Influence of HDL Subfractions on Erythrocyte Aggregation in Hypercholesterolemic Men

S.M. Razavian, V. Atger, Ph. Giral, M. Cambillau, M. Del-Pino, A.C. Simon, N. Moatti, J. Levenson, the PCVMETRA Group

Abstract Recent studies have suggested that rheological mechanisms may be involved in the pathogenesis of ischemic syndromes in hyperlipidemias. We investigated the association between erythrocyte aggregation and components of lipoproteins in the blood and hypercholesteremic, hyperlipidemic men aged 45±8 years. The rheological parameters assessed were aggregation index (AI) and disaggregation shear rate threshold (yt) as determined by laser reflectometry, plasma fibrinogen, total serum protein, and hematocrit. The lipoprotein variables included total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol and its subfractions HDL1 cholesterol and HDL2 cholesterol, apolipoprotein (apo) B, apoA-I, HDL particles containing apoA-I without apoA-II (LpA-I), and HDL particles containing both apoA-I and apoA-II (LpA-I/A-II). Covariables considered for possible confounding effects were body mass index, age, smoking behavior, fibrinogen, total serum protein, and both aggregation parameters (AI and yt) were elevated in this hypercholesterolemic population. Univariate analysis showed that both AI and yt correlated positively with fibrinogen (P<.001) and total serum protein (P<.01) and negatively with HDL cholesterol (P<.01) and LpA-I (P<.01); yt also provided a positive correlation with LpA-I/A-II (P<.05). A multivariate model analysis demonstrated that HDL2, cholesterol, LpA-I, and LpA-I/A-II also emerged as significant factors influencing erythrocyte aggregation; 60% to 68% of the variance of AI and 47% to 64% of the variance of yt could be explained by these factors. The negative relation with HDL cholesterol, containing mainly LpA-I, suggested a possible role of these HDL subfractions in modifying erythrocyte aggregation induced by fibrinogen or other macromolecules. However, since LpA-I/A-II is positively related to yt, the possible influence of HDL particles on erythrocyte interactions might result from a balance between the respective effects of heterogeneous particles. (Arterioscler Thromb. 1994;14:361-366.)

Key Words • shear rate • shear stress • blood viscosity • HDL2 cholesterol • LpA-I • LpA-I/A-II

The fluidity of blood plays an important role in the physiological behavior of the circulation, and its alteration might promote cardiovascular complications in situations associated with low-flow states.1 In this condition red blood cells (RBCs) aggregate due to the increase in the bridging force of high-molecular-weight plasma proteins.1,2 Among these proteins, some are considered to be cardiovascular risk markers (e.g., lipoproteins, fibrinogen) and are associated with increased plasma and whole-blood viscosity.3-13 Associations between rheology and components of lipoproteins in human blood are reported. Studies in patients with various forms of hyperlipoproteinemia show a concentration-dependent increase in plasma viscosity for low-density lipoprotein cholesterol (LDL-C) and chylomicrons and hence, for total triglycerides.9,10 These earlier studies are in line with the positive linear correlation of plasma viscosity with total cholesterol and apolipoprotein (apo) A-II and apoB observed in a recent study.11 Moreover, both blood viscosity11 and erythrocyte aggregation12 are negatively related to high-density lipoprotein cholesterol (HDL-C) concentration, which is known to be inversely correlated with coronary heart disease risk.2,5 However, high-density lipoprotein (HDL) is a heterogeneous population of lipoproteins separated by density into two main subfractions, HDL1 and HDL2.14 Furthermore, HDL may also be separated according to apolipoprotein composition, leading to at least two species of particles: LpA-I, which contains apoA-I without apoA-II, and LpA-I/A-II, which contains both apoA-I and apoA-II.15 These heterogeneous HDL subfractions seem to have distinct metabolic roles, but the specific effect of one or another particle on the atherosclerotic process remains unclear.16-21 In the present study we investigated the relation of lipoprotein components, and especially HDL subfractions, to the aggregation and disaggregation of RBCs in a population of asymptomatic, hypercholesterolemic, normotriglyceridermic, normotenstive subjects.

Methods

Selection of Subjects

The study group was from a cholesterol-screening program conducted at a worksite by a group of occupational health physicians (PCVMETRA: prévention cardiovasculaire en médecine du travail; see "Appendix"). This group referred employees whose total cholesterol concentration at the worksite was above 6.2 mmol/L.22,23 Among them, the subjects selected for the present study were normotensive (diastolic pressure less than 95 mm Hg) men without any symptomatic cardiovascular disease and in whom cholesterol and triglycerides at repeated measurements in the hospital were above 5.2 mmol/L and below 2 mmol/L, respectively. Thus, 60 hypercholesterolemic men who were 45±8 (mean±SD) years old and had a body mass index (weight/height²) of 24±2 kg/m² and a mean blood pressure (one third of the sum of systolic pressure and
twice the diastolic pressure) of 94±9 mm Hg entered the study. A control group of 16 normocholesterolemic men who were 43±10 years old and had a body mass index of 24±3 kg/m² and a mean blood pressure of 90±3 mm Hg participated in the study. Thirteen percent of normocholesterolemic and 41% of hypercholesterolemic subjects were smokers (P<.05). Subjects with disease or factors causing secondary hypercholesterolemia were excluded from the study. No subject had ever been treated with any lipid-lowering drug.

**Lipids, Apolipoproteins, and HDL Subfractions**

Serum total cholesterol and triglycerides were measured by using enzymatic methods.24 HDL-C was measured by an enzymatic method after the precipitation of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) by a phosphotungstic acid-MgCl₂ reagent.25,26 LDL-C was calculated according to the Friedewald formula,27 which is accurate for triglyceride levels below 4.5 mmol/L: LDL-C=total cholesterol- HDL-C- (triglycerides/2.2).

Total serum apoA-I and apoB were determined by immunonephelometry using a BNA analyzer and monospecific antibodies. Calibration was obtained by using ready-to-use plates (Sibia) with hydrated agarose gels containing monospecific antibodies. Calibration was obtained by using a lyophilized normolipidemic plasma as a secondary standard that had been assayed for LpA-I concentration with reference to the apoA-I concentration of immunofluorometry- and chromatography-isolated LpA-I. The concentration of LpA-I/II was calculated as the difference of total apoA-I determined by electroimmunossay (Sibia) with LpA-I.30 HDL₁ and HDL₃ cholesterol (HDL₁-C and HDL₃-C) concentrations were evaluated by a direct electrophoretic method in discontinuous gradient gels.31 Briefly, serum (40 μL) pre-stained with Sudan Black was electrophoresed in cylindrical tubes over successive layers of 3.5%, 6%, 13%, and 17.5% acrylamide gels in a tris(hydroxymethyl)aminomethane-glycine buffer for 4 hours at 300 V. VLDL and LDL were retained by the 3.5% and the 6% gels, respectively. HDL₁ was concentrated at the interface between the 13% and 17.5% gels, and HDL₃ migrated into the 17.5% gel. The cholesterol distribution in HDL subfractions was quantified by scanning the gels in a Preference densitometer (Sibia). The percentage of cholesterol distribution between HDL subfractions determined by densitometry was applied to total HDL-C measured after precipitation and was expressed in absolute values as millimoles per liter.

**Rheological Variables**

Plasma fibrinogen levels were determined by a thrombin clotting method.32 Plasma protein concentrations were measured with spectrophotometer. Microhematocrit was determined in duplicate by use of a Hawksley centrifuge (12 000g for 3 minutes). All tests were performed at 37°C±0.5°C. Blood

**Table 1. Comparison of Lipids, Apolipoproteins, and Lipoproteins**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normocholesterolemic Men</th>
<th>Hypercholesterolemic Men</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mmol/L</td>
<td>4.64±0.46 (n=16)</td>
<td>6.62±0.73</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.87±0.53 (n=16)</td>
<td>4.65±0.74</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.35±0.34 (n=16)</td>
<td>1.37±0.32</td>
<td>NS</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>0.97±0.43 (n=16)</td>
<td>1.33±0.38</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>1.49±0.21 (n=12)</td>
<td>1.55±0.24</td>
<td>NS</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>0.98±0.16 (n=12)</td>
<td>1.42±0.23</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>LpA-I, g/L</td>
<td>0.44±0.09 (n=8)</td>
<td>0.49±0.16</td>
<td>NS</td>
</tr>
<tr>
<td>LpA-I/II, g/L</td>
<td>0.92±0.15 (n=8)</td>
<td>0.97±0.14</td>
<td>NS</td>
</tr>
<tr>
<td>HDL₁-C, mmol/L</td>
<td>0.39±0.27 (n=8)</td>
<td>0.44±0.25</td>
<td>NS</td>
</tr>
<tr>
<td>HDL₃-C, mmol/L</td>
<td>0.84±0.09 (n=8)</td>
<td>0.93±0.15</td>
<td>NS</td>
</tr>
</tbody>
</table>

TC indicates total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; Apo, apolipoprotein; LpA-I, HDL containing apoA-I without apoA-II; LpA-I/II, HDL containing both apoA-I and apoA-II; HDL₁-C, HDL₁ cholesterol; HDL₃-C, HDL₃ cholesterol, and NS, not significant. Values are mean±SD.
was adjusted to 40%±0.5% hematocrit by removal or addition of autologous plasma.

**Determination of Erythrocyte Aggregation**

A previously validated laser technique (erythrogrammometer, SEFAM) was used to study the aggregation kinetics and disaggregation shear rate of blood samples. \(^{33,34}\) This technique measures the intensity of laser backscattered light (BSL) by a blood suspension situated in a narrow gap between two coaxial cylinders. The inner cylinder is fixed, and the outer cylinder is transparent and rotatable. The outer cylinder can be adjusted to provide shear rates from 7 to 600 s\(^{-1}\). The intensity of BSL by blood suspension is recorded as a function of time and shear rate. The variation of intensity of BSL as a function of time and shear rate allows the determination of the aggregation index (AI), which is related to the aggregation kinetics, and the disaggregation shear rate threshold \((\gamma_t)\), which is related to the shear rate needed to break up the aggregates.

**Statistical Analysis**

Data are expressed as mean±SD. Comparisons between normocholesterolemic and hypercholesterolemic subjects were performed by Student’s t test. \(^{36,37}\) Successive multivariate analyses were performed that included AI and \(\gamma_t\) as dependent variables. Age, smoking, fibrinogen, total serum proteins, and triglycerides were adjusted covariates. Lipoprotein variables were successively introduced in the multivariate models: apoA-I and apoB (model 1); LDL-C, HDL\(_2\)-C, and HDL\(_3\)-C (model 2); and LpA-I and LpA-I/A-II (model 3). The statistical analysis was performed on an Apple Macintosh computer with the use of STATVIEW (Abacus Concepts Corp) and EXCEL (Microsoft Software).

**Results**

Blood lipoprotein component data are given in Table 1. Compared with normocholesterolemic men, hypercholesterolemic men had by definition higher total cholesterol, LDL-C, and apoB but also higher triglycerides, although levels arbitrarily considered as pathological were not reached. No differences existed between the two groups in HDL-C, HDL\(_2\)-C, HDL\(_3\)-C, apoA-I, LpA-I, or LpA-I/A-II. A higher AI \((P<.001)\) and \(\gamma_t\) \((P<.001)\) were found in hypercholesterolemic patients than in normocholesterolemic subjects (Table 2). Furthermore, total serum proteins and plasma fibrinogen were increased in hypercholesterolemic men. Table 3 shows that when only nonsmokers were compared, AI, \(\gamma_t\), total serum protein, and plasma fibrinogen were significantly higher in hypercholesterolemic men. In this group of subjects no differences were observed in triglycerides values. Table 4 gives the correlation coefficients of univariate analysis for the relation of the study variables to RBC aggregation parameters in hypercholesterolemic subjects. RBC AI correlated positively with plasma fibrinogen, total serum proteins, and apoB and inversely with HDL-C, HDL\(_2\)-C, and LpA-I. ApoB was negatively correlated with LpA-I/A-II \((r=-.42; P<.01)\), and the correlation of apoB with AI disappeared at constant LpA-I/A-II values. \(\gamma_t\) correlated positively with plasma fibrinogen, total serum protein, and LpA-I/A-II and inversely with HDL-C and LpA-I. In control subjects fibrinogen

**Table 3. Comparison of Variables in Normocholesterolemic and Hypercholesterolemic Nonsmokers**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normocholesterolemic Nonsmokers</th>
<th>Hypercholesterolemic Nonsmokers</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>43±11</td>
<td>47±8</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>24.3±2.4</td>
<td>24.4±2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>45.3±2.7</td>
<td>45.1±2.3</td>
<td>NS</td>
</tr>
<tr>
<td>TSP, g/L</td>
<td>66.0±2.8</td>
<td>70.4±4.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>2.78±0.32</td>
<td>3.50±0.82</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>4.67±0.43</td>
<td>6.46±0.65</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.03±0.34</td>
<td>1.23±0.38</td>
<td>NS</td>
</tr>
<tr>
<td>AI, units</td>
<td>15.9±2.2</td>
<td>19.7±3.5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>(\gamma_t), s(^{-1})</td>
<td>46.3±4.1</td>
<td>59.7±11.9</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

\(\gamma_t\) index; TSP, total serum protein; TC, total cholesterol; TG, triglycerides; AI, aggregation index; \(\gamma_t\), disaggregation shear rate threshold; and NS, not significant. Values are mean±SD.

**Table 4. Correlations Between RBC Aggregation Parameters and Clinical and Biochemical Variables in Hypercholesterolemic Subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>AI</th>
<th>(\gamma_t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>.16</td>
<td>.05</td>
</tr>
<tr>
<td>BMI</td>
<td>.05</td>
<td>.13</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>.72*</td>
<td>.60*</td>
</tr>
<tr>
<td>TSP</td>
<td>.36†</td>
<td>.44*</td>
</tr>
<tr>
<td>TC</td>
<td>.05</td>
<td>-.09</td>
</tr>
<tr>
<td>TG</td>
<td>.12</td>
<td>.03</td>
</tr>
<tr>
<td>LDL-C</td>
<td>.15</td>
<td>-.02</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-.31†</td>
<td>-.24</td>
</tr>
<tr>
<td>HDL(_2)-C</td>
<td>-.40†</td>
<td>-.34†</td>
</tr>
<tr>
<td>HDL(_3)-C</td>
<td>-.01</td>
<td>.06</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>-.21</td>
<td>-.13</td>
</tr>
<tr>
<td>ApoB</td>
<td>-.29‡</td>
<td>.14</td>
</tr>
<tr>
<td>LpA-I</td>
<td>-.34†</td>
<td>-.33†</td>
</tr>
<tr>
<td>LpA-I/A-II</td>
<td>.15</td>
<td>.30†</td>
</tr>
</tbody>
</table>

AI indicates aggregation index; \(\gamma_t\), disaggregation shear rate threshold; RBC, red blood cell; BMI, body mass index; TSP, total serum protein; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HDL\(_2\)-C, HDL\(_2\) cholesterol; HDL\(_3\)-C, HDL\(_3\) cholesterol; Apo, apolipoprotein; LpA-I, HDL containing apoA-I without apoA-II; and LpA-I/A-II, HDL containing both apoA-I and apoA-II. Values are based on univariate analysis.

*P<.001, †P<.01, ‡P<.05.
correlated with AI (r = .49; P < .05) and yt (r = .61; P < .01). No correlations were obtained with other variables in control subjects.

The data were analyzed in a multiple regression model to determine the specific influence of lipoproteins on RBC aggregation parameters in hypercholesterolemic subjects (Table 5). Three methods of analysis were tested that systematically included age, smoking, fibrinogen, total serum protein, and triglycerides as covariates. The first model included the main protein components of HDL (apoA-I) and LDL (apoB). The second model included the cholesterol content in lipoproteins, LDL-C, HDL2-C, and HDL3-C. Finally, the third model included the apolipoprotein components of HDL, LpA-I, and LpA-I/A-II. In these models, in addition to fibrinogen and total serum protein, HDL2-C, LpA-I, and LpA-I/A-II emerged as significant factors influencing RBC AI and yt. These analyses showed that 60% to 68% of the variance of RBC aggregation and 47% to 64% of the variance of yt could be explained by these factors.

No correlations were observed between triglycerides and RBC aggregation parameters in either univariate or multivariate analysis. In addition, apoB levels were not shown in the multivariate analysis to be associated with AI independently of fibrinogen or in the absence of fibrinogen.

**Discussion**

We investigated erythrocyte aggregation and its relation with lipoprotein components in a hypercholesterolemic middle-aged male population without apparent symptoms of cardiovascular disease. Both AI and yt were elevated in this population compared with normal-cholesterol control subjects, and the aggregation parameters were strongly correlated with the concentration of fibrinogen. This result was expected, because fibrinogen is the major physiological mediator of RBC aggregation. Mean fibrinogen concentrations were indeed significantly higher in the hypercholesterolemic patients than in the normal group but were not correlated with blood lipid parameters. The association of fibrinogen with the RBC aggregation parameters cannot be explained by the differences in smoking habits between control and hypercholesterolemic subjects. The results of the analysis of the rheological data were not affected by exclusion of smokers. Furthermore, smoking did not enter as a significant categorical variable in any multiple regression analysis models that evaluated factors independently related to aggregation, yt, and fibrinogen.

In contrast to fibrinogen, little is known concerning the association of erythrocyte aggregation and plasma lipoproteins and especially HDL-C, which is reported to be inversely related with coronary heart disease incidence. The association of AI and apoB observed in hypercholesterolemic patients disappeared after controlling for age, smoking, fibrinogen, total serum proteins, triglycerides, LpA-I, and LpA-I/A-II. In addition, the AI/apoB association also disappeared when fibrinogen was not controlled, demonstrating that such an association was not unmasked in the absence of fibrinogen.

The inverse correlation of erythrocyte aggregation with HDL-C observed in our study is in good agreement with those reported in both normal subjects and patients with coronary heart disease. However, HDL is a heterogeneous class of lipoproteins, and although a specific association between HDL subclasses...
and atherosclerosis is reported, the mechanisms by which these factors are linked to cardiovascular damage are not yet clarified.\textsuperscript{5,18,19} By determining the respective roles of the main subfractions of HDL we demonstrated that the negative correlation between HDL-C and aggregation parameters was due to HDL\textsubscript{2}-C but not to HDL\textsubscript{3}-C. As shown by multiple regression, this association is independent of fibrinogen, the major determinant of erythrocyte aggregation. According to the protein definition of HDL subfractions, LpA-I, which is HDL containing apoA-I without apoA-II, was also related to aggregation parameters in a negative manner. LpA-I constitutes the majority of the HDL particles in the HDL\textsubscript{2} subtraction. Both HDL\textsubscript{2}-C and LpA-I concentrations are decreased in different events of coronary artery disease.\textsuperscript{5,19,21} The relation of HDL-C and HDL\textsubscript{2}-C with aggregation parameters cannot be explained by the classic increase in correlation between HDL-C and triglycerides, since both univariate and multivariate analyses showed that RBC aggregation parameters were not related to the triglyceride concentrations.

The observation that HDL\textsubscript{2} and LpA-I are related to a decrease in RBC aggregation may be a relevant finding concerning the mechanisms for the protective effects of these components in ischemic heart disease.\textsuperscript{17,20} Under normal circulation conditions the shear forces in medium-sized arteries are such that RBC aggregation and blood viscosity are of minor importance. However, at sites of geometric alterations (e.g., bends, angulation, flow separation, stenosis), low-flow areas, ie, low shear rates, may allow erythrocyte aggregation. Such conditions may exist in ischemic necrosis or infarction, which are well-known complications of hypercholesterolemia and atherosclerosis and might contribute to the induction or aggravation of the course of the disease. We have recently reported that the prevalence of arterial lesions in our population of hypercholesterolemic subjects reached values as high as 74\% for extracoronary plaques and 65\% for coronary calcifications.\textsuperscript{22,23,27} By modifying RBC aggregability, HDL\textsubscript{2} and LpA-I may impede the development of occlusive thrombosis at the sites of partly obstructive atherosclerotic lesions. In hypercholesterolemic subjects, the enhanced RBC aggregability leads to increased blood viscosity and the elevation of RBC shear rate threshold. This indicates that adherence forces between RBCs in the rouleaux are stronger than in normocholesterolemic subjects. The increase in RBC disaggregation shear rate might induce a vicious circle with a further slowing of the circulation. Under such conditions HDL\textsubscript{2} and LpA-I may counteract the adhesive forces of fibrinogen on RBCs in the rouleaux and prevent the promotion of occlusive thrombosis.

In contrast with LpA-I, LpA-I/A-II, which is HDL containing both apoA-I and apoA-II, appeared to be positively related to aggregation parameters and especially to the \textit{yi}. This observation is in line with results from the MONICA project\textsuperscript{1} that show a positive association between apoA-II concentrations and plasma viscosity. The association between LpA-I/A-II seems to be stronger for disaggregation shear rate than for \textit{yi}, suggesting an increased influence on the binding energy of these lipoparticles on RBC aggregation.

The difference in metabolic, functional, and clinical significance between LpA-I and LpA-I/A-II is still controversial. According to Barbaras et al.,\textsuperscript{38} LpA-I particles promote efflux of cholesterol from adipocytes, whereas LpA-I/A-II particles do not facilitate cholesterol efflux, and in fact inhibit the cholesterol efflux mediated by LpA-I. However, Johnson et al.\textsuperscript{39} report that both types of apoA-I-containing particles demonstrate equal ability to promote efflux of cholesterol from other types of cells. On the other hand, LpA-I/A-II but not LpA-I particles within HDL\textsubscript{2} are reported to be converted into HDL\textsubscript{3} by hepatic lipase.\textsuperscript{40} Thus, the contradictory effects of LpA-I and LpA-I/A-II have been previously demonstrated at different steps of the reverse cholesterol transport pathway and are not yet clarified. The clinical significance of LpA-I and LpA-I/A-II is also controversial. LpA-I but not LpA-I/A-II is reported to be inversely correlated with coronary atherosclerosis in patients studied by coronary angiography.\textsuperscript{20,21} However, other studies demonstrate that the relative concentrations of LpA-I and LpA-I/A-II do not significantly influence the discrimination of patients with high risk of coronary artery disease.\textsuperscript{31,42}

Our data suggested an opposite effect of the two types of particles on erythrocyte aggregation, and we speculated on a possible role of apoA-II in inducing increased interactions with RBCs. Both apoA-I and apoA-II exhibit amphiphilic \(\alpha\)-helical segments with distinct polar and nonpolar faces that mediate their interactions with lipids.\textsuperscript{43} However, the average net hydrophobicity of apoA-II is higher than that of apoA-I\textsuperscript{44} and probably contributes to the well-known higher affinity of apoA-II for lipids compared with apoA-I.\textsuperscript{45} The differences in conformation and lipid affinity between apoA-I and apoA-II must be considered with respect to their interactions with RBCs and deserve further investigation.

In conclusion, in the presence of high blood cholesterol the erythrocyte aggregation that occurs with macromolecules bridging the membranes of erythrocytes is strongly influenced by fibrinogen. The negative relation between erythrocyte aggregation and HDL\textsubscript{2}, mainly containing LpA-I, suggests a possible role of this lipoprotein subfraction in reducing the RBC aggregation induced by fibrinogen or other macromolecules. On the other hand, HDL containing apoA-II, ie, LpA-I/A-II, is positively related to erythrocyte aggregation. Thus, the possible influence of HDL particles on RBC interactions would result from a balance between the respective effects of heterogeneous particles. Such phenomena might help us to better understand the disparate influence of HDL particles on atherosclerosis risk and in particular its thrombotic components.

Acknowledgments

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Appendix

The PCVMETRA Group included the following individuals: P. Segond (chairman), D. Badet, C. Baylac-Lebot, P.
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