# Lack of Association Between Low HDL-cholesterol and Elevated Circulating Cellular Adhesion Molecules in Normolipidemic CAD Patients and Healthy Subjects

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**SUMMARY**

High plasma HDL-cholesterol (HDL-c) is a well-established protective factor in coronary artery disease (CAD). One of its potential protective mechanisms is the inhibition of the cytokine-induced upregulation of expression of cellular adhesion molecules (CAMs). High sCAM levels were found to be associated with low HDL-c in some studies performed mostly in hyperlipidemic subjects, but this association has not yet been investigated in CAD patients. In addition, conflicting results were obtained from *in vitro* studies that explored the proposed HDL effect on cytokine-induced CAM expression. The aim of the present case-control study was to investigate whether low HDL-c values are associated with CAM overexpression in normolipidemic CAD patients and healthy individuals, matched according to age and gender. Plasma HDL-c, sICAM-1, sVCAM-1, and sE-selectin were measured in 37 normolipidemic patients with angiographically verified coronary artery disease and in 52 healthy normolipidemic subjects. The sCAM values obtained in the subjects (patients or controls) with low HDL-c levels (< 1.03 mmol/L) were compared with the values in the subjects with high HDL-c (≥ 1.03 mmol/L). No significant difference was found between sICAM-1, sVCAM-1, and E-selectin values obtained in subjects with low and high HDL-c, either among the patients or the healthy controls. In conclusion, low HDL-c levels are not associated with CAM overexpression in normolipidemic CAD patients and healthy subjects. (Int Heart J 2005; 46: 593-600)

**Key words:** Atherosclerosis, High-density lipoproteins, E-selectin, Intracellular adhesion molecule-1, Vascular cell adhesion molecule-1

INCREASED leukocyte adhesion to the vascular endothelium and their transendothelial migration are early events in atherogenesis, mediated by cellular adhesion molecules (CAMs): intercellular adhesion molecule-1 (ICAM-1), vascular

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cell adhesion molecule-1 (VCAM-1), and E-selectin.[1] Membrane bound CAMs are expressed on endothelial cells, smooth muscle cells, and tissue macrophages.[2,3] Their soluble forms, sICAM-1, sVCAM-1, and sE-selectin circulate in plasma, and the sCAM plasma levels are correlated with CAM expression on the endothelial cell membrane.[4]

A high plasma HDL-cholesterol (HDL-c) level is an established protective factor in coronary artery disease (CAD).[5] There is evidence that the protective effect of HDL is not achieved solely by its role in reverse cholesterol transport, but also by several other mechanisms, including its antioxidative and antiinflammatory activities.[6] The cytokine-induced upregulation of CAM expression was reported to be inhibited by HDL,[7] and high circulating sCAM levels were shown to be associated with low HDL-c in several studies performed in different settings.[8-10] In the present study, we investigated the relationship between HDL-c and sCAM (sICAM-1, sVCAM-1 and sE-selectin) levels in normolipidemic CAD patients and healthy individuals who were matched according to age and gender.

## METHODS

**Study population:** A group consisting of 37 patients with angiographically established CAD with ≥ 50% of luminal narrowing was formed out of a larger group of 100 patients referred to the Institute of Cardiovascular Disease, Clinical Centre of Serbia, Belgrade, by using the following exclusion criteria: LDL-c ≥ 4.13 mmol/L and/or triglycerides ≥ 2.25 mmol/L, presence of liver or kidney disease, diabetes mellitus, obesity (body mass index [BMI] > 30 kg/m$^2$), history of alcohol abuse, or drug treatments known to affect plasma sCAMs levels. Angiograms were reviewed by two cardiologists who were not aware that a patient was enrolled in the study. The control group consisted of 52 healthy blood donors who met the same criteria used for patient selection. The study was planned according to the ethical guidelines of the Declaration of Helsinki. According to institutional guidelines, the protocol was approved by the local institutional review committee, and informed consent was obtained from each subject involved in the study.

**Apparatus, reagents, and procedures:** Blood samples were collected into evacuated tubes containing EDTA, citrate or serum separator gel, after a 12 hour fasting period. Plasma and serum were separated and multiple aliquots of each sample were stored at -80°C. The samples were thawed immediately before analyses.

HDL-cholesterol was measured by an enzymatic method using a Hitachi 704 analyzer and reagents (Roche Diagnostics, Mannheim, Germany) after precipitation of the EDTA plasma with phosphotungstic acid in the presence of magnesium ions. Circulating cellular adhesion molecules (sICAM, sVCAM, and sE-

selectin) were assayed using the ELISA technique with a Parameter Colorimetric Sandwich ELISA (R&D Systems, Minneapolis, USA) as recommended by the manufacturer and a BIOMEK 1000 Automated Laboratory Work Station (Beckmann Instruments, Inc., Fullerton, CA, USA). Serum hs-CRP levels were assayed using a latex-enhanced immunoturbidimetric method with a Quantex hs-CRP kit (BIOKIT, Barcelona, Spain) and ILAB 600 analyzer (International Laboratory, Milan, Italy). Fibrinogen was measured in citrate plasma by the Clauss method with ACL 200 (International Laboratory, Milan, Italy) and the original reagents. Total cholesterol, triglyceride, LDL-cholesterol, apolipoprotein AI, and apolipoprotein B concentrations were also measured using standard laboratory procedures.

**Statistical methods:** Unless otherwise specified, data are presented as the mean ± SD, or frequency percentages. Statistical testing of differences in the continuous variables between the groups was performed using Student's *t* test for normally distributed variables. Because the distributions of the triglyceride and hs-CRP values were skewed, a log transformation was conducted and the values obtained were compared. For these parameters, the median values are also presented. For categorical variables, group differences were examined using $2 \times 2$ contingency tables and $\chi^2$ test of significance. Group differences with $P < 0.05$ were considered statistically significant. Correlations between sCAM and HDL-c values were also examined by Spearman regression analysis.

## RESULTS

Based on the results of the HDL-c measurements, both the patients and healthy controls were divided into two groups to give a total of four groups: CAD patients with HDL-c < 1.03 mmol/L, CAD patients with HDL-c ≥ 1.03 mmol/L, controls with HDL-c < 1.03 mmol/L, and controls with HDL-c ≥ 1.03 mmol/L.

The demographic characteristics and the concentrations of circulating lipid profile parameters, hs-CRP, and fibrinogen in the patients and healthy subjects with low and high HDL-c are shown in Table I. The results are reported as the mean ± SD, or frequency percentages. Medians are also shown for triglycerides and hs-CRP, because of their skewed distributions. As expected, triglycerides were higher and apo A-I lower in the low HDL-c groups. Significantly higher hs-CRP levels were obtained in the patients than in the healthy controls, both in the low HDL-c and high HDL-c groups. The concentration of hs-CRP was lower in the subjects with higher HDL-c, but only in the group of healthy controls. A greater proportion of smokers was found in the CAD patients. The proportions of the patients receiving aspirin or ACE inhibitors are also shown, and they were not significantly different between the groups with different HDL-c levels. As only

**Table I.** Demographic Characteristics and Laboratory Data of Normolipidemic CAD Patients and Healthy Subjects Divided According to Their HDL-c Levels

| | Control group ($n$ = 52) | | Patient group ($n$ = 37) | |
|---|---|---|---|---|
| Variable | HDL-c < 1.03 mmol/L | HDL-c ≥ 1.03 mmol/L | HDL-c < 1.03 mmol/L | HDL-c ≥ 1.03 mmol/L |
| $n$ | 16 | 36 | 17 | 20 |
| $n$ (males)/$n$ (females) | 15/1 | 22/14 | 12/5 | 12/8 |
| Age, years | 48.0 ± 10.0 | 52.0 ± 8.7 | 49.4 ± 9.4 | 54.5 ± 7.3 |
| BMI, kg/m$^2$ | 26.48 ± 2.14 | 25.75 ± 3.63 | 27.50 ± 2.39 | 25.16 ± 3.22 * |
| SBP, mmHg | 136 ± 9 | 140 ± 19 | 132 ± 13 | 141 ± 20 |
| DBP, mmHg | 84 ± 7.3 | 83 ± 8.7 | 84 ± 8.3 | 87 ± 11.3 |
| Cholesterol, mol/L | 4.78 ± 0.65 | 5.7 ± 0.57 * | 4.83 ± 0.63 | 5.20 ± 0.52 |
| Triglycerides, mmol/L | 1.67 ± 0.49 (median: 1.81) | 1.34 ± 0.48 * (median: 1.34) | 1.61 ± 0.35 (median: 1.62) | 1.26 ± 0.35 * (median: 1.15) |
| HDL-cholesterol, mmol/L | 0.89 ± 0.13 | 1.24 ± 0.1.16 * $^+$ | 0.84 ± 0.14 | 1.46 ± 0.33 * |
| LDL-cholesterol, mmol/L | 3.14 ± 0.59 | 3.19 ± 0.43 | 3.26 ± 0.50 | 3.40 ± 0.45 |
| Apo A-I, g/L | 1.07 ± 0.19 | 1.49 ± 0.28 * $^+$ | 1.05 ± 0.20 | 1.20 ± 0.22 * |
| Apo B, g/L | 1.32 ± 0.47 | 1.10 ± 0.26 * $^+$ | 1.59 ± 0.50 | 1.38 ± 0.36 |
| hs-CRP, mg/L | 2.61 ± 2.77$^+$ (median: 1.62) | 1.03 ± 0.88* $^+$ (median: 0.66) | 8.08 ± 15.41 (median: 2.59) | 10.16 ± 18.88 (median: 2.87) |
| Fibrinogen, g/L | 4.77 ± 1.23 | 4.47 ± 1.22$^+$ | 5.39 ± 1.63 | 5.84 ± 1.76 |
| Smokers, % | 37.5 $^+$ | 39 $^+$ | 59 | 60 |
| Aspirin treatment, % | - | - | 23.5 | 20 |
| ACE inhibitors treatment, % | - | - | 41 | 50 |

SBP = systolic blood pressure; DBP = diastolic blood pressure. Values are given as means ± SD. *$P$ < 0.05 for HDL-c ≥ 1.03 mmol/L versus HDL-c < 1.03 mmol/L in control or patient groups; $^+P$ < 0.05 for HDL-c ≥ 1.03 mmol/L in control versus patient groups and HDL-c < 1.03 mmol/L in control versus patient groups.

**Table II.** Plasma Concentrations of sCAMs in Normolipidemic CAD Patients and Healthy Subjects Divided According to Their HDL-c Levels

| | Control group ($n$ = 52) | | Patient group ($n$ = 37) | |
|---|---|---|---|---|
| Variable | HDL-c < 1.03 mmol/L $n$ = 16 | HDL-c ≥ 1.03 mmol/L $n$ = 36 | HDL-c < 1.03 mmol/L $n$ = 17 | HDL-c ≥ 1.03 mmol/L $n$ = 20 |
| sVCAM-1, $\mu$g/L | 658.6 ± 270.21 | 678.4 ± 328.90 | 758.7 ± 289.22 | 748.9 ± 268.58 |
| sICAM-1, $\mu$g/L | 297.1 ± 110.14 | 280.1 ± 83.86 | 305.3 ± 124.25 | 253.1 ± 55.12 |
| sE-selectin, $\mu$g/L | 37.24 ± 15.35$^+$ | 35.08 ± 23.32 | 24.76 ± 15.23 | 30.27 ± 19.29 |

Values are given as means ± SD. $^+P$ < 0.05 for HDL-c < 1.03 mmol/L in control versus patient groups.

normolipidemic patients were included in the study, none received lipid-lowering drugs.

The sCAM levels did not differ significantly between the patients receiving ACE inhibitors or aspirin and the patients not treated with the drugs ($P$ > 0.05 for

all three adhesion molecules measured: sICAM-1, sVCAM-1, and sE-selectin, data not shown).

When we compared the plasma sCAM concentrations obtained in the subjects with low versus subjects with high HDL-c levels, no statistically significant differences between the sCAM values could be demonstrated either in the patients or in the control group (Table II). The correlation between sCAM and HDL-c values was also tested, but no significant correlation was found ($P > 0.05$ for all three sCAMs measured).

## DISCUSSION

Inhibition of cytokine-induced expression of CAMs by HDL was proposed to be one of several mechanisms of HDL protective function.[8] Numerous genetic and environmental factors have been shown to influence circulating sCAM levels, including hypercholesterolemia, hypertriglyceridemia, smoking, hypertension, and diabetes mellitus.

Many studies have investigated the association of sCAMs with coronary artery disease, as well as their potential significance as circulating markers of the disease. To our knowledge, the influence of low HDL-c on sCAM concentrations has not yet been studied in CAD patients. The present data show that sCAM concentrations in patients with angiographically established CAD and low HDL-c levels did not differ from the values obtained in the patients with high HDL-c (Table II). However, we also found no significant relationship between HDL-c and sCAMs in healthy controls. This is in disagreement with the results of several studies conducted by other authors in different settings, which reported the association of high sCAM with low HDL-c concentrations.[8-10] This difference could be attributed to the different study design and patient selection. In contrast with our patients and control groups of normolipidemic subjects, Lupatelli, *et al*[9] and Abe, *et al*[10] investigated the effect of low HDL-c on sCAM levels in hyperlipidemic subjects. Lupatelli, *et al*[9] found that low HDL-c was associated with higher sVCAM-1 and sICAM-1 levels, whereas Abe, *et al*[10] observed an association with three sCAMs: sVCAM-1, sICAM-1, and sE-selectin. However, Calabresi, *et al*[8] reported increased sICAM-1 and sE-selectin concentrations in both normolipidemic and hyperlipidemic subjects with low plasma HDL-c values. The difference between their findings and the results of the present study could only be attributed to the different genetic and environmental backgrounds of the subjects involved in the two studies.

The results of *in vitro* studies that investigated the inhibition of proinflammatory cytokine-induced expression of CAMs by HDL are also controversial. Cockerill, *et al*[11] demonstrated that HDL inhibits TNF$\alpha$ and IL-1$\beta$-induced

expression of cellular adhesion molecules in cultured human umbilical cord vein endothelial cells. However, Zhang, *et al*,[12] reported that no inhibitory effect of HDL on cytokine-induced CAM expression could be found in cultured human aortic endothelial cells, which is consistent with the results of the present study.

Higher concentrations of acute phase proteins (hs-CRP and fibrinogen) were found in the CAD patients than in the healthy controls, showing the presence of an inflammatory reaction in coronary artery disease (Table I). The concentration of hs-CRP, which is known to be a very sensitive marker of inflammation, was lower in the healthy subjects with higher HDL-c (HDL-c ≥ 1.03 mmol/L). This could be a consequence of the antiinflammatory activity of HDL. However, this effect is lost in coronary artery disease, since no association between high HDL-c and low hs-CRP values could be demonstrated in the CAD patients (Table I).

Several reports have appeared which studied the potential antiinflammatory effects of aspirin, ACE inhibitors, and statins, as established pharmacotherapy in patients with coronary artery disease.[13-18] Although the antiinflammatory effects of larger doses of aspirin are well-known, they are generally considered negligible at low doses commonly used in CAD patients, and the beneficial effects of aspirin are currently linked to suppression of thromboxane $A_2$-dependent platelet aggregation.[13,14] ACE inhibitors were shown to reduce inflammation, not only by inhibiting angiotensin II formation, but also by several angiotensin II-independent mechanisms.[14,16] The potential antiinflammatory activity of statins was explained by the inhibition of nuclear factor-$\kappa$B, a pivotal transcription factor regulating the expression of a variety of inflammatory genes, which leads to the reduced synthesis of inflammatory cytokines.[14] In our study, a substantial proportion of the CAD patients were treated with aspirin or ACE inhibitors (Table I). The proportions of the patients receiving ACE inhibitors and aspirin were similar in the high HDL-c and low HDL-c groups, and the levels of all three sCAMs measured in the patients receiving the drugs and the patients without therapy were not significantly different, therefore, we were unable to prove any association between either HDL-c or sCAM levels and therapy with ACE inhibitors or aspirin. We were not able to examine the possible effects of statins, since only normolipidemic patients were included in our study.

Because of the large interindividual variations in sCAM concentrations obtained in the present study (Table II), as well as by other authors,[9,17] a larger number of subjects should be included in the study to improve the significance of the results. However, coronary angiography is an invasive method that is not performed widely and without a good reason. In addition, since hyperlipidemia is a well-known risk factor for CAD, a large proportion of the patients undergoing coronary angiography were hyperlipidemic, and only normolipidemic subjects

were studied to exclude the effects of hyperlipidemia on sCAM concentrations. These two factors significantly reduced the number of patients in the study.

We do agree with recent reports[18,19] that, although endothelial dysfunction is an important feature in early as well as in advanced stages of atherosclerosis, we can not advocate its routine assessment by measuring soluble CAMs. As suggested,[19] certain aspects of laboratory limitations and costs need to be addressed as well. To conclude, low HDL-c is not associated with CAM overexpression in normolipidemic CAD patients and healthy individuals, but further studies with greater numbers of subjects are needed to draw definite conclusions and elucidate completely the relationship between HDL and cellular adhesion molecules.

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