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Original Article

Soluble Vascular Cell Adhesion Molecules May be Protective of Future Cardiovascular Disease Risk: Findings from the PREVEND Prospective Cohort Study

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***Aim*: Soluble cell adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1, E-selectin, and P-selectin, have been suggested to be associated with cardiovascular disease (CVD) risk; however, the nature and magnitude of the association between VCAM-1 and CVD risk is uncertain. We aimed to assess the association of VCAM-1 with CVD risk and determine its potential utility for CVD risk prediction.**

***Methods*: VCAM-1 concentrations were measured at baseline in the PREVEND prospective study of 2,638 participants. Hazard ratios (95% confidence intervals [CI]) and measures of risk discrimination for CVD (e.g., C-index) and reclassification (i.e., net reclassification improvement) of participants were assessed.**

***Results*: During a median follow-up of 9.9 years, 614 CVD events occurred. Plasma VCAM-1 was weakly associated with several cardiovascular risk markers. In analyses adjusted for established cardiovascular risk factors, the hazard ratio (95% CI) for CVD per 1 standard deviation increase in $\log_e$ VCAM-1 was 0.91 (0.84−0.99; $P=0.020$), which remained consistent after additional adjustment for body mass index, alcohol consumption, triglycerides, renal function, and C-reactive protein; hazard ratio (95% CI) 0.89 (0.82−0.97; $P=0.006$). Comparing the top versus bottom quintiles of VCAM-1 levels, the corresponding adjusted hazard ratios were 0.74 (0.57−0.96; $P=0.023$) and 0.70 (0.54−0.91; $P=0.007$) respectively. Adding VCAM-1 to a CVD risk prediction model containing conventional risk factors did not improve the C-index or net reclassification.**

***Conclusions*: Plasma VCAM-1 is inversely and independently associated with CVD. However, VCAM-1 provides no significant improvement in CVD risk assessment beyond conventional CVD risk factors.**

See editorial vol. 24: 791-792

Key words: Vascular cell adhesion molecule-1, Soluble cell adhesion molecules, Cardiovascular disease, Risk factor, Risk prediction

Introduction

Cardiovascular diseases (CVDs) remain the leading cause of mortality globally, accounting for over 17 million deaths each year[1)]. By 2030, almost 23.6 million people will die from CVD, mainly from coronary heart disease (CHD), which is the major manifestation of CVD[2)]. Coronary heart disease, considered to be a manifestation of a chronic inflammatory response to injury or infection, is characterized by focal lipid-rich deposits called atheroma, which is initiated by the process of atherosclerosis[3)]. Endothelial dysfunction is

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Appendix 1. STROBE 2007 Statement—Checklist of items that should be included in reports of cohort studies

Section/Topic	Item#	Recommendation	Reported on page#
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	Page 804
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 804
Introduction			
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 804-806
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 804-806
Methods			
Study design	4	Present key elements of study design early in the paper	Study Design and Participants
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Study Design and Participants
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Study Design and Participants
		(b) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Risk Factor Assessment
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Risk Factor Assessment
Bias	9	Describe any efforts to address potential sources of bias	Statistical Analyses
Study size	10	Explain how the study size was arrived at	Statistical Analyses
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Statistical Analyses
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Statistical Analyses
		(b) Describe any methods used to examine subgroups and interactions	Statistical Analyses
		(c) Explain how missing data were addressed	Not applicable
		(d) If applicable, explain how loss to follow-up was addressed	Not applicable
		(e) Describe any sensitivity analyses	Statistical Analyses
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Appendix 3
		(b) Give reasons for non-participation at each stage	Appendix 3
		(c) Consider use of a flow diagram	Appendix 3
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Results; Tables 1 and 2
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	Results
Outcome data	15*	Report numbers of outcome events or summary measures over time	Results
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Results; Table 3
		(b) Report category boundaries when continuous variables were categorized	Results; Table 3
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Results; Table 4; Figure 2

(Cont Appendix 1)

Discussion			
Key results	18	Summarise key results with reference to study objectives	Discussion - Summary of main findings
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Discussion
Generalisability	21	Discuss the generalisability (external validity) of the study results	Discussion
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 816

known to play a pivotal role in the development of atherosclerosis. Endothelial cells play an important role in the regulation of homeostasis, arterial wall structure and tension, and undisturbed blood flow[4]. Soluble cell adhesion molecules [such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, and P-selectin], which are glycoproteins integral to the cell membrane, are responsible for the adhesion of different cells onto the endothelial surface[5]. Cell adhesion molecules facilitate the attachment of circulating leucocytes to the endothelium, to sites of inflammation, and their subsequent movement and accumulation in arterial walls[6-9], all of which are processes pivotal in the development and progression of atherosclerosis[10]. E-selectin and P-selectin mediate transient rolling of leucocytes along the endothelium[11], whiles VCAM-1 and ICAM-1 mediate stronger attachment of leucocytes to the endothelium[9]. Circulating levels of these cell adhesion molecules (including VCAM-1) are associated with several cardiovascular risk factors and markers[12] and higher levels have been detected in atherosclerotic lesions[13, 14] and in patients with ischemic stroke, coronary artery disease, and CVD[15-18].

Emerging evidence indicates that these soluble cell adhesion molecules may be linked to the future risk of CVD. A number of prospective studies, based in general population settings, have generally reported positive and independent associations between CVD outcomes and circulating levels of E-selectin, P-selectin, or ICAM-1[16, 19-24]; however, the association of VCAM-1 with CVD is unclear as previous studies have mostly reported null associations between VCAM-1 and CVD outcomes[21, 23, 25]. A single study demonstrated an increased risk of cardiovascular mortality with increased levels of VCAM-1, but the association was particularly evident among subjects with a history of type 2 diabetes[26]. Given the uncertainty in the existing evidence, our primary objective was to characterize the shape and assess the magnitude and independence of the prospective association between VCAM-1 concentrations and CVD risk using a population-based sample of 2,638 participants free from pre-existing CVD at baseline. A secondary objective was to investigate the extent to which VCAM-1 concentrations could improve the prediction of first-onset CVD when added to a conventional CVD risk prediction model.

Materials and Methods

This report was conducted according to STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines for reporting observational studies in epidemiology (**Appendix 1**)[27].

Study Design and Participants

The study population, consisting of a representative sample of inhabitants living in the city of Groningen in the Netherlands, were participants in the Prevention of Renal and Vascular End-stage Disease (PREVEND) study, a longitudinal general population-based study designed to investigate the natural course of urinary albumin excretion and its relationship to renal disease and CVD. The actual PREVEND cohort (N=8,592) was recruited from inhabitants aged 28−75 years invited for pre-screening from the city of Groningen in the Netherlands. Baseline examinations and measurements were performed between 1997 and 1998. Participants with a prevalent history of CVD, liver disease, renal disease, or malignancy were excluded in the present analysis. Given that assays for VCAM-1 could only be measured in a random subset of PREVEND participants because of

Appendix 2. Baseline characteristics of participants with and without VCAM-1 measurements

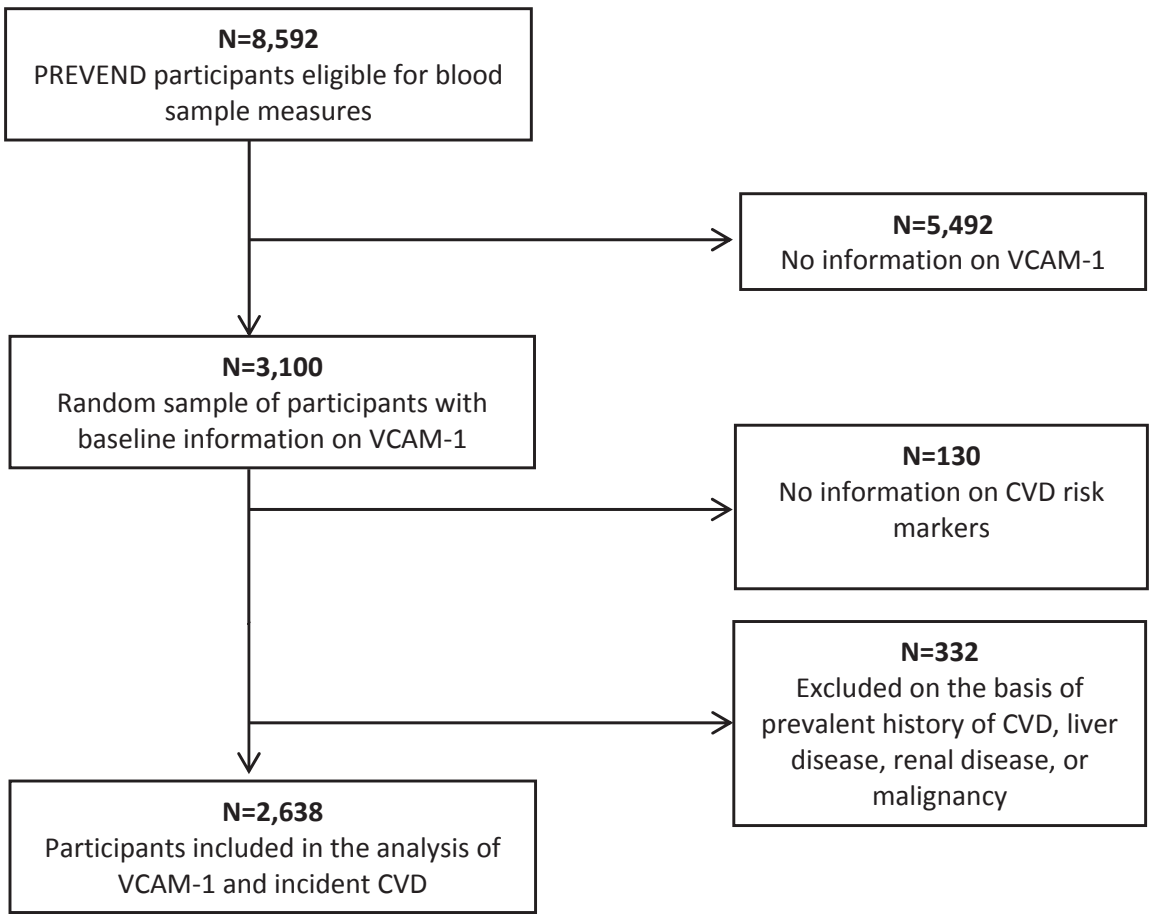
	With VCAM-1 measurements Mean (SD) median (IQR) or %	Without VCAM-1 measurements Mean (SD) or median (IQR) or %
Questionnaire		
Male	49.1	47.7
Age at survey (years)	51 (13)	47 (12)
History of diabetes	4.1	2.9
Smoking		
Current	33.7	33.7
Former	36.5	34.9
Never	29.8	31.4
Alcohol consumers	74.8	75.9
History of hypertension	12.1	8.8
Regular use of anti-hypertensive medication	13.7	9.4
Regular use of lipid-lowering medication	3.3	2.0
Physical measurements		
BMI (kg/m^2)	26.2 (4.2)	25.9 (4.3)
WHR	0.88 (0.09)	0.87 (0.09)
SBP (mmHg)	130 (21)	127 (19)
DBP (mmHg)	75 (10)	73 (10)
Lipid markers		
Total cholesterol (mmol/l)	5.72 (1.14)	5.58 (1.11)
HDL-C (mmol/l)	1.34 (0.41)	1.33 (0.40)
Triglycerides (mmol/l)	1.16 (0.85-1.69)	1.12 (0.82-1.64)
Metabolic, inflammatory, and renal function markers		
hsCRP (mg/l)	1.34 (0.57-3.06)	1.14 (0.50-2.72)
Fasting plasma glucose (mmol/l)	4.85 (1.23)	4.82 (1.03)
Creatinine (µmol/l)	82 (74-92)	82 (73-91)
Cystatine C (mg/dl)	0.82 (0.24)	0.78 (0.17)
eGFR (ml/min/1.73 m^2)	92.6 (20.0)	97.1 (19.1)
UAE (mg/24 hours)	8.04 (5.77-14.38)	9.88 (6.63-18.01)

BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); HDL-C, high-density lipoprotein cholesterol; ; hsCRP, high sensitivity C-reactive protein; IQR, interquartile range; SBP, systolic blood pressure; UAE, urinary albumin excretion; VCAM-1, vascular cell adhesion molecule-1; WHR, waist-to-hip ratio

financial constraints, our final cohort for this analysis included 2,638 participants with complete information on serum VCAM-1, relevant covariates, and cardiovascular outcomes. Participants in the PREVEND study included men and women recruited from an ethnically homogeneous population, and therefore characteristics of excluded participants were generally not different from those included in the final analyses (**Appendix 2**). The derivation of the analytic sample is reported in **Appendix 3**. The medical ethics committee of the University Medical Center Groningen duly approved the PREVEND study, which was conducted in accordance with the Declaration of Helsinki. Each participant provided written informed consent for voluntary participation, which was documented in a consent form approved by the medical ethics committee.

Risk Factor Assessment

During two outpatient visits by study participants, baseline data on sociodemographics, medical history, physical health, including anthropometric measurements, and risk markers for CVD were assessed. Blood biomarker analyses were performed on plasma and serum venous samples taken from participants after an overnight fast and 15 minutes of rest. Concentrations of VCAM-1 were determined in ethylenediaminetetraacetic acid (EDTA) plasma (that had been stored frozen at −80℃ for 15 years) using the sandwich enzyme-linked immunosorbent assay (Diaclone human sVCAM-1/CD106 ELISA, Diaclone SAS, France) with the minimal detection limit being 0.6 ng/ml. The inter-assay coefficients of variation were 5.94% and 1.44% in the lower normal and



Appendix 3. Derivation of the analytic sample

CVD, cardiovascular disease; VCAM-1, vascular cell adhesion molecule-1

higher normal range, respectively. Plasma glucose was measured by dry chemistry (Eastman Kodak, Rochester, New York). Total cholesterol, high-density lipoprotein cholesterol (HDL-C), high sensitivity C-reactive protein (hsCRP), triglycerides, serum creatinine, and serum cystatin C were measured using standard laboratory methods, which have been described previously[28-32]. Urinary albumin excretion (UAE) was calculated as the mean of two 24-hour urine collections. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) combined creatinine-cystatin C equation[33]. Type 2 diabetes mellitus and hypertension were defined as previously reported[31, 32, 34].

Outcome Assessment for CVD

The primary endpoint in our study was first-onset CVD, with incident CHD and stroke as secondary endpoints. Ascertainment of dates and causes of death were by record linkage with the Dutch Central Bureau of Statistics. Information on hospitalization for cardiovascular morbidity was retrieved from PRISMANT, the Dutch national registry of hospital discharge diagnoses[35]. All data were coded according to the *International Classification of Diseases*, Ninth Revision (ICD-9) until 01-01-2009. The data were coded according to ICD-10 codes after this date. First-onset CVD was defined as the combined endpoint of acute myocardial infarction, acute and subacute ischemic heart disease, coronary artery bypass grafting or percutaneous transluminal coronary angioplasty, subarachnoid hemorrhage, intracerebral hemorrhage, other intracranial hemorrhage, occlusion or stenosis of the precerebral or cerebral arteries, and other vascular interventions, such as percutaneous transluminal angioplasty or bypass grafting of peripheral vessels and aorta. Coronary heart disease events were defined as fatal or nonfatal myocardial infarction (MI), fatal or nonfatal ischemic heart disease (IHD), coronary artery bypass grafting (CABG), and percutaneous transluminal coronary angioplasty (PTCA). Stroke events were defined as subarachnoid

Table 1. Baseline participant characteristics overall and according to the development of incident cardiovascular disease

	Overall (*N*=2,638) Mean (SD) or median (IQR) or n (%)	Without incident CVD (*N*=2,024) Mean (SD) median (IQR) or n (%)	With incident CVD (*N*=614) Mean (SD) or median (IQR) or n (%)	*P*-value
VCAM-1 (ng/ml)	911.0 (651.9-1,231.3)	901.1 (647.6-1,220.3)	940.3 (664.3-1,261.5)	0.183
Questionnaire				
Male	1,288 (48.8)	869 (42.9)	419 (68.2)	<0.001
Age at survey (years)	51 (13)	48 (12)	60 (11)	<0.001
History of diabetes	101 (3.8)	46 (2.3)	55 (9.0)	<0.001
Smoking				
Current	887 (33.6)	622 (30.7)	265 (43.2)	
Former	967 (36.7)	731 (36.1)	236 (38.4)	<0.001
Never	784 (29.7)	671 (33.2)	113 (18.4)	
Alcohol consumers	1,970 (74.7)	1,540 (76.1)	430 (70.0)	0.003
History of hypertension	325 (12.3)	170 (8.4)	155 (25.2)	<0.001
Regular use of anti-hypertensive medication	357 (13.5)	194 (9.6)	163 (26.6)	<0.001
Regular use of lipid-lowering medication	88 (3.3)	45 (2.2)	43 (7.0)	<0.001
Physical measurements				
BMI (kg/m^2)	26.1 (4.2)	25.8 (4.1)	27.3 (4.0)	<0.0001
WHR	0.88 (0.09)	0.87 (0.09)	0.93 (0.09)	<0.0001
SBP (mmHg)	130 (21)	126 (18)	145 (23)	<0.0001
DBP (mmHg)	74 (10)	73 (9)	80 (10)	<0.0001
Lipid markers				
Total cholesterol (mmol/l)	5.72 (1.15)	5.60 (1.15)	6.11 (1.08)	<0.0001
HDL-C (mmol/l)	1.34 (0.41)	1.39 (0.41)	1.20 (0.39)	<0.0001
Triglycerides (mmol/l)	1.16 (0.85-1.69)	1.11 (0.81-1.61)	1.40 (0.99-2.03)	<0.0001
Metabolic, inflammatory, and renal function markers				
hsCRP (mg/l)	1.34 (0.57-3.08)	1.14 (0.51-2.68)	2.26 (1.07-4.97)	<0.0001
Fasting plasma glucose (mmol/l)	4.82 (1.20)	4.69 (0.96)	5.26 (1.72)	<0.0001
Creatinine (μmol/l)	82 (74-92)	81 (73-90)	89 (78-99)	<0.0001
Cystatine C (mg/dl)	0.82 (0.23)	0.79 (0.21)	0.90 (0.28)	<0.0001
eGFR (ml/min/1.73 m^2)	92.6 (20.0)	95.2 (19.6)	83.9 (19.1)	<0.0001
UAE (mg/24 hours)	8.05 (5.80-14.38)	7.20 (5.49-11.02)	14.65 (8.13-38.88)	<0.0001

BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); HDL-C, high-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; IQR, interquartile range; SBP, systolic blood pressure; UAE, urinary albumin excretion; VCAM-1, vascular cell adhesion molecule-1; WHR, waist-to-hip ratio

hemorrhage, intracerebral hemorrhage, other and unspecified intracranial hemorrhage, occlusion and stenosis of precerebral or cerebral arteries, and carotid obstruction. Further details on the definition of cardiovascular outcomes are provided in previous reports[34, 36].

Statistical Analyses

Skewed variables (e.g., VCAM-1, hsCRP, triglycerides, creatinine, and UAE) were natural logarithm ($\log_e$) transformed to achieve approximately normal distributions. Descriptive analyses were performed to summarize baseline characteristics of participants. The standard deviation (SD) of baseline log VCAM-1 concentration was 0.45, corresponding to approximately 1.6-fold higher circulating VCAM-1 (i.e., $e^{0.45}=1.57$). The cross-sectional associations of serum VCAM-1 with CVD risk markers were assessed by calculating Pearson's correlation coefficients adjusted for age and sex. Analyses of the associations between serum VCAM-1 concentrations and risk of cardiovascular outcomes involved time-to-event Cox proportional hazards models after confirmation of assumptions of proportionality of hazards[37]. Collinearity diagnostics using the variance inflation factor[38] showed no evidence of collinearity among covariates, including

Table 2. Cross-sectional correlates of VCAM-1

	Pearson correlation r (95% CI)†	Percentage difference (95% CI) in VCAM-1 levels per 1 SD higher or compared to reference category of correlate‡
Loge VCAM-1 (ng/ml)	-	-
Questionnaire		
Sex		
Female	-	Ref
Male	-	7% (4, 11)***
Age at survey (years)	0.10 (0.06, 0.13)***	4% (3, 6)***
History of diabetes		
No		
Yes		17% (7, 29)**
Smoking status		
Non-smokers	-	Ref
Current and former smokers	-	-6% (-10, -3)**
Alcohol consumption		
Non-consumers	-	Ref
Current consumers	-	-6% (-10, -2)*
History of hypertension		
No	-	Ref
Yes	-	6% (1, 12)*
Regular use of anti-hypertensive medication		
No	-	Ref
Yes	-	7% (2, 13)*
Regular use of lipid-lowering medication		
No	-	Ref
Yes	-	-5% (-14, 5)
Physical measurements		
BMI (kg/m^2)	0.01 (-0.03, 0.05)	0% (-1, 2)
WHR	-0.03 (-0.06, 0.01)	-2% (-4, 1)
SBP (mmHg)	0.01 (-0.03, 0.05)	0% (-2, 2)
DBP (mmHg)	-0.02 (-0.06, 0.02)	-1% (-3, 1)
Lipid markers		
Total cholesterol (mmol/l)	-0.08 (-0.11, 0.04)***	-4% (-5, -2)***
HDL-C (mmol/l)	-0.04 (-0.08, -0.00)*	-2% (-4, -0)*
Log triglycerides (mmol/l)	-0.01 (-0.05, 0.03)	-1% (-2, 1)
Metabolic, inflammatory, and renal function markers		
Log hsCRP (mg/l)	-0.02 (-0.06, 0.02)	-1% (-3, 1)
Fasting plasma glucose (mmol/l)	0.03 (-0.01, 0.07)	1% (-0, 3)
Log Creatinine (µmol/l)	0.06 (0.02, 0.10)*	3% (1, 5)*
Cystatine C (mg/dl)	0.08 (0.05, 0.12)***	4% (2, 6)***
eGFR (ml/min/1.73 m^2)	-0.06 (-0.10, -0.02)***	-3% (-5, -1)*
Log UAE (mg/24 hours)	0.05 (0.01, 0.08)*	2% (0, 4)*

BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); HDL-C, high-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; Ref, reference; SD, standard deviation; SBP, systolic blood pressure; UAE, urinary albumin excretion; VCAM-1, vascular cell adhesion molecule-1; WHR, waist-to-hip ratio

Asterisks indicate the level of statistical significance: *, p<0.05; **, p<0.01; ***, p<0.001; †, Pearson correlation coefficients between loge VCAM-1 and the row variables; ‡, Percentage change in VCAM-1 levels per 1 SD increase in the row variable (or for categorical variables, the percentage difference in mean VCAM-1 levels for the category versus the reference) adjusted for age and sex;

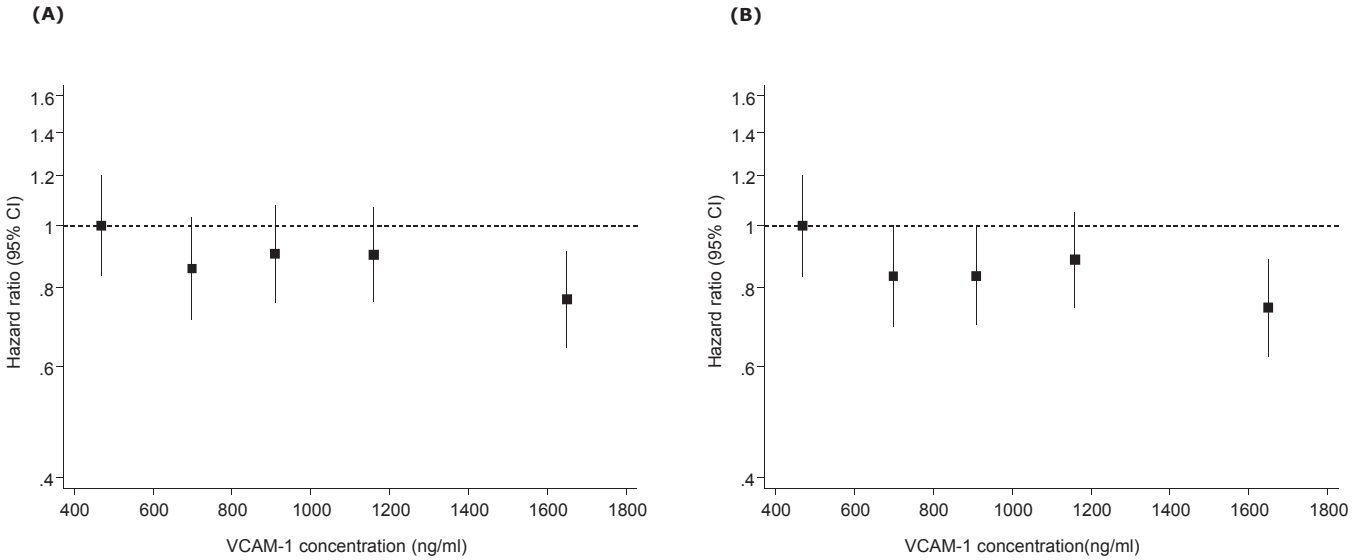


Fig. 1. Hazard ratios for incident cardiovascular disease by quintiles of baseline $\log_e$ VCAM-1 using floating absolute risks

A, adjusted for age and sex; B, adjustment as in B plus smoking status, history of diabetes, systolic blood pressure, total cholesterol, and high-density lipoprotein cholesterol

between UAE and eGFR. To characterize the shape of the association, hazard ratios (HRs) estimated within quintiles of baseline VCAM-1 concentrations relative to the bottom quintile were plotted against mean $\log_e$ VCAM-1 levels in each quintile using floating absolute risks (FARs)[39] as described in detail previously[40, 41]. Vascular cell adhesion molecule-1 was modeled as both continuous (per 1 SD) higher $\log_e$ serum VCAM-1 levels) and categorical (quintiles defined according to the baseline distribution of serum VCAM-1 level) variables. To assess the independence of the association, HRs were calculated with progressive adjustment for age and sex, other established CVD risk factors (smoking status, history of diabetes, SBP, total cholesterol, and HDL-C), potential confounders [body mass index (BMI), alcohol consumption, fasting glucose, triglycerides, eGFR, and hsCRP], and UAE. Effect modification by individual characteristics, such as age, sex, and other cardiovascular risk markers, were assessed using interaction tests. To limit potential biases due to reverse causation, we carried out subsidiary analyses that excluded the first two years of follow-up, participants on regular antihypertensive medication, and participants on regular lipid-lowering medication (statins).

To assess whether adding information on VCAM-1 to a model that included conventional cardiovascular risk factors[42] is associated with improvement in CVD risk prediction, we calculated measures of discrimination for censored time-to-event data (Harrell's C-index[43]) and reclassification. To investigate the change in C-index, we added VCAM-1 to a model based on risk factors included in the Framingham CVD Risk Score (i.e., age, sex, smoking status, SBP, total cholesterol, and HDL-C)[44]. For participants with at least 10 years of follow-up, reclassification of participants was assessed using the net-reclassification-improvement (NRI)[45] across predicted 10-year cardiovascular risk categories of low (<5%), intermediate (5 to <7.5%), and high (≥7.5%) risk[46]. Risk prediction analysis was restricted to participants without a known history of diabetes or CVD at baseline. All statistical analyses were conducted using Stata version 14 (Stata Corp, College Station, Texas).

Results

Baseline Characteristics and Correlates of VCAM-1

Data were available for 2,638 participants without a known history of CVD at baseline. The mean age of overall participants at baseline was 51 (SD 13) years, and 49% were males (**Table 1**). The mean (SD) $\log_e$ VCAM-1 was 6.79 (0.45) ng/ml. Except for VCAM-1 levels, there were significant differences in clinically relevant subgroups and levels of risk markers between participants who did and did not develop CVD. $\log_e$ VCAM-1 levels were weakly correlated with risk markers for CVD. Baseline VCAM-1 concentrations were higher by 7% in men compared with women, by 17% in people with diabetes compared with people without diabetes, and by 6% in people with a history of hypertension compared with those without a history. The levels were lower by 6% in cur-

Table 3. Association of baseline VCAM-1 concentrations with cardiovascular disease, coronary heart disease, and stroke

VCAM-1 concentration (mg/dl)	Events/ Total	Model 1		Model 2		Model 3		Model 4	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Cardiovascular disease									
Per 1 SD increase	614/2,638	0.91 (0.85 to 0.99)	0.032	0.91 (0.84 to 0.99)	0.020	0.89 (0.82 to 0.97)	0.006	0.88 (0.81 to 0.95)	0.002
Q1 (179.5-602.1)	117/528	ref		ref		ref		ref	
Q2 (603.3-800.5)	113/528	0.85 (0.66 to 1.11)	0.238	0.83 (0.64 to 1.08)	0.167	0.81 (0.63 to 1.06)	0.123	0.83 (0.64 to 1.08)	0.164
Q3 (800.6-1024.5)	124/527	0.90 (0.70 to 1.16)	0.429	0.83 (0.65 to 1.07)	0.158	0.79 (0.62 to 1.03)	0.079	0.79 (0.61 to 1.02)	0.072
Q4 (1024.8-1328.0)	130/528	0.90 (0.70 to 1.16)	0.412	0.88 (0.69 to 1.14)	0.338	0.86 (0.66 to 1.10)	0.230	0.85 (0.66 to 1.10)	0.223
Q5 (≥1328.4)	130/527	0.76 (0.59 to 0.99)	0.039	0.74 (0.57 to 0.96)	0.023	0.70 (0.54 to 0.91)	0.007	0.66 (0.51 to 0.86)	0.002
P-value for trend			0.092		0.070		0.027		0.008
Coronary heart disease									
Per 1 SD increase	388/2,638	0.86 (0.78 to 0.95)	0.002	0.85 (0.76 to 0.94)	0.001	0.84 (0.76 to 0.92)	0.001	0.82 (0.75 to 0.91)	<0.001
Q1 (179.5-602.1)	82/528	ref		ref		ref		ref	
Q2 (603.3-800.5)	67/528	0.71 (0.51 to 0.98)	0.038	0.70 (0.50 to 0.97)	0.030	0.68 (0.49 to 0.95)	0.022	0.70 (0.50 to 0.97)	0.031
Q3 (800.6-1024.5)	86/527	0.90 (0.66 to 1.21)	0.474	0.81 (0.60 to 1.10)	0.177	0.78 (0.57 to 1.06)	0.111	0.77 (0.57 to 1.05)	0.099
Q4 (1024.8-1328.0)	83/528	0.79 (0.58 to 1.08)	0.138	0.77 (0.56 to 1.05)	0.094	0.75 (0.55 to 1.03)	0.076	0.76 (0.56 to 1.04)	0.085
Q5 (≥1328.4)	70/527	0.58 (0.42 to 0.80)	0.001	0.55 (0.40 to 0.77)	<0.001	0.53 (0.38 to 0.74)	<0.001	0.50 (0.36 to 0.70)	<0.001
P-value for trend			0.008		0.004		0.002		0.001
Stroke									
Per 1 SD increase	153/2,638	0.88 (0.75 to 1.04)	0.133	0.86 (0.73 to 1.02)	0.078	0.86 (0.73 to 1.01)	0.062	0.83 (0.71 to 0.98)	0.028
Q1 (179.5-602.1)	31/528	ref		ref		ref		ref	
Q2 (603.3-800.5)	31/528	0.93 (0.56 to 1.54)	0.779	0.84 (0.51 to 1.39)	0.490	0.83 (0.50 to 1.37)	0.458	0.84 (0.51 to 1.40)	0.510
Q3 (800.6-1024.5)	27/527	0.79 (0.47 to 1.32)	0.364	0.75 (0.44 to 1.26)	0.269	0.73 (0.43 to 1.23)	0.240	0.70 (0.41 to 1.18)	0.176
Q4 (1024.8-1328.0)	34/528	0.90 (0.55 to 1.47)	0.671	0.84 (0.51 to 1.39)	0.502	0.84 (0.51 to 1.38)	0.482	0.82 (0.49 to 1.35)	0.429
Q5 (≥1328.4)	30/527	0.73 (0.44 to 1.21)	0.224	0.66 (0.39 to 1.11)	0.116	0.63 (0.37 to 1.07)	0.086	0.59 (0.35 to 1.00)	0.051
P-value for trend			0.252		0.167		0.135		0.074

CI, confidence interval; HR, hazard ratio; Q, quintile; SD, standard deviation; VCAM-1, vascular cell adhesion molecule-1
Model 1: Age and sex
Model 2: Model 1 plus smoking status, history of diabetes, systolic blood pressure, total cholesterol, and high-density lipoprotein-cholesterol
Model 3: Model 2 plus body mass index, alcohol consumption, glucose, $\log_e$ triglycerides, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation), and $\log_e$ high sensitivity C-reactive protein
Model 4: Model 3 plus $\log_e$ urinary albumin excretion

rent and former smokers compared with non-smokers and by 6% in current alcohol consumers compared with non-consumers (**Table 2**).

VCAM-1 Levels and Risk of Incident CVD

During a median follow-up of 9.9 (interquartile range, 6.8−10.8) years (22,370 person-years at risk), 614 incident CVD events were recorded. There were 153 stroke outcomes [subarachnoid hemorrhage (n=9); intracerebral hemorrhage (n=20); other and unspecified intracranial hemorrhage (n=6); occlusion and stenosis of precerebral or cerebral arteries (n=107); and carotid obstruction (n=11)] and 388 CHD outcomes [fatal or nonfatal MI (n=155); fatal or nonfatal IHD (n=119); CABG (n=51); and PTCA (n=63)].

A near log-linear inverse relationship was observed between VCAM-1 levels and CVD risk in analyses adjusted for established CVD risk factors (**Fig. 1**). **Table 3** shows the associations of VCAM-1 with cardiovascular outcomes. The HR for CVD per 1 SD increase in $\log_e$ VCAM-1 was (0.91; 95% CI, 0.85−0.99; P=0.032) in age- and sex-adjusted analyses, which remained consistent in further analyses adjusted for established cardiovascular factors (0.91; 95% CI, 0.84−0.99; P=0.020) and additional adjustment for BMI, alcohol consumption, glucose, $\log_e$ triglycerides, eGFR, and $\log_e$ hsCRP (0.89; 95% CI: 0.82−0.97; P=0.006). The association persisted in a final model adjusted for $\log_e$ UAE (0.88; 95% CI: 0.81−0.95; P=0.002). Alternatively, comparing the top versus bottom quintiles of VCAM-1 levels in similar models, the corresponding adjusted HRs were (0.76; 95% CI: 0.59−0.99; P=0.039), (0.74; 95% CI: 0.57−0.96; P=0.023), (0.70; 95% CI: 0.54−0.91; P=0.007), and (0.66; 95% CI: 0.51−0.86; P=0.002) respectively. HRs were similar in analyses that excluded the

Table 4. Hazard ratios for cardiovascular disease with first two years of follow-up, participants on regular antihypertensive medication, and participants on regular lipid-lowering medication excluded

	Events/ Total	Model 1		Model 2		Model 3		Model 4	
		Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Excluding first two years of follow-up	496/2,446	0.91 (0.84 to 1.00)	0.036	0.90 (0.82 to 0.98)	0.020	0.88 (0.81 to 0.97)	0.008	0.87 (0.79 to 0.95)	0.002
Excluding participants on regular antihypertensive medication	447/2,281	0.90 (0.82 to 1.00)	0.044	0.90 (0.82 to 0.99)	0.036	0.88 (0.80 to 0.97)	0.013	0.88 (0.80 to 0.97)	0.013
Excluding participants on regular lipid-lowering medication	566/2,550	0.92 (0.85 to 1.00)	0.053	0.91 (0.84 to 1.00)	0.035	0.90 (0.83 to 0.98)	0.015	0.88 (0.81 to 0.96)	0.004

Hazard ratios are reported per 1 standard deviation increase in $\log_e$ VCAM-1 levels; CI, confidence interval
Model 1: Age and sex
Model 2: Model 1 plus smoking status, history of diabetes, systolic blood pressure, total cholesterol, and high-density lipoprotein-cholesterol
Model 3: Model 2 plus body mass index, alcohol consumption, glucose, $\log_e$ triglycerides, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation), and $\log_e$ high sensitivity C-reactive protein
Model 4: Model 3 plus $\log_e$ urinary albumin excretion

first two years of follow-up, participants on regular antihypertensive medication, and participants on regular lipid-lowering medication (**Table 4**). The associations did not vary significantly by levels or categories of several clinically relevant individual characteristics (**Fig. 2**). In separate analyses for other cardiovascular outcomes, an inverse association was also demonstrated for CHD in analyses adjusted for several established risk factors and potential confounders; however, for stroke, the initial null association observed in analyses adjusted for established risk factors and several potential confounders became statistically significant with additional adjustment for $\log_e$ UAE (**Table 3**).

VCAM-1 and CVD Risk Prediction

A CVD risk prediction model containing risk factors based on the Framingham CVD Risk Score yielded a C-index of 0.7590 (95% CI: 0.7404−0.7776). After addition of information on VCAM-1 levels, the C-index was 0.7596 (0.7410 to 0.7783), representing a non-significant increase of 0.0006 (−0.0014 to 0.0026; $P=0.543$). There was no improvement in the classification of participants into predicted 10-year CVD risk categories (NRI: 0.00%, −0.41% to 1.42%; $P=0.996$).

Discussion

Summary of Main Findings

In this population-based study of individuals without a history of CVD at baseline, baseline VCAM-1 levels were weakly correlated with several cardiovascular risk markers. In analyses adjusted for established CVD risk factors, we observed an inverse near linear association of VCAM-1 with risk of CVD. The association remained consistent on further adjustment for several potential confounders, including UAE. The findings were consistent across several clinically relevant subgroups and levels of cardiovascular risk markers. The associations were similar in several sensitivity analyses. Similar inverse associations were observed for CHD risk. There was no statistically significant evidence of an association with stroke in analyses adjusted for established risk factors and several potential confounders; however, further adjustment for UAE strengthened the association due to a negative confounding effect of UAE on the association. This was unexpected given the positive correlation of VCAM-1 with UAE and the knowledge that albuminuria is a putative marker for endothelial dysfunction and is independently associated with increased vascular risk[47]. Indeed, further exploration of the current data showed a positive and independent association between UAE and stroke risk. Findings from the assessments of improvements in measures of risk discrimination and reclassification indicate that additional information on VCAM-1 measurements provides no significant improvement in CVD risk prediction.

Comparison with Previous Work

To our knowledge, this is the first study to assess the association between VCAM-1 and the composite outcome of CVD and to report inverse associations

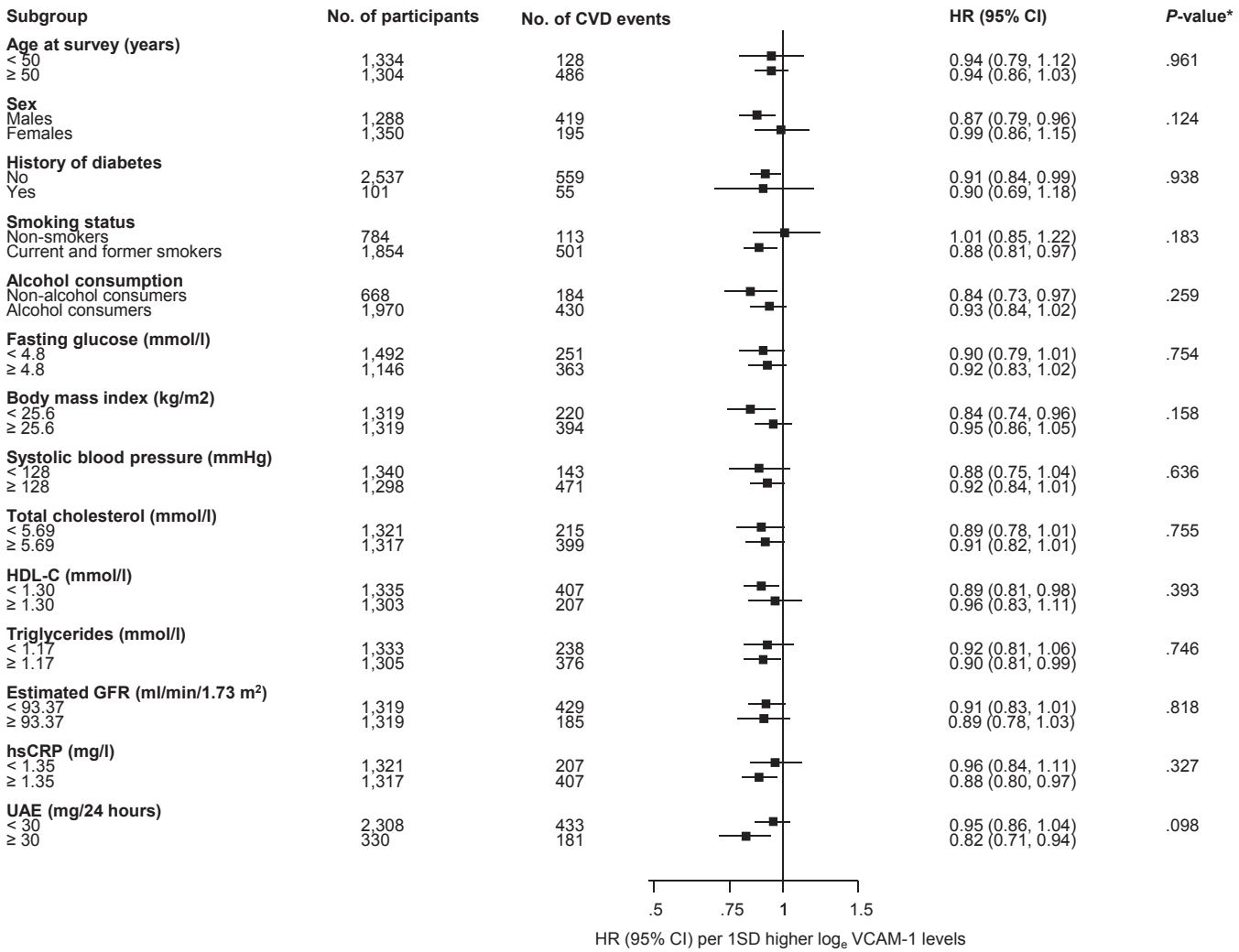


Fig. 2. Hazard ratios for cardiovascular disease, by several participant level characteristics

Hazard ratios were adjusted for age, sex, smoking status, history of diabetes, systolic blood pressure, total cholesterol, and high-density lipoprotein cholesterol; CI, confidence interval (bars); CVD, cardiovascular disease; Estimated GFR, glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; hsCRP, high sensitivity C-reactive protein; SD, standard deviation; UAE, urinary albumin excretion; *, *P*-value for interaction; cut-offs used for fasting glucose, body mass index, systolic blood pressure, total cholesterol, HDL-C, triglycerides, estimated GFR, and hsCRP are median values.

between VCAM-1 and the outcomes of CVD, CHD, and stroke. Previous studies conducted on the topic have mostly reported no evidence of any associations of VCAM-1 with cardiovascular outcomes. In analysis of the British Regional Heart Study cohort, Malik and colleagues demonstrated no statistically significant evidence of an association of VCAM-1 with CHD[25]. In addition, they conducted a pooled analysis of four studies (including their study), but this did not change the null findings. Luc and colleagues found no evidence of an association between VCAM-1 and the risk of acute coronary events in their analysis of the Epidemiological Study of Myocardial Infarction Study (PRIME), which enrolled apparently healthy men aged 50−59 years at baseline[21]. Similarly, no statistically significant evidence of an association was found between VCAM-1 and CHD risk in the Health Professional Follow-up Study[48]. On the contrary, Jager and colleagues, in a 7.4 year follow-up of the Hoorn study of 631 participants, demonstrated elevated VCAM-1 levels to be independently associated with cardiovascular mortality in subjects with type 2 diabetes[26]. However, a subset of study participants (24%) had a history of CVD at baseline, and though the study authors accounted for this in their models, stratified analysis by history of CVD was not conducted.

In our study, stratified analysis by history of diabetes showed no statistically significant evidence of effect modification. A protective effect of VCAM-1 on CVD risk was observed for participants without a history of diabetes, but the results were not statistically significant for participants with a history of diabetes. Given the small sample and low event rate for this subgroup, the null results could be attributed to low power; however, a trend towards a protective effect was observed.

Possible Explanations for Findings

Our findings would suggest that higher circulating concentrations of VCAM-1 are protective of cardiovascular outcomes. There was however no statistically significant evidence of an association of VCAM-1 with the specific outcome of stroke, which could be due to the low event rate. Though previous studies conducted in general population settings have failed to demonstrate any statistically significant association of VCAM-1 with CVD risk, an increased risk of CVD with elevated levels of VCAM-1 has been suggested, given that circulating levels of other soluble adhesion molecules (such as E-selectin, P-selectin, or ICAM-1) have been found to be positively and independently associated with CVD outcomes[16, 19-24]. Indeed, the therapeutic effect of leech enzyme extract on atherosclerosis (a traditional Chinese medicine commonly used for treating CVD) has been shown to be manifested via inhibiting the expression of adhesion molecules such as ICAM-1 and VCAM-1[49]. The mechanistic pathways for the link between elevated VCAM-1 levels and its protective effect on the development of CVD are unclear, as increased expression of soluble adhesion molecules have been suggested to play a direct role in the pathogenesis of atherosclerosis[50] via endothelial dysfunction and inflammation. These observations may reflect the differences in the functions of these adhesion molecules and suggest that there may be important pathophysiologic differences between these molecules and the genesis of atherosclerosis. E-selectin is expressed exclusively by endothelial cells and therefore may be a better marker of endothelial dysfunction, whereas ICAM-1 and VCAM-1 are expressed on a number of cells, including endothelial cells and leukocytes[51]. Whiles E-selectin and P-selectin mediate transient rolling of leucocytes along the endothelium[11], ICAM-1 and VCAM-1 allow stronger leukocyte rolling and attachment to the endothelium[9]. Intercellular adhesion molecule-1, which has been consistently demonstrated to be associated with incident vascular events, is highly expressed by endothelial cells in human atheroma, and low levels have been expressed on normal endothelium. In contrast, VCAM-1 is not expressed at all on normal endothelium, and lower levels of expression have been found in human atheroma[14, 52]. A previous study has also suggested that ICAM-1 and VCAM-1 function at opposite ends of the atherosclerosis spectrum and that their interaction with CVD involves a more complex process[48]. There is also a possibility that the null associations demonstrated in previous studies may be attributable to study design and participant characteristics such as sample size, duration of follow-up, and age and sex of participants. Compared to some previous studies[21, 48, 53], our study had a longer follow-up and a higher event rate. Given the postulated role of VCAM-1 in the early atherosclerotic process[54, 55], longer follow-up durations may be needed to demonstrate any association that we demonstrated in our study. In addition, the majority of previous studies have demonstrated these null associations in cohorts only limited to men, whereas the current study involved both men and women[21, 48, 56]. Indeed, our subgroup analyses showed no statistically significant associations in both genders, though there were trends towards protective associations. Finally, while our cohort involved relatively younger to older participants, previous studies employed middle-aged to elderly participants in their samples[21, 48, 56]. These explanations and mechanisms are however speculative and further work is warranted to identify key underlying biological mechanisms through which VCAM-1 may play a protective role in the development of CVD.

Implications of Findings

The findings of our study may have clinical implications. Irrespective of the irrelevance of the utility of VCAM-1 in CVD risk prediction, its use for CVD risk prevention and treatment may still have relevance. The overall evidence supports the possibility that elevated VCAM-1 levels might protect from CVD events in the general population. It highlights the vascular endothelium as a potential therapeutic target for the prevention and/or treatment of CVD. Circulating VCAM-1 levels may be a potential biomarker for monitoring the effects of treatment on the vascular endothelium. Nonetheless, given that this is the first study to demonstrate these findings; further studies are needed to replicate these findings.

Strengths and Limitations

This is the first simultaneous and comprehensive assessment of the association of VCAM-1 with the risk of incident composite CVD, CHD, and stroke. Other strengths include the nationally representative sample, the large endpoint numbers by most standards, the high response and long-term follow-up, and measurements on a comprehensive panel of lifestyle

and biological markers that permitted adequate adjustment for potential confounders. Reverse-causation bias was minimized as individuals with prevalent CVD at baseline were excluded from the analyses at the onset. The analyses were detailed, which included comparison of the associations at different levels or categories of vascular risk markers, several sensitivity analyses to supplement and contextualize the principal findings, and risk prediction analyses. Limitations include the potential for residual confounding due to errors in measurements of risk marker and other unmeasured confounders; absence of repeat measurements of VCAM-1 which precluded the ability to correct for regression dilution bias, which could have underestimated the association between long-term VCAM-1 levels and CVD; and analyses which were based on a predominantly white-European population, therefore the findings may not be generalizable to individuals of different ethnicities. In addition, blood samples for VCAM-1 measurements involved long-term storage which could have resulted in under-estimation of the associations due to sample degradation. However, it has been reported that VCAM-1 is not known to be influenced by repeated freeze-thaw cycles after storage[57].

In conclusion, available evidence for the first time shows that VCAM-1 is protective of incident CVD. Addition of information on VCAM-1 to conventional risk factors provides no significant improvement in CVD risk prediction in the general population. Further studies are needed to replicate this association and the etiological mechanisms underlying these data require investigation.

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Conflict of Interest

The authors report no relationships that could be construed as a conflict of interest.

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