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Soluble intercellular adhesion molecule 1 and flow-mediated dilatation are related to the estimated risk of coronary heart disease independently from each other

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Abstract

Background: Flow mediated dilatation (FMD) of the brachial artery and soluble intercellular adhesion molecule 1 (sICAM-1) are measures of distinct functions of the endothelium, reflecting nitric oxide (NO)-mediated and pro-inflammatory status, respectively. The comparative value of the two measures in relation to cardiovascular risk is unknown. *Objective:* To study and quantify the relation between these two measures, and their relative value in relation to the risk of coronary heart disease as estimated by the Framingham risk function. *Methods:* We performed a single centre population-based study of 85 men and 81 women, aged 18–73 years. Endothelial function was assessed biochemically by sICAM-1 and functionally by FMD. In addition traditional cardiovascular risk factors, CRP, leukocyte count, homocysteine and fibrinogen were determined. Analyses were performed with multivariate linear regression, adjusted for age, gender, and CRP. *Results:* Median sICAM-1 levels were 217.0 μg/l (interquartile range: 174.0–348.5). Mean FMD was 4.5% (S.D.: 3.9). The regression coefficient for the association between sICAM-1 and FMD was −3.3 μg/l (95% CI: −6.0;−0.6) per percentage rise in FMD, after adjustment for age, gender, smoking, oral contraceptives (OC) use, classical risk factors and CRP. After adjustment for CRP and sICAM-1, the estimated risk of coronary heart disease in the next 10 years varied from 1.55% (95%CI: 0.89; 2.70) in the highest quintile of FMD to 3.92% (95% CI: 2.23; 6.92) in the lowest quintile. For sICAM-1, estimated risk, adjusted for FMD and CRP varied from 1.50% (95%CI: 0.85; 2.64) in the lowest quintile of sICAM-1 to 4.15% (95%CI: 2.35; 7.34) in the highest quintile. *P*-values for trends were 0.02 and 0.01 for quintiles of FMD and quintiles of sICAM-1, respectively. *Conclusion:* These findings indicate that sICAM-1 and FMD are related in healthy individuals, independently of cardiovascular risk factors and CRP, and that they are both related to the estimated risk of coronary heart disease, independently of each other.

Keywords: Cardiovascular disease; Atherosclerosis; sICAM-1; FMD; Endothelial function; Adhesion molecules

1. Introduction

Current clinical practice in cardiovascular risk management is centered on the assessment of an individual's risk of suffering myocardial infarction or stroke. The numerous national and international guidelines on this topic are generally derived from risk algorithms like the Framingham risk function, which are based on classical

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cardiovascular risk factors. In recent years however, numerous novel markers of cardiovascular risk have been proposed. Most importantly, it has become clear that impaired endothelial function and inflammation play a central role in atherosclerosis [1–3]. Measures of endothelial function and inflammation, especially the non-invasive, are therefore expected to be of use in a more accurate stratification of risk.

Studies on endothelial dysfunction have focussed on two distinct mechanisms; dysfunction of the nitric oxide (NO)-mediated vasodilator effect of endothelium, and disruption of the equilibrium between pro- and anti-inflammatory mechanisms. Flow-Mediated Dilatation (FMD) of the brachial artery and intercellular adhesion molecule 1 (ICAM-1) are two widely used measures to assess the functionality of these two mechanisms.

Measurement of flow-mediated dilatation was first described in 1992 [4]. Since then it has been used extensively as a non-invasive marker of endothelial function. FMD is expressed as the percentage increase in the diameter of the brachial artery after distal occlusion of forearm blood-flow [4]. This is dependent on acute NO release by the endothelium [5]. Reduced FMD has been shown to be related to cardiovascular risk factors [6] in subjects free from cardiovascular disease, and to be reversible after therapeutic interventions [7,8]. Patients with coronary artery disease [9,10], peripheral arterial disease [11,12] and decreased renal function [13,14] are known to have decreased endothelial function compared with healthy individuals. Recent evidence suggests that impaired FMD is an independent prospective predictor of cardiovascular events in patients undergoing vascular surgery [15] and in hypertensive postmenopausal women [16].

ICAM-1 is a member of the cellular adhesion molecule family, which also includes VCAM and E-selectin. These molecules facilitate the rolling, adhesion and migration of leukocytes across the endothelial barrier. Each has a plasma soluble form, which can serve as a surrogate marker for increased expression of CAM's on vascular endothelial cells, and reflect inflammation and activation of endothelial cells. Soluble intercellular adhesion molecule (sICAM-1) is expressed on the surface of endothelial cells, leukocytes and smooth muscle cells in reaction to stimuli like shear stress, bacterial toxins, pro-inflammatory cytokines and oxidants [17]. Levels of sICAM-1 have been found to be related positively to age [18], systolic and diastolic blood pressure [18], hypercholesterolemia [19], hypertriglyceridemia [19] and inversely to estrogens [20]. Not all other reports, however, confirm these findings. Furthermore, associations have been found between sICAM-1 and cardiovascular mortality in both healthy individuals [21,22] and populations at high risk [21,23]. However, sICAM-1, did not have additional predictive value compared with classical risk factors [21].

Even though FMD and sICAM-1 each highlight a different function of the endothelium, one would conceptually expect impairment in both mechanisms to occur concurrently. In other words, one would expect to find raised sICAM-1 values in conjunction with low FMD values. This means that to a certain degree, sICAM-1 and FMD may convey the same information about the state of the endothelium. Individually, both FMD and sICAM-1 have been related to prospective risk of a cardiovascular event [15,16,21–23]. It is however not clear whether both measures overlap in their ability to predict cardiovascular risk, and if so, to which degree. To explore this concept further, we investigated the relation between FMD and sICAM-1, and compared their separate and joint relation to the long term risk of coronary heart disease as estimated by the Framingham risk function.

2. Subjects and methods

2.1. Subjects

The study was performed using baseline measurements of an intervention study performed at TNO Nutrition and Food Research in Zeist, The Netherlands. The full study included 379 subjects (178 men, 201 women). We report on a random subgroup of 166 subjects (85 men, 81 women) who had measurements of both FMD and sICAM-1. Volunteers were recruited from the pool of volunteers and through advertising [24]. The major inclusion criterium was an age of 18–75 years. The main exclusion criteria were pregnancy, lactation, the wish to become pregnant, serum cholesterol >7.5 and/or triglycerides >2.3 mmol/l, if not under stabilized treatment. Informed consent was obtained from all subjects. The study was performed according to ICH (International Conference on Harmonization of Technical Requirements of Registration of Pharmaceuticals for Human Use-http://www.ifpma.org/ich1.html) guidelines for good clinical practice, and was approved by an external Medical Ethical Committee. The study was executed at the Department of Nutritional Physiology of TNO Nutrition and Food Research, Zeist. The measurements were performed from June 29 to September 16, 1998.

2.2. Blood sampling

For the analysis of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, CRP and glucose, blood was collected in tubes containing clot activator and gel (Becton Dickinson, Vacutainer systems). Tubes were centrifuged within 15–30 min after collection at $2000 \times g$ for 10 min at 4 °C to obtain serum. After centrifugation serum was removed and

stored at −80 °C. For the analysis of sICAM-1, blood was collected in tubes containing lithium heparin (Becton Dickinson, Vacutainer systems). Tubes were centrifuged within 30 min after collection at approximately $2000 \times g$ for 20 min at approximately 4 °C to obtain plasma. After centrifugation plasma was removed and stored at −80 °C. Fibrinogen was analyzed in citrated plasma.

For the analysis of leukocytes, blood was collected in tubes containing EDTA.

2.3. Chemical analyses

Serum triglycerides were analyzed by enzymatic hydrolysis with subsequent enzymatic determination by colourimetry (Boehringer, Mannheim, Germany). Total cholesterol was analyzed by enzymatic conversion to a stable chromogen, detectable by colourimetry (Boehringer). HDL-cholesterol was analyzed after precipitation of apoB-containing lipoproteins with polyethylene glycol, centrifugation and enzymatic detection by colourimetry (Boehringer) and low-density lipoprotein (LDL)-cholesterol was calculated using the Friedewald formula [25]. Fibrinogen was measured according to Clauss [26] using the STA Fribrinogen kit on a STA analyzer. CRP was analyzed by an enzyme-immunoassay using polyclonal antibodies [27] (Dako, Copenhagen, Denmark) with a low detection limit to detect basal levels in serum [27]. Soluble ICAM-1 was determined in plasma with an immunoenzymometric method [28]. The leukocyte count was measured using the SYSMEX K-1000.

2.4. Flow mediated dilatation

FMD of the brachial artery was performed as described previously [4,29,30]. FMD was calculated as the maximum percentage increase in diameter of the brachial artery after ischemia of the forearm. The measurements were performed in supine position at the elbow of the right arm. The cuff was placed on the forearm, distally to the measurement site. The duration of ischemia of the forearm was 5 min. All lumen diameter measurements were done at end diastole by the use of the R-wave of the electrocardiogram. The ultrasound images were made by one technician with a 7.5 MHz linear array transducer of an Ultramark duplex scanner. All images were stored on super-VHS videotape for off-line analysis. Measurement of the endothelium-independent vasodilatation using nitroglycerine, was not performed. Further details and reproducibility data have been described elsewhere [29,31].

2.5. Potential confounders and baseline characteristics

Smoking status was obtained by a questionnaire (current smoking: yes/no). Age was determined, body weight and height measured and body mass index (BMI) calculated. Body weight was assessed by weighing the subject wearing indoor clothing, without shoes, wallet and keys. Height was measured without shoes. BMI was calculated with the formula body weight (kg)/body height squared (m^2).

Systolic and diastolic blood pressure were measured oscillometrically by a Boso Oscillomat in a supine position in the right arm after at least 5 min rest and recorded in mm Hg and beats/min, respectively.

2.6. Risk estimate

A risk function estimates an individual's probability of experiencing an event within a certain time span as a function of the individual's level of the risk indicators. Risk functions are derived from analyses on data from longitudinal (cohort) studies, in which the relative and independent contribution of certain risk factors in predicting the occurrence of the event have been evaluated. The risk function used in this study, was derived by Wilson et al. based on data from the Framingham Heart Study cohort [32] and is based on gender, age, smoking status, total cholesterol, HDL-cholesterol, blood pressure, and the presence of diabetes mellitus as risk indicators. The exact equations used to obtain the Framingham risk score are given in the original report [32].

2.7. Data analysis

Analyses were performed using SPSS for WINDOWS 10.1. In all analyses the log transformed Framingham risk score was used, due to its skewed distribution. In order to account for possible raised CRP values due to acute inflammation [33], we performed all analyses involving CRP, excluding persons with CRP values $>$ 10 mg/l ($n = 12$). Multiple linear regression models were used to establish the relation between sICAM-1, FMD and cardiovascular risk factors, adjusting for age and gender.

The association between FMD and sICAM-1 was assessed with multiple linear regression, adjusting for age, gender, smoking and use of oral contraceptives (OC). The model was additionally adjusted for LDL-cholesterol, systolic blood pressure and glucose to assess whether the relation between the two measures of endothelial dysfunction was independent of classical risk factors.

The relation between FMD, sICAM-1 and the Framingham risk estimate was assessed with linear regression. FMD and sICAM-1 were standardized

(divided by their S.D.) in order to make regression coefficients comparable. Separate models were obtained for the unadjusted relation between FMD and the Framingham risk estimate, and the relation between sICAM-1 and the Framingham risk estimate. Additionally, the relation of both measures was adjusted for the presence of the other by assessing them in a single linear regression model. Finally, the same consecutive steps were undertaken adjusting for CRP.

3. Results

Table 1 shows the characteristics of the study population. FMD values were 1.2% higher in women than in men (95% CI: 0.1; 2.4). sICAM-1 and CRP did not differ significantly between genders. Table 2 shows the associations between sICAM-1, FMD and risk factors for atherosclerosis with adjustment for age and gender. Adjusted for age and gender, sICAM-1 was positively associated with smoking, diastolic blood pressure, systolic blood pressure, LDL-cholesterol, triglycerides, glucose, leukocyte count, log CRP and fibrinogen; and negatively associated with HDL-cholesterol. Adjusted for age and gender, FMD had a significant negative associated with LDL-cholesterol and CRP.

FMD and sICAM-1 were significantly correlated with each other. The Pearsons correlation coefficient was −0.24. The age and gender adjusted regression coefficient was −4.6 μg/l (95% CI: −7.5; −1.6) per percentage rise in FMD. After additional adjustment for smoking, OC use, log CRP, LDL-cholesterol, systolic blood pressure and glucose, the regression coefficient was −3.3 μg/l (95% CI: −6.0; −0.6).

Table 1
Baseline characteristics of the study population

Number of patients	166	
Age (years)	42.5	14.3
Men (%)	51.2	
OC use (% of women)	33.3	
Smokers (%)	28.9	
Height (m)	1.8	0.1
Weight (kg)	76.4	13.9
BMI (kg/m^2)	24.6	20.0–28.3
Diastolic bloodpressure (mmHg)	73.0	9.2
Systolic bloodpressure (mmHg)	120.1	13.9
Total cholesterol (mmol/l)	5.5	0.9
LDL-cholesterol (mmol/l)	3.4	0.9
HDL-cholesterol (mmol/l)	1.6	0.4
Triglycerides (mmol/l)	1.1	0.4
Glucose (mmol/l)	5.0	0.6
C-reactive protein (mg/l)	1.3	0.3–6.9
Leukocytes ($\times 10^9$ per l)	5.5	4.0–8.3
Homocysteine (μmol/l)	11.1	3.6
Fibrinogen (g/l)	3.3	0.7
SICAM-1 (μg/l)	217.0	174.0–348.5
FMD (%)	4.5	3.9

Values are means with S.D.s for normally distributed continuous variables, medians with 10th and 90th percentiles for continuous variables with a skewed distribution and percentages for dichotomous variables. OC, oral contraceptives; LDL, low-density lipoprotein; HDL, high-density lipoprotein; sICAM-1, soluble intercellular adhesion molecule 1.

Table 3 shows the regression coefficients and 95% confidence intervals for the relation between FMD, sICAM-1 and the Framingham risk estimate. In the unadjusted model, sICAM-1 had a statistically significant positive relation to the risk of coronary heart disease, whereas FMD was significantly negatively related. When assessed simultaneously, adjusting for the presence of the other, both relations attenuated. However, sICAM-1 attenuated less than FMD, remaining significantly related to the risk of coronary heart disease, as opposed to FMD which was no longer significantly related. Additional adjustment for CRP, attenuated all relations, however, the same effects could be observed. Fig. 1 shows the mean risk of coronary heart disease, in quintiles of FMD and sICAM-1 adjusting for CRP and sICAM-1 in the case of FMD; and for CRP and FMD in the case of sICAM-1. After adjustment for CRP and sICAM-1, the estimated risk of coronary heart disease in the next 10 years varied from 1.55% (95%CI: 0.89; 2.70) in the highest quintile of FMD to 3.92% (95% CI: 2.23; 6.92) in the lowest quintile. For sICAM-1, estimated risk, adjusted for FMD and CRP varied from 1.50% (95%CI: 0.85; 2.64) in the lowest quintile of sICAM-1 to 4.15% (95%CI: 2.35; 7.34) in the highest quintile. The p-values for a trend were 0.02 and 0.01 for quintiles of FMD and quintiles of sICAM-1, respectively.

4. Discussion

The most important finding of this study is that in healthy men and women both sICAM-1 and FMD are related to the estimated risk of coronary heart disease, independently of each other. We further found a clear and stable relation of moderate strength between FMD and sICAM-1.

These findings are of interest because they shed light on the mutual relation of a functional marker (FMD) and a biochemical indicator (sICAM-1) of distinct functions of the endothelium, and on their relative value in the estimation of the risk of coronary heart disease. Despite their different nature, FMD and sICAM-1 can both be considered measures of ‘endothelial function’. This is reflected in our finding of a clear relation between the FMD and sICAM-1. The fact that we find that this relation is stable after adjustment for classical risk factors, and low-grade inflammation indicates that the estimate we found is to be ascribed to a shared reflection about the state of the endothelium,

Table 2
Relation between sICAM-1, FMD, and cardiovascular risk factors ($n = 166$)

	SICAM-1		FMD	
	Adjustment: age, gender		Adjustment: age, gender	
	β	95% confidence interval	β	95% confidence interval
Smoking	56.21	32.36; 80.06	−0.60	−1.91; 0.70
Diastolic BP	2.05	0.73; 3.37	0.00	−0.07; 0.07
Systolic BP	0.96	0.08; 1.83	0.04	−0.01; 0.08
Total cholesterol	12.12	−2.49; 26.73	−0.56	−1.32; 0.20
LDL-cholesterol	18.80	3.90; 33.70	−0.81	−1.59; −0.04
HDL-cholesterol	−48.60	−80.10; −17.11	1.02	−0.65; 2.70
Triglycerides	34.62	8.61; 60.63	−0.07	−1.45; 1.31
Glucose	21.83	2.90; 40.76	−0.56	−1.56; 0.43
BMI	1.40	−1.54; 4.33	−0.01	−0.16; 0.14
Leukocyte count	13.89	8.21; 19.57	0.11	−0.20; 0.42
CRP	11.37	5.02; 17.71	−0.40	−0.73; −0.07
Fibrinogen	25.79	9.24; 42.33	−0.81	−1.68; 0.05
Homocysteine	−0.84	−4.70; 3.03	0.09	−0.11; 0.29

rather than to confounding by a common external influence. Despite being stable, the magnitude of the relation we found is modest. This is the first indication that apart from the 'overlapping' information, FMD and sICAM-1 each carry additional, distinctive information.

It further becomes apparent that FMD and sICAM-1 each express the functionality of a different endothelial mechanism, from our comparison of the relation between each of them and the estimated risk of coronary heart disease. Both with and without accounting for the level of low-grade inflammation, we found that FMD and sICAM-1 were significantly related to the estimated risk. When we adjusted the estimate of each of the two for the overlapping information conveyed by the other, we saw some attenuation of the relations. This effect was to be expected due to the presence of a moderate degree of overlap mentioned earlier. However, as becomes clear from Fig. 1, even after accounting for the presence of the other, both measures remained related to the risk of coronary heart disease.

The relation between sICAM-1 and FMD has not previously been assessed in healthy individuals. The relation between endothelial NO-dependent mechanisms and adhesion molecules has been assessed in mouse models, in which ICAM-1 deficient mice were shown to have a reduced dilatation response to acetylcholine [34]. Studies in humans have focussed on invasive measures of NO-dependent dilatation. John et al. [35] and Lim et al. [36] studied the relation between sICAM-1 and endothelium-dependent dilatation measured by forearm arterial flow after intra-arterial infusion of acetylcholine in patients with hypercholesterolemia and diabetes, respectively. They found no significant relation between sICAM-1 levels and endothelium-dependent dilatation, and concluded that sICAM-1 cannot substitute measurement of endothelium-dependent dilatation.

Holmlund et al. [37] studied the relation between soluble adhesion molecules and endothelial function measured by venous occlusion forearm plethysmography and found that endothelial function was inversely related ($r = -0.31$) to ICAM-1, but not to VCAM-1 or E-selectin. Elhadd et al. [38] univariately evaluated the relation between FMD and sICAM-1 in a mixed group of patients with hypopituarism and controls, and found a significant correlation coefficient of −0.225. Brevetti

Table 3
Relation between sICAM-1, FMD (expressed in S.D.s) and the logarithmically transformed Framingham risk estimate

	β	95% CI	β	95% CI	Attenuation (%)
SICAM-1	0.19	0.08; 0.31	0.17	0.06; 0.29	−9.38
FMD	−0.15	−0.29; −0.02	−0.12	−0.25; 0.02	−23.53
	Unadjusted		Adjusted for each other		
SICAM-1	0.15	0.04; 0.26	0.13	0.02; 0.24	−15.13
FMD	−0.14	−0.27; −0.02	−0.11	−0.24; 0.02	−25.00%
	Adjusted for CRP		Adjusted for CRP and each other		

Regression coefficients (β) indicate the change in the log transformed Framingham risk per S.D. increase in either sICAM-1 or FMD. The last column indicates the percentage attenuation of the regression coefficient by mutually adjusting sICAM-1 and FMD for the presence of the other.

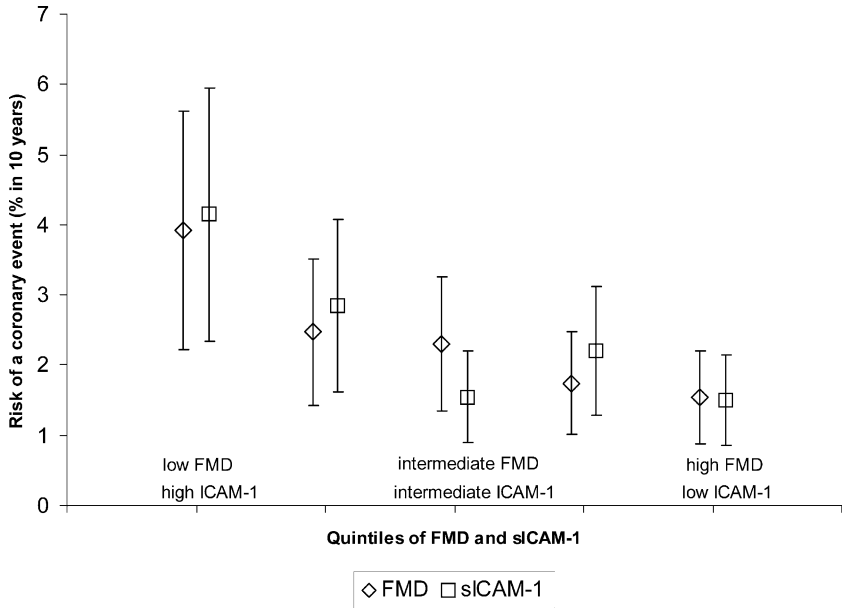

Fig. 1. Mean risk and 95% confidence intervals, of coronary heart disease as estimated by the Framingham risk algorithm, in quintiles of sICAM-1 and FMD. Values are adjusted for CRP and FMD in the case of sICAM-1 and for CRP and sICAM-1 in the case of FMD. Quintiles are presented in ascending order for FMD, and descending order for sICAM-1. Cut-off points for quintiles of FMD were 1.5, 3.1, 5.2 and 8.3%. Cut-off points for sICAM-1 were 186, 206, 232 and 294 μg/l. The *P*-values for a trend were 0.02 and 0.01 for quintiles of FMD and quintiles of sICAM-1, respectively.

et al. [12] found FMD to be significantly decreased by 3.2% in patients with sICAM-1 levels over 253 μg/l compared with those below this level in patients with peripheral arterial disease and controls. Our study has clearly confirmed that FMD and sICAM-1 are also related in healthy subjects, and has quantified the relation as one of moderate strength. We have furthermore revealed that this relationship is not influenced by adjustment for possible confounding by other risk factors, including CRP as a measure of low-grade inflammation.

In a more practical sense, the results of our study indicate that although endothelial function assessed by sICAM-1 and by FMD partly overlap, both measures carry considerable distinctive information. A limitation of our study is the cross-sectional design, in which we have estimated the risk of future coronary heart disease by an available risk function. To assess the relative value of FMD and sICAM-1 more accurately, both measures would have to be available in a cohort of patients with prospective follow-up on cardiovascular events. In the absence of this information however, our results give a first insight into the comparative value of FMD and sICAM-1 in the assessment of risk.

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