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Nahid Tabibzadeh[1,2]*, M.D., Ph.D. Sandra Wagner[3,4]*, Ph.D., Marie Metzger[4], Ph.D., Martin Flamant[5,6], M.D., Ph.D., Pascal Houillier[7,8], M.D., Ph.D., Jean-Jacques Boffa[1,2,9], M.D., Ph.D., Francois Vrtovsnik[5,10], M.D., Ph.D., Eric Thervet[7,11], M.D., Ph.D., Bénédicte Stengel[3,4], M.D., Ph.D., Jean-Philippe Haymann[1,2], M.D., Ph.D., on behalf of the NephroTest Study Group

*NT and SW equally contributed to this work.

[1] Sorbonne Université, Inserm UMR_S 1155, Paris, France

[2] Unit of Renal Physiology, AP-HP Hôpital Tenon, Paris, France

[3] CESP, Inserm U1018, Univ Paris-Saclay, Univ Paris-Sud, UVSQ, Villejuif, France

[4] FCRIN INI-CRCT, France

[5] Université Paris Diderot, Paris, France

[6] Unit of Renal Physiology, AP-HP Hôpital Bichat, Paris, France

[7] Université Paris Descartes, Paris, France

[8] Unit of Renal Physiology, AP-HP Hôpital Européen Georges Pompidou, Paris, France

[9] Unit of Nephrology, AP-HP Hôpital Tenon, Paris, France

[10] Unit of Nephrology, AP-HP Hôpital Bichat, Paris, France

[11] Unit of Nephrology, AP-HP Hôpital Européen Georges Pompidou, Paris, France

Corresponding author:

Bénédicte Stengel

CESP, Inserm U1018

16 avenue P. Vaillant Couturier

F-94807 Villejuif cedex

Abstract

Rationale & Objective. Chronic kidney disease (CKD) characterized by decreased glomerular filtration rate (GFR) is often accompanied by various degrees of impaired tubular function in the cortex and medulla. Assessment of tubular function may, therefore, be useful in establishing the severity of kidney disease and in identifying those at greater risk of CKD progression. We explored reductions in urinary concentrating ability, a well-known feature of CKD, as a risk factor for GFR decline and end-stage kidney disease (ESRD).

Study Design. Prospective longitudinal cohort study.

Setting and participants. 2,084 adult patients with CKD stages 1 to 4 from the French NephroTest Cohort Study.

Predictor. Fasting urinary osmolality (Uosm) measured by delta cryoscopy.

Outcomes. End-stage renal disease (ESRD), mortality prior to ESRD, and measured GFR (mGFR) assessed by ^{51}Cr- EDTA renal clearance.

Analytical Approach. Cause-specific hazards models were fit to estimate crude and adjusted associations of urinary osmolality with ESRD and death prior to ESRD. Linear mixed models with random intercepts were fit to evaluate the association of urinary osmolality with slope of decline in mGFR.

Results. At baseline, mean age was 58.7±15.2 (SD) years with a median mGFR of 40.2 [IQR, 29.1-54.5] ml/min and a median fasting Uosm of 502.7 ± 151.7 mOsm/kg H_2O. Baseline fasting Uosm was strongly associated with mGFR (R=0.54, p<0.001). 380 ESRD events and 225 deaths prior to ESRD occurred during a median follow-up of 5.9 years [3.8-8.2]. Patients with lower baseline fasting Uosm had a higher adjusted risk of

ESRD but not of mortality (HRs of 1.9 [95% CI, 1.2-3.0] and 0.99 [95% CI, 0.68-1.44], respectively, for the lowest versus highest tertile). Based on a mixed linear model, adjusted for baseline mGFR and clinical characteristics, patients in the lowest tertile of baseline Uosm had a steeper decline in kidney function (-4.9% ± 0.9% per year, $p<0.001$) compared to patients in the highest tertile.

Limitations. Fasting was self-reported.

Conclusions. Fasting Uosm may be a useful tool, in addition to GFR and albuminuria, for assessing non-glomerular damage in patients with CKD who are at higher risk of CKD progression.

Keywords: urine osmolality, chronic kidney disease **(**CKD), CKD progression, tubular damage; urine concentration ability, glomerular filtration rate (GFR), measured GFR (mGFR), GFR decline, end-stage renal disease (ESRD), prognostic factor, biomarker

Introduction

The ability of kidneys to concentrate urine enables the excretion of a maximal amount of osmoles in a minimal urine volume, leading to a urinary osmolality which can considerably exceed plasma osmolality. Differences among species depend on the axial length of the papilla, and human kidneys are able to raise urine osmolality to a maximum of 1200 mOsm/kg H_2O after a fasting state.[1]

Chronic kidney disease (CKD) is characterized by a decreased glomerular filtration rate (GFR) and several tubular defects including impaired urine concentration ability, especially at late stages of the kidney disease. Of note, regardless of the cause of Kidney failure, the natural history of chronic kidney failure is characterized by hyposthenuria in experimental models as well as in humans [2–4].

It is not currently known whether therapeutic interventions on urine concentration are efficient in preventing kidney function decline. Some authors suggest that decreasing urine osmolality, and consequently plasma vasopressin, would be kidney protective,[5–8] whereas others conclude that high water intake and high urine volume output could be deleterious [3,9–11].

Kidney function is usually assessed by the estimation or the measure of the GFR *i.e.* the filtration process within glomeruli, whereas nephron functions also include other structures such as tubules and peritubular capillaries. Indeed, tubules ensure several other functions, including the establishment and maintenance of a cortico-papillary concentration gradient. Moreover, many studies have shown the highly predictive value of interstitial fibrosis and tubular atrophy in CKD, irrespective of the underlying nephropathy, either in the native kidney or in allografts [12–16]. We presumed that fasting

urine osmolality, a reliable marker of maximal urine concentration, would provide a relevant assessment of tubular dysfunction independently of glomerular alteration and thus could be of prognostic value. Using a prospective cohort of stage 1-4 CKD patients, our study aimed at investigating the association of fasting urine osmolality with CKD progression (*i.e.* end stage renal disease (ESRD) and mGFR decline and pre-ESRD death. We hypothesized that lower fasting urine osmolality would be associated with worse outcomes.

Methods

Study population and design

The NephroTest cohort is a prospective multicenter study that enrolled 2,084 adult patients with all stages of CKD referred by nephrologists to three departments of physiology for extensive workups between January 2000 and December 2012 [17]. Patients were recruited and followed-up during this period until December 2013. Eligible patients were ≥18 years of age at inclusion and had neither started dialysis nor received a kidney transplant. Pregnant women were excluded. After exclusion of 62 patients with missing data for urine osmolality measurement, 123 patients with mGFR < 15 ml/min and 103 patients with a single visit and untraceable thereafter through any clinical or administrative health databases, this analysis included 1,796 patients (Figure 1). All patients provided written informed consent. The NephroTest study design was approved by an ethics committee (CCTIRS MG/CP09.503).

Data collection and measurements

Over the study period, patients underwent a total of 5199 laboratory visits with Uosm and mGFR data and a median of 2 [IQR, 1-4] visits in addition to the inclusion

visit. The median period between visits was 1.08 (IQR, 0.99-1.51) years.

During a 5-h in-person visit, a large set of clinical and laboratory data were collected. They included demographics, renal diagnosis, medical history, height and weight, blood pressure (BP), and treatments received. Height and weight were measured at the clinical examination and obesity was defined as body mass index > 30 kg/m². Diabetes was either self-reported or defined as fasting glycemia > 7 mmol/L or antidiabetic drug treatment. Smoking was self-reported. Hypertension was defined as elevated blood pressure (BP, average of 3 office measurements) ≥140/90 mmHg or the use of antihypertensive drugs. Cardiovascular disease history was defined as a history of stroke, ischemic heart disease (angioplasty, surgical coronary bypass, or myocardial infarction), or heart failure. The type of nephropathy was collected from patient's medical record. Patients were instructed to fast (not to eat or drink) from 8 p.m. the day before the admission. At each visit, fasting blood samples, 24-h urine collection and fasting urine samples were collected. Fasting Uosm was measured by delta cryoscopy (Osmometer, Radiometer, Denmark). Though the terms osmolality (osmoles per kilogram of solute) and osmolarity (osmoles per liter of solvent) differ scientifically, for the sake of simplicity, in this article Uosm refers to both. Urinary albumin-creatinine ratio (ACR) was measured in 81% of the patients, either using the 24-hour collection or a spot morning sample, and estimated from the protein-creatinine ratio (PCR) in 17% of those without urinary tract infection; it was missing in others.

At each visit, mGFR was measured by urinary ^{51}Cr-EDTA clearance (GE Healthcare, Velizy, France) and determined as the average of 5–7 consecutive 30 min clearance periods (fractionated clearances), as previously described[18].

Outcomes

The primary end-point of this study was ESRD defined as initiation of renal replacement therapy (RRT) by dialysis or pre-emptive transplantation as well as its competitive event of pre-ESRD death. The secondary end-point was mGFR over time. Information about deaths and their causes and ESRD events was obtained either from patient medical records or linkage with the national death registry and the national *REIN* (Renal Epidemiology and Information Network) registry of treated ESRD. All survival data were right-censored on 31 December 2013, or on the last visit when the patients were not found in registries.

Statistical analyses

Univariate analyses were performed with all the covariates, and all variables with $p<0.2$ were included on the multivariable analysis. Patient characteristics were then described according to gender-specific tertiles of Uosm.

First, differences between the three tertiles were tested with analysis of variance (ANOVA), the Kruskal–Wallis test or the Chi-square test, as appropriate.

Second, we performed cause-specific hazards models to estimate crude and adjusted hazard ratios (HRs) and 95% confidence intervals [HR (95% CI)] for ESRD and pre-ESRD death associated with gender-specific Uosm tertiles, using the highest tertile as the reference category. In each model, the competing events were treated as censored observations. The cause-specific approach is the most suitable to account for competing risks of concurrent events for etiological studies according to previous studies [19]. Sequential models were developed for each outcome studied with similar covariates for adjustment in both analyses: model 1 was adjusted for mGFR; model 2 included model 1

covariates and age, sex, center, ethnicity, obesity; model 3 included model 2 covariates and ACR in class (<3, 3-30, >30 mg/mmol), high blood pressure, diabetes, CV history, smoking status, ACE inhibitors or ARBs, nephropathy type, and natremia. We also tested the interaction between osmolality and type of nephropathy; and between osmolality and age. In all models, we performed tests for linear trend across tertiles of Uosm. Subsidiary analysis was performed with Uosm treated as a continuous variable. Assessment of the Schoenfeld residuals showed that none of the variables (except mGFR) violated the proportional-hazards assumption in the cause-specific hazards model for ESRD. Consequently, interaction term between mGFR and log(time) was used in model 1 to 3 for ESRD to account for time-varying effects.

Third, we used linear mixed model with random intercepts and slope to estimate mean annual decline in mGFR and study the association between baseline osmolality and mGFR in terms of mGFR slope where slope is reported in % per year. We estimated mean differences in mGFR slopes and 95% confidence interval adjusted for baseline mGFR group (<30, 30-45, >45 mL/min), as well as for the following covariates: fasting urine osmolarity, age, gender, center, ethnicity, log(PCR), elevated blood pressure, BMI, CV history, diabetes, smoking, ACE inhibitor or ARB intake. The covariance matrix for the random effects was estimated for each group of baseline mGFR separately (using group option in random statement from proc mixed (SAS 9.3), and robust sandwich variance estimators were used to estimate variances of regression coefficients. Interactions with time were tested for all covariates. Only the interaction terms that were statistically significant according to the Wald test and improved the model according to the Akaike information criteria (AIC) were included in the final model. Missing values

accounted for less than 3% of most variables used in our multivariable analyses and were replaced by median or mode, except for ethnicity and ACR for which we used a missing data class. Finally, in a sensitivity analysis, we included the 62 patients with missing data for urine osmolality measurement. Multiple imputations of our dataset were performed using all covariates of the multivariable models (n=5 imputed dataset, Fully Conditional Specification using all covariates including outcomes, maximum 20 iterations), cause-specific hazards models were fitted on each complete dataset and finally the estimated HRs were combined using Rubin's rules [20].

A two-sided P-value < 0.05 indicated statistical significance. Data analyses were performed with SAS software, version 9.3 (SAS institute, Cary, NC) and with R version 3.0.2 software (R Development Core Team, 2005).

Results

Mean age at baseline was 58.7 ±15.2 years, 67.7% were male, 21.3% had obesity (BMI≥30 kg/m^2), and 27.3% had diabetes (Table 1). Diabetic nephropathy was present in 10.1%, vascular in 26.6%, glomerular in 14.5%, interstitial in 9.2%, and polycystic kidney disease in 5.8%. In terms of serum sodium level, 65.2% of patients were in the reference range (138 to 142 mmol/L), while 18.1% had hyponatremia and 16.7% hypernatremia. Median mGFR at baseline was 40.2 (IQR, 29.1-54.5) ml/min. Baseline fasting urine osmolality (Uosm) ranged from 109 to 1114 mOsm/kg H_2O, with a mean of 502.7 ± 151.7 and a median of 482 (IQR, 401.5-591) mOsm/kg H_2O. As expected, mean fasting Uosm value was significantly higher in men than women, 514.7 ± 150.4 and 477.5 ± 151.5, respectively ($p<0.001$), justifying the use of gender-specific thresholds. Baseline fasting Uosm was positively associated with baseline mGFR in men and women

(Figure 2).

Patients in the lowest fasting Uosm tertile were older, with a higher prevalence of hypertension (p=0.01), diabetic nephropathy, polycystic kidney disease, and tubulo-interstitial nephropathy (p<0.001). They also experienced lower fasting urinary ammonium, higher 24-hour urine output, and higher ACR and PCR (p<0.001). They were more likely to receive anti-proteinuric treatments (p=0.01) and loop diuretics (p<0.001). Of note, there was no difference according to fasting Uosm tertiles for natremia.

***Hazard ratios for ESRD and death according to* fasting Uosm**

Over a median follow-up of 5.9 [IQR, 3.7-8.2] years, there were 380 ESRD events and 225 deaths before ESRD. Crude and adjusted HRs for ESRD were significantly higher in patients with lower baseline fasting Uosm (with adjusted HRs of 1.97 (95% CI, 1.26-3.08) and 1.18 (95% CI, 1.06-1.32) for the lowest vs highest tertile and per 100 mOsm/kg H_2O lower baseline Uosm, respectively; table 2). The increased risk of ESRD for low values of osmolality depended neither on the type of nephropathy nor on the age at baseline (interaction p-value = 0.4 and 0.3 respectively). Additional adjustment for urinary ammonium did not change the association (HRs of 1.91 (95% CI, 1.18-3.09) and 1.17 (95% CI, 1.03-1.33) for the lowest vs highest tertile or per 100 mosm/kg H_2O lower baseline Uosm, respectively).

Crude HRs for pre-ESRD death were significantly higher in patients with lower baseline fasting Uosm (HRs of 1.42 (95% CI, 1.03-1.96) and 1.13 (95% CI, 1.04-1.24) for the lowest vs highest tertile and per 100 mOsm/kg H_2O lower baseline Uosm, respectively). Results from sensitivity analyses showed that simple and multiple

imputations provided very similar HRs for ESRD: adjusted HRs of 1.94 (95% CI, 1.23-3.06) and 1.62 (95% CI, 1.04-2.52) for tertiles 1 and 2, respectively, vs tertile 3; and an HR of 1.20 (95% CI, 1.06-1.35) per 100 mOsm/kg H_2O lower baseline Uosm.

Crude HRs for pre-ESRD death were significantly higher in patients with lower fasting Uosm (with HRs of 1.42 (95% CI, 1.03-1.96) and 1.13 (95% CI, 1.04-1.24) for the lowest vs highest tertile and per 100 mOsm/kg H_2O lower baseline Uosm, respectively). In contrast, adjusted HRs for pre-ESRD death were not statistically significant before and after adjusting for confounders such as natremia status (with adjusted HRs of 0.99 (95% CI, 0.68-1.44) or 1.04 (95% CI, 0.92-1.17) the lowest vs highest tertile and per 100 mOsm/kg H_2O lower baseline Uosm, respectively; Table S1).

Fasting Uosm and mGFR decline over the study period

Mean annual change in mGFR was -6.1%±2.8%; the decline was steeper in patients with lower mGFR at baseline (-9.4%±0.8% per year for mGFR<30 ml/min, -6.7%±0.6% per year for 30-44 ml/min, -4.5%±0.4% per year for ≥ 45 ml/min).

In the mixed model adjusted for baseline mGFR and patient characteristics, each 100 mOsm/kgH_2O lower fasting Uosm at baseline was associated with an mGFR change of -1.0% [IQR, -1.4% to -0.6%] per year. Patients with urine osmolality in the lowest tertile had a steeper mGFR decline compared to the highest tertile (-4.9% [IQR, -6.6% to -3.2%] per year – Table 3). In this analysis PCR (log transformed) was the only other variable significantly associated with GFR decline over time.

Discussion

Our study demonstrates, in a large prospective CKD cohort, an independent relationship between fasting urine osmolality and GFR decline rate and ESRD outcome.

Impaired ability to concentrate urine is reported in CKD, especially in pre-ESRD stages, and in experimental models such as 5/6 nephrectomized rats [2–4,21–24]. However, its prevalence is unknown according to CKD stages. Our results show that if we consider a normal fasting Uosm value as being above 600 mosm/l (Osugi et al[25]), a concentrating defect is encountered in 77% of our study population: 14%, 46%, 69%, 84% and 98% of cases in CKD stage 1, 2, 3a, 3b, and 4 subgroups. This high prevalence was expected as urine concentration depends on corticopapillary osmotic gradient integrity, and aquaporin 2 expression levels within collecting duct principal cells, both of which may be altered by CKD [26].

Consistently with previous studies, we found a relationship between gender and urine osmolality. After water deprivation, higher urine osmolality and lower urine output were found in male compared to female rats in an experimental study by Wang et al [27]. Interestingly, this difference faded after gonadectomy. Additionally, human physiologic studies have shown the same difference, independent of sodium intake [28], in healthy individual as well as in CKD [29]. Higher plasma vasopressin levels were also found in men[30]. Whether these differences are due to a differential vasopressin release, thirst threshold, or renal response to vasopressin, and the influence of sexual hormones, remains poorly evaluated.

There is conflicting literature regarding the role of vasopressin V2 receptor activation, vasopressin, and vasopressin V2 receptor antagonism in CKD, and especially in ADPKD. Similar to our study, Hebert et al showed that 24-hour urine output was associated with faster eGFR decline in polycystic patients as well as in other CKD patients [9]. Interestingly, Devuyst et al. reported a defect in the concentrating ability of

patients with ADPKD before treatment with tolvaptan, a vasopressin V2 receptor antagonist that was recently approved for this indication, and an improved response to the treatment in patients with higher baseline Uosm [31]. In contrast, the link between 24-hour urine osmolality and adverse renal outcome has been described in two other studies in global CKD patients and specifically in polycystic kidney patients, respectively [8,32]. However, while fasting Uosm represents the nearest estimation of the maximal urinary concentration ability, 24-hour osmolality rather depends on salt and protein intakes.

CKD is currently assessed by GFR and albuminuria, which evaluate glomerular function [33]. However chronic kidney failure is characterized by alteration to various degrees of other kidney structures such as tubules [34,35], interstitium [36] and peritubular capillaries [37,38]. It should be emphasized that all these structures (namely large ascending limb of Henle, peritubular microcirculation, collecting ducts and interstitial cells) are involved in the onset and maintenance of cortico-papillary osmotic gradient [26]. Of note, previous studies have shown that tubular damage assessed on kidney pathology was more correlated with impaired urine concentration and acidification than glomeruli involvement [39,40], notably because of the presence of atubular glomeruli [41]. Furthermore, along with interstitial fibrosis and peritubular rarefaction, the former is associated with worst renal outcome [42–47]. Accordingly, for a given GFR, the magnitude of the gradient defect assessed by fasting Uosm should quantify tubular and peritubular capillaries damage, *i.e.* medullary function. In addition to GFR and ACR, fasting Uosm may thus provide relevant information on kidney injury and its measurement is easy to perform in a routine clinical setting. Interestingly, urine ammonium excretion, which depends both on daily acid intake and tubular function, was previously reported as an independent ESRD

risk factor [48]. We show here that fasting Uosm prognostic value is independent of urine acid excretion.

The hypothesis of an impaired abnormal vasopressin secretion could also explain low urine concentrating capacity. Indeed, hypothalamic-pituitary axis dysfunction has been reported in CKD [49]. Alternatively, though not mutually exclusive, excessive vasopressin secretion due to tubule resistance [23,50,51] has been shown to be deleterious in terms of renal outcome [6]. Our data may thus raise the issue whether increased plasma vasopressin or copeptin (which, since it is co-secreted with vasopressin, could be a surrogate marker for it [52]) in CKD could be an adaptive regulation for water balance due to impaired medulla function. Indeed, fasting Uosm remained of prognostic value independently of natremia levels. Conversely, hyponatremia, a state characterized by an impaired diluting ability of distal and collecting duct cells, did not seem to be associated with worse outcomes (i.e. ESRD or pre-ESRD mortality) in our study. Further investigations are warranted in order to address this hypothesis.

Last, the finding that fasting Uosm was an independent risk factor for ESRD, and not for mortality, strengthens the hypothesis that cortico-papillary gradient evaluation provides kidney-specific information on the magnitude of tubular and microvascular lesions. Conversely, mGFR and albuminuria, which are both reported risk factors for ESRD and cardiovascular mortality may instead measure systemic endothelial dysfunction [53].

Our study displays limitations. First, the fasting nature of Uosm was based on patient declaration and thus may neither be accurate for some individuals (ie, not reflecting the true maximum urine concentration value). However, the range of Uosm

throughout the study participants was quiet reasonable. 24-hour urine measurement errors can also introduce a bias in the parameters analyzed in our study, since patients often have confusion about the collection protocol, especially the discard of the first urine but not the last one. Second, fasting Uosm was measured by delta cryoscopy in our study, which is not broadly available. Calculated fasting Uosm (= (Urinary sodium ion + Urinary potassium ion)*2 + Urinary urea +/- Urinary glucose) was not available in our study although it is known to be an accurate estimation of measured osmolality [54]. Our findings can also only be generalized to CKD patients. Studies in the general population are warranted in order to evaluate the association of urine osmolality with renal outcome or mortality. Last, further research might allow evaluating the precise relevance of fasting Uosm in individual patient management, and assessing Uosm thresholds that would be specifically associated with adverse renal outcome.

In conclusion, our study demonstrates that fasting Uosm is associated with GFR decline and ESRD outcome in CKD patients, independent of confounding factors including baseline GFR and albuminuria. It may be a useful tool for medullary protection assessment in patients with CKD.

Supplementary Material

Table S1. Crude and adjusted HRs for mortality before ESRD according to fasting urinary osmolality.

Supplementary Material Descriptive Text for Online Delivery
Supplementary Table S1 (PDF). Crude and adjusted HRs for mortality before ESRD according to fasting urinary osmolality.

Article Information

NephroTest Study Group Collaborators: Those who contributed to data collection are Marine Livrozet, Emmanuel Letavernier, Pierre Ronco, and Hafedh Fessi (Hôpital Tenon); Emmanuelle Vidal-Petiot, Eric Daugas, and Caroline du Halgouet (Hôpital Bichat); Renaud de La Faille, Gerard Maruani, Marion Vallet, Laurence Nicolet-Barousse, Mélanie Roland, and Christian Jacquot (Hôpital Européen G. Pompidou).
Authors' Full Names and Academic Degrees:

Authors' Affiliations:
Address for Correspondence:

Authors' Contributions: design of NephroTest cohort: MF, PH, JPH, FV, ET, JJB, and BS; data collection: NT, MF, PH, JPH; design of study analyses: MM, SW, NT, JPH; performed study: MM, SW; interpretation of results: MM, SW, NT, JPH, BS. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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Table 1. Patients characteristics according to baseline urinary osmolality

	Overall* (n=1796)	**Baseline fasting urinary osmolality**				
		Tertile1 (low; n=594)	**Tertile2 (medium; n=598)**	**Tertile3 (high; n=604)**	**p-value**	**Missing data**
Age (y)	58.7±15.2	58.0±15.8	60.4±14.4	57.7±15.2	**0.01**	0
Male sex	67.7 (1216)	67.7 (402)	67.7 (398)	67.8 (416)	0.9	0
African American	12.7 (228)	12.0 (71)	11.9 (70)	14.2 (87)	0.7	82
BMI (kg/m²)	26.6±5.1	26.4±5.2	26.7±5.0	26.6±5.1	0.5	0
Former smokers	32.5 (583)	31.0 (184)	33.8 (199)	32.6 (200)	0.8	0
Current smokers	14.1 (253)	15.2 (90)	13.3 (78)	13.8 (85)		0
Diabetes	27.3 (490)	27.1 (161)	30.3 (178)	24.6 (151)	0.1	0
Hypertension	91.4 (1641)	92.6 (550)	93.0 (547)	88.6 (544)	**0.01**	0
CV history	18.2 (326)	20.2 (120)	18.7 (110)	15.6 (96)	0.2	36
Type of nephropathy					**<0.001**	0
Diabetic	10.1 (181)	10.9 (65)	12.2 (72)	7.2 (44)		
Glomerular	14.5 (260)	14.8 (88)	14.3 (84)	14.3 (88)		
Vascular	26.6 (477)	23.9 (142)	28.7 (169)	27.0 (166)		
Polycystic	5.8 (105)	7.4 (44)	6.1 (36)	4.1 (25)		
Tubulo-interstitial	9.2 (166)	13.6 (81)	7.8 (46)	6.4 (39)		
Others	33.8 (607)	29.3 (174)	30.8 (181)	41.0 (252)		
mGFR (ml/min)	40.2 (29.1-54.5)	30.7 (22.9-43.1)	36.5 (28.6-48.0)	53.7 (42.0-68.1)	**<0.001**	0
CKD stage					**<0.001**	
1	2.1 (37)	0.5 (3)	0.3 (2)	5.2 (32)		
2	16.7 (300)	6.9 (41)	9.5 (56)	33.1 (203)		
3a	22.1 (396)	15.8 (94)	19.7 (116)	30.3 (186)		
3b	31.9 (572)	28.6 (170)	41.8 (246)	25.4 (156)		
4	27.3 (491)	48.2 (286)	28.6 (168)	6.0 (37)		
Volemia	21.5 (19.6-23.6)	21.6 (19.6-23.5)	21.4 (19.5-23.7)	21.5 (19.7-23.6)	0.9	232
ACR (mg/mmol)	8.0 (1.5-47.2)	18.7 (3.5-96.9)	11.7 (1.8-57.3)	3.0 (0.8-14.2)	**<0.001**	52
Albuminuria						52
Normal to mildly increased (<3 mg/mmol)	35.1 (612)	22.4 (133)	29.3 (172)	49.8 (306)	**<0.001**	**52**
Moderately increased (3-30 mg/mmol)	32.3 (580)	31.1 (185)	33.7 (198)	32.1 (197)		
Severely increased (≥30 mg/mmol)	30.8 (553)	43.4 (258)	33.5 (197)	16.0 (98)		
PCR (mg/mmol)	25.9 (11.6-89.3)	52.3 (17.9-165.2)	32.4 (13.3-103.9)	14.7 (8.2-34.6)	**<0.001**	83
24h urinary output (l/24h)	2.0 (1.5-2.5)	2.3 (1.8-2.9)	2.0 (1.5-2.)	1.7 (1.4-2.2)	**<0.001**	67
Fasting urinary ammonium (mmol/l)	12.5 (7.6-21.0)	7.5 (4.6-10.9)	12.9 (9.2-18.7)	23.4 (15.5-32.0)	**<0.001**	445
Protein intake (g/kg/d)	1.0 (0.9-1.2)	1.0 (0.9-1.2)	1.1 (0.9-1.2)	1.0 (0.9-1.2)	0.4	241
Loop diuretics	28.4 (510)	33.7 (200)	32.8 (193)	19.1 (117)	**<0.001**	**1**
Thiazide diuretics	20.4 (366)	17.3 (103)	22.8 (134)	21.0 (129)	0.06	1

ACEi or ARBs	74.3 (1335)	75.4 (448)	77.9 (458)	69.9 (429)	**0.01**	**1**

Categorical data expressed as count (percentage); continuous data as mean ± SD or median [interquartile range] as appropriate.
BMI: body mass index, CV: cardiovascular, mGFR: measured glomerular filtration rate, ACR: albumin-creatinine ratio, PCR: protein-creatinine ratio, ACEi: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker.
* Fasting urine osmolality 482 (IQR, 401-591) mOsm/kg H_2O
Tertiles were gender-specific: T1 (low): 109-444 mOsm/kg H_2O for men, 121-402 mOsm/kgH_2O for women, T2 (medium): 445-557 mOsm/kg H_2O for men, 403-516 mOsm/kg H_2O for women, T3 (high): 558-1114 mOsm/kg H_2O for men, 517-1031 mOsm/kgH_2O for women.

Table 2. Crude and adjusted hazard ratios for ESRD according to fasting urinary osmolality.

	Per 100-mOsm/kgH_2O lower baseline fasting urinary osmolality	p-value	baseline fasting urinary osmolality			p-wald test	p for trend
			Tertile1 (low)	Tertile2 (medium)	Tertile3 (high)		
No. of events/no. at risk	380/1796		223/594	131/588	26/614		
Crude Model	1.67 (1.54-1.80)	**<0.001**	10.65 (7.09-15.99)	5.43 (3.57-8.27)	1.00 (reference)	**<0.001**	**<0.001**
Model 1	1.28 (1.15-1.41)	**<0.001**	3.03 (1.97-4.66)	2.15 (1.40-3.32)	1.00 (reference)	**<0.001**	**<0.001**
Model 2	1.26 (1.14-1.40)	**<0.001**	2.68 (1.73-4.14)	2.05 (1.33-3.17)	1.00 (reference)	**<0.001**	**<0.001**
Model 3	1.18 (1.06-1.32)	**0.004**	1.97 (1.26-3.08)	1.62 (1.04-2.52)	1.00 (reference)	**0.01**	**0.003**

Unless otherwise indicated, values shown are hazard ratios (95% confidence interval).
Model 1: adjusted for mGFR with time-depending effect
Model 2: Model 1 + age, gender, centre, ethnicity, obesity
Model 3: Model 2 + ACR category (<3, 3-30, >30 mg/mmol), high blood pressure, diabetes, CV history, smoking status, ACEi or ARBs, nephropathy type and natremia
Tertiles were gender-specific: T1 (low): 109-444 mOsm/kgH_2O for men, 121-402 mOsm/kgH_2O for women, T2 (medium): 445-557 mOsm/kgH_2O for men, 403-516 mOsm/kgH_2O for women, T3 (high): 558-1114 mOsm/kgH_2O for men, 517-1031 mOsm/kgH_2O for women.

Table 3. Linear mixed model analysis of the mean effect of fasting urinary osmolality at baseline on change over time of mGFR decline

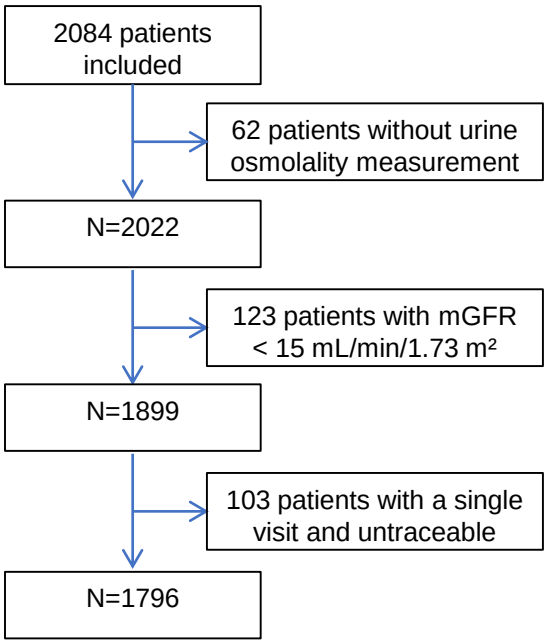
			*baseline fasting urinary osmolality**		
	Per 100-mOsm/kgH_2O lower baseline fasting urinary osmolality	p-value	Tertile1 (low)	Tertile2 (medium)	Overall p-value
Model 1	-1.4% (-1.8% to -0.9%)	**<0.001**	-4.9% (-6.6% to -3.2%)	-2.1% (-3.4% to -0.9%)	**<0.001**
Model 2	-1.0% (-1.4% to -0.6%)	**<0.001**	-4.9% (-6.6% to -3.2%)	-2.2% (-3.4% to -0.9%)	**<0.001**

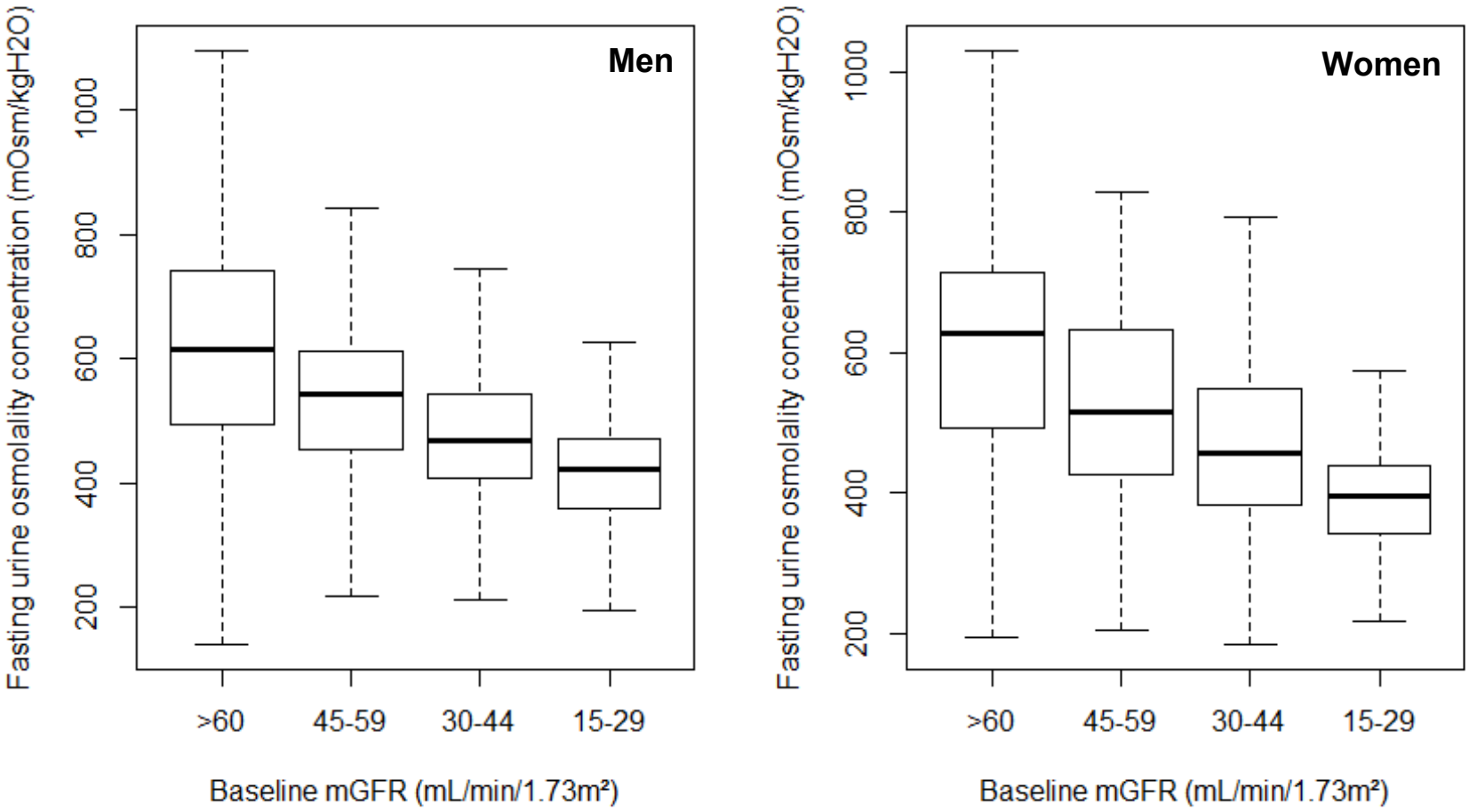
Values shown are mean difference in mGFR slope [95% CI], expressed as percent per year
*Tertile 3 (high baseline fasting osmolality) is the reference group (0 change).
Model 1: includes intercept, time, fasting urinary osmolality at baseline, GFR group and an interaction between time and fasting urinary osmolality at baseline.
Model 2: includes Model 1 variables and mean effect on log(PCR) slope and mean effect on GFR at baseline of the following covariates: age, gender, centre, ethnicity, diabetes, elevated blood pressure, BMI, cardiovascular history, smoking, and log(PCR). Interaction terms between covariates and time, except log(PCR) were non-significant and were not included in the final model.
Tertiles were gender-specific: T1 (low): 109-444 mOsm/kgH_2O for men, 121-402 mOsm/kgH_2O for women, T2 (medium): 445-557 mOsm/kg H_2O for men, 403-516 mOsm/kgH_2O for women, T3 (high): 558-1114 mOsm/kgH_2O for men, 517-1031 mOsm/kg H_2O for women

Figures

Figure 1. Study Flowchart. mGFR: measured glomerular filtration rate.

Figure 2. Baseline Fasting Uosm according to baseline CKD stages in men and women. mGFR: measured glomerular filtration rate.


2084 patients included
62 patients without urine osmolality measurement
N=2022
123 patients with mGFR < 15 mL/min/1.73 m²
N=1899
103 patients with a single visit and untraceable
N=1796


Men
Women
Fasting urine osmolality concentration (mOsm/kgH2O)
200
400
600
800
1000
>60
45-59
30-44
15-29
Baseline mGFR (mL/min/1.73m²)