Lipoproteins in Patients with Proved Coronary Artery Disease: Qualitative and Quantitative Changes in Agarose-gel Electrophoretic Patterns

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SUMMARY  The relationship between the configuration of agarose-gel lipoprotein electrophoresis patterns and the extent of coronary artery disease (CAD) was studied in consecutive patients undergoing diagnostic coronary angiography. Three groups were identified: patients with normal coronary arteries (group 1), patients with minor luminal irregularities (group 2) and patients with significant coronary artery obstructions (group 3). Densitometric scans of the electrophoretograms were studied to determine the relative proportion of lipoproteins in each major fraction and the configuration of the pre-β complex. The presence of multiple subfractions within the pre-β lipoprotein complex had a sensitivity of 66% for the presence of CAD and a specificity of 99%. A decreased level of α, lipoproteins, defined as less than 25% of total lipoproteins, occurred almost entirely in groups 2 and 3, whereas an increased level (≥ 28%) of pre-β lipoproteins was less specific for CAD. The proportions of α and pre-β lipoproteins were inversely related (r = 0.677). Although abnormalities of serum cholesterol and/or triglycerides were common in groups 2 and 3, the detailed study of lipoprotein patterns provided a more sensitive index of disordered lipoprotein metabolism. The angiographic severity of CAD was significantly related to age and to levels of serum triglycerides, α1 lipoproteins, and pre-β lipoproteins. The lipoprotein electrophoretogram, in conjunction with serum lipid levels, is a valuable tool for demonstrating abnormalities of lipoprotein metabolism associated with CAD.

THE INCIDENCE of clinical events that are a consequence of coronary atherosclerosis is clearly increased as serum lipid levels rise.1 However, coronary atherosclerosis is not limited to subjects with marked hyperlipidemia, as shown in the Framingham Study, in which 251 of 322 males who subsequently developed coronary heart disease had initial serum cholesterol levels below 250 mg/dl.1 Similarly, in a study of 500 survivors of acute myocardial infarction, only 31% had elevated levels of serum cholesterol or triglycerides.2 Therefore, predictors of coronary atherosclerosis that are more sensitive than serum lipid levels are needed.

The relationship between quantitative abnormalities of serum lipids and coronary artery disease (CAD) is more evident when coronary angiography is used to show the presence of atherosclerotic disease. This approach excludes subjects who have had either a myocardial infarction or angina pectoris on a basis other than fixed obstructions of coronary arteries secondary to atherosclerosis. Consequently, the prevalence of elevated levels of serum lipids in this very selected population ranges from 40–90%,4 and may be higher in young subjects.5

Elevations of low-density lipoproteins (LDL) are also predictive of subsequent coronary events but are considered by some investigators as no more helpful than levels of serum cholesterol.1 Recent data indicate that the level of high-density lipoprotein (HDL) cholesterol is inversely related to both the incidence and prevalence of coronary heart disease, and its discriminative function is independent of serum cholesterol level.6

Traditionally, physicians have focused on serum lipid levels when ascertaining the risk for coronary heart disease because concentrations of all serum lipoprotein classes were not readily obtained. However, lipoprotein electrophoresis is now widely available and can provide the relative proportions of the main electrophoretic lipoprotein fractions. In our modification, this method can also identify quantitative and qualitative changes within the pre-β complex. Whether these abnormalities are related to the presence or absence of CAD needs to be critically examined. In this report we describe our experience with a specific modification of agarose-gel electrophoresis in patients in whom CAD was documented by coronary angiography.

Methods

Patient Selection

Consecutive patients were selected on the basis of referral to the cardiac catheterization laboratory for diagnostic coronary angiography. The study population included subjects with chest pain of uncertain etiology, candidates for possible coronary bypass surgery and patients with valvular heart disease undergoing preoperative coronary angiography. None were taking drugs to lower serum lipids. Patients with liver disease, thyroid disease, recent myocardial infarction, alcoholism or who were in New York Heart Association class IV were excluded.

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Coronary Angiography

Coronary arteriograms were performed by the Sones technique. Radiographic imaging was performed with a cesium-iodide image intensifier and a 135-mm lens. Filming was at 30 frames/sec and the 35-mm films were viewed on a Tagaro projector. The magnification factor for the viewed films was 2.8 and the line resolving power of the system was 80 line pairs/inch. Each film was reviewed by two experienced observers. The right coronary artery, left main trunk, left anterior descending artery, left main circumflex artery, lateral ventricular branch of the circumflex and the vessel to the posterolateral wall of the left ventricle were individually graded according to the following criteria: 0 = no visible irregularity; 1 = definite irregularity but no narrowing greater than 49%; 2 = 50–74% reduction in vessel diameter; 3 = 75–99% reduction in vessel diameter; 4 = total occlusion. The sum of the grades assigned to each vessel represents an expression of the overall severity of CAD. The grading was completed without knowledge of the lipid studies. The subjects were then divided into three groups according to the results of coronary angiography: group 1 — no CAD (grade 0 in all vessels); group 2 — minimal CAD (grade 1 lesions in one or more vessels); group 3 — obstructive CAD (grade 2 lesions in one or more vessels).

Lipid Analyses

Blood samples in EDTA, as well as without anticoagulant, were obtained on the morning of the cardiac catheterization after a 14-hour fast. Total serum cholesterol was determined by a micromethod (Dow Chemical, Diagnostic Division) that incorporates the Wybinga modification. The results agree with the reference procedure of Abell.

Total serum triglycerides were measured by another micromethod (Dow Chemical, Diagnostic Division) based on an estimation of the glycerol concentrations, using enzymatic reactions with glycerol-kinase dehydrogenase and diaphorase to produce colored formazan, after triglycerides are hydrolyzed by alkali in methanol at 37°C.

Electrophoretic Method

Lipoprotein electrophoretic analyses were achieved on an agarose-gel electrophoretic system (Pol-E-Film, Pfizer Diagnostic Division). Migration was carried out in the Pol-E-Film Cassette Electrophoretic Cell with a power supply that automatically compensated for line voltage fluctuations between 90–125 V to achieve a constant output of 90 V. Pol-E-Film is a uniformly thin layer (0.15 inch) of a special agarose (1.2% agarose, 10% sucrose and 0.035% disodium EDTA) in barbital buffer (pH 8.6 and 0.05 M). The Pol-E-Films were stained with Fat Red 7B stain. Four liters of this stain were prepared by dissolving 0.9 g in absolute methanol containing stabilizer Triton-X-100. Working stain was prepared just before use. Staining was standardized by adjusting stain concentration against permanent colorimetric controls at 600 nM.

One microliter of plasma, stored at 4°C for no longer than 48 hours, was applied and electrophoresis was performed for 40 minutes at 90 V (12 V/cm). The separated lipoproteins were fixed by heating at 72°C. After 15 minutes of staining with Fat Red 7B, the plates were cleared in a mixture of MeOH and H2O (3:1) for 20 seconds, rinsed in 2% glycerol, and dried at 72°C.

The above procedure was further standardized by the concurrent use of the direct standard described by Opplt. This standard contains known quantities of ultracentrifugally isolated and purified lipoprotein classes (S = 0–400). This allows verification of the precise mobility of each ultracentrifugal class and confirmation that their mobilities are identical with each analysis.

Analysis of Electrophoretograms

The films were scanned at 520 nm on a scanning densitometer (Microzone Digital Integrator, Model R-111, Beckman Instruments, Inc., Spinco Division). The raw densitometric values were used to determine the relative proportion belonging to each major fraction (reported as percentage of the total area of the entire scan). The total counts per delimited area were also obtained and no correction factor was applied. For the analysis of relative percentage, the α-lipoprotein fraction included the α1,α2, and α3 components, the pre-β-lipoprotein complex included the subfractions pre-β1, pre-β2, and α, and the β fraction was always represented by one peak.

Mobility measurements of a particular lipoprotein were expressed by the distance (d) of migration in cm from the starting point (o) to its final position in the spectrum. This was measured from the graphic record of a densitometric scan, using a corresponding factor for enlargement, and mobility was then computed by the formula μ = dl/Vt, where μ is mobility, l is length of the agarose gel in centimeters, V is voltage in volts, and t is time in seconds. Therefore, μ is expressed in cm2/V-sec.

Each electrophoretic fraction and subfraction was identified by calculating the electrophoretic mobility. The physicochemical characteristics of each electrophoretic fraction and the physical conditions under which the fractions separate during the standard electrophoretic procedure were derived from our previous studies. In these investigations, each lipoprotein class and subclass constituting the electrophoretic pattern was isolated by separation on a molecular filter. Each isolated fraction was further analyzed by microultracentrifugation and measurements of particle size. Electrophoretic mobilities of each molecular fraction were then determined in concurrent electrophoresis with the patient’s original plasma and/or with the entire lipoprotein complex that had been preisolated by preparative ultracentrifugation at d1,l. Consequently, all fractions separable by electrophoresis, including the subfractions belonging to the pre-β complex, could be qualitatively and quantitatively measured.
Rationale for Normal Values

Because a large population of subjects with proved normal coronary arteries had never been investigated, the choice of an \( \alpha \), lipoprotein less than 25% and the pre-\( \beta \) complex greater than 28% as abnormal was based on prior comparisons of electrophoretic and ultracentrifugal analyses of plasma lipoproteins from a broad population. The small numbers in group 1 resulted in wide standard deviations and so precluded the use of two standard deviations beyond the mean as the normal range. Other levels were examined as to specificity and sensitivity but did not result in a better separation of groups.

The selection of serum cholesterol \( \geq 250 \text{ mg/dl} \) and serum triglycerides \( \geq 150 \text{ mg/dl} \) as abnormal was arbitrary. Lower cut-off values resulted in insignificant differences in prevalence between groups 1 and 3.

Statistical Methods

Two-way analysis of variance (fixed-effects model) and multiple comparisons were used to determine the significance of differences between groups in table 1. The two factors for analysis of variance were sex and the severity group (three levels). The chi-square statistic was used to assess differences between group 1 and group 3 in tables 2, 3 and 4. The correlation between lipid determinations and severity of disease was examined by using stepwise linear regression analysis. The sensitivity and specificity of serum lipid levels and lipoprotein electrophoretic abnormalities in detecting patients with CAD were defined as follows:

Sensitivity (\%) = 
\[
\frac{\text{true positives}}{\text{true positives} + \text{false negatives}} \times 100;
\]

Specificity (\%) = 
\[
\frac{\text{true negatives}}{\text{true negatives} + \text{false positives}} \times 100;
\]

Predictive value (\%) = 
\[
\frac{\text{true positives}}{\text{true positives} + \text{false positives}} + 100.
\]

Because the groups contained unequal numbers of subjects, the predictive value was calculated using the percentage of true positives and false positives in each group.

Results

Serum Lipid Data (tables 1 and 2)

The mean age of group 1 was slightly less than that of groups 2 and 3, and females were predominant in groups 1 and 2. Within each group there was no statistically discernible differences between the lipid levels for males and females. Although the levels of serum cholesterol were somewhat higher in groups 2 and 3 than in group 1, the differences were not significant. The serum triglyceride level was substantially higher in group 3 than in group 1 (\( p \leq 0.01 \)).

The proportion of group 3 patients with elevated serum lipid levels was significantly greater than the proportion in group 1 (table 2). The only abnormality of lipid levels not seen in group 1 was the combined elevation of cholesterol and triglycerides.

Description of Electrophoretic Fractions (fig. 1)

The agarose-gel electrophoretic pattern of plasma lipoproteins usually consists of three major fractions and may exhibit additional subfractions. The lipoprotein composition of the \( \alpha \) and \( \beta \) fractions is constant. The \( \alpha \) fraction contains an albumin-fat acid complex (\( \alpha_1 \)-y) with a mobility of 10.36 \( \pm \) 0.30 \( \mu \times 10^{-5} \) cm\(^2\)/V-sec and HDL\( _{2-3} \) (\( \alpha_1 \)-x) with \( \mu = 8.48 \pm 0.40 \times 10^{-6} \) cm\(^2\)/V-sec. The LDL\( _2 \) subclasses always form the \( \beta \) electrophoretic fraction (\( \mu = 2.13 \pm 0.20 \times 10^{-6} \) cm\(^2\)/V-sec).

The pre-\( \beta \) complex is far more variable and may possess one to three subfractions.\(^3\) Pre-\( \beta \) subfraction (\( \mu = 3.00 \pm 0.45 \times 10^{-6} \) cm\(^2\)/V-sec) is composed predominantly of LDL\( _1 \) and some very low density lipoproteins (VLDL), whereas the pre-\( \beta_2 \) subfraction (\( \mu = 3.83 \pm 0.30 \times 10^{-6} \) cm\(^2\)/V-sec) contains mostly VLDL; however, when chylomicrons and very low density lipoproteins (VVLDL) are present in the plasma in low concentrations, they will also migrate with the pre-\( \beta_2 \) subfraction. When the chylomicrons exceed 5% of the total lipoprotein concentration, the \( \alpha \) subfraction (\( \mu = 4.70 \pm 0.25 \times 10^{-6} \) cm\(^2\)/V-sec) separates and contains a majority of the VVLDL and chylomicron particles.

Classification of Electrophoretic Patterns

The standard lipoprotein phenotypes were classified quantitatively according to Fredrickson and Levy.\(^4\) Only IIA, IIB and IV phenotypes were found in our subjects. In addition to the classification by phenotype, one can designate subtypes based on the number of fractions within the pre-\( \beta \) complex (fig. 1). All IIB and IV patterns can be subtyped. When a pre-\( \beta \) fraction appears in phenotype IIA or in patterns that are normal (N) in all other respects, subtypes can also be designated. Subtype "a" occurs only in phenotypes IIB and IV, whereas subtype "b," which is very common in types IIB and IV, can occasionally be seen in IIA and N patterns.

Correlation of Electrophoretic Data with CAD

Mild abnormalities of the pre-\( \beta \) complex were present in 14 of 37 group 1 subjects. Thirteen had subtype IV-c and only one subject had double pre-\( \beta \) subfractions (IV-b), but none had an \( \alpha \) subfraction. In group 2, eight of 17 subjects had subtypes IV-b or IV-c and an additional three had IIB-b patterns.

In group 3, 96 of 122 subjects had pre-\( \beta \)-complex abnormalities. Multiple pre-\( \beta \) bands, and frequently the \( \alpha \) subfraction, were present in 81 patients. The subtypes in group 3 were distributed as follows: N-c (\( n = 8 \)), IIA-c (\( n = 4 \)), IIB-c (\( n = 12 \)), IV-c (\( n = 11 \)).
### Table 1. Clinical and Laboratory Data

<table>
<thead>
<tr>
<th>Group</th>
<th>Males</th>
<th>Total</th>
<th>Females</th>
<th>Proportion of lipoproteins in electrophoretic fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>α1</td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>46.6 ± 8.5</td>
<td>207 ± 33</td>
<td>105 ± 55</td>
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<tr>
<td>Males</td>
<td>16</td>
<td>48.7 ± 8.6</td>
<td>210 ± 30</td>
<td>131 ± 62</td>
</tr>
<tr>
<td>Females</td>
<td>21</td>
<td>45.1 ± 8.2</td>
<td>207 ± 36</td>
<td>85 ± 40</td>
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<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>52.2 ± 8.0</td>
<td>213 ± 41</td>
<td>157 ± 78</td>
</tr>
<tr>
<td>Males</td>
<td>7</td>
<td>53.3 ± 6.7</td>
<td>224 ± 34</td>
<td>168 ± 98</td>
</tr>
<tr>
<td>Females</td>
<td>10</td>
<td>51.5 ± 9.0</td>
<td>206 ± 45</td>
<td>149 ± 65</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>122</td>
<td>52.1 ± 7.6 §</td>
<td>222 ± 41</td>
<td>174 ± 101†</td>
</tr>
<tr>
<td>Males</td>
<td>101</td>
<td>52.1 ± 7.7</td>
<td>220 ± 39</td>
<td>174 ± 103</td>
</tr>
<tr>
<td>Females</td>
<td>21</td>
<td>52.4 ± 7.6</td>
<td>233 ± 45</td>
<td>175 ± 96</td>
</tr>
</tbody>
</table>

Values are mean ± sd.

*See Methods section.

†p < 0.01 compared with group 1.

‡p < 0.05 compared with group 1.

§ Significant sex effect, p < 0.01.

### Table 2. Prevalence of High Serum Lipid Levels

<table>
<thead>
<tr>
<th>Serum lipid variables</th>
<th>Group 1 (n = 37)</th>
<th>Group 2 (n = 17)</th>
<th>Group 3 (n = 122)</th>
<th>p (1 vs 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol ≥ 250 mg/dl</td>
<td>3 (8.1%)</td>
<td>3 (17.6%)</td>
<td>29 (23.4%)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Triglycerides ≥ 150 mg/dl</td>
<td>8 (21.6%)</td>
<td>9 (52.9%)</td>
<td>64 (52.3%)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cholesterol ≥ 250 mg/dl and Triglycerides ≥ 150 mg/dl</td>
<td>0 (0%)</td>
<td>2 (11.8%)</td>
<td>18 (14.6%)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

### Table 3. Prevalence of Pathologic Changes in Lipoprotein Electrophoretic Patterns

<table>
<thead>
<tr>
<th>Electrophoretic variables</th>
<th>Group 1 (n = 37)</th>
<th>Group 2 (n = 17)</th>
<th>Group 3 (n = 122)</th>
<th>p (1 vs 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1 lipoprotein &lt; 30%</td>
<td>8 (21.6%)</td>
<td>8 (47%)</td>
<td>77 (63.0%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>α1 lipoprotein &lt; 25%</td>
<td>2 (5.4%)</td>
<td>3 (17.6%)</td>
<td>45 (36.9%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pre-β lipoprotein complex &gt; 28%</td>
<td>6 (16.4%)</td>
<td>6 (35.3%)</td>
<td>44 (36.1%)</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>Multiple pre-β subfractions</td>
<td>1 (2.7%)</td>
<td>12 (70.5%)</td>
<td>81 (66%)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

### Table 4. Prevalence of Pathologic Changes in Both Plasma Lipids and in Lipoprotein Electrophoretic Patterns

<table>
<thead>
<tr>
<th>Lipoprotein variables</th>
<th>Group 1 (n = 37)</th>
<th>Group 2 (n = 17)</th>
<th>Group 3 (n = 122)</th>
<th>p (1 vs 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol ≥ 250 mg/dl and α1 lipoprotein &lt; 30%</td>
<td>0</td>
<td>2 (11.8%)</td>
<td>24 (19.7%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cholesterol ≥ 230 mg/dl and α1 lipoprotein &lt; 25%</td>
<td>1 (2.7%)</td>
<td>1 (5.9%)</td>
<td>24 (19.7%)</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>Cholesterol ≥ 250 mg/dl and pre-β complex &gt; 28%</td>
<td>0</td>
<td>2 (11.8%)</td>
<td>12 (9.7%)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Triglycerides ≥ 150 mg/dl and α1 lipoprotein &lt; 30%</td>
<td>4 (10.8%)</td>
<td>4 (23.5%)</td>
<td>52 (42.7%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Triglycerides ≥ 150 mg/dl and pre-β complex &gt; 28%</td>
<td>3 (8.1%)</td>
<td>6 (35.3%)</td>
<td>36 (28.8%)</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>
### Types and Subtypes in Electrophoretic Patterns of Plasma Lipoproteins Obtained in Patients with Proven CAD

<table>
<thead>
<tr>
<th>Normal Pattern</th>
<th>Types:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II A</td>
</tr>
<tr>
<td></td>
<td>II B</td>
</tr>
<tr>
<td></td>
<td>IV</td>
</tr>
</tbody>
</table>

#### Subtypes:

- **α**: HDL<sub>2,3</sub>
- **β**: LDL<sub>1</sub>, VLDL
- **γ**: LDL<sub>2</sub>, HDL<sub>1</sub>

#### Composition of Different Fractions

- **α<sub>1</sub> L**: HDL<sub>2,3</sub>
- **α<sub>2</sub> L**: Chm
- **β<sub>2</sub> L**: VLDL, VLDL
- **β<sub>1</sub> L**: LDL<sub>1</sub>, VLDL
- **β**: LDL<sub>2</sub>, HDL<sub>1</sub>

#### Figure 1. Electrophoretograms of plasma lipoproteins on agarose gel. Normal patterns and standard phenotypes are identified by relative proportions of standard fractions: α, pre-β and β. Examples of subtypes are shown in the lower section. The letters "c," "b" and "a" designate subtypes that can be delineated by the number and mobilities of the pre-β fractions. Subtype "c" has one pre-β fraction (pre-β<sub>1</sub>); "b" has two pre-β subfractions (pre-β<sub>1</sub> and pre-β<sub>2</sub>); and "a" has three subfractions (pre-β<sub>1</sub>, pre-β<sub>2</sub> and α<sub>3</sub>). Whenever a faster subfraction appears, the slower subfractions (pre-β<sub>1</sub> and pre-β<sub>2</sub>) are already present. Composition of the different fractions is illustrated to the left. HDL = high-density lipoprotein; Chm = chylomicron; LDL = low-density lipoprotein; VLDL = very low density lipoprotein; VVLDL = very low density lipoprotein.

N-b (n = 12), IIA-b (n = 3), IIIB-b (n = 16), IV-b (n = 18); N-a (n = 0), IIA-a (n = 0), IIIB-a (n = 15), IV-a (n = 17). Only six subjects (four II A and two N) had no pre-β fraction.

The percentage of the serum lipoproteins in the β area of the electrophoretic pattern was similar in all groups (table 1). Since the electrophoretic analysis described percentages of the entire spectrum of plasma lipoproteins, this relative constancy of the β fraction permitted us to study the variabilities of α<sub>1</sub> and pre-β lipoproteins (fig. 2). A significant inverse relationship between the α<sub>1</sub> and pre-β lipoprotein was evident for both group 1 and group 3 (fig. 2).

Patients with CAD (groups 2 and 3) had significantly lower α<sub>1</sub> lipoproteins and higher pre-β lipoproteins compared with group 1 (table 1, fig. 2), but only the differences between groups 1 and 3 were statistically significant. Because we used a two-way analysis of variance, the observed group effect is independent of sex effects. The anticipated higher α<sub>1</sub> lipoproteins in females was statistically discernible only in group 1 (table 1).

Two abnormalities of lipoprotein patterns, an α<sub>1</sub> lipoprotein proportion of less than 25% and multiple pre-β bands, occurred almost exclusively in groups 2 and 3 (table 3). Consequently, when groups 2 and 3 are combined, the specificities and predictive values of these phenomena for CAD are high (table 5). The presence of multiple pre-β fractions has a far stronger predictive value than elevated serum triglyceride levels and is equally sensitive. The combination of increased levels of either serum cholesterol or triglyceride with these two abnormalities of lipoprotein patterns was more common in group 3 than in group 1 (table 4). However, abnormalities of lipoprotein electrophoresis did occur in the absence of lipid abnormalities (fig. 3).

Results of the stepwise multiple regression are presented in table 6. The partial correlation of serum triglycerides with severity of CAD is statistically significant for men, women, and the combined group. The correlation of age with severity of disease is statistically significant for men and the combined group. Though the individual partial correlation coefficients of age and α<sub>1</sub> lipoprotein are not significant in women, these two variables combine with serum triglycerides to explain a significant portion of
Figure 2. (A) The relationship between the proportions of α₁ lipoproteins and pre-β lipoproteins in subjects without coronary artery disease (group 1). The broken lines indicate normal limits. (B) The same relationship is depicted for patients with significant coronary obstructions (group 3). Proportionately more of these subjects have a reduced α₁ fraction and/or elevated pre-β complex.

Table 5. Diagnostic Value of Lipid and Lipoprotein Variables for Coronary Artery Disease

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Predictive Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple pre-β fractions</td>
<td>66</td>
<td>99</td>
<td>96</td>
</tr>
<tr>
<td>α₁ lipoprotein &lt; 25%</td>
<td>35</td>
<td>96</td>
<td>87</td>
</tr>
<tr>
<td>Pre-β lipoprotein &gt; 28%</td>
<td>36</td>
<td>84</td>
<td>69</td>
</tr>
<tr>
<td>Cholesterol ≥ 250 mg/dl</td>
<td>23</td>
<td>92</td>
<td>74</td>
</tr>
<tr>
<td>Triglyceride ≥ 150 mg/dl</td>
<td>52</td>
<td>80</td>
<td>71</td>
</tr>
</tbody>
</table>

Figure 3. A comparison of the prevalence of lipid and lipoprotein electrophoretic abnormalities in patients free of coronary disease (group 1) and those with significant disease (group 3). Normal is defined as cholesterol < 250 mg/dl, triglycerides < 150 mg/dl, α fraction > 25%, pre-β fraction < 28%, and the absence of multiple pre-β bands.

Discussion

Qualitative and quantitative changes in the pre-β region were the most frequent electrophoretic characteristics in our patients with CAD, and were even detected in more than half of the subjects in group 3 who had normal serum lipid levels. The most readily discernible qualitative abnormality was the presence of multiple pre-β bands. It is therefore important to consider the lipoprotein classes participating in this altered configuration of the pre-β complex under pathologic conditions.

Physochemical studies of lipoprotein classes and associations of classes have enhanced our understanding of their electrophoretic mobilities and the conditions for their electrophoretic separation. Fractions having a mobility faster than β₁ (i.e., in the β₂ and α₂ regions) contain chylomicrons, VVLDL and some VLDL.

Although chylomicrons (−S₁,₂₁ 10,000–1,000) have been observed to remain at the electrophoretic origin and cause a trail, they do migrate and affect not only the broadness and asymmetry of the β₁,₂ region, but also form the distinct α₂ subfraction. VLDL and LDL₁ are the predominant components of the pre-β fraction. When VLDL (−S₁,₂₁ 400–70, size 600–400 Å), which have only a slightly faster mobility than LDL₁ (−S₁,₂₁ 70–42, size 400–250 Å), are present in large amounts, the pre-β complex will often assume a plateau pattern. If LDL₁ is the major component of the β₁ fraction, this fraction may then separate poorly from the β fraction, and, consequently, the β fraction...
appears asymmetric on the anodic side of the curve. These data indicate that changes in the pre-\(\beta\) complex reflect a variety of derangements in the metabolism of particulate fat.

Multiple pre-\(\beta\) bands can sometimes be recognized by simple inspection of the electrophoreogram. However, precise mobility measurements of stained lipoprotein bands can only be obtained from the densitometric scan. Knowledge of the mobilities is essential for the correct interpretation of electrophoreograms: normal and "fast" \(\beta\) fractions can be distinguished and the fractions participating in the pre-\(\beta\) complex can be identified. The densitometric scan permits the planimetric reconstruction of the main \(\beta\) fraction, when it is asymmetric, and the "hidden" pre-\(\beta\), subfraction can be more readily identified (fig. 1, patterns II\(\beta\)c, II\(\beta\)a and IV\(\beta\)).

Either the presence of two or three fractions in the pre-\(\beta\) complex or an increased accumulation of lipoproteins in the pre-\(\beta\) complex indicates an altered sequence of particulate fat metabolism. Multiple pre-\(\beta\) fractions were very common in our patients with CAD, but only one patient without CAD had such a pattern. Although Papadopoulos and Bedynek\textsuperscript{19} described a greater incidence in their normal subjects, data from Scandinavia and the U.S. support our finding that multiple pre-\(\beta\) bands identify a metabolic abnormality strongly associated with CAD.\textsuperscript{20-22}

A decrease in the level of \(\alpha_1\) lipoproteins was also common in patients with CAD. Because the proportions of \(\alpha_1\) and pre-\(\beta\) lipoproteins were inversely related, we cannot distinguish whether the reduction in \(\alpha_1\) lipoproteins was a primary phenomenon or secondary to disordered metabolism of chylomicrons and VLDL. The inverse relationship between HDL and VLDL has also been observed in runners\textsuperscript{28} as well as during therapy for type IV hyperlipidemia.\textsuperscript{24} Until the factors that regulate HDL synthesis are understood, we cannot resolve whether a reduction in HDL is a primary phenomenon or a secondary consequence of specific metabolic trends.

The importance of decreased levels of \(\alpha_1\) lipoproteins in our patients with severe CAD is underscored by recent epidemiologic studies of the inverse relationship between serum levels of HDL cholesterol and the incidence of coronary heart disease.\textsuperscript{5, 11} These observations are true for both men and women and remain significant when corrected for other known risk factors. The prevalence of CAD in a variety of population groups also increases as HDL cholesterol levels fall below 35 mg/dl.\textsuperscript{28} It is apparent that the HDL cholesterol level can provide risk data that are not evident from serum lipid levels. Our clinical study indicates that similar risk information is also available from the densitometric scan of the lipoprotein electrophotogram performed on agarose gel. A reduction in total HDL is reflected in a reduced proportion of \(\alpha_1\) lipoproteins (less than 25%). Although the abnormality occurred predominantly in patients with an elevation of serum lipids, it also occurred in eight patients with normal levels of serum lipids.

An approximate estimate of HDL, as well as other major lipoprotein classes, can be obtained from the electrophoretogram if one applies corrections for the staining characteristics of each fraction or subfraction. Specific indexes, based on the differential staining of fatty acids, cholesterol esters, triglycerides and phospholipids, have been proposed by Opplt and Musil,\textsuperscript{26} Hatch et al.\textsuperscript{27} and Wong et al.\textsuperscript{28} These approaches provide a reasonable quantitation of \(\alpha_1\) lipoproteins or HDL\(_{2,3}\), but are far less precise in the evaluation of other fractions because their lipid composition varies under pathologic conditions. This is an inherent problem in all methods that measure a lipid level in a fraction or class to estimate the actual quantity of lipoprotein. Correction factors are based on average lipid content, yet the actual lipid content may be dissimilar in different patients.

Coronary angiography is a precise diagnostic tool that permits recognition of early atherosclerotic lesions that have not yet resulted in any clinical

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**Table 6. Regression Analysis in Relationship to Severity of Coronary Artery Disease: Partial Correlation Coefficients**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women (n = 52)</th>
<th>Men (n = 124)</th>
<th>Both (n = 176)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.278 (p &gt; 0.1)</td>
<td>0.146 0.025 &lt; (p &lt; 0.05)</td>
<td>0.229 0.001 &lt; (p &lt; 0.005)</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>0.170 (p &gt; 0.1)</td>
<td>0.002 (p &gt; 0.1)</td>
<td>0.059 (p &gt; 0.1)</td>
</tr>
<tr>
<td>Serum triglycerides</td>
<td>0.367 0.005 &lt; (p &lt; 0.01)</td>
<td>0.176 0.05 &lt; (p &lt; 0.06)</td>
<td>0.267 (p &lt; 0.001)</td>
</tr>
<tr>
<td>(\alpha_1) lipoprotein %</td>
<td>-0.297 (p &gt; 0.1)</td>
<td>-0.087 (p &gt; 0.1)</td>
<td>-0.228 (p &gt; 0.1)</td>
</tr>
<tr>
<td>pre-(\beta) (\alpha_1) lipoprotein %</td>
<td>0.257 (p &gt; 0.1)</td>
<td>0.037 (p &gt; 0.1)</td>
<td>0.156 (p &gt; 0.1)</td>
</tr>
<tr>
<td>Variables used in regression of severity of disease ((p &lt; 0.05))</td>
<td>Age, triglycerides, (\alpha_1) lipoprotein %</td>
<td>Age, triglycerides, (\alpha_1) lipoprotein %, pre-(\beta) lipoprotein %</td>
<td>Age, triglycerides, (\alpha_1) lipoprotein %, pre-(\beta) lipoprotein %</td>
</tr>
</tbody>
</table>
evidence of CAD. Recognition of patients with minimal disease is essential when attempting to characterize metabolic changes in patients with atherosclerosis. Our data indicate that subjects with minimal disease have a significantly greater prevalence of serum lipid and lipoprotein abnormalities than patients with normal coronary vessels. The importance of these signs of abnormal metabolism is emphasized by the observation that subjects with even minimal coronary irregularity have a higher incidence of subsequent coronary events than those with entirely normal vessels.29 Similarly, recognition of subjects with entirely normal vessels defines a group with almost no cardiac mortality29 and a low prevalence of abnormal lipoprotein metabolism.

When CAD is documented by coronary angiography, the coronary disease groups have consistently exhibited higher lipid levels than the controls, but the prevalence and the severity of these variations in quantities do differ between studies.4,6,8,30 The highest serum lipid levels also tend to be associated with a greater extent of coronary disease.30-33 Similar data were obtained by Jenkins and co-workers, who extended the observations to cholesterol levels of the major lipoprotein classes.34 Severity of CAD was directly related to LDL cholesterol, VLDL cholesterol and LDL triglyceride and inversely related to HDL cholesterol. Likewise, the proportion of alpha lipoproteins was inversely related to extent of CAD in our study, but the partial correlation coefficient was not significant for this as a single variable. Although we also found that age and serum triglyceride levels were related to the extent of CAD, an association with serum cholesterol levels was not evident. Perhaps the latter observation relates to the moderate decline in serum cholesterol levels that has occurred in the general population of the U.S. since 1970.35

The lack of strong correlations between the abnormalities of lipids and lipoproteins and the extent of CAD defined angiographically is not surprising. The inherent difficulties in quantitating the precise extent of CAD are formidable. Further, there are many other factors, apart from disorders of lipid and lipoprotein metabolism, that influence the degree of atherosclerosis and the severity of an obstructive lesion. In addition, the severity of any lipoprotein derangement varies with time and in many cases may be less evident when clinical symptoms appear.

Although marked hypercholesterolemia is a strong predictor of initial CAD events, large proportions of our study population and the general population who develop symptomatic CAD have serum cholesterol levels similar to those found in our disease-free group. This finding suggests that we need to gain understanding of additional metabolic markers associated with CAD. Agarose-gel lipoprotein electrophoresis can be used to identify distinctive features that reflect altered lipoprotein metabolism. Pathologic electrophoretic patterns are significantly more prevalent in a population with asymptomatic and early coronary artery atherosclerosis and probably reflect metabolic disturbances that contribute to atherosclerosis and are not just a consequence of advanced CAD.

References


Lipoproteins in patients with proved coronary artery disease: qualitative and quantitative changes in agarose-gel electrophoretic patterns.
R C Bahler, J J Opplt and D M Waggoner

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