Plasma soluble adhesion molecules and endothelium-dependent vasodilation in early human atherosclerosis

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ABSTRACT

Levels of soluble cellular adhesion molecules are increased in patients with atherosclerosis, and have been found to predict coronary heart disease. Therefore these molecules have been suggested to represent laboratory markers for inflammation and activation of endothelial cells. Impaired endothelium-dependent vasodilation has been demonstrated to be an early marker of atherosclerosis. We hypothesized that soluble adhesion molecules are related to impaired endothelium-dependent vasodilation and may serve as an early marker of atherosclerosis. Patients (n = 52) with moderate and uncomplicated hypercholesterolaemia [low-density lipoprotein (LDL)-cholesterol 4.89 ± 1.26 mmol/l] were compared with healthy controls (n = 43; LDL-cholesterol 2.44 ± 0.79 mmol/l). Endothelium-dependent vasodilation of the forearm vasculature was assessed by intra-arterial infusion of acetylcholine (12 and 48 l g/min). Forearm blood flow was measured by venous occlusion plethysmography. Plasma concentrations of the soluble forms of ICAM-1 (intercellular cell adhesion molecule-1), VCAM-1 (vascular cell adhesion molecule-1) and E-selectin were measured by ELISA. Hypercholesterolaemic patients had impaired endothelium-dependent vasodilation in comparison with healthy controls (forearm blood flow after 48 l g/min acetylcholine: 21.3 ± 10.6 and 30.4 ± 16.3 ml/min/100 ml −1 respectively; P = 0.002). Plasma concentrations of soluble adhesion molecules were not different between hypercholesterolaemic patients and controls (ICAM-1, 196 ± 56 and 180 ± 38 ng/ml respectively; VCAM-1, 431 ± 137 and 405 ± 65 ng/ml respectively; E-selectin, 39 ± 17 and 37 ± 12 ng/ml respectively). Moreover, levels of soluble adhesion molecules were not correlated with endothelium-dependent vasodilation. Thus, in hypercholesterolaemic patients without clinical atherosclerosis, levels of soluble adhesion molecules were not elevated in comparison with healthy controls. In addition, these markers of endothelial inflammation were not related to impaired endothelium-dependent vasodilation. Our data indicate that measurement of levels of soluble adhesion molecules cannot replace assessment of endothelium-dependent vasodilation in detection of early hypercholesterolaemic atherosclerosis.

INTRODUCTION

Atherosclerosis has been supposed to represent an inflammatory disease [1]. Thus the adherence of circulating leucocytes to endothelial cells and the subsequent trans-endothelial migration to the vascular intima is one of the key events in the initiation and progression of atherosclerosis [2,3]. These processes are mediated chiefly

Key words: adhesion molecules, atherosclerosis, endothelial function, LDL-cholesterol, nitric oxide.
Abbreviations: CAM, cellular adhesion molecule; HDL, high-density lipoprotein; (s)ICAM-1, (soluble) intercellular cell adhesion molecule-1; LDL, low-density lipoprotein; 1-NMMA, Nω-monomethyl-L-arginine; n.s., not significant; sE-selectin, soluble E-selectin; (s)VCAM-1, (soluble) vascular cell adhesion molecule-1.
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by different cellular adhesion molecules (CAMs), which are expressed on the surface of vascular endothelial cells in response to pro-inflammatory cytokines, oxidants or oxidized low-density lipoprotein (LDL)-cholesterol [4, 5]. Increased expression of CAMs has been described on endothelial cells, smooth muscle cells and macrophages in human atherosclerotic plaques [6–8]. CAMs are also present in the circulation as soluble forms, which lack membrane-spanning and cytoplasmic domains that are present in the membrane-bound forms [9]. Plasma concentrations of these soluble forms of CAMs have been noted to be elevated in certain pathological conditions, such as sepsis, autoimmune diseases and allograft rejection, in which tissue expression of the membrane-bound forms of CAMs is also known to be up-regulated [10–12]. In addition, elevated plasma levels of these soluble molecules have been demonstrated in patients with atherosclerosis or in patients at risk for atherosclerosis, such as dyslipidaemic or hypertensive patients [13–16]. Recently, two prospective studies have provided evidence that plasma concentrations of soluble CAMs may be useful as a marker for the risk of subsequent vascular disease many years in advance of the development of coronary heart disease [17, 18]. Thus the concentration of soluble CAMs may serve as a surrogate marker for an increased expression of CAMs on vascular endothelial cells, and may reflect inflammation and activation of endothelial cells as an early step in the atherosclerotic process.

Impaired function of the vascular endothelium is another early sign of atherosclerosis that occurs long before the formation of atherosclerotic lesions [19]. The endothelium plays a major role in determining vascular tone through the production and release of different vasodilator and vasoconstrictor substances that control the activity of the underlying smooth muscle layer [20]. Nitric oxide, the most important endothelium-derived vasodilatory substance, has several anti-atherogenic properties, such as maintaining vasodilation, and inhibiting platelet aggregation, leucocyte adhesion and proliferation of smooth-muscle cells [21]. Impaired bioavailability of NO has been demonstrated to be responsible for impaired endothelium-dependent vasodilation in hypercholesterolaemia and other states at risk for cardiovascular diseases [22–26]. Thus impairment of endothelium-dependent vasodilation through disturbances in the l-arginine/nitric oxide pathway plays an early and central role in the pathogenesis of atherosclerosis. However, the assessment of endothelium-dependent vasodilation is difficult and is restricted to clinical research laboratories. In contrast, measurements of soluble CAMs in plasma of patients can be easily performed using standardized ELISA techniques [17, 18].

In the present study we hypothesized that plasma levels of soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble E-selectin (sE-selectin) are elevated in hypercholesterolaemic patients with impaired endothelium-dependent vasodilation, in comparison with healthy controls. The aim of the study was to determine whether these markers of endothelial inflammation are related to endothelium-dependent vasodilation as a marker of endothelial cell function, and might therefore be useful as a substitute for the assessment of endothelium-dependent vasodilation in early atherosclerosis.

METHODS

Study population
Patients were eligible for the study if they were aged between 18 and 70 years, had a history of polygenic hypercholesterolaemia, a serum LDL-cholesterol level of $\geq 4.12$ mmol/l and a serum triacylglycerol level of $\leq 3.4$ mmol/l, and if they were not receiving cholesterol-lowering medication. Exclusion criteria were as follows: pregnant or lactating women, patients who were active smokers or had given up less than 1 year ago, familial hypercholesterolaemia, secondary hyperlipoproteinaemia, liver or kidney disease (aspartate transaminase and alkaline phosphatase, bilirubin and serum creatinine $>150$ % of upper normal limit), diabetes mellitus [glycated haemoglobin (HbA1c) $>7$ % or fasting oral glucose $>140$ mg/dl], vascular abnormalities in the forearm vasculature, history of cerebrovascular accident, myocardial infarction, unstable angina, congestive heart failure (NYHA III/IV), history or clinical signs of peripheral artery disease or carotid artery disease, any other history of atherosclerosis or ischaemic events, patients with arterial hypertension (diastolic blood pressure $\geq 95$ mmHg or systolic blood pressure $\geq 160$ mmHg), patients on any blood-pressure-lowering agent, lipid-lowering medication, steroids, immunosuppressive agents or non-steroidal anti-inflammatory drugs. A total of 52 patients with hypercholesterolaemia were enrolled in the study.

In addition, 43 healthy subjects (all non-smokers) with normal serum LDL-cholesterol levels ($95\pm 31$ mg/dl), normal blood pressure (diastolic $78\pm 9$ mmHg) and normal fasting blood sugar ($<6.1$ mmol/l) were examined and served as a control group to the hypercholesterolaemic patients. The baseline characteristics of the hypercholesterolaemic patients and the healthy controls are shown in Table 1.

Study design
The study was approved by the Clinical Investigation and Ethics Committee of the University of Erlangen-Nürnberg, and has been carried out in accordance with the Declaration of Helsinki (1989) of the World Medical
Assessment of forearm blood flow
Forearm blood flow and responses to different vasoactive drugs were assessed by forearm plethysmography at baseline and again after treatment, as described previously [22]. In brief, an intra-arterial line was inserted into the brachial artery of the left arm, then subjects rested for 10 min at 4°C. Drugs were infused intra-arterially at the rate of 2 ml/min, to allow forearm blood flow to return to resting levels. Forearm blood flow was obtained from an average of sequential doses of 12 and 48 μg/min, to assess endothelium-dependent vasodilatation; (2) sodium nitroprusside (3200 ng/ml), to test endothelium-independent vascular relaxation; and (3) Nω-monomethyl-L-arginine (1-NMMA) (2 and 4 μmol/min), to test the basal production and release of NO. Before each intervention, saline was infused for 15 min at a flow rate of 2 ml/min, to allow forearm blood flow to return to resting levels. Forearm blood flow was obtained from an average of measurements recorded for 9 s out of every 15 s during the final 2 min at baseline and of each infusion period. No significant changes in blood pressure or heart rate were observed during drug administration.

Statistical analysis
All statistical analysis was carried out using SPSS software [27]. Laboratory values are expressed as means of absolute values ± S. D. in the text, and as means ± S.E.M. in Figures. A sample size of n = 30 in each group was calculated prior to the study to detect a difference in the plasma ICAM-1 concentration of 20% between hypercholesterolaemic patients and healthy controls (normal values approx. 200 ng/ml), assuming a standard deviation of 40 ng/ml with a type I error of α = 0.01 and a type II error of β = 0.1. Analysis of variance (ANOVA) with the Bonferroni correction was used to compare the hypercholesterolaemic patients with the control group. Linear correlation analysis (Pearson) was used to test correlations between CAMs and forearm blood flows after the 60 min before the study began. Forearm vascular responsiveness to vasoactive agents was assessed by venous-occlusion plethysmography (EC 5R Plethysmograph; Hokanson, Bellevue, WA, U.S.A.). Drugs were infused intra-arterially at the rate of 2 ml/min. The following substances were administered (each dose was infused intra-arterially for 5 min): (1) acetycholine, at sequential doses of 12 and 48 μg/min, to assess endothelium-dependent vasodilatation; (2) sodium nitroprusside (3200 ng/ml), to test endothelium-independent vascular relaxation; and (3) Nω-monomethyl-L-arginine (1-NMMA) (2 and 4 μmol/min), to test the basal production and release of NO. Before each intervention, saline was infused for 15 min at a flow rate of 2 ml/min, to allow forearm blood flow to return to resting levels. Forearm blood flow was obtained from an average of measurements recorded for 9 s out of every 15 s during the final 2 min at baseline and of each infusion period. No significant changes in blood pressure or heart rate were observed during drug administration.

Table 1 Baseline characteristics of hypercholesterolaemic patients and healthy controls
Values are means ± S.D.; n.s., not significant.

<table>
<thead>
<tr>
<th>Hypercholesterolaemic patients (n = 52)</th>
<th>Controls (n = 43)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52 ± 10</td>
<td>38 ± 13</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>31/21</td>
<td>25/18</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170 ± 9</td>
<td>173 ± 8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74 ± 11</td>
<td>69 ± 11</td>
</tr>
<tr>
<td>Body mass index</td>
<td>25.7 ± 2.9</td>
<td>22.9 ± 2.4</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.86 ± 0.16</td>
<td>1.82 ± 0.18</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>133 ± 16</td>
<td>122 ± 13</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>86 ± 11</td>
<td>78 ± 9</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>7.29 ± 1.39</td>
<td>4.00 ± 0.98</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>4.89 ± 1.26</td>
<td>2.44 ± 0.79</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.50 ± 0.41</td>
<td>1.01 ± 0.73</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/l)</td>
<td>1.86 ± 0.92</td>
<td>1.00 ± 0.51</td>
</tr>
<tr>
<td>Lipoprotein (a) (mg/dl)</td>
<td>24 ± 26</td>
<td>22 ± 22</td>
</tr>
</tbody>
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intra-arterial infusion of vasoactive drugs. Partial correlation was used to control for other confounding variables, such as LDL-cholesterol, age and blood pressure. In addition, we arbitrarily classified all study participants into tertiles according to their endothelium-dependent vasodilation after acetylcholine at 48 μg/min. ANOVA was applied to test differences among tertiles. Two-sided P-values are given throughout the text. A two-sided P-value of < 0.05 was considered to be significant.

RESULTS

Baseline characteristics
Baseline characteristics of the hypercholesterolaemic patients and the healthy controls are given in Table 1.

![Figure 1](plasma_concentrations.png)

Table 2 Forearm blood flow at baseline and after the administration of intra-arterial acetylcholine, sodium nitroprusside or L-NMMA in hypercholesterolaemic patients and controls
Values are means ± S.D.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Hypercholesterolaemic patients (n = 52)</th>
<th>Controls (n = 43)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.96 ± 1.26</td>
<td>4.31 ± 1.52</td>
<td>n.s.</td>
</tr>
<tr>
<td>12 μg/min</td>
<td>11.94 ± 6.82</td>
<td>18.81 ± 11.79</td>
<td>0.001</td>
</tr>
<tr>
<td>48 μg/min</td>
<td>21.33 ± 10.40</td>
<td>30.40 ± 16.38</td>
<td>0.002</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.07 ± 1.89</td>
<td>5.70 ± 1.88</td>
<td>n.s.</td>
</tr>
<tr>
<td>3200 ng/min</td>
<td>15.14 ± 4.60</td>
<td>16.55 ± 4.23</td>
<td>n.s.</td>
</tr>
<tr>
<td>L-NMMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>6.32 ± 2.22</td>
<td>6.79 ± 2.65</td>
<td>n.s.</td>
</tr>
<tr>
<td>2 μmol/min</td>
<td>5.03 ± 1.27</td>
<td>4.76 ± 1.62</td>
<td>n.s.</td>
</tr>
<tr>
<td>4 μmol/min</td>
<td>5.16 ± 1.72</td>
<td>5.40 ± 2.10</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Hypercholesterolaemic patients had significantly greater serum levels of total cholesterol and LDL-cholesterol than control subjects. Triacylglycerol levels, although not markedly elevated in the hypercholesterolaemic group, were also different between the two groups. Control subjects were younger and had a lower body weight and lower blood pressures than hypercholesterolaemic patients, whose body weight and blood pressures were still in the normal range. There were no differences in gender distribution between hypercholesterolaemic patients and controls.

Markers of endothelial inflammation
The plasma concentrations of sICAM-1 were 196 ± 56 ng/ml in the hypercholesterolaemic group and 180 ± 38 ng/ml in the control group (difference not significant (n.s.)) (Figure 1). Concentrations of sVCAM-1 were 431 ± 137 ng/ml in the hypercholesterolaemic group and 405 ± 65 ng/ml in the control group (n.s.). sE-selectin plasma concentrations were 39 ± 17 ng/ml in the hypercholesterolaemic group and 37 ± 12 ng/ml in the control group (n.s.). The sE-selectin concentration was correlated with the high-density lipoprotein (HDL)-cholesterol level (r = 0.20, P < 0.05). Soluble CAMs were not correlated with concentrations of total cholesterol or LDL-cholesterol (all r < 0.15; n.s.).

Markers of endothelial function
Prior to the administration of acetylcholine, baseline forearm blood flow was 3.96 ± 1.26 ml·min⁻¹·100 ml⁻¹ in the hypercholesterolaemic group, whereas it was 4.31 ± 1.52 ml·min⁻¹·100 ml⁻¹ in the control group (n.s.). Intra-arterial administration of acetylcholine caused an increase in forearm blood flow in both groups.
Soluble adhesion molecules and endothelial function

Figure 2  Forearm blood flows (FBFs) at baseline and after the infusion of acetylcholine

Acetylcholine was infused at 12 μg/min (Ach 12) and 48 μg/min (Ach 48). Forearm blood flow was measured in the control group (●; n = 43) and in the hypercholesterolaemic patients (■; n = 52).

(P < 0.001). The increase in forearm blood flow in response to acetylcholine was lower in hypercholesterolaemic patients than in control subjects for both doses of acetylcholine (P < 0.002) (Table 2; Figure 2). The serum LDL-cholesterol concentration was correlated with the maximum forearm blood flow response to acetylcholine at 48 μg/min (r = 0.33; P = 0.001) (Figure 3).

In contrast, the administration of sodium nitroprusside as an endothelium-independent vasodilator provoked similar vasodilator responses in hypercholesterolaemic patients as in healthy controls (Table 2).

Prior to the administration of l-NMMA, baseline forearm blood flow was 6.3 ± 2.2 ml·min⁻¹·100 ml⁻¹ in the hypercholesterolaemic group, whereas it was 6.7 ± 2.7 ml·min⁻¹·100 ml⁻¹ in the control group (n.s.). Intra-arterial administration of l-NMMA caused a decrease in forearm blood flow in both groups, with no differences between groups (Table 2).

Relationships between markers of endothelial inflammation and function

Plasma levels of sICAM-1, sVCAM-1 and sE-selectin were not found to be related to forearm blood flow after the infusion of acetylcholine at 12 or 48 μg/min in the whole study population. Table 3 gives the correlation coefficients in the whole study population, along with the corresponding P-values; Figure 4, as an example, shows the relationship of the plasma concentrations of sICAM-1 (left panel) and sVCAM-1 (right panel) to forearm blood flow after the administration of acetylcholine at 48 μg/min. Correlation coefficients between CAM levels and endothelium-dependent vasodilation did not change when controlled for LDL-cholesterol, age and baseline blood pressure. In addition, plasma concentrations of sICAM-1, sVCAM-1 and sE-selectin were not related to blood flow responses after the intra-arterial infusion of l-NMMA (Table 3). All study participants were arbitrarily classified into tertiles according to their endothelium-dependent vasodilation after administration of acetylcholine at 48 μg/min. No differences were found between these tertiles with respect to levels of soluble CAMs (Figure 5).

Subsequently, we analysed the group of hypercholesterolaemic patients only. Again, no correlations were found between forearm blood flow measurements after the infusion of acetylcholine and serum levels of sICAM-1, sVCAM-1 or sE-selectin (all r < 0.1; n.s.).

Finally, we examined only those hypercholesterolaemic patients who had low blood flow responses to
Table 3  Correlation coefficients and corresponding P values for the correlation between plasma concentrations of sICAM-1, sVCAM-1 and sE-selectin and the blood flow responses to acetylcholine and L-NMMA in the whole study population (n = 95)

<table>
<thead>
<tr>
<th>Agent</th>
<th>sICAM-1</th>
<th>sVCAM-1</th>
<th>sE-selectin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 μg/min</td>
<td>0.04</td>
<td>0.68</td>
<td>—0.02</td>
</tr>
<tr>
<td>48 μg/min</td>
<td>—0.02</td>
<td>0.82</td>
<td>—0.16</td>
</tr>
<tr>
<td>L-NMMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 μmol/min</td>
<td>—0.19</td>
<td>0.23</td>
<td>—0.13</td>
</tr>
<tr>
<td>4 μmol/min</td>
<td>—0.02</td>
<td>0.82</td>
<td>—0.04</td>
</tr>
</tbody>
</table>

DISCUSSION

In the present study we have shown that soluble CAMs, such as sICAM-1, sVCAM-1 and sE-selectin, were not increased in hypercholesterolaemic patients with significant...
cantly impaired endothelium-dependent vasodilation but with no clinical signs of overt atherosclerosis and no cardiovascular risk factors other than hypercholesterolaemia. In addition, plasma concentrations of soluble CAMs were not related to the degree of impairment of endothelium-dependent vasodilation in hypercholesterolaemic patients and healthy controls. Therefore soluble CAMs do not appear to be clinically useful to indicate impaired endothelial function in this group of patients with early atherosclerosis.

No controversy exists about the importance of interactions among endothelial cells, monocytes and T-cells for the inflammatory response present in developing and established atherosclerosis [1]. These cell–cell interactions are mainly mediated by CAMs expressed on the surface of endothelial cells [6–8]. CAMs can be cleaved from the surface of endothelial cells, and soluble CAMs can be measured in the serum as markers of a sustained inflammatory response. In different sequelae of athero-sclerosis, increased concentrations of CAMs have been reported in previous studies, and it has been hypothesized that CAMs may reflect inflammation and activation of endothelial cells as an early step in the atherosclerotic process [14,15,17,18,28,29].

Plasma concentrations of soluble CAMs in our patients and controls were in the lower normal range reported in previous studies for healthy controls [11,16–18]. Smoking, diabetes mellitus, arterial hypertension, hypertriglyceridaemia and clinical atherosclerosis have been demonstrated to increase concentrations of soluble CAMs [13,16–18,30,31]. However, none of these factors were present in our study population. There was a difference in age between patients and controls. It has been shown that plasma concentrations of soluble VCAM-1 increase with age [18,32]. However, this age difference may have even favoured an elevation of CAMs in our hypercholesterolaemic patients. According to a power calculation prior to the study, our study had a power of 90% to detect a clinically relevant 20% elevation of s-ICAM-1 compared with the values in healthy controls. However, no such elevation in soluble CAMs could be detected in our study population.

In contrast with our results, elevated concentrations of soluble CAMs have been demonstrated in patients with dyslipidaemia and hypertriglyceridaemia, and it has been postulated that increased plasma levels of soluble CAMs may be related to the extent of endothelial activation and endothelial dysfunction induced by dyslipidaemia [13,30]. However, patients investigated in these studies had marked elevations of LDL-cholesterol [13] or severely elevated triacylglycerol levels [13,30]. Even more important, most of the patients studied had additional cardiovascular risk factors, such as hypertension, diabetes mellitus and tobacco use, and/or a history of vascular disease. Patients who had clinical evidence of atherosclerosis or more than one cardiovascular risk factor had the highest plasma concentrations of soluble CAMs in these trials.

In the present study we focused on a patient population early in the process of developing atherosclerosis. Our patients had only moderately elevated LDL-cholesterol levels and normal triacylglycerol levels, and no clinical detectable signs of atherosclerosis. However, our hypercholesterolaemic patients had impaired endothelium-dependent vasodilation in comparison with a healthy control group, as demonstrated by a decreased arterial vasodilatory capacity to intra-arterial infusion of acetylcholine (Figure 2). Not surprisingly, endothelium-dependent vasodilation was significantly correlated with the LDL-cholesterol level (Figure 3).

Impaired endothelium-dependent vasodilation is widely accepted to be an early sign of developing atherosclerosis, detectable long before the formation of atherosclerotic lesions in different groups at risk for cardiovascular disease [19,22–26,33,34], although it has to be emphasized that no follow-up data are yet available concerning the development of atherosclerosis in patients with impaired endothelium-dependent vasodilation. In our patients with severely impaired endothelium-dependent vasodilation, concentrations of soluble CAMs were not significantly elevated in comparison with patients with preserved endothelium-dependent vasodilation and with healthy controls. Thus our data from patients who had not yet developed clinical atherosclerosis suggest that impaired endothelium-dependent vasodilation appears earlier in the atherosclerotic process than the elevation of soluble CAMs, which have been suggested to serve as biochemical markers of subclinical atherogenesis [13–16,18,28,29]. Our data indicate that measurement of soluble CAMs cannot be used as a substitute for the measurement of endothelium-dependent vasodilation to identify patients in whom the atherosclerotic process is at an early stage.

E-selectin is expressed exclusively by endothelial cells, but ICAM-1 and VCAM-1 are expressed by a variety of cell types, including intimal smooth muscle cells, as well as by endothelial cells. Although the origins, metabolism and functional significance of soluble forms of CAMs are not fully understood, they have been suggested to represent a surrogate marker for an increased expression of CAMs on vascular endothelial cells and to reflect inflammation and activation of vascular endothelium [13,15–17]. The nature of impaired endothelium-dependent vasodilation and decreased bioavailability of NO has not yet been elucidated. Decreased formation of NO by injured endothelial cells, accumulation of endogenous inhibitors of NO synthesis, increased oxidative inactivation of NO by increased superoxide anion production and defects in the NO/cGMP signalling pathway are only some of the possible mechanisms which may lead to impaired endothelial function [25,26,35–37]. Inflammation of the vascular wall may contribute to decreased
NO formation, as well as to increased free radical formation [1]. Although soluble CAMs may not be sensitive enough to indicate minor local endothelial inflammation, our findings in humans suggest that severe endothelial inflammation with the consequence of elevated levels of soluble CAMs is not necessarily involved in the development of impaired endothelium-dependent vasodilation in our patients.

In conclusion, we have demonstrated in a study population of hypercholesterolaemic patients with impaired endothelium-dependent vasodilation that soluble CAMs were not elevated in comparison with a healthy control group. In addition, these markers of endothelial inflammation were not related to endothelium-dependent vasodilation as a marker of endothelial function. Our data indicate that measurement of levels of soluble CAMs cannot substitute for assessment of endothelium-dependent vasodilation in identifying early atherosclerosis.

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