between all 3 subfractions \( \rho_{G} = 0.001 \) and did not reject complete pleiotropy \( \rho_{P} = 1.0 \) between HDL1-C and HDL2-C \( \rho_{G} = -0.82 \). Within the Chow dietary environment, shared genes account for 38% to 67% of the genetic variance in the 3 subfractions. In data obtained from animals after the HCSF dietary challenge, only the genetic correlation between HDL1-C and HDL2-C \( \rho_{G} = -0.62 \) was significant \( P<0.01 \). Within the HCSF dietary environment, shared genes account for about 38% of the genetic variance in these 2 subfractions. Likelihood ratio tests revealed significant additive genetic correlations, indicative of pleiotropy, between the same HDL subfractions measured in both the Chow diet and HCSF diets. Up to 90% of the genetic variance in levels of HDL1-C in the 2 dietary environments is attributable to the shared additive effects of the same genes. The same is true for the HDL2-C subfraction. For HDL2-C, more than 72% of

diets. Significant sex effects were found only for HDL2-C measured on the HCSF diet. For both sexes, age contributed significantly to variation in HDL1-C and HDL2-C levels on the Chow diet, but on the HCSF diet, age influenced variation in HDL1-C and HDL2-C only in females and in HDL3-C only in males. Nursery and percent \( P \) h cynocephalus admixture (β subspecies) effects were significant only for HDL3-C measured on the HCSF diet.

The hypothesis of different means in the 2 dietary environments for each subfraction was also addressed. Likelihood ratio tests rejected the hypothesis \( \mu_{\text{Chow}} = \mu_{\text{HCSF}} \) for HDL1-C \( \chi^2 = 63.48, P<0.00001 \) and HDL2-C \( \chi^2 = 224.47, P<0.00001 \) but not for HDL3-C \( \chi^2 = 0.04, P=0.81 \).

Values are mean ± SEM. Hexovariate quantitative genetic analysis was performed on log transformation of 3HDL-C subfractions in 942 pedigreed baboons in 2 dietary environments. Chow indicates low fat, low cholesterol; HCSF, high cholesterol, high saturated fat; \( \mu \), estimated trait mean; \( \sigma \), estimated phenotypic standard deviation for a trait; \( h^2 \), estimated narrow sense heritability for a trait; and \( \beta \), estimated mean effect on a trait for covariate.

Indicators of significant difference determined by means of Likelihood Ratio Test between \( \chi^2 \) (in likelihoods) of models in which parameter value to be tested is fixed at zero are compared to those in hexovariate polygenic models in which the parameter value was estimated.

\( \dagger P<0.025 \); \( \ddagger P<0.001 \).
subfractions measured in the Chow diet than in those measured in the HCSF environment. In the Chow diet, genes shared by HDL₁-C and the other 2 subfractions account for a greater proportion of both their genetic and residual phenotypic variances than do genes shared by HDL₂-C and HDL₃-C. Within the HCSF dietary environment, this relationship does not obtain. Only genes shared by HDL₁-C and HDL₃-C account for more than 5% of the residual phenotypic variance in subfraction pairs measured in animals on the HCSF diet (18.5% and 14.3%, respectively).

Because maximum likelihood estimates of genetic correlations between the same subfraction measured on the 2 diets were either 1.0 or, in the case of HDL₂-C, not significantly different from 1.0, the proportions of the residual phenotypic variance attributable to shared genes approximated the diet-specific heritability estimates of the subfractions. Compared with the Chow diet estimates, the proportion of the residual phenotypic variance due to shared genes for HDL₁-C, but reduced by more than half for HDL₂-C and only slightly for HDL₃-C.

The environmental correlations between the 3 HDL-C subfractions exhibit very similar patterns in the 2 diets (Table 3). On both Chow and HCSF diets, the correlations between HDL₁-C and HDL₃-C are of greatest magnitude. Approximately 25% of the variance due to unmeasured, nongenetic factors in these 2 subfractions is accounted for by factors that are shared in both the Chow and HCSF environments. This proportion does not exceed 3.9% for any of the other subfraction pairs within each diet. There is less difference between the environmental correlations obtained when comparing each of the same subfractions across the 2 diets. For example, at $r_{PE}=0.30$, the environmental correlation between the 2 HDL₂-C measures is about 50% higher than those for the other 2 subfractions. On average, shared unmeasured, nongenetic factors account for slightly more than 3% (HDL₁-C) to 9% (HDL₃-C) of the environmental variance in subfraction concentration between diets.

The product of the squared environmental correlation ($r_{E}^2$) and the quantity $1-h^2$ provides an estimate of the proportion of the residual phenotypic variance for a phenotype that is attributable to the effects of unmeasured shared genes (Table 4). Shared genes account for a greater proportion of the residual phenotypic variance in all subfractions measured in the Chow diet than in those measured in the HCSF environment.
Within Chow diet

<table>
<thead>
<tr>
<th>Subfraction Pair</th>
<th>( \rho^2_E )</th>
<th>( \rho^2_E \times (1-h^2) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL₁-C</td>
<td>0.011</td>
<td>0.007</td>
</tr>
<tr>
<td>HDL₂-C</td>
<td>0.022</td>
<td>0.014</td>
</tr>
<tr>
<td>HDL₃-C</td>
<td>0.039</td>
<td>0.022</td>
</tr>
</tbody>
</table>

Within HCSF diet

<table>
<thead>
<tr>
<th>Subfraction Pair</th>
<th>( \rho^2_E )</th>
<th>( \rho^2_E \times (1-h^2) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL₁-C</td>
<td>0.010</td>
<td>0.005</td>
</tr>
<tr>
<td>HDL₂-C</td>
<td>0.252</td>
<td>0.130</td>
</tr>
<tr>
<td>HDL₃-C</td>
<td>0.004</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Between diets, same subfraction

<table>
<thead>
<tr>
<th>Subfraction Pair</th>
<th>( \rho^2_E )</th>
<th>( \rho^2_E \times (1-h^2) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL₁-C Chow</td>
<td>0.038</td>
<td>0.024</td>
</tr>
<tr>
<td>HDL₂-C Chow</td>
<td>0.090</td>
<td>0.050</td>
</tr>
<tr>
<td>HDL₃-C Chow</td>
<td>0.032</td>
<td>0.025</td>
</tr>
</tbody>
</table>

\( \rho^2_E \) indicates proportion of \( \sigma^2_e \) due to shared environmental effects; \( \rho^2_E \times (1-h^2) \), proportion of \( \sigma^2_e \) due to shared environmental effects.

**Discussion**

The utility of estimating genetic correlations between ostensibly correlated phenotypes is well established in quantitative genetics. For the most part, such estimates have provided means for assessing the causes of observed phenotypic associations and the likelihoods of correlated responses of trait pairs to selective breeding in experimental and agricultural populations. In this study, we have used both established and novel multivariate extensions to basic quantitative genetics theory and methods to detect and characterize the genetic contributions to phenotypic associations between serum levels of 3 different but interrelated lipoprotein subfractions measured in 2 dietary environments. And although the baboon differs from humans with respect to characteristic HDL subfraction profile (in baboons, HDL₁ and HDL₂ predominate, whereas HDL₂ and HDL₃ are the major components of human HDL), the relevance of these analyses to human atherosclerosis is that they address the genetic influences on quantitative variation in HDL subfraction profiles and, by extension, on the metabolic pathways that may produce this variation.

Using a maximum likelihood-based hexavariate quantitative genetic analysis strategy applied to data from 942 pedigreed captive baboons, we have assessed the relative contributions of genes, shared genes, and shared nongenetic factors to normal phenotypic variation in serum levels of 3 HDL-C subfractions measured in 2 dietary environments. Further, we have tested these data for evidence of interaction between the 2 diets and the detected genes. We interpret the results of our analyses to indicate that variation in dietary cholesterol and fat intake influences quantitative variation in serum concentrations of the 3 HDL-C subfractions through effects on the expression of genes responsible for normal variation in levels of these 3 subfractions. In addition to demonstrating the existence of these genotype by diet interactions, the results of our analyses also provide indications of their magnitude and nature.

Our observations of significant increases in mean levels of HDL₁-C and HDL₂-C, but not of HDL₃-C, in response to the HCSF diet are consistent with those reported by others, including observations of increased HDL₃-C and no change in HDL₁, in response to dietary supplementation with n-3 fatty acids in humans; an association between increased formation of HDL particles and n-6 γ-linolenic acid–enriched evening primrose oil in rabbits; and increased HDL levels mediated by the effects of dietary fat on the fractional catabolic rate of HDL cholesterol ester in the human apoA1 mouse. We note, however, that significant changes in phenotypic means are not necessary preconditions for the subsequent detection of a genotype by diet interaction. In fact, genotype by diet interaction can occur in the absence of significant changes in phenotype means. This would be the case, for example, if responses of specific genotypes were in opposite directions with respect to the original mean and their changes effectively canceled the effect of one another on the population mean.

As noted above, the influence of genes on quantitative variation in levels of HDL-C and its subfractions is well accepted. The mean of the 6 heritability estimates in this study, 0.32, is within the range of heritability estimates reported for total HDL-C levels in numerous human twin and family studies, between 0.16 and 0.79 (reviewed previously).

Observation of pleiotropy between the different HDL-C subfraction levels within the basal Chow diet is not entirely unexpected. A few studies have reported observations consistent with pleiotropic relationships between the HDL subclasses. One study of data from 116 human probands undergoing coronary arteriography at an early age, plus 676 relatives, detected possible pleiotropic effects of a locus, which also accounts for 33% of the variation in HDL₁-C levels, on total HDL concentration and size. A multivariate segregation analysis of data from the Donner Laboratory Family Study detected evidence for a major locus for apoA1 serum levels that exerts pleiotropic effects on the relative distribution of HDL subfractions. Also, a study of 717 individuals from 26 families in the San Antonio Family Heart Study reported significant shared additive genetic effects on the distributional patterns of apoA1 among HDL subclasses. However, detecting significant pleiotropic interactions between the 3 subfractions and determining that the magnitudes of these interactions are diet-specific are novel outcomes of this study. In this nonhuman primate model for dietary and genotypic interactions with atherosclerosis risk factors, shared genes account for a greater proportion of the genetic variance in the 3 subfractions under a low cholesterol, low fat diet than under an HCSF diet. On the Chow diet, much of the genetic variance and a moderate amount of the residual phenotypic variance in the 3 different subfractions is attributable to the effects of shared genes. In contrast, on the HCSF diet, only HDL₁-C and HDL₂-C exhibit significant shared genetic effects. We interpret these differences as evidence for HCSF diet-induced alterations of the pleiotropic relationships among the 3 subfractions.

Comparison of the shared genetic effects on each of the 3 HDL-C subfractions measured in 2 different dietary environments provides additional information regarding this possible genotype by diet effect. Between dietary environments, shared genes account for nearly all of the genetic variance and
moderate amounts of the phenotypic variance in each of the 3 subfractions. That is, for the most part, the detected significant changes in mean levels of 2 of the HDL-C subfractions are not caused by the additive effects of different genes in different dietary environments. Rather, we suggest that these differences are attributable to changes in expression of the shared genes in response to the HCSF diet.

The significant genetic contribution to the response to diet for HDL1-C and HDL2-C, the 2 subfractions that also exhibit significantly different mean levels under the 2 diets, provides further support for this suggestion. Specifically, the high cholesterolemia, high saturated fat levels of the HCSF diet are associated with a change in gene expression such that the genetic variance for the HDL1-C subfraction is increased whereas that for the HDL2-C subfraction is decreased. We conclude that some genes within the suite of genes influencing phenotypic variation in total HDL-C may exert dissimilar effects on different HDL subfractions in response to diet.

Shared, unmeasured environmental factors account for only moderate proportions of the residual environmental and phenotypic variances in HDL1-C and HDL2-C within dietary environments, and small to negligible proportions between dietary environments. Because these “environmental” correlations reflect shared effects of unmeasured, noninherited factors, it is unlikely that our inferences regarding additive genetic pleiotropy are biased upward or would be diminished by the subsequent elucidation, and inclusion in our models, of one or more of these factors. It is more likely that such additions to our analyses would only serve to increase the signal to noise ratio of the detected shared additive genetic effects.

We expect to follow up on these observations to obtain additional indications of the genetic, environmental, and metabolic contributions to the changes in the pleiotropic relationships among the 3 subfractions under the HCSF diet. A study in which many of these same animals have been challenged with a third diet that is low in cholesterol but high in saturated fats has begun to yield insights regarding the differential effects of cholesterol and saturated fat on the genes influencing lipemic response in LDL-C levels. Additional, we have shown elsewhere 68–70 that knowledge of both the pleiotropic interactions and shared effects of unmeasured nongenetic factors on pairs of phenotypes can be exploited to detect and localize specific genes influencing quantitative variation in those traits. Using the pleiotropy detected between HDL-C subfractions in these pedigreed baboons on 2 diets, we are initiating multivariate, multipoint variance component whole genome linkage screens 71 to identify chromosomal regions harboring genes influencing lipemic response to a high cholesterolemia, high fat diet.

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Pleiotropy and Genotype by Diet Interaction in a Baboon Model for Atherosclerosis: A Multivariate Quantitative Genetic Analysis of HDL Subfractions in Two Dietary Environments

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