Endothelial Dysfunction Contributes to Renal Function–Associated Cardiovascular Mortality in a Population with Mild Renal Insufficiency: The Hoorn Study

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Mildly impaired renal function is associated with cardiovascular morbidity and mortality. There are indications that endothelial dysfunction and/or chronic inflammation, which play an important role in atherothrombosis, are present in early stages of renal insufficiency. This study investigated whether and to which extent endothelial dysfunction and inflammation were related to renal function and contributed to renal function–associated cardiovascular mortality in a population-based cohort ($n = 613$), aged 50 to 75 yr, that was followed with a median duration of 12.5 yr. During follow-up, 192 individuals died (67 of cardiovascular causes). At baseline, renal function was estimated with serum creatinine, the Cockcroft-Gault formula, and the Modification of Diet in Renal Disease equation of GFR (eGFR). Endothelial function was estimated by plasma von Willebrand factor, soluble vascular cell adhesion molecule-1, and the urinary albumin-creatinine ratio. Inflammatory activity was estimated by plasma C-reactive protein and soluble intercellular adhesion molecule-1. Renal function was mildly impaired (mean eGFR 68 ± 12 ml/min per 1.73 m^2) and independently associated with von Willebrand factor (standardized β −0.09; 95% confidence interval [CI] −0.18 to −0.002; $P < 0.05$), soluble vascular cell adhesion molecule-1 (standardized β −0.14; 95% CI −0.22 to −0.05; $P < 0.01$), and albumin-creatinine ratio (standardized β −0.15; 95% CI −0.23 to −0.08; $P < 0.001$) but not with markers of inflammatory activity. Renal function was inversely associated with cardiovascular and all-cause mortality. The relative risk for cardiovascular mortality but not all-cause mortality associated with renal function decreased from 1.22 to 1.12 per 5 ml/min per 1.73 m^2 decrease of eGFR after adjustment for markers of endothelial dysfunction. In conclusion, endothelial dysfunction was related to renal function and contributed to the excess in cardiovascular mortality in this population-based cohort with mild renal insufficiency.

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Chronic kidney disease (CKD) is a prevalent health problem, involving >10% of the general population of the United States (1). CKD, especially ESRD, is strongly associated with the occurrence of cardiovascular disease (2). As recently demonstrated, even mildly impaired renal function is associated with cardiovascular morbidity (3,4) and mortality (3,5). Although CKD is associated with several risk factors for cardiovascular disease, such as male gender, older age, hyperhomocysteinemia, hypertension, smoking, diabetes, obesity, and pre-existing cardiovascular disease, the excess cardiovascular risk in CKD is not fully explained by this clustering of conventional risk factors.

In atherogenesis, endothelial dysfunction and inflammation are important and interrelated early steps (6). Indeed, several biochemical markers of endothelial dysfunction and inflammatory activity have been shown to be independent risk factors of cardiovascular morbidity and mortality (7–11). It is unknown whether the excess cardiovascular risk in CKD is (partially) attributable to endothelial dysfunction and inflammation. There is evidence that a decreased GFR is associated with endothelial dysfunction as well as inflammatory activity (12–14). However, there is little evidence that a low GFR is associated with endothelial dysfunction and inflammation in the general population and, if so, whether any such associations can explain the association of a low GFR with risk for cardiovascular disease.

In view of these considerations, we investigated, in a prospective, population-based study (*1*) whether estimates of GFR were cross-sectionally associated with markers of endothelial dysfunction and inflammatory activity at the baseline examination and (*2*) whether endothelial dysfunction and inflammatory activity were involved in the excess mortality associated with a low GFR.

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Materials and Methods

Participants

The study population consisted of an age-, gender-, and glucose tolerance–stratified sample of the Hoorn Study, a population-based study of glucose tolerance and other cardiovascular risk factors in a 50- to 75-yr-old general white population conducted from 1989 to 1992, as described previously (15).

Briefly, 2484 people (71% of those invited) participated. All participants, except for those who had previously diagnosed diabetes and were treated with oral glucose-lowering agents or insulin, underwent an oral glucose tolerance test (OGTT) according to the World Health Organization guidelines (16). For reasons of efficiency, participants with a 2-h postload glucose ≥7.5 mmol/L, all participants with type 2 diabetes, and a random sample of participants with a 2-h postload glucose <7.5 mmol/L stratified by age and gender were invited within 4 wk for a second visit to investigate glucose intolerance–related complications (709 invited, 631 [89%] of whom participated).

These participants underwent a second OGTT (except those who already used blood glucose–lowering agents; $n = 67$). On the basis of the mean of the two OGTT, glucose tolerance status was divided into three categories according to the 1999 World Health Organization criteria: Normal glucose metabolism, impaired glucose metabolism, and type 2 diabetes (17). Participants in our study population thus represented a stratified random sample of all participants in the initial cohort.

The Hoorn Study was approved by the Ethical Review Committee of the VU University Medical Center. Informed consent was obtained from all participants.

Baseline Laboratory and Clinical Assessments

C-reactive protein (CRP) and soluble intercellular adhesion molecule-1 (sICAM-1) were measured as markers of inflammatory activity, and soluble vascular cell adhesion molecule-1 (sVCAM-1), von Willebrand factor (vWf), and microalbuminuria were measured as markers of endothelial dysfunction. After an overnight fast, blood was drawn from an antecubital vein. We measured blood concentrations of vWf, sVCAM-1, CRP, sICAM-1, total (free plus bound) homocysteine, glucose, creatinine, albumin, urea nitrogen, and lipids, as described elsewhere (10,15). The urinary albumin-creatinine ratio (ACR) was calculated.

Creatinine clearance was estimated (eCC) by the Cockcroft-Gault formula [(140 − age) × (1.23 × body weight/creatinine), amplified by 0.85 if female] (18). GFR was estimated (eGFR) by the Modification of Diet in Renal Disease (MDRD) equation [170 × (creatinine)$^{-0.999}$× (age)$^{-0.176}$× (serum urea nitrogen)$^{-0.170}$× (albumin)$^{0.318}$× (0.762 if patient is female) (1.180 if patient is black)] (MDRD equation is given in traditional units. To convert to International System units, multiply creatinine in mg/dl by 88.4, urea in mg/dl by 0.357, and albumin in g/dl by 10.) (19). eCC and eGFR were expressed in ml/min per 1.73 m^2 body surface area. BP was measured as the mean of four measurements performed on two different occasions, using a random-zero sphygmomanometer under standardized conditions. Participants were classified as current cigarette smokers or nonsmokers. Body mass index (BMI) and waist-to-hip ratio were calculated as described elsewhere (15). Cardiovascular disease was defined as coronary artery disease, cerebrovascular disease, and/or peripheral arterial disease.

We excluded individuals for whom no data were available for calculation of the renal function estimates ($n = 18$), leaving 613 individuals available for analysis. From these individuals, data on vWf and sVCAM-1 were missing in 21, data on ACR in 23, and data on CRP and sICAM-1 in 23.

Follow-Up

Data on the participants' vital status on January 1, 2004, were collected from the mortality register of the municipality of Hoorn. Of 51 participants who had moved out of town, information was obtained from the local municipalities. Of the 613 included participants, one was lost to follow-up. For the other 612 participants, we determined whether death had occurred during follow-up and, if so, the date when death occurred. For all participants 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 *International Classification of Diseases, Ninth Revision* (20). Cardiovascular mortality was defined as codes 390 to 459.

Statistical Analyses

All analyses were performed with the SPSS, version 11.5 (SPSS Inc., Chicago, IL). Variables are presented as mean ± SD, number (percentage of the total), or, in case of a skewed distribution, the median and the interquartile range.

For descriptive purposes, the group was divided in tertiles according to the eGFR. P value for trend over tertiles was calculated with univariate ANOVA for continuous variables and with χ^2 test for binary variables. Pearson test was used to assess correlation coefficients.

To study whether biochemical markers of endothelial dysfunction and inflammatory activity were related independently to estimates of renal function, regression analyses were performed with three different models. In the first model—because of the stratification procedure—multivariate regression analysis was performed with adjustment for age, gender, and glucose tolerance status. In the second model, we additionally adjusted for potential confounders, *i.e.*, previous cardiovascular disease, systolic BP, current smoking, waist-to-hip ratio, total cholesterol, and homocysteine. Finally, we adjusted the associations between renal function and endothelial dysfunction markers for markers of inflammation, and *vice versa*. Variables that did not have a normal distribution of the residuals (vWf, ACR, and CRP) were transformed into their natural logarithm (ln) for a better fit of the data. Results are described as standardized β with 95% confidence intervals (CI). Multivariate Cox regression analyses were performed to assess whether the associations of renal function with all-cause mortality and cardiovascular mortality were independent of potential confounding or mediating factors.

Adjustment for endothelial function was performed by adding each marker of endothelial dysfunction separately to the model and by adding vWf, sVCAM-1, and ACR simultaneously. Because microalbuminuria has been shown previously to have a heterogeneous association with endothelial dysfunction in the Hoorn Study (21,22), we also adjusted for sVCAM-1 and vWf only. In an additional analysis, sICAM-1 was added to vWf, sVCAM-1, and ACR, because it has been suggested that sICAM-1 may reflect endothelial dysfunction as well as inflammatory activity. Adjustment for inflammatory activity was performed by adding CRP and sICAM-1 (separately and simultaneously) to the model.

Results are described as relative risks (RR; hazard ratios) with 95% CI associated with a decrease in eGFR and eCC of 5 ml/min per 1.73 m^2 and increase in serum creatinine concentration of 5 μmol/L. Two-sided $P < 0.05$ was considered to reflect statistical significance.

Results

Table 1 shows the baseline characteristics of the study population according to tertiles of eGFR. In the whole study population, eGFR was 68 ± 12 ml/min per 1.73 m^2, eCC was 74 ±

Table 1. Baseline characteristics of all participants[a]

	Tertiles of eGFR			*P* Value (Trend)
	First (n = 204) >72 ml/min per 1.73 m²	Second (n = 205) 63 to 72 ml/min per 1.73 m²	Third (n = 204) <63 ml/min per 1.73 m²	
eGFR (ml/min per 1.73 m²)	80 ± 7	68 ± 3	55 ± 8	<0.001
eCC (ml/min per 1.73 m²)	86 ± 16	74 ± 12	62 ± 14	<0.001
Serum creatinine (μmol/L)	82 ± 11	90 ± 12	104 ± 24	<0.001
Male gender (n [%])	133 (65)	109 (53)	135 (66)	0.84
Age (yr)	62 ± 7	63 ± 7	67 ± 6	<0.001
Normal glucose metabolism (n [%])	77 (38)	94 (46)	79 (39)	0.84
Impaired glucose metabolism (n [%])	56 (28)	60 (29)	57 (28)	0.91
Type 2 diabetes (n [%])	71 (35)	51 (25)	68 (33)	0.75
BMI (kg/m²)	26.7 ± 3.6	26.8 ± 3.5	28.1 ± 4.4	<0.001
Waist-to-hip ratio (cm/cm)	0.94 ± 0.09	0.90 ± 0.08	0.92 ± 0.08	<0.01
Total cholesterol (mmol/L)	6.5 ± 1.2	6.7 ± 1.2	6.7 ± 1.2	0.06
HDL cholesterol (mmol/l)	1.3 ± 0.4	1.3 ± 0.3	1.3 ± 0.3	0.98
Lipid-lowering medication (n [%])	5 (2)	1 (0)	3 (1)	0.41
Systolic BP (mmHg)	138 ± 20	136 ± 19	144 ± 19	<0.01
Diastolic BP (mmHg)	83 ± 10	82 ± 10	83 ± 10	0.82
Pulse pressure (mmHg)	55 ± 15	54 ± 13	62 ± 16	<0.001
BP-lowering medication (n [%])	52 (25)	40 (20)	77 (40)	<0.01
Hypertension (n [%])	108 (53)	90 (44)	136 (67)	<0.01
Current smokers (n [%])	63 (31)	52 (25)	41 (20)	<0.05
Previous cardiovascular disease (n [%])	47 (23)	43 (21)	52 (26)	0.56
Homocysteine (μmol/L)	11.2 ± 4.2	12.5 ± 5.9	13.9 ± 6.3	<0.001
vWf (IU/ml)	110 (88–162)	108 (74–166)	134 (89–188)	<0.05
sVCAM-1 (μg/L)	1304 ± 423	1325 ± 368	1512 ± 515	<0.001
Urinary ACR (mg/mmol)	0.77 (0.54 to 1.21)	0.87 (0.59 to 1.28)	0.99 (0.65 to 1.72)	<0.05
C-reactive protein (mg/l)	1.75 (0.88 to 3.44)	1.49 (0.59 to 3.02)	2.13 (1.02 to 4.27)	0.74
sICAM-1 (μg/L)	495 ± 190	462 ± 149	488 ± 146	0.67

[a]Values for continuous variables are presented as mean ± SD or median (interquartile range). *P* value for trend was calculated with univariate ANOVA for continuous variables and with χ^2 test for dichotomous variables. Hypertension was defined as a BP ≥140 mmHg systolic and/or ≥90 mmHg diastolic and/or the current use of BP-lowering medication. eGFR, estimated GFR; eCC, estimated creatinine clearance; BMI, body mass index; vWf, Von Willebrand factor; sVCAM-1, soluble vascular cell adhesion molecule-1; ACR, albumin-creatinine ratio; sICAM-1, soluble intercellular adhesion molecule-1.

17 ml/min per 1.73 m², and serum creatinine was 92 ± 19 μmol/L.

Relationship between Renal Function and Endothelial Function

Table 2 shows that eGFR was independently associated with vWf, sVCAM-1, and ACR in all models (see also Figure 1, A through C).

Relationship between Renal Function and Inflammatory Activity

None of the inflammatory markers was related independently to any of the estimates of renal function (Table 3 and Figure 1, D and E). In model 1, a significant positive association was found between CRP and eCC, which, however, was not present after adjustment for BMI (standardized β 0.03; 95% CI −0.07 to 0.14; $P = 0.58$), one of the dependent variables in models 2 and 3. BMI was higher in participants with a higher eGFR (Table 1) and strongly positively related with CRP (model 3: 0.20; 95% CI 0.11 to 0.28; $P < 0.001$).

Renal Function, Endothelial Dysfunction and Inflammatory Activity in Relation to Cardiovascular and All-Cause Mortality

Median duration of follow-up was 12.5 yr (interquartile range 9.9 to 13.2). During follow-up, 192 of 613 individuals died (67 of cardiovascular disease and 31 of unknown causes). Renal function, whether expressed as eGFR, eCC, or serum creatinine, was inversely associated with risk for cardiovascular and all-cause mortality. After adjustment for age, gender, glucose tolerance, systolic BP, and previous cardiovascular disease, the RR (95% CI) of cardiovascular and all-cause mortality associated with a decrease of 5 ml/min per 1.73 m² of eGFR were 1.22 (1.09 to 1.36) and 1.12 (1.05 to 1.20), respectively (Tables 4 and 5). The

Table 2. Relation of renal function with markers of endothelial function[a]

Model	vWf		sVCAM-1		Urinary ACR	
	Stand. β	95% CI	Stand. β	95% CI	Stand. β	95% CI
1: Age, gender, glucose tolerance status						
eGFR	−0.11[b]	−0.20 to −0.02	−0.23[d]	−0.32 to −0.15	−0.20[d]	−0.28 to −0.12
eCC	−0.02	−0.15 to 0.10	−0.15[c]	−0.24 to −0.05	−0.13[c]	−0.23 to −0.02
serum creatinine	0.07	0.00 to 0.14	0.25[d]	0.17 to 0.34	0.27[d]	0.19−0.35
2: Model 1 + previous cardiovascular disease, systolic BP, current smoking, BMI, total cholesterol, homocysteine						
eGFR	−0.10[b]	−0.19 to −0.001	−0.18[d]	−0.27 to −0.10	−0.14[c]	−0.22 to −0.06
eCC	−0.05	−0.18 to 0.07	−0.16[c]	−0.28 to −0.05	−0.17[c]	−0.28 to −0.06
serum creatinine	0.05	0.00 to 0.15	0.18[d]	0.09 to 0.27	0.17[d]	0.09 to 0.25
3: Model 2 + C-reactive protein, sICAM-1						
eGFR	−0.09[b]	−0.18 to 0.00	−0.14[c]	−0.22 to −0.05	−0.15[d]	−0.23 to −0.08
eCC	−0.05	−0.17 to 0.08	−0.11[b]	−0.22 to −0.01	−0.18[c]	−0.29 to −0.07
serum creatinine	0.04	−0.03 to 0.11	0.11[b]	0.02 to 0.19	0.18[d]	0.10 to 0.25

[a]vWf, urinary ACR, and C-reactive protein were transformed to natural logarithm (ln). Stand., standardized; CI, confidence interval.
[b]$P < 0.05$; [c]$P < 0.01$; [d]$P < 0.001$.

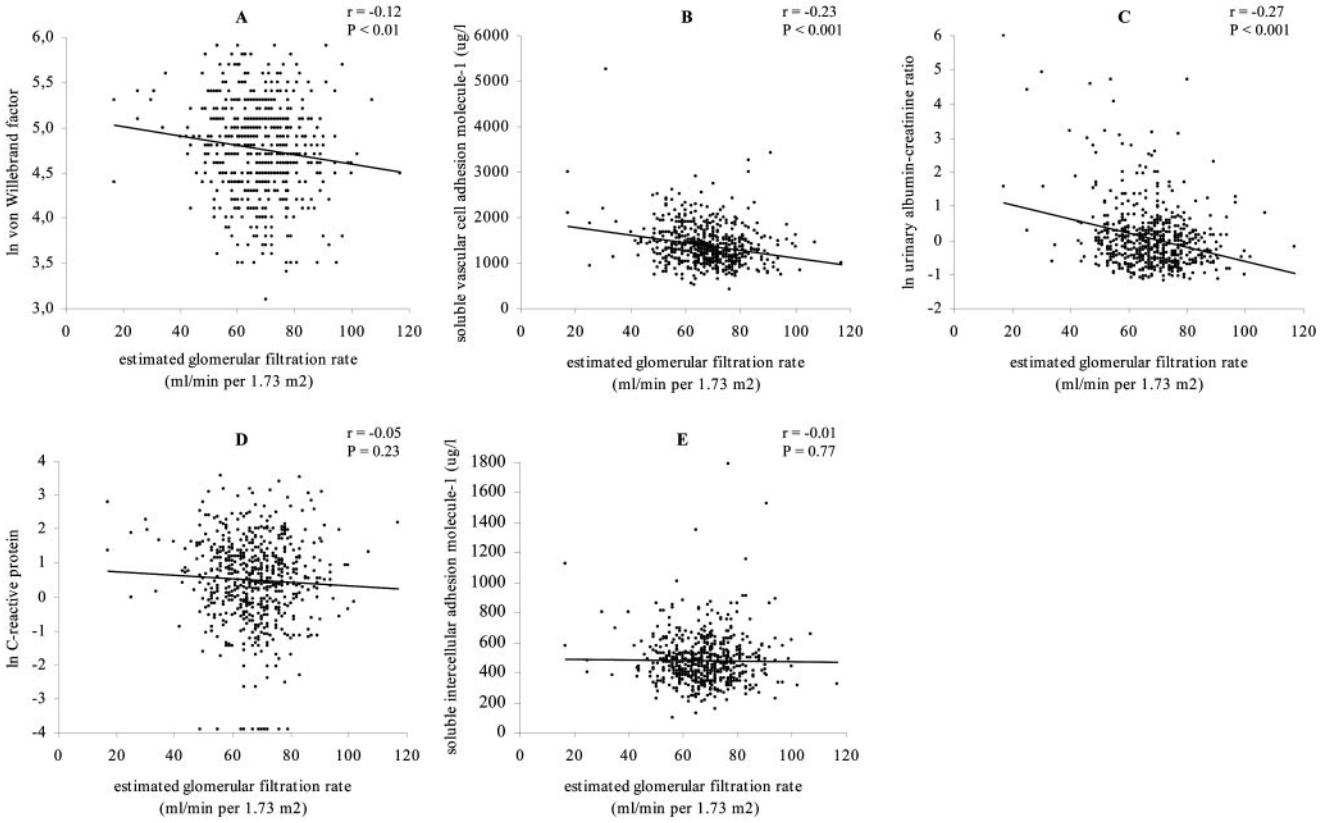

Figure 1. Relations between estimated GFR and markers of endothelial dysfunction (von Willebrand factor [A], soluble vascular cell adhesion molecule-1 [B], and urinary albumin-creatinine ratio [C]) and inflammatory activity (C-reactive protein [D], soluble intercellular adhesion molecule-1 [E]). r, correlation coefficient.

relationship between eGFR and cardiovascular mortality is shown in Figure 2. The association was stronger for eGFR than for eCC or serum creatinine (Table 4).

When vWf, sVCAM-1, and ACR were added individually to the model, the RR for cardiovascular mortality associated with renal function decreased from 1.22 to 1.21, 1.16, and 1.17, respectively (Table 4, models 3 through 5). When the three markers were added simultaneously, the RR decreased from 1.22 to 1.12 (Table 4, model 6). Adjustment for vWf and sVCAM-1 without ACR decreased the RR from 1.22 to 1.16 (Table 4, model 7). After addition of sICAM-1 to the three endothelial markers, the RR decreased from 1.22 to 1.13 (Table 4, model 8). The RR for all-cause

Table 3. Relation of renal function with markers of inflammatory activity[a]

Model	C-Reactive Protein		sICAM-1	
	Stand. β	95% CI	Stand. β	95% CI
1: Age, gender, glucose tolerance status				
eGFR	0.00	−0.07 to 0.08	−0.02	−0.10 to 0.07
eCC	0.14[b]	0.04 to 0.25	−0.03	−0.12 to 0.07
serum creatinine	0.03	−0.07 to 0.12	0.07	−0.01 to 0.16
2: Model 1 + previous cardiovascular disease, systolic BP, current smoking, BMI, total cholesterol, homocysteine				
eGFR	0.04	−0.05 to 0.13	0.02	−0.08 to 0.11
eCC	0.04	−0.06 to 0.15	−0.03	−0.15 to 0.09
serum creatinine	−0.01	−0.09 to 0.08	0.01	−0.08 to 0.11
3: Model 2 + vWf, sVCAM-1, urinary ACR				
eGFR	0.07	−0.03 to 0.16	0.06	−0.03 to 0.15
eCC	0.07	−0.06 to 0.20	0.02	−0.14 to 0.10
serum creatinine	−0.04	−0.12 to 0.05	−0.03	−0.12 to 0.06

[a]C-reactive protein, vWf, and urinary ACR were transformed to natural logarithm (ln).
[b]$P < 0.01$.

Table 4. Relative risks of cardiovascular mortality associated with estimates of renal function[a]

Model	eGFR (RR [95% CI] Associated with a Decrease of 5 ml/min per 1.73 m^2)	eCC (RR [95% CI] Associated with a Decrease of 5 ml/min per 1.73 m^2)	Serum Creatinine (RR [95% CI] Associated with an Increase of 5 μmol/L)
1: Age, gender, glucose tolerance status	1.22[b] (1.09 to 1.36)	1.10[c] (1.01 to 1.21)	1.11[b] (1.07 to 1.16)
2: Model 1 + previous cardiovascular disease, systolic BP	1.22[b] (1.09 to 1.36)	1.12[c] (1.02 to 1.23)	1.11[b] (1.06 to 1.16)
3: Model 2 + vWf	1.21[d] (1.08 to 1.35)	1.12[c] (1.02 to 1.23)	1.10[b] (1.06 to 1.15)
4: Model 2 + sVCAM-1	1.16[d] (1.04 to 1.30)	1.08 (0.99 to 1.19)	1.09[b] (1.04 to 1.14)
5: Model 2 + urinary ACR	1.17[d] (1.04 to 1.31)	1.09 (0.99 to 1.19)	1.09[b] (1.04 to 1.14)
6: Model 2 + vWf, sVCAM-1, ACR	1.12[c] (1.00 to 1.26)	1.07 (0.98 to 1.17)	1.08[d] (1.02 to 1.13)
7: Model 6 − urinary ACR	1.16[c] (1.04 to 1.30)	1.09 (0.99 to 1.19)	1.09[b] (1.04 to 1.14)
8: Model 6 + sICAM-1	1.13[c] (1.01 to 1.27)	1.06 (0.97 to 1.17)	1.07[d] (1.02 to 1.13)
9: Model 2 + C-reactive protein	1.22[d] (1.09 to 1.36)	1.12[c] (1.02 to 1.23)	1.11[b] (1.06 to 1.16)
10: Model 2 + sICAM-1	1.20[d] (1.08 to 1.34)	1.10 (1.00 to 1.21)	1.10[b] (1.05 to 1.15)
11: Model 2 + C-reactive protein, sICAM-1	1.20[d] (1.07 to 1.34)	1.10[c] (1.00 to 1.22)	1.10[b] (1.05 to 1.15)

[a]vWF, urinary ACR, and C-reactive protein were transformed to natural logarithm (ln). RR, relative risk.
[b]$P < 0.001$; [c]$P < 0.05$; [d]$P < 0.01$.

mortality associated with renal function was not materially affected by these markers of endothelial dysfunction (Table 5). Adjustment for markers of inflammatory activity did not change substantially the RR for cardiovascular (Table 4) or all-cause (Table 5) mortality associated with renal function. Sequential inclusion of smoking status, BMI (or hip-to-waist ratio), total cholesterol (or HDL cholesterol), use of lipid-lowering medication, and homocysteine as independent variables in model 2 of the Cox regression analysis did not materially change the effect of adjustment for the different markers of endothelial function and/or inflammation on the renal function–associated cardiovascular and all-cause mortality (data not shown).

Additional Analyses

Because age and gender are part of the Cockcroft-Gault formula and the MDRD equation and were added to the analyses with serum creatinine as key independent variable, all models were tested without these (stratification) variables. This did not materially influence our results (data not shown).

In the multivariate and Cox regression analyses, replacement of systolic BP by diastolic BP, pulse pressure, presence of hypertension, or use of BP-lowering medication did not materially change the relation between renal function and markers of endothelial dysfunction or inflammatory activity (data not shown). In addition, we repeated all analyses after exclusion of

Table 5. Relative risks for all-cause mortality associated with estimates of renal function

Model	eGFR (RR [95% CI] Associated with a Decrease of 5 ml/min per 1.73 m^2)	eCC (RR [95% CI] Associated with a Decrease of 5 ml/min per 1.73 m^2)	Serum Creatinine (RR [95% CI] Associated with an Increase of 5 μmol/L)
1: Age, gender, glucose tolerance status	1.12[b] (1.04 to 1.19)	1.03 (0.98 to 1.09)	1.08[c] (1.04 to 1.11)
2: Model 1 + previous cardiovascular disease, systolic BP	1.12[b] (1.05 to 1.20)	1.04 (0.98 to 1.09)	1.08[c] (1.04 to 1.11)
3: Model 2 + vWf	1.11[b] (1.04 to 1.19)	1.04 (0.99 to 1.10)	1.07[c] (1.04 to 1.11)
4: Model 2 + sVCAM-1	1.11[b] (1.04 to 1.19)	1.04 (0.98 to 1.10)	1.08[c] (1.04 to 1.11)
5: Model 2 + urinary ACR	1.10[b] (1.03 to 1.18)	1.04 (0.99 to 1.10)	1.07[c] (1.04 to 1.11)
6: Model 2 + vWf, sVCAM-1, ACR	1.10[b] (1.03 to 1.18)	1.05 (0.99 to 1.11)	1.07[c] (1.03 to 1.11)
7: Model 6 − urinary ACR	1.10[b] (1.03 to 1.18)	1.04 (0.98 to 1.10)	1.07[c] (1.04 to 1.11)
8: Model 6 + sICAM-1	1.10[b] (1.03 to 1.18)	1.04 (0.98 to 1.10)	1.07[c] (1.03 to 1.10)
9: Model 2 + C-reactive protein	1.12[b] (1.05 to 1.20)	1.04 (0.98 to 1.10)	1.08[c] (1.04 to 1.11)
10: Model 2 + sICAM-1	1.11[b] (1.04 to 1.19)	1.03 (0.98 to 1.09)	1.07[c] (1.04 to 1.11)
11: Model 2 + C-reactive protein, sICAM-1	1.11[b] (1.04 to 1.19)	1.04 (0.98 to 1.10)	1.07[c] (1.04 to 1.11)

[a]vWF, urinary ACR, and C-reactive protein were transformed to natural logarithm (ln).
[b]$P < 0.01$.
[c]$P < 0.001$.

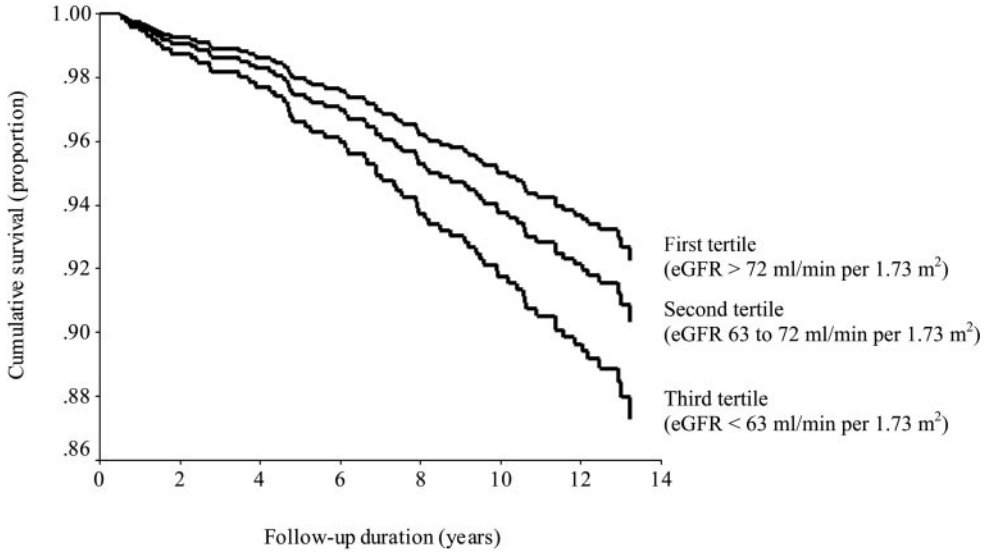

Figure 2. Proportion of participants without cardiovascular death according to tertiles of estimated GFR (eGFR), adjusted for age, gender, and glucose tolerance status (Cox regression analysis).

the four patients with CKD stage 4 (eGFR 15 to 29 ml/min per 1.73 m^2) (1), which again did not materially change the results.

Further analyses to exclude the presence of effect modification by the presence of diabetes, older age (>65 yr), male gender, or hypertension confirmed the consistency of the results, as the associations between renal function and biochemical markers of inflammatory activity and endothelial dysfunction and between eGFR and mortality were similar regardless of the presence or absence of these states (data not shown).

Discussion

This population-based study had three main findings. First, mild impairment of renal function was independently associated with endothelial dysfunction. Second, we confirm and extend our previous finding (5) of an association between mild impairment of renal function and cardiovascular mortality (and to a lesser extent all-cause mortality) that was independent of conventional risk factors and that persisted during up to 13 yr of follow-up. Third, endothelial dysfunction seemed to be involved in this renal function–associated cardiovascular mortality.

An increasing number of studies show that individuals with even mildly impaired renal function are at high risk for cardiovascular morbidity and mortality, independent of traditional risk factors (2). We have proposed that endothelial dysfunction and/or inflammation may be (partially) responsible for this phenomenon (14). This was based on the general view that

changes in endothelial cell properties and increased inflammation play an important role in the initiation and the progression of the atherothrombotic process (6) and on observations that patients with renal dysfunction show evidence of endothelial dysfunction and inflammation (13,14).

This study demonstrates that endothelial dysfunction is related to renal function in an elderly general population in whom renal function was only mildly impaired but cannot establish whether endothelial dysfunction causes impairment of GFR or *vice versa* or that a third variable causes both. Endothelial dysfunction may be an important mechanism linking mildly impaired renal function to cardiovascular disease, because healthy endothelium normally has antiatherothrombotic properties, such as promotion of vasodilation and inhibition of vascular smooth muscle cell proliferation, thrombosis, and inflammatory activity. Many of these functions are mediated by the release of compounds with specific biologic properties, such as nitric oxide, proteins involved in hemostasis and fibrinolysis, adhesion molecules, and selectins. The plasma concentrations of such substances are thought to reflect endothelial function status. vWf, which has prothrombogenic properties through its involvement in platelet adhesion and aggregation and in blood coagulation (23), has been suggested to be a marker of generalized endothelial dysfunction (24). VCAM-1 is involved in the recruitment of mononuclear blood cells into the vascular wall (25). Soluble VCAM-1 in plasma is derived from shedding of endothelial cells and has also been related to endothelial dysfunction (26). In the absence of significant excretion of sVCAM-1 in the urine (27), plasma sVCAM-1 concentration can be regarded as a reflection of endothelial release. Finally, microalbuminuria is often regarded as a marker of generalized endothelial dysfunction and has been shown to be independently associated with impaired endothelium-dependent vasodilation (28). In accordance with the concept that these markers reflect endothelial dysfunction, vWf, sVCAM-1, and (micro)albuminuria all have been associated with increased risk for cardiovascular and all-cause mortality (7–9).

This is the first population-based study to show that mildly impaired renal function is associated with endothelial dysfunction. Instead, some studies have suggested that microalbuminuria is associated with glomerular *hyper*filtration (29,30). For example, in the PREVEND study, individuals with microalbuminuria, as compared with those with normoalbuminuria, had a higher mean creatinine clearance (97 *versus* 90 ml/min per 1.73 m^2) (29). In contrast, we observed a *linear* inverse relationship between eGFR and ln ACR. This discrepancy may be related to the higher mean age and the lower mean eGFR in the current compared with these previous studies (29,30).

In our study, no significant relation was found between renal function and inflammation, as estimated with sICAM-1 and CRP. In patients with more advanced predialysis renal insufficiency, plasma sICAM-1 (14,31) and CRP (14,32) have been shown to be elevated. The relationship between mildly impaired renal function and CRP is less clear. In another community-based, nondiabetic population, Stuveling *et al.* (33) found that CRP was related with both diminished and high creatinine clearance (*i.e.*, a U-shaped relationship). As in our study, the positive relationship between CRP and eCC disappeared after adjustment for BMI. This is probably due to the strong positive relationship between CRP and BMI (34,35), which may be explained by the finding that IL-6, a regulator of the synthesis of CRP in the liver, has been shown to be secreted by human subcutaneous adipose tissue (36). As neither serum creatinine nor eGFR was significantly related with CRP, it is unlikely that a true association between CRP and eCC was masked by overadjustment for BMI.

Mild renal function impairment was associated with cardiovascular and all-cause mortality during up to 13 yr of follow-up, in a way that was independent of conventional risk factors for cardiovascular disease. Recent studies, with a shorter follow-up, have also demonstrated that mildly decreased GFR is an independent risk factor for cardiovascular disease (3,4). Compared with our previous study of the same cohort (5), this study consisted of older individuals, had a longer follow-up and more mortality cases, and, therefore, more power to identify and quantify influences of individual risk factors on cardiovascular and all-cause mortality. A major and new finding of our study is that the association of renal function and cardiovascular mortality was attenuated after adjustment for endothelial function, suggesting that the relationship may be partially explained by endothelial dysfunction.

It is likely that the change in RR for cardiovascular mortality associated with a decrease in eGFR of 5 ml/min per 1.73 m^2 from 1.22 to 1.12, which was caused by adjustment for biochemical markers of endothelial dysfunction, is an underestimation of the true impact of endothelial dysfunction in individuals with mildly impaired renal function. The markers of endothelial dysfunction that we used reflect only selected aspects of a complex biologic entity; for example, these markers contain no (or at most very indirect) information on endothelial nitric oxide synthesis, which is known to be impaired in individuals with impaired renal function (37) and which is likely to play an important role in the association between impaired renal function and atherothrombosis (38). In this respect, it is noteworthy that the three endothelial function markers that we used (vWf, sVCAM-1, and ACR) contributed *mutually independently* to the association between impaired renal function and cardiovascular mortality, suggesting that these various aspects of endothelial dysfunction all are relevant. Furthermore, misclassification of the 31 patients who had an unknown cause of death may have caused underestimation of the impact of endothelial dysfunction on cardiovascular mortality.

We used ACR as a marker of endothelial dysfunction. However, it has been suggested that microalbuminuria may sometimes reflect increased albumin excretion by the kidney without generalized endothelial dysfunction (21,39). An alternative explanation of our data, therefore, is that decreased eGFR and increased ACR are related to cardiovascular mortality through similar mechanisms that (in part) have nothing to do with endothelial dysfunction. We cannot exclude this, but the RR of cardiovascular mortality decreased also when we adjusted for endothelial function assessed by vWf and sVCAM-1 without ACR, although to a lesser extent.

In this study, there was no relation between markers of

inflammation and renal function or renal function–associated mortality. However, such relations may have been underestimated, because only CRP and sICAM-1 were used as parameters of inflammation. Although both are predictors of cardiovascular disease (8,10,11), they may only partially reflect the state of chronic inflammation. Whether other important markers of inflammation (*e.g.*, TNF-α, IL-6, secretory phospholipase A_2) explain renal insufficiency–associated cardiovascular mortality requires further investigation. A limitation of our data is that we studied a relatively healthy, elderly white population. Therefore, it remains to be investigated whether the results of our study can be generalized to individuals who are younger, nonwhite, or at high risk for cardiovascular morbidity and mortality.

Conclusion

In this population-based study, endothelial dysfunction but not inflammation was related to renal function and seemed to contribute to cardiovascular mortality in mild renal insufficiency. The results of this study may be clinically relevant because endothelial dysfunction is potentially reversible (40). In the prevention of cardiovascular disease in mild renal insufficiency, targeting of endothelial dysfunction therefore might be an early treatment goal.

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