

# High Protein Intake Associates with Cardiovascular Events but not with Loss of Renal Function

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## ABSTRACT

The long-term effects of higher dietary protein intake on cardiovascular and renal outcomes in the general population are not clear. We analyzed data from 8461 individuals who did not have renal disease and participated in two or three subsequent screenings (6.4-yr follow-up) in a prospective, community-based cohort study (Prevention of Renal and Vascular ENd-stage Disease [PREVEND]). We calculated daily protein intake from 24-h urinary urea excretion (Maroni formula) and used Cox proportional hazard models to analyze the associations between protein intake, cardiovascular events, and mortality. We used mixed-effects models to investigate the association between protein intake and change in renal function over time. The mean  $\pm$  SD daily protein intake was  $1.20 \pm 0.27$  g/kg. Protein intake was significantly associated with cardiovascular events during follow-up. The associations seemed U-shaped; compared with intermediate protein intake, individuals with either higher or lower protein intake had higher event rates. All-cause mortality and noncardiovascular mortality also were significantly associated with protein intake; individuals with low protein intake had the highest event rates. We found no association between baseline protein intake and rate of renal function decline during follow-up. In summary, in the general population, high protein intake does not promote accelerated decline of renal function but does associate with an increased risk for cardiovascular events.

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Protein restriction is often prescribed to slow the progression of renal failure in patients with chronic kidney disease (CKD). The Modification of Diet in Renal Disease (MDRD) study, designed to clarify the role of protein restriction in CKD, supports the role of dietary protein restriction but provides no conclusive evidence for renoprotection.<sup>1,2</sup> Meta-analyses on this topic also do not provide convincing results. Uncertainty about the optimal level and duration of dietary protein restriction, together with the possibility of publication bias, which has been suggested by the authors of two meta-analyses,<sup>3,4</sup> make it questionable whether low-protein diets should be applied to patients with CKD. In the Nurses' Health Study, the influence of daily protein intake (assessed with a food frequency questionnaire) on the long-term course of renal function

was investigated in individuals with normal renal function and in individuals with mild renal insufficiency. In this study, high protein intake was associated with accelerated renal function decline in individuals with a baseline renal function  $<80$  ml/min per  $1.73$  m<sup>2</sup> but not in women with normal renal function.<sup>5</sup>

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A few studies have been published on the effects of protein intake on all-cause mortality, in particular on cardiovascular morbidity and mortality. Interest in these effects was based on the fact that in Western populations, dietary pattern changes toward diets with high protein intake. Indeed, low-carbohydrate diets, which frequently contain a high amount of protein, have become very popular to prevent obesity.<sup>6–10</sup> In one of these studies, carried out in a cohort of Swedish women, high protein intake in middle-aged women was associated with a higher cardiovascular mortality.<sup>11</sup> These findings, however, were not in line with the results of the Nurses' Health Study, which showed a decreased risk for coronary heart disease in individuals with high protein intake.<sup>12,13</sup> The effect of high protein intake on both renal function and cardiovascular mortality and morbidity in the general population is thus not well known; therefore, the aim of our study was to investigate the associations between protein intake and cardiovascular and renal outcomes in a cohort derived from the general population.

## RESULTS

Mean  $\pm$  SD daily protein intake was  $1.20 \pm 0.27$  g/kg for the total population,  $1.21 \pm 0.27$  g/kg for men, and  $1.18 \pm 0.26$  g/kg for women. Daily protein intake varied widely between individuals, with the 5th and 95th percentiles being 0.80 to 2.66 g/kg. Of the 8461 individuals, 502 had an estimated GFR (eGFR)  $<60$  ml/min per  $1.73$  m<sup>2</sup>. The characteristics of our study population according to the gender-stratified quintiles

of protein intake are listed in Table 1. In the highest compared with the lowest quintiles of protein intake, individuals were heavier and had higher BP, cholesterol, plasma glucose, urinary albumin, and sodium excretion. In contrast, the percentage of smokers was lowest in the quintile with the highest protein intake, as was the prevalence of a previous cardiovascular disease history. eGFR did not differ over the quintiles of protein intake.

Mean follow-up for cardiovascular events was  $7.0 \pm 1.6$  yr, resulting in 59,240 person-years of follow-up. The incidence of cardiovascular events during follow-up was highest in the first and last quintiles of protein intake (Table 2). The association between protein intake (used as a continuous variable) and cardiovascular event rate seemed to be significant and not linear but U-shaped. The quadratic term of protein intake could be added to these Cox regression models (crude, adjusted for age and gender and adjusted for known cardiovascular risk factors [all  $P < 0.05$ ]). Figure 1 shows the graphic representation of the associations under investigation; for these figures, we divided protein intake in quintiles. Of note, the  $P$  values in the figures refer to the associations with protein intake as a continuous variable included in the models. Figure 1A shows the graphic representations of the association between protein intake and cardiovascular event rate, corrected for age and gender and also corrected for known cardiovascular risk factors. After these adjustments, this association still remained statistically significant. To check whether the association between protein intake and the incidence of cardiovascular events was confounded by the amount of sodium intake or body mass index (BMI), we also adjusted the model for

**Table 1.** Baseline characteristics per gender-stratified quintile of daily protein intake<sup>a</sup>

Characteristic	Gender-Stratified Quintiles of Daily Protein Intake (g/kg)					P for Trend
	First	Second	Third	Fourth	Fifth	
Male	0.26 to 0.99	0.99 to 1.13	1.13 to 1.26	1.26 to 1.42	1.42 to 3.27	
Female	0.39 to 0.96	0.96 to 1.10	1.10 to 1.22	1.22 to 1.38	1.38 to 2.88	
N	1692	1692	1693	1692	1692	
Age (yr) <sup>b</sup>	49.0 (13.3)	50.0 (13.3)	49.7 (12.9)	50.0 (12.4)	50.2 (11.4)	0.010
Smoking (%)	51.4	40.0	34.4	32.7	31.9	$<0.001$
Cardiovascular disease history (%)	13.8	12.1	11.9	9.7	10.1	$<0.001$
Weight (kg) <sup>b</sup>	75.1 (13.4)	76.3 (13.2)	77.3 (13.8)	79.6 (14.0)	82.8 (15.5)	$<0.001$
BMI (kg/m <sup>2</sup> ) <sup>b</sup>	24.6 (3.8)	25.3 (3.6)	25.7 (3.8)	26.6 (4.0)	28.2 (4.2)	$<0.001$
SBP (mmHg) <sup>b</sup>	127 (21)	129 (21)	129 (21)	130 (20)	131 (20)	$<0.001$
DBP (mmHg) <sup>b</sup>	73 (10)	74 (10)	74 (10)	74 (10)	75 (10)	$<0.001$
Cholesterol (mmol/L) <sup>b</sup>	5.6 (1.2)	5.6 (1.1)	5.6 (1.1)	5.7 (1.1)	5.7 (1.1)	$<0.001$
Triglycerides (mmol/L) <sup>c</sup>	1.2 (0.9 to 1.7)	1.1 (0.8 to 1.7)	1.1 (0.8 to 1.7)	1.2 (0.8 to 1.7)	1.2 (0.9 to 1.8)	NS
Glucose (mmol/L) <sup>b</sup>	4.7 (1.0)	4.8 (1.0)	4.9 (1.2)	4.9 (1.1)	5.1 (1.5)	$<0.001$
CRP (mg/L) <sup>c</sup>	1.3 (0.5 to 3.0)	1.2 (0.6 to 2.9)	1.2 (0.5 to 2.9)	1.3 (0.5 to 2.9)	1.5 (0.6 to 3.2)	NS
UAE (mg/24 h) <sup>c</sup>	7.8 (5.4 to 14.2)	8.9 (6.0 to 16.1)	9.5 (6.4 to 16.7)	10.0 (6.6 to 19.3)	11.4 (7.4 to 23.6)	$<0.001$
Urinary urea excretion (mmol/24 h) <sup>b</sup>	233 (53)	303 (48)	349 (52)	398 (61)	487 (88)	$<0.001$
Urinary sodium excretion (mmol/24 h) <sup>b</sup>	106 (39)	127 (40)	141 (43)	154 (46)	180 (54)	$<0.001$
eGFR (ml/min per $1.73$ m <sup>2</sup> ) <sup>b</sup>	81.2 (15.7)	80.1 (15.1)	80.4 (14.2)	80.2 (13.7)	81.1 (13.8)	NS

<sup>a</sup>CRP, C-reactive protein; DBP, diastolic BP; IQR, interquartile range; SBP, systolic BP; UAE, urinary albumin excretion.

<sup>b</sup>Mean (SD).

<sup>c</sup>Median (IQR).

**Table 2.** Numbers of cardiovascular events, all-cause mortality, and noncardiovascular mortality and mean change in renal outcome per year, and accompanying event rates per quintile of daily protein intake

Parameter	Gender-Stratified Quintiles of Daily Protein Intake (g/kg)					P for Trend
	First (0.26 to 0.99)	Second (0.96 to 1.13)	Third (1.10 to 1.26)	Fourth (1.22 to 1.42)	Fifth (1.38 to 3.27)	
N	1692	1692	1693	1692	1692	
Cardiovascular events (n)	121	116	109	101	119	<0.001
Event rate (no. of events per 1000 patient-years)	10.4	9.9	9.1	8.5	9.9	
All-cause mortality (n)	123	93	79	77	71	<0.001
Event rate (no. of events per 1000 patient-years)	10.3	7.7	6.4	6.3	5.8	
Non-cardiovascular mortality (n)	94	65	52	45	48	<0.001
Event rate (no. of events per 1000 patient-years)	7.8	5.4	4.2	3.7	3.9	
Change in eGFR (ml/min per 1.73 m <sup>2</sup> /yr)	-0.45	-0.46	-0.50	-0.41	-0.41	NS

these variables. These adjustments did not materially affect our findings.

We next investigated the association of protein intake with all-cause mortality and noncardiovascular mortality. Duration of follow-up for these variables was  $7.2 \pm 1.3$  yr. The unadjusted results in Table 2 and the graphic representation of the adjusted Cox regression models in Figure 1, B and C, show a negative association between protein intake and all-cause and noncardiovascular mortality. Adjustment for age and gender, known cardiovascular risk factors, sodium intake, and BMI did not change these results. These associations were also analyzed in Cox regression models treating protein intake as a continuous variable. In all of these models, protein intake was significantly (negatively) associated with the incidence of all-cause and noncardiovascular mortality. The *P* values in Figure 1, B and C, refer to the results of these models.

The change in renal function over time did not differ between the quintiles of protein intake (Table 2). To investigate further the relation between the change in renal function, we analyzed our data in a mixed-effects model. In the univariate model, no significant association was found between the amount of protein intake and the change in renal function. Adjustment for age and gender and also further adjustment for known cardiovascular risk factors and for sodium intake and BMI did not change these results (Figure 1D). The *P* values in Figure 1D refer to the results of the mixed-effects models in which protein intake was treated as a continuous variable.

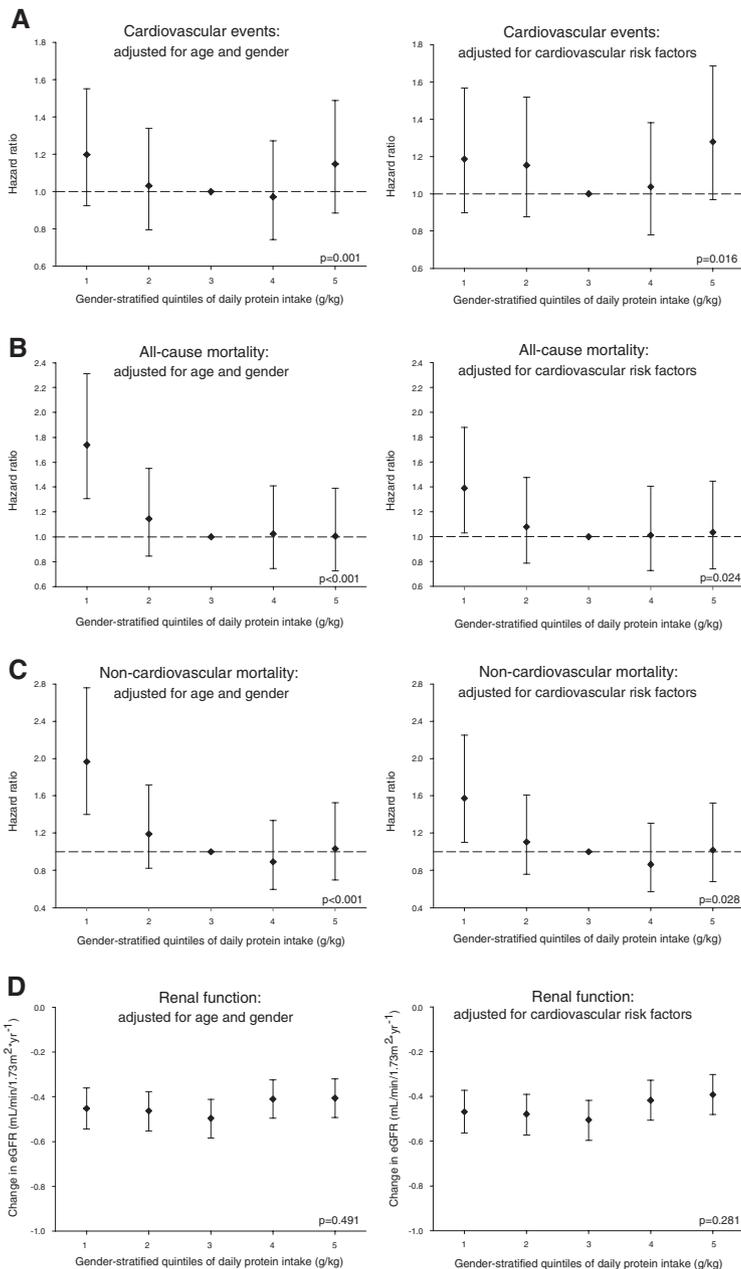
We repeated our analyses by including only individuals with a stable amount of protein intake. We also repeated our analyses with the amount of protein intake corrected for measured body weight instead of "ideal" body weight. These sensitivity analyses did not change our results essentially. Of note, in the final model, including protein intake, age, gender, and cardiovascular risk factors, daily sodium intake was not associated with the incidence of cardiovascular events, all-cause mortality, noncardiovascular mortality, or renal function decline. All regression analyses were also performed with inverse weighting to correct for the oversampling of individuals with an elevated urine albumin excretion. The results we obtained with these weighted analyses were in general comparable with the results of the unweighted analyses; however, the associa-

tion between protein intake and noncardiovascular mortality corrected for the cardiovascular risk factors was no longer statistically significant in the weighted analyses. Furthermore, we checked our full models for effect modification. For that purpose, we added to our models interaction terms between protein intake and all of the confounders we adjusted for in our analyses (age, gender and cardiovascular risk factors). Interaction terms with *P* < 0.05 were considered to be significant. No significant interactions were found.

## DISCUSSION

In this study, we show that, in the general population, a higher protein intake is associated with a higher incidence of cardiovascular events. This effect remained significant after adjustment for cardiovascular risk factors. Second, individuals with the lowest protein intake also had a higher cardiovascular event rate. In the latter group, however, noncardiovascular and all-cause mortality were also higher. We could not show an effect of protein intake on the progression of renal function loss.

The association between high protein intake and high incidence of cardiovascular morbidity and mortality is in agreement with some<sup>11</sup> but in contrast with other data.<sup>13</sup> A study of 42,237 Swedish women, 30 to 49 yr of age, showed that a high-protein diet was associated with a higher risk for death from cardiovascular causes, whereas the association they found between high protein intake and all-cause mortality was NS, as in our study. The unfavorable effect of a high-protein diet in the Swedish study, however, was limited to the subgroup of women in the age of 40 to 49 yr.<sup>11</sup> In contrast, in the 80,082 women of the Nurses' Health Study, aged 34 to 59 yr, a lower risk for ischemic heart disease was found in individuals with a higher intake of total protein.<sup>13</sup> There are several reasons to interpret the differences in the described impact of a high-protein diet on cardiovascular events. First, the cited studies were performed only on women, whereas we included both genders; however, we found no interaction between gender and protein intake with outcome. Second, the cited studies had a more narrow age range of individuals included than our study. Again, however, in our study, no interaction was found



**Figure 1.** (A through D) Associations between protein intake (for these figures divided in quintiles) and cardiovascular events (A), all-cause mortality (B), noncardiovascular mortality (C), and change in renal function (D). (Left) Data adjusted for age and gender. (Right) Data with additional adjustment for known cardiovascular risk factors. The *P* values are based on the Cox proportional hazard and mixed-effects models in which protein intake is treated as a continuous variable. These models are described in detail in the Results section.

between age and protein intake with outcome. Third, the cited studies used food frequency questionnaires or 24-h recalls instead of the Maroni formula to investigate protein intake. It is known that questionnaire-based methods are associated with both random and systematic error.<sup>14,15</sup> Fourth, we are not aware on the type of protein ingested. It has been reported, for

instance, that the intake of vegetable and animal proteins have different effects on cardiovascular outcome,<sup>16</sup> although data from the Nurses' Health Study showed that both animal and vegetable proteins contributed similarly.<sup>13</sup> Fifth, it may be that high protein intake is accompanied by other changes in the diet, such as a lower carbohydrate and fiber intake,<sup>17</sup> a higher salt intake, and/or a change in the total energy intake; therefore, the association we found between high protein intake and high cardiovascular event rate may be (partly) accounted for by effects of other nutrients whose intake is correlated with protein intake or by a change in total energy intake. For instance, in one study, salt intake by itself, also independent of BP, was found to be associated with a higher cardiovascular event rate.<sup>18</sup> Although we are not informed on the carbohydrate and fiber content of the diet or on total energy intake, we have data on salt intake (indirectly obtained from daily sodium excretion) and BMI (as proxy for energy intake). In our study protein intake seemed to be positively associated with salt intake. We found, however, that adjustment for differences in salt intake or BMI did not influence our conclusions. Finally, the cited studies expressed protein intake as percentage of total energy intake but did not offer precise information on the impact of protein intake itself.

There are several possible explanations for the association we found between high protein intake and increased incidence of cardiovascular events. It is likely that the intake of more protein causes a change in cardiovascular risk factors, which in turn causes a higher cardiovascular event rate. Recently, it was indeed described that a diet high in protein and low in carbohydrate is associated with an increased prevalence of the metabolic syndrome.<sup>19</sup> In line with these data, our results also show increases in most components of the metabolic syndrome and in cardiovascular risk factors over the quintiles of protein intake. This can be only part of the explanation, however, because the association between high protein intake and cardiovascular event rate is still significant after adjustment for cardiovascular risk factors. We surprisingly found that low protein intake was also associated with an increase in cardiovascular events. Because low protein intake was also associated with a high incidence of all-cause and of noncardiovascular mortality, however, we suggest the observed effects of low protein intake to be due to malnutrition induced by poor health status of this group.<sup>20</sup> In line with that assumption is that body weight was lowest in this group, they contained the largest number of smokers, and they more often had a family history of cardiovascular disease. Again, however, the effect remained significant after adjustment for these factors.

Our finding of the absence of an effect of protein intake on renal outcome in individuals with normal renal function is compatible with results of the Nurses' Health Study.<sup>5</sup> Thus far, most other studies investigating the effect of protein intake on renal outcome have been performed on individuals with CKD<sup>1,2</sup> and are therefore difficult to compare with this study. We investigated the association between daily protein intake and renal outcome in a large population-based cohort. Although the range of daily protein intake between these individuals varied widely from 0.3 to 3.3 g/kg, we did not find a relation between protein intake and rate of renal function loss over time, even in the subgroup of individuals with an eGFR <60 ml/min per 1.73 m<sup>2</sup> and/or individuals with microalbuminuria. It has to be emphasized, though, that our study is observational in design. Our data therefore do not exclude the possibility that lowering of protein intake in some individuals would favor their renal prognosis.

For this study, we estimated protein intake using the Maroni formula,<sup>21</sup> not using food frequency questionnaires. The use of this formula will be called a strength by some because of the objective way of measuring protein intake and a limitation by others because of the lack of detailed information about food intake (*e.g.*, kind of protein and amount of energy intake). It has been proved, however, that this formula is a valid method to estimate actual protein intake,<sup>22</sup> whereas it is also known that questionnaire-based methods are associated with both random and systematic error.<sup>14,15</sup> Moreover, the average amount of protein we found in our population is comparable with the values reported in another study using the Maroni formula to estimate the amount of protein intake in a Japanese community-based cohort.<sup>23</sup> Another limitation of this study is that the protein intake we measure is susceptible to fluctuations because it reflects protein intake of only a few days before the urine collection; therefore, we based our protein intake on the average of two 24-h urine collections. In addition, we performed a sensitivity analysis including only individuals with a small difference in protein intake between the first and the second screening rounds. The results of these analyses were comparable with the primary analyses. Furthermore, if fluctuations in measured protein intake would play a role, then they would "dilute" our results, whereas the most important conclusion in our study is that high protein intake is significantly associated with an increased risk for cardiovascular events. In general, a limitation of all large cohort studies is lack of detailed information on some of the potential confounders. This may limit the ability of the covariate adjustment to control for confounding. For instance, 24-h urinary sodium excretion may not fully reflect an individual's long-term sodium intake. Another limitation of our study may be 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 individuals have a higher rate of renal function decline; therefore, in our analyses, the impact of risk factors may have been underestimated. By using mixed-effects model analyses, we took into account the potential bias induced by

loss to follow-up, because, in these analyses, available data of individuals who attended only one or two screenings are used. Last, concerning the association between protein intake and change in renal function, we cannot exclude that longitudinal changes in creatinine generation during follow-up, resulting from changes in muscle mass or dietary habits, may have influenced the levels of serum creatinine and thus the estimated changes in eGFR.

One of the strengths of this study is the use of a large community-based cohort study with three eGFR measurements during follow-up and the availability of detailed information on cardiovascular risk factors and confounders. Furthermore, to our knowledge, it is one of the first studies investigating the effect of daily protein intake on both cardiovascular and renal outcomes in the general population.

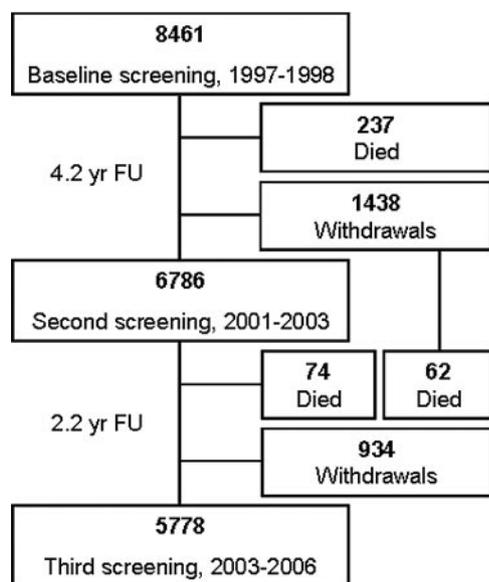
In conclusion, we showed that high protein intake is associated with a higher incidence of cardiovascular events in the general population. This is clinically relevant as dietary patterns in the general population change toward more protein intake. In our cohort, with relatively normal renal function at baseline, we could not find unfavorable effects of a high-protein diet on renal outcome.

## CONCISE METHODS

### Study Design and Population

The analyses are based on data of individuals who participated in the first three screening rounds of the Prevention of Renal and Vascular End-stage Disease (PREVEND) study. This is a prospective cohort study, designed to investigate the impact of urinary albumin excretion on renal and cardiovascular outcomes in the general population. Details of the study protocol have been published elsewhere.<sup>24,25</sup> In summary, in 1997 through 1998, all inhabitants of the city of Groningen (Netherlands) aged between 28 to 75 yr were sent a questionnaire and a vial to collect a first morning void urine sample. Of these individuals, 40,856 (47.8%) responded and sent a vial to a central laboratory for urinary albumin and urinary creatinine assessment. From these 40,856 individuals, the PREVEND cohort was selected with the aim to create a cohort enriched for the presence of high albuminuria. After exclusion of patients with type 1 diabetes and pregnant women, all individuals with urinary albumin concentration  $\geq 10$  mg/L ( $n = 7768$ ) were invited, 6000 of whom participated. Furthermore, a randomly selected cohort group with urinary albumin concentration <10 mg/L ( $n = 3394$ ) was invited, 2592 of whom participated. These 8592 individuals form the actual PREVEND cohort.

At approximately 3-yr intervals, participants in this study are invited to visit an outpatient department for measurements concerning their health. In total, 8592 participants completed the baseline screening in 1997 through 1998. For this study, we excluded individuals who indicated having a known renal disease ( $n = 22$ ) and/or missing data on protein intake ( $n = 109$ ) during the first screening round, leaving data of 8461 individuals for analysis. Data on mortality and morbidity are available from these 8461 individuals. During the interval between the first and the third screenings, 373 individuals died (Figure 2). The



**Figure 2.** Outline of the PREVEND study cohort; first three screenings.

second screening took place from 2001 through 2003 and the third from 2003 through 2006. Overall, 5778 individuals completed the third screening round. Data on the slope of renal function over time are available from individuals who completed two or three screening rounds. The PREVEND study is approved by the local medical ethics committee and conducted in accordance with the guidelines of the Declaration of Helsinki. All participants gave written informed consent.

### Measurements and Definitions

Each screening round consisted of two visits to an outpatient department separated by approximately 3 wk. Participants filled out a questionnaire on demographics; cardiovascular and renal history; smoking status; and the use of oral antidiabetic, antihypertensive, and lipid-lowering drugs. Smoking was defined as current smoking or cessation of smoking <1 yr before the baseline screening (first screening round). Information on drug use was completed with data from community pharmacies. During both study visits per screening round, BP was measured in the right arm every minute for 10 and 8 min, respectively, with an automatic Dinamap XL Model 9300 series device (Johnson-Johnson Medical, Tampa, FL). For systolic and diastolic BP, the mean of the last two recordings from each of the two visits was used. Anthropometric measurements were performed, and fasting blood samples were taken. In addition, individuals collected urine for two consecutive periods of 24 h. Concentrations of total cholesterol, HDL cholesterol, triglycerides, plasma glucose, C-reactive protein, and urinary urea and sodium were measured using standard methods. Dietary protein intake was calculated by the method of Maroni and colleagues.<sup>21,22</sup> in each of the two 24-h urine collections obtained during the first screening round. For each individual, we expressed the estimates of dietary intake per kilogram of “ideal” or “desirable” body weight, derived from the BMI equation ( $\text{weight}/\text{height}^2$ ), and obtained by multiplying the squared value of height times a reference BMI value of 22. Dietary sodium intake was calculated based on the

daily urinary excretion of sodium in the two 24-h urine collections and expressed per kilogram of ideal body weight. Urinary albumin concentration was determined by nephelometry (Dade Behring Diagnostic, Marburg, Germany), and urinary albumin excretion was given as the mean of the two 24-h urinary excretions obtained during the first screening round. Serum creatinine was measured by dry chemistry (Eastman Kodak, Rochester, NY), with intra- and interassay coefficients of variation of 0.9 and 2.9%, respectively. eGFR was calculated using the MDRD study equation, taking into account gender, age, race, and serum creatinine.<sup>26</sup>

### Outcome Variables

For cardiovascular outcome, we used the combined incidence of cardiovascular morbidity and cardiovascular mortality after the first screening round. Data on mortality were received through the municipal register. Cause of death was obtained by linking the number of the death certificate to the primary cause of death as coded by the Dutch Central Bureau of Statistics. Noncardiovascular mortality was defined as mortality not related to any of the cardiovascular causes mentioned at the end of this paragraph. Information on hospitalization for cardiovascular morbidity was obtained from PRISMANT, the Dutch national registry of hospital discharge diagnoses. The validity of this database has been shown to be good, with 84% of the primary diagnoses and 87% of the secondary diagnoses matching the diagnoses found in the patient chart.<sup>27,28</sup> All data were coded according to the *International Classification of Diseases, Ninth Revision* (ICD-9) and the classification of interventions. For this study, cardiovascular events were defined as acute myocardial infarction (ICD-9 code 410), acute and subacute ischemic heart disease (ICD-9 411), subarachnoid hemorrhage (ICD-9 430), occlusion or stenosis of the precerebral (ICD-9 433) or cerebral arteries (ICD-9 434), coronary artery bypass grafting or percutaneous transluminal coronary angioplasty, and other vascular interventions such as percutaneous transluminal angioplasty or bypass grafting of aorta and peripheral vessels.

Survival time was defined for cardiovascular outcome as the period from the date of urine collection to the date of first cardiovascular event or December 31, 2005 (end of follow-up). For all-cause mortality, survival time was defined as the period from the date of urine collection to the date of death or December 31, 2005 (end of follow-up). In case a person moved to an unknown destination, the date on which the person was removed from the municipal registry was used as censored date.

Data on renal outcome were obtained from the yearly decline of renal function as calculated by the slope of a linear regression line fitted between the two or three serial estimates of GFR using least squares regression.

### Statistical Analyses

Continuous data are reported as means  $\pm$  SD. For skewed distributions, the median and interquartile range are presented. All *P* values are two-tailed. *P* < 0.05 was considered statistically significant. Baseline characteristics of our study population are shown with the population subdivided into gender-corrected quintiles of protein intake (Table 1). Differences in the characteristics among protein intake quintiles were tested for statistical significance by  $\chi^2$  analysis for di-

chotomous data or ANOVA for continuous data. We performed mixed-effects models with random intercepts and random slopes to investigate the association between protein intake and renal function decline. Such a model estimates the rate of change in eGFR over time within and between individuals, taking into account correlations within individuals and time, including also individuals with only one or two eGFR measurements.<sup>29</sup> To study the impact of protein intake on cardiovascular events and all-cause mortality, we used Cox proportional hazard analyses. We performed all analyses univariate, corrected only for age and gender and corrected additionally for known cardiovascular risk factors, for sodium intake, and finally for BMI. The traditional known cardiovascular risk factors we used for adjustment were positive cardiovascular disease history, smoking, systolic BP, cholesterol/HDL cholesterol ratio, triglycerides, and plasma glucose. In each model, we tested for presence of nonlinear associations by adding the quadratic term of protein intake to the model. A nonlinear association was considered significant at  $P < 0.05$ . We tested possible effect modification by implementing interaction terms. The Cox proportional hazards assumptions were checked and met. The models were also tested for collinearity. For Figure 1, we performed all analyses treating the gender-stratified quintiles of protein intake as categorical factors. All analyses were conducted with the use of the statistical package SPSS 14.0 (SPSS, Chicago, IL).

### Sensitivity Analyses

First, we performed a sensitivity analysis repeating our analyses including only individuals with a stable protein intake. We defined stable protein intake as a difference in protein intake between the first and second screening rounds of  $<10\%$ . Second, we performed our analyses using protein intake corrected for measured body weight instead of ideal body weight. Last, we performed all regression analyses using the “weight” function in R 2.5.0.

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### DISCLOSURES

None.

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