Two Major Loci Control Variation in β-Lipoprotein Cholesterol and Response to Dietary Fat and Cholesterol in Baboons


Abstract—We explored the genetic control of cholesterolemic responses to dietary cholesterol and fat in 575 pedigreed baboons. We measured cholesterol in β-lipoproteins (low density lipoprotein cholesterol [LDLC]) in blood drawn from baboons while they were consuming a baseline (low in cholesterol and fat) diet, a high–saturated fat (lard) diet, and a high-cholesterol, high–saturated fat diet. In addition to baseline levels (LDLC_{Base}), we analyzed two variables for diet response: LDLC_{RF}, which represents the LDLC response to increasing dietary fat (ie, high-fat diet minus baseline), and LDLC_{RC}, which represents the LDLC response to increasing dietary cholesterol level (ie, high-cholesterol, high-fat diet minus high-fat diet). Heritabilities (h^2) of the 3 traits were 0.59 for LDLC_{Base}, 0.14 for LDLC_{RF}, and 0.59 for LDLC_{RC}. In addition, LDLC_{Base} and LDLC_{RC} had a significant genetic correlation (ie, r\_G=0.54), suggesting that 1 or more genes exert pleiotropic effects on the 2 traits. Segregation analyses detected a single major locus that accounted for nearly all genetic variation in LDLC_{RC} and some genetic variation in LDLC_{Base} and LDLC_{RF} and confirmed the presence of a different major locus that influences LDLC_{Base} alone. Preliminary linkage analyses indicated that neither locus was linked to the LDL receptor gene, a likely candidate locus for LDLC. Detection of these major loci with large effects on the LDLC response to dietary cholesterol in a nonhuman primate offers hope of detecting and ultimately identifying similar loci that determine LDLC variation in human populations. (Arterioscler Thromb Vasc Biol. 1998;18:1061-1068.)

Key Words: LDL ■ diet ■ genetics ■ baboons

The role of plasma LDLC in predicting risk of coronary heart disease is well established,1 and dietary fat and cholesterol are recognized as the major environmental determinants of LDLC concentration.2 However, cholesterolemic responses to dietary lipids vary among individuals, in both humans and experimental animals, and much recent effort has been devoted to explaining this variability by physiological and, ultimately, genetic mechanisms.3

We began to search for genes controlling responsiveness to dietary lipids in baboons having high and low plasma cholesterol and lipoprotein levels after a 7-week challenge diet enriched in saturated fat and cholesterol. Both the basal and challenge levels of LDL and HDL were highly heritable.4 Subsequent studies focused on detecting and identifying the responsible genes by molecular5–8 and statistical9–11 genetic strategies. A previous study suggested that the response to dietary cholesterol and to dietary saturated fat might be controlled by separate genes in baboons.12 In the present study, we subjected baboons to a dietary challenge protocol that enabled us to analyze the separate responses to dietary cholesterol and saturated fat.

Methods

Animals

Baboons (Papio hamadryas sensu lato13,14) were maintained at the Southwest Foundation for Biomedical Research, a facility certified by the American Association for the Accreditation of Laboratory Animal Care, under conditions approved by the institutional animal care and use committee.

We studied the effects of dietary fat and cholesterol in 575 pedigreed baboons, primarily olive (P h anubis) and yellow (P h cynocephalus), and their hybrids. Of these baboons, 47% (8%) had been reared in the nursery on artificial human infant formula,10,15 Average age (in years) of the baboons at the outset of the experiment was 9.0 (SD, 6.8; range, 2.2 to 28.5).

The pedigreed baboons represented the genetic diversity of 187 founders not known to be related. There were 209 males and 366 females in 28 sire families, with a total of 191 dams and their offspring. These families provided a large number of pairwise relationships for genetic analyses, including 1129 first-degree, 6099 second-degree, 2114 third-degree, and 633 fourth-degree relative pairs.
Diet Protocol
All baboons were subjected to the same 4-step diet challenge protocol as follows: (1) Animals were fed a baseline monkey diet (Wayne Teklad), low in fat (~4% of calories) and cholesterol (0.03 mg/kcal), for at least 2 months before the baseline blood sample was drawn. (2) They were then fed a diet high in saturated fat (40% of calories from lard) and cholesterol (1.7 mg/kcal) for 7 weeks before the high-cholesterol, high-fat–diet blood sample was drawn. (3) They were then fed the baseline diet again for 7 weeks to provide a “washout” period between challenge diets. (4) After the washout, the animals were fed a high fat–only diet (40% of calories from lard, 0.03 mg cholesterol per kcal) for 7 weeks before the high fat–diet blood sample was taken. We previously reported results of a pilot study with 60 animals, which indicated that LDLC values had returned to the initial baseline levels by the end of the baseline diet washout period.

For each of the diet treatments, blood samples were drawn from the femoral vein after baboons were fasted overnight and immobilized with ketamine (10 mg/kg). The blood was allowed to clot and the femoral veins were punctured. The blood was allowed to clot and the blood sample was taken. We previously reported results of a pilot study with 60 animals, which indicated that LDLC values had returned to the initial baseline levels by the end of the baseline diet washout period.

Measurements of LDLC
Cholesterol concentrations were measured enzymatically with a reagent supplied by Boehringer Mannheim Diagnostics and a Ciba-Corning Express Plus clinical chemistry analyzer. Cholesterol in HDL was determined in the supernatant after precipitation of apoB-containing lipoproteins by use of heparin-Mn2+ at 4°C. Concentrations of cholesterol in apoB-containing lipoproteins (LDLC) were estimated as the difference between total serum and HDL cholesterol measurements of cholesterol in apoB-containing lipoproteins (LDLC) were estimated as the difference between total serum and HDL cholesterol levels. Cholesterol in baboon apoB-containing lipoproteins resides primarily in LDL particles; other relatively minor contributors of cholesterol to this LDLC value include VLDL, IDL, and Lp(a). Average coefficients of variation for control products in these assays were 2.2% and 4.6% for total cholesterol and HDL cholesterol, respectively.

Three LDLC concentration variables were derived from these data and used in the genetic analyses: (1) LDLCBaseline, the LDLC level in the baseline blood sample; (2) LDLCResponse, a variable representing LDLC response to dietary fat and calculated as the difference between the high-fat and baseline blood sample values; and (3) LDLCResponseToCholesterol, a variable representing LDLC response to dietary cholesterol in the high-fat environment and calculated as the difference between the high-cholesterol, high-fat–diet and the high-fat–diet blood sample values. Figure 1 gives frequency histograms for these 3 variables. LDLCResponseToCholesterol was not normally distributed, so this variable was logarithmically normalized (natural) transformed before analysis (after first adding a constant value to make all variables positive).

Statistical Genetic Analyses
Quantitative Genetic Analyses
We used univariate quantitative genetic analysis to assess heritability and to evaluate covariate effects on the LDL traits. Effects of potential covariates (eg, age, sex, weight, etc) were simultaneously estimated. Significant covariates were determined by comparing a series of submodels, in which the covariate effect was removed, to the most general model in which it was included. We retained all covariates for which the $\chi^2$ associated with the likelihood ratio test was significant at the 0.10 level.

We used multivariate quantitative genetic analysis to calculate genetic correlations (\(\rho_g\)) among the LDLC phenotypes and to estimate the magnitude of pleiotropic effects of underlying genes. Large genetic correlations between traits imply that the same genes influence both traits. Hypotheses regarding the extent of pleiotropic effects (ie, \(\rho_p=0\) for no pleiotropy or \(\rho_p=1.0\) for complete pleiotropy) were evaluated using likelihood ratio tests. The total phenotypic correlation (\(\rho_t\)) was estimated as \(\rho_t = \sqrt{\rho_g^2 + \rho_p^2 - 2 \rho_g \rho_p \cdot \rho_p} \), where \(\rho_g^2\) and \(\rho_p^2\) are heritabilities for traits 1 and 2, respectively, and \(\rho_p\) is the environmental correlation.

Segregation Analyses
To detect and estimate the contribution of individual genes to LDLC levels, we employed complex segregation analysis by using the computer program PAP. We compared selected submodels that represented different transmission hypotheses with an unrestricted general model that permitted a mixture of as many as 3 normal phenotypic distributions. The mixture of distributions can be interpreted to reflect genotypes, or types, that result from 2 discrete factors. Relative frequencies (under the assumption of Hardy-Weinberg equilibrium) and means for each type were estimated, and a common SD for the residual phenotypic distributions was assumed. Residual nonindependence among relatives due to kinship was estimated by the polygenic heritability (\(h^2\)). The transmission probabilities for 1 factor by individuals of different genotypes were estimated in the general model.

We tested against the most general model several classes of submodels, including a single-distribution (polygenic) model, a multiple-distribution (environmental) model, and a mendelian model (transmission probabilities, \(\tau\), fixed at their mendelian expectations: \(\tau_{AA}=1\), \(\tau_{2A}=0.5\), and \(\tau_{AA}=0\)). Each submodel was compared with the unrestricted general model by using likelihood ratio test statistics obtained as twice the difference between the natural-log likelihoods of the 2 models. These test statistics approximate a \(\chi^2\) distribution with degrees of freedom equal to the difference in the numbers of parameters between the 2 models. The best model is one that has the fewest estimated parameters and is not significantly worse than the most general model.

We have developed automated methods that search the likelihood surface for various models of inheritance. This approach enables us...
TABLE 1. Trait Means (μ), SDs, Heritabilities (h²), and Covariate Effects (β) in Univariate Quantitative Genetic Analyses of 575 Baboons

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LDLC&lt;sub&gt;base&lt;/sub&gt;</th>
<th>LDLC&lt;sub&gt;RF&lt;/sub&gt;</th>
<th>In LDLC&lt;sub&gt;RC&lt;/sub&gt;*</th>
</tr>
</thead>
<tbody>
<tr>
<td>μ, mmol/L</td>
<td>0.91±0.07</td>
<td>0.35±0.03</td>
<td>1.77±0.01</td>
</tr>
<tr>
<td>SD</td>
<td>0.41±0.01</td>
<td>0.43±0.01</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td>h²</td>
<td>0.59±0.07</td>
<td>0.14±0.06</td>
<td>0.59±0.08</td>
</tr>
<tr>
<td>β&lt;sub&gt;sex&lt;/sub&gt;</td>
<td>0.24±0.06</td>
<td>0.02±0.00</td>
<td>0.002±0.003</td>
</tr>
<tr>
<td>β&lt;sub&gt;age-males&lt;/sub&gt;</td>
<td>−0.03±0.01</td>
<td>0.003±0.001</td>
<td>0.002±0.003</td>
</tr>
<tr>
<td>β&lt;sub&gt;age-females&lt;/sub&gt;</td>
<td>0.003±0.001</td>
<td>0.000±0.003</td>
<td>−0.004±0.001</td>
</tr>
</tbody>
</table>

Only those covariates giving a P<0.10 were estimated.
*Before log transformation, 5.0 mmol/L was added to ensure that all recalculated values were positive and to facilitate calculation. Given are the transformed values (except for h²).

Univariate Quantitative Genetic Analyses

Table 1 gives the parameter estimates for each of the traits resulting from univariate quantitative genetic analyses. We tested for the effects of sex, age, and the square of age in males and females; weight; nursery rearing; and subspecies admixture; only those covariates giving a P value <0.10 were retained in the final model for each trait. Two potential covariates found in other studies, breast fed versus formula fed and subspecies admixture, did not satisfy the requirement and were not retained in subsequent models for any of the traits.

LDLC<sub>base</sub> values averaged 0.91 mmol/L in these animals. Nearly 60% of residual phenotypic variation in LDLC<sub>base</sub> was additive genetic (ie, h²=0.59, P<0.000001), and significant covariates included sex, linear and quadratic effects of age, and body weight. Increasing fat in the diet caused a 39% increase of LDLC to 1.26 mmol/L, but only a small portion of this increase was due to the additive effects of genes (h²=0.14 for LDLC<sub>RF</sub>, P=0.001). Increasing cholesterol level in the high-fat diet caused a near doubling of LDLC (from 1.26 to 2.27 mmol/L). This increase was strongly genetic (h²=0.59 for LDLC<sub>RC</sub>, P<0.00001), and weight was a significant covariate.

Major Locus Pleiotropy

We employed bivariate segregation analysis to determine whether the major gene for 1 trait had any effect on phenotypic variation for a second trait. In brief, this model considers the effect of a single trait locus on 2 traits simultaneously. The hypothesis that the major gene influences a second trait was tested by comparing the likelihood of a model in which genotypic means were estimated for both phenotypes (unrestricted model) to a model in which genotypic means were estimated for the first trait and a single mean was estimated for the second trait (restricted model). Major gene pleiotropy is indicated if the likelihood of the former model is significantly higher than that of the latter.

To further evaluate the pleiotropic actions of both genes simultaneously and to estimate the proportions of phenotypic variance explained by each locus on each trait, we also performed a 2-locus, bivariate segregation analysis. In this model, the effects of 2 loci were estimated on each of 2 traits simultaneously. Under this more general framework, we tested several models, including the following: (1) 1 locus affects both traits, (2) 2 loci are present and 1 locus affects the first trait and the other locus affects the second trait, and (3) the most general model, in which both loci have effects on both traits. We also tested whether the 2 major genes were linked by estimating the likelihood of a model in which recombination frequency (θ) was <0.5 (ie, the loci are linked) versus a model in which θ was fixed at 0.5 (the loci are not linked). As described previously, the models were compared using the likelihood ratio test, and the best model was the one with the fewest parameters estimated that was not significantly different from the most general model.

Results

Univariate Quantitative Genetic Analyses

Table 1 gives the parameter estimates for each of the traits resulting from univariate quantitative genetic analyses. We tested for the effects of sex, age, and the square of age in males and females; weight; nursery rearing; and subspecies admixture; only those covariates giving a P value <0.10 were retained in the final model for each trait. Two potential covariates found in other studies, breast fed versus formula fed and subspecies admixture, did not satisfy the requirement and were not retained in subsequent models for any of the traits.

LDLC<sub>base</sub> values averaged 0.91 mmol/L in these animals. Nearly 60% of residual phenotypic variation in LDLC<sub>base</sub> was additive genetic (ie, h²=0.59, P<0.000001), and significant covariates included sex, linear and quadratic effects of age, and body weight. Increasing fat in the diet caused a 39% increase of LDLC to 1.26 mmol/L, but only a small portion of this increase was due to the additive effects of genes (h²=0.14 for LDLC<sub>RF</sub>, P=0.001). Increasing cholesterol level in the high-fat diet caused a near doubling of LDLC (from 1.26 to 2.27 mmol/L). This increase was strongly genetic (h²=0.59 for LDLC<sub>RC</sub>, P<0.00001), and weight was a significant covariate.

Bivariate Quantitative Genetic Analyses

There was a positive phenotypic correlation between LDLC<sub>base</sub> and LDLC<sub>RC</sub> (Table 2). This phenotypic correlation was caused by a strong genetic correlation (ρ<sub>g</sub>=0.54), which explained 29% of the covariance (ie, h²=0.29 in the 2 traits). The phenotypic correlations of LDLC<sub>RF</sub> with LDLC<sub>base</sub> and with LDLC<sub>RC</sub> were small or negative due to strong negative environmental correlations.

Segregation Analysis of LDLC Response to Dietary Cholesterol

Segregation analysis (Table 3) indicated that a major gene, herein labeled the R locus, affected LDLC response to dietary cholesterol (ie, LDLC<sub>RC</sub>). The single-distribution polygenic model was strongly rejected when compared with the multiple-distribution unrestricted general model (P<0.00001). Likewise, a multiple-distribution model that allowed for polygenic effects (the environmental model) was also rejected (P<0.000001). The multiple-distribution model in which the underlying distributions reflected phenotypes (the mendelian model) described the data as well as the unrestricted model (P=0.58). Estimates of the transmission parameters in the unrestricted model (1, 0.43, and 0.12) are similar to those expected under mendelian transmission (1, ½, and 0, respectively). Furthermore, the residual heritability for the mendelian model was low (h²=0.15±0.09), suggesting that genetic variation at the R locus accounted for most of the genetic control of LDLC<sub>RC</sub>. Figure 2 presents a frequency histogram for LDLC<sub>RC</sub> together with the 3 underlying distri-
butions for each of the major gene genotypes as estimated from segregation analysis.

Analyses of Pleiotropic Effects

Previously, we detected a major gene for LDLC Base, herein termed the B locus, by using a different set of baboons. We wished to determine whether the R gene for LDLC RC concentrations was the same as the B gene previously detected for LDLC Base. First, we performed segregation analysis to confirm the presence of a major gene affecting LDLC Base in this group of animals and to estimate the major gene parameters. The environmental and polygenic models were rejected (data not shown), but not the mendelian model, and the parameter estimates were similar to those previously reported.11

Next, we conducted a 1-locus, bivariate segregation analysis to test the hypothesis that the B locus for LDLC Base exerts pleiotropic effects on LDLC RC. Because the R locus for LDLC RC explains virtually all of the genetic variation in the trait, results of this analysis would indicate whether only one major gene exerts pleiotropic effects on both LDLC Base and LDLC RC. Table 4 presents the results of this analysis. The B locus for LDLC Base does not explain a significant proportion of variation in LDLC RC (P = 0.37). We also tested for significant pleiotropic effects of the LDLC RC major locus (R) on LDLC Base. The R locus for LDLC RC accounted for a small but significant proportion of variation in LDLC Base (P = 0.0003) (Table 4). In addition, allowing for major locus pleiotropy on the 2 traits reduces the residual heritability for LDLC Base (from 0.59 to 0.45) as well as the residual genetic correlation (from 0.42 to 0.29) (Table 4).

On the basis of these results, we tested by 2-locus, bivariate segregation analyses whether only 1 locus was affecting both traits, 2 loci were affecting both traits, or there was some intermediate effect (Table 5). In comparison with the general model (No. 1) in which both loci affect both traits, the 1-locus models (No. 5 and 6), in which 1 of the loci (either B or R) affects both traits, were strongly rejected. Likewise, the 2-locus model (No. 4), in which the B locus affects only LDLC Base and the R locus affects only LDLC RC, was rejected. However, the model (No. 2) in which the B locus affects LDLC Base only but the R locus affects both LDLC Base and LDLC RC was not significantly different from the most general model. This model was the one suggested by the results of the 1-locus, bivariate analyses. Furthermore, by comparing the best 2-locus, bivariate model, in which recombination was estimated to a model in which recombination was fixed at 1/2, we found no evidence for linkage between the B and R loci (P = 0.21, results not shown). Using the best 2-locus, bivariate model, we estimated the mean genotypic effects on LDLC Base for the 9 possible genotype combinations of the two loci. On average, bbrr individuals had a 2.3-fold higher LDL than did BBRR individuals (Table 6).

Figure 2. Frequency histogram for LDLC RC with distributions representing the 3 LDLC RC major locus genotypes RR, Rr, and rr. LDL concentrations were logarithmically transformed (after adding 5 mmol/L) before segregation analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>General</th>
<th>Environmental</th>
<th>Mendelian</th>
<th>Polygenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f_B )</td>
<td>0.74</td>
<td>1.00</td>
<td>0.73</td>
<td>(1)</td>
</tr>
<tr>
<td>( \tau_{RR} )</td>
<td>1*</td>
<td>0.95</td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td>( \tau_{Rr} )</td>
<td>0.43</td>
<td>(= ( \tau_{RR} ))</td>
<td>(0.5)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>( \tau_{rr} )</td>
<td>0.12</td>
<td>(= ( \tau_{RR} ))</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>( \mu_{RR} )</td>
<td>1.71</td>
<td>1.75</td>
<td>1.71</td>
<td>1.77</td>
</tr>
<tr>
<td>( \mu_{Rr} )</td>
<td>1.80</td>
<td>2.08</td>
<td>1.79</td>
<td>(= ( \mu_{RR} ))</td>
</tr>
<tr>
<td>( \mu_{rr} )</td>
<td>2.11</td>
<td>1.42</td>
<td>2.11</td>
<td>(= ( \mu_{RR} ))</td>
</tr>
<tr>
<td>SD</td>
<td>0.11</td>
<td>0.13</td>
<td>0.11</td>
<td>0.16</td>
</tr>
<tr>
<td>( h^2 )</td>
<td>0.20</td>
<td>0.57</td>
<td>0.15</td>
<td>0.59</td>
</tr>
<tr>
<td>( \beta_{weight} )</td>
<td>-0.004</td>
<td>-0.002</td>
<td>-0.004</td>
<td>-0.004</td>
</tr>
<tr>
<td>( \chi^2 )</td>
<td>...</td>
<td>61.7</td>
<td>2.0</td>
<td>100.2</td>
</tr>
<tr>
<td>( P )</td>
<td>...</td>
<td>&lt;0.000001</td>
<td>0.58</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

Values in parentheses are those fixed in the models.
*Parameter estimated at the boundary.
†Estimated as \(-2\) in likelihood for each model compared with the unrestricted general model.

TABLE 3. Comparison of Parameter Estimates of Frequencies (\( f \)), Transmission Probabilities (\( \tau_{RR} \), \( \tau_{Rr} \), and \( \tau_{rr} \)), Means (\( \mu_{RR} \), \( \mu_{Rr} \), and \( \mu_{rr} \)), SDs, Heritabilities (\( h^2 \)), Covariate Effects (\( \beta_{weight} \)), \( \chi^2 \), and Probabilities (\( P \)) for Models Tested in Segregation Analyses of LDLC RC Data From 572 Baboons

By guest on October 15, 2017 http://atvb.ahajournals.org/ Downloaded from
Although the heritability of LDLC RF was low ($h^2 = 0.14$), we also tested whether the $B$ or $R$ locus had pleiotropic effects on LDLC RF. We found no strong consistent effects of the $B$ locus on LDLC RF (data not shown), but we did find a significant ($P < 0.004$) pleiotropic effect of the $R$ locus on LDLC RF (Table 4). The $R$ locus accounted for much of the additive genetic variance in LDLC RF, as evidenced by the decrease in the estimated residual heritability from $0.12 \pm 0.05$ to $0.03 \pm 0.05$. In addition, the genetic correlation between the 2 traits dropped from $0.69 \pm 0.39$ (Table 2, no major gene was included) to $-0.34 \pm 0.67$ (when the $R$ locus was included). This result implies that much of the genetic correlation between the 2 traits was attributable to the $R$ major gene.

### Components of Variance for 3 LDLC Traits

For each trait we estimated the proportion of total phenotypic variance attributable to various factors (Table 7). For the trait LDLC Base, the major gene for LDLC Base ($B$ locus) accounted for '27% of total phenotypic variance. Because this gene behaves as a dominant-recessive locus, some of its effects are not detected as additive (ie, not included in the heritability estimate). Approximately 3% of total phenotypic variance is accounted for by the major locus for LDLC RC (the $R$ locus), which exerts a pleiotropic effect on LDLC Base. Residual polygenic effects account for '38% of total phenotypic variance, covariates explained '22% (residual error) was not explained by the model for LDLC Base.

### TABLE 4. Single-Locus, Bivariate Segregation Analyses to Test for Pleiotropic Effects of the Major Loci for LDLC Base and LDLC RC

<table>
<thead>
<tr>
<th>LDLC Base Major Locus ($B$)</th>
<th>LDLC RC Major Locus ($R$)</th>
<th>LDLC RC Major Locus ($R$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>No Pleiotropy</td>
<td>With Pleiotropy</td>
</tr>
<tr>
<td>$f_B$</td>
<td>0.74</td>
<td>0.73</td>
</tr>
<tr>
<td>$\mu_{BB}$</td>
<td>0.92</td>
<td>0.89</td>
</tr>
<tr>
<td>$\mu_{Bb}$ ($= \mu_{BB}^*$)</td>
<td>0.89</td>
<td>0.97</td>
</tr>
<tr>
<td>$\mu_{bb}$</td>
<td>1.80</td>
<td>1.70</td>
</tr>
<tr>
<td>SD</td>
<td>0.37</td>
<td>0.38</td>
</tr>
<tr>
<td>$h^2$</td>
<td>0.67</td>
<td>0.65</td>
</tr>
</tbody>
</table>

The same covariates as indicated in Table 1 were included in each model, but none of the estimates differed between the contrasting models for pleiotropy and so are not presented in this table.

*The $B$ allele appears to be dominant over the $b$ allele.11

†Estimated as $-2$ in likelihood for the model allowing no pleiotropy on the second trait, compared with the unrestricted model allowing pleiotropy.

### TABLE 5. Results of 2-Locus, Bivariate Segregation Analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>$f_B^*$</th>
<th>$f_R^*$</th>
<th>LDLC Base Affects Trait</th>
<th>LDLC RC Affects Trait</th>
<th>$\chi^2$</th>
<th>df</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Two-locus (general)</td>
<td>0.72</td>
<td>0.84</td>
<td>Yes</td>
<td>Yes</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>2. Two-locus</td>
<td>0.72</td>
<td>0.87</td>
<td>Yes</td>
<td>No</td>
<td>2</td>
<td>2.6</td>
<td>0.28</td>
</tr>
<tr>
<td>3. Two-locus</td>
<td>0.74</td>
<td>0.85</td>
<td>Yes</td>
<td>No</td>
<td>2</td>
<td>10.9</td>
<td>0.004</td>
</tr>
<tr>
<td>4. Two-locus</td>
<td>0.74</td>
<td>0.88</td>
<td>Yes</td>
<td>No</td>
<td>4</td>
<td>12.7</td>
<td>0.013</td>
</tr>
<tr>
<td>5. One-locus</td>
<td>0.74</td>
<td>(1)</td>
<td>Yes</td>
<td>Yes</td>
<td>4</td>
<td>48.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6. One-locus (1)</td>
<td>0.86</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>4</td>
<td>95.5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Frequencies of the $B$ and $R$ alleles were estimated or fixed (in parentheses) in each model.
is much greater than the differences among inbred and recombinant strains of mice and among swine with apoB variants. Physiological mechanisms associated with dietary responsiveness have been studied extensively in squirrel monkeys, African green monkeys, rhesus monkeys, and baboons, but no single gene affecting responsiveness to dietary cholesterol has been identified in any nonhuman primate. Given the close phylogenetic relatedness of baboon and human species and their similarity in the physiological processes of lipoprotein metabolism, this major locus for response to dietary cholesterol may also exist in humans. Discovery of such a gene in humans would be valuable in identifying diet-susceptible people or, potentially, suggesting new strategies for developing drugs targeted to control plasma LDL.

We do not yet know the identity of the major locus that controls response to dietary cholesterol in baboons. An obvious candidate is the \(LDLR\), which is regulated by intracellular cholesterol levels. In a previous study, we found a polymorphic site in intron 17 of baboon \(LDLR\) that was associated with serum LDL and apoB levels on both the basal and high-cholesterol, high–saturated fat diets. We tested for linkage of this \(LDLR\) polymorphism with the \(R\) locus in these baboon families. However, we found no evidence of linkage (log of the odds score was \(-0.9\); D.L.R. et al., unpublished observations, 1997) and conclude that the major gene for response to dietary cholesterol is not \(LDLR\).

Other earlier studies that focused on high and low LDL–responding baboons showed that high responders had increased cholesterol absorption, apoB production, and conversion of VLDL to LDL. High responders also had lower plasma levels of 27-hydroxycholesterol and lower hepatic levels of sterol 27-hydroxylase protein and activity. These latter observations suggest that a locus affecting bile acid metabolism may be a candidate for the LDL became major gene.

### Relationship of LDL Response to Fat and to Cholesterol

The major locus affecting response to dietary cholesterol also affects responsiveness to dietary saturated fat under the conditions of this study and accounts for most of the genetic correlation between the traits, as well as most of the heritability for each of the 2 traits. Thus, the data suggest that the product of the \(R\) locus, which affects both diet response and baseline levels of LDL, is central to LDL metabolism.

A much greater interindividual variability has been observed in the response of baboons to dietary cholesterol compared with the response to saturated fat, and the same observation was made in this study. Dietary cholesterol has dominated most animal model studies of diet-induced hyperlipidemia, whereas its role in human hyperlipidemia is considered small relative to that of saturated fatty acids. A possible explanation for the differences in responsiveness to dietary cholesterol among animal species (including humans) is greater interspecies variation in the frequencies of alleles for genes affecting cholesterol response compared with those affecting fat response. That is, animals of all species show a

### Summary of the Results

The LDL response to dietary cholesterol was strongly heritable \((h^2=0.59)\), whereas response to dietary fat was only weakly heritable \((h^2=0.14)\). A major gene \((R\) locus\) explained about half the phenotypic variation in LDL base. In addition, the \(R\) locus exerted pleiotropic effects on 2 other related traits: \(R\) explained \(\approx3\%\) of the variation in LDL base and \(\approx6\%\) of the phenotypic variation (and almost all of the additive genetic variation) in LDL RF. The previously described major gene \((B\) locus\) for LDL base, however, explained variation in LDL only and in neither of the response variables. Thus, we have evidence for 2 separate major loci that regulate LDL levels.

### Nature of the Major Locus for Cholesterol Responsiveness

The magnitude of the effect of the \(R\) locus on responsiveness is similar to that of the \(7\alpha\)-hydroxylase mutation in the cholesterol-resistant rabbit and the unidentified genetic variant responsible for the JAX rabbit. The effect

### TABLE 6. Genotype Means for LDL base (in mmol/L)

<table>
<thead>
<tr>
<th>LDL base</th>
<th>Genotype (BB)</th>
<th>Genotype (Bb)</th>
<th>Genotype (bb)</th>
<th>Marginals</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLC base</td>
<td>0.86</td>
<td>0.88</td>
<td>1.75</td>
<td>1.30</td>
</tr>
<tr>
<td>LDLC RC</td>
<td>1.03</td>
<td>1.05</td>
<td>1.91</td>
<td>1.47</td>
</tr>
<tr>
<td>LDLC RF</td>
<td>1.10</td>
<td>1.12</td>
<td>1.98</td>
<td>1.54</td>
</tr>
</tbody>
</table>

### TABLE 7. Decomposition of Phenotypic Variance for LDL base, LDL RC, and LDL RF

<table>
<thead>
<tr>
<th>Component of Variance</th>
<th>Trait Proportion of Trait Variance in %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDL base</td>
</tr>
<tr>
<td>Genetic</td>
<td></td>
</tr>
<tr>
<td>Major locus for LDL base ((B))</td>
<td>27</td>
</tr>
<tr>
<td>Major locus for LDL RC ((R))</td>
<td>3</td>
</tr>
<tr>
<td>Residual genetic</td>
<td>38</td>
</tr>
<tr>
<td>Covariates</td>
<td>10</td>
</tr>
<tr>
<td>Residual error (unexplained)</td>
<td>22</td>
</tr>
</tbody>
</table>

*Not estimated because of nonsignificant effects as described elsewhere.
consistent response to dietary fat, whereas the proportion of animals responding to dietary cholesterol varies considerably across species. This explanation suggests that, although lower in frequency, polymorphisms associated predominantly with responsiveness to dietary cholesterol may also exist in human populations.

**Genetic Control of LDLₐ₀**

The previously described major locus for LDLₐ₀ was confirmed in this study of a different group of baboons. This B locus influences more than a quarter of the variation in LDLₐ₀, but exerts no detectable effects on the response to dietary components. The identity of this locus is also unknown, although our preliminary results suggest that, like the R locus discussed above, this locus is not linked to the candidate locus LDLₐ₀ (log of the odds score was ~0.7; D.L.R. et al, unpublished observations, 1997). There remains substantial genetic variation in LDLₐ₀ (eg, ~38% of LDLₐ₀) that is not accounted for by either the R or the B locus. Included in this set of genes is the effect of variation at the LDLₐ₀ locus, which explains ~6% of total phenotypic variation in this trait. Thus, in this study, we have detected the actions of at least 2 different loci that exert substantial effects on LDLₐ₀ variation and that may also play important roles in LDLₐ₀ variation in humans.

**Acknowledgments**

This work was supported in part by grant HL28972 from National Institutes of Health, Bethesda, Md. The authors are grateful to the veterinary staff who conducted the diet experiment and obtained the blood samples for these analyses. For technical assistance and data management, we thank Jim Bridges, Debbie Christian, and Mary L. Sparks.

**References**

23. Hasstedt SJ. Pedigree Analysis Package v. 3.0. Salt Lake City, Utah: Department of Human Genetics, University of Utah Medical Center; 1989.
24. Hasstedt SJ. Pedigree Analysis Package, v. 4.0. Salt Lake City, Utah: Department of Human Genetics, University of Utah Medical Center; 1994.
33. Van Zutphen LFM, Fox RR. Strain differences in response to dietary cholesterol varies considerably across species. This explanation suggests that, although lower in frequency, polymorphisms associated predominantly with responsiveness to dietary cholesterol may also exist in human populations.
Two Major Loci Control Variation in β-Lipoprotein Cholesterol and Response to Dietary Fat and Cholesterol in Baboons


doi: 10.1161/01.ATV.18.7.1061

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/18/7/1061

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/