Apolipoprotein A-I, A-II and C-II in Black and White Residents of Evans County

HERMAN A. TYROLER, M.D., GERARDO HEISS, M.D., PH.D., GUSTAV SCHONFELD, M.D., GERALD COOPER, M.D., SIEGFRIED HEYDEN, M.D., PH.D., AND CURTIS G. HAMES, M.D.

SUMMARY Plasma levels of lipids, lipoprotein-cholesterol and three major apolipoproteins (ApoA-I, A-II and C-II) were studied in 318 black and white males and females randomly sampled in Evans County, Georgia. Black-white differences in lipid and lipoprotein-cholesterol concentrations were observed, with low-density fractions higher in whites and high-density fractions higher in blacks. Plasma levels of ApoA-I but not ApoA-II were higher in blacks than in whites and in females than in males. ApoC-II concentrations were lower in black than in white men and women.

Black-white differences in atherogenic lipoprotein fractions were statistically explained (in the sense of association, not necessarily of causal process) by the differences in ApoC-II concentrations between the race groups. Black-white differences in antiatherogenic high-density lipoprotein-cholesterol were greater than statistically predicted by differences in ApoA lipoprotein levels. The findings are indicative of black-white differences in lipoprotein composition.

THE RECENT REDISCOVERY of an inverse association between levels of plasma high-density lipoprotein (HDL) and coronary heart disease prevalence1, 2 and incidence3-4 has renewed interest in factors that influence the balance between atherogenic low-density lipoproteins (LDL) and antiatherogenic HDL.7 Female hormones,6, 8 alcohol consumption10-16 and strenuous physical activity16-20 are associated with raised levels of HDL, while cigarette smoking10, 17, 21 and obesity1, 22-24 are associated with lower levels of HDL. Black children have higher levels of HDL than whites in the southern U.S.25 and in the urban North.26 HDL-cholesterol (HDL-C) is lower in coronary heart disease cases than in controls in both black and white males.27 Black residents of Evans County have a lower prevalence and incidence28 of coronary heart disease than white males. Black males in Evans County had a lower risk of coronary heart disease despite a markedly higher prevalence of hypertension in black than white males and persisted controlling for age, total serum cholesterol, smoking and blood pressure by logistic risk-function analysis.29 The possibility that some of the lower risk of coronary heart disease in black males in Evans County was attributable to HDL-C was also suggested by the finding of significantly higher levels of HDL-C and significantly lower levels of very low density lipoprotein cholesterol (VLDL-C) and total plasma triglycerides in black than white males, all free of coronary heart disease, controlling for levels of total plasma cholesterol.23

Cholesterol represents only one of several chemical moieties in HDL, since HDL is composed of about 50% apoproteins and about 50% lipids. Apoproteins A-I and A-II (ApoA-I and ApoA-II) constitute about 90% of the mass of HDL protein, while ApoA-I and ApoA-II are present only in trace amounts of the other lipoprotein density fractions.20 Thus, ApoA-I and ApoA-II also represent valid indexes of plasma HDL levels. Apolipoprotein C-II (ApoC-II) serves a central role as a functional apoprotein in intravascular triglyceride metabolism as a modulator of the activity of lipoprotein lipase.31, 32 In this study, we report on the distributions of these important apoproteins in a sample of the black and white residents of Evans County who were free of coronary heart disease.

Methods

Fasting plasma was drawn from an age-sex-stratified random sample of all black and white survivors of the original 1960 Evans County cohort, free of coronary heart disease and residing in Evans County in 1977. This report is based on 82 white males, 68 black males, 95 white females and 73 black females. Total cholesterol of plasma and cholesterol of lipoprotein fractions were determined by a partially automated CDC Abell-Kendall cholesterol reference method.33 Triglyceride measurements were performed by the CDC reference chromotropic triglyceride method.34 Triolein was used as the standard. The lipoprotein fractions were prepared by an ultracentrifugal procedure specified by the Laboratory of Molecular Disease of the National Heart, Lung, and Blood Institute35 and by the laboratory manual of the Lipid Research Clinics Program.36 The concentration of LDL-C was calculated as the difference between the concentration of the 1.006 infranatant fraction and HDL-C. LDL-C was determined on the plasma supernate after precipitating LDL and VLDL by heparin–manganese chloride procedure.36 The manganese did not interfere with determination of HDL-C.
TABLE 1. Mean Plasma Total Cholesterol, Triglycerides, and Lipoprotein Fraction Cholesterol by Race and Sex

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black (mg/dl)</td>
<td>White (mg/dl)</td>
<td>Difference B-W</td>
<td>Black (mg/dl)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>56.5 ± 2.3</td>
<td>45.8 ± 1.5</td>
<td>10.7</td>
<td>59.3 ± 2.0</td>
</tr>
<tr>
<td>LDL-C</td>
<td>122.0 ± 5.5</td>
<td>137.6 ± 4.1</td>
<td>-15.6</td>
<td>131.1 ± 5.5</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>22.2 ± 1.6</td>
<td>26.2 ± 1.6</td>
<td>-4.0</td>
<td>21.1 ± 1.6</td>
</tr>
<tr>
<td>Total C</td>
<td>200.7 ± 4.9</td>
<td>209.6 ± 4.6</td>
<td>-8.9</td>
<td>211.5 ± 5.4</td>
</tr>
<tr>
<td>TG</td>
<td>111.2 ± 8.1</td>
<td>132.1 ± 7.9</td>
<td>-20.9</td>
<td>105.5 ± 7.9</td>
</tr>
<tr>
<td>Age</td>
<td>54.2 ± 1.5</td>
<td>54.8 ± 1.4</td>
<td>-0.6</td>
<td>52.1 ± 1.6</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

Abbreviations: C = cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low density lipoprotein; TG = triglyceride.

TABLE 2. Mean Lipoprotein Apoproteins A-I, A-II, C-II by Race and Sex

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black (mg/dl)</td>
<td>White (mg/dl)</td>
<td>Difference B-W</td>
<td>Black (mg/dl)</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>122.5 ± 3.9</td>
<td>110.4 ± 3.9</td>
<td>12.1</td>
<td>134.9 ± 4.4</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>39.0 ± 1.5</td>
<td>38.2 ± 1.5</td>
<td>0.8</td>
<td>37.7 ± 1.3</td>
</tr>
<tr>
<td>ApoA-I/ApoA-II (mg/dl)</td>
<td>3.4 ± 0.1</td>
<td>3.1 ± 0.1</td>
<td>0.3</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>ApoC-II</td>
<td>4.9 ± 0.4</td>
<td>7.0 ± 0.4</td>
<td>-2.1</td>
<td>4.7 ± 0.3</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
Table 3. Intraindividual Correlations Between ApoA-I, A-II, and High-density Lipoprotein-Cholesterol (HDL-C)

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black</td>
<td>White</td>
</tr>
<tr>
<td>ApoA-I · HDL-C</td>
<td>0.44†</td>
<td>0.40†</td>
</tr>
<tr>
<td>ApoA-II · HDL-C</td>
<td>0.30†</td>
<td>0.45†</td>
</tr>
<tr>
<td>ApoA-I · ApoA-II</td>
<td>0.11</td>
<td>0.27*</td>
</tr>
</tbody>
</table>

*p < 0.01.
†p < 0.001.

(p = 0.03) in black than white men. Similar, smaller differences were present for ApoA-I between black and white women. Expressed as percentage difference (i.e., mean black-white apoprotein difference divided by black values × 100) ApoA-I was 9.9% higher for black than for white men, and 6.4% higher for black than for white women. Because ApoA-I levels were higher in blacks but ApoA-II levels were about the same in blacks and whites, ApoA-I/ApoA-II ratios tended to be higher in blacks.

Although not the major purpose of this study, male-female differences in the apoproteins should be noted. ApoA-I was significantly higher in women than men, both black and white; ApoA-II did not differ significantly between men and women in either race, although ApoA-II was slightly higher for men in both races. Therefore, ApoA-I/ApoA-II ratios were higher in women than in men.

ApoC-II levels were significantly (p = 0.0001) lower in black men than in white men by 43%. Similar but nonsignificant (p = 0.15) changes were found in black women (15% lower than white women).

The intraindividual Pearson product-moment correlation coefficients of the components of HDL are listed in table 3. ApoA-I and ApoA-II were both positively correlated with HDL-C and with each other. Each of these correlation coefficients, ranging from 0.27-0.46, was significantly different from zero (p < 0.01) except for the correlation of ApoA-I with ApoA-II in black males. Similar patterns of correlation were obtained for the group as a whole (n = 307). There were no consistent correlations between either ApoA-I or ApoA-II and VLDL or LDL lipids or total plasma ApoC-II levels.

Table 4. Intraindividual Correlations* of ApoC-II with Plasma Lipid and Lipoprotein-Cholesterol Levels by Race and Sex

<table>
<thead>
<tr>
<th></th>
<th>TG</th>
<th>VLDL-C</th>
<th>Total C</th>
<th>LDL-C</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoC-II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black men</td>
<td>0.44†</td>
<td>0.42†</td>
<td>0.35‡</td>
<td>0.22†</td>
<td>-0.08</td>
</tr>
<tr>
<td>White men</td>
<td>0.59‡</td>
<td>0.52‡</td>
<td>0.46‡</td>
<td>0.41†</td>
<td>-0.25†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>TG</th>
<th>VLDL-C</th>
<th>Total C</th>
<th>LDL-C</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoC-II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black women</td>
<td>0.50†</td>
<td>0.48†</td>
<td>0.33†</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>White women</td>
<td>0.63‡</td>
<td>0.63‡</td>
<td>0.62‡</td>
<td>0.33†</td>
<td>-0.01</td>
</tr>
</tbody>
</table>

*Pearson’s product-moment correlation coefficient.
†p < 0.01.
‡p < 0.001.
Abbreviations: TG = triglyceride; C = cholesterol; VLDL = very low density lipoprotein; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

(ApoC-II was most strongly associated with total triglyceride levels and VLDL-C and, to a lesser extent, with total cholesterol and LDL-C, and weakly or not at all with HDL-C. The magnitude of the correlation of ApoC-II with the lipids ranked similarly from high to low in each race-sex group. The correlation of ApoC-II with total triglycerides and VLDL-C as well as with total cholesterol and LDL-C was higher for whites than for blacks.

Having demonstrated the higher levels of ApoA-I, the association of ApoA-I and ApoA-II with HDL-C and with each other, the lower levels of ApoC-II in blacks than in whites, and the associations of ApoC-II with VLDL and LDL lipids, the next set of analyses was directed to quantifying how much of the observed black-white differences in lipids was attributable statistically to the black-white differences in levels of apoproteins.

Covariance analysis was used to adjust the observed levels of lipids for the differences in apoproteins for each race-sex group and then to contrast the adjusted lipid values in blacks and whites (table 5). Black-white differences in HDL-C in men were decreased from 10.7 mg/dl to 8.3 mg/dl after adjusting for Apo-A-I, a 22% reduction; however, the residual difference of 8.3 mg/dl was still large and significant (p < 0.005). ApoA-II considered alone as a covariate did not reduce the black-white difference in HDL-C, nor did it contribute additionally to the effect of Apo-A when the sum of ApoA-I and ApoA-II was treated as a covariate.

The effect of controlling for the level of ApoC-II was to produce major decreases in black-white differences in LDL-C, total C, VLDL-C and triglyceride in males, such that none of the adjusted differences was significantly different from zero. None of the black-white lipid differences in women were significantly different from zero, although the triglyceride difference was large (~20.4 mg/dl). After controlling for ApoC-II the black-white triglyceride difference was reduced to ~6.9 mg/dl, a 66% decrease.

Given that ApoC-II and triglyceride values are closely correlated in plasma, ApoC-II was then used as the dependent variable in the covariance model and
log triglyceride, race and age as the other terms of the equation. The results suggest that the difference in ApoC-II levels between black and white men were consistent in large part with differences in triglycerides, but that other factors different in blacks and whites also made an important contribution. Similar results were obtained by matching black and white men for triglycerides and age (table 6). In the 42 matched pairs significant differences persisted in ApoC-II levels, despite almost identical values for triglycerides and VLDL-C. The results of the variance and matched analyses may be summed up as follows: The different ApoA-I and ApoC-II levels in black and white males are not statistically totally accounted for by differences in lipoprotein cholesterol, lipids and age. As demonstrated earlier, however, male black-white differences in total cholesterol, LDL-C, VLDL-C and triglycerides are statistically accounted for by the black-white differences in ApoC-II.

**Table 5. Mean Black-White Differences Observed and Covariance Adjusted for Apoproteins**

<table>
<thead>
<tr>
<th></th>
<th>Male Observed</th>
<th>Male Adjusted</th>
<th>Female Observed</th>
<th>Female Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C</td>
<td>10.7</td>
<td>8.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C†</td>
<td>10.7</td>
<td>10.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C‡</td>
<td>10.7</td>
<td>8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C§</td>
<td>-15.6</td>
<td>-8.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL-C§</td>
<td>-4.0</td>
<td>-0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total-C§</td>
<td>-8.9</td>
<td>-1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG§</td>
<td>-20.9</td>
<td>-3.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for A-I.  
†Adjusted for A-II.  
‡Adjusted for A-I and A-II.  
§Adjusted for C-II.

Abbreviations: C = cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low density lipoprotein; TG = triglyceride.

**Discussion**

This community-based study has disclosed black-white differences in mean levels of ApoA-I and ApoC-II and statistical associations between levels of apoproteins and levels of plasma total cholesterol, triglycerides and lipoprotein cholesterol. The data also demonstrate black-white differences in levels of HDL-C that are not totally attributable statistically to differences in ApoA-I, suggesting that the HDL composition of blacks may be different from that of whites. Similarly, there are black-white differences in the atherogenic lipoprotein fractions (VLDL and LDL) that are statistically explainable (in the sense of association, not necessarily of causal process) by the differences in ApoC-II. However, residual, statistically unexplained differences in ApoC-II, adjusting for levels of lipoproteins, again suggested that there may be black-white differences in lipoprotein composition.

Obviously, any conclusions must be qualified by the fact that the measured levels of apoproteins, lipids and lipoprotein density fraction cholesterol represent single, one-time concentrations that result from an extremely large number of interacting biochemical and metabolic processes and that the associations reported are cross-sectional and statistical rather than the results of experimental perturbation produced in individuals. Nevertheless, the findings should stimulate more "metabolically" oriented research to explain the black-white differences reported herein.

There were significant black-white differences in HDL-C, especially in men. However, only about one-fifth of the black-white differences in HDL-C is attributable statistically to differences in levels of ApoA-I. In addition, there are suggestive differences in ApoA-I/ApoA-II ratios by race. This raises the possibility of black-white differences in compositional properties of HDL, e.g., differences in the relative proportions of HDL2 and HDL3. The ratios of ApoA-I to ApoA-II are higher in HDL2 than HDL3, and women are known to have higher levels of HDL2 than HDL3. This would be expected to produce higher...
ApoA-I/ApoA-II ratios in women than in men, an expectation borne out by the data (table 2). Thus, the tendency for higher ApoA-I/ApoA-II ratios in blacks, particularly among the men, could mean that HDL may be relatively higher among blacks. This hypothesis, which could explain some of the racial differences in VLDL levels in terms of the metabolic interactions between VLDL and HDL, needs further testing.

The differences in ApoC-II were not explained by the factors tested. This suggests that some or all of the lipoproteins of white men contain more ApoC-II than do the lipoproteins of black men. This is surprising in view of the higher triglyceride levels of whites. If ApoC-II levels are critical in regulating VLDL catabolism, one would have expected that the excess of ApoC-II in plasma would be associated with lower levels of triglycerides. However, our findings are compatible with those of Breckenridge et al. They report that the complete absence of ApoC-II results in hypertriglyceridemia, but suboptimal ApoC-II levels are compatible with normal triglycerides. Thus, ApoC-II levels under usual circumstances do not appear to be rate-limiting in VLDL triglyceride catabolism.

Lower triglyceride levels have been observed in blacks than in whites during childhood in both the rural South and urban North, and in both males and females in adulthood. These differences persist after controlling for the potentially confounding effects of obesity. The association of triglycerides with ApoC-II was positive, and the ApoC-II difference was of a magnitude appropriate for the black-white differences in triglycerides. The contribution of behavioral, environmental and genetic factors to the observed differences requires identification and quantification to provide insight to black-white differences in atherosogenesis.

References
Risk Factors and Angiographic Coronary Artery Disease: A Report from the Coronary Artery Surgery Study (CASS)

RONALD E. VLIETSTRA, M.B., CH.B., ROBERT L. FRYE, M.D., RICHARD A. KRONMAL, PH.D., DALICE A. SIM, M. PHIL., FELIX E. TRISTANI, M.D., THOMAS KILLIP III, M.D., AND PARTICIPANTS IN THE CORONARY ARTERY SURGERY STUDY

SUMMARY The findings for 14 risk variables were correlated with the results of coronary arteriography in 8807 patients registered in the interinstitutional Coronary Artery Surgery Study (CASS). Discriminant-function analysis revealed that age, sex, cigarette smoking and the level of blood cholesterol best distinguished between the groups with (6688 patients) and without (2119 patients) coronary artery disease. A family history of coronary artery disease and the presence of hypertension or diabetes were of additional, but less, discriminating value. The relative risk for coronary artery disease in patients with the combination of cigarette smoking and an elevated cholesterol level was high (> 4) in females age 55 years or younger and in males age 35 years or younger. Few females age 45 years or younger (seven of 97) had coronary artery disease when both of these risk factors were absent. In spite of these correlations, only limited gains accrued from the use of discriminant-function analysis in correctly allocating patients into disease and nondisease groups. This indicates that, while certain factors are significantly correlated with coronary angiographic findings, their value for predicting the presence of coronary artery disease is limited.

PREVIOUS SURVEYS1-4 of large, asymptomatic populations have established a firm relationship between clinical risk factors and the development of clinical manifestations of coronary artery disease (CAD). Characteristics such as age, sex, cigarette smoking, serum cholesterol level, hypertension and diabetes have identified groups at higher risk for later occurrence of angina, myocardial infarction and cardiac death. However, long-term follow-ups of asymptomatic groups have revealed that many subjects have remained free of manifestations of CAD even when multiple risk factors were present.2-5 The lack of specificity and low sensitivity of some of the clinical end points used in those studies, such as angina and sudden death, may have masked stronger associations between risk factors and CAD. Also, the risk factors may be related more to the clinical manifestations than to the CAD. Werk6 has reviewed problems in the conduct and interpretation of large clinical trials that study risk factors.

Coronary arteriography provides a precise anatomic delineation of the presence or absence of CAD during life and, if a large enough sample is available, allows risk factors to be correlated with the precise diagnosis. Accordingly, the registry data from the interinstitutional Coronary Artery Surgery Study (CASS), sponsored by the National Institutes of Health (NIH), were reviewed to correlate clinical risk factors with arteriographically defined coronary anatomy. This study was designed to determine whether, in patients considered for coronary arteriography, profiles of high and low incidence of CAD can be defined on the basis of risk factor data alone.

Methods

Since October 1974, clinical, laboratory and angiographic data have been collected in a standardized fashion and entered into a registry from consecutive patients undergoing coronary arteriography for clinically suspected CAD at 15 participating clinical centers of CASS. Patients with significant
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H A Tyroler, G Heiss, G Schonfeld, G Cooper, S Heyden and C G Hames

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