

Association of a Low-Protein Diet With Slower Progression of CKD



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Introduction: Reducing protein intake is recommended for slowing chronic kidney disease (CKD) progression, but assessment of its true effectiveness is sparse.

Methods: Using the Maroni formula, we assessed dietary protein intake (DPI) from 24-hour urinary urea excretion in 1594 patients (67% men and 33% women) with CKD, 784 of whom also had 7-day food records. Cause-specific hazard ratios (HRs) and 95% confidence intervals for the competing risks of DPI-associated end-stage renal disease (ESRD) or death were estimated in 1412 patients with baseline glomerular filtration rate ≥ 15 ml/min per 1.73 m², measured by ⁵¹Cr-EDTA renal clearance (mGFR).

Results: Overall, mean DPI estimated from urea excretion was 1.09 \pm 0.30 g/kg of body weight per day (range = 0.34–2.76); 20% of patients had values > 1.3 g/kg per day, and 1.9% had values < 0.6 g/kg per day. Urea excretion and food records produced similar estimates of mean DPI. The lower the mGFR, the lower the mean DPI. Over a median follow-up of 5.6 years, there were 319 ESRD events and 189 pre-ESRD deaths. After adjusting for relevant covariates, each 0.1 g/kg daily higher baseline urea excretion-based DPI or food record-based DPI was associated with an HR for ESRD of 1.05 (95% confidence interval 1.01–1.10) or 1.09 (95% confidence interval 1.04–1.14), respectively. HRs were stronger in patients with baseline mGFR < 30 ml/min per 1.73 m². There was no association with mortality. The mean age of the patients was 59 \pm 15 years, and mean body mass index was 26.6 \pm 5.2 kg/m².

Conclusion: In this prospective observational study, the lower the baseline DPI, the slower the progression toward ESRD. Most importantly, the absence of threshold for the relation between DPI and ESRD risk indicates that there is no optimal DPI in the range observed in this cohort.

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Despite numerous clinical trials and observational studies, the effectiveness of lowering dietary protein intake (DPI) to slow the progression of chronic kidney disease (CKD) remains controversial,¹ although it is well established that protein restriction is beneficial for reducing uremic symptoms and proteinuria in patients with CKD.² However, insufficient protein intake, particularly in older patients, can result in

malnutrition.³ Considering both the potential benefits and harms, current guidelines suggest “lowering protein intake to 0.8 g/kg per day in adults with glomerular filtration rate (GFR) <30 ml/min per 1.73 m² [...] [and] avoiding high protein intake > 1.3 g/kg per day in adults with CKD at risk of progression.”⁴

Several randomized controlled trials (RCTs) have investigated the effects of reducing DPI on CKD progression; the largest of these was the Modification of Diet in Renal Disease (MDRD) Study. Although its primary results were inconclusive,⁵ several secondary analyses tend to support the conclusion that successful DPI restriction has a beneficial effect on the rate of GFR decline, proteinuria, and end-stage renal disease (ESRD) onset.^{5–9} The long-term follow-up of this study, however, also revealed a higher risk of death associated with very low DPI.⁹ The last updated Cochrane meta-analysis, based on 10 RCTs and 2000 patients with nondiabetic CKD randomized to either low (0.6 g/kg per day) or very low (0.3–0.6 g/kg per day) DPI versus standard DPI (> 0.8 g/kg per day), showed a 32% reduction of the combined outcome of ESRD or mortality associated with reduced DPI.¹⁰ Adherence to low DPI was poor in all these RCTs, however, as it is in individuals with CKD not participating in RCTs.¹¹ Further nutritional studies are still needed to characterize the optimal level of protein restriction in CKD patients, in terms of compliance, efficacy, and safety.

We therefore investigated the relation of baseline DPI with subsequent GFR decline and the risks for ESRD and death over a 6-year period in the NephroTest cohort of patients with CKD (all stages). Our goal was to document whether or not there were thresholds above or below which DPI was beneficial or deleterious for ESRD risk and below which it may be deleterious for survival. DPI was assessed by 2 independent methods: measurement of 24-hour urinary urea excretion (DPI-UE) in all patients, and a 7-day food record (DPI-FR) in nearly half of them. Measured GFR (mGFR) was determined by urinary clearance of ⁵¹Cr-EDTA.

METHODS

Patients

NephroTest is a prospective cohort including 1835 adult patients (≥ 18 years of age) with all stages of CKD and any type of kidney disorder who were referred by nephrologists to 3 French physiology departments for extensive annual workups between 2000 and 2010, as previously described.¹² Patients on dialysis or living with a kidney transplant and pregnant women were excluded. All patients signed written informed consent before inclusion. The ethics committee of the French

Research Ministry approved the study design (CCTIRS MG/CP09.503).

Measurements

At baseline, all patients underwent clinical examination and provided data about their medical history and treatment. They also provided samples for various laboratory measurements, including 24-hour urine to measure daily urine volume, creatinine clearance (24-hour Ccreat), urinary urea concentration (by spectrophotometer at 340 nm; AU680 Beckman Coulter France, Villepinte, France), protein, and albumin. Daily excretion rates of urea and creatinine, and albumin-to-creatinine ratio (UACR) were calculated. Diabetes was defined as either fasting glycemia ≥ 7 mmol/l or anti-diabetic treatment, or reported diabetes, and hypertension by either a blood pressure > 140/90 mm Hg or antihypertensive treatment.

At baseline and each follow-up visit, urinary clearance of ⁵¹Cr-EDTA (mGFR) and plasma creatinine concentration were determined as the average of 5 to 7 consecutive 30-min clearance periods (30-min Ccreat), as previously described.¹³ We compared 24-hour Ccreat to 30-minute Ccreat taken as the gold standard to estimate the percentage of under and over 24-hour urine collection using the following equation: (1 – Ccreat ratio) × 100, where Ccreat ratio = 24-hour Ccreat ÷ 30-minute Ccreat (both expressed in ml/min). Nine patients with percentage in absolute values exceeding 100%, that is, Ccreat ratio > 2, were excluded. Distribution of the Ccreat ratio is shown in [Supplementary Figure S1](#).

Dietary Protein Intake Assessment

Protein intake at baseline was estimated from 24-hour urinary urea excretion (UE) according to the Maroni formula,¹⁴ as follows:

$$\text{DPI-UE (g per d)} = 6.25 \times [\text{UUN (g per d)} + 0.031 \times \text{body weight (kg)}]$$

where UUN (urinary urea nitrogen) = 0.028 × UE (mmol/24 h).

Before calculating UUN, all UE values were multiplied by the reverse of the Ccreat ratio in order to reduce measurement errors resulting from either under or over 24-hour urine collection. Finally, DPI was normalized to body weight and expressed in grams per kilogram per day. After excluding 221 patients with missing data for UE or Ccreat measures as well as 20 outliers for either urea excretion (> 800 mmol/24 h or < 30 mmol/24 h), Ccreat ratio (> 2), or DPI (> 3 g/kg per day), we analyzed baseline data for 1594 patients with DPI-UE ([Figure 1](#)). In a subgroup of 784 patients from 1 center, DPI was also estimated by dietitians, based on 7-day food records

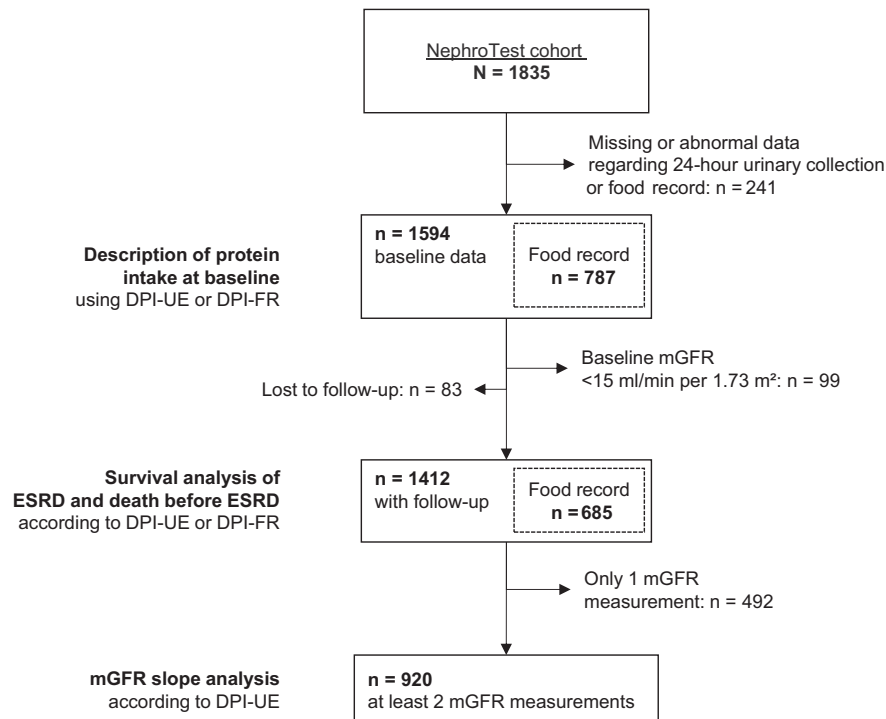


Figure 1. Study flow chart. DPI-FR, dietary protein intake estimated from 7-day food record; DPI-UE, dietary protein intake estimated from 24-hour urinary urea excretion; ESRD, end-stage renal disease; mGFR, measured glomerular filtration rate.

(DPI-FR) completed by the patients during the week before the 24-hour urine collection.

Outcomes

The primary endpoints of this study were ESRD, defined as initiation of dialysis or preemptive transplantation, and pre-ESRD mortality. Events were identified either from medical records or through linkage with the national Renal Epidemiology and Information Network (REIN) and death registries.¹⁵ After excluding 99 patients with CKD stage 5 at baseline, and 83 lost to follow-up, 1412 patients were followed up through 31 December 2013. We also studied mGFR decline as a secondary endpoint in 920 patients with at least 2 mGFR measurements over the study period (Figure 1).

Statistical Analyses

We first described baseline characteristics in the overall sample of 1594 patients and compared these characteristics between patients with and without 7-day food records. We then studied mean DPI-UE and DPI-FR according to mGFR level, stratified into 5 classes (< 15, 15–29, 30–44, 45–59, and ≥ 60 ml/min per 1.73 m²). We also calculated the percentages of DPI-UE (in 3 classes: < 0.8, 0.8–1.3, and > 1.3 g/kg per day) by mGFR class and mean DPI-UE according to patient characteristics. Agreement between DPI-UE and DPI-FR was analyzed with Bland–Altman plots.¹⁶

Second, in the 1412 patients with CKD stages 1 to 4 at baseline, we used cause-specific Cox models to estimate crude hazard ratios (HR) and 95% confidence intervals of ESRD and death before ESRD according to DPI-UE treated continuously in decigrams per kilogram per day, and in categories (< 0.8, 0.8–1.3, > 1.3 g/kg per day). We used an intent-to-treat approach and analyzed hazard ratios (HRs) associated with baseline DPI-UE. In these models, the competing events of ESRD and death before ESRD were treated as censored observations. The cause-specific approach has been shown to be most appropriate for accounting for competing risks of concurrent events in etiological studies.¹⁷ HRs were then adjusted for age, gender, origin (sub-Saharan Africa, other), smoking, body mass index, diabetes, elevated blood pressure (> 140/90), renin-angiotensin system inhibitor (RASi) use, history of cardiovascular disease, UACR (<3, 3–29, ≥ 30 mg/mmol), serum albumin, and center. We assessed the Cox model assumption of proportional hazards, which was met for all covariates except GFR; this result led us to stratify, rather than adjust for, mGFR in 6 classes in the multivariate model for ESRD. We tested interactions between diabetes, RASi use, UACR and DPI-UE in the relation with ESRD risk, and estimated HR of ESRD by subgroups stratified by baseline mGFR. Penalized splines were used in fully adjusted Cox models to represent the functional relationship between DPI-UE and ESRD risk. We also conducted 2

sensitivity analyses. One estimated HR for ESRD associated with DPI-UE based on uncorrected UE values in a subgroup of 726 patients with reliable 24-hour urine collection, defined by a urine collection bias $< |15\%|$, that is, a Ccreat ratio between 0.85 and 1.15, a restrictive definition that minimized measurement errors. The other estimated HRs associated with both DPI estimates in the 784 patients with both measurements. In the latter subgroup, we adjusted for total energy intake, by applying the residual method to protein intake estimated from the food records.¹⁸

Third, in the 920 patients with at least 2 mGFR measurements, we used a linear mixed model with random intercepts and slopes to study the association between baseline DPI-UE and mGFR slope in milliliters per minute per year. We estimated β and SDs adjusted for baseline mGFR (< 30 , $30\text{--}44$, ≥ 45 ml/min per 1.73 m²), as well as for the above covariates and the number of mGFR measurements. The covariance matrix for the random effects was estimated for each group of baseline mGFR separately, and robust sandwich variance estimators were used to estimate variances of regression coefficients. Interactions with time were tested for all covariates. Only those that were statistically significant according to the Wald test and improved the model according to the Akaike Information Criterion (AIC) were included in the final model. All analyses were performed with SAS version 9.3 software (SAS Institute, Cary, NC).

RESULTS

Baseline Patient Characteristics

Among the 1594 patients, 59% were men and 14% were from sub-Saharan Africa or the French West Indies (Table 1). About 20% were obese (body mass index ≥ 30 kg/m²), 25% had diabetes, and more than a third had high blood pressure. Half of them had CKD stage 3, and 33% CKD stage 4 or 5. All patients had DPI-UE estimated at baseline; in 1 of the 3 study centers, which included 784 patients, they also had DPI-FR. Patients with and without DPI-FR did not differ significantly in regard to demographic characteristics or most clinical data, except diabetes, which was more common in patients with DPI-FR. However, mGFR tended to be lower and UACR tended to be higher in patients with than without DPI-FR.

Agreement Between the 2 Dietary Protein Intake Estimates

In the subgroup of patients whose DPI was estimated by both methods, mean DPI-UE and DPI-FR were very similar (1.13 ± 0.34 vs. 1.12 ± 0.34), respectively (Table 1). There was no bias between the 2 methods (mean difference = -0.02 ± 0.40 g/kg per day; 95% CI = -0.05

Table 1. Patient characteristics, overall and by subgroup

	All patients (N = 1594)	Without FR (n = 810)	With FR (n = 784)
Demographic characteristics			
Men (%)	66.6	66.2	67.1
Age (yr)	59.0 \pm 15.2	58.5 \pm 15.4	59.6 \pm 14.9
African origin (%)	13.5	14.7	12.3
Current smoking (%)	14.0	13.0	15.1
Clinical characteristics			
Body mass index (kg/m ²)	26.6 \pm 5.2	26.5 \pm 5.2	26.6 \pm 5.1
Diabetes ^a (%)	26.5	21.0	32.3
History of CVD ^b (%)	18.4	16.7	20.2
Systolic BP (mm Hg)	136 \pm 20	137 \pm 21	136 \pm 20
Diastolic BP (mm Hg)	75 \pm 12	76 \pm 12	74 \pm 12
Hypertension ^c (%)	91.7	90.0	93.5
Elevated BP ^d (%)	37.5	37.6	37.5
RASi use (%)	77.4	76.9	77.9
Laboratory measurements			
mGFR (ml/min per 1.73 m ²)	38.2 [26.6–53.4]	40.2 [27.3–54.0]	36.5 [25.6–52.2]
UACR (mg/mmol)	8.9 [1.7–45.3]	7.3 [1.3–41.9]	10.8 [2.3–48.9]
UACR ≥ 30 mg/mmol (%)	31.8	29.8	34.0
Serum albumin (g/l)	39.3 \pm 4.5	39.9 \pm 4.1	38.7 \pm 4.7
Nutritional characteristics			
Protein intake (g/kg per d)			
Estimated from 24-h UE	1.09 \pm 0.3	1.06 \pm 0.24	1.13 \pm 0.34
Estimated from 7-day FR			1.12 \pm 0.34
Energy intake ^e (kcal/kg per d)			26 \pm 8

Data are mean \pm SD, median [interquartile range], or percentage.

BP, blood pressure; CVD, cardiovascular disease; FR, food record; mGFR, measured glomerular filtration rate; RASi, renin-angiotensin system inhibitor; UACR, urinary albumin-to-creatinine ratio; UE, urea excretion.

^aDiabetes defined as fasting glycemia > 7 mmol/l or antidiabetic treatment or reported diabetes.

^bHistory of CVD includes myocardial infarction, angioplasty/coronary artery bypass graft, stroke, heart failure.

^cHypertension defined as BP $> 140/90$ mm Hg or antihypertensive treatment.

^dElevated BP defined as systolic BP > 140 mm Hg or diastolic BP > 90 mm Hg.

^eEnergy intake estimated from food record.

to 0.01), with no variation in difference according to DPI level, but the Bland–Altman plot showed relatively poor agreement (Supplementary Figure S2).

Dietary Protein Intake According to Patient Characteristics and mGFR at Baseline

Mean DPI-UE was 1.09 ± 0.30 g/kg per day (5th–95th percentiles: 0.68–1.62). It was lower with older age, higher body mass index, serum albumin, and total energy intake, but was not associated with gender, origin, smoking, diabetes status, or UACR (Table 2).

In the overall cohort, the lower the mGFR, the lower the mean DPI-UE (P for trend < 0.01); a similar trend was observed for DPI-FR (P for trend < 0.01) (Figure 2). Overall, 20% of the patients had DPI-UE > 1.3 g/kg

Table 2. Dietary protein intake estimated from 24-hour urinary urea excretion (in g/kg per day) according to patient characteristics

	n	DPI-UE Mean ± SD	P value
Gender			
Men	1062	1.10 ± 0.28	0.1
Women	532	1.08 ± 0.31	
Age (yr)			
<50	428	1.13 ± 0.29	0.006
50–59	347	1.09 ± 0.30	
60–69	359	1.08 ± 0.29	
≥70	460	1.07 ± 0.31	
African origin			
No	1327	1.10 ± 0.30	0.07
Yes	207	1.06 ± 0.27	
Current smoking			
No	1371	1.09 ± 0.30	0.3
Yes	223	1.11 ± 0.29	
Body mass index (kg/m²)			
<19	56	1.30 ± 0.37	<.0001
19–24	611	1.16 ± 0.30	
25–29	585	1.07 ± 0.26	
≥30	342	0.97 ± 0.26	
Diabetes^a			
No	1166	1.10 ± 0.29	0.6
Yes	423	1.09 ± 0.31	
History of CVD^b			
No	1301	1.09 ± 0.29	0.6
Yes	293	1.08 ± 0.31	
Elevated BP^c			
No	962	1.11 ± 0.29	0.002
Yes	578	1.06 ± 0.30	
RASi use			
No	343	1.11 ± 0.31	0.3
Yes	1174	1.09 ± 0.29	
UACR (mg/mmol)			
<3	517	1.10 ± 0.28	0.2
3–29	542	1.11 ± 0.31	
≥30	494	1.07 ± 0.30	
Serum albumin (g/l)			
<35	223	1.04 ± 0.30	<.0001
35–39	637	1.06 ± 0.30	
≥40	691	1.14 ± 0.29	
Energy intake (kcal/kg per d)^d			
<21.6	260	1.04 ± 0.31	<.0001
21.6–27.8	261	1.12 ± 0.32	
≥27.9	262	1.23 ± 0.35	

BP, blood pressure; CVD, cardiovascular disease; DPI-UE, dietary protein intake estimated from 24-hour urinary urea excretion; RASi, renin-angiotensin system inhibitor; UACR, urinary albumin-to-creatinine ratio.

^aDiabetes defined as fasting glycemia >7 mmol/l, antidiabetic treatment, or reported diabetes.

^bHistory of CVD includes myocardial infarction, angioplasty/coronary artery bypass graft, stroke, heart failure.

^cElevated BP defined as systolic BP > 140 mm Hg or diastolic BP > 90 mm Hg.

^dEnergy intake estimated from 7-day food records in the subgroup with food records and urea excretion measurements.

per day and 14% had DPI-UE < 0.8 g/kg per day. Only 31 patients (1.9%) had DPI-UE < 0.6 g/kg per day. The prevalence of high DPI-UE (> 1.3 g/kg per day) was higher and that of low DPI-UE (<0.8 g/kg per day) lower at high than at low mGFR (Figure 3).

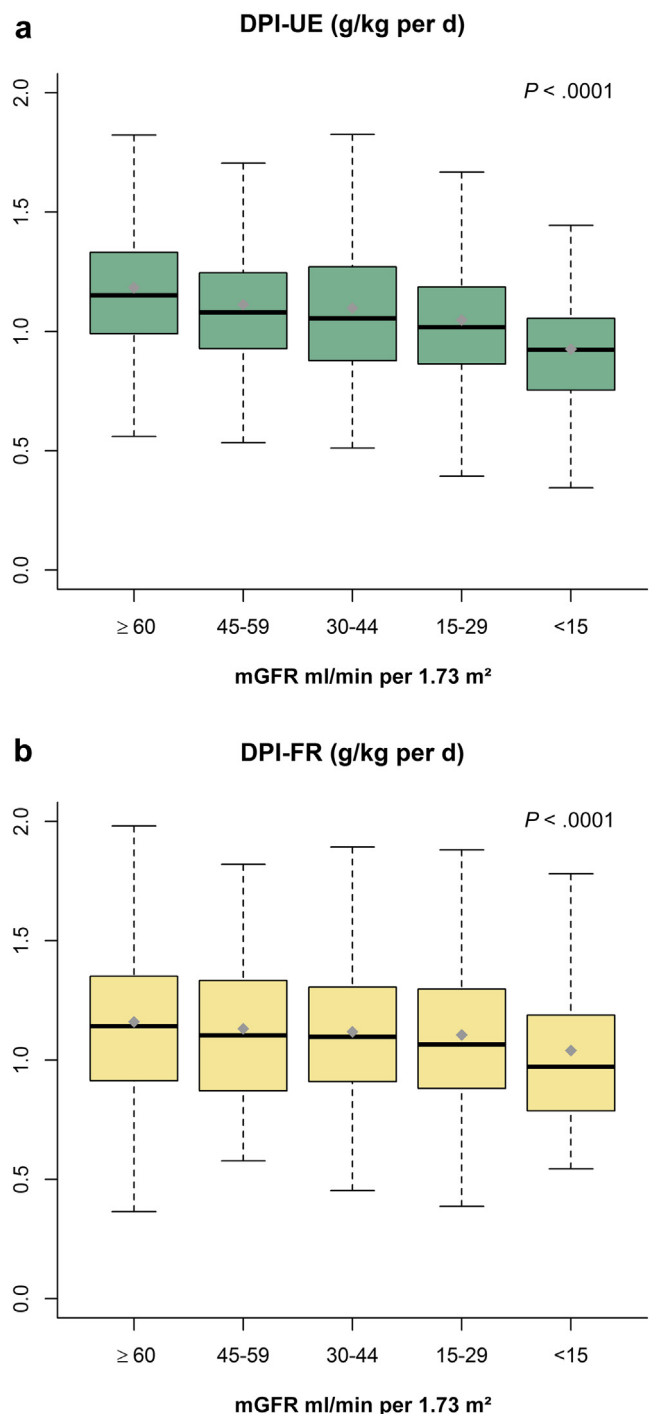


Figure 2. Distribution of dietary protein intake (DPI) in grams per kilogram per day according to measured glomerular filtration rate (mGFR). (a) DPI estimated from 24-hour urinary urea excretion (UE) in the overall sample (N = 1594). (b) DPI estimated from 7-day food record (FR) in the subsample (n = 784). Diamonds represent mean values; boxes show the median values and the interquartile range. Whiskers extend to 1.5 times the interquartile range.

Hazard Ratios for ESRD and Pre-ESRD Death, According to Baseline Dietary Protein Intake

Over a median follow-up of 5.6 years (interquartile range [IQR] = 3.6–7.8), there were 319 ESRD events and 189 deaths before ESRD. The crude hazard ratio

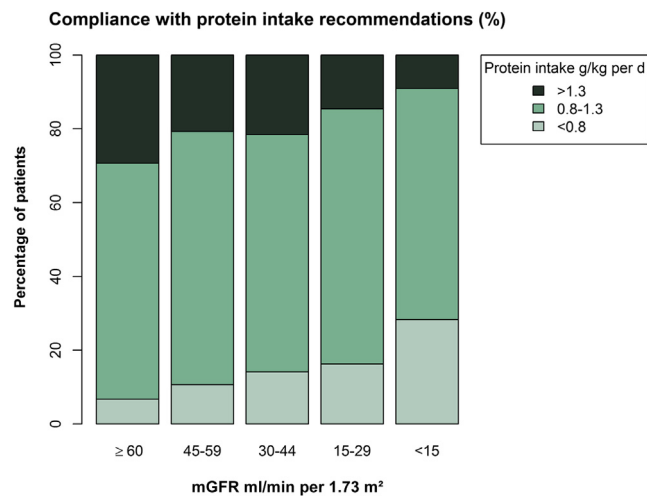


Figure 3. Percentage of patients with low, normal, or excess dietary protein intake (in grams per kilogram per day), according to measured glomerular filtration rate (mGFR).

(HR) for ESRD was significantly higher in patients with diabetes, high blood pressure, cardiovascular disease, elevated albuminuria, and in smokers than in those without these conditions, but not with higher DPI-UE, whether analyzed continuously or in classes (Table 3 and Supplementary Table S1). After accounting for baseline mGFR, however, the HR for ESRD associated with 0.1 g/kg per day higher DPI-UE was statistically significant and did not change after further adjustment for the above covariates. Penalized spline regression showed that both DPI-UE and DPI-FR had a linear no-threshold relation with ESRD risk (Figure 4). Further adjustment for sodium intake estimated from 24-hour urine slightly attenuated the HR for ESRD associated with DPI-UE (1.04, 95% confidence interval [CI] = 0.9–1.09), but not that with DPI-FR (HR = 1.09, 95% CI = 1.04–1.14). There was no significant interaction with diabetes, RASi use, or UACR in the relation between DPI-UE and ESRD risk (all interaction P values > 0.35). The HR for ESRD associated with 0.1 g/kg per day higher DPI-UE nevertheless tended to be slightly higher in patients with than without diabetes (HR = 1.08, 95% CI = 1.01–1.17, vs. HR = 1.04, 95% CI = 0.99–1.09, respectively). Analyses by baseline mGFR showed increasing HR for ESRD with higher DPI-UE or DPI-FR in both subgroups (Supplementary Figure S3), but adjusted HR associated with 0.1 g/kg per day higher DPI-UE was stronger in patients with mGFR < 30 than \geq 30 ml/min per 1.73 m² (HR = 1.09, 95% CI = 1.03–1.15, n = 409, P = 0.002, vs. HR = 1.03, 95% CI 0.96–1.10, n = 1003, P = 0.41).

Sensitivity analyses conducted in the patient subgroup with reliable urine collection (see Materials and Methods) as well as in that with both DPI estimates yielded similar findings (Table 3). In the latter subgroup,

Table 3. Hazard ratios (95% confidence intervals) for ESRD in 1412 patients with CKD stages 1 to 4 at baseline

	n	Crude ESRD HR	Adjusted ESRD HR ^a
All patients	1412		
DPI-UE (per 0.1 g/kg per d)		0.98 (0.94–1.02)	1.05 (1.01–1.10)
DPI-UE in classes			
<0.8 versus 0.8–1.3 g/kg per d		1.08 (0.77–1.51)	0.89 (0.62–1.27)
>1.3 versus 0.8–1.3 g/kg per d		0.86 (0.65–1.13)	1.20 (0.89–1.62)
In patients with reliable 24-h urine collection ^b			
DPI-UE (per 0.1 g/kg per d)	726	0.95 (0.88–1.01)	1.08 (1.01–1.16)
Uncorrected DPI-UE (per 0.1 g/kg per d)	726	0.92 (0.86–0.99)	1.06 (0.99–1.14)
In the subsample with food records			
DPI-UE (per 0.1 g/kg per d)	685	0.97 (0.93–1.01)	1.04 (0.99–1.09)
DPI-FR (per 0.1 g/kg per d)	685	1.04 (0.98–1.09)	1.09 (1.04–1.14)

Creat, creatinine clearance; CKD, chronic kidney disease; DPI-FR, dietary protein intake estimated from 7-day food records; DPI-UE, dietary protein intake estimated from 24-hour urinary urea excretion; ESRD, end-stage renal disease; HR, hazard ratio.

^aAdjusted for age, gender, body mass index (< 19, 20–24, 25–29, \geq 30), origin (sub-Saharan Africa, other), urinary albumin-to-creatinine ratio (<3, 3–29, and \geq 30 mg/mmol), elevated blood pressure (>140/90), history of cardiovascular disease (including myocardial infarction, angioplasty/coronary artery bypass graft, stroke, heart failure), current smoking, angiotensin-converting enzyme inhibitor or angiotensin receptor blocker treatment, serum albumin, and center. Cox models were stratified for baseline mGFR levels into 6 classes (15–19, 20–29, 30–39, 40–49, 50–59, \geq 60 ml/min per 1.73 m²).

^bReliability of 24-h urine collection was defined as an absolute value of urine collection bias [$1 - (24\text{-h Ccreat} \div 30\text{-min Ccreat})$] < 15%, that is, a Ccreat ratio between 0.85 and 1.15. In this analysis, DPI-UE was estimated using uncorrected 24-h urinary urea excretion (uncorrected DPI-UE), otherwise it was based on 24-h urinary urea excretion multiplied by the reverse of the Ccreat ratio as in all other analyses.

further adjustment for total calorie intake slightly reduced its HR to 1.06 (95% CI = 1.00–1.13; P = 0.04). In this model, the HR for ESRD associated with total calorie intake also rose significantly (HR = 1.06, 95% CI = 1.02–1.10) per 100 kcal higher per day.

In contrast, the HR for pre-ESRD death associated with low DPI-UE (<0.8 g/kg per day) was not statistically significant either before or after adjusting for confounders (Supplementary Table S2). Of note, older patients, men, those with diabetes, low body mass index, or a history of cardiovascular disease, and smokers were at higher risk for death, whereas African patients and those with higher mGFR and serum albumin had lower risk.

Baseline Dietary Protein Intake and Subsequent mGFR Decline

In 920 patients with at least 2 visits (median number [IQR], 3 [2–5] per patient) over a mean follow-up of 4.0 \pm 2.6 years, the mean mGFR decline was -1.46 ± 0.09 ml/min per year; it was steeper in patients with a higher mGFR at baseline (Table 4). In the mixed model adjusted for baseline mGFR and patient characteristics, each 0.1 g/kg per day higher DPI-UE at baseline was

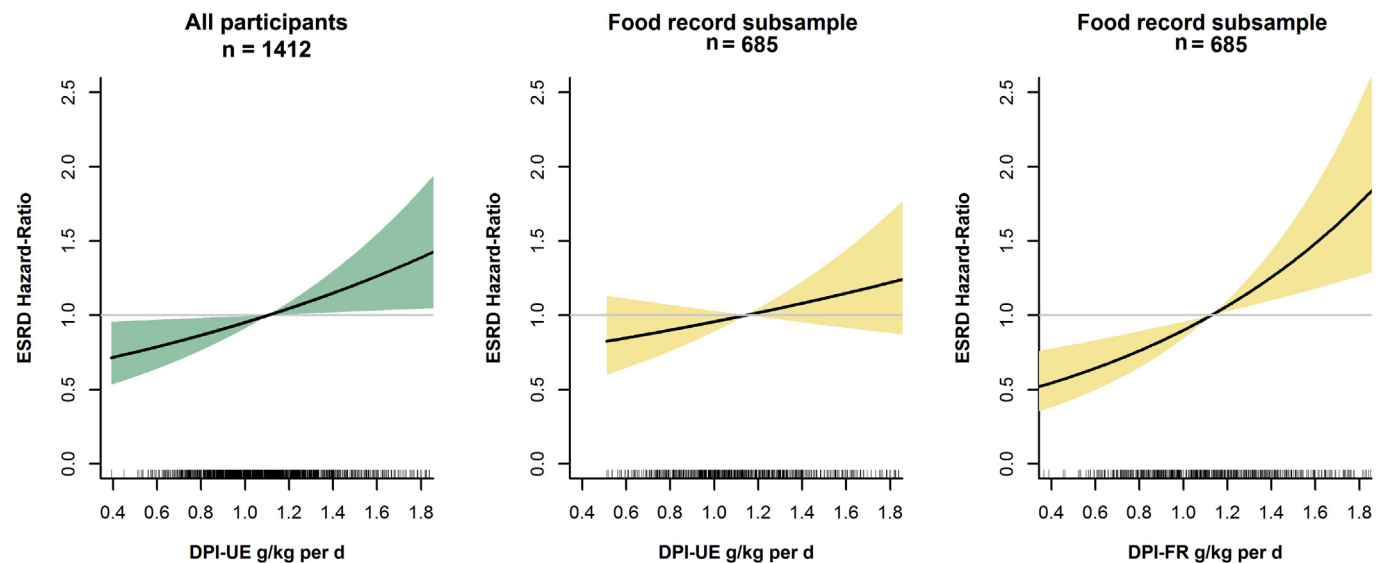


Figure 4. Hazard ratio for end-stage renal disease (ESRD) according to dietary protein intake estimated from 24-hour urinary urea excretion (UE) or 7-day food records (FR). Continuous lines represent predictions with penalized splines in Cox models (95% confidence intervals in color). Ticks on the x-axis represent the distribution of dietary protein intake (DPI). Hazard ratios are adjusted for age, gender, body mass index (< 19, 20–24, 25–29, \geq 30), origin (sub-Saharan Africa, other), urinary albumin-to-creatinine ratio (<3, 3–29, and \geq 30 mg/mmol), elevated blood pressure (> 140/90), history of cardiovascular disease (including myocardial infarction, angioplasty/coronary artery bypass graft, stroke, heart failure), current smoking, angiotensin-converting enzyme inhibitor or angiotensin receptor blocker treatment, serum albumin, and center. Cox models were stratified for baseline mGFR levels into 6 classes (15–19, 20–29, 30–39, 40–49, 50–59, \geq 60 ml/min per 1.73 m²).

associated with a loss of kidney function that was faster by 0.064 ± 0.031 ml/min per year ($P = 0.039$). Baseline mGFR, proteinuria, diabetes, and mean blood pressure were also significantly associated with the rate of GFR decline in this analysis.

DISCUSSION

This study's major new finding is that the increased risk of progression to ESRD associated with higher DPI is linear and has no threshold. This indicates that there is no optimal level of protein intake in CKD with regard

to mGFR decline, at least within the range observed in this study (0.34–2.76 g/kg per day). This study also showed that the risk of ESRD associated with higher DPI did not significantly differ by diabetes status, but may be stronger at low GFR. The value of these findings is strengthened by the consistency of the risk estimates provided by the 2 independent methods used to assess DPI and the accurate measurement of GFR to assess kidney function and its decline over time. In this cohort of patients with optimal CKD management including dietary counseling, we observed that only a few (14%) met the recommended goal of DPI < 0.8 g/kg per day, and a significant number (20%) had DPI exceeding the upper recommended limit of 1.3 g/kg per day. Nonetheless, DPI was lower at lower mGFR values. Low DPI levels were not associated with greater mortality in this range, but very few patients (<2%) had a DPI < 0.6 g/kg per day, the threshold below which lowering DPI may become deleterious.

Our results confirm and extend several previous studies showing that lowering DPI benefits CKD patients.^{2,10,19–21} In a Cochrane Review of adults with CKD and without diabetes from 10 RCTs, Fouque and Laville reported a risk of kidney failure that was 32% lower among patients with reduced, compared to higher, protein intake.¹⁰ Among patients with type 1 diabetes, Hansen *et al.* showed that moderate dietary protein restriction was associated with a risk of ESRD or death 77% lower than with standard protein intake.²² Another Cochrane Review concluded that

Table 4. Baseline dietary protein intake and other factors associated with mGFR slope over time: linear mixed model

	$\beta \pm$ SD	P
Slope (ml/min per yr), ^a according to baseline mGFR		
\geq 45 ml/min	-1.899 ± 0.182	<.0001
30–44 ml/min	-1.418 ± 0.165	<.0001
<30 ml/min	-1.160 ± 0.158	<.0001
Other baseline factors associated with mGFR slope over time ^b		
DPI-UE (per 0.1 g/kg per d)	-0.064 ± 0.031	0.03
Diabetes	-0.486 ± 0.213	0.02
Log protein-to-creatinine ratio	-0.758 ± 0.093	<.0001
Mean blood pressure	-0.017 ± 0.009	0.05

mGFR, measured glomerular filtration rate; DPI-UE, dietary protein intake estimated from 24-h urea excretion.

^aCrude slope.

^bAdjusted β for baseline mGFR (<30, 30–44, \geq 45 ml/min), age, gender, origin (sub-Saharan Africa, other), diabetes status, continuous mean blood pressure, urinary protein-to-creatinine ratio (log-transformed), center, and number of mGFR measurements.

reducing protein intake appeared to slightly slow progression to renal failure in patients with type 1 and type 2 diabetes, but not statistically significantly so.²³ In the meta-analysis by Pedrini *et al.*, protein restriction in CKD patients both with and without diabetes was associated with slower progression of kidney disease.²¹ However, results from the original MDRD trial were mixed.^{5,7–9} Among patients with moderate renal disease (baseline GFR 25–55 ml/min per 1.73 m²), GFR declined more slowly and proteinuria was lower in those assigned to a low-protein diet than in those with a usual diet,^{5,6} but the groups did not differ significantly in terms of ESRD risk or all-cause mortality in the long term.⁸ As Levey *et al.* explained in 1999,⁷ however, the initial GFR reduction observed in the first 4 months after patients with moderate renal disease start a low-protein diet is simply the (short-term and) normal response of the kidney (whether healthy or diseased) to a reduction in protein intake. The mean GFR decline in the subsequent 32 months was significantly slower ($P = 0.009$) in these patients than in those assigned to the usual protein diet. In contrast, among patients with advanced renal disease (baseline GFR 13–24 ml/min per 1.73 m²), GFR did not decline more slowly for those on a very low-protein diet supplemented with keto acids compared with those on a low-protein diet,^{5,6} and a long-term follow-up study even observed an excess risk of death.⁹ Our study showed no significant excess mortality in patients achieving a DPI < 0.8 g/kg per day but very few (< 2%) achieved levels as low as 0.6 g/kg per day, as in the MDRD study. Further studies have shown that a keto acid-supplemented very-low-protein diet has a beneficial effect on rate of progression among advanced CKD patients,² on dialysis initiation,^{2,24} and on long-term outcome.^{2,19} Variable adherence to dietary protein restrictions may explain the differences between studies. Indeed, intense nutrition education has been shown to be more effective than standard dietary counseling in reducing protein intake.²⁵

Our findings are also in line with those from observational studies in the general population. In the Nurses' Health Study, high protein intake was associated with a greater decline in kidney function among women with mild CKD, compared to those with normal renal function.²⁰ Other data from this study showed that a Western dietary pattern, defined by high intake of red and processed meats, saturated fats, and sweets, was also associated with significantly higher risks of microalbuminuria and of rapid kidney function decline than among women eating less of these foods.²⁶ This finding is consistent with that from the Framingham Heart Study showing higher risk for developing adverse kidney measures in participants with low

adherence to meat and legume consumption recommendations.²⁷ Likewise, in the population-based Gubbio study, which included men and women aged 45 to 64 years, high protein intake was associated with a higher estimated GFR at baseline and greater GFR decline over time.²⁸ Moreover, higher protein intake was recently associated with a higher risk of ESRD in the Singapore Chinese Health Study, a population-based cohort of Chinese adults followed up for 15 years.²⁹ Interestingly, red meat intake showed a strong dose-dependent relation with ESRD risk, whereas intake of poultry, fish, eggs, and dairy products were not associated with ESRD risk.

The mechanism underlying the deleterious effect of high DPI on CKD progression is well understood. Whereas carbohydrate and lipid metabolism produce only CO₂ and H₂O, which are easily excreted by the lungs and kidneys, protein metabolism leads to the production of nitrogen end products (urea, ammonia, uric acid, etc.) and strong acids that are excreted by the kidney and concentrated in urine at much higher levels than in plasma. A protein meal or an amino acid infusion have been shown to induce a rapid and reversible increase in GFR that is thought to depend on inhibition of tubuloglomerular feedback, involving the renin-angiotensin system and nitric oxide, and possibly influenced by vasopressin and glucagon.³⁰ Hyperfiltration induces a vicious circle because of the work overload imposed on the remaining nephrons in terms of filtration and reabsorption of the extra solutes filtered (including sodium and other electrolytes, glucose, amino acids, and small proteins). This progressive insult leads to ESRD.³¹ Our finding that the HR for ESRD associated with higher DPI was stronger at lower GFR may reflect a potentially stronger impact of this work overload when renal function is reduced. In contrast, lowering protein intake during experimental kidney disease is associated with a reverse protective effect, for example, reduced hyperfiltration and less severe and less progressive renal insult, as reported during the first 4 months of the MDRD study.^{7,8} It is important to emphasize that although the present study is very useful in solidifying our understanding about the deleterious renal impact of high protein intake (i.e., protein intake that is higher than what is physiologically necessary for most people), it cannot inform about a lower safety threshold, and the risks and benefits of very-low-protein diet, because of the few number of patients with a DPI < 0.6 g/kg per day in this study.

Major strengths of this study include its large sample size, the extensive patient phenotyping including a number of laboratory measurements performed with reference methods, as well as the

availability of 2 independent methods for assessing DPI, namely, 24-hour urinary urea excretion and a 7-day food record.¹² A key advantage of this study is that GFR was measured by a validated method and not estimated from serum creatinine concentration, which may be affected by protein intake.³² It should be noted that our findings about the association of DPI with CKD progression were consistent regardless of whether we used ESRD incidence or mGFR decline as the outcome. Several studies have raised questions about the accuracy and validity of questionnaires assessing dietary intake.^{33,34} Although 24-hour urine urea excretion is a reliable biomarker, it is rarely used in epidemiological studies to assess protein intake. In this study, we found that both methods provided similar mean DPI at the population level, despite their relatively poor agreement at the individual level according to Bland–Altman plots. The consistent risk estimates for ESRD and mortality yielded by these 2 methods strengthen our findings.

This study also has limitations. First, it is an observational study and thus precludes any causal interpretation. In particular, it is difficult to disentangle whether the association of lower DPI with lower GFR observed at baseline resulted from successful dietary advice or from spontaneous reduction of DPI with GFR decline. Nevertheless, observational studies have some advantages over clinical trials, notably the ability to study a large range of DPI levels without predefined risk thresholds. They are therefore complementary to and reinforce findings from clinical trials. Moreover, several rodent studies have clearly established causality between the level of protein intake and CKD progression.³⁵ Second, we cannot rule out the possibility that low DPI may mask the symptoms of uremia and thus delay the need to start dialysis, although without slowing the rate of CKD progression. The association between lower DPI at baseline and subsequent steeper kidney function decline nonetheless suggests that DPI affects CKD progression. Finally, total energy intake was not available in all patients, but we were able to show in a subgroup that adjusting for total energy intake did not abolish the associations observed between DPI and the outcomes.

In conclusion, our study shows that usual dietary protein intake, whether measured by urea excretion or 7-day food records in these patients under nephrology care, is higher than expected at each stage of CKD; this finding reflects poor adherence to the recommendations. The linear no-threshold relation shown in this study between either method of DPI assessment and both the onset of kidney failure and steeper mGFR decline strengthens the evidence that a moderately low DPI is beneficial for reducing CKD progression. The

lack of impact on mortality reflects the safety of low DPI, at least in the range observed in this study, for example, in patients with DPI > 0.6 g/kg per day. Overall, these findings underline the importance of dietary counseling and reasonable protein intake reduction to preserve kidney function in CKD patients.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary Material. Figure S1 shows the distribution of creatinine clearance ratio (24-hour Ccreat ÷ 30-minute Ccreat). Figure S2 shows the study of the agreement between the 2 dietary protein intake estimates. Figure S3 shows the measured glomerular filtration rate (mGFR) subgroup analysis of end-stage renal disease (ESRD) risk according to dietary protein intake estimated from 24-hour urinary urea excretion or 7-day food records. Table S1 shows the hazard ratios for ESRD in 1412 patients with chronic kidney disease stages 1 to 4 at baseline. Table S2 shows the hazard ratios for death before ESRD in 1412 patients with chronic kidney disease stages 1 to 4 at baseline.

Supplementary material is linked to the online version of the paper at www.kireports.org.

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