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Published in:
Kidney International

Publication date:
2008

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Gender differences in predictors of the decline of renal function in the general population

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We sought to identify predictors of the decline in renal function, especially those that are modifiable, in the 5488 participants of the prospective, community-based cohort study PREVEND who completed three visits during a mean follow-up of 6.5 years. The change in renal function was used as the outcome and this was calculated as the linear regression of three estimated GFR measurements obtained during follow-up. Risk factors, known to influence renal outcome in patients with primary renal diseases, were used as potential predictors in multivariate regression analyses. High systolic blood pressure and plasma glucose were found to be independent predictors for an accelerated decline in function for both genders. In males, albuminuria was the strongest independent predictor for renal function decline, whereas in females albuminuria was univariately associated only after adjustment for age. The direction of the association between cholesterol/HDL ratio and decline of renal function differed by gender. Surprisingly, in males, waist circumference was an independent predictor and positively associated with renal function outcome. These studies show that there are gender differences in the standard predictors of the decline in renal function.

Kidney International (2008) **74,** 505–512; doi:10.1038/ki.2008.200; published online 21 May 2008

KEYWORDS: albuminuria; renal function; predictors; gender; PREVEND

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Received 28 September 2007; revised 5 March 2008; accepted 11 March 2008; published online 21 May 2008

Chronic kidney disease (CKD) is a growing public health problem worldwide.[1] In 2000, approximately 300,000 patients had end-stage renal disease (ESRD) in the United States alone, and this number is expected to double by the year 2010.[2] Furthermore, the earlier stages of CKD are expected to be about 80 times more prevalent.[3] Given these expectations, it is evidently important to identify risk factors of renal function decline. Such factors can be implemented in screening programs to identify subjects at high risk of renal function decline, who may benefit from early preventive treatment.

Most studies that have been performed on this topic have reported on predictors of the development of CKD (estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2) or ESRD. However, the adverse consequences of renal insufficiency appear not to be limited to those whose renal function falls below a certain threshold. For instance, even subjects with relatively minor impairment of renal function are already at increased risk of cardiovascular disease.[4–8] The Hoorn study, a prospective population-based study including subjects with an eGFR ranging from 17 to 117 ml/min/1.73 m^2 reported that a 5 ml/min/1.73 m^2 lower eGFR was associated with a 26% increase in the risk of cardiovascular death over the entire range of baseline renal function.[4] Therefore, we aimed to investigate predictors of renal function decline, especially modifiable ones, in subjects over a broad eGFR range. For this analysis, we used data of subjects who participated in a community-based prospective cohort study. As outcome variable we calculated for each participant the slope through three eGFR values over time. Multivariate regression analysis was applied to identify variables that were associated with renal function decline.

RESULTS

Mean follow-up of the 5488 subjects in this analysis was 6.5 years (35,500 person-years of follow-up). Baseline characteristics of the overall study population are given in Table 1. Of note, the results of multivariate linear regression analyses showed that gender was a strong effect modifier, because a significant interaction term was found between urinary

Table 1 | Baseline characteristics for the overall study population and for males and females separately

	Overall (*N*=5488)	Males (*N*=2770)	Females (*N*=2718)	*P*-value
Age (years)	49 (12)	50 (12)	48 (11)	<0.001
Smoking (%)	34.5	34.3	34.7	NS
Positive family history (%)	31.3	31.1	31.4	0.003
Waist circumference (cm)	88.1 (12.7)	93.3 (10.8)	82.8 (12.3)	<0.001
SBP (mm Hg)	128 (19)	133 (18)	123 (19)	<0.001
Antihypertensive medication (%)	14.5	15.5	13.5	0.012
ACEi/A2A medication (%)	4.2	5.1	3.2	<0.001
Cholesterol/HDL ratio	4.6 (1.8)	5.2 (1.9)	4.0 (0.5)	<0.001
Triglycerides (mmol/l)†	1.1 (0.8–1.7)	1.3 (0.9–1.9)	1.0 (0.8–1.5)	<0.001
Lipid lowering medication (%)	6.2	7.0	5.4	<0.001
Glucose (mmol/l)	4.9 (1.2)	5.0 (1.3)	4.7 (1.0)	<0.001
Antidiabetic medication (%)	1.3	1.4	1.2	NS
CRP (mg/l)†	1.20 (0.53–2.74)	1.16 (0.52–2.49)	1.24 (0.53–3.03)	0.001
UAE (mg/24 h)†	9.0 (6.2–15.3)	10.0 (6.8–18.8)	8.1 (5.8–12.9)	<0.001
Urinary urea excretion (mmol/24 h)	361 (103)	397 (105)	324 (88)	<0.001
Urinary sodium excretion (mmol/24 h)	143 (50)	159 (52)	127 (42)	<0.001
eGFR (ml/min/1.73 m^2)	80.7 (13.9)	83.8 (14.3)	77.5 (12.7)	<0.001

ACEi, angiotensin converting enzyme inhibitors; A2A, angiotensin-II antagonists; CRP, hs C-reactive protein; eGFR, estimated glomerular filtration rate (MDRD); HDL, high-density lipoprotein; SBP, systolic blood pressure; NS, not significant; UAE, urinary albumin excretion.
Values are given as mean (s.d.), or median (interquartile range) in case of skewed data (†) distribution. Statistical analyses, to test the differences between males and females, were performed with *t*-test, Mann–Whitney test in case of skewed distribution, or χ^2 test in case of categorical variables.

albumin excretion (UAE) and gender ($P<0.001$), and between cholesterol/high-density lipoprotein (HDL) ratio and gender ($P=0.005$) versus the change in eGFR over time. The statistical significance of these interaction terms indicate that the association between UAE versus outcome and cholesterol/HDL ratio versus outcome is not similar in males and females. Therefore, we stratified all further analyses by gender. Consequently, Table 1 shows also baseline characteristics for males ($n=2770$) and females ($n=2718$) separately. At baseline, males had a significantly higher waist circumference, systolic blood pressure, percentage ACEi/A2A treatment, cholesterol/HDL ratio, percentage lipid lowering treatment, triglycerides, plasma glucose, and a higher UAE, urea, and sodium excretion and also a higher eGFR. For males, the range of UAE is 1.18–2960 mg/24 h and for females it is 1.0–3610 mg/24 h. For males, the eGFR values are between 23.2 and 155.9 ml/min/1.73 m^2 and for females the values are between 21.9 and 136.3 ml/min/1.73 m^2. The mean eGFR slope over time in males was -0.55 ± 1.47 ml/min/1.73 m^2/year, and in females it was -0.33 ± 1.41 ml/min/1.73 m^2/year ($P<0.001$ for males versus females). The mean serum creatinine levels during the three screening rounds were 83.6 ± 14.4, 84.9 ± 19.0, and 85.1 ± 22.9 μmol/l, respectively.

Table 2 shows the results of the univariate linear regression analyses, and Table 3 shows the effect of correction for age and baseline eGFR. In both males and females, systolic blood pressure, plasma glucose, and UAE were significantly and negatively associated with slope of renal function, indicating that a higher systolic blood pressure, plasma glucose and UAE were associated with a larger decline in eGFR. Other variables were only associated with renal function decline in one of the genders. For instance, Ln CRP was only associated with renal function decline in females. Surprisingly, some variables were associated differently in the two genders: in males, lower waist circumference and a lower cholesterol/HDL ratio predicted accelerated renal function decline, whereas in females an opposite association was found.

Tables 4 and 5 present the results of the gender-specific multivariate linear regression models. A higher (absolute) standardized beta value indicates a stronger association between the independent variable and the outcome change in eGFR over time. In males, a higher systolic blood pressure, plasma glucose, and UAE were associated with more renal function decline. In contrast, a higher waist circumference and cholesterol/HDL ratio were associated with less renal function decline over time. The quadratic terms of UAE and cholesterol/HDL ratio were significant in the linear regression model. In females, results were slightly different, insofar that only systolic blood pressure, plasma glucose, and cholesterol/HDL ratio were associated with more renal function decline, whereas triglycerides were found to be associated with less renal function decline. In this model, inclusion of the quadratic term of SBP was significant.

Figure 1 presents the graphical interpretation of the associations between independent variables and eGFR slope that were identified by multivariate regression analysis. More renal function decline is observed in the higher range of systolic blood pressure, glucose, and UAE, both in males and in females. The curves for cholesterol/HDL ratio and waist circumference versus change in eGFR over time, however, show opposite patterns for males and females.

A priori-defined sensitivity analyses were performed. To investigate whether inclusion of only subjects with reliable slopes influences the results, we excluded the 773 males and 812 females with observed eGFR values that were not within the 99% confidence interval of the expected eGFR value. The results obtained were only slightly different. In males, the cholesterol/HDL ratio was not significantly associated with change in renal function, whereas in females,

Table 2 | Univariate associations between baseline characteristics and change in renal function during follow-up (assessed as slope through three eGFR values over time per individual)

	Males (*N*=2770)		Females (*N*=2718)	
	Standardized beta	*P*-value	Standardized beta	*P*-value
Smoking	0.005	NS	−0.003	NS
Positive family history	−0.003	NS	−0.010	NS
Waist circumference	0.052	0.006	−0.023	NS
SBP	−0.074	<0.001	−0.046	0.016
Cholesterol/HDL ratio	0.069	<0.001	−0.049	0.011
Ln_Triglycerides	0.054	0.005	0.015	NS
Glucose	−0.135	<0.001	−0.104	<0.001
Ln_CRP	−0.026	NS	−0.028	NS
Ln_UAE	−0.126	<0.001	−0.038	0.045
Urinary urea excretion	0.025	NS	−0.025	NS
Urinary sodium excretion	0.021	NS	−0.027	NS

CRP, hs C-reactive protein; eGFR, estimated glomerular filtration rate (MDRD); HDL, high density lipoprotein; NS, not significant; SBP, systolic blood pressure; UAE, urinary albumin excretion.
In case of skewed distribution, data were Ln-transformed to obtain normal data distribution. A negative standardized beta indicates that the higher the value of the variable under study, the more negative the slope of renal function over time is.

Table 3 | Associations between baseline characteristics and change in renal function during follow-up (assessed as slope through three eGFR values over time per individual), with correction for baseline eGFR and age.

	Male (*N*=2770)		Female (*N*=2718)	
	Standardized beta	*P*-value	Standardized beta	*P*-value
Smoking	0.027	NS	0.018	NS
Positive family history	−0.005	NS	0.002	NS
Waist circumference	0.048	0.014	−0.039	0.046
SBP	−0.092	<0.001	−0.074	<0.001
Cholesterol/HDL ratio	0.052	0.005	−0.056	0.004
Ln_Triglycerides	0.029	NS	−0.010	NS
Glucose	−0.126	<0.001	−0.094	<0.001
Ln_CRP	−0.017	NS	−0.038	0.041
Ln_UAE	−0.145	<0.001	−0.046	0.012
Urinary urea excretion	0.020	NS	−0.026	NS
Urinary sodium excretion	0.022	NS	−0.022	NS

CRP, hs C-reactive protein; eGFR, estimated glomerular filtration rate (MDRD); HDL, high density lipoprotein; NS, not significant; SBP, systolic blood pressure; UAE, urinary albumin excretion.
In case of skewed distribution, data were Ln-transformed to obtain normal data distribution. A negative standardized beta indicates that the higher the value of the variable under study, the more negative the slope of renal function over time.

Table 4 | Multivariate model for males explaining change in renal function during follow-up (assessed as slope through three eGFR values over time per individual)

	Male (*N*=2770)	
	Standardized beta	*P*-value
Ln_UAE^2	−0.581	<0.001
Cholesterol/HDL ratio^2	0.129	<0.001
Waist circumference	0.102	<0.001
Glucose	−0.096	<0.001
SBP	−0.064	0.003

eGFR, estimated glomerular filtration rate (MDRD); HDL, high-density lipoprotein; SBP, systolic blood pressure; UAE, urinary albumin excretion.
Variables included in the multivariate prediction model are statistically significant associated with slope of renal function over time. The model is adjusted for baseline eGFR, age, and the use of medication. In case quadratic terms of variables proved to contribute significantly to the model (indicated with ^{2}), the single term was also forced into the model. For reasons of clarity, only results with respect to the quadratic term are shown in the table. In case of skewed distribution, data were Ln-transformed to obtain normal data distribution. A negative standardized beta indicates that the higher the value of the variable under study, the more negative the slope of renal function over time. The variables are ranked on the order of the standardized beta.

Table 5 | Multivariate model for females explaining change in renal function during follow-up (assessed as slope through three eGFR values over time per individual)

	Female (*N*=2718)	
	Standardized beta	*P*-value
SBP^2	−0.359	0.026
Glucose	−0.067	0.002
Cholesterol/HDL ratio	−0.061	0.014
Ln_Triglycerides	0.052	0.038

eGFR, estimated glomerular filtration rate (MDRD); HDL, high-density lipoprotein; SBP, systolic blood pressure.
Variables included in the multivariate prediction model are statistically significant associated with slope of renal function over time. The model is adjusted for baseline eGFR, age, and the use of medication. In case, quadratic terms of variables proved to contribute significantly to the model (indicated with ^{2}), the single term was also forced into the model. For reasons of clarity, only results with respect to the quadratic term are shown in the table. In case of skewed distribution, data were Ln-transformed to obtain normal data distribution. A negative standardized beta indicates that the higher the value of the variable under study, the more negative the slope of renal function over time. The variables are ranked in order of the standardized beta.

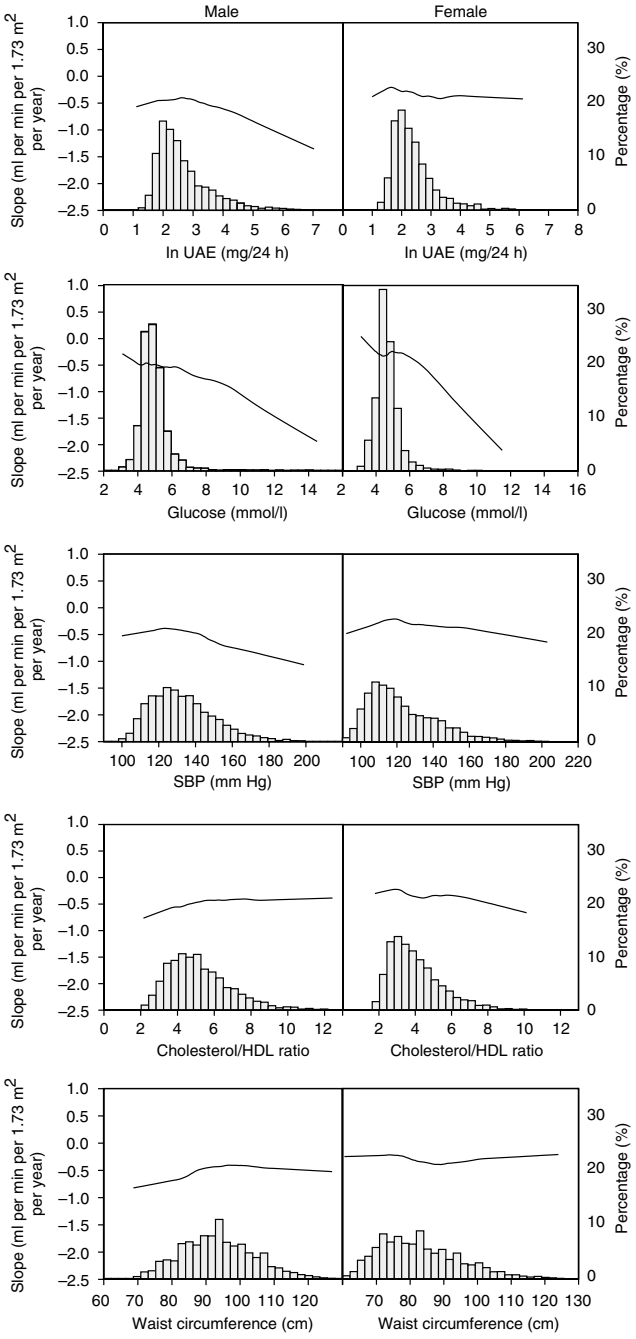

Figure 1 | Graphical representation of the association between risk predictors and change in renal function during follow-up (assessed as slope through three eGFR values over time per individual). The Loess method (locally weighted polynomial regression analysis)[52] is used to plot these associations. Using this method makes it possible to show a possible nonlinear relationship, as the plots are 'distribution free'. The histograms (expressed as percentage) present the distributions of the predictors. HDL, high-density lipoprotein; SBP, systolic blood pressure; UAE, urinary albumin excretion.

only triglycerides were not found to be associated with outcome anymore. The sensitivity analysis performed with a mixed effects model with random intercepts and random slopes resulted in models with the same variables included. Additionally, we investigated the potential role of hormonal status. For this purpose, we repeated the multivariate linear regression analysis only in postmenopausal females ($N = 1107$). Similar results were obtained as in the overall group of females. Furthermore, we performed an analysis using a relative instead of absolute measure for renal function decline and an analysis using slopes through the reciprocals of serum creatinine values as outcome variables. The results of these analyses were essentially similar to our primary analyses, as were the results of the analyses performed in a subcohort representative for the general population.

DISCUSSION

In this study, we investigated which modifiable risk factors are associated with change in renal function during follow-up in a community-based study cohort. We found different results for males versus females. In males, UAE was the strongest independent predictor of greater renal function decline, together with plasma glucose and systolic blood pressure. In contrast, waist circumference and cholesterol/HDL ratio were associated with a better renal function outcome. In females, systolic blood pressure and plasma glucose were independent risk predictors of renal function decline, whereas triglycerides were associated with better renal prognosis.

The interest in identification of modifiable risk factors of renal function decline is increasing. Such risk factors may be used to estimate a subject's risk of future renal function decline and may also form the basis for preventive intervention. The mean eGFR decline we found in this study is low, probably not pathological and does not warrant intervention. However, the goal of this study was to identify predictors of accelerated renal function loss. Most observational studies investigating this issue apply 'threshold' analysis, using a cut-off value to indicate that subjects reach a certain stage of CKD. Most common cut-off values are the incidence of ESRD (defined as start of renal replacement therapy) or *de novo* K/DOQI CKD stage 3 or 4 (defined as eGFR below 60 or 30 ml/min/1.73 m^2).[9–12] This study applies a 'slope' analysis. The choice of slope versus threshold analysis has received scant attention, but has important implications. This is illustrated by the following theoretical example. It is known that in obese subjects GFR values estimated with the modification of diet in renal disease (MDRD) formula are considerably lower than their true GFR because, in general, obesity is associated with more muscle mass.[13] Therefore, obese subjects at similar baseline true GFR and at similar rate of true GFR loss as nonobese subjects will reach an MDRD formula-based eGFR threshold of 30 or 60 ml/min/1.73 m^2 earlier than their nonobese counterparts. This suggests that obesity is a risk factor for renal function decline, whereas a slope analysis would not have led to this conclusion. One might consider that application of an eGFR-independent threshold, such as the occurrence of ESRD, might circumvent this problem. However, obesity has

been found to be associated with better survival in subjects in renal replacement therapy.[14] In case this would also be true for K/DOQI CKD stage 4, obese subjects would survive 'preferentially'. This will result in the observation that the proportion of obese subjects that reaches ESRD is higher than in the general population, again leading to the possible incorrect conclusion that obesity is associated with worse renal function outcome. For these reasons, together with the fact that we wanted to study risk factors of renal function decline over the entire eGFR range, we adopted a slope-based analysis with change in renal function during follow-up as outcome parameter. We also performed our analyses using slopes based on the reciprocals of serum creatinine values and using relative change in eGFR as outcome parameters. These analyses resulted in the identification of the same predictors, making our results convincing.

Applying a slope-based analysis, we found in both males and females higher systolic blood pressure and higher plasma glucose to be major determinants of change in renal function. This is in line with other studies investigating community-based populations, but applying threshold analysis. High plasma glucose has been shown to be a risk factor of the development of CKD[15] and ESRD.[16] The same holds true for high blood pressure.[12,17–19] Interestingly, similar to our study, a study performed in Maryland, USA, showed in both males and females a strong relationship between systolic blood pressure and the development of CKD, with the relationship being strongest in females and the cumulative incidence of ESRD increasing exponentially in the more severe stages of hypertension.[20] Of note, we found a difference of 10 mmHg in systolic blood pressure levels between males and females. This result is in line with other community-based studies.[21–24]

We found UAE to be the best predictor of renal function decline in males. This association was independent of the effects of systolic blood pressure and plasma glucose. Although most evidence on the impact of urinary albumin leakage and renal prognosis is based upon data on overt proteinuria in subjects with nondiabetic[25,26] and diabetic[27,28] renal disease, there is also evidence that lower amounts of protein leakage predict renal function decline in the general population. Iseki *et al.*[29] showed that subjects with trace dipstick-positive proteinuria already have an increased incidence of ESRD during follow-up, as did the MRFIT study.[10] In a previous analysis, we found in the Prevention of Renal and Vascular End-stage Disease (PREVEND) study that, after 4.2 years of follow-up, subjects with microalbuminuria progress more frequently to an eGFR below a threshold of 60 ml/min/1.73 m^2 than subjects without microalbuminuria.[30] Remarkably, this study found that UAE was not an independent predictor for renal function decline in females. However, in females, the negative association between UAE and renal function decline was significant when tested univariately, and also when corrected for baseline eGFR and age. We repeated our analyses using the albumin/creatinine ratio with correction for age and gender.[31,32] The results of these analyses also showed that albuminuria was an independent predictor for renal function decline in males, but not in females. Most studies concerning predictors of renal function decline do not report gender differences in outcome. Possibly, this was not studied specifically, and therefore gender differences may have remained unnoticed. Furthermore, we should take into account that we applied a slope-based analysis, in contrast to other studies. Indeed, when we performed a threshold analysis and investigated which subjects developed an eGFR less than 60 ml/min/1.73 m^2 during follow-up, we also found no significant interaction between gender and UAE on outcome ($P = 0.53$). However, our data on gender-specific renal effects are in line with a previous report showing that males have a higher UAE for a given age, plasma glucose, and BMI than females.[33]

Another gender difference was observed with respect to the impact of cholesterol/HDL ratio on change in renal function. In females, a higher cholesterol/HDL ratio was associated with more renal function decline, whereas we found the opposite in males. Literature on cholesterol as an independent predictor for renal function impairment is not consistent, with some community-based studies finding total cholesterol and/or triglycerides not to be an independent predictor for the onset of CKD[9] or ESRD,[34] whereas others found these variables to be associated with worse renal outcome.[34–36] A possible explanation for these inconsistent results might be differences in length of follow-up, with especially studies with longer follow-up suggesting that high cholesterol and/or triglycerides influence renal function outcome negatively.[36,37]

Surprisingly, we found that a greater waist circumference was not associated with a worse renal prognosis. In fact, in males, waist circumference was associated with a better renal prognosis. Several other community-based studies reported an association between a higher BMI and increased risk for CKD.[11,38,39] As mentioned before, these contradictory findings might be due to the fact that in this study a slope-based analysis is used, whereas other studies applied a threshold analysis. When we analyzed which subjects developed an eGFR less than 60 ml/min/1.73 m^2 during follow-up, we did not find a positive association between waist circumference and change in renal function, neither in the overall population, nor when we analyzed males separately. Another explanation may again be duration of follow-up. Data from the Okinawa screening project in Japan showed that 10 years follow-up was not sufficient to establish a relationship between BMI and the risk of developing ESRD,[40] whereas data of 17 years follow-up did show such an association.[38] Another study with longer duration of follow-up (13.2 years) also showed an association between obesity and renal prognosis.[41] Thus, it could be that the follow-up of 6 years in our study is not sufficient to find a negative association between waist circumference and renal function decline. However, the fact that we found even a positive association between waist circumference and change in renal

function in males in our slope-based analyses is surprising, and worth further study.

Strengths of this study are the use of a large prospective community-based cohort, with three eGFR estimates available. Our study is one of the first studies that investigates risk factors of renal function decline by a slope-based analysis. Of course, also limitations should be kept in mind. First, the relatively short follow-up in our study and the fact that there are only three eGFR measurements available may have influenced the precision of the calculated slope. Therefore, we performed a sensitivity analysis, including only subjects with 'reliable' slopes. This did not essentially affect our results. Second, by using the calculated slope, we assume a linear change in renal function over time. Although it is generally used in nephrology, it is questionable whether this assumption of linearity is correct. However, on these data (three values available), it is appropriate to assume a linear change.[42] Third, our results may be biased by the influence of loss to follow-up. Participants who died or were lost to follow-up for other reasons may have been in a worse condition of health. In general, such subjects have a higher rate of renal function decline. Therefore, in our analyses, the impact of risk factors may have been underestimated. To investigate the potential bias induced by loss to follow-up, we performed a mixed effects models analysis, which takes into account also data available of subjects who attended only one or two screenings. This again did not influence our main conclusions. Fourth, we used the MDRD formula to estimate the GFR. It is known that the use of the MDRD formula has shortcomings.[43,44] Estimating GFR with the MDRD formula may introduce bias.[45] As this bias is systematic, that is, constant in a particular subject, this bias is expected not to influence our analyses, as we use change in eGFR as outcome parameter, and not the absolute value of eGFR. In addition, at this moment, there is no other feasible method to estimate GFR, and the MDRD-based GFR estimates are easy to interpret for clinicians. Fifth, we studied a relatively healthy Caucasian population. Our findings may therefore not be valid for other populations.

In conclusion, this community-based observational population study investigated which modifiable variables were associated with change in renal function during follow-up, applying a slope-based analysis. Results differed between males and females. High systolic blood pressure and plasma glucose were found to be independent predictors for worse renal outcome in both males and females. In males, UAE was identified as the strongest independent predictor for renal function decline. In females, UAE was only univariately, and after adjustment for age, associated with change in renal function. The direction of the association between cholesterol/HDL ratio and change in renal function was different in males and females. In males, predictors independently associated with a better renal function outcome were waist circumference and cholesterol/HDL ratio, whereas in females this was higher triglycerides. Our findings suggest that, in future studies, possible gender-specific risk predictors for renal function decline should be taken into account. Furthermore, these data may help to make renal risk prediction scores to identify subjects in the general population at risk of renal function decline, who may benefit from early preventive intervention.

MATERIALS AND METHODS

Study design and population

The analyses are based on data of subjects who participated in the first three screening rounds of the PREVEND study. This is a prospective cohort study, designed to investigate the impact of UAE on renal and cardiovascular outcome in the general population. In 1997–1998, the participants of the PREVEND cohort were selected from 40,856 inhabitants of the city of Groningen, the Netherlands. Selection was based on the albumin concentration in a spot morning urine sample to obtain a cohort enriched for the presence of elevated albuminuria levels. At approximately 3-year intervals, participants in this study are invited to visit an outpatient department for measurements concerning their health status. Details of the study protocol have been published elsewhere.[46,47]

In total, 8592 participants completed the first screening round in 1997–1998. Approximately 6.5 years later, from 2003 to 2006, the third screening round took place. During the interval between the first and the third screening round, 377 subjects died and 2415 patients were lost to follow-up, however, with vital status known (Figure 2). Thus, 5862 subjects completed the third screening round. For this study, we excluded subjects who indicated in a questionnaire to have a renal disease during the first screening round ($n=22$). Subjects with missing information on eGFR in one of the three screening rounds were also excluded ($n=352$), leaving 5488 subjects for analysis. The PREVEND study was approved by the local medical ethics committee and conducted in accordance with the Declaration of Helsinki Principles. All participants gave written informed consent.

Measurements and definitions

Each screening round consisted of two visits to an outpatient department separated by approximately 3 weeks. Participants filled

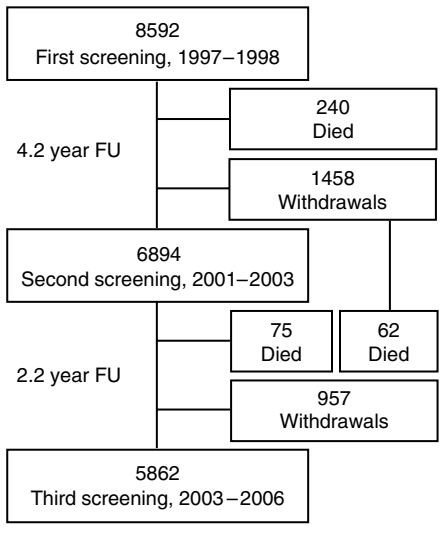

Figure 2 | Flowchart of the PREVEND study.

out a questionnaire on demographics, cardiovascular and renal history, smoking status, menstrual status, and the use of oral antidiabetic, antihypertensive, and lipid-lowering drugs. A positive family history of cardiovascular disease was defined as having a first-degree family member who experienced a cerebrovascular accident, myocardial infarction, or intervention for peripheral vascular disease before the age of 65 years. Postmenopausal status for females was defined as the absence of menstruation for at least 6 months before the first screening. Smoking was defined as current smoking, or cessation of smoking less than a year before the baseline screening. Information on drug use was completed with data from community pharmacies. During both study visits per screening round, blood pressure was measured in the right arm, every minute for 10 and 8 min, respectively, by an automatic Dinamap XL Model 9300 series device (Johnson-Johnson Medical INC, Tampa, FL, USA). For systolic blood pressure, the mean of the last two recordings from each of the two visits was used. Anthropometrical measurements were performed, and fasting blood samples were taken. In addition, subjects collected urine for two consecutive periods of 24 h. Concentrations of total cholesterol, HDL-cholesterol, triglycerides, glucose, C-reactive protein, and urinary urea and sodium were measured using standard methods. Serum creatinine was measured by dry chemistry (Eastman Kodak, Rochester, NY, USA), with intra- and interassay coefficients of variation of 0.9 and 2.9%, respectively. Urinary albumin concentration was determined by nephelometry (Dade Behring Diagnostic, Marburg, Germany), and UAE was given as the mean of the two 24 h urinary excretions. eGFR was estimated using the MDRD study equation, taking into account gender, age, race, and serum creatinine.[48]

Statistical analyses

Baseline characteristics of subjects included in this analysis are given in Table 1. Continuous data are reported as the mean and standard deviation. For skewed distributions, the median and interquartile range are presented. To identify risk factors of renal function decline, we performed linear regression analyses. For all our regression models, we used change in eGFR over time as outcome variable. This variable was defined for each subject as the slope of the linear regression line through their three eGFR measurements that were obtained at the consecutive screening rounds in the PREVEND study. As possible predictors of renal function decline, all variables tested are enlisted in Table 1, as these have all been suggested to influence renal outcome in patients with known renal disease. When necessary, these variables were Ln-transformed to obtain normal data distribution. First, we performed univariate regression analysis. Second, we repeated this analysis, with correction for age and gender, as these factors are nonmodifiable. We also corrected for baseline eGFR to reduce the effect of regression to the mean. Third, we performed forward multivariate regression analysis. A *P*-value of 0.05 was adopted as the entry criterion for including variables in the regression model. Because of the reasons described above, gender, age, and baseline eGFR were forced in the model. As the use of medication interfering with the variables under study (for example, antihypertensives) may influence results, we corrected the multivariate models (Tables 4 and 5) for the use of medication. All variables under study were tested for possible nonlinear associations by adding quadratic terms to the multivariate regression model and to test their inclusion for statistical significance. Furthermore, we explored possible effect modification by implementing interaction terms, for all variables that significantly contributed to the multivariate regression model in the model. It was *a priori* decided that, in case a significant interaction was to be found, all models were to be built taking into account this interaction. Lastly, graphical representations were made of the final models, showing the relation between the most important predictors and the change in renal function. For this analysis, the mean slope of renal function over time was calculated per quintile of each predicting variable.

All analyses were conducted with the statistical package SPSS 14.0 (SPSS, Chicago, IL, USA). A *P*-value of 0.05 or less was adopted to indicate statistical significance.

Sensitivity analyses

Various sensitivity analyses were performed. First, we performed a sensitivity analysis including only subjects in whom a 'reliable' slope of renal function over time could be calculated. To judge slopes as reliable, we calculated the theoretical 99% confidence interval of the expected eGFR value for every observed eGFR measurement. This confidence interval is determined by intrapatient day-to-day coefficient of variation (CV) in true GFR, and by measurement error in serum creatinine. The CV in true GFR and creatinine measurement in our institution has previously been shown to be 2.2 and 1.1%, respectively.[49,50] The overall CV for the expected GFR can be calculated according to the formula $\sqrt{((\text{CV true GFR})^2 + (\text{CV creatinine})^2)}$ and the 99% confidence interval. In case all three observed eGFR values of a subject were within the calculated 99% confidence intervals of the expected eGFR values, we defined the slope as reliable (males $n = 2007$, females $n = 1911$). Second, we performed sensitivity analyses to test whether loss to follow-up influenced results. For this reason, we repeated our analyses using a mixed effects model with random intercepts and random slopes. Such a model estimates the rate of change in eGFR over time, including also subjects with only one or two eGFR measurements.[42] Third, we repeated our analyses using the following outcomes: (a) percentage change in eGFR and (b) slopes through the reciprocals of serum creatinine. Fourth, to investigate whether the enrichment for albuminuria in our cohort influenced the results, we performed our analyses in a subcohort representative for the general population ($N = 2269$). A detailed description how this cohort was formed has been published previously.[51]

ACKNOWLEDGMENTS

We thank Dade Behring (Marburg, Germany) for supplying equipment (Behring Nephelometer II) and reagents for nephelometric measurement of urinary albumin concentration. The PREVEND Study has been made possible by grants of the Dutch Kidney Foundation.

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