Elevated Markers of Endothelial Dysfunction Predict Type 2 Diabetes Mellitus in Middle-Aged Men and Women From the General Population

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Objective—Using the Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)/Cooperative Research in the Region of Augsburg (KORA) database, we investigated prospectively whether increased levels of soluble E-selectin (sE-selectin), soluble intercellular adhesion molecule 1 (sICAM-1), and von Willebrand factor (vWF), all considered to be sensitive markers of endothelial dysfunction, are associated with an increased risk of incident type 2 diabetes mellitus.

Methods and Results—In a case–cohort study, concentrations of adhesion molecules were measured in stored samples of 532 case subjects and 1712 noncase subjects. VWF was measured in a subsample with available plasma samples (n=191 case and 580 noncase subjects). Men and women with elevated levels of sE-selectin had a significantly increased risk of type 2 diabetes after multivariable adjustment. Hazard ratios (95% CIs) comparing tertile extremes of sE-selectin were 2.63 (1.79 to 3.88) and 1.71 (1.07 to 2.75) for men and women, respectively. Elevated levels of sICAM-1 were also associated with an increased risk of type 2 diabetes; however, the association was not independent of other diabetes risk factors including E-selectin [hazard ratio (95% CI) for tertile 3 versus tertile 1: men, 1.32 (0.89 to 1.96); women, 1.03 (0.64 to 1.67)]. In this study, vWF was not associated with risk of type 2 diabetes.

Conclusions—These data support a role of endothelial dysfunction in the etiology of type 2 diabetes. (***Arterioscler Thromb Vasc Biol*. 2006;26:398-405.)**

Key Words: endothelial dysfunction ■ type 2 diabetes ■ adhesion molecules ■ von Willebrand factor

Endothelial dysfunction is regarded as a key event in the development and progression of atherosclerosis.[1,2] Whereas insulin resistance and its related conditions, such as obesity, hyperglycemia, dyslipidemia, hypertension, altered coagulation, and fibrinolysis are considered to be important causes of impaired endothelial function, another hypothesis suggests that endothelial dysfunction represents the root cause of insulin resistance and the associated features of the insulin resistance syndrome.[3,4] According to this hypothesis, endothelial dysfunction could be the mechanism explaining the strong association observed between atherosclerotic cardiovascular disease (CVD) and type 2 diabetes mellitus (DM), a common consequence of a prolonged state of insulin resistance. It is suggested that dysfunction of the endothelium in large and medium-sized arteries plays a central role in atherogenesis, whereas dysfunction at the peripheral vascular endothelium at the arteriolar and capillary level plays the primary role in the pathogenesis of the insulin resistance syndrome.[3]

Endothelial dysfunction or damage can be approximated through measurement of biomarkers released from endothelial cells, such as cellular adhesion molecules, like soluble E-selectin (sE-selectin), soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and von Willebrand factor (vWF).[5,6] In several cross-sectional studies, increased levels of markers of endothelial dysfunction have been observed in subjects with DM.[7–12] However, because the temporal sequence of events cannot be assessed in cross-sectional studies, these studies are not very informative concerning the question of whether endothelial dysfunction is a cause or a consequence of DM. Prospective studies that provide evidence for the temporality of events are scarce and have provided partly inconsistent results.[13–15]

To additionally elucidate the role of endothelial dysfunction in the pathogenesis of type 2 DM and to assess possible sex differences, we examined the association between 3 markers of endothelial dysfunction (sE-selectin, sICAM-1,

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and vWF) and incident type 2 DM in a large, population-based study in middle-aged men and women.

Methods

Study Population

The data presented were derived from the population-based Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)/Cooperative Research in the Region of Augsburg (KORA) studies conducted between 1984 and 2002. The MONICA Augsburg project was part of the multinational World Health Organization MONICA project.[16] Three independent, cross-sectional, population-based surveys covering the city area of Augsburg (Germany) and 2 adjacent counties were conducted in 1984–1985 (S1), 1989–1990 (S2), and 1994–1995 (S3) to estimate the prevalence and distribution of cardiovascular risk factors among men and women aged 25 to 64 (S1) or 25 to 74 years (S2 and S3). The study was approved by the local authorities, and all of the participants provided written informed consent. The total number of participants was 13 427 (6725 men and 6702 women). All of the subjects were prospectively followed within the frame of KORA. The present study was restricted to subjects aged 35 to 74 years at baseline, because the incidence of type 2 DM is low in younger subjects. Altogether, 10 718 persons (5382 men and 5336 women) of this age range participated in ≥1 of the 3 baseline surveys. After exclusion of 1187 subjects with missing blood samples, 509 participants with self-reported prevalent DM, 14 subjects with incident DM other than type 2 DM (eg, type 1 or secondary diabetes), 30 subjects with self-reported incident DM where the diagnosis could not be validated, 988 subjects without follow-up information, and 54 subjects with a follow-up time of <1 year, the source population for the present study was composed of 7936 subjects (3894 men and 4042 women). The sample sizes of the source population by survey were 1156 (S1), 1463 (S2), and 1275 (S3) for men and 1339 (S1), 1438 (S2), and 1265 (S3) for women.

Starting with the source population as the full cohort of interest, we applied a case–cohort design. This design involves the selection of a random sample or a stratified random sample of the entire cohort and the assembly of covariate histories only for this random sample and for all cases that developed in the entire cohort.[17] In the present study, the stratified random sample of the cohort of interest, called here the subcohort, contained 1885 subjects (1018 men and 867 women) and was selected for each of women and men stratifying by survey. From these, we excluded 41 men and 11 women with missing values on markers of endothelial dysfunction or any of the covariables used in the present analysis leading to a subcohort of 1833 subjects (977 men and 856 women). The sample sizes for the subcohort by survey were 395 (S1), 376 (S2), and 206 (S3) for men and 345 (S1), 300 (S2), and 211 (S3) for women. These final stratum-specific sample sizes were used together with the stratum-specific sizes of the cohort of interest to compute sampling fractions, and the inverse of the sampling fractions yield the survey and sex-specific sampling weights: 2.93, 3.89, and 6.19 for men and 3.88, 4.79, and 5.99 for women.

A total of 555 incident cases of type 2 DM (329 men and 226 women) were observed between participants' study start dates and December 31, 2002. Of these, 19 men and 4 women were excluded because of incomplete information on any of the markers of endothelial dysfunction or other covariables, leaving 310 men and 222 women with incident type 2 DM for the final analyses. Because 75 male and 46 female cases were also part of the randomly drawn subcohort, the present analysis was composed of total of 2244 participants (310 men with incident DM, 222 women with incident DM, 902 male noncases, and 810 female noncases).

Ascertainment of DM at Follow-Up

To determine the incidence of DM, a written follow-up questionnaire was sent to all of the participants of the 3 baseline surveys in 1997–1998 and in 2002–2003. Furthermore, all of the subjects who participated in the first survey were invited to participate in a follow-up examination conducted in 1987–1988. At follow-up, all of the subjects were asked whether they had DM and whether the disease had been diagnosed by a physician. In addition, the year of diagnosis was assessed. Cases with self-reported incident DM were validated by a questionnaire mailed to the treating physician or medical chart review. For deceased subjects without a previously reported diagnosis of diabetes, hospital records were searched for and/or the last treating physician was asked about a history of diabetes. If the participant had suffered from diabetes, type of diabetes and date of diagnosis were ascertained. All of the deceased participants for whom no information concerning previous development of diabetes could be obtained were excluded from the analysis.

Assessment of Risk Factors for CVD and DM

Standardized interviews were conducted by trained medical staff (mainly nurses) to assess information concerning sociodemographic variables, smoking habits, leisure time physical activity level, alcohol consumption, and parental history of DM. In addition, participants underwent standardized medical examinations, including collection of a nonfasting venous blood sample. All of the assessment procedures and standard laboratory methods used to determine total serum cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) have been described elsewhere in detail.[18–20] Serum and plasma samples stored at −80°C were used to analyze C-reactive protein (CRP) and markers of endothelial dysfunction. CRP concentrations were measured using a high-sensitivity immunoradiometric assay (range, 0.05 to 10 mg/L) (S1: men aged 45 to 64; S3)[21] or a high-sensitivity latex-enhanced nephelometric assay on a BN II analyzer (S1: men aged 35 to 44 and all women; S2) (Dade Behring). Both methods gave similar results when the same samples were analyzed.[22] SE-selectin, sICAM-1, and vWF were measured with commercially available ELISAs [R&D Systems (sE-selectin), Diaclone (sICAM-1), and Hemochrom (vWF)]. The intraassay and interassay coefficients of variation of quality control test sera for CRP and markers of endothelial dysfunction were as follows: CRP-immunoradiometric assay: 4% and 12.0%; CRP nephelometric assay: 2.5% and 5.1%; sE-selectin: 3.3% and 6.3%; sICAM-1: 2.3% and 4.7%; and vWF: 6.1% and 16.3%, respectively. Levels of vWF were measured for participants of S2 only, because measurement is only possible in citrate plasma, and the necessary plasma samples had not been stored for subjects who participated in S1 or S3. Among the 852 participants from S2, 90.5% (n=771) had available values for vWF.

Statistical Methods

Means or proportions for baseline demographic and clinical characteristics were computed using the SAS macros SURVEYREG[23] or SURVEYFREQ,[24] which estimated SEs appropriate to the sampling scheme. Tests of differences between subjects with and without incident DM stratified for sex were based on these procedures. In the case of nonnormality, tests were carried out with log-transformed variables, and results were presented as geometric means with antilogs of SEs of the adjusted log means.

To measure the relationship between markers of endothelial dysfunction and CRP, weighted Pearson correlation coefficients were calculated using the SAS procedure CORR, and SURVEYREG was used to assess statistical significance. Cox proportional hazards analysis was used to assess the impact of sE-selectin, sICAM-1, and vWF on the incidence of DM during follow-up time. Because of the case-cohort design, SEs were corrected using a SAS macro with a "sampling weight" approach developed by Barlow.[25] The weighted tertiles of each marker of endothelial dysfunction in the subcohort were used to classify subjects in different risk groups. A test for trend was conducted assigning the median of each category to all subjects in the category and including this variable in the Cox regression analysis. Results are presented for each category of marker of endothelial dysfunction as hazard ratios (HRs) together with 95% CIs and *P* values based on robust variance estimates using the Barlow macro.

For all of the statistical analyses, a *P* value <0.05 was considered to be statistically significant. All of the evaluations were performed

TABLE 1. Weighted Means (SE) and Proportions of Demographic and Clinical Characteristics for Subjects Without and With Incident DM (n=2244)

	Men			Women		
Characteristics	Cases	Noncases	*P* Value	Cases	Noncases	*P* Value
No.	310	902	...	222	810	...
Age, y*	56.1 (0.6)	51.8 (0.4)	<0.001	56.2 (0.6)	51.7 (0.4)	<0.001
Education <12 y (%)	74.5	66.0	0.005	92.8	83.6	<0.001
BMI, kg/m2*	29.7 (0.2)	27.1 (0.1)	<0.001	30.9 (0.3)	26.4 (0.2)	<0.001
WC, cm*‡	103.4 (0.8)	95.3 (0.4)	<0.001	94.3 (1.0)	82.6 (0.5)	<0.001
WHR*‡	0.966 (0.004)	0.926 (0.003)	<0.001	0.857 (0.006)	0.805 (0.003)	<0.001
Systolic BP, mm Hg*	142.5 (1.0)	134.7 (0.6)	<0.001	142.3 (1.3)	130.6 (0.7)	<0.001
Diastolic BP, mm Hg*	85.4 (0.7)	83.4 (0.4)	0.007	84.5 (0.8)	79.6 (0.4)	<0.001
Actual hypertension, %	65.8	43.9	<0.001	69.4	35.9	<0.001
Ratio TC/HDL-C*	6.0 (0.1)	5.0 (0.1)	<0.001	5.0 (0.1)	3.9 (0.1)	<0.001
CRP, mg/L†	2.1 (1.1)	1.4 (1.0)	<0.001	3.3 (1.1)	1.3 (1.0)	<0.001
Level of low physical activity, %	65.8	56.8	0.005	75.7	61.3	<0.001
Smoking status, %			0.02			0.38
Never smoker	22.6	30.2		67.1	64.6	
Former smoker	42.3	40.4		17.6	16.2	
Current smoker	35.1	29.4		15.3	19.2	
Alcohol intake, %			0.09			0.02
0 g/day	20.0	15.9		51.8	41.4	
0.1 to 39.9/19.9 g/day§	44.5	51.4		32.9	37.2	
≥ 40/20 g/day§	35.5	32.7		15.3	21.4	
Parental history of DM, %			0.008			<0.001
Negative	47.7	58.1		41.9	61.1	
Positive	24.5	18.5		33.3	21.2	
Unknown	27.7	23.4		24.8	17.8	
Current HRT, %‖			...	8.2	11.7	0.20
Current use of OC, %#			...	7.7	15.2	0.08
sE-selectin, ng/mL*	78.3 (2.6)	59.5 (1.0)	<0.001	64.6 (2.1)	50.2 (0.8)	<0.001
sICAM-1, ng/mL*	879.5 (18.5)	792.8 (11.1)	<0.001	812.1 (19.3)	730.0 (9.4)	<0.001
vWF, %*††	136.8 (6.7)	122.3 (4.0)	0.06	137.1 (8.9)	128.2 (4.1)	0.37

Weights: cases=1; noncases=1/sfrac with sfrac=subcohort/full cohort without cases for each sex and survey. HRT indicates hormone replacement therapy; BP, blood pressure; OC, oral contraceptives; IQR, interquartile range.

*Arithmetic mean (SE).

†Geometric mean (SE)

‡Only for survey 2 and 3

§For men 0.1 to 39.9 g/day and ≥40 g/day; for women 0.1 to 19.9 g/day and ≥20 g/day.

‖Only for women aged ≥50 years (n=606) with no current use of OC.

#Only for women aged <50 years (n=414) with no current HRT.

††Only for n=771 participants (439 men, 332 women).

with the statistical software package SAS (Version 8.02 for Unix and Version 9.1 for Windows [procedure SURVEYFREQ], SAS-Institute Inc).

Results

Table 1 shows selected baseline characteristics and biomarker concentrations of subjects who developed incident type 2 DM during the follow-up period (cases) and those who remained free of DM (noncases). As expected, cases were older, less educated, had a higher body mass index (BMI), higher systolic and diastolic blood pressures, a higher TC/HDL-C ratio, higher CRP concentrations, were less physically active during leisure time (significant for women only), and were more likely to have actual hypertension and a positive parental history of DM than noncases. Moreover, diabetes status was significantly associated with smoking status in men and with alcohol consumption in women. In addition, cases had significantly higher mean concentrations of sE-selectin and sICAM-1 than noncases in men and women, respectively [men: sE-selectin (ng/mL): 78.3 versus 59.5, $P<0.001$; sICAM-1 (ng/mL): 879.5 versus 792.8, $P<0.001$; women: sE-selectin (ng/mL): 64.6 versus 50.2, $P<0.001$; sICAM-1 (ng/mL): 812.1 versus 730.0, $P<0.001$]. Levels of vWF were not significantly different between cases and

TABLE 2. Associations Between Markers of Endothelial Dysfunction and CRP in the Randomly Sampled Subcohort* Using Weighted Pearson Correlation

Variable	sE-selectin	sICAM-1	vWF	Log CRP
Men				
sE-selectin	—	0.34‡	−0.01	0.13‡
sICAM-1		—	0.25§	0.27‡
vWF†			—	0.18‡
Log CRP				—
Women				
sE-selectin	—	0.35‡	−0.04	0.23‡
sICAM-1		—	0.02	0.23‡
vWF†			—	0.18§
Log CRP				—

*n=1833 (856 women, 977 men).
†Only available for n=616 subjects (341 men, 275 women).
‡$P<0.001$.
§$P<0.01$.

noncases in either men or women (men: 136.8 versus 122.3%; women: 137.1 versus 128.2%).

Markers of endothelial dysfunction correlated only modestly with C-reactive protein and other markers of endothelial dysfunction (Table 2). The largest correlations were seen between sE-selectin and sICAM-1 ($r=0.34$ in men and 0.35 in women; $P<0.001$).

The adhesion molecules sE-selectin and sICAM-1 both significantly predicted incident type 2 DM in crude or age- and survey-adjusted Cox proportional hazards models in men and women (Table 3, models 1 and 2). Associations for sE-selectin were attenuated considerably by additional adjustment for BMI but remained significant in men and in women (Table 3, model 3). The respective HRs and 95% CIs comparing the upper tertile versus the lower tertile of sE-selectin were 3.44 (2.46 to 4.83; model 2) and 2.83 (1.99 to 4.04; model 3) for men and 2.79 (1.87 to 4.17; model 2) and 1.82 (1.18 to 2.81; model 3) for women. Additional adjustment for other DM risk factors, including smoking status; alcohol intake; physical activity; systolic blood pressure; ratio of TC/HDL-C; and a parental history of DM, CRP, and sICAM-1 (Table 3, models 4 and 5) had a relatively small impact on estimated HRs.

In contrast to sE-selectin, the association between sICAM-1 and incident type 2 DM became nonsignificant after additional adjustment for BMI in women [HR for models 2 and 3: 1.98 (1.33 to 2.95) and 1.24 (0.80 to 1.91), respectively] but changed less in men [2.07 (1.50 to 2.85) and 1.83 (1.30 to 2.59), respectively]. Additional adjustment for other DM risk factors did not appreciably affect HRs for sICAM-1 in women, but attenuated HRs in men and the observed HRs finally became nonsignificant in the fully adjusted model, which also included sE-selectin as a covariate [model 5: 1.32 (0.89 to 1.96)]. vWF was not significantly associated with incident type 2 DM in men or women. In women, additional adjustment for use of oral contraceptives or hormone replacement therapy in addition to the DM risk factors described above had virtually no impact on associations between markers of endothelial dysfunction and incident DM (data not shown).

To determine potential confounding effects of abdominal body fat [estimated by waist-to-hip ratio (WHR) and waist circumference (WC)] as compared with total adipose mass (estimated by BMI), we performed additional analyses in a subsample of 335 cases (207 men and 128 women) and 1035 noncases (543 men and 492 women) where measurements of WHR and WC were available. Results showed that adjustment for WHR or WC, in addition to the other DM risk factors, only slightly affected HRs for incident DM. In men, HRs and 95% CIs before and after adjustment for WHR were 2.47 (1.48 to 4.12) versus 2.47 (1.49 to 4.11) for sE-selectin and 1.71 (1.01 to 2.90) versus 1.70 (1.01 to 2.88) for sICAM-1. Respective HRs for women were 1.63 (0.81 to 3.31) versus 1.44 (0.69 to 2.99) and 1.14 (0.55 to 2.37) versus 1.03 (0.49 to 2.18). HRs were similar if WC was included in the models instead of WHR (data not shown).

In order to ensure that the observed associations were not caused by undiagnosed prevalent cases of DM at baseline, we performed additional analyses excluding subjects with ≤5 years of follow-up (n=383). HRs estimated from these multivariable adjusted models were slightly higher for sE-selectin in men and women [HR (95% CI) comparing tertile extremes in men: 2.84 (1.80 to 4.49); in women: 2.05 (1.20 to 3.52)] and lower for sICAM-1 in men [1.03 (0.65 to 1.64)] compared with those observed in the whole study sample (see Table 3). In women, HRs for the third tertile of sICAM-1 were virtually not affected, but HRs for the second tertile [1.37 (0.79 to 2.38)] were somewhat higher after exclusion of subjects with ≤5 years of follow-up. We performed additional analyses after the exclusion of subjects with prevalent myocardial infarction, prevalent stroke, or incident myocardial infarction (n=194) to ensure that results were not confounded by the presence of CVD at baseline. Exclusion of these subjects had virtually no impact on the observed associations in women. In men, the association between sE-selectin and incident type 2 diabetes increased slightly, whereas the association between sICAM-1 and incident type 2 DM was slightly attenuated (data not shown).

Discussion

In this large, population-based prospective case–cohort study of middle-aged men and women, elevated baseline levels of 2 markers of endothelial dysfunction (sE-selectin and sICAM-1) were significantly associated with the risk of developing type 2 DM. In particular, sE-selectin was a strong independent predictor of incident DM after adjustment for obesity and other clinical and lifestyle parameters in both men and women. In fully adjusted analyses, men in the highest tertile of sE-selectin had a 2.6-fold increased risk of developing type 2 DM compared with men in the lowest tertile. Women in the highest tertile had a 1.7-fold increased risk of DM. Levels of sICAM-1 were not independently associated with type 2 DM in the fully adjusted model, which also included sE-selectin, and plasma concentrations of vWF were not significantly associated with the risk of type 2 DM at all.

TABLE 3. HRs for the Risk of Developing Type 2 DM According to Baseline Levels of sE-selectin, sICAM-1, and vWF for Men and Women (n=2244)

	Men				Women			
Variables	T1	T2	T3	*P* for Trend	T1	T2	T3	*P* for Trend
Tertiles of sE-selectin								
Median (IQR), ng/mL	36.4 (11.7)	55.9 (8.1)	82.0 (29.0)		30.0 (9.1)	47.9 (8.8)	71.3 (21.3)	
No. cases/noncases	60/308	80/309	170/285		42/281	57/271	123/258	
Model 1*: HR	1.00	1.38	3.01	<0.001	1.00	1.37	3.29	<0.001
(95% CI)	(Ref.)	(0.96 to 1.99)	(2.18 to 4.17)		(Ref.)	(0.90 to 2.10)	(2.25 to 4.83)	
P value		0.08	<0.001			0.14	<0.001	
Model 2†: HR	1.00	1.47	3.44	<0.001	1.00	1.33	2.79	<0.001
(95% CI)	(Ref.)	(1.02 to 2.14)	(2.46 to 4.83)		(Ref.)	(0.86 to 2.04)	(1.87 to 4.17)	
P value		0.04	<0.001			0.20	<0.001	
Model 3‡: HR	1.00	1.32	2.83	<0.001	1.00	1.12	1.82	0.003
(95% CI)	(Ref.)	(0.89 to 1.96)	(1.99 to 4.04)		(Ref.)	(0.71 to 1.75)	(1.18 to 2.81)	
P value		0.16	<0.001			0.63	0.006	
Model 4§: HR	1.00	1.28	2.79	<0.001	1.00	1.09	1.72	0.01
(95% CI)	(Ref.)	(0.84 to 1.94)	(1.91 to 4.09)		(Ref.)	(0.67 to 1.78)	(1.07 to 2.75)	
P value		0.25	<0.001			0.74	0.02	
Model 5‖ HR	1.00	1.18	2.63	<0.001	1.00	1.08	1.71	0.02
(95% CI)	(Ref.)	(0.77 to 1.82)	(1.79 to 3.88)		(Ref.)	(0.66 to 1.78)	(1.07 to 2.75)	
P value		0.44	<0.001			0.75	0.03	
Tertiles of sICAM-1								
Median	539.1	753.6	1034.0		506.2	690.7	940.0	
(IQR), ng/mL	(134.9)	(84.4)	(258.0)		(126.1)	(79.2)	(247.8)	
No. cases/noncases	78/311	82/304	150/287		50/278	69/272	103/260	
Model 1*: HR	1.00	1.09	2.16	<0.001	1.00	1.42	2.24	<0.001
(95% CI)	(Ref.)	(0.78 to 1.54)	(1.59 to 2.93)		(Ref.)	(0.96 to 2.12)	(1.55 to 3.25)	
P value		0.61	< 0.001			0.08	< 0.001	
Model 2†: HR	1.00	1.06	2.07	<0.001	1.00	1.39	1.98	<0.001
(95% CI)	(Ref.)	(0.75 to 1.49)	(1.50 to 2.85)		(Ref.)	(0.93 to 2.08)	1.33 to 2.95	
P value		0.76	< 0.001			0.11	< 0.001	
Model 3‡: HR	1.00	1.04	1.83	<0.001	1.00	1.16	1.24	0.38
(95% CI)	(Ref.)	(0.72 to 1.49)	(1.30 to 2.59)		(Ref.)	(0.75 to 1.79)	(0.80 to 1.91)	
P value		0.84	< 0.001			0.51	0.35	
Model 4§: HR	1.00	0.92	1.50	0.02	1.00	1.06	1.16	0.53
(95% CI)	(Ref.)	(0.62 to 1.37)	(1.02 to 2.19)		(Ref.)	(0.67 to 1.70)	(0.72 to 1.88)	
P value		0.69	0.04			0.80	0.54	
Model 5‖ HR	1.00	0.86	1.32	0.14	1.00	1.05	1.03	0.91
(95% CI)	(Ref.)	(0.58 to 1.28)	(0.89 to 1.96)		(Ref.)	(0.65 to 1.68)	(0.64 to 1.67)	
P value		0.47	0.17			0.85	0.90	
Tertiles of vWF								
Median (IQR), %	59.5 (28.5)	110.9 (31.4)	183.4 (71.6)		65.6 (27.0)	117.2 (25.5)	181.0 (59.4)	
No. cases/noncases	31/109	53/102	43/101		20/87	18/91	26/90	
Model 1*: HR	1.00	1.67	1.43	0.25	1.00	0.91	1.31	0.38
(95% CI)	(Ref.)	(1.01 to 2.76)	(0.85 to 2.40)		(Ref.)	(0.45 to 1.81)	(0.69 to 2.49)	
P value		0.05	0.18			0.78	0.41	
Model 2†: HR	1.00	1.41	0.99	0.72	1.00	0.57	0.83	0.85
(95% CI)	(Ref.)	(0.84 to 2.39)	(0.57 to 1.73)		(Ref.)	(0.27 to 1.21)	(0.41 to 1.69)	
P value		0.20	0.97			0.14	0.61	

TABLE 3. Continued

	Men				Women			
Variables	T1	T2	T3	*P* for Trend	T1	T2	T3	*P* for Trend
Model 3‡: HR	1.00	1.29	0.82	0.34	1.00	0.73	0.99	0.89
(95% CI)	(Ref.)	(0.73 to 2.30)	(0.45 to 1.52)		(Ref.)	(0.33 to 1.63)	(0.47 to 2.07)	
P value		0.38	0.53			0.44	0.98	
Model 4§: HR	1.00	1.31	0.85	0.39	1.00	0.93	1.00	0.98
(95% CI)	(Ref.)	(0.67 to 2.57)	(0.42 to 1.71)		(Ref.)	(0.34 to 2.52)	(0.41 to 2.45)	
P value		0.44	0.64			0.89	1.00	
Model 5‖ HR	1.00	1.31	0.87	0.45	1.00	0.93	1.10	0.79
(95% CI)	(Ref.)	(0.66 to 2.60)	(0.44 to 1.74)		(Ref.)	(0.33 to 2.64)	(0.42 to 2.90)	
P value		0.44	0.70			0.89	0.85	

HRs were estimated by Cox proportional hazard model. Correction for SEs was made by SAS macro ROBPHREG using the method by Barlow. Tertiles of the weighted distributions in the subcohort, stratified for sex, were used. Models contained continuous variables unless indicated otherwise; markers of endothelial dysfunction were coded as tertiles using indicator variables. IQR indicates interquartile range.

*Model 1: crude.

†Model 2: adjusted for age and survey (where applicable).

‡Model 3: adjusted for age, survey (where applicable), and BMI.

§Model 4: adjusted for age, survey (where applicable), BMI, smoking status (never smoker, former smoker, current smoker), alcohol consumption (0, 0.1 to 39.9, ≥40 g/day for men; 0, 0.1 to 19.9, ≥20 g/day for women), physical activity (inactive, active), systolic blood pressure, TC/HDL-C, parental history of DM (negative, positive, unknown), CRP.

‖Model 5: adjusted for variables in model 4+sICAM-1 (for sE-selectin), sE-selectin (for sICAM-1), and sE-selectin and sICAM-1 (for vWF).

¶n=771 subjects.

Previous studies examining the association between markers of endothelial dysfunction and incident DM have been partly inconsistent. The present study confirms previous data from the Nurses Health Study, which have demonstrated that sE-selectin was a significant and independent predictor of incident type 2 DM in middle-aged women,[15] and extends these results by showing that sE-selectin is also an independent predictor of DM in middle-aged men. The results of the present and the Nurses Health Study are, however, in contrast to results obtained in Pima Indians.[14] In this highly selected population of Indian men and women, Krakoff et al[14] did not observe a significant association between sE-selectin and incident type 2 DM in univariate or multivariable-adjusted analyses.

The previously shown independent association between sICAM-1 and incident DM in middle-aged women[15] could not be confirmed in the present study. Although we saw such an association in middle-aged men in the model adjusted for various risk factors, the association became nonsignificant when sE-selectin was included in the model. Krakoff et al[14] did not find an association between sICAM-1 and incident DM in a smaller sample of men and women.

The results of the present study concerning vWF are in contrast to results from the Atherosclerosis Risk in Communities study, which have shown that vWF was positively associated with incident type 2 DM in women.[13] This difference could possibly be explained by the fact that measurements of vWF were only available for a relatively small number of subjects in the present study. Our negative findings in men are in line with results from the Atherosclerosis Risk in Communities study.[13] Overall, the results of the present study are in line with a recent study, which has shown that decreased flow–mediated dilation of the brachial artery measured by high-resolution ultrasound is associated with an increased risk of type 2 diabetes in postmenopausal women.[26]

Endothelial dysfunction could be the underlying pathogenetic mechanism explaining the strong link observed among inflammation, atherosclerosis, and insulin resistance, including its clinical end points CVD and DM. Whereas dysfunction of the endothelium in the large and medium-sized arteries plays a crucial role in atherogenesis, dysfunction at the arteriolar and capillary level could be the cause of insulin resistance and the associated features of the insulin resistance syndrome.[3] Endothelial dysfunction is characterized by an imbalance of vasoconstrictors and vasodilators produced by endothelial cells and can be directly determined through imaging of endothelium-dependent vasodilation. An alternative approach, which is particularly suitable for large epidemiologic studies, is the measurement of plasma levels of markers of an "activated" endothelium.[27] These markers include cellular adhesion molecules (CAMs) such as E-selectin and ICAM-1 or vWF.[5,28] E-selectin and ICAM-1 mediate the adhesion and transmigration of leukocytes to the vascular endothelium.[29] Soluble forms are found in plasma and in cytokine-stimulated human endothelial cell tissue culture supernatants and are regarded as surrogate markers of cellular expression.[30,31] vWF mediates platelet adhesion to the subendothelium and acts as a cofactor for coagulation factor VIII in thrombus formation.[32]

Our observation that high levels of CAMs were associated with an increased risk for incident DM underscores the association between impaired endothelial function and insulin resistance. That is, under conditions of endothelial dysfunction, the vasodilating and antiinflammatory effects of insulin via the NO pathway are impaired.[3,4] Thus, a state of endothelial dysfunction could diminish insulin action through reduced capillary perfusion of skeletal muscle, adipose tissue,

or islets. In addition, reduced endothelial permeability impairs insulin delivery to the interstitium and could thereby limit insulin action.[33]

The strengths of our study include its population-based prospective design, the large number of incident cases, a long follow-up period, and detailed assessment of potential confounding factors. However, several limitations also need to be considered. First, selection bias because of missing blood samples and incomplete follow-up information could have influenced the results. However, there is little evidence that lends support to the assumption that the association between markers of endothelial dysfunction and DM is different between the 2 groups; baseline levels of the major cardiovascular risk factors differed only slightly between subjects that had to be excluded because of missing blood samples, missing follow-up data, or other reasons and the source population used for the case–cohort study. Also, baseline levels of markers of endothelial dysfunction were similar in subjects with available follow-up information and those excluded because of missing follow-up data. Second, fasting plasma glucose concentrations at baseline were not available for the whole study population. Thus, we may have included some subjects with prevalent diabetes in our analyses, which could, in theory, have caused spurious positive associations. However, most of these subjects probably would have been diagnosed during the first years of follow-up, and exclusion of cases diagnosed within the first 5 years of follow-up did not materially affect the results. Also, in a subgroup of subjects from the subcohort (n=413) where HbA1c and random glucose levels had been measured at baseline only 7 (1.7%) had either a HbA1c level >6.5% or a random glucose level of ≥200 mg/dL (≥11.1 mmol/L). Third, incident cases of DM were initially identified by self-report. Thus, we may have missed subjects with undiagnosed DM. Misclassification of incident cases at follow-up would, however, have biased our results toward the null and, thus, cannot explain the positive associations that were observed. Finally, markers of endothelial dysfunction were only measured at a single point in time at baseline, which could have lead to some misclassification of exposure.

In conclusion, in this prospective study on the role of markers of endothelial dysfunction in the prediction of type 2 DM, we found that sE-selectin was a strong and independent risk factor in both men and women. Elevated levels of sICAM-1 were also associated with an increased risk in men and women; however, associations became nonsignificant after multivariable adjustment for other DM risk factors, including sE-selectin. Our observations support a role for endothelial dysfunction in the etiology of type 2 DM and provide new aspects for the prevention of this disease. Modification of lifestyle factors, like reduction of body weight[34] or increased physical exercise,[35] or specific pharmacological agents, such as angiotensin-converting enzyme inhibitors,[36] statins,[37] and glitazones,[38] or substances that increase HDL-C[39] or apolipoprotein A1[40] could improve endothelial function and thereby lower the risk of type 2 DM. Finally, the development of specific CAM blockers might offer promising possibilities in the future.

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Elevated Markers of Endothelial Dysfunction Predict Type 2 Diabetes Mellitus in Middle-Aged Men and Women From the General Population

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