Serum Apolipoproteins A-I and B as Markers of Coronary Artery Disease Risk in Early Life: The Bogalusa Heart Study

Sathanur R. Srinivasan and Gerald S. Berenson

The functional properties of the apolipoproteins (apoB and apoA-I) governing lipoprotein metabolism make these variables conceptually important as markers for coronary artery disease risk. This aspect was examined in a biracial (black–white) population of children as part of the Bogalusa Heart Study. White children, especially boys, showed lower concentrations of apoA-I and ratios of cholesterol to apoB within low-density lipoprotein (LDL) than did black children. Persistence of apoB concentrations over time, coupled with its strong linkage with apoB gene locus, underscores the value of detecting apoB excess early in life. Further, the impact of apoE genotypes on apoB and apoA-I levels is already evident in childhood. We found that, as a screening test for detecting increased LDL cholesterol, apoB is superior to total cholesterol. Low values for apoA-I concentrations, the apoA-I to apoB ratio, and LDL cholesterol to apoB ratio in children are strongly related to parental incidence of myocardial infarction; no such relationship is seen with respect to lipoprotein cholesterol. Thus, expanding screening strategies might be useful for identifying individuals with adverse apolipoprotein profiles early in life.

Indexing Terms: epidemiology/race-related effects/sex- and age-related effects/pediatric chemistry/genetic studies

Initial clinical and epidemiologic studies established a relation between plasma total cholesterol and coronary artery disease (CAD) risk. Later studies have focused on the atherogenicity of the concentration of cholesterol in different lipoprotein classes. Increased cholesterol in the low-density lipoprotein (LDL) fraction and decreased cholesterol in the high-density lipoprotein (HDL) fractions have been associated with increased CAD risk. As a consequence of better understanding of lipoprotein composition and metabolism in relation to CAD over the last decade, attention is being increasingly focused on the protein components of lipoproteins, known as apolipoproteins or apoproteins (apos), as markers of CAD. However, such studies have been confined mainly to the adult population. Given the current recognition that the pathologic precursors of CAD originate in childhood, this report presents relevant observations from our long-term epidemiologic study of cardiovascular risk factors in children and young adults, the Bogalusa Heart Study.

Apolipoproteins A-I and B: Background

ApoA-I and apoB are of particular relevance to atherogenesis in the general population. ApoB serves as an essential structural component of chylomicrons, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins, and LDL. By being a ligand for the LDL receptor, apoB (i.e., B-100) facilitates cholesterol delivery to the tissues (2). In addition, apoB promotes cholesterol accumulation in the arterial tissue through being modified by oxidation and (or) specific binding to extracellular matrix proteoglycans (7, 8). In contrast, apoA-I, the structural constituent of HDL, mediates efflux of cholesterol from the membrane of peripheral cells (9). In addition, apoA-I functions as an activator of lecithin–cholesterol acyltransferase, a key enzyme in the reverse transport of cholesterol from the peripheral tissues to the liver (10). Thus, plasma concentrations of these two apolipoproteins and their relative proportion may reflect cholesterol transport to and from the peripheral tissues, including the arterial wall.

Race- and Sex-Related Differences in Childhood

With respect to apoA-I, race-related difference (blacks > whites) is seen only among boys (11). Studies in adults show similar differences (12). The sex-related changes in prepubertal children indicate a tendency for black girls to have lower apoA-I, and for girls of both races to have higher apoB concentrations than boys do. The characteristic adult pattern of relatively low apoA-I and high apoB in men vs women seems to emerge during the latter part of sexual maturation. That the continued decrease of apoA-I during sexual maturation is seen only in white males makes this group particularly vulnerable for future CAD.

Persistence of Apolipoprotein Concentrations Over Time

As with LDL cholesterol and HDL cholesterol, a single determination of apoB and apoA-I concentrations at an earlier point in childhood is modestly predictive of the concentrations in individuals 4 years later (14). For all race and sex groups, apoB tracks better than apoA-I (Fig. 1). For example, 31% of those in the lowest quintile for apoA-I were still in this rank 4 years later. Whereas 50% of those individuals whose apoB concentrations were in the highest quintile at year 1 remained in this quintile at year 4. Another 22% originally in the highest quintile drifted into the fourth quartile (60th–80th percentile) at follow-up. Hence, >70% of those with increased apoB in youth tended to remain so on follow-up.
The magnitude of tracking can be influenced by analytical measurement error and intraindividual biological variation such as diet and other lifestyle variables. Despite these limitations, however, the tracking phenomenon based on a single measurement is particularly important because it may serve as a practical indicator for future CAD risk.

Relation of Cholesterol to ApoB and ApoA-I Within Lipoproteins

LDL and HDL are heterogeneous in terms of both size and composition (14, 15). Unlike apoB and apoA-I, the lipid components are readily amenable for exchange between lipoprotein classes by the transfer process. Therefore, the amount of cholesterol within LDL and HDL molecules can vary widely. Measuring the ratios of cholesterol to apoB and apoA-I in LDL and HDL, respectively, offers one of the means whereby variability in these lipoproteins can be examined on a population basis.

The composition of LDL and HDL varies markedly among children (11, 16–18). For example, the ratio of cholesterol to apoB in LDL varies from 1.03 (10th percentile) to 1.37 (90th percentile), and the ratio of cholesterol to apoA-I in HDL varies from 0.27 (10th percentile) to 0.57 (90th percentile). Obviously, therefore, lipoprotein cholesterol concentration does not correspond to lipoprotein concentration. Moreover, the observed variability in lipoproteins may have a bearing on susceptibility to CAD later in life. A disproportionate increase in apoB relative to cholesterol in LDL reflects small, dense particles, which are highly atherogenic (19–21). In general, LDL particles in white children are apoB-enriched relative to those of black children. Thus, white males may have enhanced risk of CAD because they also have lower apoA-I concentrations and a lower HDL cholesterol to apoA-I ratio (a measure of HDL$_2$ subfraction), especially following sexual maturation.

It has been generally considered that concentrations of serum triglycerides (VLDL) determine the characteristics of LDL and HDL (22). However, variability in the ratio of cholesterol to apoB in LDL of children is primarily related to serum HDL cholesterol rather than to triglycerides (Fig. 2) (18). In contrast, the variability in the ratio of HDL cholesterol to apoA-I is primarily related to serum triglyceride concentrations, as one would expect (16). Changes in characteristics of LDL and HDL have been attributed to the bidirectional transfer of triglycerides and cholesteryl ester between these lipoproteins and VLDL, as mediated by lipid transfer proteins (23). However, the low postabsorptive serum concentrations of triglycerides (median value: 0.53 g/L) found in children may not be the driving force for alterations in the cholesterol to apoB ratio in LDL. Instead, an efficient catabolism of triglyceride-rich lipoproteins can promote the formation of large, buoyant HDL$_2$ from the small, dense HDL$_3$ precursor particles (24); this results in reduced translocation of cholesteryl ester from large, buoyant LDL and HDL to triglyceride-rich lipoproteins, in exchange for triglycerides. Therefore, the variability in the ratio of cholesterol to apoB in LDL in children may reflect the postprandial triglyceride metabolism, which in turn affects the concentrations of HDL cholesterol, especially HDL$_2$ cholesterol. These observations support the earlier suggestion that the relationships of LDL and HDL to CAD risk are interdependent (25).

ApoB Screening to Detect Increased LDL Cholesterol

In terms of screening for CAD risk factors, serum total cholesterol measurement is an effective tool for detecting increased LDL cholesterol in adults because the correlation between these two variables is very high ($r = 0.84–0.88$) (26). In the pediatric population, however, the usefulness of serum total cholesterol is limited because children have higher HDL cholesterol and lower LDL cholesterol concentrations than adults do, and the correlation between total cholesterol and LDL cholesterol is lower ($r = 0.73–0.75$) (27). This confounding effect of HDL cholesterol is eliminated when measurement of apoB is used as a screening test to detect...
high concentrations (≥95th percentile) of LDL cholesterol in children (28). When we used this approach to screen a total population of children (28), the sensitivity was higher for apoB than for serum total cholesterol at all percentile cutoff points examined (Table 1). The specificities of each screening test were comparable. The positive predictive values for the two tests were comparable between 75th and 90th percentiles. At the 95th percentile, the positive predictive value of the apoB test exceeded that of the cholesterol test (72% vs 50%).

When we measured serum total cholesterol to screen the same population, the children who were missed (false negatives) had lower HDL cholesterol and higher ratios of LDL cholesterol to HDL cholesterol than those who were correctly classified (true positives) (Table 2). Thus, the children “missed” when measurement of total cholesterol was used as a screening test might actually be at greater risk than the children who were identified. In contrast, when measurement of apoB was used as a screening test, HDL cholesterol concentrations no longer acted as a confounder in those with false-negative results. Thus, the children in whom increased LDL cholesterol values were detected by apoB screening were those at greatest risk for CAD.

Influence of Candidate Genes on ApoB and ApoA-I Concentrations

Pedigree study. The genes for most of the major proteins involved in lipoprotein metabolism have now been examined in populations for associations with quantitative lipoprotein traits related to CAD, with mixed results (29–31). Although association studies can be useful in detecting possible genetic factors influencing a trait, the power of these studies may be greatly affected by unknown characteristics of the population.

Maximum likelihood linkage analyses, powerful tools for the detection of linkage, were applied to a large pedigree of 200 members sampled from a six-generation family with adverse lipoprotein phenotype (high VLDL cholesterol and LDL cholesterol and low HDL cholesterol) and excess CAD and diabetes (32–34); the family gave no evidence of a single-gene defect causing familial dyslipidemia. The candidate genes tested were apoB, apoA-I, lipoprotein lipase, hepatic lipase, and cholesteryl ester transfer protein. Haptoglobin was used as a marker for lecithin–cholesterol acyltransferase and cholesteryl ester transfer protein due to its genetic proximity on chromosome 16 and the lack of known polymorphism within the lecithin–cholesterol acyltransferase gene. The genetic markers corresponding to these candidate genes were examined with 11 different, two-allele restriction fragment length polymorphisms and an apoB locus variable number of tandem repeats (VNTR) polymorphism. The values of allele frequencies compared reasonably well with those in other studies of caucasians.

ApoB gene (PvuII digestion site) and cholesteryl ester transfer protein gene (Taq(B) digestion site) showed association with apoB concentrations. Further, linkage between apoB concentration and polymorphism of the apoB gene defined by the two restriction digests EcoRI and PvuII was supported by a lod score of 3.3, whereas inclusion of the VNTR typings yielded a lod score of 2.3. None of the other candidate genes showed positive evidence of linkage to apoB concentrations. The apoA-I

---

**Table 1. Screening test characteristics of serum total cholesterol and apoB for detecting children with high concentrations of LDL cholesterol.**

<table>
<thead>
<tr>
<th>Cutoff point: age-, race-, sex-specific percentiles</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>PPV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>75th</td>
<td>97</td>
<td>79</td>
<td>18</td>
</tr>
<tr>
<td>80th</td>
<td>93</td>
<td>84</td>
<td>22</td>
</tr>
<tr>
<td>85th</td>
<td>85</td>
<td>89</td>
<td>27</td>
</tr>
<tr>
<td>90th</td>
<td>80</td>
<td>94</td>
<td>41</td>
</tr>
<tr>
<td>95th</td>
<td>48</td>
<td>98</td>
<td>50</td>
</tr>
</tbody>
</table>

Source: The Bogalusa Heart Study. PPV, Positive predictive value.

**Table 2. Mean concentrations of serum lipoprotein cholesterol in children with above-normal LDL cholesterol concentrations as classified by total cholesterol vs apoB screening tests.**

<table>
<thead>
<tr>
<th>Total cholesterol test</th>
<th>True pos. a</th>
<th>False neg. b</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL chol, g/L</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>LDL chol, g/L</td>
<td>1.66</td>
<td>1.49 c</td>
</tr>
<tr>
<td>HDL chol, g/L</td>
<td>0.50</td>
<td>0.31 c</td>
</tr>
<tr>
<td>LDL chol/HDL chol</td>
<td>5.7</td>
<td>7.2 d</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ApoB test</th>
<th>True pos. a</th>
<th>False neg. b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>0.18</td>
<td>0.10 c</td>
</tr>
<tr>
<td>VLDL chol, g/L</td>
<td>1.62</td>
<td>1.45 c</td>
</tr>
<tr>
<td>LDL chol, g/L</td>
<td>0.38</td>
<td>0.46 d</td>
</tr>
<tr>
<td>HDL chol, g/L</td>
<td>6.7</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Source: The Bogalusa Heart Study.

a Positive screening test results: ≥95th age-, race-, and sex-specific percentile of serum total cholesterol or apoB; results ≤95th percentile are negative.

b With respect to LDL cholesterol (≥95th percentile).

c,d Significantly different from true positives: c P <0.0001; d P <0.05.
gene locus, unlike the apoB gene locus, failed to show any association or linkage to apoA-I concentrations.

Effect of apoE genotype. Apolipoprotein E is a constituent of serum triglyceride-rich lipoproteins and HDL. This apolipoprotein, in tandem with apoB, plays an important physiologic role in the regulation of overall cholesterol homeostasis and concentration of lipoproteins (2, 35). In humans, there are three common allelic variants at the structural gene locus for apoE, the e2, e3, and e4 alleles yielding six genotypes or phenotypes. Extensive data on frequencies of apoE genotypes and their impact on serum lipoprotein concentrations are available in several populations, but such information in the black population has begun to emerge only recently (36–38).

Black–white divergences in relative frequencies of the apoE alleles and the impact of apoE genotypes on serum lipoprotein variables including apoB and apoA-I in early life were examined in 8- to 17-year-old children (39). ApoE allele frequencies showed a significant black–white difference, but no sex difference. A lower frequency of the e3 allele in black children (than that in white children) was associated with higher frequencies of both e2 and e4 alleles. As in white adults, the e2 allele in white children is associated with lower concentrations of total cholesterol, LDL cholesterol, and apoB, and the e4 allele is associated with higher values for these variables (Fig. 3). Black children showed a similar trend for these variables, but to a lesser degree. In addition, the e2 allele was associated with higher HDL cholesterol and apoA-I only in black children. Recent studies suggest that the e4 allele is associated with an increased risk for CAD independently of the LDL cholesterol concentration (40–42). Therefore, determination of the apoE genotype early in life may be helpful regarding CAD risk.

Relation of ApoB and ApoA-I Concentrations in Children to Parental Myocardial Infarction

Although the concentrations of apoB and apoA-I have been more strongly related to CAD than have the corresponding lipoprotein cholesterol fractions (4, 5), the discriminant value of these apolipoproteins in absolute terms appears to be less than that of their ratio (apoA-I/apoB) (43, 44). In addition, above-normal concentrations of LDL apoB with normal concentrations of LDL cholesterol also occur in patients with CAD (19). These observations raise the question as to whether apoB and apoA-I concentrations can be used to predict future CAD among individuals in a general population.

Prospective studies have not been conclusive in determining the relative merit of apoB and apoA-I concentrations vs lipoprotein cholesterol concentrations in predicting CAD in the general population (45, 46). However, given the familial nature of CAD, relevant information can be obtained by using parental disease as a surrogate measure of the offspring’s risk. Therefore, child–parent associations were assessed in a total population of children of school age (47). Notably, children whose fathers had had a myocardial infarction were more likely to be white, to smoke cigarettes, to be older, and to be more obese than were children whose fathers did not report myocardial infarction.

Children whose father reported having had an infarction had relatively low mean concentrations of apoA-I and low ratios of LDL cholesterol to apoB along with high apoB/apoA-I ratios (Table 3). The observed difference in LDL cholesterol to apoB ratio indicates a variation in LDL characteristics between the two groups.

<table>
<thead>
<tr>
<th>Table 3. Concentrations (mean ± SD) of apolipoproteins and lipoprotein cholesterol in children, according to paternal myocardial infarction.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable*</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>Apolipoprotein, g/L</td>
</tr>
<tr>
<td>ApoB</td>
</tr>
<tr>
<td>ApoA-I</td>
</tr>
<tr>
<td>Lipoprotein choL, g/L</td>
</tr>
<tr>
<td>VLDL</td>
</tr>
<tr>
<td>LDL</td>
</tr>
<tr>
<td>LDL choL/apoB</td>
</tr>
<tr>
<td>LDL choL/HDL choL</td>
</tr>
<tr>
<td>Source: The Bogalusa Heart Study.</td>
</tr>
<tr>
<td>* Values for the variables were adjusted for race, sex, age, subcapular skinfold thickness, cigarette smoking, alcohol consumption, and oral contraceptive use before statistical evaluation.</td>
</tr>
<tr>
<td>b,c Significantly different from children of men without myocardial infarction:</td>
</tr>
<tr>
<td>b P &lt; 0.05; c P &lt; 0.01.</td>
</tr>
</tbody>
</table>
These differences existed independently of the offspring's race, sex, age, body fat, history of smoking, alcohol intake, and use of oral contraceptives. Despite the smaller number of reported maternal myocardial infarctions, offspring of those parents also tended to have an increased apoB/apoA-I ratio. In contrast, neither the concentrations of lipoprotein cholesterol nor the ratio of LDL cholesterol to HDL cholesterol of the offspring was related to myocardial infarction in either parent.

The prevalence of paternal myocardial infarction did not show any consistent pattern over quintiles of LDL cholesterol, VLDL cholesterol, or the ratio of LDL cholesterol to HDL cholesterol (Fig. 4). In contrast, gradients in the relation of paternal myocardial infarction were evident over quintiles of both apoB to apoA-I ratio (a positive association) and LDL cholesterol to apoB ratio (a negative association). Thus, adverse concentrations of apoB and apoA-I and an apoB-enriched LDL pattern are evident long before clinical disease begins.

Comments

The above findings from a free-living population of children have implications for preventive cardiology. Children with high apoA-I concentrations and low ratios of apoA-I to apoB and of LDL cholesterol to apoB appear to be at increased risk for future CAD because adverse values for these variables in the offspring are strongly related to parental disease. Relatively high persistence of apoB concentrations over time coupled with the strong linkage of these concentrations with the apoB gene locus underscore the value of detecting apoB excess early in life. Further, the impact of apoE genotypes on apoB and apoA-I is evident early in life. We conclude that, as a screening test, apoB is superior to total cholesterol in identifying children with increased LDL cholesterol. Thus, screening strategies might be expanded to identify individuals with adverse apoB and apoA-I profiles and apoE genotype, especially those from high-risk families with a history of premature CAD.

The Bogalusa Heart Study is a joint effort of many investigators and staff, whose contribution is gratefully acknowledged. We especially thank the Bogalusa school system, teachers, parents, and most importantly the children and young adults of the community of Bogalusa, LA. We thank the Bogalusa Heart Study field staff and the Core Laboratory staff. This research is supported by funds from the National Heart, Lung, and Blood Institute of the US Public Health Service, Early Natural History of Atherosclerosis 5R01 HL38844.

References
16. Freedman DS, Srinivasan SR, Webber LS, Berenson GS. Divergent levels of high density lipoprotein cholesterol and apo-