Elevated Soluble Cellular Adhesion Molecules in Subjects With Low HDL-Cholesterol

Laura Calabresi, Monica Gomaraschi, Barbara Villa, Laura Omoboni, Camille Dmitrieff, Guido Franceschini

Abstract—The purpose of this study was to investigate whether the expression of cellular adhesion molecules (CAMs) is enhanced in individuals with low HDL cholesterol (HDL-C). Plasma levels of soluble vascular cell adhesion molecule-1 (sVCAM-1), intercellular adhesion molecule-1 (sICAM-1), and E-selectin (sE-selectin) were measured in subjects with low (below the 10th percentile for the Italian population), average, or high (above the 90th percentile) HDL-C. Average sICAM-1 and sE-selectin levels were significantly higher in two groups of 65 individuals with low HDL levels, either hyperlipidemic (320.5±16.0 and 61.4±3.5 ng/mL) or normolipidemic (309.6±13.0 and 60.0±2.7 ng/mL), than in subjects with average HDL levels, either hyperlipidemic (267.0±10.1 and 50.4±2.8 ng/mL) or normolipidemic (257.9±5.4 and 51.1±2.4 ng/mL), or with high HDL levels (254.8±10.2 and 52.5±3.2 ng/mL). No significant difference was found in the plasma sVCAM-1 concentration. HDL-C was inversely correlated with sICAM-1 and sE-selectin in the low-HDL subjects (r²=0.087 and 0.035, P=0.0007 and 0.033, respectively), but not in individuals with normal or elevated HDL-C (r²=0.012 and 0.006). A fenofibrate-induced increase of HDL-C in 20 low-HDL subjects was associated with a significant reduction of plasma sICAM-1 and sE-selectin concentrations. An increased CAMs expression may be a mechanism by which a low plasma HDL level promotes atherogenesis and causes acute atherothrombotic events. (Arterioscler Thromb Vasc Biol. 2002;22:656-661.)

Key Words: HDL ■ intracellular adhesion molecule-1 ■ E-selectin ■ vascular cell adhesion molecule-1 ■ atherosclerosis

Epidemiological studies have clearly established a strong inverse correlation between the concentration of plasma HDL-cholesterol (HDL-C) and the incidence of ischemic heart disease (IHD).1 Accordingly, the HDL-C level was added to the US National Cholesterol Education Program algorithm for the prevention of IHD,2 and plasma HDL is now considered a major target for innovative therapeutic approaches to IHD.3,4 There are several potential mechanisms that may be involved in the cardioprotective function of HDL. HDL is thought to remove excess cholesterol from the arterial wall, and drive it to the liver for excretion.5 HDL also has the capacity to directly and beneficially affect several cellular processes involved in the formation, progression and outbreak of a vascular lesion.6 The contribution of these various mechanisms to HDL protection against the development of IHD in humans is still unknown.

Adhesion of leukocytes to the vascular endothelium, and subsequent transmigration into the intima, are key events in the pathogenesis of atherosclerosis.7 Vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin are cellular adhesion molecules (CAMs) that are expressed on the endothelial cell membrane and mediate the adhesion and transmigration of leukocytes to vascular endothelium.8 The expression of these CAMs is enhanced by a variety of pro-atherogenic stimuli, including inflammatory cytokines and reactive oxygen species.9 Increased CAMs expression has been indeed observed in animal models of human atherosclerosis,10 and in human atherosclerotic tissues.11,12 Soluble shedded forms of CAMs (sCAMs) are found in plasma, and their concentration may be regarded as a surrogate marker of cellular expression.13 Indeed, the amount of sCAMs released by cultured endothelial cells is correlated with their cell surface expression,14 and raised plasma sCAMs levels have been found in a variety of pathological conditions in which cell-surface expression is also increased.13 The concentration of sCAMs was reported to be elevated in patients with IHD,15 atherosclerosis,16,17 hyperlipidemia,18,19 and diabetes,20 and a high plasma sCAMs level was found associated with increased risk of future coronary events.17,21-23

HDL has been recently shown to inhibit the upregulation of CAMs expression in cultured endothelial cells induced by different stimuli, such as lipopolysaccharide, tumor necrosis factor-α, or interleukin-1.24,25 To investigate whether this in vitro finding may have clinical significance, we measured the plasma concentration of three different sCAMs in individuals with low, average, or high plasma HDL-C levels.
After an overnight fast, blood was collected into tubes containing Na₂-EDTA (final concentration 1 mg/mL), and plasma was prepared by low-speed centrifugation. Aliquots were immediately frozen at −80°C for subsequent sCAMs determination. The measurement of plasma lipids was performed by using standard enzymatic techniques; HDL-C was determined after precipitation of apolipoprotein B–containing lipoproteins, and LDL-C was calculated by using the Friedewald’s formula. Plasma levels of sVCAM-1, sICAM-1, and sE-selectin were determined by using commercially available monoclonal antibody-based ELISAs (R&D Systems). The assay was performed in duplicate for each sample. The operator was blinded for sample classification. Intra-assay and inter-assay coefficient of variations for all measured sCAMs was <4.7% and <8.0%.

Statistical Analyses

Results are reported as mean±SEM, if not otherwise stated. Differences among groups or treatments were evaluated by using one-way or repeated-measurements ANOVA, with post hoc evaluation by the Newman-Keuls test. For categorical variables, group differences were examined with the use of 2×2 contingency tables and a χ² test of significance. Simple and multivariate regression analyses were performed to assess the association between parameters, and the significance of the correlations was determined by the F parameter. In the forward stepwise regression, the independent parameters were included one at a time starting with the parameter which had the highest correlation with the dependent variable, the plasma sCAM concentration; additional parameters were included only if a significant increase in goodness of fit was achieved. Logarithmic transformation was performed on individual data when values were not normally distributed. The goodness of fit of plasma sCAMs levels to HDL-C was determined by a sum-of-squares best-fit analysis comparing a straight line to a hyperbolic curve; the simpler equation was chosen unless the more complex equation fit significantly better with P<0.05. Group differences or correlations with P<0.05 were considered statistically significant.

Results

Two groups of 65 Low-HDL subjects were recruited among those attending our Lipid Clinic (Low-HDL-LC), or the Blood Service of the Niguarda Hospital (Low-HDL-BD). They were compared with 65 individuals with high HDL levels, and with two groups of controls, from the Lipid Clinic (Controls-LC), or the Blood Service (Controls-BD). There was a prevalence of women among High-HDL subjects compared with the other groups, that were matched for sex; the mean age was higher in the High-HDL group and lower, as expected, in Controls-BD (Table 1). Clinical evaluation of the five groups showed minor differences in BMI, this being

### Methods

**Subjects**

The study includes five groups of 65 individuals with low (below the 10th percentile for age- and sex-matched Italian subjects), average (between the 45th and the 55th percentiles), or high (above the 90th percentile) plasma HDL-C. To investigate the effect of low HDL on plasma sCAMs concentration, independent of that of hyperlipidemia, which has been previously associated with enhanced CAMs expression, two groups of age-sex-matched subjects with low HDL-C were recruited either among patients referred to our Lipid Clinic (Low-HDL-LC), or among normolipidemic (LDL-C 200 mg/dL) patients referred to our Lipid Clinic (Controls-LC), or among normolipidemic (LDL-C 160 mg/dL or triglycerides<200 mg/dL) blood donors attending the Blood Service of the Niguarda Hospital (Low-HDL-BD). Subjects with secondary causes of low HDL-C, such as liver/kidney disease, thyroid dysfunction, diabetes mellitus, obesity (body mass index [BMI] >30 kg/m²), a history of alcohol abuse, or drug treatments known to affect plasma HDL levels were excluded. Two groups of sex-matched individuals with average HDL-C acted as controls; these two groups were randomly recruited either among hyperlipidemic (LDL-C>160 mg/dL and triglycerides>200 mg/dL) patients referred to our Lipid Clinic (Controls-LC), or among normolipidemic blood donors (Controls-BD). A fifth group of subjects with high HDL-C was recruited among patients referred to our Lipid Clinic (High-HDL). A detailed medical history, with particular emphasis on cardiovascular and metabolic diseases, history of smoking, and drug treatments, was collected for all subjects. Individuals with chronic inflammatory disorders were excluded from the study.

A pilot study to test whether a drug-induced increase of HDL-C resulted in opposite changes in plasma sCAMs levels was conducted in 20 consecutive Low-HDL subjects, who were randomly assigned to receive an HDL-raising agent, comirazonized fenofibrate (200 mg/dL), or a corresponding placebo for two periods of 8 weeks each, according to a double-blind crossover protocol. Plasma lipid and sCAMs levels were measured at baseline and at the end of each treatment period.

All subjects gave an informed consent, and the study protocol was approved by the Institutional Review Board.

**Measurements**

After an overnight fast, blood was collected into tubes containing Na₂-EDTA (final concentration 1 mg/mL), and plasma was prepared by low-speed centrifugation. Aliquots were immediately frozen at −80°C for subsequent sCAMs determination. The measurement of plasma lipids was performed by using standard enzymatic techniques; HDL-C was determined after precipitation of apolipoprotein B–containing lipoproteins, and LDL-C was calculated by using the Friedewald’s formula. Plasma levels of sVCAM-1, sICAM-1, and sE-selectin were determined by using commercially available monoclonal antibody-based ELISAs (R&D Systems). The assay was performed in duplicate for each sample. The operator was blinded for sample classification. Intra-assay and inter-assay coefficient of variations for all measured sCAMs was <4.7% and <8.0%.

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**Results**

Two groups of 65 Low-HDL subjects were recruited among those attending our Lipid Clinic (Low-HDL-LC), or the Blood Service of the Niguarda Hospital (Low-HDL-BD). They were compared with 65 individuals with high HDL levels, and with two groups of controls, from the Lipid Clinic (Controls-LC), or the Blood Service (Controls-BD). There was a prevalence of women among High-HDL subjects compared with the other groups, that were matched for sex; the mean age was higher in the High-HDL group and lower, as expected, in Controls-BD (Table 1). Clinical evaluation of the five groups showed minor differences in BMI, this being

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics of Subjects With Low-HDL and High-HDL and of Control Subjects</th>
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<tbody>
<tr>
<td>No.</td>
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<tr>
<td>Sex, F/M</td>
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<td>Age, y</td>
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<tr>
<td>BMI, kg/m²</td>
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<td>Systolic BP, mm Hg</td>
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<td>Diastolic BP, mm Hg</td>
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<tr>
<td>LDL cholesterol, mg/dL</td>
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<tr>
<td>HDL cholesterol, mg/dL</td>
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<tr>
<td>Triglycerides, mg/dL</td>
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<td>Glucose, mg/dL</td>
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Data are mean±SEM. *Significantly different from Controls-BD (at least P<0.05).
...which showed similar average values. The average LDL-C level was significantly lower in Controls-BD, intermediate in the Low-HDL groups, and higher in High-HDL and Controls-LC. Blood glucose was significantly higher in the Low-HDL-LC, but not in the Low-HDL-BD, subjects than in the other groups.

The median and average plasma concentrations of the investigated sCAMs, sVCAM-1, sICAM-1, and sE-selectin were higher in the two Low-HDL groups compared with controls, both hyperlipidemic (Controls-LC) and normolipidemic (Controls-BD) (Figure 1, Table 2). These differences were all significant, except for sVCAM-1. Plasma sICAM-1 and sE-selectin levels were similarly elevated in Low-HDL subjects with normal or high total cholesterol and triglyceride values (Low-HDL-BD and Low-HDL-LC, respectively) (Figure 1, Table 2). No significant differences in plasma sCAMs were found in the Low-HDL groups between female and male subjects (not shown). Median and average plasma sCAMs levels were similar among High-HDL and controls (Figure 1, Table 2).

To further explore the “independent” effect of low-HDL on plasma sCAMs concentrations, stepwise multiple regression analyses were performed with data from all subjects, sICAM-1 and sE-selectin as dependent variables, and all the independent variables listed in Table 1. A low HDL-C was the strongest independent predictor of a higher sICAM-1 ($F=28.2, P<0.0001$), followed by high LDL-C ($F=13.7, P=0.0003$), and age ($F=5.4, P=0.0213$). A low HDL-C ($F=15.3, P=0.0001$) and a high total cholesterol ($F=5.0, P=0.0262$) were joint predictors of higher sE-selectin. No other variables entered the final predictive sICAM-1 and sE-selectin models.

A lower plasma HDL-C was a significant univariate predictor of higher sICAM-1 ($r^2=0.087, P=0.0007$) and sE-selectin ($r^2=0.035, P=0.033$) in the Low-HDL groups but not in individuals with normal or elevated HDL-C concentrations ($r^2=0.012$ and 0.006, respectively). When all data were considered together, the relationship between HDL-C and either sICAM-1 or sE-selectin was best fit by a hyperbolic ($r^2=0.149$ and $r^2=0.052$) rather than linear ($r^2=0.077$ and $r^2=0.038$) equation, with minor changes of plasma sICAM-1 and sE-selectin concentrations occurring at HDL-C values above 40 mg/dL (Figure 2). No significant correlation was found between HDL-C and sVCAM-1, either in the whole series or in separate groups.

Finally, to investigate whether an increase of plasma HDL-C in Low-HDL subjects is associated with an opposite change in sCAMs concentrations, 20 consecutive Low-HDL subjects were given fenofibrate or a corresponding placebo.

![Figure 1. Box plot of sVCAM-1, sICAM-1, and sE-selectin concentrations in the plasma of hyperlipidemic or normolipidemic subjects with low HDL-C (Low-HDL-LC and Low-HDL-BD), of hyperlipidemic or normolipidemic subjects with average HDL-C (Controls-LC and Controls-BD), and of subjects with high HDL-C (High-HDL). The plot displays median values with the 25th and 75th percentiles; capped bars indicate the 10th and 90th percentiles. Each group consists of 65 individuals.](http://atvb.ahajournals.org/)

### TABLE 2. Plasma Levels of Soluble Cell Adhesion Molecules in Subjects With Low-HDL and High-HDL and in Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Low-HDL-LC</th>
<th>Low-HDL-BD</th>
<th>High-HDL</th>
<th>Controls-LC</th>
<th>Controls-BD</th>
</tr>
</thead>
<tbody>
<tr>
<td>sVCAM-1</td>
<td>666.1±42.0</td>
<td>629.0±31.3</td>
<td>598.1±32.5</td>
<td>597.6±24.5</td>
<td>613.3±21.4</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>320.5±16.0*</td>
<td>309.6±13.0*</td>
<td>254.8±10.2</td>
<td>267.0±10.1</td>
<td>257.9±5.4</td>
</tr>
<tr>
<td>sE-selectin</td>
<td>61.4±3.5*</td>
<td>60.0±2.7*</td>
<td>52.5±3.2</td>
<td>50.4±2.8</td>
<td>51.1±2.4</td>
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Data are in ng/mL, mean±SEM.
*Significantly different from Controls-BD (at least $P<0.05$).
for 8 weeks, in a double-blind crossover study. As expected, plasma lipid levels did not change after placebo; fenofibrate treatment raised plasma HDL-C levels by 21% and reduced total cholesterol and triglyceride concentrations by 8% and 41%, respectively (Table 3). The fenofibrate-induced sHDL increase was accompanied by significant reductions of plasma sICAM-1 and sE-selectin, but not of sVCAM-1 (Table 3). The decrease of sICAM-1 correlated inversely with the HDL-C rise ($r^2=0.50, P=0.0005$), and directly with the triglyceride reduction ($r^2=0.38, P=0.0071$). The decrease of sE-selectin correlated inversely with the increase of HDL-C ($r^2=0.33, P=0.0082$), but not with the triglyceride reduction ($r^2=0.12, P=0.13$).

Previous studies have shown that patients with severe elevations of plasma LDL-C or triglycerides have increased levels of sCAMs. Those patients also had subnormal plasma HDL-C, and that may have contributed to the increased plasma sCAMs levels. A low HDL-C often occurs in the presence of other metabolic abnormalities, such as high triglycerides, obesity, and some degree of glucose intolerance. These complex metabolic interrelationships may complicate the search for the “independent” effect of low HDL-C on elevated sCAMs concentrations. This issue has been addressed here by recruiting a group of healthy blood donors with low HDL but normal triglycerides, BMI, and blood glucose. Their average plasma sICAM-1 and sE-selectin were remarkably similar to those of Low-HDL subjects recruited in a Lipid Clinic, who had higher average triglycerides, BMI, and blood glucose. Their average plasma sICAM-1 and sE-selectin were higher than controls. However, the difference among low HDL-C groups was not statistically significant. In summary, the present data show that (1) individuals with low HDL-C levels have elevated plasma concentrations of sICAM-1 and sE-selectin, but with high HDL-C levels have sCAMs concentrations similar to healthy controls; (2) HDL-C is inversely and significantly associated with sICAM-1 and sE-selectin in individuals with low plasma HDL-C, but not in those with normal or elevated HDL-C; (3) the impact of HDL-C on plasma sICAM-1 and sE-selectin concentrations is independent of triglyceride and LDL-C levels; (4) a drug-induced increase of plasma HDL-C in Low-HDL subjects is associated with a significant reduction of both sICAM-1 and sE-selectin levels. All together, these findings indicate that plasma HDL effectively modulate sICAM-1 and sE-selectin levels in vivo.

The present study is based on the hypothesis that the levels of sCAMs may serve as surrogate markers that reflect the expression of CAMs by the vascular endothelium. Indeed, increased levels of sCAMs have been observed in a variety of clinical conditions in which detailed pathology studies have shown an increased CAMs expression on endothelial cells. Therefore, the present data imply that a low plasma HDL-C level is associated with ICAM-1 and E-selectin overexpression in the vascular endothelial wall. This assumption is fully consistent with the results of in vitro experiments showing that the cytokine-induced expression of CAMs in cultured endothelial cells is blunted, in a dose-dependent manner, by the addition of plasma-derived or synthetic HDL, with maximal inhibition occurring at HDL concentrations similar to those found in the plasma of healthy individuals.

**TABLE 3.** Plasma Levels of HDL Cholesterol and sCAMs in 20 Low-HDL Subjects Before (Baseline) and After 8 Weeks of Treatment with Placebo or Fenofibrate

<table>
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<th>Baseline</th>
<th>Placebo</th>
<th>Fenofibrate</th>
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<tbody>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>254.3±12.0</td>
<td>257.6±15.0</td>
<td>234.8±8.9</td>
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<tr>
<td>Triglycerides, mg/dL</td>
<td>303.5±43.7</td>
<td>331.6±49.5</td>
<td>178.7±18.7*</td>
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<tr>
<td>HDL cholesterol, mg/dL</td>
<td>33.3±1.0</td>
<td>33.5±1.5</td>
<td>40.2±1.4*</td>
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<tr>
<td>sVCAM-1, ng/mL</td>
<td>631.4±43.1</td>
<td>631.5±41.7</td>
<td>612.0±36.7</td>
</tr>
<tr>
<td>sICAM-1, ng/mL</td>
<td>319.4±14.2</td>
<td>321.4±14.3</td>
<td>278.3±13.1*</td>
</tr>
<tr>
<td>sE-selectin, ng/mL</td>
<td>59.8±4.2</td>
<td>60.0±4.2</td>
<td>53.8±3.7*</td>
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</table>

Data are mean±SEM.

*Significantly different from baseline and placebo (at least $P<0.05$).
not achieve statistical significance. This result may be explained by the different pattern of CAMs expression in various cell types and tissues. VCAM-1 is unique in that its expression is more prevalent in the intima of atherosclerotic plaques than in nonatherosclerotic segments. ICAM-1 and E-selectin are mostly expressed in endothelial cells, and their expression is enhanced by a variety of pro-inflammatory stimuli. Indeed, a significant correlation was found between sVCAM-1, but not sICAM-1 and sE-selectin, levels and the extent of atherosclerosis, as assessed by angiography. It is noteworthy that a high plasma sICAM-1 and sE-selectin, but not sVCAM-1, concentration has been identified as a risk factor for future myocardial infarction in initially healthy people, whereas sVCAM-1 has a strong predictive value in patients with atherosclerotic lesions. Elevated sVCAM-1 levels may reflect the presence of advanced atherosclerotic disease, whereas high sICAM-1 and sE-selectin might be markers of a chronic inflammatory condition predisposing to atherosclerosis development. The differential effect of Low-HDL on sCAMs levels suggests it is the low HDL that causes endothelial dysfunction that leads to increased ICAM-1 and E-selectin expression and plasma levels, rather than the elevated sCAMs being the consequence of extensive preclinical atherosclerosis induced by the low HDL-C levels. Indeed, HDL was able to inhibit cytokine-induced CAMs expression in cultured endothelial cells and to prevent the binding of monocytes to target endothelial cells. Moreover, HDL reversed an impaired endothelial function, both in vitro and in vivo. The plasma levels of sICAM-1 and sE-selectin increase in the course of an acute myocardial infarction, and this higher level of adhesion molecules may be a mechanism by which a low plasma HDL concentration not only promotes atherogenesis but also causes and worsens acute atherothrombotic events. This hypothesis, which would explain the beneficial effects of the current HDL-raising agents in preventing vessels narrowing and reducing acute ischemic events, prompts the development of innovative and specific HDL-based therapies for the prevention and treatment of cardiovascular disease.

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References


