C-reactive protein and e-selectin levels are related to vasodilation in resistance, but not conductance arteries in the elderly
The prospective investigation of the Vasculature in Uppsala Seniors (PIVUS) study

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Abstract

Background: Divergent results have emerged in the past when relating single markers of inflammation to measures of vascular reactivity. The aim of the present study is to relate a wide range of inflammatory markers to vasoreactivity in both resistance and conductance arteries.

Methods: In the Prospective Investigation of the Vasculature in Uppsala Seniors (the PIVUS study), endothelium-dependent vasodilation was evaluated by the invasive forearm technique with acetylcholine given in the brachial artery (EDV), the brachial artery ultrasound technique with measurement of flow-mediated dilatation (FMD) and the pulse wave analysis method with beta-2 receptor agonist (terbutaline) provocation in 1016 subjects aged 70. A panel of 14 inflammatory markers, including cytokines, chemokines, adhesion molecules, CRP, sCD40 ligand and leukocyte count, was measured.

Results: After adjustment for gender and coronary risk factors, EDV was independently related to CRP levels and e-selectin in an inverse way (p < 0.006 for both). FMD was not significantly related to any marker of inflammation after adjustment. Endothelium-independent vasodilation evaluated by the invasive forearm technique with sodium nitroprusside was also found to be related to both CRP and e-selectin in an inverse way (p = 0.005 and p = 0.045, respectively).

Conclusion: Acetylcholine-induced vasodilation in the forearm, but not FMD, was inversely related to CRP and e-selectin levels independently of traditional risk factors in elderly subjects. As also endothelium-independent vasodilation was related to CRP and e-selectin, general vasoreactivity in resistance arteries seems to be effected by low-grade inflammation in elderly subjects.

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Keywords: Endothelium; Vasodilation; Inflammation; CRP; Selectins; Cytokines; Adhesion molecules

1. Introduction

It is today well established that inflammation is a key feature in the development of atherosclerosis [1], and modestly elevated levels of different markers of inflammation, such as CRP and leukocyte count, have repeatedly been shown to predict future cardiovascular events in general populations [2,3].

Endothelium-dependent vasodilation is a characteristic feature of the vasculature and impaired endothelium-dependent vasodilation is seen early in the atherosclerotic
process [4]. Also a defect endothelium-dependent vasodilation has been shown to predict future cardiovascular events [5,6].

Since the first report in year 2000 of an inverse relationship between CRP levels and endothelium-dependent vasodilation in a sample of patients with coronary artery disease [7], a number of studies with conflicting results have been presented regarding the relationship between CRP levels and endothelium-dependent vasodilation [8–15]. These discrepant results might in part be explained by the fact that many of these studies were rather small and may therefore only have power to detect very close relationships. Another reason for the divergent findings might be that different techniques have been used to evaluate endothelium-dependent vasodilation and that these techniques do not carry the same information [16,17].

Also the relationships between a number of other inflammatory markers, such as cytokines and adhesion molecules, and endothelium-dependent vasodilation have been studied [8,9,14,18], but no study has evaluated the effects of a wide range of inflammatory markers on vasoreactivity in both resistance and conductance arteries in the same study.

The Prospective Investigation of the Vasculature in Uppsala Seniors (the PIVUS study) was conducted with the primary aim to evaluate three different tests of endothelium-dependent vasodilation in the peripheral circulation in more than 1000 subjects aged 70 living in the community of Uppsala, Sweden [19]. We here report one of the aims of the study, namely to evaluate the relationships between a panel of multiple markers of general inflammation, such as cytokines, chemokines, adhesion molecules, and measurements of endothelium-dependent vasodilation in both resistance and conductance vessels. The hypothesis tested was that several of these inflammatory markers were related to vasoreactivity independently of established coronary risk factors.

2. Materials

2.1. Subjects

Eligible were all subjects aged 70 living in the community of Uppsala, Sweden. The subjects were chosen from the register of community living and were invited in a randomized order from the start of the study in April 2001 to the last included subject in June 2004. The subjects received an invitation by letter within 1 months of their 70th birthday in order to standardize for age. Of the 2025 subjects invited, 2016 subjects were invited giving a participation rate of 50.1%.

The study was approved by the Ethics Committee of the University of Uppsala and the participants gave informed consent.

2.2. Baseline investigation

The participants were asked to answer a questionnaire about their medical history, smoking habits and regular medication.

All subjects were investigated in the morning after an overnight fast. No medication or smoking was allowed after midnight. After recordings of height, weight, abdominal and hip circumference, an arterial cannula was inserted in the brachial artery for blood sampling and later regional infusions of vasodilators. During the investigation, the subjects were supine in a quiet room maintained at a constant temperature.

Blood pressure was measured by a calibrated mercury sphygmomanometer in the non-cannulated arm to nearest mmHg after at least 30 min of rest and the average of three recordings was used. Lipid variables and fasting blood glucose were measured by standard laboratory techniques.

Framingham risk score was calculated and used as a comprehensive measure of the major cardiovascular risk factors [20].

Subjects with CRP > 10 or leukocyte count >10 (n = 44) were excluded from the analysis in order not to include subjects with infections, chronic inflammatory disorders or some hematologic malignancies, which might distort the analysis. Characteristics of the sample are given in Tables 1 and 2. Of the women, 231 were on hormone replacement therapy.

As the participation rate in this cohort was only 50%, we carried out an evaluation of cardiovascular disorders and

Table 1

<table>
<thead>
<tr>
<th>Total sample</th>
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<tbody>
<tr>
<td>n</td>
</tr>
<tr>
<td>Females (%)</td>
</tr>
<tr>
<td>Height (cm)</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>Waist circumference (cm)</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Waist/hip ratio</td>
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<td>SBP (mmHg)</td>
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<td>DBP (mmHg)</td>
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<tr>
<td>Heart rate (beats/min)</td>
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<tr>
<td>Serum cholesterol (mmol/l)</td>
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<tr>
<td>LDL-cholesterol (mmol/l)</td>
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<tr>
<td>HDL-cholesterol (mmol/l)</td>
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<tr>
<td>Serum triglycerides (mmol/l)</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
</tr>
<tr>
<td>Current smoking (%)</td>
</tr>
<tr>
<td>Framingham risk score</td>
</tr>
<tr>
<td>EDV (%)</td>
</tr>
<tr>
<td>EIDV (%)</td>
</tr>
<tr>
<td>FMD (%)</td>
</tr>
<tr>
<td>Change in RI (%)</td>
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</table>

Means are given ±S.D. or as median and 10th and 90th percentiles in parentheses. SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; EDV, endothelium-dependent vasodilation (invasive forearm technique); EIDV, endothelium-independent vasodilation (invasive forearm technique); FMD, flow-mediated dilatation; RI, reflectance index.
medications in 100 consecutive non-participants. The prevalence of cardiovascular drug intake, history of myocardial infarction, coronary revascularization, antihypertensive medication, statin use and insulin treatment were similar to those in the investigated sample, while the prevalence of diabetes, congestive heart failure and stroke tended to be higher among the non-participants (see Table 2 for details).

3. Methods

3.1. The invasive forearm technique

Forearm blood flow (FBF) was measured by venous occlusion plethysmography (Elektromedicin, Kullavik, Sweden) with the strain-gauge technique. FBF was calculated from at least five consecutive recordings.

An arterial cannula was placed in the brachial artery. Resting FBF was measured 30 min after cannula insertion, and local intra-arterial drug-infusions were given during 5 min for each dose with a 20 min washout period between the drugs. The infused dosages were 25 and 50 μg/min for Acetylcholine (Clin-Alpha, Switzerland) and 5 and 10 μg/min for SNP (Nitropress, Abbot, UK). The drugs were given in a random order at a maximal rate of 1 ml/min.

Endothelium-dependent vasodilation with this technique (EDV) was defined as FBF during infusion of 50 μg/min of acetylcholine minus resting FBF divided by resting FBF. Endothelium-independent vasodilation (EIDV) was defined as FBF during infusion of 10 μg/min of SNP minus resting FBF divided by resting FBF.

Cannulation of the artery was not performed in the 3% of the subjects who were on regular treatment with Warfarin and in another 10% cannulation failed. The reproducibility (coefficient of variation, CV) for EDV and EIDV were 8–10% [21].

3.2. The brachial artery ultrasound technique

The brachial artery was assessed by external B-mode ultrasound imaging 2–3 cm above the elbow (Acuson XP128 with a 10 MHz linear transducer, Acuson Mountain View, CA, USA), according to the International Brachial Artery Task Force [22]. A cuff was placed below the elbow and inflated to a pressure at least 50 mmHg above systolic blood pressure for 5 min. FMD was defined as the maximal brachial artery diameter recorded between 30 and 90 s following cuff release minus diameter at rest divided by the diameter at rest, using electronic callipers for measurements. FMD was successfully evaluated in 97% of the participants.

The reproducibility (CV) was 3% for baseline brachial artery diameter and 29% for FMD [16].

3.3. Pulse wave analysis

A micromanometer tipped probe (Sphygmocor, Pulse Wave Medical Ltd., Australia) was applied to the radial artery and 10 pulse waves were used for analyses. After a baseline recording, terbutaline was subcutaneously administered (0.25 mg) and the maximal response after 15 and 20 min was used. In previous studies, the augmentation index (Alx) derived from the first reflected wave in systole was used [23,24]. We have previously validated the relative height of the first diastolic reflected wave (the reflection index, RI) [25]. Here, we report both changes in Alx and RI as relative changes from baseline following terbutaline. The changes in the RI and Alx could successfully be evaluated in 86% of the sample. Terbutaline was not given to subjects with frequent ectopic beats or atrial fibrillation.

The reproducibility (CV) for the changes in RI and Alx were 9.4 and 16%, respectively [25]. Due to better reproducibility, RI was chosen as the primary variable for the pulse wave based method.

Each vasodilatory technique was performed and evaluated by one unique technician throughout the study not being aware of other data. A more detailed description of the study sample and techniques has previously been presented [19].

3.4. Inflammatory markers

High sensitive CRP was measured in human serum by an ultrasensitive particle enhanced immunoturbidimetric assay (Orion Diagnostica, Espoo, Finland) on a Konelab 20...
autoanalyzer (Thermo Clinical Labsystems, Espoo, Finland). The inter-assay coefficient of variation was 3.2%.

Cytokines, chemokines and adhesion molecules were analyzed on the Evidence® array biochip analyzer (Randox Laboratories Ltd., Crumlin, UK) [26].

Plasma levels of sCD40L were measured in citrated blood using the Elisa technique (Bender MedSystems, Burlingame, CA). Intra- and inter-assay coefficients of variation were 6.9–15.0% and 8.0–16%, respectively.

The functional sensitivity for the different inflammatory markers were as follows: IL-2: 4.1 pg/ml; IL-6: 0.3 pg/ml; IL-8: 1.5 pg/ml, INF-gamma: 1.8 pg/ml, TNF-alpha: 1.8 pg/ml, MCP-1: 19.4 pg/ml, ICAM-1: 18.6 pg/ml, VCAM-1: 3.1 pg/ml, e-selectin: 3.1 pg/ml, p-selectin: 11.2 pg/ml, l-selectin: 32.8 pg/ml, CRP: 0.1 mg/l, leukocyte count: 0.2.

Also IL-1-alpha, IL-1-beta, IL-4 and IL-10 were included in the Evidence® array biochip cytokine panel, but were found to have insufficient sensitivity for measurements in the present sample and were therefore not evaluated.

3.5. Statistics

Non-normally distributed variables, such as EDV, FMD and most of the cytokines, were log-transformed in order to achieve a normal distribution. Cytokines with a value of zero were then given a positive value just above zero (such as 0.00001), but well below the lower detection limit of the assay, for not being lost in the transformation process.

In the first step of analysis, univariate relationships between vasodilatory and inflammatory variables were evaluated by Spearman’s correlation coefficient. In the next step, the independent relationships between the vasodilatory variables and the inflammatory variables were evaluated with gender and Framingham risk score as confounding variables. Four separate backward stepwise multiple regression models with the four vasodilatory variables as dependent variables. Four separate backward stepwise multiple regression models to assure adjustment for these two important confounders. In each of those models, gender and the Framingham score were used as independent variables together with the inflammatory variables being significant in the univariate analysis presented in Table 4. After creation of these models, the non-significant inflammatory variables were removed in a backward stepwise fashion. Gender and the Framingham score were however always retained in the models to assure adjustment for these two important founders.

Two-tailed significance values were given with $p<0.05$ regarded as significant. StatView (SAS Inc., NC, USA) was used for calculations.

4. Results

The medians and 10th–90th percentile for the inflammatory variables are given in Table 3, while the markers of endothelium-dependent vasodilation are presented in Table 1.

### Table 3

<table>
<thead>
<tr>
<th>Marker</th>
<th>Median</th>
<th>10th–90th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-alpha (pg/ml)</td>
<td>3.7</td>
<td>2.3–7.1</td>
</tr>
<tr>
<td>IL-2 (pg/ml)</td>
<td>3.6</td>
<td>2.6–14.3</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>4.1</td>
<td>1.2–66.1</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>6.4</td>
<td>3.1–13.6</td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>386</td>
<td>250–569</td>
</tr>
<tr>
<td>Interferon-gamma (pg/ml)</td>
<td>1.6</td>
<td>0–3.8</td>
</tr>
<tr>
<td>ICAM-1 (mg/l)</td>
<td>344</td>
<td>265–470</td>
</tr>
<tr>
<td>VCAM-1 (mg/l)</td>
<td>518</td>
<td>394–695</td>
</tr>
<tr>
<td>e-Selectin (mg/l)</td>
<td>14.7</td>
<td>8.5–23.0</td>
</tr>
<tr>
<td>p-Selectin (mg/l)</td>
<td>101</td>
<td>70–134</td>
</tr>
<tr>
<td>l-Selectin (mg/l)</td>
<td>706</td>
<td>570–890</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>1.2</td>
<td>0.4–3.9</td>
</tr>
<tr>
<td>sCD40L (ng/ml)</td>
<td>0.16</td>
<td>0–0.43</td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>5.5</td>
<td>4.0–7.8</td>
</tr>
</tbody>
</table>

IL, interleukin; TNF, tumour necrosis factor; MCP, monocyte chemotactic protein; ICAM, intracellular adhesion molecule; VCAM, vascular adhesion molecule; CRP, C-reactive protein.

### 4.1. Univariate relations between indices of vasodilation and inflammatory markers

When the inflammatory variables were related to the indices of endothelium-dependent vasodilation in univariate analysis, TNF-alpha, IL-6, interferon-gamma, ICAM-1, e-selectin, CRP and leukocyte count were significantly related to EDV, while IL-8, e-selectin, CRP and l-selectin were related to the change in RI. Only IL-1 beta and p-selectin were related with FMD. ICAM-1, e-selectin, CRP and leukocyte count were significantly related to EIDV (see Table 4 for details).

Almost identical results were obtained if the 44 subjects with CRP >10 or leukocyte count >10 were included in the analysis (data not shown).

### Table 4

<table>
<thead>
<tr>
<th>Markers</th>
<th>Spearman’s rank correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDV</td>
<td>EIDV</td>
</tr>
<tr>
<td>TNF-alpha</td>
<td>0.07</td>
</tr>
<tr>
<td>IL-2</td>
<td>0.04</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.11</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.03</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.06</td>
</tr>
<tr>
<td>Interferon-gamma</td>
<td>0.07</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>0.10</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>0.04</td>
</tr>
<tr>
<td>e-Selectin</td>
<td>0.11</td>
</tr>
<tr>
<td>p-Selectin</td>
<td>0.02</td>
</tr>
<tr>
<td>l-Selectin</td>
<td>0.01</td>
</tr>
<tr>
<td>CRP</td>
<td>0.14</td>
</tr>
<tr>
<td>sCD40L</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Note that a low value for the change in RI denotes a good vasodilatory response. A correlation coefficient $>0.07$ corresponds to a $p$-value $<0.05$, the predefined value for entry in further multivariate models. See Table 2 for abbreviations.

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4.2. Independent relations between indices of vasodilation and inflammatory markers

Multiple regression analysis were thereafter performed with EDV, FMD, the change in RI and EIDV as dependent variables in four different models with the inflammatory markers with a p-value <0.05 in the univariate analysis given above together with gender and Framingham score as independent variables (see Table 5).

In the model in which EDV was the dependent variable, CRP (p = 0.0047, Fig. 1), e-selectin (p = 0.0060, Fig. 1) and Framingham score (p = 0.037) were independently related to EDV in an inverse way. CRP and e-selectin were still significantly associated with EDV in a similar manner when adjustments also were performed for potentially confounding medications, such as ACE-inhibitors, angiotensin-II receptor blockers, statins or aspirin. Also when we excluded all subjects with any cardiovascular medication, CRP and e-selectin were still significantly associated with EDV, although the p-values were higher due to loss of almost half of the sample (details not shown). The same was seen when excluding all women on hormone replacement therapy.

When the change in RI was used as dependent variable, gender (p < 0.0001), CRP (p = 0.015) and l-selectin (p = 0.011) were independent predictors, while there was only a tendency for e-selectin (p = 0.081) (see Table 5 for details). CRP and l-selectin were still significantly associated with EDV in a similar manner when adjustments also were performed for potentially confounding medications, such as ACE-inhibitors, angiotensin-II receptor blockers, statins or aspirin. Also when we excluded all subjects with any cardiovascular medication, CRP and l-selectin were still significantly associated with EDV, although the p-values were higher due to loss of almost half of the sample (details not shown). The same was seen when excluding all women on hormone replacement therapy.

Regarding FMD, only Framingham score was independently related to this marker of vasoreactivity (regression coefficient −0.015, p = 0.0071).

In the model in which EIDV was the dependent variable, CRP (p = 0.0047), e-selectin (p = 0.045) and Framingham score (p = 0.025) were independently related to EIDV (see Table 5 for details). However, after adjustment for confounding medications, such as ACE-inhibitors, angiotensin-II receptor blockers, statins or aspirin, or when we excluded all subjects with any cardiovascular medications and hormone replacement therapy, e-selectin lost in significance and CRP was the only proinflammatory marker being related to EIDV.
5. Discussion

The present study showed that endothelium-dependent vasodilation evaluated by the invasive forearm technique, but not FMD, was inversely related to CRP levels and e-selectin independently of coronary risk factors in a large population sample of elderly subjects. A similar pattern was seen for EIDV and the change in RI, suggesting the relationships between CRP levels, e-selectin and vasodilation not to be endothelium-specific, but rather reflecting that general vasoreactivity in resistance arteries to be effected by low-grade inflammation in elderly subjects.

5.1. CRP and endothelium-dependent vasodilation

The above-mentioned findings might in part explain the somewhat discrepant results previously published regarding the relations between CRP and endothelium-dependent vasodilation [7–15]. First, the relationship presented in the present study was highly significant and consistent, but with a moderate correlation coefficient. Thus, small studies in the past may not have been powered to detect such relationship. We have ourselves previously concluded that CRP was not related to EDV in a sample of 59 healthy middle-aged subjects [14]. However, the correlation coefficient between CRP and EDV in that study was similar to the one found in the present study, but the previous study was not powered to detect such relationship.

Secondly, CRP was only related to endothelium-dependent vasodilation when evaluated with the invasive forearm technique, but not when evaluated with FMD. The majority of the previously published studies which have shown a significant inverse correlation between CRP and endothelium-dependent vasodilation have been carried out with the invasive forearm technique [7–9], while most of the studies using FMD have not been able to demonstrate such a relationship [13,18]. In the Framingham study, FMD was inversely related to CRP in univariate analysis, but not after correction for traditional risk factors [18]. Thus, it seems as if inflammation evaluated by CRP levels could have different influences on endothelium-dependent vasodilation in the resistance vessels evaluated by acetylcholine stimulation and in a conductance vessel evaluated by FMD in the brachial artery. It has previously been shown that these two techniques does not correlate and thus carries different information regarding endothelium-dependent vasodilation [16,17,19].

This discrepancy in the information on vasoreactivity between EDV and FMD might have multiple origins. First, we study change in blood flow in one case and a diameter change in the other case. Since blood flow is determined by both blood velocity and the diameter of the vessel along with heart rate, it is obvious that differences may occur since FBF is a more complex variable for which diameter is only one determinant. Second, regarding FMD the change in wall shear stress is the driving force for vasodilation, while muscarinic receptor activation by acetylcholine will induce vasodilation in the other model. Therefore, we compare different stimuli for dilatation in the two models. Third, we compare larger arteries vs. smaller arteries.

It has experimentally been shown that nitric oxide is a more dominant endogenous vasodilator in the larger arteries compared with resistance arteries, in which also a yet unidentified endothelium-derived hyperpolarizing factor (EDHF) is liberated and induce dilatation. Thus, several differences between EDV and FMD exist, but it is yet not known which of these mechanisms that explain the discrepant results regarding the different associations found between inflammatory markers and the two models of vasoreactivity.

It is also not clear if CRP in this clinically normal range have any direct effect on the endothelium, or if this marker of inflammation merely serves as a marker. In favour of the first idea are findings that endothelial cells generate less nitric oxide, the major endothelium-dependent vasodilator, and that the life-span of endothelial progenitor cells are shortened when exposed to CRP [27–30].

5.2. Adhesion molecules and endothelium-dependent vasodilation

In univariate analysis, both ICAM-1 and e-selectin were related to EDV with a similar power, but only e-selectin was related to EDV in the multivariate model including CRP, gender and coronary risk. This is not in agreement with our previous study in a small sample of healthy middle-aged subjects, in which ICAM-1 was more closely related to EDV than e-selectin [14]. Also another study has reported a relationship between vasodilation and ICAM-1 [18]. Since ICAM-1 and e-selectin both are adhesion molecules being interrelated and associated with future cardiovascular events with a similar power [31], future studies are needed in order to clarify if e-selectin is superior to ICAM-1 in the prediction of endothelium-dependent vasodilation. Although not significant in the multiple analyses, the finding that e-selectin was related to the change in RI, another model of endothelium-dependent vasodilation mainly in resistance vessels, supports the importance for e-selectin in endothelium-dependent vasodilation.

The only previous study investigating the relationship between e-selectin and EDV did not find any relationship [32]. That study was however conducted in hypertensive patients, a group known to have a general activation in adhesion molecules, which might disclose such relationship.

Unexpectedly, l-selectin was related to the change in RI in an opposite way compared to CRP and e-selectin. If this means that l-selectin could have endothelium-protective properties or if this merely is a chance finding remains to be elucidated in future studies.
5.3. Cytokines, chemokines and endothelium-dependent vasodilation

Although other studies have reported associations between single cytokines and endothelium-dependent vasodilation [9,18], no other study has evaluated a number of these important inflammatory mediators. In univariate analysis, some of the major proinflammatory cytokines, like TNF-alpha and IL-6, were inversely related to EDV as expected, but the power of these markers were lost in the multiple models in favour of CRP and e-selectin. It might thus be that endothelium-dependent vasodilation is more related to the levels of the down-stream products of these cytokines, such as CRP and e-selectin, rather than to the cytokine levels in themselves.

Another explanation might be that analysis of several of the cytokines resulted in many observations below the detection limit of the assay. Such variables with a large number of observations with the same value are less powerful in statistical analysis and the effects of several of the cytokines might therefore have been underestimated.

5.4. Leukocyte count and endothelium-dependent vasodilation

It was already shown in 1974 that an increased leukocyte count could predict cardiovascular events [33], and recently a relationship between leukocyte count and FMD has been presented being independent of traditional cardiovascular risk factors [34]. In the present study, the leukocyte count was inversely related to EDV and EIDV in the univariate analysis, but lost power when adjusted for coronary risk factors and other inflammatory markers.

5.5. sCD40 ligand and endothelium-dependent vasodilation

Soluble CD40 ligand has previously been found to be associated with a poor outcome in patients with unstable CHD [35–37], and with biochemical markers of endothelial activation [38]. In this study however no relationship was seen with endothelium-dependent vasodilation. This might be in line with a recent population study in which sCD40 ligand was not related to subclinical atherosclerosis, while CRP was [39].

5.6. Flow-mediated vasodilation

We did not found any consistent relationships between CRP or other markers of inflammation and FMD. One explanation might be that FMD is rather low in this age-group even in healthy subjects making it hard to show any differences between those with high and those with low levels of different risk factors, as suggested by Wendelhag et al. [40]. It has also recently been shown by Witte et al. that a reduced arterial compliance in the elderly limits flow-mediated vasodilation to the extent that is it hard to relate FMD to major risk factors in the elderly [41]. The finding that a reduced arterial compliance in the elderly affects FMD has also been reproduced in the present cohort, but no such effect of a poor arterial compliance on EDV was found [42].

5.7. Endothelium-independent vasodilation

Also EIDV evaluated by the invasive forearm technique was significantly related to CRP and e-selectin. While many investigators have not found EIDV to be related to coronary heart disease and different cardiovascular risk factors [43,44], others have [45,8]. EIDV is regarded to mainly represent structural changes in the vascular wall or dysfunction of the vascular smooth muscle cells, both common alterations in the elderly. Thus, a long-standing history of mild inflammation might not only affect EDV, but also EIDV.

5.8. Arterial stiffness

Arterial compliance is often reduced in the elderly. Since this will increase blood flow velocity, a primary determinant of wall shear stress, this will effect nitric oxide production. Arterial stiffness is also associated with mild inflammation [46]. Thus, arterial stiffness might therefore be an important confounder for the relationships found between vasoreactivity and inflammatory markers. However, when we used pulse pressure as a marker for arterial stiffness and added that marker to the multiple models presented in Table 5, pulse pressure was not significant in any of the models and the relationships between the indices of vasoreactivity and inflammatory markers were only marginally changed and still significant indicating that arterial stillness was not a major confounder in this study.

5.9. Strength of the associations

In the present study most of the significant associations show correlation coefficients being rather modest (0.10–0.15), despite being highly significant, and the biological relevance might be questioned. It should however be emphasized that most such relationships are attenuated with age and higher values are usually seen in younger subjects with less disorders and medications. Therefore, also these lower correlation coefficients are worth to be reported in the elderly, especially then the relationships are in a plausible direction.

Furthermore, it is well known that all major traditional risk factors are related to vasoreactivity. In the multiple models in Table 5, Framingham risk score, a powerful measure of the traditional risk factors, show a lower p-value than CRP in all of the models. Thus, at least for CRP, the relationship is more closely related to vasoreactivity than a combined measure of the traditional risk factors. So, even if the correlation coefficients are quite low, the relationships found between mild
inflammation and vasodilation in resistance arteries could well be of clinical importance.

5.10. Limitation of the study

The present sample is limited to Caucasians aged 70, so caution should be used in drawing conclusions for other ethnic and age-groups.

The present study had a moderate participation rate. However, an analysis of non-participants showed the present sample to be fairly representative of the total population regarding most cardiovascular disorders and drug intake.

EIDV was only assessed by one of the methods for practical and ethical reasons not to prolong the investigation procedure, as we would have had to give GNT before terbutaline and would have had to wait for the withdrawal of the GNT effect. Furthermore, we have previously shown that EIDV evaluated by SNP infusion in the brachial artery and GNT provoked change in brachial artery diameter are closely related [16], so additional measurements of EIDV would probably not add substantial information to the study.

6. Conclusion

Acetylcholine-induced vasodilation in the forearm, but not FMD, was inversely related to CRP levels and e-selectin independently of traditional risk factors in elderly subjects. As also endothelium-independent vasodilation was related to CRP and e-selectin, general vasoreactivity mainly in resistance arteries seems to be effected by low-grade inflammation in elderly subjects.

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