

Comparison of Different Measures of Urinary Protein Excretion for Prediction of Renal Events

Hiddo J. Lambers Heerspink,* Ron T. Gansevoort,[†] Barry M. Brenner,[‡] Mark E. Cooper,[§] Hans Henrik Parving,^{||} Shahnaz Shahinfar,[¶] and Dick de Zeeuw*

*Department of Clinical Pharmacology, and [†]Division of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; [‡]Brigham and Women's Hospital and Harvard School of Medicine, Boston, Massachusetts; [§]Baker IDI Heart and Diabetes Research Institute, Melbourne, Australia; ^{||}Department of Medical Endocrinology, University Hospital of Copenhagen, Copenhagen, Denmark; and [¶]Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

ABSTRACT

There are many methods to screen for abnormal amounts of proteinuria to identify patients at risk for progression of renal disease, but which method best predicts renal risk is unknown. Here, we analyzed a subset of 701 patients with type 2 diabetes and nephropathy participating in the Reduction of Endpoints in Non Insulin Dependent Diabetes Mellitus with the Angiotensin II Antagonist Losartan (RENAAL) trial to compare the ability of urinary protein excretion (UPE) and urinary albumin excretion (UAE) from a 24-hour urine collection and urinary albumin concentration (UAC) and the albumin:creatinine ratio (ACR) from a first-morning void in predicting renal events. The primary outcome measure was the time to a doubling of serum creatinine or end-stage renal disease. During follow-up, 202 events occurred. The hazard ratios for the risk of a renal outcome (95% CIs) associated with 1-SD increment in the log-transformed measures were 3.16 (2.60 to 3.86) for UAE, 3.02 (2.53 to 3.62) for UPE, 3.23 (2.67 to 3.91) for UAC, and 4.36 (3.50 to 5.45) for ACR. The area under the ROC curve was significantly higher for ACR compared with the other measures. In conclusion, measurement of the albumin:creatinine ratio in a first-morning void is the superior method to predict renal events in patients with type 2 diabetes and nephropathy.

J Am Soc Nephrol 21: ●●●-●●●, 2010. doi: 10.1681/ASN.2010010063

Increased levels of proteins in the urine have been clearly established as an important determinant for renal complications in various populations and settings.¹⁻³ Screening for increased protein excretion has therefore been advocated to identify individuals at risk for renal disease progression in a timely manner. However, there is still continuing uncertainty as to how urine should be collected and which urinary proteins should be specifically measured for prediction of renal events.⁴

From a historical perspective, initial recommendations for urinary protein assessment included the measurement of 24-hour total protein excretion. When it was discovered that even small amounts of albumin predicted renal and cardiovascular disease progression, the total protein assays were replaced

by albumin assays because the former was not sensitive enough to detect quantities of albumin in the normal or just above the normal range.

With respect to urine collection procedures, 24-hour collections were initially advocated because of the circadian rhythm of urinary protein excretion.⁵ However, 24-hour urine collection is a cumbersome

Received January 15, 2010. Accepted March 28, 2010.

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Hiddo J. Lambers Heerspink, Department of Clinical Pharmacology, University Medical Center Groningen, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands. Phone: +31 50 363 2809; Fax: +31 50 363 2812; E-mail: H.J.Lambers.Heerspink@med.umcg.nl

Copyright © 2010 by the American Society of Nephrology

Table 1. Baseline characteristics

N	701
Age (years)	60.4 (7.3)
Men, n (%)	436 (62.2)
Caucasian, n (%)	343 (46.1)
African American, n (%)	144 (20.5)
Hispanic, n (%)	192 (27.4)
Serum albumin (g/dl)	3.8 (0.4)
BMI (kg/m ²)	30.1 (6.3)
Diastolic BP (mmHg)	81.5 (10.5)
Systolic BP (mmHg)	151.3 (20.1)
Hemoglobin (mg/dl)	12.4 (1.9)
HbA1c (%)	8.6 (1.7)
Total cholesterol (mg/dl)	225.0 (53.4)
Creatinine (mg/dl)	1.85 (0.5)
eGFR (ml/min per 1.73 m ²)	40.7 (13.0)
24-hour urine:	
albumin excretion (mg/24 h)	1378 (620 to 2897)
protein excretion (mg/24 h)	2290 (1121 to 4870)
creatinine excretion (g/24 h)	1.3 (0.8)
creatinine concentration (g/L)	0.67 (0.3)
First-morning void:	
albumin concentration (mg/L)	663 (285 to 1370)
albumin:creatinine ratio (mg/g)	1043 (470 to 2359)
creatinine concentration (g/L)	0.68 (0.3)

Data are given as means (SD) or medians (interquartile ranges) in case of skewed data distribution. eGFR, estimated GFR.

some procedure and subject to collection errors. This has led professional organizations to advise collection of untimed urine samples and report the urinary albumin concentration or albumin:creatinine ratio. These recommendations are based on numerous cross-sectional studies demonstrating good correlations of urinary albumin concentration and albumin:creatinine ratio in untimed urine samples and urinary albumin excretion in 24-hour urine collection.⁶⁻⁸ However, none of these studies have assessed and indeed compared the relative importance and discriminatory abilities of different proteinuria measures in predicting the risk for renal events.⁹

The objective of this study was therefore to assess and compare the ability of various proteinuria measures (including proteinuria *versus* albuminuria and 24-hour *versus* early morning sampling) to predict renal events, as determined by a doubling of serum creatinine (DsCR) or end-stage renal disease (ESRD), in patients with diabetes and nephropathy participating in the RENAAL trial.

RESULTS

The baseline characteristics of the 701 participants who collected both a first-morning void and a 24-hour urine are presented in Table 1. During follow-up, 202 (28.8%)

doubling of serum creatinine or ESRD endpoints were recorded.

Baseline proteinuria measures showed a positive continuous relationship with the risk for DsCR or ESRD. The effect size of the first-morning void albumin:creatinine ratio was higher than the other proteinuria measures (Figure 1). There was no consistent interaction among the four proteinuria measures and age, gender, race, and treatment allocation. When the data were analyzed according to quintiles of baseline proteinuria measures, a stepwise increase in the risk for DsCR or ESRD was observed (Figure 2). The first-morning void albumin:creatinine ratio demonstrated the steepest increase in the risk for DsCR or ESRD compared with the other proteinuria measures.

For prediction of DsCR or ESRD, the area under the curve (AUC) of the first-morning void albumin:creatinine ratio was significant higher compared with the three other proteinuria measures (Table 2). The AUC of the first-morning void albumin:creatinine ratio was also largest in age-, gender-, and race-specific subgroups, although not statistically significant in all subgroups, which is possibly because of the small number of events in some subgroups. The AUC for urinary albumin concentration in a first-morning void was equal to 24-hour urinary albumin excretion and protein excretion and no difference was observed between 24-hour urinary albumin excretion and protein excretion. Finally, the AUC for 24-hour urinary albumin:creatinine ratio and protein:creatinine ratio were 0.82 (0.78 to 0.85) and 0.82 (0.79 to 0.86), respectively, which was not statistically significantly different from the first-morning void albumin:creatinine ratio.

The relative integrated discrimination improvement (RID) statistics are presented in Table 3. Significant increases of 39.5% of the RID were observed when baseline 24-hour urinary albumin excretion was replaced by first-morning void albumin:creatinine ratio. These data indicate an improvement in the predictive value when a model includes first-morning void albumin:creatinine ratio instead of 24-hour urinary albumin excretion. The RID score marginally changed when 24-hour urinary albumin excretion was replaced by 24-hour urinary protein excretion or by first-morning void urinary

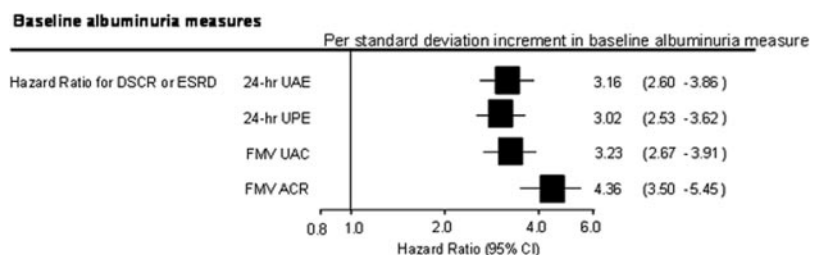


Figure 1. Increased risk for renal events per standard deviation increment in baseline first morning void albumin:creatinine ratio compared to other proteinuria measures. Boxes represent the point estimate (HRs) and the horizontal bars the 95% CI. ACR, albumin:creatinine ratio; FMV, first-morning void; UAE, urinary albumin excretion; UPE, urinary protein excretion; UAC, urinary albumin concentration. Log-transformed SD: UAE = 1.1; SD UPE = 1.0; SD UAC = 1.1; SD ACR = 1.1.

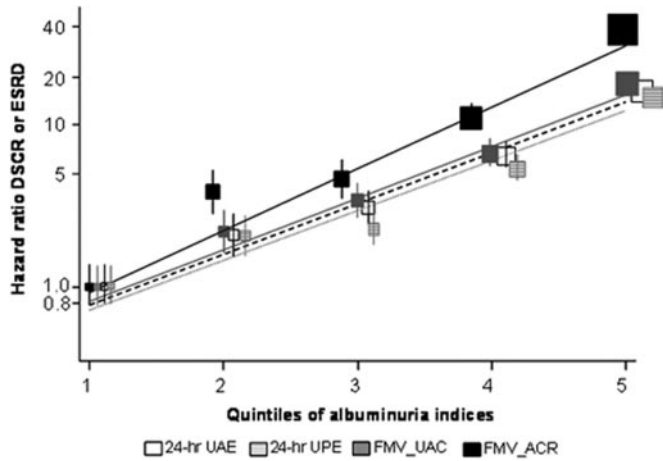


Figure 2. Increased risk for renal events by quintiles of baseline first morning void albumin:creatinine ratio compared to other proteinuria measures. Boxes represent the point estimate (HRs) and the vertical bars its 95% CI. The size of the boxes are proportional to the number of events. ACR, albumin:creatinine ratio; FMV, first-morning void; UAE, urinary albumin excretion; UPE, urinary protein excretion; UAC, urinary albumin concentration.

albumin concentration. The predictive performance for DsCR or ESRD was significantly improved when the first-morning void albumin:creatinine ratio was used instead of first-morning void urinary albumin concentration.

The risk for DsCR or ESRD increased statistically significantly for each SD decrement in urinary creatinine measure, after adjustment for age, gender, race, and treatment allocation (Figure 3). The three urinary creatinine measures remained statistically significantly associated with DsCR or ESRD even after adjustment for a range of renal risk markers, including

Table 3. RIDI in classification for predictive scores for doubling of serum creatinine or ESRD

	Doubling Serum Creatinine or End-Stage Renal Disease	
	RIDI Estimate (%)	P
24-hour UAE versus FMV ACR	39.5	<0.0001
24-hour UAE versus 24-hour UPE	1.0	0.684
24-hour UAE versus FMV UAC	6.1	0.154
FMV UAC versus FMV ACR	31.5	<0.0001

The RIDI compares a model including 24-hour urinary albumin excretion with the equivalent model in which 24-hour urinary albumin excretion is replaced by either 24-hour urinary protein excretion, first-morning void urinary albumin concentration, or first-morning void albumin:creatinine ratio. Finally, a model with first-morning void urinary albumin concentration is compared with a model including first-morning void albumin:creatinine ratio. For each line, the models replace the second listed proteinuria measure with the first mentioned proteinuria measure. Models were adjusted for age, gender, race, and treatment allocation. ACR, albumin:creatinine ratio; FMV, first-morning void; UAC, urinary albumin excretion; UAE, urinary albumin excretion; UPE, urinary protein excretion.

systolic and diastolic BP, estimated GFR, urinary albumin excretion hemoglobin, HbA1c, serum albumin, and smoking status.

DISCUSSION

This study provides a comprehensive overview of the performance of different proteinuria measures derived from 24-hour and first-morning void collections in predicting renal events. The albumin:creatinine ratio derived from a first-morning void displayed the strongest association with the risk for renal events. Receiver operator characteristics (ROC) comparison and RIDI analyses showed that the predictive value of the first-

Table 2. Area under the ROC curve and 95% CIs for the prediction of the composite of doubling of serum creatinine or end-stage renal disease based on baseline proteinuria measures

	Subjects/Events	Doubling of Serum Creatinine or End-Stage Renal Disease			
		24-Hour Urine		First-Morning Void	
		UAE (mg/24 h)	UPE (mg/24 h)	UAC (mg/L)	ACR (mg/g)
Overall	701/202	0.78 [0.74, 0.82]	0.78 [0.75, 0.82]	0.79 [0.75 to 0.83]	0.82 [0.79, 0.86] ^{a,b,c}
Subgroups					
Gender					
men	436/107	0.76 [0.71, 0.81]	0.76 [0.70, 0.81]	0.77 [0.72, 0.82]	0.79 [0.73, 0.84]
women	265/95	0.81 [0.76, 0.87]	0.83 [0.77, 0.88]	0.82 [0.77, 0.87]	0.86 [0.82, 0.91] ^{a,c}
Age					
≤61.0 years	351/120	0.78 [0.72, 0.83]	0.79 [0.74, 0.84]	0.80 [0.75, 0.85]	0.84 [0.79, 0.88] ^{a,b,c}
>61.0 years	350/82	0.77 [0.71, 0.83]	0.76 [0.70, 0.83]	0.77 [0.71, 0.83]	0.80 [0.75, 0.86]
Race					
Caucasian	323/75	0.76 [0.70, 0.82]	0.77 [0.71, 0.83]	0.79 [0.73, 0.85]	0.79 [0.74, 0.85]
African American	144/33	0.75 [0.64, 0.86]	0.76 [0.65, 0.86]	0.77 [0.68, 0.87]	0.83 [0.74, 0.91]
Hispanic	192/78	0.78 [0.71, 0.85]	0.78 [0.71, 0.85]	0.78 [0.71, 0.85]	0.82 [0.75, 0.88] ^{a,b}
Gender, age, and race adjusted	701/202	0.79 [0.75, 0.83]	0.79 [0.75, 0.83]	0.80 [0.76 to 0.83]	0.82 [0.79, 0.86] ^{a,b,c}

Bonferroni correction was applied in AUC comparison to adjust for multiple testing. ACR, first-morning void albumin:creatinine ratio; AUC, area under the ROC curve; UAE, 24-hour urinary albumin excretion; UPE, 24-hour urinary protein excretion.

^aP < 0.01 versus UAE.

^bP < 0.01 versus UPE.

^cP < 0.01 versus UAC.

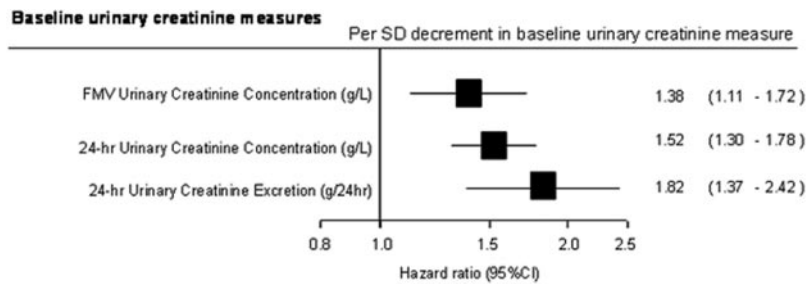


Figure 3. Higher risk for renal events for each standard deviation decrement in baseline urinary creatinine measure. Boxes represent hazard ratios and the horizontal bars its 95% CI. SD urinary creatinine concentration FMV, 0.32; creatinine concentration 24 hours, 0.28; creatinine excretion 24 hours, 0.83. FMV, first-morning void.

morning void albumin:creatinine ratio was significantly higher than 24-hour urinary albumin excretion or protein excretion and confirmed the superiority of the first-morning void albumin:creatinine ratio in predicting renal events.

Standardization of proteinuria measures will improve the use of urinary albumin for detecting and monitoring kidney disease. Currently, various methods for collecting urine and reporting urinary albumin are recommended by different treatment guidelines. Indeed, surveys conducted in different countries have shown that a broad range of methods to collect urine and choice of measurement procedure are applied in clinical practice.^{10–12} The different methods of urinary albumin assessment may be confusing for clinicians and hamper the use of urinary albumin for managing kidney disease. In line with the conclusion of a recent conference on the standardization of urinary albumin, we recommend that first-morning voids be used and that urinary albumin be reported as the albumin:creatinine ratio to detect and monitor kidney disease.⁴

There is a plausible rationale for expecting greater predicting abilities of the albumin:creatinine ratio in a first-morning void compared with urinary albumin concentration. Urinary albumin concentration depends on hydration status such that a higher urinary output lowers urinary albumin concentration and *vice versa*. Because the albumin concentration is divided by urinary creatinine concentration, which is assumed to be excreted with relative constancy over 24 hours, the ratio adjusts for changes in hydration status. This augments the ability of the albumin:creatinine ratio to predict renal events.

Of interest, our data show that the albumin:creatinine ratio predicted outcome even better than 24-hour urinary albumin excretion. Because of the circadian rhythm of proteinuria, which furthermore differs between patients, we anticipated that proteinuria measures from first-morning void urine samples would predict outcome, at the very best, as good as 24-hour urinary albumin excretion. There may be two explanations for our unexpected finding. First, the albumin:creatinine ratio not only depends on urinary albumin concentration but also on urinary creatinine concentration. Decreased urinary creatinine excretion reflects lower muscle mass and may indicate poor health. Indeed, we demonstrated an association be-

tween urinary creatinine excretion and the risk for DsCR or ESRD, which was independent of age, gender, race, and other risk markers for renal disease progression. Hence, an explanation for the superior predictive performance of the albumin:creatinine ratio is that it incorporates the predictive value of poor health, as reflected by low muscle mass and decreased urinary creatinine excretion. Indeed, a recent study established that decreased urinary creatinine excretion is associated with increased risk for all cause mortality in the general population.¹³ It should be noted that these re-

sults do not negate the predictive value of urinary albumin itself. Urinary albumin concentration and urinary albumin excretion were strongly correlated with the renal endpoint, albeit the albumin:creatinine ratio demonstrated the strongest association. An alternative explanation is that patients could have made collection errors during the 24-hour urine collection, which will negatively affect the predictive performance of the 24-hour urinary albumin excretion. Indeed, when we adjusted for urinary creatinine excretion, the predictive performance of the albumin:creatinine ratio and protein:creatinine ratio in the 24-hour urine samples was similar to that seen with the albumin:creatinine ratio in the first-morning void.

Collection of a first-morning void is less cumbersome than a 24-hour sample. This implies that a first-morning void is favored for clinical practice even when no difference in predictive performance would be observed. This study demonstrated that the first-morning void albumin:creatinine ratio showed a stronger association with the risk for DsCR or ESRD than 24-hour urinary albumin excretion, although the numerical differences in the hazard ratio and area under the ROC curve among the proteinuria measures were modest. We therefore advocate measurement of the albumin:creatinine ratio in a first-morning void.

Little information is available on the prognostic significance of albuminuria and proteinuria measures for renal disease progression. The results of this analysis demonstrate that both measures perform equally well in predicting renal outcome. If specific proteins other than albuminuria display a higher association with renal outcomes requires further exploration.

How do these results compare with the literature? Only one study has investigated the value of different proteinuria measures as predictors of decline of renal function in patients and this was in patients with nephropathy who did not have diabetes.¹⁴ These results are in line with our own study in patients with diabetes and demonstrate that the protein:creatinine ratio in a first-morning void in individuals with chronic kidney disease who do not have diabetes was at least as reliable as 24-hour urinary protein excretion in predicting the rate of decline in renal function and risk for progression to end-stage renal disease. In that study, however, albuminuria was not assessed and

thus no comparison could be made between albuminuria and proteinuria.

Although the RENAAL trial included a diverse range of patients with type 2 diabetes with reduced GFR and macroalbuminuria, the results cannot be directly applied to individuals without diabetes or nephropathy. However, a previous study demonstrated that the predictive value of the albumin:creatinine ratio for cardiovascular morbidity and mortality in the general population is slightly better than 24-hour urinary albumin excretion.¹⁵ These results together suggest that the albumin:creatinine ratio is a feasible and potentially more practical alternative to 24-hour urinary albumin excretion in various populations. Total protein concentrations were not measured in first-morning void urine sample and no comparison could be made between protein:creatinine and albumin:creatinine ratios derived from a first-morning void. However, because there was no difference between 24-hour albumin:creatinine and protein:creatinine ratios, it is not unlikely that the predictive ability of protein:creatinine and albumin:creatinine ratios in a first-morning void are similar.

In conclusion, for predicting renal disease progression in patients with type 2 diabetes and nephropathy, collecting first-morning void urine samples and measuring the albumin:creatinine ratio appear to be superior when compared with measuring 24-hour urinary albumin excretion. These results are clinically important because they imply that collection of first-morning voids, which is clearly more convenient than collecting a 24-hour urine, can be used for assessment of proteinuria.

CONCISE METHODS

RENAAL Study Design

The RENAAL study was a double-blind, randomized, placebo-controlled trial that was designed to evaluate the renoprotective effects of a losartan-based antihypertensive regimen compared with a traditional BP-lowering regimen in patients with type 2 diabetes, hypertension, and nephropathy. The study design, inclusion and exclusion criteria, and results have been reported elsewhere.^{16,17} In brief, participants were considered to have type 2 diabetes if they were over 30 years old at time of diagnosis of diabetes, had no history of ketoacidosis, and did not use insulin therapy within 6 months after diagnosis. A serum creatinine between 1.3 and 3.0 mg/dl (1.5 to 3.0 mg/dl for males more than 60 kg), urinary albumin:creatinine ratio from a first-morning specimen of at least 300 mg/g, HbA1c <12%, and age between 31 and 70 years were part of the inclusion criteria. All patients collected, at the randomization visit and every 3 months visit, a first-morning void urine sample for albumin and creatinine assessment. In addition, a random sample of 701 patients collected 24-hour urine samples for quantification of 24-hour albumin and total protein excretion. Urinary albumin and creatinine were measured by nephelometry (Beckman Array) and total protein by colorimetry (Olympus colorimetry) in a central laboratory. Measurements were conducted in fresh urine samples within 48 hours after collection.

The primary outcome measure of the RENAAL trial was defined as the time to the composite of a confirmed DsCR from the baseline

level, ESRD (defined as the need for long-term dialysis or renal transplantation) or all cause death. All potential endpoints were adjudicated by a blinded endpoint committee using rigorous guideline definitions.¹⁷ The combined endpoint of doubling of serum creatinine or ESRD was a prespecified endpoint and is used for this analysis.¹⁷ Information on the clinical endpoints was collected during a mean follow-up duration of 3.4 years with a range of 2.3 to 4.6 years. The RENAAL trial was conducted in accordance with the principles outlined in the Declaration of Helsinki. All patients signed informed consent. The protocol was approved by all relevant ethics committees.

Statistical Analysis

To compare the predictive abilities of the various proteinuria measures, we used three different analyses. First, we estimated the hazard ratio (HR) and 95% CI for a 1-SD higher level of the log-transformed urinary albumin and urinary creatinine measure at baseline by using Cox proportional hazard regression models. The multivariate Cox model was adjusted for age, gender, race, and treatment allocation. For further exploration of the HR profile, HRs (95% CI) for the participants according to quintiles in log-transformed proteinuria measures were calculated, with the HR in the first quintile used as the common reference to compute the HR (95% CI) for the remainder of the categories. The variance of each quintile of proteinuria measure was calculated by using the absolute floating risk method.¹⁸ The regression line for the risk estimates according to quintiles of baseline proteinuria measures was fitted using inverse variance weighting. The albumin:creatinine ratio depends on urinary creatinine excretion and thus on muscle mass. Muscle mass differs between sexes and ethnicities and declines with age. To investigate the effect of gender, race, and age, interaction terms for age, gender, race, and treatment allocation and also proteinuria measurements were included in the Cox proportional hazard models. Second, ROC curves were used to compare the ability of proteinuria measures to discriminate between patients who developed an event and those who did not. Nonparametric methods were used to compare area under the ROC curves. To investigate the effect of gender, race, and age on the discriminative abilities of the different proteinuria measures, we analyzed men and women, patients of different ethnicities, and patients above and below the median value of age separately. In additional analyses, age, gender, race, and treatment allocation adjusted ROC curves were calculated.

Third, the RIDI was calculated. The RIDI measures the percentage of increased discrimination when an extra variable is added to a prediction model.¹⁹

Because 24-hour urine collection procedures are subject to collection errors, we calculated in sensitivity analyses if adjustment for 24-hour urinary creatinine excretion improved the discriminative ability of 24-hour urinary albumin and protein excretion. Furthermore, the albumin:creatinine ratio depends on both urinary albumin and urinary creatinine excretion. Lower urinary creatinine excretion may be a consequence of reduced production caused by low muscle mass. Because low muscle mass is often associated with poor health, low urinary creatinine excretion may be indicative of general morbidity and associated with the risk for renal events. We therefore studied in another analysis the relation between urinary creatinine excretion and

DsCR and ESRD. All analyses were conducted with SAS version 9.1 and Stata version 10.0.

DISCLOSURES

The RENAAL study was sponsored by Merck & Co, Inc. Drs. Brenner, Cooper, Parving, and de Zeeuw are members of the RENAAL Steering Committee and have received grants from Merck. Dr. Shahinfar has been an employee of Merck. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit the manuscript for publication.

REFERENCES

1. de Zeeuw D, Remuzzi G, Parving HH, Keane WF, Zhang Z, Shahinfar S, Snapinn S, Cooper ME, Mitch WE, Brenner BM: Proteinuria, a target for renoprotection in patients with type 2 diabetic nephropathy: Lessons from RENAAL. *Kidney Int* 65: 2309–2320, 2004
2. Ninomiya T, Perkovic V, de Galan BE, Zoungas S, Pillai A, Jardine M, Patel A, Cass A, Neal B, Poulter N, Mogensen CE, Cooper M, Marre M, Williams B, Hamet P, Mancia G, Woodward M, Macmahon S, Chalmers J: Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes. *J Am Soc Nephrol* 20: 1813–1821, 2009
3. van der Velde M, Halbesma N, de Charro FT, Bakker SJ, de Zeeuw D, de Jong PE, Gansevoort RT: Screening for albuminuria identifies individuals at increased renal risk. *J Am Soc Nephrol* 20: 852–862, 2009
4. Miller WG, Bruns DE, Hortin GL, Sandberg S, Aakre KM, McQueen MJ, Itoh Y, Lieske JC, Seccombe DW, Jones G, Bunk DM, Curhan GC, Narva AS: Current issues in measurement and reporting of urinary albumin excretion. *Clin Chem* 55: 24–38, 2009
5. Hansen HP, Hovind P, Jensen BR, Parving HH: Diurnal variations of glomerular filtration rate and albuminuria in diabetic nephropathy. *Kidney Int* 61: 163–168, 2002
6. Jermendy G, Farkas K, Nadas J, Daroczy A, Peterfai E: Practical aspects of measuring microalbuminuria in diabetic patients. *Diabetes Nutr Metab* 14: 195–200, 2009
7. Guy M, Borzomato JK, Newall RG, Kalra PA, Price CP: Protein and albumin-to-creatinine ratios in random urines accurately predict 24 h protein and albumin loss in patients with kidney disease. *Ann Clin Biochem* 46: 468–476, 2009
8. Witte EC, Lambers Heerspink HJ, de Zeeuw D, Bakker SJ, de Jong PE, Gansevoort R: First morning voids are more reliable than spot urine samples to assess microalbuminuria. *J Am Soc Nephrol* 20: 436–443, 2009
9. Gansevoort RT, Brinkman J, Bakker SJ, De Jong PE, de Zeeuw D: Evaluation of measures of urinary albumin excretion. *Am J Epidemiol* 164: 725–727, 2006
10. Fagnani F, Souchet T, Labeled D, Gaugris S, Hannedouche T, Grimaldi A: Management of hypertension and screening of renal complications by GPs in diabetic type 2 patients (France-2001). *Diabetes Metab* 29: 58–64, 2003
11. Edge JA, Swift PG, Anderson W, Turner B: Diabetes services in the UK: Fourth national survey; are we meeting NSF standards and NICE guidelines? *Arch Intern Med* 90: 1005–1009, 2005
12. Aakre KM, Thue G, Subramaniam-Haavik S, Bukve T, Morris H, Muller M, Lovrencic MV, Plum I, Kallion K, Aab A, Kutt M, Gillery P, Schneider N, Horvath AR, Onody R, Oosterhuis W, Ricos C, Perich C, Nordin G, Sandberg S: Postanalytical external quality assessment of urine albumin in primary health care: an international survey. *Clin Chem* 54: 1630–1636, 2008
13. Oterdoom LH, Gansevoort RT, Schouten JP, de Jong PE, Gans RO, Bakker SJ: Urinary creatinine excretion, an indirect measure of muscle mass, is an independent predictor of cardiovascular disease and mortality in the general population. *Atherosclerosis* 207: 534–540, 2009
14. Ruggenenti P, Gaspari F, Perna A, Remuzzi G: Cross sectional longitudinal study of spot morning urine protein:creatinine ratio, 24 hour urine protein excretion rate, glomerular filtration rate, and end stage renal failure in chronic renal disease in patients without diabetes. *BMJ* 316: 504–509, 1998
15. Lambers Heerspink HJ, Brantsma AH, de Zeeuw D, Bakker SJ, de Jong PE, Gansevoort RT: Albuminuria assessed from first-morning-void urine samples versus 24-hour urine collections as a predictor of cardiovascular morbidity and mortality. *Am J Epidemiol* 168: 897–905, 2008
16. Brenner BM, Cooper ME, de Zeeuw D, Grunfeld JP, Keane WF, Kurokawa K, McGill JB, Mitch WE, Parving HH, Remuzzi G, Ribeiro AB, Schluchter MD, Snavely D, Zhang Z, Simpson R, Ramjit D, Shahinfar S: The losartan renal protection study—rationale, study design and baseline characteristics of RENAAL (Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan). *J Renin Angiotensin Aldosterone Syst* 1: 328–335, 2000
17. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 345: 861–869, 2001
18. Easton DF, Peto J, Babiker AG: Floating absolute risk: An alternative to relative risk in survival and case-control analysis avoiding an arbitrary reference group. *Stat Med* 10: 1025–1035, 1991
19. Pencina MJ, D'Agostino RB Sr., D'Agostino RB Jr., Vasan RS: Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med* 27: 157–172, 2008