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Effects of Diet on Genetic Regulation of Lipoprotein Metabolism in Baboons

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Abstract

Several measures of lipoprotein phenotype are significant predictors of cardiovascular risk. Although such lipoprotein phenotypes are under strong genetic control, it is not clear to what extent they are controlled by the same - and by different - genes and whether these relationships may be altered in different dietary environments. Therefore, we measured six lipoprotein traits (three LDL traits - LDLC and apoB concentrations and LDL size - and three HDL traits - HDLC and apoA1 concentrations and HDL size) on each of three diets differing in level of fat and cholesterol. In bivariate analyses, all but two metabolically related trait pairs were genetically correlated, though none were completely correlated, implying additive genetic effects by both pleiotropic and unique genes. In comparing genetic correlations for the same pair of traits across diet, we detected evidence of diet effects on genetic control of these metabolically related traits; specifically, increasing level of dietary cholesterol was associated with a significant decrease in the genetic correlation of apoA1 with HDL size, and a significant increase in the genetic correlations of LDL size with LDLC and apoB. The results suggest a complex network of genes affecting lipoprotein metabolism: the genes may exert both unique and pleiotropic effects; the genes may exert detectable effects in many or only in specific dietary environments.

INTRODUCTION

Despite many improvements in therapeutic approaches and lifestyle recommendations, cardiovascular disease (CVD) remains the leading killer of men and women in the US and Europe. CVD is a multifactorial disease, with a multiplicity of etiologies and risk factors associated with clinical manifestations. Perhaps the most commonly-recognized risk factors for CVD are various dyslipidemias, including high circulating levels of total and low-density lipoprotein cholesterol (LDLC) and low levels of high-density lipoprotein cholesterol (HDLC).

LDLs and HDLs actually are themselves heterogeneous mixtures of lipid-bearing particles. Heterogeneity includes aspects of composition (both lipid and protein), particle size, and

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metabolism. A number of approaches have been explored to separate LDLs and HDLs into their component subclasses that might better predict associated CVD risk.

Lipoprotein metabolism is regulated by environmental factors, especially diet, and by a complex array of genes that encode apolipoproteins and other proteins directly involved in lipoprotein metabolism, as well as proteins governing metabolism in general [1–4]. A more thorough understanding of the factors governing lipoprotein metabolism and their interactions may provide useful insights into approaches to modulating CVD risk associated with dyslipidemia.

The pedigreed baboon model closely resembles the human situation in many key respects, including the pathology of atherosclerotic lesions and the metabolism of lipoproteins [5]; the rich diversity of pedigree relationships in our colony offer the prospect of teasing out genetic and environmental factors that control of lipoprotein metabolism and associated risk of CVD. A number of years ago, our group initiated studies of diet-genotype interactions using a dietary paradigm that employed a standardized shift in level of dietary fat and cholesterol [6,7]. The ultimate goal of this research has been to improve our understanding of the complex network of factors, particularly genetic, that influence the traits and their responses to specific dietary changes.

The purpose of the present study was to characterize the genetic control of lipoprotein predictors of CVD and to ascertain the effects of changing the dietary environment. In particular, we have sought evidence of diet effects on genetic control of coordinated variation in these metabolically-related traits.

METHODS

Animals and diet protocol

We subjected 716 baboons to a standard diet protocol that involved feeding them each of three defined diets, differing in levels of fat and cholesterol, for seven weeks prior to taking a blood sample. The diets included: Basal, low in fat and cholesterol; HFHC, high in fat and cholesterol; and HFLC, high in fat but low in cholesterol. The basal diet was from Harlan-Teklad (Madison WI) and contained 7% (w/w) fat and 0.02 mg/g cholesterol; the two high-fat diets were constructed from a defatted meal to which was added lard (to 40%, w/w); the two high-fat diets differed by whether cholesterol (to 6.4 mg/g) was added or not, as described previously [8]. Animals were fasted overnight, immobilized with ketamine, and bled from the femoral vein to obtain the three diet samples. Blood was allowed to clot and subjected to low-speed centrifugation to isolate serum samples which were prepared in multiple single-use aliquots [9] and stored at −80°C until use.

Baboons were maintained by the veterinary resources staff in the Southwest Primate Research Center at Southwest Foundation for Biomedical Research. This institution is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The protocol was approved by the Institutional Animal Care and Use Committee and conformed to the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Measurement of lipid and lipoprotein traits

Serum cholesterol levels were measured enzymatically in a clinical chemistry analyzer using commercial reagents [10]. ApoB-containing lipoproteins were precipitated with heparin-Mn^{+2} [11]; HDLC was measured in the supernatant and then LDLC was calculated as the difference between total and HDL cholesterol values. Because a small amount of cholesterol in very large HDLs, that accumulate in some baboons, is also precipitated under these

conditions, we used gradient gel electrophoresis to adjust HDLC and LDLC values for this infrequent contaminant as described [12]. Non-denaturing acrylamide gradient gel electrophoresis was performed as described [13,14] in order to resolve LDLs and HDLs on the basis of size. Lipoprotein cholesterol was stained with Sudan black B and global measures of LDL and HDL size distributions – median diameters – were determined as described [13]: HDL median diameter (Hmed) was defined as the diameter where half the HDL absorbance (8.2–24 nm) was on smaller, and half was on larger, particles; LDL median diameter (Lmed) was similarly determined for the LDL size range (24–36 nm). Concentrations of apolipoproteins A1 (ApoA1) and B (ApoB) were quantified immunoturbidometrically by use of commercial reagents in a clinical chemistry analyzer [15,16].

Statistical genetic analyses

All statistical genetic analyses described below were done using routines in the software package SOLAR [17]. The 18 lipid and lipoprotein traits were each pre-adjusted for the effects of a standard set of covariates (age, sex, and weight) that have proven to be significant in many previous studies, even if one or more was not significant in a specific instance. The residual was then normalized by fitting to a Gaussian distribution and standardized such that mean and standard deviation were zero and one, respectively. These are the traits that were subjected to further analyses.

Pedigree-based quantitative genetic analyses were implemented in SOLAR. Univariate analyses were performed to estimate the proportions of phenotypic variance explained by covariates and the additive effects of genes (heritability, h^2, calculated as σ^2_G/σ^2_P). Bivariate models included estimates of heritability for both traits and the phenotypic correlation was partitioned into the additive genetic (ρ_G) and residual environmental (ρ_E) correlations, using the formula: $\rho_P = \rho_G \cdot \sqrt{h^2_1} \cdot \sqrt{h^2_2} + \rho_E \cdot \sqrt{(1-h^2_1)} \cdot \sqrt{(1-h^2_2)}$ [18]. We tested for diet-genotype interactions for each trait measured on two diets by testing either for absence of complete pleiotropy or for equality of genetic variances; thus, we compared the $\log_e$ likelihoods of the full model to nested ones in which we fixed $\rho_G=1$ or $\sigma^2_G(\text{Trait 1}) = \sigma^2_G(\text{Trait 2})$, respectively. A significant difference between the models implied diet-genotype interaction. We tested for pleiotropic additive genetic effects on different traits measured on the same diet by comparing the $\log_e$ likelihoods of the full model to those for models in which ρ_G was constrained to be zero; a significant difference between the two models implied pleiotropy. Similarly, we also tested the significance of ρ_E and ρ_P.

We tested for diet effects on additive genetic correlations in quadrivariate analyses by two approaches. The models included the same pair of traits measured on each of two diets differing in level of either fat or cholesterol. As an exploratory step, we first used a two sample t-test and treated the genetic correlation between two lipoprotein-related traits as a summary statistic (maximum likelihood estimates of the standard error were used to obtain the appropriate standard deviation for each correlation). We then tested the hypothesis that this statistic was the same in two dietary environments. Alternately, we compared the $\log_e$ likelihood of a full model to that for a model in which the genetic correlation for the two traits was constrained to be the same on each of the two diets. For either test, a significant difference between additive genetic correlations on the two diets implied that the magnitude of pleiotropic effects was altered by diet composition.

RESULTS

Lipid and lipoprotein traits

The sample included 452 female and 264 male baboons; average ages and weights were 14.4 y and 18.7 kg for females and 11.4 y and 29.0 kg for males. We obtained data on a global particle size distribution trait (median diameter) and two concentration variables (cholesterol and apolipoprotein) for HDLs and LDLs for these baboons fed three diets. Table 1 shows the proportions of trait variance attributable to the standard set of covariates (age, sex, and weight) and to the additive effects of genes for these traits on each of the diets. On average, the selected covariates explained 9% of the raw phenotypic variance, but these values ranged from 1–27%. Thus, the bulk of total trait variance remained after covariate adjustment. Each of the covariate-adjusted traits was significantly heritable in these models and, on average, about half the total phenotypic variance (range 27–69%) was attributable to the additive effects of genes. The residual variance not explained in the models averaged ~40% in this study.

Tests of diet effects on genetic regulation of LDL and HDL traits

Each of the six traits was investigated for the possibility of diet effects on genetic variance (i.e., diet-genotype interaction). Given the expectation that genetic effects on a trait in two environments will be the same, we can posit two criteria that should be true and that can be tested in bivariate analyses: 1) the genetic correlation (ρ_G) between a trait on different diets should equal one (i.e., complete pleiotropy) and 2) the genetic variances (σ^2_G) on each diet should be identical. The violation of either of these criteria constitutes *prima facie* evidence of diet-genotype interaction. We tested the hypothesis of complete pleiotropy by comparing the likelihood of a model in which ρ_G was fixed at 1 to a model in which ρ_G was freely estimated. We tested the second hypothesis by comparing the likelihood of a model in which the genetic standard deviations for each trait were constrained to be equal to a model in which they were freely estimated. Based on these criteria, changing dietary fat level significantly affected genetic control of HDLC, whereas changing dietary cholesterol level significantly affected genetic control of each trait except Hmed (Table 2). Thus, all but one of the six traits showed evidence of diet-genotype interaction for the two dietary perturbations that were tested in this study.

Tests of genetic contribution to metabolic correlations

To explore diet-genotype interactions affecting metabolic pathways, we investigated traits reflecting different aspects of each lipoprotein class; such traits were expected to be metabolically related. Thus, we studied, within diet, the intercorrelations of the three HDL (Hmed, HDLC, apoA1) and LDL (Lmed, LDLC, and apoB) traits. We conducted bivariate analyses for each of the three possible trait pairs and Table 3 lists some of the parameters estimated in the models, including heritabilities of the two traits and their phenotypic, environmental, and genetic correlations (ρ_P, ρ_E, ρ_G, respectively). The phenotypic correlations averaged 0.49 but were quite variable, ranging from 0.022 to 0.864. Phenotypic correlations were strongest in each case for the pair of concentration variables and correlations involving lipoprotein size were consistently stronger for cholesterol, compared to apolipoprotein, concentrations. Generally, correlations were stronger for HDL traits, by comparison with LDL traits.

The genetic correlations averaged 0.64 and ranged from 0.028 to 0.922. The genetic correlations followed the same pattern as for the phenotypic correlations. Particularly noteworthy were the substantially lower significance levels observed for the correlations of size with concentration variables among LDL traits, compared to HDL traits. Nevertheless, all but two of the genetic correlations were significant at the 0.05 level, implying that

pleiotropic genes (or sets of genes) were responsible for an important component of covariation in these lipoprotein traits. However, there was strong evidence in each case against complete pleiotropy for all correlations (data not shown), implying that additional genes uniquely influenced variation in each trait. In some cases, the strength of a genetic correlation appeared to differ across diets. For example, the genetic correlation for Bmed and LDLC on HFLC diet was 0.257, but was much higher (0.600) on the HFHC diet. Therefore, we explored the possibility that diet might affect the genetic control of lipoprotein metabolism (i.e., genetic correlations among related traits) using quadrivariate analyses.

Tests of diet-genotype interaction effects on lipoprotein metabolism

Each trait reflects a summation of metabolic pathways that influence it. Some of the pathways may be unique to the trait, but others may influence several related traits in common, as is suggested above by the significant intercorrelations among traits. In the absence of diet-genotype interactions, we would expect the genetic correlations (i.e., the proportions of shared genetic variance) for pairs of traits to be the same across diets and this hypothesis was tested in quadrivariate analyses that included the same pair of lipoprotein traits from each of two diets that differed in the level of either fat or cholesterol. Table 4 gives estimates of the within-diet genetic correlations for each of the trait pairs and these values were similar to those obtained in the bivariate models (i.e., compare to Table 3). In addition, *P*-values from two tests of the hypothesis that dietary composition had no effect on trait genetic correlations are given in Table 4: 1) a two-sample t-test and 2) a $\log_e$ likelihood ratio test (see methods section). Although two of the latter models failed to converge, the results of both tests are in agreement that changing level of dietary fat had no effect on the genetic correlations, suggesting that genes exerted similar pleiotropic effects on these traits in the two dietary environments. However, the genetic correlations for three of the trait pairs were significantly altered when the level of dietary cholesterol was changed (i.e., HFLC vs. HFHC). Specifically, the correlations for Bmed/ApoB and Bmed/LDLC were significantly strengthened, and the correlation for Hmed/ApoA1 was significantly reduced, in the high-cholesterol environment (*P*-values ranged from 0.00004 to 0.008). Similar tests of the environmental correlations, however, revealed no significant diet effects on these correlations (data not shown).

DISCUSSION

It has long been known that high circulating levels of LDLs and low circulating levels of HDLs are strongly associated with risk of CVD and, for nearly as long, it has been recognized that these major classes of lipoproteins are each quite heterogeneous [19]. Lipoprotein particle heterogeneity results from variation in type and proportion of various lipid and protein components of the particle and interindividual heterogeneity stems from variation in concentrations of this broad diversity of lipoprotein species. Recent mass spectrometry studies of isolated HDL particles have identified an average of one or more molecules per particle of as many as 48 different proteins to occur on HDL [20]. However, some of these proteins may occur in particle-specific clusters [21]. A number of approaches have been developed to separate the major lipoprotein classes into subfractions that might be more directly related to atherogenesis and risk of CVD.

In this study we have analyzed, within each lipoprotein class, concentration measures of cholesterol and protein plus a measure of lipoprotein size distribution. We have chosen cholesterol concentration because of its frequent associations with CVD across numerous studies [22]. We have chosen apoA1 and apoB concentrations as the dominant and defining protein components of HDLs and LDLs, respectively, recognizing that there are many other component proteins whose individual variation may or may not be adequately indicated by

the predominant protein. A number of studies, however, suggest that concentrations of these two class-defining apolipoproteins are stronger predictors of CVD than are cholesterol concentrations [23,24]. This may be due in part to the fact that protein concentrations are more closely associated with particle concentrations, which drive diffusion into the intima, than are cholesterol concentrations [25]. We have chosen median diameter as a global indicator of lipoprotein size distributions; this trait reflects aspects of lipid/protein ratio and particle concentration. Variation in particle size phenotypes has been reported to be associated with CVD risk in several prospective studies [26–30], though whether such variation satisfies the additional criterion of independence is less certain [31,32]. Therefore, we believe these six traits measure a number of different aspects of lipoprotein phenotype and potentially they reflect most, but undoubtedly not all, metabolic pathways that influence lipoprotein variation and the associated risk of CVD.

As was expected [7], each of the six traits was strongly heritable, with genes explaining approximately half the total phenotypic variance in this study. This approximate level of heritability held for each of three diets – differing in levels of fat and cholesterol – that were tested in this study. To assess diet effects on genetic control of lipoprotein metabolism, we first investigated effects on individual traits measured on two diets. Not surprisingly [7,10,18,33], traits were very strongly correlated across diets; ρ_G ranged from 0.97 to 1.00 for traits measured on basal and HFLC diets (differing in level of fat) and from 0.84 to 0.98 for traits measured on HFLC and HFHC diets (differing in level of cholesterol). Highly significant genetic correlations notwithstanding, we found evidence of diet-genotype interaction for HDLC with changing levels of fat and for all but Hmed with changing levels of cholesterol, suggesting the metabolic pathways responsible for variation in each of these traits are governed both by common and, to some extent, unique sets of genes in the contrasting dietary environments. The results also suggested that altering the level of dietary cholesterol (in the high fat environment) was more likely to affect genetic control of lipoprotein metabolism.

To further characterize the genetic regulation of these traits, we next investigated the intercorrelations of metabolically related traits within each diet. Accordingly, we conducted bivariate genetic analyses that paired, within lipoprotein class, each of the three related traits. It is noteworthy that all but two of the 18 ρ_G were significant at the P=.05 level and that 14 were significant at the P=.001 level. The square of ρ_G gives an estimate of the proportion of genetic variance that is shared between each pair of traits and in this study that proportion exceeded 50% for more than half the 18 pairwise comparisons. The product of proportion of genetic variance shared and heritability (i.e., $\rho_G 2*h2$) yields an estimate of the proportion of the total residual phenotypic variance that is attributable to shared genes. After adjusting for covariate effects, we calculate that such shared genetic variance accounts for an average 26% of total phenotypic variance (range 0–58%). These results demonstrate that a significant and substantial component of lipoprotein metabolism is under the control of pleiotropic genes jointly influencing multiple related traits. A genetic basis for covariation of lipoprotein traits has also been reported previously in human subjects, notably to help explain the atherogenic lipoprotein phenotype component of metabolic syndrome [34–36].

We addressed the question of diet-genotype interaction for lipoprotein metabolism (as reflected in pleiotropic traits) using quadrivariate analyses. In these analyses we tested whether the genetic correlation for a trait pair differed significantly, depending on the dietary environment. Dietary effects on proportion of shared genetic variance were interpreted as evidence of diet-genotype interaction. Pleiotropic genes, influencing multiple related traits, are presumed to be fundamentally important to the metabolism of the major lipoprotein classes. Our findings did not support any significant effects on pleiotropic genetic effects due to changes in level of dietary fat. However, we detected significant

effects of changes in levels of dietary cholesterol such that increasing dietary cholesterol tended to decrease the degree of genetic correlation between HDL size and concentration but to increase the genetic correlations between LDL size and concentration.

In a recent study, we identified a number of loci exerting pleiotropic effects on subsets of 126 baboon lipoprotein traits [7]. For some traits the predominant locus effects on the trait was the same for each diet [for example, the QTLs for Lp(a) concentration and PON1 activity]. However, we also identified some loci whose effects were predominant only on specific diets. For example, a locus on chromosome 1 exerted significant effects on LDLC levels on the two low cholesterol diets, but not the HFHC diet (the primary QTL in this case was located on chromosome 19; see http://baboon.sfbrgenetics.org/Bab_SupplementalData/Rainwater2009.php). Furthermore, we localized a pleiotropic QTL for LDLC and Lp-PLA_2 (i.e., lipoprotein-associated phospholipase A_2) to the baboon orthologue of human chromosome 2p when animals were fed basal diet, but to chromosome 19 when fed HFHC diet [33,37]. Teasing out a gene whose expression is responsive to dietary environment may provide useful insights into lipoprotein metabolism and potential methods to modify CVD risk associated with dyslipidemia.

Several limitations of this study restrict our ability to generalize the results. (1) The diet was very consistent, but lard is a complex mixture of fatty acids. Although certainly pertinent to human diet, we were unable to distinguish the effects of specific fatty acids that could potentially be particularly important to lipoprotein metabolism and risk of CVD. (2) We measured only a limited subset of clinical characteristics - those which have proven significant predictors of variation in lipoprotein traits in previous studies. Because the residual unexplained variance averaged ~40% in our models, it is likely we have not measured all relevant clinical indicators of metabolic status that are important to lipoprotein metabolism. It is possible that identification of these additional clinical indicators in future studies will further clarify our models and interpretations.

Overall, our results indicate the existence of a complex network of genetic effects on lipoprotein metabolism: some genes influence variation in multiple traits within a lipoprotein class and some appear to exert effects only on specific traits; some genes influence traits in several dietary environments and some appear to exert significant effects only on specific diets. We found that the proportion of shared genetic variance was surprisingly high for most lipoprotein traits and it averaged 26% of total phenotypic variance in this study. We tested two perturbations of the dietary environment: increasing levels of fat and cholesterol. Generally, we found more consistent evidence of diet-genotype interactions for individual traits when changing levels of dietary cholesterol, compared to dietary fat. Similarly, changing levels of dietary cholesterol exerted significant effects on pleiotropy for several trait pairs. These findings, implying complex diet effects on the network of genes influencing lipoprotein metabolism, are likely to pertain similarly to lipoprotein metabolism in free-living humans, even if not readily detectable. It will be an objective of our future studies to identify the genes and characterize the pathways that underlie the present observations.

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Reference List

1. Bossé Y, Pérusse L, Vohl MC. Genetics of LDL particle heterogeneity: from genetic epidemiology to DNA-based variations. J Lipid Res 2004;45:1008–1026. [PubMed: 15060093]
2. Sviridov D, Nestel PJ. Genetic factors affecting HDL levels, structure, metabolism and function. Curr Opin Lipidol 2007;18:157–163. [PubMed: 17353664]
3. Kathiresan S, Musunuru K, Orho-Melander M. Defining the spectrum of alleles that contribute to blood lipid concentrations in humans. Curr Opin Lipidol 2008;19:122–127. [PubMed: 18388691]
4. Holleboom AG, Vergeer M, Hovingh GK, Kastelein JJ, Kuivenhoven JA. The value of HDL genetics. Curr Opin Lipidol 2008;19:385–394. [PubMed: 18607186]
5. Rainwater, DL.; VandeBerg, JL. Baboon model for dyslipidemia and atherosclerosis. In: VandeBerg, JL.; Williams-Blangero, S.; Tardiff, SD., editors. The Baboon in Biomedical Research. Springer; New York: 2009. p. 225-236.
6. Kushwaha RS, McGill HC Jr. Diet, plasma lipoproteins and experimental atherosclerosis in baboons (*Papio* sp.). Human Reproduction Update 1998;4:420–429. [PubMed: 9825856]
7. Rainwater DL, Cox LA, Rogers J, VandeBerg JL, Mahaney MC. Localization of multiple pleiotropic genes for lipoprotein metabolism in baboons. J Lipid Res 2009;50:1420–1428. [PubMed: 19270339]
8. Wang XL, Rainwater DL, Mahaney MC, Stocker R. Cosupplementation with vitamin E and coenzyme Q10 reduces circulating markers of inflammation in baboons. Am J Clin Nutr 2004;80:649–655. [PubMed: 15321805]
9. Cheng ML, Woodford SC, Hilburn JL, VandeBerg JL. A novel system for storage of sera frozen in small aliquots. J Biochem Biophys Methods 1986;13:47–51. [PubMed: 3772018]
10. Rainwater DL, Kammerer CM, Carey KD, Dyke B, VandeBerg JF, Shelledy WR, Moore PH Jr, Mahaney MC, McGill HC Jr, VandeBerg JL. Genetic determination of HDL variation and response to diet in baboons. Atherosclerosis 2002;161:335–343. [PubMed: 11888516]
11. Lipid Research Clinics Program. Lipid and Lipoprotein Analysis. (DHEW Publ. No. (NIH). Vol. 1. U.S. Government Printing Office; Washington D.C: 1974. Manual of Laboratory Operations; p. 75-628.p. 56
12. Cheng ML, Kammerer CM, Lowe WF, Dyke B, VandeBerg JL. Method for quantitating cholesterol in subfractions of serum lipoproteins separated by gradient gel electrophoresis. Biochem Genet 1988;26:657–681. [PubMed: 3242488]
13. Rainwater DL, Moore PH Jr, Shelledy WR, Dyer TD, Slifer SH. Characterization of a composite gradient gel for the electrophoretic separation of lipoproteins. J Lipid Res 1997;38:1261–1266. [PubMed: 9215553]
14. Rainwater DL, Moore PH Jr, Gamboa IO. Improved method for making nondenaturing composite gradient gels for the electrophoretic separation of lipoproteins. J Lipid Res 2004;45:773–775. [PubMed: 14729853]
15. Rainwater DL, Kammerer CM, Cox LA, Rogers J, Carey KD, Dyke B, Mahaney MC, McGill HC Jr, VandeBerg JL. A major gene influences variation in large HDL particles and their response to diet in baboons. Atherosclerosis 2002;163:241–248. [PubMed: 12052470]
16. Kammerer CM, Rainwater DL, Cox LA, Schneider JL, Mahaney MC, Rogers J, VandeBerg JL. Locus controlling LDL cholesterol response to dietary cholesterol is on baboon homologue of human chromosome 6. Arterioscler Thromb Vasc Biol 2002;22:1720–1725. [PubMed: 12377755]
17. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 1998;62:1198–1211. [PubMed: 9545414]
18. Mahaney MC, Blangero J, Rainwater DL, Mott GE, Comuzzie AG, MacCluer JW, VandeBerg JL. Pleiotropy and genotype by diet interaction in a baboon model for atherosclerosis - A multivariate quantitative genetic analysis of HDL subfractions in two dietary environments. Arterioscler Thromb Vasc Biol 1999;19:1134–1141. [PubMed: 10195946]
19. Gofman JW, Glazier F, Tamplin A, Strisower B, De Lalla O. Lipoproteins, coronary heart disease, and atherosclerosis. Physiol Rev 1954;34:589–607. [PubMed: 13185756]
20. Vaisar T, Pennathur S, Green PS, Gharib SA, Hoofnagle AN, Cheung MC, Byun J, Vuletic S, Kassim S, Singh P, Chea H, Knopp RH, Brunzell J, Geary R, Chait A, Zhao XQ, Elkon K,

Marcovina S, Ridker P, Oram JF, Heinecke JW. Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. J Clin Invest 2007;117:746–756. [PubMed: 17332893]

21. Davidson WS, Silva RAGD, Chantepie S, Lagor WR, Chapman MJ, Kontush A. Proteomic analysis of defined HDL subpopulations reveals particle-specific protein clusters: relevance to antioxidative function. Arterioscler Thromb Vasc Biol 2009;29:870–876. [PubMed: 19325143]
22. LaRosa JC, Hunninghake D, Bush D, Criqui MH, Getz GS, Gotto AM Jr, Grundy SM, Rakita L, Robertson RM, Weisfeldt ML. The cholesterol facts. A summary of the evidence relating dietary fats, serum cholesterol, and coronary heart disease. A joint statement by the American Heart Association and the National Heart, Lung, and Blood Institute. The Task Force on Cholesterol Issues, American Heart Association. Circulation 1990;81:1721–1733. [PubMed: 2184951]
23. Contois JH, McConnell JP, Sethi AA, Csako G, Devaraj S, Hoefner DM, Warnick GR. Apolipoprotein B and cardiovascular disease risk: position statement from the AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices. Clin Chem 2009;55:407–419. [PubMed: 19168552]
24. Thompson A, Danesh J. Associations between apolipoprotein B, apolipoprotein AI, the apolipoprotein B/AI ratio and coronary heart disease: a literature-based meta-analysis of prospective studies. J Intern Med 2006;259:481–492. [PubMed: 16629854]
25. Otvos JD, Collins D, Freedman DS, Shalaurova I, Schaefer EJ, McNamara JR, Bloomfield HE, Robins SJ. Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. Circulation 2006;113:1556–1563. [PubMed: 16534013]
26. Gardner CD, Fortmann SP, Krauss RM. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA 1996;276:875–881. [PubMed: 8782636]
27. Stampfer MJ, Krauss RM, Ma J, Blanche PJ, Holl LG, Sacks FM, Hennekens CH. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA 1996;276:882–888. [PubMed: 8782637]
28. Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Després JP. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. Circulation 1997;95:69–75. [PubMed: 8994419]
29. Campos H, Moye LA, Glasser SP, Stampfer MJ, Sacks FM. Low-density lipoprotein size, pravastatin treatment, and coronary events. JAMA 2001;286:1468–1474. [PubMed: 11572739]
30. Arsenault BJ, Lemieux I, Despres JP, Gagnon P, Wareham NJ, Stroes ES, Kastelein JJ, Khaw KT, Boekholdt SM. HDL particle size and the risk of coronary heart disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. Atherosclerosis 2009;206:276–281. [PubMed: 19268944]
31. Sacks FM, Campos H. Clinical review 163: Cardiovascular endocrinology: Low-density lipoprotein size and cardiovascular disease: a reappraisal. J Clin Endocrinol Metab 2003;88:4525–4532. [PubMed: 14557416]
32. Musunuru K, Orho-Melander M, Caulfield MP, Li S, Salameh WA, Reitz RE, Berglund G, Hedblad B, Engstrom G, Williams PT, Kathiresan S, Melander O, Krauss RM. Ion mobility analysis of lipoprotein subfractions identifies three independent axes of cardiovascular risk. Arterioscler Thromb Vasc Biol 2009;29:1975–1980. [PubMed: 19729614]
33. Vinson A, Mahaney MC, Diego VP, Cox LA, Rogers J, VandeBerg JL, Rainwater DL. Genotype-by-diet effects on co-variation in Lp-PLA2 activity and LDL cholesterol concentration in baboons fed an atherogenic diet. J Lipid Res 2008;49:1295–1302. [PubMed: 18334716]
34. Edwards KL, Mahaney MC, Motulsky AG, Austin MA. Pleiotropic genetic effects on LDL size, plasma, triglyceride, and HDL cholesterol in families. Arterioscler Thromb Vasc Biol 1999;19:2456–2464. [PubMed: 10521376]
35. Hokanson JE, Langefeld CD, Mitchell BD, Lange LA, Goff DC Jr, Haffner SM, Saad MF, Rotter JI. Pleiotropy and heterogeneity in the expression of atherogenic lipoproteins: the IRAS Family Study. Hum Hered 2003;55:46–50. [PubMed: 12890925]

36. Kullo IJ, de AM, Boerwinkle E, McConnell JP, Kardia SL, Turner ST. Pleiotropic genetic effects contribute to the correlation between HDL cholesterol, triglycerides, and LDL particle size in hypertensive sibships. Am J Hypertens 2005;18:99–103. [PubMed: 15691623]

37. Vinson A, Mahaney MC, Cox LA, Rogers J, VandeBerg JL, Rainwater DL. A pleiotropic QTL on 2p influences serum Lp-PLA2 activity and LDL cholesterol concentration in a baboon model for the genetics of atherosclerosis risk factors. Atherosclerosis 2008;196:667–673. [PubMed: 17767937]

Table 1

For each trait on the three diets is given the proportions of total phenotypic variance associated with the standard set of covariates, additive effects of genes (h^2), and the residual.*

	Basal			HFLC			HFHC		
Trait	**Covariates**	**h^2**	**Residual**	**Covariates**	**h^2**	**Residual**	**Covariates**	**h^2**	**Residual**
Hmed	0.039	0.512	0.449	0.023	0.579	0.398	0.006	0.589	0.405
HDLC	0.026	0.669	0.305	0.091	0.630	0.279	0.076	0.599	0.325
ApoA1	0.056	0.529	0.415	0.042	0.441	0.517	0.032	0.376	0.592
Bmed	0.046	0.265	0.689	0.081	0.367	0.552	0.047	0.527	0.426
LDLC	0.240	0.561	0.199	0.180	0.538	0.282	0.018	0.686	0.296
ApoB	0.274	0.334	0.392	0.263	0.360	0.377	0.127	0.562	0.311

* Covariate effects were estimated using the raw untransformed traits and, as described in the Methods section, the residual was normalized and standardized prior to conducting quantitative genetic analysis to estimate the heritability; the proportions are scaled to total phenotypic variance.

Table 2

Results from bivariate analyses of traits measured in different dietary environments; given are heritabilities (h^2), genetic correlations (ρ_G), and genetic variances (σ^2_G) for each trait in the model and the *P*-values associated with the tests for complete pleiotropy and equality of genetic variance.*

Increase Dietary Fat:			**Test for complete pleiotropy:**				**Test for equal σ^2_G:**		
Basal	**HFLC**		**h^2**			**P-value: ρ_G=1**	**σ^2_G**		**P-value: $\sigma^2_{G(1)} = \sigma^2_{G(2)}$**
Trait1	**Trait2**	**N**	**Trait1**	**Trait2**	**ρ_G**		**Trait1**	**Trait2**	
Hmed	Hmed	715	0.532	0.562	0.977	5.8E-02	0.443	0.458	7.8E-01
HDLC	HDLC	715	0.696	0.668	0.976	**1.4E-02**	0.566	0.533	5.7E-01
ApoA1	ApoA1	716	0.556	0.438	0.984	2.6E-01	0.510	0.407	1.3E-01
Bmed	Bmed	715	0.293	0.380	0.998	9.5E-01	0.275	0.352	1.6E-01
LDLC	LDLC	715	0.640	0.630	0.965	5.2E-02	0.509	0.501	9.0E-01
ApoB	ApoB	716	0.425	0.466	1.000	1.0E+00	0.349	0.377	4.6E-01
Increase Dietary Cholesterol:			Test for complete pleiotropy:				Test for equal σ^2_G:		
HFLC	HFHC		h^2			P-value: ρ_G=1	σ^2_G		P-value: $\sigma^2_{G(2)} = \sigma^2_{G(3)}$
Trait2	Trait3	N	Trait2	Trait3	ρ_G		Trait2	Trait3	
Hmed	Hmed	715	0.567	0.570	0.982	9.2E-02	0.463	0.466	9.6E-01
HDLC	HDLC	715	0.694	0.609	0.926	**1.9E-05**	0.566	0.521	5.7E-01
ApoA1	ApoA1	716	0.470	0.364	0.849	**8.5E-04**	0.442	0.357	4.0E-01
Bmed	Bmed	715	0.300	0.548	0.970	2.1E-01	0.272	0.458	**1.3E-02**
LDLC	LDLC	715	0.619	0.691	0.874	**1.6E-06**	0.486	0.496	8.8E-01
ApoB	ApoB	716	0.450	0.622	0.842	**1.0E-07**	0.364	0.467	1.4E-01

*σ^2_G, h^2 (σ^2_G/σ^2_P), and ρ_G were estimated using routines in SOLAR; P-values in bold were significant at the P<0.05 level

Table 3

Bivariate results for HDL and LDL size and concentration variables on three diets; given are residual heritabilities for each trait, the phenotypic (ρ_P), environmental (ρ_E), and genetic (ρ_G) correlations, and an indication of the *P*-value for tests of the hypothesis of $\rho_X = 0$.

Trait1	Trait2	Diet	N	h2 Trait1	h2 Trait2	ρ_P	ρ_E	ρ_G
Hmed	HDLC	Basal	715	0.499	0.685	0.641***	0.433**	0.803***
Hmed	ApoA1	Basal	715	0.516	0.528	0.387***	0.108	0.643***
HDLC	ApoA1	Basal	715	0.689	0.492	0.718***	0.486**	0.901***
Hmed	HDLC	HFLC	715	0.570	0.689	0.658***	0.287	0.883***
Hmed	ApoA1	HFLC	715	0.575	0.413	0.384***	0.058	0.729***
HDLC	ApoA1	HFLC	715	0.689	0.409	0.740***	0.585**	0.922***
Hmed	HDLC	HFHC	715	0.586	0.632	0.567***	0.243	0.776***
Hmed	ApoA1	HFHC	715	0.592	0.371	0.251***	0.084	0.443*
HDLC	ApoA1	HFHC	715	0.636	0.360	0.757***	0.701***	0.877***
Bmed	LDLC	Basal	713	0.285	0.738	0.248***	0.104	0.444*
Bmed	ApoB	Basal	712	0.276	0.460	0.022	−0.048	0.147
LDLC	ApoB	Basal	714	0.735	0.477	0.706***	0.458**	0.903***
Bmed	LDLC	HFLC	715	0.396	0.667	0.292***	0.356**	0.257*
Bmed	ApoB	HFLC	715	0.402	0.492	0.071	0.106	0.028
LDLC	ApoB	HFLC	715	0.651	0.484	0.748***	0.564**	0.906***
Bmed	LDLC	HFHC	715	0.544	0.697	0.510***	0.378*	0.600***
Bmed	ApoB	HFHC	715	0.559	0.643	0.324***	0.262*	0.369**
LDLC	ApoB	HFHC	715	0.697	0.628	0.864***	0.758***	0.921***

*$P<.05$;

**$P<.001$;

***$P<.000001$

Table 4

Results of quadrivariate genetic analyses of the same pair of traits in two dietary environments to test effects of increasing dietary fat and cholesterol; given are N, $\rho_G \pm$SE for the trait pairs compared across diet, and *P*-values for the tests of the hypothesis that the two within-diet correlations are the same.

Increase Dietary Fat:

Basal		**HFLC**			**ρ_G**		***P*-value:**	
Trait1	**Trait2**	**Trait3**	**Trait4**	**N**	**T1/T2**	**T3/T4**	**t-test**	**LRT***
Hmed	HDLC	Hmed	HDLC	715	0.803±0.035	0.883±0.035	1.00E-01	-
Hmed	ApoA1	Hmed	ApoA1	715	0.644±0.094	0.708±0.091	6.20E-01	4.27E-01
HDLC	ApoA1	HDLC	ApoA1	715	0.903±0.035	0.925±0.035	6.61E-01	5.31E-01
Bmed	LDLC	Bmed	LDLC	713	0.303±0.123	0.314±0.118	9.47E-01	9.18E-01
Bmed	ApoB	Bmed	ApoB	712	0.076±0.138	0.063±0.133	9.44E-01	8.93E-01
LDLC	ApoB	LDLC	ApoB	714	0.922±0.039	0.900±0.034	6.82E-01	6.07E-01

Increase Dietary Cholesterol:

HFLC		HFHC			ρ_G		*P*-value:	
Trait1	Trait2	Trait3	Trait4	N	T1/T2	T3/T4	t-test	LRT*
Hmed	HDLC	Hmed	HDLC	715	0.851±0.046	0.762±0.070	2.71E-01	7.95E-02
Hmed	ApoA1	Hmed	ApoA1	715	0.717±0.092	0.423±0.094	**2.61E-02**	**8.26E-03**
HDLC	ApoA1	HDLC	ApoA1	715	0.922±0.094	0.877±0.091	7.27E-01	-
Bmed	LDLC	Bmed	LDLC	715	0.191±0.130	0.608±0.073	**5.40E-03**	**4.20E-05**
Bmed	ApoB	Bmed	ApoB	715	0.004±0.133	0.386±0.099	**2.14E-02**	**1.34E-03**
LDLC	ApoB	LDLC	ApoB	715	0.904±0.035	0.924±0.021	6.29E-01	5.46E-01

*LRT, $\log_e$ likelihood ratio test; *P*-values in bold were significant at the 0.05 level and dashes indicate models that failed to converge.