Relation of High-Density Lipoprotein Subfractions and Apolipoprotein E Isoforms to Coronary Disease

Peter W. F. Wilson

Although a variety of methods have become available for the determination of high-density lipoprotein (HDL) subfractions in plasma, a review of published data from nine studies of coronary disease outcomes and 10 investigations of coronary artery disease severity do not suggest that measurement of HDL2-cholesterol (C) and HDL3-C offer any advantage in the prediction of coronary disease over the determination of total HDL-C alone. Apolipoprotein E is typically present in plasma as one of six isoforms, the six being encoded by three common alleles, \( \varepsilon_2 \), \( \varepsilon_3 \), and \( \varepsilon_4 \). The \( \varepsilon_3 \) allele is the most common, the \( \varepsilon_4 \) allele has been reported to be associated with higher cholesterol concentrations, and the \( \varepsilon_2 \) and \( \varepsilon_4 \) alleles are both associated with higher triglyceride concentrations. Clinical and arteriographic studies of coronary disease suggest that vascular disease risk is increased among persons with the \( \varepsilon_4 \) allele.

**Indexing Terms:** cholesterol/triglycerides/vascular disease risk

It was suggested in the 1960s that high-density lipoprotein (HDL) was involved in reverse cholesterol transport and the removal of cholesterol from peripheral tissues.\(^1\) Since that time, several population-based studies have attempted to assess the potential role of HDL and its subfractions as biomarkers that might identify persons at risk for coronary heart disease (CHD). Methods developed over the past two decades to assess the role of HDL subfractions include ultracentrifugation, polyacrylamide gel electrophoresis, gradient gel electrophoresis, immunoelectrophoresis, double precipitation, and fractional esterification rates (1–5). Early investigators measured HDL subfractions by ultracentrifugation, and showed that both HDL2 and HDL3 were highly associated with CHD and the severity of atherosclerosis (6, 7). Similar studies over the years usually replicated these results, but not in all instances (1). Although the data showed that both HDL2 and HDL3 were associated with CHD, the results did not suggest that these measurements significantly improved prediction of CHD more than determination of HDL-C alone.

The isoforms of apolipoprotein E (apoE) have interested lipid researchers for the past two decades, and three alleles (\( \varepsilon_2 \), \( \varepsilon_3 \), and \( \varepsilon_4 \)) are responsible for the six common isoforms of this apoprotein. Whereas some reports documented that a rare dyslipidemia, hyperlipoproteinemia type III, was associated with \( \varepsilon_2 \) homozygosity and the occurrence of premature CHD, others demonstrated that the \( \varepsilon_4 \) allele was associated with increased concentrations of LDL cholesterol (LDL-C) and the \( \varepsilon_2 \) allele with lower LDL-C (8). Relations between the apoE alleles and triglycerides have also been observed, both \( \varepsilon_2 \) and \( \varepsilon_4 \) having been associated with higher triglyceride concentrations (9). Coronary disease studies have tended to emphasize an increased risk for CHD in association with the \( \varepsilon_4 \) allele; less information is available concerning the \( \varepsilon_2 \) allele and CHD.

**HDL Subfractions, CHD, and Coronary Artery Disease (CAD)**

This review will consider the reports included by Müller a few years ago (1), as well as more recently published data. In one of the earliest studies, using analytical ultracentrifuge data from the Donner Laboratories, Gofman et al. (6) showed that HDL2 mass was 32% lower (\( P = 0.01 \)), and HDL3 mass was 8% lower (\( P = 0.02 \)) among 38 men ages 20–66 years with CHD compared with 1961 controls (Fig. 1). Significant associations with CHD were obtained for HDL2 by rate zonal ultracentrifugation and serial preparative ultracentrifugation in several clinical studies (10, 11). The HDL3 association with CHD was present in some reports (6, 12, 13), but not in all (11, 14) (Table 1).

The technology of HDL subfraction determinations evolved to include preparative ultracentrifugation of plasma after initial precipitation of low- and very-low-density lipoprotein (LDL and VLDL) particles by heparin–MnCl\(_2\). Several studies showed an association be-

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**Fig. 1.** Mean HDL mass and coronary disease incidence in men ages 20–66 years, followed up by Donner Laboratories. Adapted from Gofman et al. (6).

\(^1\) Nonstandard abbreviations: HDL-C, LDL-C, high- and low-density lipoprotein cholesterol; VLDL, very-low-density lipoprotein; CHD, coronary heart disease; CAD, coronary artery disease; and apo, apolipoprotein.

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between low HDL$_2$-C and CHD or CAD by this method (13, 15, 16), but the HDL$_3$-C association was less consistent (13, 16).

The next evolution was the development of double precipitation assays, in which a first step precipitated the particles containing apoB (VLDL and LDL), and HDL-C was measured in the supernate. A second step precipitated HDL$_2$, leaving the cholesterol in HDL$_3$ to be measured (17, 18). As first developed, heparin–Mn$^{2+}$ was used for the initial step and dextran–Mg$^{2+}$ for the subsequent step. The amount of HDL$_2$-C removed was related to the concentration of Mn$^{2+}$, as well as the concentration and molecular mass of the dextran sulfate used (17). Investigators concluded that dextran sulfate with a molecular mass of 15 000 Da was preferable. To validate the double precipitation methods, the authors took into account that an earlier standard to measure HDL$_2$, sequential ultracentrifugation, also included lipoprotein(a) and other apoB-containing lipoproteins, and thus systematically overestimated HDL$_2$. In linear regression models to predict the HDL subfractions by the double precipitation methods (y) in comparison with sequential ultracentrifugation (x), HDL$_2$-C was estimated with r = 0.88 ($r^2 = 0.60, P < 0.0001$), and HDL$_3$-C was estimated with r = 0.61 ($r^2 = 0.36, P < 0.0001$) (17). The $r^2$ estimates the degree of variance explained by the newer method; thus, the accuracy of the double precipitation technique appeared to be only 60% for HDL$_2$-C and 36% for HDL$_3$-C, in comparison with that of ultracentrifugation.

Respective mean concentrations of HDL-C subfractions in middle-aged men and women, estimated with the double precipitation method (17), were typically 310 and 350 mg/L for HDL$_2$-C (significantly different between the sexes; $P < 0.001$), and 140 and 200 mg/L for HDL$_3$-C (also significant, $P < 0.001$) (Table 2) (17). The higher HDL$_2$-C values for women were considered possible reasons for the lower CHD rates among women, and several investigators tested for associations between the HDL subfractions and personal characteristics. For instance, abdominal obesity (19, 20), alcohol intake (21), exercise (17, 22, 23), and estrogen replacement (24) often showed higher correlations with HDL$_2$-C concentrations than with HDL-C, and lower correlations between these characteristics and HDL$_3$-C were typical.

Results using the double precipitation methods and modifications (6, 11–14, 25–28) have generally shown (Table 1) that lower concentrations of HDL$_2$-C and HDL$_3$-C were associated with increased risk of CHD in larger studies (14, 26–28). Left unanswered in most of these studies is an estimate of the relative impact that total HDL-C concentration exerted on CHD risk compared with that of the concentration of its subfractions. One study (27) looked into this aspect in greater detail by examining the adjusted odds for myocardial infarction according to quartile of the HDL measurement. In comparison with the lowest quartile of the lipoprotein cholesterol measured, the multivariable-adjusted CHD risk gradients for the higher quartiles respectively were 0.37, 0.21, and 0.15 for HDL-C; 0.62, 0.43, and 0.17 for HDL$_2$-C; and 0.74, 0.33, and 0.32 for HDL$_3$-C (27). Burri et al. concluded that HDL$_2$-C and HDL$_3$-C were significantly associated with myocardial infarction, but demonstrated no advantage in CHD prediction beyond conventional HDL-C determinations.

Investigators have also reported significant associations between HDL subfractions and the degree of CAD determined at cardiac catheterization. These reports, often based upon clinical series and frequently lacking a true case-control design, are summarized in Table 3 (7, 15–17, 29–32). The results parallel those for the CHD studies (1). Similar degrees of association with the severity of CAD were typically observed for HDL-C, HDL$_2$-C, and HDL$_3$-C, although the associations were not always

<table>
<thead>
<tr>
<th>Study</th>
<th>Event</th>
<th>No. of cases</th>
<th>No. of non-cases</th>
<th>Sex</th>
<th>Method*</th>
<th>HDL</th>
<th>HDL$_2$</th>
<th>HDL$_3$</th>
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<td>Gofman et al. (6)</td>
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<td>38</td>
<td>1961</td>
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<td>1702</td>
<td>Men</td>
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<td>ns</td>
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<td>246</td>
<td>246</td>
<td>Men</td>
<td>Dbl ppt</td>
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<td>0.02</td>
<td>&lt;0.0001</td>
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<tr>
<td>Buring et al. (27, 28)</td>
<td>MI</td>
<td>283</td>
<td>275</td>
<td>Men, women</td>
<td>Dbl ppt</td>
<td>&lt;0.0001</td>
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MI, myocardial infarction; ns, not significant.

* Ultra ppt, combination of ultracentrifugation and precipitation; Dbl ppt, double precipitation; Ultra serial, serial ultracentrifugation; Ultra anal, analytical ultracentrifugation; Ultra RZ, rate zonal ultracentrifugation.

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Table 1. Clinical CHD and HDL subfractions.

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<th>Event</th>
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<th>No. of non-cases</th>
<th>Sex</th>
<th>Method*</th>
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MI, myocardial infarction; ns, not significant.

* Ultra ppt, combination of ultracentrifugation and precipitation; Dbl ppt, double precipitation; Ultra serial, serial ultracentrifugation; Ultra anal, analytical ultracentrifugation; Ultra RZ, rate zonal ultracentrifugation.

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Table 2. Concentrations of HDL-C subfractions in men and women.

<table>
<thead>
<tr>
<th>Number</th>
<th>HDL-C</th>
<th>HDL$_2$-C</th>
<th>HDL$_3$-C</th>
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<tbody>
<tr>
<td>Men</td>
<td>273</td>
<td>458 ± 117</td>
<td>79 ± 19</td>
</tr>
<tr>
<td>Women</td>
<td>180</td>
<td>555 ± 140*</td>
<td>143 ± 78</td>
</tr>
</tbody>
</table>

* Significantly different from men ($P < 0.001$).

Source: Gidez et al. (17).
Table 3. Arteriographic severity of CAD and HDL subfractions.

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of cases</th>
<th>No. of non-cases</th>
<th>Sex</th>
<th>Method</th>
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<td>21</td>
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<td>Gidez et al. (17)</td>
<td>43</td>
<td>135</td>
<td>Men</td>
<td>Dbl ppt</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>82</td>
<td>Women</td>
<td>Dbl ppt</td>
</tr>
<tr>
<td>Brook et al. (29)</td>
<td>10</td>
<td>10</td>
<td>Men</td>
<td>Ultra serial</td>
</tr>
<tr>
<td>Levy et al. (30)</td>
<td>323</td>
<td>—</td>
<td>Men</td>
<td>Ultra anal</td>
</tr>
<tr>
<td>Wallentin and</td>
<td>74</td>
<td>—</td>
<td>Men</td>
<td>Ultra ppt</td>
</tr>
<tr>
<td>Sundin (16)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Schmidt et al. (31)</td>
<td>69</td>
<td>14</td>
<td>Men</td>
<td>Dbl ppt</td>
</tr>
<tr>
<td>Kempen et al. (7)</td>
<td>43</td>
<td></td>
<td>Men, women</td>
<td>Ultra single</td>
</tr>
<tr>
<td>Drexel et al. (32)</td>
<td>87</td>
<td>28</td>
<td>Men</td>
<td>Dbl ppt</td>
</tr>
</tbody>
</table>

Ultra single, single-spin density ultracentrifugation. Other methods and abbreviations as in Table 1.

statistically significant. Because each study defined the degree of CAD severity in a different way, it is not possible to effectively summarize the data with overall summary estimates or to compare the relative merit of HDL-C vs HDL subfractions as CAD determinants.

Newer methods to assess HDL subfractions reveal different compositions. For instance, a polyacrylamide gradient gel electrophoretic technique can identify the subspecies HDL_{2a} and HDL_{2b} within the HDL_{2} subfraction and HDL_{3a}, HDL_{3b}, and HDL_{3c} within the HDL_{3} subfraction (3). In addition, immunoelectrophoresis has been used to identify HDL particles that contained apoA-I only and those that contained both apoA-I and apoA-II (4, 33, 34). Preliminary data suggest that concentrations of the apoA-I-only particles, but not the apoA-I:A-II particles, are decreased in patients with CAD (35). The fractional esterification rate of HDL and the concentration of HDL particles with pre-β mobility on gradient gel electrophoresis have also been suggested as biochemical measures that might provide information on the HDL subfractions and reverse cholesterol transport (2, 36, 37).

Many reasons may underlie the variations in associations between the HDL subfractions and CHD reported in earlier studies. The degree of true association between HDL subfractions and the coronary endpoint may vary randomly from one study to the next, or may be attributed to HDL-C assay characteristics such as within-subject variation, within-batch precision, and between-batch precision; all of these characteristics were seldom reported (1). Clinical aspects of studies should also be considered. Lower HDL-C concentrations appear to be a risk factor for CHD, and an increase of HDL-C concentrations, obtained with lipid-altering medications, has been associated with a decreased risk for CHD in clinical trials (38, 39). In addition, a decreased concentration of HDL-C among coronary cases in case-control studies may be partly attributed to the use of medications such as thiazide diuretics and beta blockers, which may depress HDL-C contents. This effect may result in ascribing a greater role than appropriate for the association of low HDL-C with CHD in such studies (40). It is reasonable to expect that concentrations of HDL subfractions may be lowered by the use of such medications, but little information is available.

The clinical utility of HDL subfraction determinations is left incompletely answered. Apparently, subfraction determinations by ultracentrifugation or double precipitation do not significantly improve CHD prediction once total HDL-C concentrations, obtained by conventional methods, are known. Current research is active concerning the determination of CHD and HDL subfractions by newer techniques such as immunoelectrophoresis and fractional esterification, but whether or not these research methods will help the clinician to predict or treat CHD is not known.

ApoE Isoforms, Lipids, and CHD

ApoE isoforms appear to be important modulators of plasma lipid concentrations. The isoforms may be determined from plasma (41), or from DNA analysis with polymerase chain reaction methodology (42). The six common phenotypes, listed in descending order of frequency, with approximate population frequencies, are denoted 3/3 (60%), 3/4 (20%), 2/3 (14%), 4/4 (3%), 2/4 (2%), and 2/2 (0.5%) (43). Similar frequencies of the apoE alleles have been reported for both sexes, and geographic comparisons have shown that the e4 allele is slightly more common among Finns and less common among Asians than in most Europeans (43, 44). About 60% of Caucasian populations have the 3/3 phenotype; the remaining 40% have one or both of the e2 or e4 alleles.

The relative impact of the e2 and e4 alleles on various plasma lipids has been considered by several investigators, as recently reviewed (43). Compared with that for the apoE 3/3 phenotype, mean cholesterol concentrations were lower for the 2/2 and 2/3 phenotypes, higher for the 3/4 and 4/4 phenotypes, and not different for the 2/4 phenotype (9). Taking into account the counterbalancing influences of the e2 and e4 alleles on cholesterol concentration, the e2 allele was associated with total cholesterol values that typically ranged from 1 to 2 mg/L lower than for the e3, and the e4 allele was associated with concentrations 0.5–1.0 mg/L higher (43). No consistent impact of the various apoE phenotypes on the
mean concentrations of HDL-C was observed, but both \( e2 \) and \( e4 \) appeared to be associated with a tendency toward higher triglycerides (9).

Investigators have subsequently studied coronary risk and atherosclerosis in association with the apoE alleles (8, 43). The apoE isofrom data were abstracted from published reports of several coronary and angiographic studies to estimate the \( e2 \) and \( e4 \) allele frequencies among cases and non-cases. The apoE allele frequencies allowed calculation of the relative odds for CHD according to the \( e4 \) and \( e2 \) alleles (45–49), as summarized in Fig. 2. Both studies with CHD endpoints (8, 50–54) and those for CAD (44, 55–57) are shown. The \( e4 \) allele was associated with an increased odds for CHD in five of six CHD studies (two were statistically significant) and in three of four angiographic studies (one was statistically significant). Similarly, the \( e2 \) allele was associated with CHD in three of six CHD reports (two were statistically significant) and in two of four angiographic studies (none statistically significant). Although a formal metaanalysis was not performed (45, 46), the data suggest that the \( e4 \) allele was mildly associated with increased risk of CAD and CHD, and that the \( e2 \) allele had less of an impact.

The apoE alleles appear to increase the relative odds for CHD and CAD largely by way of their association with abnormal lipids. The \( e4 \) allele was first described as positively associated with LDL-C, but associations with other lipids should also be considered; e.g., Dallongeville et al. (9) have described higher triglycerides associated with both the \( e2 \) and \( e4 \) alleles. The latter finding would also help to explain why the presence of the \( e2 \) allele is not associated with cardioprotection, even though the LDL-C concentrations are lower than for the \( e3 \) or \( e4 \) alleles. The role of screening for the presence of apoE alleles to identify persons at greater risk for CHD is not established at this time, but other avenues of research, such as whether the \( e2 \) or \( e4 \) allele might determine a response to lipid-lowering diets or medications or might predispose toward insulin resistance, are active at this time (58, 59).

References
1. Miller NE. Associations of high-density lipoprotein subclasses and apolipoproteins with ischemic heart disease and coronary arteriosclerosis [Review]. Am Heart J 1987;113:588–97.


