Low Density Lipoprotein Cholesterol and Whole Blood Viscosity*

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ABSTRACT

Whole blood viscosity (WBV) was measured in a normal population and was analyzed in relation to packed cell volume, (hematocrit, PCV), fibrinogen, white blood cell count (WBC), platelet count, and plasma lipids, including total cholesterol, triglycerides, high density lipoprotein cholesterol (HDLc) and low density lipoprotein cholesterol (LDLc). Conventional assays were used for all blood and lipid measurements. Whole blood viscosity was measured under disaggregating conditions with a disposable, porous bed viscometer. As expected, the strongest correlation was seen between WBV and PCV ($r = 0.78, p < 0.001$). Significant positive correlations also were demonstrated between WBV and cholesterol ($r = 0.22, p < 0.001$), triglycerides ($r = 0.14, p < 0.001$) and LDLc ($r = 0.21, p < 0.001$). A significant negative correlation was found between HDLc and WBV ($r = -0.20, p < 0.001$). Correlation analysis by sex showed only the correlation of LDLc was significant for both men and women. A stepwise multiple regression analysis of WBV indicated that LDLc, fibrinogen (Fbg) and

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platelet (Pit) counts correlated independently of PCV to WBV. The equation derived from multiple regression and partial correlation analysis was:

\[ \text{WBV (mPa} \cdot \text{sec)} = -9.317 + 0.0047 (\text{LDLc}) + 0.381 (\text{PCV}) + 0.00152 (\text{Pit}) + 0.0021 (\text{Fbg}) \]

The calculated mean specific contribution of PCV was 90.8 percent, LDLc 3.5 percent, and fibrinogen 3.3 percent to observed mean WBV. This study shows that LDLc is the principal lipoprotein independently influencing whole blood viscosity and its effect is similar in magnitude to fibrinogen. Further studies to elucidate the mechanism and clinical significance of the effects of LDLc on WBV are indicated.

Introduction

The role of blood viscosity in the pathogenesis of cardiovascular disease has been the study of several recent epidemiological studies.\(^{1,2,3,4,5,6,7,8}\) In these studies, abnormal hemorheologic parameters have been shown to be associated with hypercholesterolemia. Moreover, a recent report on the blood rheology after LDL apheresis using dextran sulfate cellulose absorption\(^9\) demonstrated that selective extracorporeal LDL cholesterol elimination resulted in a 32 percent fall in whole blood viscosity (WBV), standardized to a hematocrit of 45 percent suggesting that LDLc reduction may influence WBV.

Increases in serum cholesterol and triglycerides that correlate with increased plasma viscosity may be due to the direct effect of lipoproteins on plasma viscosity.\(^3\) Plasma with chylomicronemia similarly may elevate plasma viscosity. A multivariate analysis of three major cardiovascular risk markers, cholesterol, blood pressure, and smoking showed that these risk factors had both independent as well as additive effects on blood viscosity.\(^4\) In the present study, the relation between whole blood viscosity and plasma lipoproteins has been investigated. In addition to measurements of total cholesterol and triglycerides, measurement of high density lipoprotein cholesterol (HDLc) was performed and low density lipoprotein cholesterol level (LDLc) was calculated.

The results to be presented confirm that there is a statistically significant correlation between cholesterol and triglycerides with whole blood viscosity. The results further show that this relation is mainly owing to the influence of LDLc cholesterol, and that the magnitude of this effect is similar to fibrinogen.

Methods

Subjects

The specifics of the selection and interviewing of subjects has been described previously.\(^{10,11}\) Briefly, the evaluation effort of the Pawtucket Heart Health Program includes biennial risk factor surveys. Households in Pawtucket, RI, and in a comparison city in southeastern Massachusetts were randomly selected and visited by a trained surveyor. One resident aged 18–64 was then randomly selected from each household for interview concerning cardiovascular disease risk factors, blood pressure measurement, and phlebotomy. In all, 982 individuals were studied. The average age was 41.3 ± 14.5 (range 18–73). There were 43.5 percent males and 56.7 percent females. This population was 89.5 percent Caucasian and consisted of 91.3 percent non-smokers.

Laboratory Measurements

Total cholesterol,\(^{12}\) triglyceride,\(^{13}\) and high density lipoprotein cholesterol\(^{14}\)
measurements were performed on serum. Low density lipoprotein cholesterol (LDLc) was calculated using the following formula:

\[
LDLc = \left[ \text{total cholesterol} - \frac{\text{triglyceride}}{5} \right] - \text{HDLc}
\]

Blood cell parameters and whole blood viscosity (WBV) were measured on whole blood drawn into a test tube containing K3 EDTA. White blood cell count, red blood cell count, hemoglobin, hematocrit, and platelet count were measured on a Coulter T540.* RBC indices were calculated using standard formulae. Fibrinogen levels were measured on an automated Coag-a-mate \(\times 2\)† using a thrombin-time method. Whole blood viscosity was measured using a porous bed viscometer (PBV)\(^{15}\) after warming the blood and the viscometers for 30 minutes at 37°C. Using the PBV, the time in seconds required for 0.1 ml of blood to pass through the porous bed is measured. Using identical devices the flow time for 0.1 ml of 10.0 centipoise calibration liquid at 37°C to pass through the porous bed is 27.5 ± 0.5 seconds. Therefore, each second of measured flow time is equivalent to 0.364 ± 0.06 centipoise.\(^{15}\)

* Coulter Electronics, Hialeah, FL 33012.
† General Diagnostics, Morris Plains, NJ 07950.

Since one centipoise is essentially equivalent to one milliPascal-second (mPa · sec), our measurements were converted as follows:

Flow time in seconds × 0.364 = whole blood viscosity in mPa · sec\(^{15}\)

Blood collected for the various laboratory parameters was refrigerated overnight at 4°C and delivered to the laboratory for testing the morning following collection. Previous studies have established that there was no significant change in WBV measured with the porous bed viscometer when samples were collected in EDTA or stored overnight at 4°C.\(^{15}\)

**Statistical Analysis**

The mean and standard deviation (mean ± SD) are reported for each parameter measured. A one way factorial analysis of variance (ANOVA) was used to compare groups. Stepwise multiple regression analysis was done to select those variables which made the most statistically significant contribution to the predictable variance of WBV. These variables were analyzed in a multiple regression analysis and partial correlation coefficients were calculated. In all cases \(p < 0.05\) was considered significant. The tables and figures indicate the exact number (N) of analyses of each statistical comparison. To determine if variances in hematocrit related to the parameters studied might have influenced any observed positive or negative correlations (e.g., higher hematocrits associated with higher cholesterol levels), a final analysis with all hematocrits corrected to 45 percent was performed.

**Results**

Results of blood lipoproteins and blood counts in the total population of 982 and the population grouped by gender (43.5 percent males, 56.7 percent females) are shown in table I. There were no significant differences in age, total cholesterol, LDLc, or WBC between men and women enrolled in this study. The HDLc was significantly higher in women (50.3 vs. 41.8 mg/dl, \(p < 0.001\)). Triglycerides were significantly lower in women than in men (149.8 mg/dl vs. 207.1 mg/dl, \(p < 0.001\)). Fibrinogen levels were higher and hematocrit and WBV lower in women than men (all \(p < 0.0001\)). Overall, there were positive correlations between WBV and Hct, cholesterol, and
Analysis of Variance of Parameters of Normal Volunteers by Gender

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Male</th>
<th>Female</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>982.</td>
<td>425.</td>
<td>557.</td>
<td></td>
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<tr>
<td>Age</td>
<td>41.3 ± 14.5</td>
<td>40.7 ± 14.7</td>
<td>41.7 ± 14.4</td>
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</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>207.8 ± 45.5</td>
<td>209.4 ± 44.7</td>
<td>206.7 ± 46.2</td>
<td>NS</td>
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<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>47.0 ± 13.0</td>
<td>41.8 ± 11.8</td>
<td>50.3 ± 13.0</td>
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<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>125.9 ± 37.9</td>
<td>124.7 ± 39.5</td>
<td>126.8 ± 36.7</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>174.6 ± 156.0</td>
<td>207.1 ± 135.3</td>
<td>149.8 ± 166.0</td>
<td>0.001</td>
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<tr>
<td>WBC (x 10⁶ /ml)</td>
<td>7.7 ± 2.2</td>
<td>7.74 ± 2.2</td>
<td>7.73 ± 2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.0 ± 1.4</td>
<td>15.0 ± 1.1</td>
<td>13.2 ± 1.1</td>
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<tr>
<td>Hematocrit (vol %)</td>
<td>41.3 ± 3.9</td>
<td>44.1 ± 3.1</td>
<td>39.1 ± 2.9</td>
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<tr>
<td>Platelet (x 10⁹ /ml)</td>
<td>277.8 ± 68.2</td>
<td>269.0 ± 64.5</td>
<td>284.6 ± 70.2</td>
<td>0.001</td>
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<tr>
<td>Fibrinogen (mg/dl)</td>
<td>268.8 ± 81.6</td>
<td>252.2 ± 73.4</td>
<td>281.5 ± 85.2</td>
<td>0.001</td>
</tr>
<tr>
<td>WBV mPa • sec</td>
<td>8.0 ± 1.9</td>
<td>9.0 ± 1.9</td>
<td>7.2 ± 1.6</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Statistics given as mean ± standard deviation

ANOVA = analysis of variance

LDLc and a significant negative correlation between WBV and HDLc (figures 1–4).

A stepwise multiple regression analysis using WBV as a dependent variable and Hct, Chol, HDLc, Tri, LDLc, WBC, Plt, Fbg, and Hgb as independent variables indicated that LDLc, Fbg, and Hct contributed the most statistically to the variance of WBV. These four parameters were then regressed against WBV in a multiple regression. In this analysis, the F-value was 390.89, p < 0.001, and adjusted r² was 0.619. The partial correlation coefficients of these parameters are presented in table II. The equation for whole blood viscosity giving the best fit to the multiple regression assays using all significantly associated parameters (table II) was:

\[
\text{WBV} = -9.317 + 0.0047 (\text{LDLc}) + 0.381 (\text{PCV}) + 0.00152 (\text{Plt}) + 0.0021 (\text{Fbg}).
\]

The means of the population of 982 individuals were inserted in the equation to give:

\[
\text{WBV} = -9.317 + 0.0047 (125.9) + 0.381 (41.3) + 0.00152 (277.8) + 0.0021 (268.8).
\]

This equation reduced to \(\text{WBV} = -9.317 + 17.3\). This produced a mean \(\text{WBV} = 8.0 \text{ mPa} \cdot \text{sec}\) which corresponded exactly to the measured mean WBV of the 982 samples (table I) demonstrating the mathematical validity of the equation. By multiplying each of the coefficients by the means of the parameters, the percentage of each parameter's contribution to mean whole blood viscosity was calculated to be: \(\text{Hct} = 90.8\) percent, \(\text{LDLc} = 3.5\) percent, \(\text{Plt} = 2.4\) percent, \(\text{Fbg} = 3.3\) percent.

A final analysis was done to determine if the correlations between LDLc and whole blood viscosity were related to some concomitant variation in hematocrit. The whole blood viscosity corrected to a hematocrit of 45 percent (see Methods) showed the \(r\) values for the relation between whole blood viscosity and cholesterol to be 0.22 (\(p < 0.001\)), for HDL 0.15 (\(p < 0.001\)), for triglycerides 0.124, (\(p < 0.001\)), and for fibrinogen 0.74 (\(p < 0.001\)).
The corrected correlation for LDLc and whole blood viscosity was 0.21, (p < 0.001). These correlations were similar to those with whole blood viscosity as directly measured (figures 1–4) and indicated the correlations were not due to concomitant variations in the hematocrit related to the parameter studied. The r values for the correlation of LDLc and WBV in men and women were 0.24 and 0.25, respectively. When the WBV correlated with HDLc (figure 4) was analyzed by gender, no significant correlation was observed (data not shown).

Discussion

Positive correlations were observed between WBV and cholesterol, triglycerides and LDLc, and a negative correlation between WBV and HDLc. When analyzed by gender, the correlations between WBV and LDLc and cholesterol were significant for both sexes, while the correlation between WBV and triglyceride was significant only for women. The explanation for the positive correlations observed between WBV and LDLc may lie in direct effects of the LDLc macromolecules on plasma viscosity as well as the enhancement of red blood cell interactions by large lipoprotein molecules. Previous studies have demonstrated that intrinsic red blood cell factors such as microcytosis or macrocytosis may enhance or diminish whole blood viscosity respectively, but these factors do not offer any explanation for the correlation between WBV and lipids observed in the present study. Levy et al have demonstrated that platelet membrane stiffness is related to mem-

\[ r = 0.78, \ N = 982, \ p < 0.001 \]

![Graph](image-url)
brane lipoprotein concentration. Low density lipoprotein cholesterol is subject to oxidation as are red blood cell membrane associated lipids; thus, higher levels of cholesterol and LDLc may favor increased oxidation of the red blood cell membrane lipids. This process may lead to stiffening of the red blood cell membrane akin to the normal RBC aging process. Further studies of red blood cell membrane deformability in relevance to LDLc concentration will be needed to verify whether this explanation actually accounts for the correlation between increased WBV and increased LDLc.

Our results confirm previous studies that have shown a positive correlation between total cholesterol level and whole blood viscosity and extend these observations by demonstrating that LDLc is the basis for the correlation. Our findings are consistent with the report of Koenig et al who found that plasma viscosity has a positive correlation with total cholesterol as well as apoprotein B. The r values for these variables for men and women were 0.23 and 0.24, respectively, similar to our correlation coefficients of 0.24 for men and 0.25 for women between LDLc and WBV. Thus, our finding that LDLc and total cholesterol correlate significantly with whole blood viscosity may also be related to the effect of apolipoprotein B on plasma viscosity. Further studies are needed to assess the influence of LDLc on red blood cell interactions before the known effect of the LDLc on plasma viscosity can be related to the influence of LDLc on whole blood viscosity.
High density lipoprotein cholesterol is associated with an improved outlook in coronary artery disease.\textsuperscript{22,23,24} Our finding of a negative correlation between HDLc and whole blood viscosity suggests the possibility that the protective effect of HDLc may be partially explained through its association with lower whole blood viscosity. The recent study of Koenig et al. also found a negative correlation between HDLc, total cholesterol and plasma viscosity.\textsuperscript{4} An overall negative correlation was found of HDLc and WBV, but when the data were analyzed by gender, this correlation, unlike the LDLc WBV correlation, was no longer significant. In our opinion, the most likely explanation to account for the overall negative correlation between HDLc and WBV (figure 4) is that HDLc levels are higher and WBV lower in women which causes a fortuitous negative association present only when all data are plotted but not found when the data were analyzed separately by sex (table I). In contrast, the positive correlation of LDLc and WBV was significant for the entire group (figure 3) as well as for each gender when analyzed separately (table I).

The results of this study show a statistically significant correlation between LDLc and WBV. Low density lipoprotein cholesterol is established as a risk factor for coronary artery disease, and the correlation between LDLc and WBV raises the question as to whether or not some of the deleterious effects of increased LDLc may be related to increases in WBV. To date, there has been no study of intervention to reduce WBV in patients with coronary artery disease, but the results of the
Figure 4. The relation between whole blood viscosity and serum high density lipoprotein levels in a normal population.

The present study suggests that this issue merits further study. The PCV may be lowered in patients with polycythemia vera by inducing a state of reduced iron stores by phlebotomy. One approach that could be taken to determine whether or not WBV mediates some of the risk of increased LDLc levels would be to take a selected group of patients with histories of high LDLc levels and render them hypoferremic by phlebotomy to lower their PCV and WBV. It could then be ascertained by follow up whether or not those assigned to phlebotomy fared better with respect to cardiovascular complications than the group not phlebotomized.

Finally, this study demonstrates that LDLc has a magnitude of effect similar to fibrinogen on WBV and fibrinogen is a well recognized independent risk factor for coronary disease. Further studies of WBV in patients with pathologically elevated LDLc should be performed to determine the magnitude of the effect of elevated LDLc on WBV as well as the clinical significance of this interaction and any additional effects related to concomitant variation in fibrinogen levels.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial Correlation</th>
<th>Beta</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLc</td>
<td>0.150</td>
<td>0.095</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.141</td>
<td>0.090</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platelet</td>
<td>0.088</td>
<td>0.055</td>
<td>0.007</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.777</td>
<td>0.774</td>
<td>&lt;0.001</td>
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</table>
Acknowledgment

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References