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Endothelial Dysfunction and Low-Grade Inflammation Explain Much of the Excess Cardiovascular Mortality in Individuals With Type 2 Diabetes

The Hoorn Study

Jolien de Jager, Jacqueline M. Dekker, Adriaan Kooy, Piet J. Kostense, Giel Nijpels, Rob J. Heine, Lex M. Bouter, Coen D.A. Stehouwer

Objective—The mechanisms responsible for the increased cardiovascular disease risk that accompanies type 2 diabetes (T2D) remain poorly understood. It is commonly held that endothelial dysfunction and low-grade inflammation can explain, at least in part, why deteriorating glucose tolerance is associated with cardiovascular disease. However, there is no direct evidence for this contention.

Methods and Results—In this population-based study (n=631), T2D was cross-sectionally associated with both endothelial dysfunction and low-grade inflammation, whereas impaired glucose metabolism (IGM) was associated only with low-grade inflammation. These findings were independent of other risk factors that accompany T2D or IGM. During a follow-up of 11.7 years (median; range 0.5 to 13.2 years), low-grade inflammation was associated with a greater risk of cardiovascular mortality (hazard ratio, 1.43 [95% CI, 1.17 to 1.77] per 1 SD difference). For endothelial dysfunction, the association with cardiovascular mortality was stronger in diabetic (hazard ratio, 1.87 [95% CI, 1.43 to 2.45]) than in nondiabetic individuals (hazard ratio, 1.23 [95% CI, 0.86 to 1.75]; *P* interaction=0.06). Finally, T2D-associated endothelial dysfunction and low-grade inflammation explained ≈43% of the increase in cardiovascular mortality risk conferred by T2D.

Conclusions—These data emphasize the necessity of randomized controlled trials of strategies that aim to decrease cardiovascular disease risk by improving endothelial function and decreasing low-grade inflammation, especially for T2D patients. **(*Arterioscler Thromb Vasc Biol*. 2006;26:1086-1093.)**

Key Words: epidemiology ■ diabetes mellitus ■ endothelium ■ inflammation ■ mortality

Up to 75% of individuals with type 2 diabetes (T2D) will die of cardiovascular disease.[1] However, the mechanisms responsible for the high cardiovascular disease risk that accompanies T2D and possibly impaired glucose metabolism (IGM; ie, impaired fasting glucose and (or) impaired glucose tolerance)[2] remain poorly understood. There is strong evidence that conventional risk factors, such as hypertension, obesity, and dyslipidemia, cannot fully explain the high cardiovascular disease risk associated with deteriorated glucose tolerance.[3]

It is commonly held that endothelial dysfunction and low-grade inflammation, 2 key features in the pathophysiology of atherothrombosis,[4–7] can explain, at least in part, why deteriorated glucose tolerance is associated with cardiovascular disease.[2,8] However, there is no direct evidence for this contention, and several important issues have remained unresolved. First, it is not clear to what extent the associations of IGM and T2D on the one hand with endothelial dysfunction and low-grade inflammation on the other are independent of other risk factors associated with deteriorated glucose tolerance. Second, it is not known whether associations of endothelial dysfunction and low-grade inflammation with cardiovascular disease are independent of other conventional cardiovascular risk factors and indicators, nor to what extent these associations overlap or represent distinct pathways. If these pathways are distinct, then associations of endothelial dysfunction and low-grade inflammation with cardiovascular disease will be expected to be mutually independent. Finally, it is not known to what extent associations of IGM and T2D with cardiovascular disease are in fact accounted for by IGM- and T2D-associated endothelial dysfunction and low-grade inflammation.

We addressed these questions in the Hoorn Study, a prospective population-based cohort study of glucose tolerance and cardiovascular disease.[9,10]

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Materials and Methods

General Study Design

This study was part of the Hoorn Study, a population-based cohort study of glucose tolerance and cardiovascular disease in a white population in Hoorn, The Netherlands, of which the baseline measurement was performed from October 1989 to February 1992.[9,10] Briefly, a random sample of all men and women 50 to 75 years of age was drawn from the municipal population registration office of Hoorn; 2484 individuals participated (response rate 71%). The present study population is an age-, sex-, and glucose-tolerance–stratified random subsample (n=631; response rate 89%) in whom an extensive investigation of diabetes complications was performed.[9,10] For the present analyses, we used the 1999 World Health Organization criteria and classified individuals as having normal glucose metabolism (NGM), IGM, or T2D based on 2 glucose tolerance tests.[11]

The Hoorn Study was approved by the ethical review committee of the VU University Hospital. Written informed consent was obtained from all participants.

Baseline Investigations

We considered plasma levels of von Willebrand factor (vWF) and soluble vascular adhesion molecule-1 (sVCAM-1) as markers of endothelial function[12–14] and plasma levels of C-reactive protein (CRP) and soluble intercellular adhesion molecule-1 (sICAM-1) as markers of low-grade inflammation.[15,16] Microalbuminuria was not used as a marker of endothelial function because we have shown previously that microalbuminuria in the Hoorn Study is heterogeneous in terms of its association with endothelial dysfunction.[17]

Markers of Endothelial Dysfunction and Low-Grade Inflammation

Concentrations of vWF, sVCAM-1, CRP, and sICAM-1 were assessed in deep frozen (−70°C) heparin plasma samples. vWF, sVCAM-1, and sICAM-1 were estimated in duplicate by ELISA.[18–20] For vWF and sVCAM-1, no plasma samples were available for 21 subjects. Concentrations of CRP were measured with a highly sensitive sandwich enzyme immunoassay, as described previously.[18] For CRP and sICAM-1, no plasma samples were available for 23 subjects.

Other Measurements

We obtained an ankle-brachial blood pressure index (n=631) and a resting ECG (n=625).[9,10] Subjects were classified as having cardiovascular disease when they had a history of myocardial infarction or had an ECG with a Minnesota code 1.1 to 1.3, 4.1 to 4.3, 5.1 to 5.3, or 7.1 or had undergone coronary bypass surgery or angioplasty, or had an ankle-brachial pressure index <0.9 in either leg, or had undergone a peripheral arterial bypass or nontraumatic amputation. In addition, we obtained data on blood pressure, weight, height, body mass index, waist-to-hip ratio, glycohemoglobin, serum creatinine, homocysteine, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, smoking habits, and the use of medication. Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula[21] except when the triglyceride level was >4.55 mmol/L (n=23). Hypertension was defined as a blood pressure ≥140 mm Hg systolic and/or ≥90 mm Hg diastolic and/or the current use of antihypertensive medication. Subjects were classified as current cigarette smokers or nonsmokers. The glomerular filtration rate was calculated according to Levey et al.[22]

Follow-Up

For each subject, we determined whether or not death had occurred during follow-up, and if so, the date at which death occurred. Data on the subjects' vital status on January 1, 2003, were collected from the mortality register of the municipality of Hoorn. Of 51 subjects who had moved out of town, information on vital status was obtained from the new local municipalities. For all subjects who had 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.[23] Cardiovascular mortality was defined as codes 378 and 390 to 459. Information on cause of death could not be obtained for 33 (19%) of the deceased subjects, and 1 subject was lost to follow-up.

Statistical Analyses

Because markers of endothelial dysfunction and inflammatory activity show marked intraindividual (day-to-day) variation and because we measured these markers only once, the associations (if any) of endothelial dysfunction and inflammatory activity with other variables will tend to be underestimated. As a result of this, statistical power will be diminished. To address this concern, we created mean SD scores (z scores) for markers of endothelial dysfunction and chronic low-grade inflammation and used these in regression analyses as described below. For each subject, each variable was expressed as SDs of difference from the population mean, which was calculated using all available data on the separate markers (n=608 and 610 [of 631] for the endothelial dysfunction and inflammation z scores, respectively). The z scores were calculated as the mean of these SD scores as follows: (1) endothelial dysfunction z score=[vWF + sVCAM-1]/2 and (2) inflammation z score=[CRP + sICAM-1]/2.

To assess to what extent the associations of T2D and IGM on the one hand with endothelial dysfunction and low-grade inflammation on the other were independent of other risk factors known to be associated with deteriorated glucose tolerance, we performed linear regression analyses. Endothelial dysfunction or inflammation z scores were entered as dependent variables and T2D and IGM as independent variables, with adjustment for potential confounders. Results are described as regression coefficients (β) with 95% CIs.

To assess associations of markers of endothelial dysfunction and low-grade inflammation with risks of cardiovascular and all-cause mortality, we performed Kaplan–Meier and Cox proportional hazards multiple regression analyses. Because of the stratification procedure, we first adjusted for age, sex, and glucose tolerance status in all models and, subsequently, for other potential confounders. Variables measured on a continuous scale were used as such in the regression models except for levels of sVCAM-1, CRP, and systolic and diastolic blood pressure because of their nonlinear association with mortality. Therefore, a high level of sVCAM-1 was defined as in the upper tertile (>1485 ng/mL); data on CRP were log-transformed before analysis; and systolic and diastolic blood pressure were defined as high (≥140 mm Hg and ≥90 mm Hg, respectively) or low. In spite of the nonlinear association of sVCAM-1 (all-cause) and CRP (both all-cause and cardiovascular) with mortality, additional analysis showed that associations between the z scores and mortality were nevertheless best described as linear. To evaluate a possible interaction between glucose tolerance status and endothelial dysfunction or low-grade inflammation, Cox regression analyses were performed with glucose tolerance status, the endothelial dysfunction or inflammation z score, their product term, age, and sex in the model. A significant hazard ratio for the product term was considered indicative for interaction of glucose tolerance status with either low-grade inflammation or endothelial dysfunction. Results are described as relative risks (hazard ratios) with 95% CIs.

To assess to what extent associations of T2D and IGM with cardiovascular mortality could in fact be explained by T2D- and IGM-associated endothelial dysfunction and low-grade inflammation, we performed Cox regression analyses without and with adjustments for the endothelial dysfunction and inflammation z scores and without and with potential interaction terms. Percentages explained were calculated using the regression coefficients instead of hazard ratios because of the logarithmic character of the hazard ratio. All models were fitted comparing T2D and IGM to NGM, and T2D to IGM separately.

Two-sided P values <0.05 were considered statistically significant except for the interaction analyses, where P values <0.10 were used.

TABLE 1. Baseline Characteristics of the Study Population According to Glucose Tolerance Status

	NGM	IGM	T2D	*P* (trend)
No. (males/females)	258 (126/132)	179 (91/88)	194 (87/107)	
Conventional risk factors				
Age, y	63±7	64±7	66±7	0.001
Hemoglobin A_{1c}, % of hemoglobin	5.3±0.5	5.6±0.5	7.1±1.8	<0.001
Hypertension, %	25	43	55	<0.001
Diastolic blood pressure, mm Hg	81±10	84±10	83±10	0.05
Systolic blood pressure, mm Hg	133±18	142±20	144±19	<0.001
Current smokers, %	30	22	24	0.15
Body mass index, kg/m^2	25.9±3.3	27.6±3.7	28.7±4.4	<0.001
HDL cholesterol, mmol/L	1.4±0.4	1.3±0.4	1.1±0.3	<0.001
LDL cholesterol, mmol/L	4.6±1.0	4.6±1.0	4.3±1.1	0.003
Triglycerides, mmol/L	1.3 (1.0 to 1.8)	1.6 (1.2 to 2.3)	2.0 (1.3 to 2.8)	<0.001
Homocysteine, umol/L	11.2 (9.2 to 14.3)	12.2 (9.7 to 14.9)	11.1 (9.0 to 13.5)	0.47
Glomerular filtration rate, mL/min*	68.0±11.3	67.9±11.1	67.4±13.8	0.60
Prievious cardiovascular disease, %	17	23	31	0.001
Markers of endothelial dysfunction and chronic low-grade inflammation				
vWF, %	106 (90 to 131)	115 (93 to 150)	148 (108 to 176)	<0.001
sVCAM-1, ng/mL	1316±377	1363±420	1497±540	<0.001
Endothelial dysfunction *z* score, SD	−0.23±0.85	−0.05±0.93	0.34±1.15	<0.001†
CRP, mg/L	1.30 (0.84 to 1.98)	2.11 (1.03 to 3.05)	2.43 (1.63 to 356)	<0.001
sICAM-1, ng/mL	448±123	489±166	520±195	<0.001
Inflammation *z* score, SD	−0.29±0.87	0.07±1.01	0.32±1.05	<0.001‡

Data are mean±SD or median (interquartile range). Mean SD scores (*z* scores) for markers of endothelial dysfunction and chronic low-grade inflammation were created. For each subject, each variable was expressed as SDs of difference from the population mean, which was calculated using all available data on the separate markers (n=608 and 610 [of 631] for the endothelial dysfunction and inflammation *z* scores, respectively). The *z* scores were calculated as the mean of these SD scores as follows: (1) endothelial dysfunction *z* score = [vWF+sVCAM-1]/2; (2) inflammation *z* score = [CRP+sICAM-1]/2.

*According to the Modification of Diet in Renal Disease Study Group (MDRD) formula; †IGM vs NGM, $P=0.050$; T2D vs IGM, $P<0.0001$; T2D vs NGM, $P<0.0001$; ‡IGM vs NGM, $P<0.0001$; T2D vs IGM, $P=0.021$; T2D vs NGM, $P<0.0001$.

Results

The median duration of follow-up was 11.7 years (range 0.5 to 13.2 years). After follow-up, 174 (55 NGM, 43 IGM, and 76 T2D) of the 631 subjects had died, of whom 66 (38%; 17 NGM, 16 IGM, and 33 T2D) had died of cardiovascular disease. Table 1 shows the baseline characteristics of the study population according to glucose tolerance status.

Glucose Tolerance Status Is Associated With Endothelial Dysfunction and Low-Grade Inflammation

Table 2 and Figure I (available online at http://atvb.ahajournals.org) show that T2D was significantly associated with both endothelial dysfunction and low-grade inflammation, whereas IGM was associated only with low-grade inflammation.

Higher Levels of Markers of Endothelial Dysfunction and Low-Grade Inflammation Are Associated With Greater Mortality Risks

Figure 1 and Table 3 show that in general, higher levels of markers of endothelial dysfunction, low-grade inflammation, and their *z* scores were associated with greater mortality risks. For example, the hazard ratio of cardiovascular and all-cause mortality associated with the inflammation *z* score were 1.43 (1.17 to 1.77) and 1.27 (1.10 to 1.47) per SD difference. For endothelial dysfunction, the associations with cardiovascular and all-cause mortality were stronger in diabetic than in nondiabetic individuals (*P* interaction=0.064 and 0.028; Table 3). For example, the cardiovascular mortality hazard ratio associated with the endothelial dysfunction *z* score was 1.87 (1.43 to 2.45) for diabetic individuals compared with 1.23 (0.86 to 1.75) in nondiabetic individuals. For all-cause mortality, the hazard ratio was 1.41 (1.16 to 1.72) in diabetic individuals and 1.09 (0.87 to 1.36) in nondiabetic individuals. Results were similar when individual markers were used instead of the endothelial dysfunction and inflammation *z* scores (data not shown). Table I shows that adjustment for potential confounders (hypertension, smoking, LDL cholesterol, HDL cholesterol, triglycerides, previous cardiovascular disease, body mass index, homocysteine, and glomerular filtration rate) did not markedly change the associations of the endothelial dysfunction and inflammation *z* scores with cardiovascular and all-cause mortality.

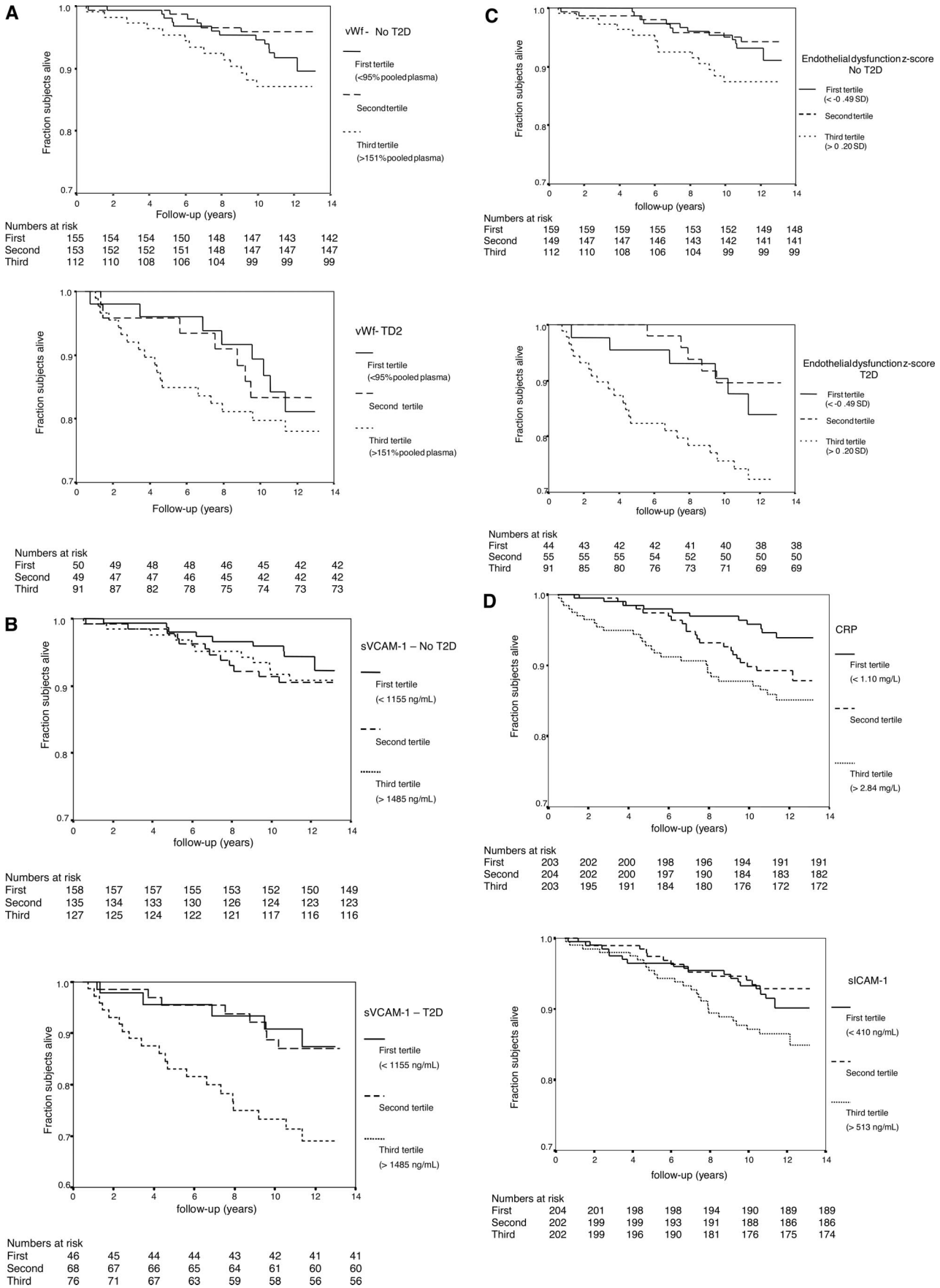
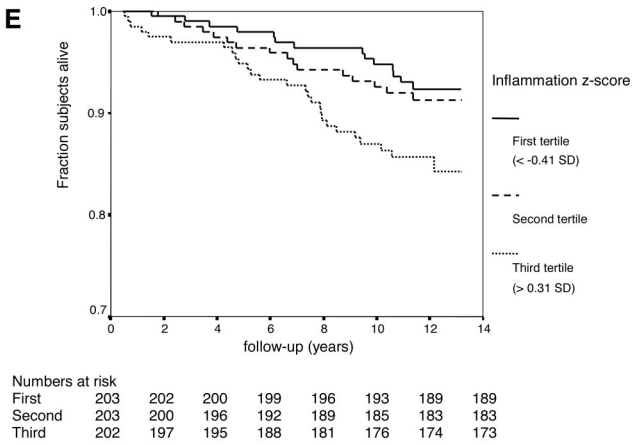


Figure 1. Cardiovascular survival (Kaplan–Meier method) according to markers of endothelial dysfunction and chronic low-grade inflammation.



T2D type 2 diabetes; No T2D no type 2 diabetes (NGM + IGM); NGM normal glucose metabolism; IGM impaired glucose metabolism

Figure 1. Continued.

Endothelial Dysfunction and Low-Grade Inflammation Explain Much of the Cardiovascular Mortality Risks Associated With T2D

Table II (available online at http://atvb.ahajournals.org) shows that T2D was significantly associated with both cardiovascular and all-cause mortality (2.74 [1.52 to 4.92] and 1.90 [1.34 to 2.69], respectively), but IGM was not (1.25 [0.63 to 2.48] and 1.05 [0.70 to 1.56], respectively). Adjustment for the endothelial dysfunction and low-grade inflammation *z* scores reduced the magnitude of the association between T2D and cardiovascular mortality by 34% and 25%, respectively. Together, the *z* scores explained 43% of the cardiovascular mortality risk associated with T2D (Table II; Figure 2). Results were similar when adjusted for the individual markers instead of the endothelial dysfunction and inflammation *z* scores (data not shown). Adjustment for traditional risk factors such as hypertension, smoking, LDL cholesterol, body mass index, and previous cardiovascular disease did not reduce the magnitude of the association between T2D and cardiovascular mortality to this extent (Table II).

Additional Analyses

Exclusion of individuals with impaired fasting glucose (n=29) did not affect the results (data not shown). The following additional adjustments also did not materially affect our results: analyses using waist or waist-to-hip ratio instead of body mass index; analyses using the creatinine clearance according to the Cockcroft–Gault criteria instead of the glomerular filtration rate; and analyses that adjusted for microalbuminuria. Hazard ratios remained constant over time (data not shown).

Discussion

Our study on endothelial dysfunction and low-grade inflammation in individuals without and with T2D had 3 main findings. First, T2D was associated with both endothelial dysfunction and low-grade inflammation, whereas IGM was associated only with low-grade inflammation. These findings were independent of other risk factors that accompany T2D or IGM. Second, endothelial dysfunction and low-grade inflammation were associated with greater risks of cardiovascular mortality, especially in T2D. Third, T2D-associated endothelial dysfunction and low-grade

TABLE 2. Associations Between Glucose Tolerance Status and the Endothelial Dysfunction and Inflammation *z* Scores

		Regression Coefficient (ß)			
		Endothelial Dysfunction *z* Score		Inflammation *z* Score	
Model	Added Variables	T2D vs NGM	IGM vs NGM	T2D vs NGM	IGM vs NGM
1	Age and sex	0.521*†	0.153	0.594*‡	0.357*
		(0.337 to 0.705)	(−0.034 to 0.341)	(0.409 to 0.779)	(0.168 to 0.545)
2	Age, sex, hypertension, smoking, LDL cholesterol,	0.395*†	0.098	0.490*	0.313§
	and prior cardiovascular disease	(0.204 to 0.585)	(−0.091 to 0.286)	(0.301 to 0.678)	(0.127 to 0.500)
3	Model 2 and body mass index	0.350*‡	0.080	0.390*	0.265§
		(0.155 to 0.546)	(−0.110 to 0.269)	(0.198 to 0.583)	(0.079 to 0.451)
4	Model 2 and HDL cholesterol	0.360*‡	0.080	0.392*	0.267§
		(0.164 to 0.556)	(−0.080 to 0.270)	(0.200 to 0.584)	(0.082 to 0.452)
5	Model 2 and triglycerides	0.391*‡	0.100	0.384*	0.269§
		(0.191 to 0.591)	(−0.090 to 0.291)	(0.188 to 0.579)	(0.083 to 0.455)
6	Model 2 and homocysteine	0.414*‡	0.083	0.496*	0.309§
		(0.225 to 0.603)	(−0.104 to 0.269)	(0.307 to 0.685)	(0.122 to 0.495)
7	Model 2 and glomerular filtration rate ‖	0.431*‡	0.097	0.486*	0.325 §
		(0.243 to 0.618)	(−0.090 to 0.283)	(0.294 to 0.677)	(0.134 to 0.515)

Regression coefficients ß and 95% CIs were obtained by linear regression analysis with endothelial dysfunction or inflammation *z* scores as dependent variables and IGM or T2D as independent variables. Model 1: Adjusted for stratification variables; model 2: adjusted for stratification variables and hypertension, smoking, and previous cardiovascular disease; models 3 through 6, plus adjusted for other potential confounders. The same analysis was also performed with NGM and T2D as independent variables to compare T2D with IGM.

*$P<0.0001$ vs NGM; †$P<0.0001$ vs IGM; ‡$P<0.025$ vs IGM; §$P<0.025$ vs NGM; other P values>0.1.

‖According to the MDRD formula.

TABLE 3. Hazard Ratios of Cardiovascular and All-Cause Mortality Associated With Markers of Endothelial Dysfunction and Chronic, Low-Grade Inflammation, and With Potential Confounders

	Contrast for Which Hazard Ratio Is Presented	Cardiovascular Mortality Hazard Ratio (95% CI) n=66		All-Cause Mortality Hazard Ratio (95% CI) n=174
Markers of endothelial dysfunction and chronic low-grade inflammation				
vWF, %	Per SD increase*	T2D	1.31 (1.00 to 1.72)	1.10 (0.91 to 1.34)
		No T2D	1.23 (0.88 to 1.73)	1.26 (1.04 to 1.54)
sVCAM-1, ng/mL	High vs low†	T2D	2.87 (1.42 to 5.80)	2.16 (1.37 to 3.41)
		No T2D	1.05 (0.51 to 2.17)	0.72 (0.46 to 1.14)
Endothelial dysfunction *z* score, SD	Per SD increase*	T2D	1.87 (1.43 to 2.45)	1.41 (1.16 to 1.72)
		No T2D	1.23 (0.86 to 1.75)	1.09 (0.87 to 1.36)
CRP, mg/L	Doubling‡	1.63 (1.04 to 2.57)		1.27 (0.97 to 1.67)
sICAM-1, ng/mL	Per SD increase*	1.28 (1.09 to 1.50)		1.22 (1.08 to 1.37)
Inflammation *z* score, SD	Per SD increase*	1.43 (1.17 to 1.77)		1.27 (1.10 to 1.47)
Potential confounders				
Male sex, %	Yes vs no	1.46 (0.90 to 2.38)		1.62 (1.20 to 2.19)
Age, y	Per SD increase*	2.22 (1.65 to 2.98)		1.93 (1.61 to 2.29)
T2D, %	T2D vs NGM	2.74 (1.52 to 4.92)		1.90 (1.34 to 2.69)
IGM, %	IGM vs NGM	1.25 (0.63 to 2.48)		1.05 (0.70 to 1.56)
Hemoglobin A_{1c}, % of hemoglobin	Per SD increase*	1.12 (0.89 to 1.42)		1.17 (1.00 to 1.36)
Hypertension, %	Yes vs no	1.97 (1.17 to 3.29)		1.58 (1.16 to 2.15)
Diastolic blood pressure, mm Hg	High vs low†	1.08 (0.61 to 1.91)		1.10 (0.78 to 1.57)
Systolic blood pressure, mm Hg	High vs low†	1.66 (1.00 to 2.75)		1.32 (0.96 to 1.82)
Current smokers, %	Yes vs no	1.62 (0.93 to 2.83)		1.65 (1.18 to 2.30)
Body mass index, kg/m^2	Per SD increase*	1.11 (0.87 to 1.42)		1.13 (0.97 to 1.32)
HDL cholesterol, mmol/L	Per SD decrease*	1.18 (0.85 to 1.65)		1.05 (0.88 to 1.25)
LDL cholesterol, mmol/L	Per SD increase*	1.12 (1.08 to 1.17)		1.02 (0.88 to 1.18)
Triglycerides, mmol/L	Per SD increase*	1.16 (0.91 to 1.48)		1.18 (1.05 to 1.32)
Homocysteine, umol/L	Per SD increase*	1.09 (0.93 to 1.29)		1.05 (0.92 to 1.19)
Glomerular filtration rate, mL/min§	Per SD decrease*	1.58 (1.22 to 2.05)		1.32 (1.12 to 1.57)
Previous cardiovascular disease	Yes vs no	2.25 (1.37 to 3.70)		1.74 (1.26 to 2.38)

Hazard ratios and 95% CIs were obtained by Cox regression analysis after adjustment for age, sex, and glucose tolerance status, except when analyses were stratified for glucose tolerance status because of interaction between glucose tolerance status and endothelial dysfunction, or when glucose tolerance status was the variable under consideration.

*SDs for vWF, 67.6%; for endothelial dysfunction *z* score, 1; for sICAM-1, 162.9 ng/mL; for inflammation *z* score, 1; for age, 7.2 years; for hemoglobin A_{1c}, 1.3%; for body mass index, 4.0 kg/m^2; for HDL cholesterol, 0.4 mmol/L; for LDL cholesterol, 1.1 mmol/L; for triglycerides, 1.3 mmol/L; for homocysteine, 5.8 μmol/L; and for glomerular infiltration rate, 12.1 mL/min.

†sVCAM-1 high ≥1485 ng/mL, low <1484 ng/mL; diastolic blood pressure high ≥90 mm Hg, low <90 mm Hg; systolic blood pressure high ≥140 mm Hg, low <140 mm Hg.

‡Data on CRP were log transformed before analysis because of its nonlinear association with mortality.

§According to the MDRD formula.

inflammation explained ≈43% of the greater cardiovascular mortality risk conferred by T2D.

Strengths of our study include its population-based design, the long follow-up (up to 13 years), the limited loss to follow-up, and the extensive characterization of participants at baseline. In addition, the results were robust and consistent across the markers of endothelial dysfunction and inflammation used.

As expected, T2D was associated with both endothelial dysfunction and low-grade inflammation.[12,18,24] In contrast, IGM was associated only with low-grade inflammation, which is in agreement with previous studies that have shown a much clearer association of IGM with low-grade inflammation than with endothelial dysfunction,[25–29] which, to some extent, appears to depend on the endothelial function marker used.[28–36] Together, these data suggest that endothelial dysfunction is not universal in IGM and may depend on other factors not identified in these studies.

Endothelial dysfunction and low-grade inflammation were associated with higher risks of cardiovascular mortality, consistent with previous studies.[7,18–20,37] Importantly, we show that for endothelial dysfunction, these associations were stronger in diabetic than in nondiabetic individuals, were independent of other cardiovascular risk factors, remained present during up to 13 years of follow-up, and appeared mutually independent,[7,37] indicating that

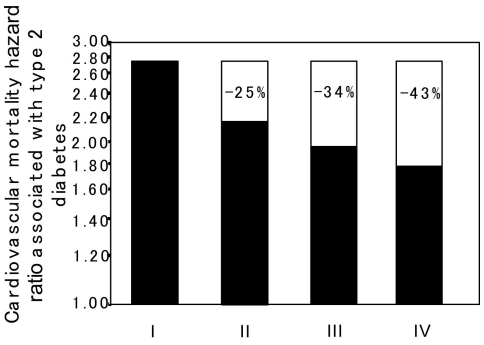


Figure 2. Hazard ratio of cardiovascular mortality associated with T2D after adjustment for stratification variables (I), low-grade inflammation (II), endothelial dysfunction (III), and both low-grade inflammation and endothelial dysfunction (IV).

they may represent largely distinct pathways of disease and therefore distinct targets for intervention.

Both endothelial dysfunction and low-grade inflammation appeared to explain parts of the increased mortality risks associated with T2D. However, the role of endothelial dysfunction seems especially relevant because of its interaction with T2D. Together, our data suggest that treatments to improve the cardiovascular prognosis of individuals with T2D should focus on improving endothelial function and decreasing chronic inflammation. The causes of endothelial dysfunction and low-grade inflammation in T2D remain incompletely understood and may include not only obesity, hypertension, dyslipidemia, insulin resistance, and hyperglycemia (the metabolic syndrome), but also advanced glycation end products.[12] In addition, endothelial dysfunction and low-grade inflammation may precede and contribute to the occurrence of T2D.[38]

In the present study, IGM was not clearly associated with an increased mortality risk (although the confidence limits show that we could not exclude any such associations with great certainty), and we therefore could not test the influence of endothelial dysfunction or low-grade inflammation. Other reports from the Hoorn Study have shown that 2-hour postload plasma glucose concentrations do predict cardiovascular and all-cause mortality but mostly in the diabetic range.[2,39] Other studies on IGM and risk of mortality have reported inconsistent results.[40–46]

Our study has several limitations. First, its relatively small size and consequently limited power may have obscured more subtle associations. Second, the incomplete assessment of endothelial function and inflammatory activity may have increased nondifferential misclassification, leading to an underestimation of the hazard ratios presented here. However, our results were robust and consistent with previous experience. Third, we used sICAM-1 as a marker of inflammation, although sICAM-1 can be regarded as a marker of both endothelial function and inflammation.[16,47] However, to classify sICAM-1 as a marker of inflammation can be considered the most conservative alternative because sICAM-1 is not selectively derived from endothelial cells but originates from leukocytes as well. However, sICAM-1 upregulation is driven by inflammatory cytokines such as tumor necrosis factor-α and interleukin-8, resulting in the activation of nuclear factor κB.[48] Importantly, additional analyses showed that our conclusions remain unchanged when sICAM-1 is classified as a marker of endothelial function (data not shown). Fourth, we studied white individuals, and the results therefore are not necessarily valid for other ethnicities. Fifth, an assumption in the construction of the z scores is that its components are equally important, which is not necessarily true. Nevertheless, z scores have the considerable merit of increased precision, as demonstrated by the smaller CIs of the z scores compared with those of the individual markers and, possibly, of increased validity because these z scores address various aspects of endothelial dysfunction and inflammation, respectively. Finally, because traditional risk factors were measured only once, we may, to some extent, have underestimated their associations with mortality, although previous analyses from the Hoorn Study have shown that traditional risk factors, even if measured only once, do in fact predict mortality.[49]

In conclusion, we have shown that T2D is associated with both endothelial dysfunction and low-grade inflammation, whereas IGM is associated only with low-grade inflammation, that endothelial dysfunction and low-grade inflammation are associated with greater risks of cardiovascular mortality, especially in T2D, and that T2D-associated endothelial dysfunction and low-grade inflammation can explain ≈43% of the higher cardiovascular mortality risk conferred by T2D. These data emphasize the necessity of randomized controlled trials of strategies that aim to decrease cardiovascular disease risk by improving endothelial function and decreasing low-grade inflammation, especially in T2D, for which endothelial dysfunction is particularly ominous and for which both endothelial dysfunction and low-grade inflammation are highly prevalent.

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References

1. Pyorala K, Laakso M, Uusitupa M. Diabetes and atherosclerosis: an epidemiologic view. *Diabetes Metab Rev*. 1987;3:463–524.
2. de Vegt F, Dekker JM, Ruhe HG, Stehouwer CD, Nijpels G, Bouter LM, Heine RJ. Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: the Hoorn study. *Diabetologia*. 1999;42:926–931.
3. Laakso M. Hyperglycemia and cardiovascular disease in type 2 diabetes. *Diabetes*. 1999;48:937–942.
4. Munro JM, Cotran RS. Biology of disease: the pathogenisis of atherosclerosis: atherogenesis and inflammation. *Lab Invest*. 1988;58:249–261.
5. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med*. 1999;340:115–126.
6. Alexander RW. Inflammation and coronary artery disease. *N Engl J Med*. 1994;331:468–469.
7. Stehouwer CDA, Gall MA, Twisk JWR, Knudsen E, Emeis JJ, Parving HH. Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. *Diabetes*. 2002;51:1157–1165.
8. Saydah SH, Loria CM, Eberhardt MS, Brancati FL. Subclinical states of glucose intolerance and risk of death in the U.S. *Diabetes Care*. 2001;24:447–453.

9. Beks PJ, Mackaay AJ, de Neeling JN, de Vries H, Bouter LM, Heine RJ. Peripheral arterial disease in relation to glycaemic level in an elderly Caucasian population: the Hoorn study. *Diabetologia*. 1995;38:86–96.
10. Jager A, Kostense PJ, Ruhé HG, Heine RJ, Nijpels G, Dekker JM, Bouter LM, Stehouwer CD. Microalbuminuria and peripheral arterial disease are independent predictors of cardiovascular and all-cause mortality, especially among hypertensive subjects: five-year follow-up of the Hoorn study. *Arterioscler Thromb Vasc Biol*. 1999;19:617–624.
11. Alberti KG, Zimmet PZ. Definition, diagnosis, and classification of diabetes mellitus and its complications, part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*. 1998;15:539–553.
12. Stehouwer CDA, Lambert J, Donker AJM, van Hinsbergh VWM. Endothelial dysfunction and pathogenisis of diabetic angiopathy. *Cardiovasc Res*. 1997;34:55–68.
13. Mannucci PM. Von Willebrand factor: a marker of endothelial damage? *Arterioscler Thromb Vasc Biol*. 1998;18:1359–1362.
14. Vapaatalo H, Mervaala E. Clinically important factors influencing endothelial function. *Med Sci Monit*. 2001;7:1075–1085.
15. Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. *Circ Res*. 2001;89:763–771.
16. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*. 2000;342:836–843.
17. Stehouwer CDA, Yudkin JS, Fioretto P, Nosadini R. How heterogeneous is microalbuminuria? The case for 'benign' and 'malignant' microalbuminuria. *Nephrol Dial Transplant*. 1998;13:2751–2754.
18. Jager A, van Hinsbergh VW, Kostense PJ, Emeis JJ, Yudkin JS, Nijpels G, Dekker JM, Heine RJ, Bouter LM, Stehouwer CD. Von Willebrand factor, C-reactive protein, and 5-year mortality in diabetic and non-diabetic subjects. The Hoorn study. *Arterioscler Thromb Vasc Biol*. 1999;19:3071–3078.
19. Jager A, van Hinsbergh VW, Kostense PJ, Emeis JJ, Nijpels G, Dekker JM, Heine RJ, Bouter LM, Stehouwer CD. Increased levels of soluble vascular cell adhesion molecule 1 are associated with risk of cardiovascular mortality in type 2 diabetes: the Hoorn study. *Diabetes*. 2000;49:485–491.
20. Becker A, van Hinsbergh VW, Jager A, Kostense PJ, Dekker JM, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD. Why is soluble intercellular adhesion molecule-1 related to cardiovascular mortality? *Eur J Clin Invest*. 2002;32:1–8.
21. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499–502.
22. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med*. 1999;130:461–470.
23. World Health Organization. International Classification of Diseases, 9th ed, Vol. 1 and 2. Geneva, Switzerland: WHO; 1977.
24. Ford ES. Body mass index, diabetes, and C-reactive protein among US adults. *Diabetes Care*. 1999;22:1971–1977.
25. Eposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, Quagliaro L, Ceriello A, Giugliano D. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation*. 2002;106:2067–2072.
26. Tan KC, Wan NM, Tam SC, Janus ED, Lam TH, Lam KS. C-reactive protein predicts the deterioration of glycemia in Chinese subjects with impaired glucose tolerance. *Diabetes Care*. 2003;26:2323–2328.
27. Choi KM, Lee J, Lee KW, Seo JA, Oh JH, Kim SG, Kim NH, Choi DS, Baik SH. Comparison of serum concentrations of C-reactive protein, TNF-α, and IL 6 between elderly Korean women with normal and impaired glucose tolerance. *Diabetes Res Clin Pract*. 2004;64:99–106.
28. Ferri C, Desideri G, Baldoncini R, Bellini C, De Angelis C, Mazzocchi C, Santucci A. Early activation of vascular endothelium in nonobese, nondiabetic essential hypertensive patients with multiple metabolic abnormalities. *Diabetes*. 1998;47:660–667.
29. Caballero AE, Arora S, Saouaf R, Lim SC, Smakowski P, Park JY, King GL, LoGerfo FW, Horton ES, Veves A. Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. *Diabetes*. 1999;48:1856–1862.
30. Yanik CS, Naik SS, Raut KN, Khade AD, Bhat DS, Nagarkar VD, Deshpande JA, Shelgikar KM. Urinary albumin excreation rate (AER) in newly-diagnosed type 2 Indian diabetic patients is associated with central obesity and hyperglycaemia. *Diabetes Res Clin Pract*. 1992;17:55–60.
31. Matsumoto K, Miyake S, Yano M, Ueki Y, Tominaga Y. High serum concentrations of soluble E-selectin in patients with impaired glucose tolerance with hyperinsulinemia. *Atherosclerosis*. 2000;152:415–420.
32. Blüher M, Unger R, Rassoul F, Richter V, Paschke R. Relation between glycaemic control, hyperinsulinaemia, and plasma concentrations of soluble adhesion molecules in patients with impaired glucose tolerance or type II diabetes. *Diabetologia*. 2002;45:210–216.
33. Leurs PB, Stolk RP, Hamulyak K, van Oerle R, Grobbee DE, Wolffenbuttel BH. Tissue factor pathway inhibitor and other endothelium-dependent hemostatic factors in elderly individuals with normal or impaired glucose tolerance and type 2 diabetes. *Diabetes Care*. 2002;25:1340–1345.
34. Pontiroli AE, Pizzocri P, Koprivec D, Vedani P, Marchi M, Arcelloni C, Paroni R, Esposito K, Giugliano D. Body weight and glucose metabolism have a different effect on circulating levels of ICAM-1, E-selectin, and endothelin-1 in humans. *Eur J Endocrinol*. 2004;150:195–200.
35. Viswanathan V, Snelhalatha C, Nair MB, Ramachandran A. Markers of endothelial dysfunction in hyperglycaemic Asian Indian subjects. *J Diabetes Complications*. 2004;18:47–52.
36. Henry RM, Ferreira I, Kostense PJ, Dekker JM, Nijpels G, Heine RJ, Kamp O, Bouter LM, Stehouwer CD. Type 2 diabetes is associated with impaired endothelium-dependent, flow-mediated dilation, but impaired glucose metabolism is not: The Hoorn Study. *Atherosclerosis*. 2004;174:49–56.
37. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, Lowe GD, Pepys MB, Gudnason V. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med*. 2004;350:1387–1397.
38. Stehouwer CDA. Endothelial dysfunction in diabetic nephropathy: state of the art and potential significance for non-diabetic renal disease. *Nephrol Dial Transplant*. 2004;19:778–781.
39. de Vegt F, Dekker JM, Stehouwer CDA, Nijpels G, Bouter LM, Heine RJ. Similar 9-year mortality risks and reproducibility for the World Health Organization and Am Diabetes Association glucose tolerance categories: the Hoorn study. *Diabetes Care*. 2000;23:40–44.
40. The DECODE study group. Glucose tolerance and mortality: comparison of WHO and American Diabetic Association diagnostic criteria. *Lancet*. 1999;354:617–621.
41. Tominaga M, Eguchi H, Manaka H, Igarashi K, Kato T, Sekikawa A. Impaired glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose: the Fungata Diabetes Study. *Diabetes Care*. 1999;22:920–924.
42. Jarrett R. The cardiovascular risk associated with impaired glucose tolerance. *Diabet Med*. 1996;13:S15–S19.
43. Piemonti L, Calori G, Mercalli A, Lattuada G, Monti P, Garancini MP, Costantino F, Ruotolo G, Luzi L, Perseghin G. Fasting plasma leptin, tumor necrosis factor-a receptor 2, and monocyte chemoattracting protein 1 concentration in a population of glucose-tolerant and glucose-intolerant women: impact on cardiovascular mortality. *Diabetes Care*. 2003;26:2883–2889.
44. Rajala U, Koskela P, Keinanen-Kiukaanniemi S. Hyperglycemia as a risk factor of mortality in a middle-aged Finnish population. *J Clin Epidemiol*. 2001;54:470–474.
45. Gabir MM, Hanson RL, Dabelea D, Imperatore G, Roumain J, Bennett PH, Knowler WC. Plasma glucose and prediction of microvascular disease and mortality: evaluation of 1997 American Diabetes Association and 1990 World Health Organization criteria for diagnosis of diabetes. *Diabetes Care*. 2000;23:1113–1118.
46. Hiltunen L, Läärä E, Kivelä S-L, Keinänen-Kiukaanniemi S. Glucose tolerance and mortality in an elderly Finnish population. *Diabetes Res Clin Pract*. 1998;39:75–81.
47. Brevetti G, Martone VD, de Cristofano T, Corrado S, Silvestro A, Di Donato AM, Bucur R, Scopacasa F. High levels of adhesion molecules are associated with impaired endothelium-dependent vasodilation in patients with peripheral arterial disease. *Thromb Haemost*. 2001;85:63–66.
48. Karatzis EN. The role of inflammatory agents in endothelial function and their contribution to atherosclerosis. *Hellenic J Cardiol*. 2005;46:232–239.
49. Henry RM, Kostense PJ, Bos G, Dekker JM, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD. Mild renal insufficiency is associated with increased cardiovascular mortality: the Hoorn Study. *Kidney Int*. 2002;62:1402–1407.