Plasma levels of total high density lipoprotein cholesterol (HDL) and its subfractions (HDL$_2$ and HDL$_3$) were measured in 366 healthy Caucasian males; these values were related to a number of coronary risk factors. On univariate statistical analysis, total HDL was negatively correlated with cigarette consumption, body mass index, and serum triglycerides, and positively associated with level of physical activity and alcohol consumption. HDL$_2$ showed an inverse relationship with cigarette consumption, body mass index, triglycerides, and systolic blood pressure and a positive relationship with age. HDL$_3$ was negatively correlated with cigarette smoking, body mass index, and triglycerides and positively associated with exercise level and alcohol consumption. Total HDL and HDL$_2$ were inversely related to coronary risk rating, but HDL$_3$ showed no significant correlation. Many of these relationships became nonsignificant after allowing for the effects of other variables. In particular, none of the HDL measurements correlated significantly with risk score after allowing for the effect of triglycerides. There is insufficient evidence at present to recommend the inclusion of HDL subfractions as routine screening tests for heart disease. 

Methods

Subjects

The study population consisted of Caucasian males attending a health screening center in north London. Subjects with a medical history of heart disease or diabetes or with a current complaint of angina, and those with an abnormal fasting glucose level were excluded from the study. Also excluded were subjects with electrocardiographic evidence of ischemic heart disease and those taking antihypertensive or lipid-lowering medication. We were left with a final sample of 366 healthy men.

The screening process, which has been described in detail elsewhere, included a detailed medical history and physical examination, chest x-ray and 12-lead electrocardiogram, and collection of blood for a number of biochemical and hematological measurements. Blood pressure was taken using a random-zero (Gelman Hawksley) sphygmomanometer, the value recorded being the mean of two measurements. Stage V end-point was used for the diastolic pressure. Weight (kg) and height (m) were recorded for each subject, and body mass index (BMI) was calculated as weight/height$^2$. Cigarette smoking, alcohol consumption, and exercise level were assessed by a computerized questionnaire. Ex-smokers and those smoking only pipes or cigars were coded as non-cigarette smokers. Teetotalers (10 subjects) were omitted from the alcohol analysis. All procedures were approved by the ethical committee of BUPA Medical Research, and informed consent was obtained from each subject.

The subjects fasted overnight for 14 hours. Whole blood
Triglycerides were determined by the differential dextran sulphate precipitation method of Gidez et al.\textsuperscript{23} Although the double precipitation method lacks specificity, it is convenient and correlates well with ultracentrifugation methods of HDL subfractionation.\textsuperscript{23, 24, 25} A major difficulty is in determining the best concentration of dextran sulphate for the optimal separation of HDL\textsubscript{2} and HDL\textsubscript{3}. The recommended final concentrations ranged from 0.87 g/l for serum\textsuperscript{25} to 1.3 g/l for plasma.\textsuperscript{23} We previously advocated that the optimal concentration of dextran sulphate should be experimental-ly derived for each new batch of reagent.\textsuperscript{26} For this study, we used a final concentration of 1.3 g/l. Clotted blood samples were also collected for the determination of serum cholesterol and triglycerides. These were measured by enzymatic methods\textsuperscript{27, 28} on a Centrifichem centrifugal analyzer.

### Statistical Analysis

The statistical analysis was done on an AMDAHL 470 V7/B computer, using the Statistical Package for the Social Sciences.\textsuperscript{29} The associations between HDL subfractions and the continuous variables were assessed by means of Pearson product-moment correlation coefficients, and F-tests were used for discrete variables. Univariate tests for linear trend were carried out by analysis of variance, and the effects of confounding variables were allowed for by means of partial correlation, after taking the logarithms of systolic and diastolic blood pressure, triglycerides, and BMI and applying a square-root transformation to the number of cigarettes, the exercise level, and the alcohol consumption.

### Results

The mean age of the subjects was 45.4 years (SD 10.5 years). The mean values of total HDL, HDL\textsubscript{2}, and HDL\textsubscript{3} were 1.23 ± 0.31 mmol/l, 0.42 ± 0.24 mmol/l, and 0.81 ± 0.15 mmol/l, respectively.

Table 1 shows the univariate relationships between total HDL and its subfractions and various coronary risk factors. For BMI, blood pressure, and triglycerides, the sample has been divided into approximate quarters (Q1 to Q4) on the basis of the distributions of these measurements. Table 2 shows the partial Pearson correlation coefficients between the three HDL measurements and each variable after allowing for the effects of all the other variables. Thus, for example, the correlation between total HDL and age is 0.184 after allowing for the effects of smoking, exercise, alcohol consumption, body mass index, blood pressure, and triglycerides.

#### Age

Both total HDL and HDL\textsubscript{2} showed a positive association with age. HDL\textsubscript{3} and age were uncorrelated.

#### Cigarette Smoking

All three measurements showed a decrease in the mean level associated with cigarette smoking. However, these effects became nonsignificant after controlling for other variables, in particular triglycerides.

#### Exercise

The total HDL and HDL\textsubscript{2} showed increases in the mean levels associated with physical activity. After multivariate analysis, this was only significant in the case of HDL\textsubscript{3}.

#### Alcohol Consumption

In each case there was an association between the mean level and alcohol consumption, but only for HDL\textsubscript{2} did...
a clear pattern emerge: increased alcohol consumption led to increased mean HDL\textsubscript{3} levels.

**Body Mass Index**

All three measurements showed a negative correlation with body mass index (BMI). The association was much stronger for HDL\textsubscript{2} than for HDL\textsubscript{3}. However, after adjustment for the effects of other variables, these correlations ceased to be significant. As with cigarette smoking, this was due mainly to the confounding effect of triglycerides.

**Blood Pressure**

After multivariate analysis there remained a weak positive correlation between diastolic blood pressure and both total HDL and HDL\textsubscript{3}. Systolic pressure was not related to either fraction.

**Triglycerides**

There was a strong negative correlation with all three measurements, the association being much greater for HDL\textsubscript{2} than for HDL\textsubscript{3}. This remained true after allowing for the effects of all other variables.

**Risk Score**

A cumulative risk score was calculated for each subject based on weight, diastolic blood pressure, cigarette smoking, total cholesterol, and exercise. Full details have been published elsewhere\textsuperscript{6} and are summarized in Table 3. A score of 0, 1, or 2 was allocated for each measurement, and the sum of these individual scores was taken as the cumulative risk rating. The possible range was from 0 (low risk) to 10 (high risk). Because of the small numbers, subjects scoring 5 or higher were grouped together. Table 4 shows the univariate relationships between mean HDL, HDL\textsubscript{2}, and HDL\textsubscript{3} levels and cumulative risk score, and Figure 1 presents these results graphically. Total HDL and HDL\textsubscript{2} both showed an inverse relationship with increasing risk score. For HDL\textsubscript{3} there was no correlation. Once again, however, after adjusting for triglyceride levels, the correlations between risk and all three HDL measurements became nonsignificant (Table 2).

**Discussion**

Few studies have reported on the association between HDL subfractions and coronary risk factors. Haffner et al.\textsuperscript{19} studied the effects of smoking, alcohol, and adiposity on HDL subfractions in a group of 33 men and 17 women. They found that HDL\textsubscript{2} correlated much more strongly with BMI and triglycerides than did HDL\textsubscript{3}. We have confirmed these findings in a sample of 366 healthy men, although in our study the correlations with body mass index were secondary to the correlation with triglyceride levels. They also found that alcohol consumption was positively correlated with HDL\textsubscript{3}, but not with HDL\textsubscript{2}. Haskell et al.\textsuperscript{30} have demonstrated the same effect in a group of 77 middle-aged men, and have also shown\textsuperscript{71} that cessation and resumption of moderate alcohol intake affects HDL\textsubscript{3} levels, whereas HDL\textsubscript{2} remains unchanged. Because of the considerable evidence that coronary heart disease relates to changes in HDL\textsubscript{2} rather than total HDL,\textsuperscript{32-35} these authors go on to suggest that the lower incidence of heart disease in moderate drinkers as compared with nondrinkers\textsuperscript{38-40} may be mediated through a mechanism unrelated to HDL. In our study, likewise, the only consistent relationship with alcohol intake was an increase in HDL\textsubscript{3} with increasing consumption. The association with HDL\textsubscript{2} was much weaker.

Table 2. Partial Correlation Coefficients

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Cigarettes</th>
<th>Exercise</th>
<th>Alcohol</th>
<th>BMI</th>
<th>Systolic BP</th>
<th>Diastolic BP</th>
<th>Triglyceride</th>
<th>Risk score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total HDL</td>
<td>0.184\textdagger</td>
<td>-0.056</td>
<td>0.045</td>
<td>0.254\textdagger</td>
<td>-0.059</td>
<td>-0.088</td>
<td>0.105\textdagger</td>
<td>-0.502\textdagger</td>
<td>0.011</td>
</tr>
<tr>
<td>HDL\textsubscript{2}</td>
<td>0.251\textsection</td>
<td>-0.010</td>
<td>-0.026</td>
<td>0.156\textdagger</td>
<td>-0.038</td>
<td>-0.064</td>
<td>0.029</td>
<td>-0.556\textsection</td>
<td>0.012</td>
</tr>
<tr>
<td>HDL\textsubscript{3}</td>
<td>-0.009</td>
<td>-0.091</td>
<td>0.119\textasterisk</td>
<td>0.261\textsection</td>
<td>-0.058</td>
<td>-0.076</td>
<td>0.150\textdagger</td>
<td>-0.212\textsection</td>
<td>-0.002</td>
</tr>
</tbody>
</table>

BMI = body mass index; BP = blood pressure.
\textdagger p < 0.05; \textsection p < 0.025; \textdagger p < 0.005; \textsection p < 0.001.

Table 3. Risk-Rating Score Sheet

<table>
<thead>
<tr>
<th>Factor</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative weight (%)</td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>0</td>
</tr>
<tr>
<td>100–120</td>
<td>1</td>
</tr>
<tr>
<td>&gt;120</td>
<td>2</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td></td>
</tr>
<tr>
<td>&lt;90</td>
<td>0</td>
</tr>
<tr>
<td>90–110</td>
<td>1</td>
</tr>
<tr>
<td>&gt;110</td>
<td>2</td>
</tr>
<tr>
<td>Cigarette smoking (no/day)</td>
<td></td>
</tr>
<tr>
<td>Nonsmoker or exsmoker</td>
<td>0</td>
</tr>
<tr>
<td>1–20</td>
<td>1</td>
</tr>
<tr>
<td>&gt;20</td>
<td>2</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td></td>
</tr>
<tr>
<td>&lt;6.7</td>
<td>0</td>
</tr>
<tr>
<td>6.7–7.8</td>
<td>1</td>
</tr>
<tr>
<td>&gt;7.8</td>
<td>2</td>
</tr>
<tr>
<td>Exercise</td>
<td></td>
</tr>
<tr>
<td>Very active</td>
<td>0</td>
</tr>
<tr>
<td>Active or slightly active</td>
<td>1</td>
</tr>
<tr>
<td>Inactive</td>
<td>2</td>
</tr>
</tbody>
</table>

Relative weight was defined as actual weight as a percentage of expected weight, where expected weight was calculated from a regression equation based on the individual's age and height. BP = blood pressure.
Table 4. Mean Total HDL and Subfractions Related to Cumulative Risk Rating (Univariate Analysis)

<table>
<thead>
<tr>
<th>Cumulative risk rating</th>
<th>No</th>
<th>Total HDL</th>
<th>HDL₂</th>
<th>HDL₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>46</td>
<td>1.30 ± 0.33§</td>
<td>0.47 ± 0.25 §</td>
<td>0.83 ± 0.15 (NS)</td>
</tr>
<tr>
<td>1</td>
<td>91</td>
<td>1.29 ± 0.33</td>
<td>0.48 ± 0.25</td>
<td>0.82 ± 0.14</td>
</tr>
<tr>
<td>2</td>
<td>92</td>
<td>1.23 ± 0.32</td>
<td>0.43 ± 0.25</td>
<td>0.81 ± 0.14</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>1.23 ± 0.29</td>
<td>0.41 ± 0.20</td>
<td>0.82 ± 0.16</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>1.15 ± 0.27</td>
<td>0.36 ± 0.20</td>
<td>0.79 ± 0.14</td>
</tr>
<tr>
<td>≥5</td>
<td>34</td>
<td>1.06 ± 0.26</td>
<td>0.28 ± 0.18</td>
<td>0.78 ± 0.15</td>
</tr>
</tbody>
</table>

Values are mmol/l ± SD.
Test for trend: §p < 0.001; NS = not significant.

Berg et al.41 investigated the changes in HDL subfractions induced by a single period of extended physical exercise, and concluded that exercise "induces an increased formation of HDL particles of lower density from HDL particles of higher density." This is in agreement with the findings of Nye et al.42 who studied 17 men over the course of a 10-week training program and found that HDL₂ rose, HDL₃ fell, and consequently total HDL remained unchanged. On the other hand, many studies have found higher levels of total HDL in active as opposed to inactive subjects.7-9 43 and in a review article on the effect of exercise on plasma HDL44 the authors concluded that "the increase in plasma high density lipoprotein appears to be the result largely of an increase in the less dense HDL₂ subfraction." In this study, however, we found significantly higher levels of HDL₃, but not HDL₂, in active subjects.

The higher mean levels of total HDL and HDL₂ in men over age 55 years in our study may be due to either an enrichment of this group by subjects with high levels, or a depletion of those with low levels—the so-called 'survivor effect.'

The much stronger negative correlation between triglycerides and HDL₂ than between triglycerides and HDL₃ is in agreement with earlier findings16, 19, 23, 45 and may be due to the derivation of HDL₂ from HDL₃ by its interaction with VLDL, as proposed by Patsch et al.17

The results depicted in Figure 1 confirm the importance of HDL₂ as the "active" subfraction in heart disease. As the risk weighting increases, total HDL and HDL₂ levels decrease almost in parallel, whilst HDL₃ remains virtually unchanged. However, the fact that these relationships become nonsignificant when the effects of other variables are taken into account suggests that HDL per se may not be an independent risk factor for heart disease. This was the conclusion reached by Shaper et al.46 who used multivariate methods in a prospective study of 7735 middle-aged men. In our study, the effect of HDL on risk score and on most individual risk factors was mediated by its association with triglyceride—although the value of this measurement itself as an independent risk factor has been queried by some.46, 47 However, despite the lack of independence, total HDL remains a useful summary indicator of CHD risk. It is a relatively simple and inexpensive assay to perform, and plays an important role in any battery of screening tests. On the other hand, the evidence from our study would suggest that the increase in predictive power gained by measuring HDL subfractions is marginal and insufficient to justify the additional expense of including them as routine tests.

It should be stressed that our conclusions are based on a cross-sectional analysis that correlates HDL subfractions with generally accepted risk factors. We deliberately excluded from the study people with heart disease because the aim of health screening is to detect individuals at risk before clinical symptoms become manifest; thus we were interested in the correlations within a distribution of

Figure 1. Mean total high density lipoproteins (HDL) and subfractions by cumulative risk score (univariate analysis).
HDL SUBFRACTIONS AND CORONARY RISK

Robinson et al.

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7. Gordon A. A. Ferns Is grateful to the Wellcome Trust for a Pathology


Index Terms: lipoproteins • HDL cholesterol • smoking • alcohol drinking • exertion • blood pressure • body weight • triglycerides
High density lipoprotein subfractions and coronary risk factors in normal men.
D Robinson, G A Ferns, E A Bevan, J Stocks, P T Williams and D J Galton

Arterioscler Thromb Vasc Biol. 1987;7:341-346
doi: 10.1161/01.ATV.7.4.341

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