HDL Cholesterol and Other Lipids in Coronary Heart Disease

The Cooperative Lipoprotein Phenotyping Study

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SUMMARY The relation between coronary heart disease (CHD) prevalence and fasting lipid levels was assessed by a case-control study in five populations with a total of 6859 men and women of black, Japanese and white ancestry drawn from subjects aged 40 years and older from populations in Albany, Framingham, Evans County, Honolulu and San Francisco.

In each major study group mean levels of high density lipoprotein (HDL) cholesterol were lower in persons with CHD than in those without the disease. The average difference was small — typically 3–4 mg/dl — but statistically significant. It was found in most age-race-sex specific groups. The inverse HDL cholesterol-CHD association was not appreciably diminished when adjusted for levels of low density lipoprotein (LDL) cholesterol and triglyceride. LDL, total cholesterol and triglycerides were directly related to CHD prevalence; surprisingly, these findings were less uniformly present in the various study groups than the inverse HDL cholesterol-CHD association.

DURING THE PAST TWO DECADES considerable progress has been made delineating the role of the plasma lipoproteins in the development of coronary heart disease (CHD). Interest has focused chiefly on the very low density and low density lipoproteins (VLDL and LDL); there has been relatively little interest in the role of the high density lipoproteins (HDL), which ordinarily carry about 20% of the total plasma cholesterol. (In electrophoretic terms, HDL and LDL correspond to alpha and beta, while VLDL corresponds to prebeta.) The neglect of HDL cholesterol is curious since as early as 1951 Barr et al. reported that healthy men had higher levels of alpha (or high density) lipoprotein than did men with CHD.1 This early observation was confirmed in subsequent cross-sectional studies;2-7 moreover, women, who have less CHD than men, were noted to have higher levels of this lipoprotein.8

The Cooperative Lipoprotein Phenotyping Study of subjects drawn from epidemiologic studies of five diverse populations provides an excellent data base for examining the role of the various lipid fractions in coronary heart disease. In this report fasting levels of HDL, LDL and total cholesterol, and triglyceride are related to CHD prevalence.

Methods

Data from five study populations participating in the Cooperative Lipoprotein Phenotyping Study served as the basis for this report. The overall design and methods of these studies, all of which were derived from ongoing prospective studies of cardiovascular disease, have been described previously.10-18 Briefly they were: a population of male Civil Service employees in Albany, New York; a general population of black and white men and women in Evans County, Georgia; a general population of men and women in Framingham, Massachusetts; and general populations of men of Japanese ancestry living in Honolulu and San Francisco. In Albany and Framingham entire cohorts were invited to participate in the Cooperative Lipoprotein Phenotyping Study. The other three studies invited only random samples of their total study population but supplemented this by calling in all study subjects who were known from prior examinations to have CHD. Details of the design are given elsewhere.18 The CHD cases from each study were contrasted with non-cases (controls) from the same study population.

Plasma was obtained after an overnight fast of at least 12 hours. The fast was confirmed by a query at the time the participant appeared. An additional check on fasting was made by refrigerating an aliquot of plasma overnight and examining it the following day. If a surface wisp of chylomicrons appeared, it was presumed that the participant had not fasted. Only fasting specimens were used.

The procedures for preparing specimens were specified by the Laboratory of Molecular Disease of the National Heart and Lung Institute, which also provided training in the procedures. The participating laboratories used common primary standards as well as specimens from common pools supplied from the Lipid Standardization Laboratory of the Center for Disease Control for standardizing cholesterol and triglyceride measurements. These specimens were then used for determining levels of total, HDL, LDL and VLDL cholesterol and triglycerides. Lipid determination for the San Francisco and Honolulu studies were made at a common laboratory (in San Francisco); otherwise each study had its own laboratory. Details of collection, preparation, and lipid determination methods together with particulars respecting quality control have been published previously.18

CHD cases were defined in each center according to the established study criteria.8-18 Sixty-five per cent of the cases had definite myocardial infarction (MI) diagnosed by ECG; 48% had angina pectoris (AP) based on the Rose questionnaire or clinical assessment; and 7% had coronary in-

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Table 1. Mean HDL Cholesterol Levels and Number of Persons in Study Groups by Age, Race and Sex

<table>
<thead>
<tr>
<th>Study group</th>
<th>Age groups</th>
<th>Mean HDL cholesterol (mg/dl)</th>
<th>Number of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Albany</td>
<td>Framingham</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>48.3</td>
<td>48.4</td>
</tr>
<tr>
<td></td>
<td>50-59</td>
<td>48.7</td>
<td>45.5</td>
</tr>
<tr>
<td></td>
<td>60-69</td>
<td>49.6</td>
<td>45.5</td>
</tr>
<tr>
<td></td>
<td>70+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. The Relation of HDL Cholesterol to CHD Prevalence

<table>
<thead>
<tr>
<th>Age</th>
<th>Total</th>
<th>Albany</th>
<th>Framingham</th>
<th>Honolulu</th>
<th>San Francisco</th>
<th>Evans County-white</th>
<th>Evans County-black</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-49</td>
<td></td>
<td>-6.05**</td>
<td>-13.08</td>
<td>-3.20</td>
<td>-3.01**</td>
<td>-17.19**</td>
<td></td>
</tr>
<tr>
<td>50-59</td>
<td></td>
<td>-3.17**</td>
<td>-2.61</td>
<td>-3.01**</td>
<td>-4.22**</td>
<td>-10.07* -10.95*</td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td></td>
<td>-4.03**</td>
<td>-2.14</td>
<td>-6.23**</td>
<td>-3.88</td>
<td>-5.86 50.00</td>
<td></td>
</tr>
<tr>
<td>70+</td>
<td></td>
<td>-4.11**</td>
<td>-5.16</td>
<td>-4.93*</td>
<td>-7.59* -18.89*</td>
<td>-6.42 -1.33</td>
<td></td>
</tr>
<tr>
<td>All ages</td>
<td></td>
<td>-3.81**</td>
<td>-2.93*</td>
<td>-4.85*</td>
<td>-3.78** -3.34</td>
<td>-7.96* 16.32</td>
<td></td>
</tr>
</tbody>
</table>

Results

Table 1 gives the mean levels of HDL cholesterol by age, race and sex in the five study populations. Levels for women are about 10 mg/dl higher than for men and mean levels for blacks about 10 mg/dl higher than for whites. Although the black populations are very small, sex trends are consistent, i.e., black women have mean levels approximately 10 mg/dl higher than black men.

HDL Cholesterol Level in CHD Cases

The mean differences in HDL cholesterol levels between persons with CHD and those without are given in table 2. For all studies combined the differences are significant at a 5% level for the age group 40-49 and at a 1% level for the three remaining age groups. Mean HDL cholesterol levels for persons with CHD are consistently lower than for persons without CHD. This is true not only across the studies, but (with one minor exception) within each population when taken over all age groups. In fact, except in instances where the numbers in certain age groups were too small for construction of reliable statistics, this differential was also noted for individual age and sex groups.

A lower HDL cholesterol level was evident for both angina pectoris and myocardial infarction. When the data for all random samples of men 50-69 were pooled, persons without CHD had higher levels than those with either angina pectoris (AP) or myocardial infarction (MI) and both contrasts were statistically significant. The contrast was greater for AP than MI, AP cases having lower HDL levels than MI cases. The difference was statistically significant at a 5% level.

Levels of Total and LDL Cholesterol, and Triglyceride, in CHD Cases

The relations of other lipids to CHD prevalence are shown in table 3. For most study groups, the mean level in subjects with CHD is higher than the mean level in those without disease. The magnitude of the average differences for total and
L DL cholesterol, over all ages and across the five studies, is about 6 mg/dl; for triglyceride it is about 21 mg/dl. The associations shown in table 3, however, are less uniform than those shown in table 2 for HDL cholesterol and CHD.

Prevalence Rates by Lipid Level

The prevalence of CHD by level of HDL cholesterol is given in table 4 for the pooled studies. Prevalence rates for subjects with very low levels are about twice those for subjects with intermediate levels. There is no discernible gradient when subjects with intermediate levels are compared with those having high levels.

Correlations Among Lipids

To assess the association of HDL cholesterol to other lipids known to be related to CHD, correlation coefficients were computed. The correlations between HDL cholesterol and other cholesterol fractions are of a low order of magnitude, but their direction is consistent across populations; the associations of HDL cholesterol with total cholesterol are all positive, and those with LDL cholesterol are all negative. There is a moderately strong negative correlation between HDL cholesterol and triglyceride, which is also consistent across populations. Triglyceride has a small negative correlation with LDL cholesterol (except for women), and a moderate positive correlation with total cholesterol. By contrast, there is a very strong positive correlation between LDL and total cholesterol.

These intercorrelations are critical in assessing the case-control data. The multivariate linear discriminant function provides a statistical method useful for disentangling the role of the three lipids relative to CHD prevalence, taking these intercorrelations into account.

Results of discriminant analysis of CHD cases versus non-cases using HDL and LDL cholesterol and triglycerides are given in table 5. Bivariate analysis, in which the coefficient for each lipid is adjusted for age, may be contrasted with multivariate analysis, in which the coefficient for each lipid is adjusted for age and the other two lipids. Standardized bivariate coefficients for HDL cholesterol are negative and significantly different from zero at a 5% level (or less) in five of the six major study groups. The coefficients are slightly diminished in absolute magnitude in the multivariate analysis, but the inverse CHD-HDL cholesterol association persists in all but one of the studies. LDL and triglyceride have positive coefficients in both bivariate and multivariate analysis, although in the bivariate case they are not as consistently significantly different from zero as was HDL cholesterol.

When the data for men 50-69 were pooled, standardized discriminant coefficients for HDL and LDL cholesterol were almost the same in absolute magnitude, indicating similarly strong associations with CHD prevalence; whereas no association between triglyceride and CHD was found (table 5). Bivariate and multivariate results for each lipid from the pooled sample were almost identical.

To explore this further, two-way tables were prepared for each pair of lipids (HDL cholesterol, LDL cholesterol and triglycerides) (figs. 1-3). For each lipid, levels were chosen so as to divide the populations into three groups of roughly
Table 5. Discriminant Analysis of CHD Cases versus Noncases among Persons 50-69, Using Specified Lipids

<table>
<thead>
<tr>
<th>Study group</th>
<th>Bivariate standardized coefficients</th>
<th>Multivariate standardized coefficients</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HDL</td>
<td>LDL</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albany</td>
<td>-0.19*</td>
<td>0.20*</td>
<td>0.16*</td>
</tr>
<tr>
<td>Framingham</td>
<td>-0.37**</td>
<td>0.06</td>
<td>0.29**</td>
</tr>
<tr>
<td>Honolulu</td>
<td>-0.30**</td>
<td>0.95**</td>
<td>0.06</td>
</tr>
<tr>
<td>San Francisco</td>
<td>-0.09</td>
<td>0.29</td>
<td>0.43*</td>
</tr>
<tr>
<td>Evans County, white</td>
<td>-0.68**</td>
<td>0.59*</td>
<td>0.56*</td>
</tr>
<tr>
<td>Pooled random sample</td>
<td>-0.24**</td>
<td>0.20**</td>
<td>0.11*</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Framingham</td>
<td>-0.26*</td>
<td>0.29*</td>
<td>0.39**</td>
</tr>
</tbody>
</table>

*P < 0.05.  **P < 0.01.

Note: Bivariate includes age and specified lipids. Multivariate includes age and the three lipids.

equal size. While this analytical method is not as powerful as multivariate discriminant analysis it is sensitive to special interactions that are not allowed for in discriminant analysis. Data for men aged 50-69 were pooled from all five studies. Data for blacks and for women were omitted, since they were substantially different (table 1). Because Albany, Framingham and Honolulu had most of the CHD cases this part of the analysis is dominated by their results.

There is a very regular increase of CHD prevalence rates with increasing LDL cholesterol level at each level of HDL cholesterol (fig. 1). Similarly, for each level of LDL cholesterol, subjects with low levels of HDL cholesterol had a greater CHD prevalence rate than subjects with moderate or high levels of HDL cholesterol. The inverse relationship between HDL cholesterol and CHD, when taken over the three levels of LDL cholesterol, is significant \( P < 0.001 \) by a method of Mantel,\(^1\) as are the positive trends of CHD prevalence on LDL cholesterol level. While the HDL trends are not completely regular, the irregularities can be attributed to sampling variability and are not evidence of significant interaction between HDL and LDL.

Figure 2 presents a similar analysis of CHD prevalence in men by levels of triglyceride and LDL cholesterol for the pooled data. The prevalence of CHD among subjects with low levels of triglyceride are similar to those of subjects with intermediate or high levels; i.e., there is no discernible trend of CHD prevalence by triglyceride level when HDL level is taken into account. However, an inverse association between CHD prevalence and HDL cholesterol within each of the three triglyceride levels is apparent and when taken over the three levels is significant at a level of less than 1%.

Cross-classification of triglyceride with LDL cholesterol level (fig. 3) leads to the conclusion that either lipid has a statistically significant association with CHD prevalence when the other is held constant. In particular, there is a statistically significant trend of CHD prevalence rates by triglyceride level when LDL cholesterol level is taken into account whereas figure 2 shows that this trend is not seen when HDL level is taken into account.

In general, then, when contingency tables are constructed for the three lipids considered two at a time, HDL and LDL cholesterol emerge as consistently significant factors in CHD prevalence whereas triglyceride is less consistently a significant factor. Multivariate discriminant analysis for the pooled sample yields similar logistic regression coefficients for HDL and LDL cholesterol but for triglyceride no statistically significant relation to CHD was demonstrable in multivariate analysis.

Figure 1. Prevalence of CHD by levels of LDL and HDL cholesterol in men aged 50-69. Pooled data from Cooperative Lipoprotein Phenotyping Studies.

Figure 2. Prevalence of CHD by levels of triglyceride and HDL cholesterol in men aged 50-69. Pooled data from Cooperative Lipoprotein Phenotyping Studies.
Discussion

Two forms of analysis were used. In tables 2, 3 and 5 analyses are made for each specific study. This has the advantage of assuring that population and laboratory differences are fully allowed for. Since specimens from cases and noncases were intermingled in the local laboratories, the comparisons of their means were unbiased. In table 4 and figures 1–3, the data for men in the age range 50–69 from the main study populations are pooled. Such pooling could, conceivably, introduce artifactual features but does have the advantages accruing from a larger data base and simplifying the presentation. Moreover, the results are consistent with the study-specific analyses.

The analyses using cross-classification are supplemented by the analytically more powerful discriminant analysis. Not only are the results of the two approaches consistent, but the results of analysis by cross-classification tend to confirm the linear assumptions of the discriminant model.

The most important finding that emerges from these data concerns the inverse relationship between HDL cholesterol and CHD prevalence. The basic observation was made in several early studies1–4 but this report is the first describing a wide diversity of populations and using modern quantitative techniques for measuring lipoproteins. The results show that the inverse HDL-CHD association is characterized by a high degree of generality and strength.

The generality of the observation is striking in view of the small magnitude of the difference in mean level of HDL cholesterol between subjects with CHD and those without the disease. The difference is typically 3–4 mg/dl, about 7% of value, and less than the usual technical error of the measurement, but it is found in all age groups studied, in practically all populations studied, for both categories of CHD studied, and within and between sexes. In fact, the uniformity of the finding even exceeds that for the direct relationship observed among the study groups between total cholesterol concentration and CHD prevalence.

The independence of the association between HDL cholesterol and CHD has been a point of concern because there is a moderate inverse association between HDL cholesterol and triglyceride.5, 8, 10 In the studies reported here, co-variance among the various lipid factors was controlled both by analysis of cross-tabulations and by discriminant analyses. Both analytic approaches leave no doubt that the inverse association between HDL cholesterol and CHD largely persists even when other lipid factors are considered; that is, knowledge of HDL cholesterol appears to provide risk information beyond that available from the usual lipid risk factors.

The strength of the inverse association between HDL cholesterol and CHD is such that there is a twofold gradient of CHD prevalence between subjects at the higher and lower ends of the distribution of HDL cholesterol concentration. Examination of pooled data for men aged 50–69 shows that this gradient of risk occurs in the lower half of the HDL cholesterol concentration distribution. Thus, excess CHD is associated with a low level of HDL cholesterol, but there appears to be no advantage to having a higher than average level. However, prospective data from Framingham (unpublished) show a decreasing CHD incidence even at above-average levels.

HDL cholesterol levels may provide partial explanation of hitherto unexplained population differences in CHD prevalence and incidence rates. Rates are lower in Evans County blacks than whites, unexplained by the major risk factors of blood pressure and serum cholesterol.20 The distinctly higher HDL cholesterol levels in blacks than whites may in part account for this. On the other hand, Honolulu Japanese men have only half the CHD incidence of men in Framingham14 but the HDL cholesterol levels of these two populations are practically the same.

The epidemiologic data presented in this report include only information on prevalence of prior episodes of CHD. The question of a precursive association can only be answered by prospective studies of the relationship of HDL cholesterol to CHD incidence. Such a study was reported in 1966 by Gofman who found that lower HDL levels were followed by greater CHD incidence among young men.24 A similar prospective finding among middle-aged Israeli men has also been reported.26 Unpublished data from Framingham also show a lower HDL cholesterol level preceding the appearance of CHD.

The finding that HDL cholesterol concentration is inversely related to subsequent development of CHD supports but does not prove the possibility that HDL elevation may prevent the development of CHD. If this were the case, there would be potential for identifying factors that could favorably influence health by elevating HDL levels. The fragmentary information on what maneuvers will lead to an increase in HDL cholesterol levels suggest that physical activity,21, 22 weight loss23 and a low carbohydrate intake24, 25 may be beneficial.

If high HDL levels are shown to be protective, the mechanism may be the one proposed by Miller and Miller.18 They suggested that plasma HDL is a transport mechanism for carrying cholesterol from the peripheral tissues to the liver where it is catabolized and excreted. Hence, higher levels of HDL cholesterol would be associated with less atherosclerosis. Some evidence supporting this hypothesis was presented, but as yet, there has not been sufficient test of its validity.

The data in this report show a direct relationship between fasting plasma triglyceride concentration and prevalence of
CHD. This relationship is found in most of the study groups but when other lipids are considered is only equivocally significant. However, even this limited finding is important since controversy still exists concerning whether triglyceride concentration is a risk factor for CHD, separate from its association with cholesterol. However, it should be noted that we have not adjusted for co-variance between triglyceride concentration and other relevant variables such as body weight and the presence of diabetes.

While the full implications of these findings remain for future work to elucidate, the virtue of partitioning total cholesterol in assessing CHD risk is unequivocally demonstrated. Clearly if one fraction (HDL cholesterol) has a negative association with the risk of CHD while the other two (LDL and VLDL cholesterol) have positive associations with CHD risk, then the arithmetic sum, i.e., total cholesterol, must be a less sensitive indicator of risk than an appropriately weighted algebraic sum. (From a practical point of view fasting triglyceride is the appropriate method of measuring VLDL cholesterol since practically all the fasting triglyceride is carried in the VLDL portion and the two measures are strongly correlated.) However, the laboratory making the measurements must have good precision; otherwise the rate of misclassification may be intolerably high. Still there is now no question from a scientific point of view that partitioning total cholesterol is preferable to a single measure of total cholesterol in assessing CHD risk; and a lipid profile based on HDL and LDL cholesterol and triglyceride is a logically preferable method of measuring the CHD risk associated with lipid characteristics. The appropriate statistical weighting, however, is yet to be determined and should come from prospective data rather than case-control studies.

References
HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study.
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