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Increased Levels of Soluble Vascular Cell Adhesion Molecule 1 Are Associated With Risk of Cardiovascular Mortality in Type 2 Diabetes
The Hoorn Study

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Membrane-bound vascular cell adhesion molecule 1 (VCAM-1) allows the tethering and rolling of monocytes and lymphocytes as well as firm attachment and transendothelial migration of leukocytes. Soluble forms of VCAM (sVCAM-1) may serve as monitors of increased expression of membrane-bound VCAM-1 and thus may reflect progressive formation of atherosclerotic lesions. Levels of sVCAM-1 have been found to be increased among type 2 diabetic as compared with nondiabetic subjects. To study the association of plasma sVCAM-1 concentration and risk of cardiovascular and all-cause mortality among nondiabetic and diabetic subjects, we investigated an age-, sex-, and glucose-tolerance–stratified sample ($n = 631$) of a population-based cohort aged 50–75 years that was followed prospectively. Plasma levels of sVCAM-1 were determined in frozen –70°C baseline samples. After 7.4 years (mean) of follow-up, 107 (17%) subjects had died (42 of cardiovascular causes). In the entire group, increased sVCAM-1 levels were significantly associated with increased risk of cardiovascular mortality (relative risks [RRs] per 100 ng/ml sVCAM-1 increase, 1.10 [1.05–1.15] after adjustment for age, sex, and glucose tolerance status). This RR was somewhat diminished by further adjustment for the presence of hypertension and cardiovascular disease; levels of total, HDL, and LDL cholesterol and homocysteine; the presence of microalbuminuria (a putative marker of endothelial dysfunction); levels of von Willebrand factor (a marker of endothelial dysfunction) and C-reactive protein (a marker of low-grade inflammation); and estimates of glomerular filtration rate. However, the RR remained statistically significant. The RR among type 2 diabetic subjects was 1.13 (1.07–1.20) per 100 ng/ml sVCAM-1 increase after adjustment for age and sex, which was somewhat higher but not significantly different from the RR in nondiabetic subjects (P value for interaction term, 0.12). Further adjustment for other risk factors gave similar results. In conclusion, levels of sVCAM-1 are independently associated with the risk of cardiovascular mortality in type 2 diabetic subjects and therefore might be useful for identifying subjects at increased cardiovascular risk. Increased plasma sVCAM-1 levels may reflect progressive formation of atherosclerotic lesions, or sVCAM-1 itself may have bioactive properties related to cardiovascular risk. Our data, however, argue against the hypotheses of sVCAM-1 levels simply being a marker of endothelial dysfunction, of low-grade inflammation, or of an impaired renal function. *Diabetes* 49:485–491, 2000

Adherence of circulating leukocytes to endothelium and their subsequent transmigration into the arterial intima is an early step in the formation of atherosclerotic lesions (1). The recruitment of leukocytes into tissues is dependent on a cascade of events mediated through a diverse family of cellular adhesion molecules that are expressed on the surface of vascular endothelial cells (2,3).

Membrane-bound vascular cell adhesion molecule 1 (VCAM-1) is expressed mainly on endothelial cells, smooth muscle cells, and tissue macrophages (4,5) and allows the tethering and rolling of monocytes and lymphocytes, as well as firm attachment and transendothelial migration of leukocytes (6–9). Endothelial expression of VCAM-1 occurs on human atherosclerotic plaques (10,11) and has been shown to be an early manifestation of experimental cholesterol-induced atherosclerosis (12,13).

Soluble forms (sVCAM-1) have been detected in plasma (14,15). Secretion of sVCAM-1 is reported to be indicative of the expression of membrane-bound VCAM-1 (16). Although the pathophysiological role of these soluble forms is unclear, it has been hypothesized that sVCAM-1 levels may serve as a monitor of expression of membrane-bound VCAM-1. Increased levels thus may reflect progressive formation of atherosclerotic lesions (14,17). In addition, recent cross-sectional studies showed sVCAM-1 concentration to be positively associated

with carotid artery intima-media thickness (18–20) and with the severity of peripheral arterial disease assessed by angiography (18,21). In prospective studies, however, high levels of sVCAM-1 could not be demonstrated to be associated with risk of cardiovascular events (22,23), which is in contrast with findings on other adhesion molecules, such as intercellular adhesion molecule 1 and E-selectin (22,24).

Most (20,25–29), but not all (30,31), studies have shown sVCAM-1 levels to be increased among type 2 diabetic subjects as compared with nondiabetic subjects, and sVCAM-1 levels have been shown to be independently associated with carotid artery intima-media thickness in type 2 diabetes patients (29). In view of these considerations, we investigated the association of sVCAM-1 concentration and risk of cardiovascular and all-cause mortality among nondiabetic and type 2 diabetic subjects in a prospective, population-based cohort study.

RESEARCH DESIGN AND METHODS

General study design. The study population consisted of an age-, sex-, and glucose-tolerance–stratified sample of the Hoorn Study, a population-based cohort study of disturbances of glucose tolerance in a Caucasian population aged 50–75 years conducted from October 1989 to February 1992, which has been described previously (32,33). Briefly, 2,484 Caucasian subjects (71% of those invited) participated. All subjects, except previously diagnosed diabetic subjects treated with oral glucose-lowering agents or insulin, underwent an oral glucose tolerance test (OGTT) according to the World Health Organization guidelines (33). Subjects with a 2-h postload glucose 7.5 mmol/l, all subjects with type 2 diabetes, and a random sample of subjects with a 2-h postload glucose <7.5 mmol/l stratified by age and sex were invited within 4 weeks for a second visit to investigate glucose-intolerance–related complications (709 were invited, of whom 631 [89%] participated). These subjects underwent a second OGTT (except those who already used glucose-lowering agents; n = 67). On the basis of the mean of the two OGTTs, glucose tolerance was divided into three categories (32,33): normal glucose tolerance (n = 288), impaired glucose tolerance (n = 170), and type 2 diabetes (n = 173). Subjects with normal or impaired glucose tolerance or type 2 diabetes in the present study population thus represented a stratified random sample of all subjects with normal or impaired glucose tolerance or type 2 diabetes in the initial cohort. Because the exact sampling procedure is known, we can back-calculate the prevalences of glucose tolerance categories and of associated variables, such as ischemic heart disease, in the initial cohort (n = 2,484) from those in the second sample (n = 631) by "direct standardization," as previously described in detail (32,33).

From these subjects, we obtained an ankle-brachial blood pressure index (n = 631) and a resting electrocardiogram (n = 625 [32,33]). Subjects were classified as having cardiovascular disease when they had a history of myocardial infarction; had an electrocardiogram with a Minnesota code 1.1–1.3, 4.1–4.3, 5.1–5.3, or 7.1; had undergone coronary bypass surgery or angioplasty; had an ankle-brachial pressure index <0.9 in either leg; and/or had undergone a peripheral arterial bypass or amputation.

sVCAM-1. Baseline concentrations of sVCAM-1 were assessed in deep-frozen (–70°C) heparin plasma samples. The mean duration of storage was 8.3 years (range 7.3–9.4). No plasma was available for 21 subjects. Concentrations of sVCAM-1 were measured in duplicate by enzyme-linked immunosorbent assay (ELISA) kits (Bender MedSystems, Wenen, Austria [Cat. #BMS232]); reference plasma values in 111 healthy subjects, as provided by the manufacturer, are 1,090 (237 ng/ml; range 675–1,693). We measured sVCAM-1 levels in 27 healthy volunteers and found a mean level of 966 ng/ml. The mean variation between duplicate plasma samples in the assay was 3.4%. The interassay variation is 14%. To limit experimental variation, all samples of the study were assayed simultaneously, in duplicate, on the same day. For comparability with other studies, it should be noted that the same pooled plasma contained 471 ng/ml sVCAM-1 when assayed by the Amersham (Buckinghamshire, U.K.) sVCAM-1 kit and 416 ng/ml when assayed by the Biosource (Nivelles, Belgium) sVCAM-1 kit.

Other measurements. We obtained data on blood pressure, weight, height, BMI, and smoking habits (32,33). Hypertension was defined as diastolic pressure 95 mmHg, systolic pressure 160 mmHg, and/or the use of antihypertensive drugs (33). Current smoking was defined as currently smoking cigarettes and/or cigars. We also obtained data on plasma GHb; fasting insulin, serum creatinine, homocysteine, total cholesterol, HDL cholesterol, and triglyceride levels; and urinary albumin and creatinine levels (32–34). Serum levels of urea nitrogen concentration were determined in samples stored at –70°C by a kinetic ultraviolet assay from

Roche Diagnostics (Mannheim, Germany). Serum albumin levels were assessed using the bromcresol purple method. LDL cholesterol was calculated by the Friedewald formula (33), except when triglyceride level was >4.55 mmol/l (n = 23). The glomerular filtration rate was estimated in two ways: 1) from the Cockroft-Gault formula for creatinine clearance (33) and 2) from the formula proposed by the Modification of Diet in Renal Disease Study Group (MDRD formula [35]): $GFR = 170 \cdot [\text{serum creatinine level (mg/dl)}]^{-0.726} \cdot [\text{age}]^{-0.176} \cdot [0.762 \text{ if patients is female}] \cdot [1.180 \text{ if patient is black}] \cdot [\text{serum urea nitrogen level (mg/dl)}]^{-0.170} \cdot [\text{serum albumin level (g/dl)}]^{+0.318}$. Microalbuminuria was defined as an albumin-to-creatinine ratio >2.0 mg/mmol (33). Concentrations of von Willebrand factor (vWF) and C-reactive protein (CRP) were assessed as previously described (36).

Follow-up. Data on the subjects' vital status on 1 January 1999 were collected from the mortality register of the municipality of Hoorn. For the 49 subjects who had moved out of town, information on vital status was obtained from the new local municipalities. For each subject, we determined whether or not death had occurred during follow-up, and if so, the date at which death occurred. For all subjects who died, the cause of death was extracted from the medical records of the general practitioner and the hospital of Hoorn and classified according to the ninth edition of the International Classification of Diseases (32,34). Cardiovascular mortality was defined as codes 390–459 and cancer mortality as codes 140–240. Information on cause of death could not be obtained for 16 (15%) of the deceased subjects, and 1 subject was lost to follow-up.

All participants gave informed consent for this study, which was approved by the local ethics committee.

Statistical analyses. All analyses were performed with SPSS 7.5 for Windows 95. The associations between sVCAM-1 levels and cardiovascular risk factors (such as smoking and hypertension) and risk indicators (such as microalbuminuria and vWF and CRP levels) were tested by linear regression analyses with sVCAM-1 levels (log-transformed because of a better fit of the regression model) as dependent variable and risk factors or indicators as independent variables, all adjusted for age, sex, and glucose tolerance status (unless this was the variable under consideration). Survival over the follow-up duration was calculated by Kaplan-Meier curves for different groups, and differences were tested by the log-rank test. To assess associations of cardiovascular risk factors and risk indicators with risks of cardiovascular and all-cause mortality, we performed a Cox proportional hazards multiple regression analysis, in all cases—because of the stratification procedure—with adjustment for age, sex, and glucose tolerance status. Results are described as relative risks (RRs) (hazard ratios) with 95% CIs.

Risk factors and risk indicators measured on a continuous scale were used as such in the regression models, except for BMI and levels of HDL cholesterol, CRP, and vWF, because the association of these variables with mortality was nonlinear. Therefore, a low level of HDL cholesterol was defined as a level <0.9 mmol/l (32); a high level of vWF or CRP was defined as a level in the upper tertile (>1.56 IU/ml and >2.84 mg/l, respectively [36]); and obesity was defined as a BMI >27kg/m^2 for men and >26 kg/m^2 for women (32). Levels of fasting insulin and triglyceride were log-transformed because of a better fit of the regression model. sVCAM-1 was entered into the regression models as a continuous variable, because the association with mortality was linear. To assess whether the associations of sVCAM-1 with mortality were independent, regression analyses were adjusted primarily for all variables that were statistically significantly associated with cardiovascular mortality, except for microalbuminuria, levels of vWF and CRP, and estimates of glomerular filtration rate; and secondarily, for variables significantly associated with sVCAM-1 levels, or microalbuminuria (a putative marker of endothelial dysfunction), or levels of vWF (a marker of endothelial dysfunction), or levels of CRP (a marker of low-grade inflammation), or estimates of glomerular filtration rate. To evaluate a possible interaction between sVCAM-1 levels and risk factors or indicators significantly associated with mortality with regard to cardiovascular or all-cause mortality, Cox regression analyses were performed with the risk factor or indicator of interest, sVCAM-1, their product term, and age, sex, and glucose tolerance status in the model. A significant RR for the product term was considered an interaction of sVCAM-1 with that risk factor or indicator. Two-sided P values <0.05 were considered statistically significant.

RESULTS

Table 1 (two columns on the left) shows the baseline characteristics of the study population. The median (range) of sVCAM-1 levels was 1,305 (417–5,260) ng/ml. The percentage of subjects with a sVCAM-1 level in the highest tertile (>1,484 ng/ml) per glucose tolerance status was 27% for normal glucose tolerance, 37% for impaired glucose tolerance, and 41% for type 2 diabetes.

The mean ± SD duration of follow-up was 7.4 ± 1.7 years, with a range of 0.5–9.2 years. After 7.4 years of follow-up, 107

TABLE 1
Baseline characteristics and RR of cardiovascular and all-cause mortality associated with risk factors or risk indicators

	Baseline characteristics		Mortality associated with the indicated difference in risk factor or indicator	
	Percentage, mean ± SD, or median (interquartile range)			RR (95% CI)
Risk factor		Difference in risk factor or indicator	Cardiovascular mortality	All-cause mortality
Male sex (%)	48	Yes vs. no	1.35 (0.73–2.48)	1.40 (0.95–1.93)
Age (years)	64 ± 7	Per 5-year increase	1.69 (1.29–2.24)	1.62 (1.37–1.91)
GHb (% of hemoglobin)	5.9 ± 1.3	Per 1% of hemoglobin increase	1.14 (0.93–1.42)	1.16 (1.01–1.33)
Fasting insulin (pmol/l)	84 (63–119)	Per 10% increase*	1.03 (0.97–1.10)	1.03 (0.99–1.08)
Type 2 diabetes (%)	27	Yes vs. no	2.76 (1.35–5.65)	2.38 (1.52–3.71)
BMI (kg/m^2)	27.2 ± 4.0	High vs. low†	1.91 (0.93–3.92)	1.41 (0.92–2.17)
Total cholesterol (mmol/l)	6.6 ± 1.2	Per 1.0 mmol/l increase	1.23 (0.97–1.56)	1.11 (0.96–1.30)
LDL cholesterol (mmol/l)	4.5 ± 1.1	Per 1.0 mmol/l increase	1.27 (0.97–1.65)	1.10 (0.93–1.31)
HDL cholesterol (mmol/l)	1.3 ± 0.4	Low vs. high‡	2.74 (1.31–5.71)	1.87 (1.13–3.09)
Triglycerides (mmol/l)	1.6 (1.2–2.2)	Per 10% increase*	1.06 (0.99–1.13)	1.04 (1.01–1.08)
sVCAM-1 (ng/ml)	1,305 (417–5,260)	Per 100 ng/ml increase	1.10 (1.05–1.15)	1.04 (1.00–1.08)
vWF (IU/ml)	1.37 ± 0.70	High vs. low§	1.95 (1.04–3.64)	1.67 (1.13–2.49)
CRP (mg/l)	1.75 (0.83–3.80)	High vs. low§	2.02 (1.08–3.80)	1.49 (1.00–2.21)
Homocysteine (µmol/l)	12.6 ± 5.8	Per 5 µmol/l increase	1.14 (0.99–1.31)	1.08 (0.95–1.21)
Hypertension (%)	39	Yes vs. no	2.79 (1.41–5.50)	1.66 (1.11–2.48)
Current smokers (%)	28	Yes vs. no	1.74 (0.88–3.42)	1.86 (1.23–2.81)
Microalbuminuria (%)	11	Yes vs. no‖	3.38 (1.71–6.68)	1.73 (1.05–2.84)
Creatinine clearance (ml/min)¶	78.5 ± 18.1	Per 5 ml/min increase	0.88 (0.79–0.97)	0.94 (0.88–1.01)
Glomerular filtration rate (ml · min^{-1} · 1.73 m^{-2})#	67.8 ± 12.1	Per 5 ml · min^{-1} · 1.73 m^{-2}	0.72 (0.64–0.81)	0.94 (0.88–1.01)
Prior cardiovascular disease (%)	24	Yes vs. no	3.79 (2.01–7.18)	2.11 (1.42–3.14)

n = 631 for all subjects. Prior cardiovascular disease was defined as a history of myocardial infarction and/or Minnesota code 1.1–1.3, 4.1–4.3, 5.1–5.3, or 7.1 on the electrocardiogram, coronary bypass operation and/or angioplasty and/or ankle-brachial pressure index <0.90 and/or peripheral arterial bypass or amputation. RR (95% CI) was obtained with Cox regression analyses of cardiovascular and all-cause mortality associated with continuous or dichotomous variables after adjustment for age, sex, glucose tolerance status (impaired glucose tolerance and type 2 diabetes), except when this was the variable under consideration. *Log-transformed; †>27 vs. 27 kg/m^2 for males and >26 vs. 26 kg/m^2 for females; ‡<0.9 vs 0.9 mmol/l; §upper tertile vs. lower tertiles (>1.56 IU/ml for vWF and >2.84 mg/l for CRP levels); ‖albumin-to-creatinine ratio 2.0 vs. >2.0 mg/mmol; ¶according to the Cockcroft-Gault formula; #according to the MDRD formula.

(49 type 2 diabetic subjects) of the 631 subjects had died, of whom 42 (39%; 21 type 2 diabetic subjects) had died of cardiovascular disease. Subjects who died had higher levels of sVCAM-1 than did those who survived (1,534 ± 639 vs. 1,355 ± 395 ng/ml, respectively). Table 1 (three columns on the right) shows RRs of mortality associated with risk factors and risk indicators.

Associations of sVCAM-1 level with cardiovascular risk factors and indicators. After adjustment for age, sex, and glucose tolerance status (unless this was the variable under consideration; Table 2), sVCAM-1 levels were significantly associated with the following: male sex; age; levels of fasting insulin, vWF, CRP, and homocysteine; and the presence of type 2 diabetes, hypertension, and microalbuminuria. sVCAM-1 levels were inversely associated with total, LDL, and HDL cholesterol levels, current smoking, and estimates of glomerular filtration rate, and they were borderline significantly associated with the presence of cardiovascular disease. sVCAM-1 levels were not significantly associated with BMI or levels of GHb and triglycerides after adjustment for age, sex, and glucose tolerance status (data not shown).

sVCAM-1 level and risk of cardiovascular mortality. Subjects with sVCAM-1 levels in the upper and the middle tertiles had about a threefold and twofold increased risk of car-

diovascular mortality, respectively, as compared with subjects with sVCAM-1 levels in the lowest tertile (Fig. 1). The test for trend for increasing sVCAM-1 levels expressed per tertile was significant (P = 0.04), suggesting a linear relationship.

In the entire group, sVCAM-1 levels were significantly associated with increased risk of cardiovascular mortality after adjustment for age, sex, and glucose tolerance status (RR per 100 ng/ml sVCAM-1 increase, 1.10 [1.05–1.15]; Table 3). Further adjustment for the presence of hypertension, cardiovascular disease, current smoking, microalbuminuria, levels of fasting insulin, total, LDL, and HDL cholesterol, homocysteine, vWF, and CRP, and estimates of glomerular filtration rate slightly diminished the RRs (Table 3, first column). Adjustment for BMI and levels of GHb and triglycerides (added to model 2 in Table 3) did not materially change the results (data not shown).

The RRs of cardiovascular mortality associated with increased sVCAM-1 levels were not significantly different in the presence as compared with the absence of type 2 diabetes (P value for interaction term 0.12). The RRs, if anything, seemed somewhat stronger among type 2 diabetic subjects (Table 3). Again, adjustment for BMI and levels of GHb and triglycerides (added to model 2 in Table 3) did not materially change the results (data not shown).

TABLE 2
sVCAM-1 levels: cross-sectional associations with cardiovascular risk factors or risk indicators

	β	SE(β)	P
Sex (M vs. F)	0.023	0.01	0.03
Age (per 1-year increase)	0.0024	0.001	0.001
Fasting insulin (per 10% increase)*	0.00029	0.011	0.009
Type 2 diabetes (yes vs. no)	0.053	0.013	<0.0005
Total cholesterol (per 1.0 mmol/l increase)	–0.018	0.004	<0.0005
LDL cholesterol (per 1.0 mmol/l increase)	–0.018	0.005	<0.0005
HDL cholesterol (per 1.0 mmol/l increase)	–0.048	0.016	0.03
vWF (per 1.0 IU/ml increase)	0.00041	<0.0005	<0.0005
CRP (per 10% increase)*	0.00012	0.0004	0.004
Homocysteine (per 1.0 µmol/l increase)	0.0033	0.001	0.001
Hypertension (yes vs. no)	0.022	0.011	0.04
Current smoking (yes vs. no)	–0.0023	0.012	0.04
Microalbuminuria (yes vs. no)	0.053	0.017	0.002
Creatinine clearance (per 1.0 ml/min increase)†	–0.00082	0.000	0.02
Glomerular filtration rate (per 1.0 ml · min^{-1} · 1.73 m^{-2} increase)‡	–0.0024	0.000	<0.0005
Prior cardiovascular disease (yes vs. no)	0.023	0.012	0.06

Regression coefficients (β), standard errors [SE(β)], and P values were obtained by linear regression analyses with sVCAM-1 levels (log transformed because of a better fit of the regression model) as dependent and risk factors or indicators as independent variable, all adjusted for age, sex, and glucose tolerance status (unless this was the variable under consideration). Prior cardiovascular disease was defined as described in the legend to Table 1; *log-transformed; †according to the Cockcroft-Gault formula; ‡according to the MDRD formula.

The RR of all-cause mortality associated with sVCAM-1 levels was somewhat less than the RR of cardiovascular mortality (RR per 100 ng/ml sVCAM-1 increase, 1.04 [1.00–1.08]; Table 3). Further adjustment for risk factors or indicators did not affect the results (Table 3).

Additional analyses. To assess whether the association between cardiovascular mortality and increased levels of sVCAM-1 varied over time, we performed analyses with different lengths of follow-up duration. The RRs of cardiovascular mortality for increased sVCAM-1 levels were similar over time (Fig. 2).

To investigate whether the association of sVCAM-1 levels and risk of cardiovascular mortality among diabetic subjects was independent of impaired renal function, analyses were performed after exclusion of subjects with 1) creatinine clearance <80 ml/min according to the Cockcroft-Gault formula (n = 84) and 2) glomerular filtration rate <80 ml · min^{-1} ·

1.73 m^{-2} according to the MDRD formula (n = 83). The RRs of cardiovascular mortality for a 100 ng/ml increase in sVCAM-1 levels among diabetic subjects did not change materially (e.g., in model 1 in Table 3, RR 1.13 [1.06–1.19] before and 1.12 [0.98–1.27] after exclusion of subjects with creatinine clearance <80 ml/min).

To investigate whether the RR of cardiovascular mortality for sVCAM-1 levels was similar among different risk groups, we performed analyses with interaction terms added (see RESEARCH DESIGN AND METHODS). None of the variables associated with cardiovascular mortality (i.e., age; levels of HDL cholesterol, vWF, and CRP; and the presence of hypertension, microalbuminuria, or cardiovascular disease) showed a significant interaction with sVCAM-1 levels (data not shown). Repeating the analyses for all-cause mortality gave similar results.

Malignancies can influence levels of circulating adhesion molecules. Levels of sVCAM-1 among subjects who died of
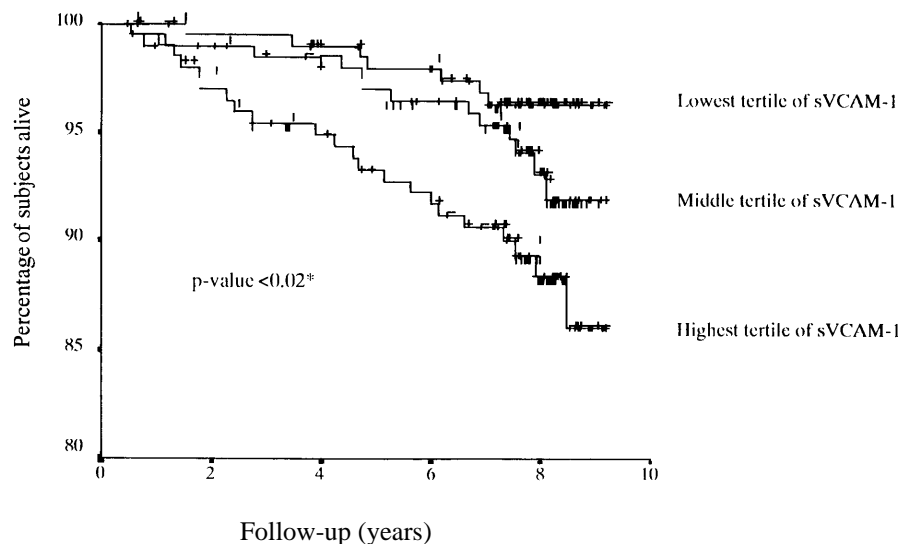

FIG. 1. Cardiovascular survival (Kaplan-Meier curves) according to plasma sVCAM-1 in the lowest (417–1,154 ng/ml), middle (1,155–1,484 ng/ml), and highest (1,485–5,260 ng/ml) tertiles. *Log-rank test for difference in survival for different groups; |censored data.

TABLE 3
RR of cardiovascular and all-cause mortality associated with sVCAM-1 concentration after adjustment for potentially confounding risk factors or risk indicators

Model	Added variables	Cardiovascular mortality (n = 42)		All-cause mortality (n = 104) (all subjects, n = 608)
		All subjects (n = 608)	Diabetic subjects (n = 166)	
1	Age, sex, glucose tolerance status	1.10 (1.05–1.15)	1.13 (1.07–1.20)	1.04 (1.00–1.08)
2	Model 1 and hypertension, cardiovascular disease, and low HDL cholesterol level*	1.08 (1.03–1.13)	1.12 (1.06–1.19)	1.04 (1.00–1.08)
3	Model 2 and levels of fasting insulin*, homocysteine, and total and LDL cholesterol and current smoking	1.08 (1.02–1.13)	1.11 (1.04–1.19)	1.03 (0.99–1.08)
4	Model 2 and microalbuminuria*	1.06 (1.01–1.12)	1.10 (1.03–1.18)	1.03 (0.99–1.07)
5	Model 2 and vWF*	1.07 (1.02–1.12)	1.12 (1.05–1.20)	1.03 (0.99–1.07)
6	Model 2 and CRP*	1.07 (1.02–1.13)	1.13 (1.06–1.20)	1.04 (1.00–1.08)
7a	Model 2 and creatinine clearance*	1.06 (1.01–1.12)	1.11 (1.04–1.18)	1.03 (0.99–1.07)
7b	Model 2 and glomerular filtration rate*	1.06 (1.01–1.11)	1.10 (1.03–1.17)	1.03 (0.99–1.07)

Data are RR (95% CI) of mortality for a 100 ng/ml increase of sVCAM-1 concentration, as obtained with Cox multiple regression analyses. The rationale of Models 1–7 is described in RESEARCH DESIGN AND METHODS. *Defined as described in Table 1.

malignancies as compared with those who died of other causes were not significantly different (1,349 ± 549 vs. 1,388 ± 444 ng/ml, respectively; P = 0.60). Thirty-nine subjects (36%) died of cancer. After adjustment for age, sex, and glucose tolerance status, sVCAM-1 was not significantly associated with cancer mortality (RR per 100 ng/ml sVCAM-1 increase, 0.93 [0.85–1.02]).

DISCUSSION

The most important novel finding of this study is that it showed sVCAM-1 levels to be significantly associated with risk of cardiovascular mortality, particularly among type 2 diabetic subjects. The risk increase did not vary with time. In accordance with previous studies, we showed sVCAM-1 levels to be significantly associated with male sex, age, fasting insulin levels, vWF level, the presence of type 2 diabetes, hypertension, and microalbuminuria, and, inversely, with levels of total, LDL, and HDL cholesterol and estimates of glomerular filtration rate (18,20–22,27,37). These variables, however, did not materially affect the association between sVCAM-1 levels and risk of cardiovascular mortality (Table 3). Thus, levels of sVCAM-1 were significantly and independently associated with risk of cardiovascular mortality.

The pathophysiological pathway through which sVCAM-1 level is associated with risk of cardiovascular mortality is unclear. The most commonly held view is that increased plasma sVCAM-1 levels indicate increased expression of membrane-bound VCAM-1 on endothelial and smooth muscle cells and on macrophages, and thus may reflect progressive formation of atherosclerotic lesions (14,17). Alternatively, increased sVCAM-1 levels might simply be a marker of an acute phase response, reflecting the progressive low-grade vessel wall inflammation that plays a pivotal role in the pathogenesis of atherothrombotic disease (1,38,39). Accordingly, several cytokines that can induce an acute phase reaction in response to pro-inflammatory antigens strongly increased the expression of VCAM-1 on cultured endothelial cells (40,41). On the other hand, previous clinical studies did not demonstrate an association of sVCAM-1 levels with lev-

els of acute phase reactants such as CRP (21,27). Although we did find a moderately strong association between sVCAM-1 and CRP levels, we showed that the association between sVCAM-1 levels and risk of cardiovascular mortality was independent of CRP levels, suggesting that sVCAM-1 levels increase cardiovascular risk through a pathway different from that of an acute phase response. Third, increased levels of sVCAM-1 could reflect generalized endothelial dysfunction, since we and others showed high sVCAM-1 levels to be associated with high vWF levels (18,22,31) and the presence of microalbuminuria (18), i.e., potential markers of generalized endothelial dysfunction (42–44). However, we showed sVCAM-1 levels to be associated with the risk of cardiovascular mortality independent of vWF levels and of microalbuminuria, which argues against the idea of increased sVCAM-1 levels being a marker of generalized endothelial dysfunction. However, because endothelial dysfunction is not a single entity, we cannot exclude the possibility that increased levels of sVCAM-1 reflect endothelial dysfunction different from that reflected by increased levels of vWF or the presence of microalbuminuria. Fourth, increased levels of sVCAM-1 can
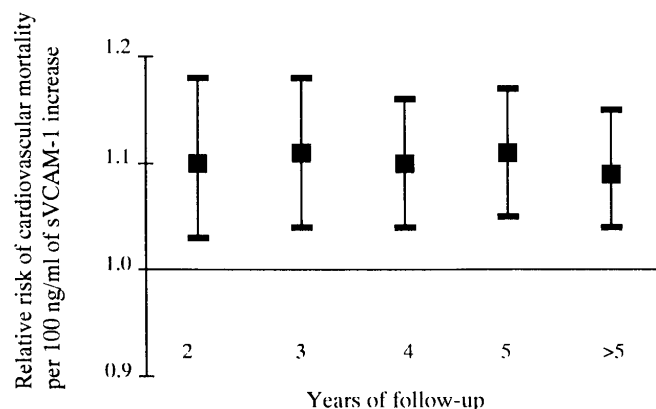

FIG. 2. RR and 95% CI of cardiovascular mortality associated with a 100 ng/ml sVCAM-1 increase.

be explained not only by an increased synthesis/shedding but also by impaired clearance of sVCAM-1 molecules. Although little is known about the route of elimination of these molecules, an important role of the kidney has been suggested, since levels of sVCAM-1 were strongly associated with serum creatinine levels among subjects with chronic renal failure not receiving dialysis (45,46). Nevertheless, we found sVCAM-1 levels to be associated with risk of cardiovascular mortality independent of estimates of glomerular filtration rate. Thus, although impaired renal function might cause increased levels of sVCAM-1, this appears not to be the explanation for the association of sVCAM-1 levels with cardiovascular risk. Finally, sVCAM-1 itself may have bioactive properties related to cardiovascular risk. For example, Koch et al. (47) have recently shown that sVCAM-1 has pro-angiogenic properties. Taken together, several pathophysiological mechanisms may explain the association between sVCAM-1 levels and risk of cardiovascular mortality. Our data argue against the hypotheses of sVCAM-1 levels simply being a marker of an acute phase response, of endothelial dysfunction, or of an impaired renal function.

In accordance with previous findings (20,24–29), we demonstrated levels of sVCAM-1 to be higher among diabetic than among nondiabetic subjects, suggesting a stronger stimulation of VCAM-1 synthesis among diabetic as compared with nondiabetic subjects. In an environment with long-standing high glucose concentrations, lipids and proteins will undergo nonenzymatic glycation and oxidation, resulting in the successive formation of successively reversible Amadori products (such as GHb) and irreversible metabolites, so-called advanced glycated end products (AGEs) (48,49). However, we, like others (25,30,50), could not demonstrate a significant association between levels of sVCAM-1 and GHb. Moreover, AGEs have been found to increase VCAM-1 expression by activating its promotor through a nuclear factor-κB–like transcriptional factor (51,52). This suggests that increased levels of AGEs rather than increased levels of GHb could be the explanation for the higher levels of sVCAM-1 among type 2 diabetic as compared with nondiabetic subjects. These issues need further study.

The present study has several limitations. The levels of sVCAM-1 were measured once, which may have led to nondifferential misclassification and therefore underestimation of the RRs associated with mortality. This study was too small to establish with certainty whether there is an interaction of sVCAM-1 levels and the presence of type 2 diabetes with regard to cardiovascular risk.

In conclusion, we have shown that the level of sVCAM-1 is strongly and independently associated with cardiovascular and all-cause mortality among type 2 diabetic subjects. This could be of clinical relevance, since therapeutic modalities to lower VCAM-1 expression have been proposed. Antioxidants have been shown to inhibit the expression of membrane-bound VCAM-1 (53), whereas administration of anti–VCAM-1 antibodies has been shown to diminish transendothelial passage of leukocytes (54). Furthermore, lowering levels of AGEs by aminoguanidine has been shown to inhibit VCAM-1 expression (55). Thus, therapeutic strategies can lower VCAM-1 expression and, in parallel, sVCAM-1 levels (16), which may prevent progression of cardiovascular disease. These issues, however, need further study.

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