Cross-Sectional Study of Soluble Intercellular Adhesion Molecule-1 and Cardiovascular Risk Factors in Apparently Healthy Men

Luis E.P. Rohde, Charles H. Hennekens, Paul M. Ridker

Abstract—An elevated plasma concentration of the soluble intercellular adhesion molecule-1 (sICAM-1) is associated with increased risk for future coronary events. However, data exploring the interrelations of sICAM-1 with known cardiovascular risk factors are sparse. We determined sICAM-1 levels in 948 middle-aged men with no prior history of cardiovascular disease. sICAM-1 levels increased with age (P<0.001) and were significantly associated with smoking (P<0.001), hypertension (P<0.05), and frequent alcohol consumption (P=0.006). Positive correlations were observed between sICAM-1 and triglycerides (r=0.15; P<0.001), fibrinogen (r=0.21; P<0.001), tissue-type plasminogen activator antigen (r=0.17; P<0.001), and total homocysteine (r=0.09; P=0.02); whereas a negative correlation was observed for high density lipoprotein cholesterol (r=−0.15; P<0.001). Overall, plasma concentrations of sICAM-1 increased with increasing prevalence of usual cardiovascular risk factors; mean plasma concentrations were 231, 236, 245, 257, and 312 ng/mL for those subjects with 0, 1, 2, 3, and >4 risk factors, respectively (P<0.01 for trend). In multivariate analysis, age, smoking status, diabetes, systolic blood pressure, positive family history of coronary disease, and serum levels of total homocysteine and fibrinogen were all independently associated with sICAM-1 levels (all P≤0.05). sICAM-1 levels are associated with several established cardiovascular risk factors. Further studies will be needed to evaluate whether these associations reflect the role of sICAM-1 as a marker of preclinical atherosclerosis, and whether such interrelations might have a causal basis. (Arterioscler Thromb Vasc Biol. 1999;19:1595-1599.)

Key Words: adhesion molecules inflammation risk factors atherosclerosis

Cellular adhesion molecules mediate the attachment and transmigration of leukocytes across the endothelial surface, and are hypothesized to play an important role in the initiation of atherosclerosis. Focal expression of adhesion molecules has been demonstrated in atherosclerotic lesions, and precedes leukocyte infiltration, and seems to be mediated in part by modified lipoproteins or their constituents. Several small studies have demonstrated that plasma concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) are elevated in atherosclerotic syndromes, and recent prospective studies indicate that elevated baseline levels of sICAM-1 are associated with increased risk for future coronary events. However, despite these provocative data, little is known about potential relations between circulating adhesion molecules and established cardiovascular risk factors.

We explored potential associations between the soluble adhesion molecule sICAM-1 and a series of lipid and nonlipid cardiovascular risk factors among 948 apparently healthy men participating in the Physicians’ Health Study. Specifically, we evaluated for evidence of association between sICAM-1 levels and age, smoking status, blood pressure, alcohol use, exercise frequency, body mass index, total cholesterol, HDL cholesterol (HDL-C), triglycerides, Lp(a), fibrinogen, tissue-type plasminogen activator antigen, and total homocysteine levels.

Methods

We evaluated sICAM-1 levels in a cross-section of apparently healthy middle-aged men participating in the Physician’s Health Study. In brief, the Physician’s Health Study was a randomized, double-blind, placebo-controlled 2×2 factorial design trial of aspirin and β-carotene among US male physicians aged 40 to 84 years. Exclusion criteria included a history of myocardial infarction, stroke, or transient ischemic attack; cancer; current renal or liver disease; peptic ulcer or gout; contraindication to aspirin consumption; current use of aspirin, other platelet-active drugs, or nonsteroidal anti-inflammatory agents; and current use of vitamin A or β-carotene supplement.

As described elsewhere, eligible participants were asked to provide baseline blood plasma samples. Kits including edetic acid tubes and plastic collection vials were sent to each doctor along with instructions for blood drawing. Participants were asked to have their blood drawn and centrifuged, and have the plasma returned (accompanied by a cold pack) by overnight courier. Participants also reported on baseline cardiovascular risk factors of age, smoking status, height, weight, systolic and diastolic blood pressure, history of hypercholesterolemia, diabetes, exercise frequency, and alcohol use.

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Of the 22,071 men randomized onto the Physician’s Healthy Study, 68% (14,916) provided baseline blood samples. Of these men, 948 had previously been selected to participate in a nested case-control study of sICAM-1 and myocardial infarction. Stored plasma for each of these participants was thawed and assayed for sICAM-1 with a commercially available ELISA, based in purified proteins and polyclonal antibodies, according to manufacturer’s recommendations (R&D Systems, Inc). Plasma concentrations of total cholesterol, HDL-C, Lp(a), fibrinogen, tissue-type plasminogen activator antigen, and total plasma homocysteine were also assayed. Spearman correlation coefficients were calculated to evaluate for evidence of association between sICAM-1 levels and body mass index, systolic and diastolic blood pressure, total cholesterol, HDL-C, triglycerides, Lp(a), fibrinogen, tissue-type plasminogen activator antigen, and total homocysteine. The Student’s t test or 1-way ANOVA were computed to compare sICAM-1 levels according to the presence or absence of other cardiovascular risk factors, such as hypertension, smoking status, diabetes, exercise frequency, and alcohol use. In addition, sICAM-1 levels were categorized into 4 groups according to quartiles of the distribution. The significance of any differences in the lipid and nondlipid plasma-based risk factors on the categorized sICAM-1 quartiles were computed using 1-way ANOVA. Stepwise logistic regression analysis was used to determine independent correlates of sICAM-1 levels. A 2-tailed P value <0.05 was considered statistically significant.

Results
Figure 1 shows the distribution of sICAM-1 levels among the study participants. Overall, sICAM-1 levels ranged from 59 to 675 ng/mL and were normally distributed. Mean and median sICAM-1 levels for this group were 238 and 229 ng/mL, respectively.

sICAM-1 levels increased significantly with age (r=0.15; P<0.001). Unadjusted and age-adjusted correlation coefficients between sICAM-1 levels and measured cardiovascular risk factors are shown in Table 1. There were significant positive associations of sICAM-1 levels with systolic and diastolic blood pressure (r=0.001 and r=0.004, respectively), triglycerides (P<0.001), fibrinogen (P<0.001), tissue-type plasminogen activator antigen (P<0.001), and total homocysteine (P=0.02). A significant negative correlation with HDL-C (P<0.001) was also observed. Adjustment for age did not substantially alter these correlations. sICAM-1 levels also increased with increasing body mass index (P=0.02); clinically obese patients (body mass index >27.8 kg/m²) had sICAM-1 levels of 251±68 ng/mL, compared with 235±68 ng/mL for nonobese patients (P<0.01).

Table 2 shows mean sICAM-1 levels according to the presence or absence of several self-reported cardiovascular risk factors. Plasma concentrations of sICAM-1 were significantly higher in subjects with a history of hypertension (P<0.05), and among frequent smokers of alcohol (P=0.02). Analyses according to smoking status demonstrated that sICAM-1 levels increased in a stepwise fashion for never, past, and current smokers (Figure 2, top). In addition, among current smokers, there was a significant positive association between the number of cigarettes smoked per day and sICAM-1 (Spearman rank coefficient, r=0.22; P=0.01; Figure 2, bottom).

As shown in Figure 3, plasma concentration of sICAM-1 also increased in a stepwise fashion across incremental levels.

<table>
<thead>
<tr>
<th>Risk Factor</th>
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<tbody>
<tr>
<td>History of hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>725</td>
<td>235±68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>218</td>
<td>246±71</td>
<td>0.05</td>
<td></td>
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<tr>
<td>History of diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>909</td>
<td>237±68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35</td>
<td>258±73</td>
<td>0.08</td>
<td></td>
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<tr>
<td>Smoking</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Never</td>
<td>420</td>
<td>222±56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>386</td>
<td>238±67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>140</td>
<td>284±85</td>
<td>&lt;0.001</td>
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<tr>
<td>Exercise frequency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;once/week</td>
<td>640</td>
<td>235±70</td>
<td></td>
<td></td>
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<tr>
<td>&lt;once/week</td>
<td>298</td>
<td>244±66</td>
<td>0.06</td>
<td></td>
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<tr>
<td>Alcohol use</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Never or rarely</td>
<td>163</td>
<td>230±58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weekly or monthly</td>
<td>553</td>
<td>236±70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily</td>
<td>230</td>
<td>248±71</td>
<td>0.02</td>
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</table>

TABLE 1. Unadjusted and Age-Adjusted Correlation Coefficients Between Soluble ICAM-1 Levels and Measured Cardiovascular Risk Factors in a Cross-Sectional Study of Apparently Healthy Middle-Aged Men
of both systolic and diastolic blood pressure. A significant inverse association was observed between exercise frequency and sICAM-1 stratified by quartiles; those who exercised 1–3 per week were less likely to have sICAM-1 levels in the highest quartile (P = 0.001 for trend; Figure 4).

To further evaluate the associations between sICAM-1 and plasma-based risk factors, we compared levels of several lipid and nonlipid risk factors according to quartiles of sICAM-1 (Table 3). In these analyses, significant associations were observed between mean sICAM-1 and triglycerides, fibrinogen, and HDL-C levels.

To assess the cumulative impact of multiple risk factors on plasma concentration of sICAM-1, we evaluated levels of sICAM-1 according to the presence or absence of older age, diabetes, smoking, hypertension, and dyslipidemia. As shown in Figure 5, sICAM-1 levels progressively increased as the prevalence of these risk factors increased. Specifically, levels of sICAM-1 were 231 ± 73, 236 ± 76, 246 ± 63, 257 ± 52, and 312 ± 141 ng/mL for those with 0, 1, 2, 3, and >4 risk factors, respectively (P < 0.01 for trend).

Finally, in multivariate analyses, age >50 years (P < 0.01), smoking status (P < 0.001), diabetes (P = 0.02), systolic blood pressure (P < 0.01), positive family history of coronary disease (P = 0.04), and serum levels of total homocysteine (P = 0.02) and fibrinogen (P = 0.05) were found to be independent correlates of sICAM-1. Together, these variables explained 28% of the variance in sICAM-1.

**Discussion**

The primary function of the cellular adhesion molecules is to promote adhesion and transmigration of circulating leukocytes across the endothelial wall, steps hypothesized to be critical in atherogenesis. Recently, baseline levels of at least one cellular adhesion molecule, sICAM-1, have been found to predict the future occurrence of myocardial infarction. However, despite these provocative findings, data describing potential interrelations between sICAM-1 and traditional cardiovascular factors are scant.

In this cross-sectional survey of apparently healthy middle-aged men, we found that sICAM-1 levels were positively associated with several established cardiovascular risk factors, including age, smoking, hypertension, diabetes, and serum levels of triglycerides, fibrinogen, homocysteine, and tissue-type plasminogen activator antigen; sICAM-1 levels were inversely associated with HDL-C. The absolute sizes of these correlations were modest, but statistically significant. Moreover, the range of correlations observed for sICAM-1 were similar in magnitude to the range of correlations observed in these data for several other well-established cardiovascular risk factors, including total cholesterol and HDL-C.

Most prior studies evaluating relationships between sICAM-1 and traditional cardiovascular risk factors have been limited by small sample size and have often resulted in inconsistent findings. For example, our data involving 948 study subjects described graded relationships between sICAM-1 and increasing levels of blood pressure, an intriguing finding because experimental studies suggest that hypertension may enhance the responsiveness of the endothelium to factors that promote leukocyte adhesion, and that the
release of sICAM-1 from strained endothelium occurs in time- and strain-dependent manner. By contrast, Caterina et al. found no relation between sICAM-1 and uncomplicated hypertension; their observations, however, were limited to a group of 11 hypertensive patients and 11 normotensive controls.

Cigarette smoking has also been associated with abnormal endothelial function and increased leukocyte adhesion. Immunohistochemical expression of ICAM-1 is significantly increased in peripheral pulmonary vessels of smokers compared with nonsmokers. Experimental studies using cigarette smoke condensate indicate an increase in adherence of human monocytes to cultured endothelial cells, concomitantly with enhanced expression of ICAM-1 and ELAM-1. Our results with regard to cigarette consumption reinforce the concept that the detrimental effects of smoking may be mediated, in part, by expression of adhesion molecules.

Clinical evidence also suggests that inflammation triggered in response to alcohol or its metabolites may be an important step in the tissue damage of alcoholic liver disease. Bautista et al. have recently demonstrated that alcohol-fed rats have increased expression of ICAM-1 on neutrophils. The significant association between frequent alcohol use with plasma concentration of sICAM-1 observed in our study extends these prior experimental studies to human subjects and raises the possibility of new mechanisms linking alcohol, atherogenesis, and cardiovascular morbidity.

Similarly, we are aware of no prior data describing the effect of exercise frequency on circulating levels of cellular adhesion molecules, although one small report suggests that intense exercise may increase levels of several adhesion molecules. The observation in the current study, that men who exercise regularly are more likely to have lower sICAM-1 levels, is consistent with the known beneficial effects of regular physical activity.

Experimental studies suggest that modified lipoproteins, or their constituents, modulate expression of adhesion molecules. Cokerill et al. have demonstrated in cultured endothelial cells that HDL inhibits cytokine-induced expression of endothelial VCAM-1, ICAM-1, and E-selectin. In addition, lipid apheresis in patients with familial hypercholesterolemia has been associated with concomitant decreases in circulating adhesion molecule levels. Although small clinical studies have suggested that increased levels of adhesion molecules are found in patients with dyslipidemia, a recent report from a large cohort of patients derived from the Atherosclerosis Risk in Communities study failed to demonstrate any significant association between sICAM-1 with triglycerides, total cholesterol, LDL-C, and HDL-C levels. The significant associations between sICAM-1 with triglycerides and HDL-C levels observed in our study, however, support the concept that lipid metabolism may play a role in the modulation of cellular adhesion molecules. Finally, the associations between fibrinogen, tissue-type plasminogen antigen, and total homocysteine level with sICAM-1 observed in this study underscore the complex interrelation between the coagulation system, leukocyte adhesion, and atherogenesis. Recent experimental studies suggest that fibrinogen mediates leukocyte adhesion to the vascular endothelium through an ICAM-1 dependent pathway.

**Figure 4.** Distribution of subjects that exercise less (white bar) or more (black bar) than once per week according to quartiles of sICAM-1 concentration. Study participants who exercise >1× per week tend to be in the lowest quartile (P<0.01 for trend).

**TABLE 3.** Plasma-Based Cardiovascular Risk Factors by Quartiles of Soluble ICAM-1 in a Cross-Sectional Study of Apparently Healthy Middle-Aged Men

<table>
<thead>
<tr>
<th>Quartiles of sICAM-1</th>
<th>60–193 ng/mL</th>
<th>194–228 ng/mL</th>
<th>229–267 ng/mL</th>
<th>267–676 ng/mL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>221±37</td>
<td>223±40</td>
<td>216±36</td>
<td>220±36</td>
<td>0.4</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>49±13</td>
<td>49±12</td>
<td>48±13</td>
<td>44±13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>174±152</td>
<td>168±116</td>
<td>163±97</td>
<td>211±137</td>
<td>0.007</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>242±65</td>
<td>256±83</td>
<td>289±110</td>
<td>283±81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>t-PA (ng/mL)</td>
<td>10.3±5.7</td>
<td>11.0±7.9</td>
<td>11.5±7.7</td>
<td>12.5±7.7</td>
<td>0.15</td>
</tr>
<tr>
<td>Lp(a) (mg/dL)</td>
<td>186±224</td>
<td>184±194</td>
<td>174±196</td>
<td>182±192</td>
<td>0.9</td>
</tr>
<tr>
<td>Total homocysteine (µmol/L)</td>
<td>10.5±2.8</td>
<td>10.9±4.0</td>
<td>11.5±5.1</td>
<td>11.4±4.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>
In conclusion, the current data indicate that levels of sICAM-1 are significantly associated with several established cardiovascular risk factors, an intriguing observation because plasma concentrations of sICAM-1 are associated with increased risk of future myocardial infarction. Further studies will be required to determine whether these associations reflect the role of sICAM-1 as a marker of preclinical atherosclerotic disease, and to evaluate whether such relationships might have a causal basis.

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