High-density Lipoprotein Subfractions
in Normolipidemic Patients
with Coronary Atherosclerosis

J.G. Brook, M.R.C.P., M. Aviram, D.Sc., A. Viener, M.D., E. Shilansky, M.D.,
and W. Markiewicz, M.D.

SUMMARY High-density lipoprotein (HDL) levels were studied in 10 male patients with severe coronary
atherosclerosis and in 10 well-matched controls. All subjects were normolipidemic, and the presence of a
disease or other factor influencing HDL levels was excluded. Very low density lipoprotein and low-density
lipoprotein levels were similar in both groups, but HDL concentration was significantly lower in the patient
group. Analysis of HDL subfractions revealed that both HDL₁ and HDL₃ concentrations were significantly
lower in the patient group. The composition of both HDL subfractions was also altered in the patient group:
an increased cholesterol-to-protein ratio was found. These data strengthen the evidence in support of an
important and independent role for HDL in the pathogenesis of coronary atherosclerosis. It appears that
both HDL₂ and HDL₄ are implicated and that both the concentration and composition of HDL are
important.

CORONARY heart disease (CHD) is the main cause of
death in the Western world. The pathogenesis of CHD
is not clear, and the interrelationships of many factors
appear to have a role. Hyperlipidemia increases the
risk for CHD, but about half of the patients with CHD
have normal plasma lipid levels. An inverse relation-
ship between CHD and high-density lipoprotein
(HDL) levels has been established for both the chole-
terol and apoprotein components of the lipoprotein. HDL, however, is not a homogeneous moiety, and
the concentration and composition of the HDL subclasses
in CHD have hardly been studied. Gofman et al. determined the HDL₁ and HDL₃ concentrations of the
lipoproteins by analytic ultracentrifugation. Concentra-
tions of both subfractions were depressed in CHD
patients, but because most of the patients were hyper-
lipidemic, interpretation of the results proved difficult.

A positive cholesterol balance in the cells of the primary atherosclerotic lesion may be responsible for
the evolution of this lesion into an advanced plaque, and HDL may remove cholesterol from extrahepatic
tissues and thus prevent its accumulation. We there-
fore decided to study HDL concentration and composi-
tion in normolipidemic CHD patients. Patients with
definite angiographic evidence of the disease were
selected. Both the lipid and protein components of HDL
and its subfractions were determined.

Methods

Patients

Ten men, ages 38–64 years (mean 55 years), who
had undergone coronary angiography for symptomatic
CHD and were shown to have an occlusion of more
than 70% of the diameter of at least one major coronary
artery and who also fulfilled the following criteria were
selected for study. None gave a history of hyperlipide-
mia, alcoholism, diabetes mellitus, or kidney, liver or
throid disorders. None was following a special diet,
taking drugs, had suffered a recent acute illness, or had
a recent drastic change in physical activity. Obese pa-
tients were excluded. Levels of fasting blood sugar,
urea nitrogen, plasma creatinine, uric acid,
GOT, alkaline phosphatase, anti-thyroid hormone,
and thyroxin, fasting plasma cholesterol and triglycerides,
and hematocrit and platelet concentration were all
normal.

The control group comprised 10 men, ages 33–65
years (mean 53 years), attending the outpatient depart-
ment for a periodic health examination. They all ful-
filled the above criteria and none complained of angina
pectoris. All had normal resting ECGs and normal
standard ergometric exercise tests.

Both patients and controls were Ashkenazi Jews
originating from Eastern Europe. All had sedentary
jobs, but the control subjects had a higher socioeco-
nomic status.

Five of the patients were smokers; four had smoked
20–30 cigarettes daily for 20–40 years and one smoked
10–20 cigarettes a day for 9 years. The four smokers in
the control group had all smoked 20–30 cigarettes daily
for 20–40 years. Four persons in each group had
hypertension, defined as diastolic pressure above 95
mm Hg. The hypertensives in both groups had a sys-
tolic pressure of 140–180 mm Hg and a diastolic pres-
sure of 95–110 mm Hg. The weekly alcohol intake of
both groups was minimal.

Lipoprotein fractions were determined from blood
draws of each subject after a 14-hour fast on two separate occasions. Sodium EDTA was the
anticoagulant and plasma cholesterol and triglyceride
levels were determined by centrifugal autoanalyzer
(Electronucleonics Gemsae). Lipoproteins were iso-
lated by preparative ultracentrifugation. Potassium
bromide was used for increasing densities: very low
density lipoproteins (VLDL), d < 1.006; low-density
lipoprotein (LDL), d = 1.006–1.063; HDL, d = 1.063–1.210; HDL₂, d = 1.063–1.125; and HDL₃, d = 1.125–1.210. Lipoprotein purity was determined

From the Lipid Research Unit and Department of Cardiology, Rambam Medical Center and Faculty of Medicine, Technion, Haifa, Israel.
Address for correspondence: Dr. J.G. Brook, Lipid Research Unit, Rambam Medical Center, Haifa, Israel.
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by immunodiffusion. The cholesterol and protein contents of each fraction were determined, the former by the autoanalyzer technique and the latter by the procedure of Lowry et al.9 The standard deviations between the two groups for each variable were compared by the F test.10 When no differences in standard deviation were demonstrated, the t test was used to examine the difference in the mean values from the two groups.

Results

The patient and control groups were well matched as regards total cholesterol and total triglyceride levels (table 1). The cholesterol concentration was 175–233 mg/dl in the patient group and 175–230 mg/dl in the control group. The triglyceride concentration was 84–140 mg/dl in the patients and 68–140 mg/dl in the controls.

Table 2 depicts the cholesterol, triglyceride and protein concentrations of the lipoprotein classes in the two groups. No significant differences were found in either the lipid or protein concentrations of VLDL or LDL. All components of the HDL fraction, however, were significantly lower in the CHD group. HDL cholesterol was 39% lower, HDL triglyceride 40% lower and HDL protein 53% lower than in the control group. All components of both HDL2 and HDL3 were also reduced (table 3). The HDL2 cholesterol was 34% lower, HDL2 triglyceride 34% lower and HDL2 protein 50% lower than in the control group. The differences in the HDL3 fraction were greater: cholesterol was 46% lower, triglycerides 36% lower and protein 55% lower than in the controls. The patient group also had changes in the composition of the HDL2 and HDL3 subfractions. The patients had a higher cholesterol-to-protein ratio in both HDL subfractions than the controls. This difference was more pronounced for HDL2, but the changes in both subfractions achieved a similar level of significance (p < 0.01).

The HDL2/HDL3 ratio for each component of these subclasses was compared in the patient and control groups (table 4). In the patient group, this ratio tended to increase (NS). No correlation was found between the extent of the coronary disease, the quality of the left ventricular function and the HDL levels in our patient group.

Discussion

Our data confirm the inverse relationship between CHD and plasma HDL levels that has been extensively reported by others.11 Our work has differed from most others in that the presence of CHD was proved angiographically in all of our patients; in only one other reported study have HDL levels been evaluated in patients with CHD determined angiographically.12 Furthermore, we attempted to emphasize the independent nature of the HDL variable by studying two homogenous groups that we hoped differed only with regard to the presence of CHD. Because our control patients were asymptomatic and had had normal ECGs and stress tests, coronary angiography could not be ethically justified. As a result, CHD might have been underdiagnosed in this group. Plasma lipid and lipoprotein levels other than HDL were normal in both groups. The presence of factors known to influence HDL levels precluded the selection of patients as control subjects for the study. The high prevalence of hypertension and smoking in the population prevented individuals with these problems from being excluded, but the number of smokers and hypertensive subjects was similar in both groups. Recently, a positive correlation has been established between socioeconomic status and HDL levels.13 Our control group consisted of executives who had a high socioeconomic status. This factor might have introduced a negative bias into the study.

In most studies, HDL levels have been determined from the concentration of HDL cholesterol alone as measured by the heparin-manganese-chloride precipitation method.14 On occasion, apolipoprotein A1 has been selected as representative of the HDL concentration.3 However, it remains far from clear whether the CHD "protective factor" inherent in HDL correlates best with HDL cholesterol or HDL protein levels or with the different components of the subclasses HDL2 and HDL3. We used preparative ultracentrifugation to isolate HDL and its subclasses and attempted to be comprehensive in determining the concentrations of the cholesterol, triglyceride and protein in each component. To our knowledge, this type of analysis has not been reported previously. HDL2 and HDL3 levels have been determined by analytic ultracentrifugation.
TABLE 3. High-density Lipoprotein Subfractions

<table>
<thead>
<tr>
<th></th>
<th>HDL-2 (mg/dl)</th>
<th></th>
<th>HDL-3 (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chol</td>
<td>Trig</td>
<td>Prot</td>
</tr>
<tr>
<td>Control</td>
<td>27 ± 10</td>
<td>7.5 ± 2</td>
<td>72 ± 2</td>
</tr>
<tr>
<td>Patients</td>
<td>18 ± 6</td>
<td>5 ± 2</td>
<td>36 ± 12</td>
</tr>
</tbody>
</table>

$p < 0.005$  $p < 0.05$  $p < 0.01$  $p < 0.01$

Values are mean ± sd.

Abbreviations: See table 2.

TABLE 4. Ratio of High-density Lipoprotein-2 to High-density Lipoprotein-3

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.22 ± 0.42</td>
<td>1.07 ± 0.51</td>
<td>0.33 ± 0.08</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>1.50 ± 0.23</td>
<td>1.11 ± 0.53</td>
<td>0.36 ± 0.07</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± sd.

in CHD patients in one reported study only. Levels of both subclasses were depressed, but the method did not determine changes in any of the components.

Our patients with CHD had low HDL levels; this finding was unrelated to the extent of disease. Cholesterol, triglyceride and protein components of both total HDL and HDL subclasses were universally depressed. Differences in HDL composition in the patient group were also apparent: the ratio of cholesterol to protein was increased in the HDL2 and HDL3 subfractions. The careful selection of our subjects suggested that these findings were independent of any other variable. LDL and VLDL levels and the cholesterol, triglyceride and protein composition of our subjects were normal, and CHD patients did not differ from controls. There was no correlation between HDL and its components and the components of the other lipoproteins. The normal levels of the VLDL and LDL proteins in our patients did not conform with the findings of Sniderman et al., who found increased apolipoprotein B levels in their normolipidemic CHD patients. It thus appears that in our selected patients, the low HDL levels represent a primary phenomenon, perhaps genetically determined, since correlations with other variables were not found.

The protective effect of HDL has been described as being due to the role of this lipoprotein in "reversed cholesterol transport," whereby excess cholesterol is taken up by the HDL and removed by the liver. Alternatively, HDL may reduce delivery of cellular cholesterol by impairing LDL binding to the cell surface. Another mechanism relates to the role of HDL as a scavenger during chylomicron and VLDL hydrolysis, whereby HDL availability would determine the amount and kind of "remnants" taken up by the macrophages of the arterial wall. None of these mechanisms have been proved, but in our group of CHD patients with their low VLDL and LDL levels, delivery of excess cholesterol does not appear to be the problem. The low HDL levels, however, could result in impaired cholesterol removal or give rise to the accumulation of "remnants" after catabolism of triglyceride-rich particles (TRP) such as chylomicrons and VLDL. The accumulation of these remnants might promote atherosclerosis. An increase in the HDL2/HDL3 ratio is said to indicate increased TRP degradation, during which the HDL2 particle accumulates surface remnants from the triglyceride particles and is converted into HDL3. HDL2 and HDL3 levels were decreased in our patients, indicating possible impairment of TRP degradation and accumulation of remnants.

In this study, we focused attention on the independent relationship between low HDL levels and established CHD. All components of the HDL moiety were reduced and some alterations in HDL composition were observed. These findings indicate disturbance of HDL metabolism, but the mechanisms underlying the relationship between these changes and the development and propagation of atherosclerosis remain unclear and await further study.

References

Sex Differences in Obstructive Coronary Artery Disease in Patients 65 Years of Age or Older with Angina Pectoris

ROBERT J. BOUCEK, M.D., RENZO ROMANELLI, M.D., WILLIAM H. WILLIS, JR., M.D., and WINSTON A. MITCHELL, M.D.

SUMMARY The symptoms, ECGs, exercise stress responses, left ventricular perfusion and function, and the topography of obstructive coronary artery disease (≥ 70% cross-sectional stenosis in the left main, left anterior descending, circumflex, and right coronary arteries and their major branches) were analyzed in 200 patients 65 years of age or older with angina pectoris. Males showed a significantly higher incidence of stenosis of the left main and the left circumflex coronary arteries and poorer left ventricular perfusion than females. One-vessel obstructive disease was found in one-fifth of the aged patients of each sex with angina pectoris.

CORONARY Atherosclerosis begins earlier and advances more rapidly in men than in women. Sex differences are maximal in the fifth decade, but diminish after the seventh decade.1,2 Because coronary artery surgery is an increasingly common recommendation for geriatric (≥ 65 years) patients with angina pectoris, the atherosclerotic topography and its consequences on ventricular perfusion and function are important information in planning therapy. We therefore studied 100 patients of each sex who were 65 years of age or older and had angina pectoris.

Materials and Methods

Records were reviewed of 200 consecutive patients 65 years of age or older, principally of Caucasian origin, drawn from the middle and upper economic strata who were studied at Loma Linda University Medical Center from 1972 to 1981. The patients were of diverse ethnic and religious backgrounds and all underwent cardiac evaluation because of angina pectoris. Each patient received a complete cardiac workup, which included cardiac catheterization and coronary arteriography by the Judkins technique.3 Sixty males and 48 females underwent exercise stress testing on a computer-controlled treadmill with 3-minute work loads, beginning with 1.7 mph at a 5% grade and increasing to 1.7 mph at a 10% grade, 2.5 mph at 16% grade, 3.4 mph at 14% grade, and 4.2 mph at a 16% grade. The exercise was continued to the point of fatigue, chest pain or dyspnea. Criteria of a positive response included horizontal ST-segment depression or elevation > 1 mm in lead I and > 1.5 mm in leads II and III and in the precordial leads. Selective left ventriculography was performed using simultaneous biplane recording; volumetric calculations were derived by computerized motion analysis.

The extent of arterial narrowing was estimated from multiple projections (right anterior oblique, left anterior oblique and lateral) and expressed as a percentage of cross-sectional area in the left main coronary (LCA), the anterior descending (LAD), the circumflex (Cx), and right coronary (RCA) arteries and their major branches. A reduction ≥ 70% of the cross-sectional area of the vessel lumen was considered hemodynamically significant. Details of these procedures have been described.4

Resting myocardial scintigrams were performed on 180 patients after selective injection (at coronary arteriography) of technetium-99m microspheres (2.6–3.4 μCi) into the left and right coronary arteries. Projections corresponding to those of arteriography itself were obtained with an Anger camera, and then integrated in a data acquisition, storage, processing, and display system developed by Adams et al.5 The images were photographed separately as a series of 10 color-coded isocount contours in spectral sequence. The channel with maximal counts was arbitrarily assigned the color red, and successive 10% decreases in counts were recorded as serial color gradations toward the
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