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Abstract: BACKGROUND: The importance of vasopressin and/or urine concentration in various kidney, cardiovascular, and metabolic diseases has been emphasized recently. Due to technical constraints, urine osmolality (Uosm), a direct reflect of urinary concentrating activity, is rarely measured in epidemiologic studies. METHODS: We analyzed 2 possible surrogates of Uosm in 4 large population-based cohorts (total n = 4,247) and in patients with chronic kidney disease (CKD, n = 146). An estimated Uosm (eUosm) based on the concentrations of sodium, potassium, and urea, and a urine concentrating index (UCI) based on the ratio of creatinine concentrations in urine and plasma were compared to the measured Uosm (mUosm). RESULTS: eUosm is an excellent surrogate of mUosm, with a highly significant linear relationship and values within 5% of mUosm (r = 0.99 or 0.98 in each population cohort). Bland-Altman plots show a good agreement between eUosm and mUosm with mean differences between the 2 variables within ±24 mmol/L. This was verified in men and women, in day and night urine samples, and in CKD patients. The relationship of UCI with mUosm is also significant but is not linear and exhibits more dispersed values. Moreover, the latter index is no longer representative of mUosm in patients with CKD as it declines much more quickly with declining glomerular filtration rate than mUosm. CONCLUSION: The eUosm is a valid marker of urine concentration in population-based and CKD cohorts. The UCI can provide an estimate of urine concentration when no other measurement is available, but should be used only in subjects with normal renal function.

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Keywords
Sodium · Potassium · Urea · Circadian rhythm · Water balance · Chronic kidney disease

Abstract
Background: The importance of vasopressin and/or urine concentration in various kidney, cardiovascular, and metabolic diseases has been emphasized recently. Due to technical constraints, urine osmolality (U_{osm}), a direct reflect of urinary concentrating activity, is rarely measured in epidemiologic studies. ***Methods:*** We analyzed 2 possible surrogates of U_{osm} in 4 large population-based cohorts (total n = 4,247) and in patients with chronic kidney disease (CKD, n = 146). An estimated U_{osm} (eU_{osm}) based on the concentrations of sodium, potassium, and urea, and a urine concentrating index (UCI) based on the ratio of creatinine concentrations in urine and plasma were compared to the measured U_{osm} (mU_{osm}). ***Results:*** eU_{osm} is an excellent surrogate of mU_{osm}, with a highly significant linear relationship and values within 5% of mU_{osm} (r = 0.99 or 0.98 in each population cohort). Bland-Altman plots show a good agreement between eU_{osm} and mU_{osm} with mean differences between the 2 variables within ±24 mmol/L. This was verified in men and women, in day and night urine samples, and in CKD patients. The relationship of UCI with mU_{osm} is also significant but is not linear and exhibits more dispersed values. Moreover, the latter index is no longer representative of mU_{osm} in patients with CKD as it declines much more quickly with declining glomerular filtration rate than mU_{osm}. ***Conclusion:*** The eU_{osm} is a valid marker of urine concentration in population-based and CKD cohorts. The UCI can provide an estimate of urine concentration when no other measurement is available, but should be used only in subjects with normal renal function.

Introduction

The interest in the influence of the antidiuretic hormone or vasopressin (AVP) as a significant player in various kidney, cardiovascular, and metabolic diseases has been revived recently [1–4]. The availability of non-peptide, orally active selective AVP receptor antagonists (vaptans) [5, 6] and of a reliable ELISA for the measurement of copeptin, a validated surrogate of AVP [7, 8], has opened the door for a number of studies addressing the AVP/thirst pathway and osmoregulation in general (see review in [9]).

Independent of the well-known contribution of antidiuretic hormone to various forms of water disorders, recent epidemiological studies have shown significant associations between indices of the AVP/hydration system

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Table 1. Demographic information about the different cohorts

Cohort	Croatia-Korcula	GS:SFHS Aberdeen	GS:SFHS Glasgow	SKIPOGH day	SKIPOGH night	CKD Necker
Number	463	554	2,305	925	Idem	146
Sample type	Spot	Spot	Spot	Day period	Night period	24 h
Age, years	58 (19–87)	57 (19–88)	53 (18–93)	47 (18–90)	Idem	64 (17–86)
Gender: M/W, %	41/59	43/57	41/59	47/53	Idem	59/41
BMI, kg/m^2	27.97±0.21	27.22±0.22	26.97±0.21	25.03±0.15	Idem	24.16±0.30
mU_{osm}, mosm/kg H_2O	668±10	524±10	540±5	457 (110–1,174)	541 (67–1,304)	396±13
eU_{osm}, mosm/L	664±9	526±11	547±5	450 (118–1,142)	513 (61–1,223)	381±12
UCI	165±4	119±3	117±2	114±2	145±3	41.6±3.6
U_{urea}, mmol/L	285±5	225±5	250±3	227±4	296±5	177±6
U_{Na}, mmol/L	113.8±2.2	79.3±2.0	83.9±1.0	94.8±1.6	93.4±1.6	68.7±2.8
U_K, mmol/L	65.9±1.6	63.5±1.5	64.9±0.8	47.4±0.8	32.7±0.6	33.3±1.3
eGFR, mL/min/1.73 m^2	83.4±1.1	92.2±0.7	89.1±0.4	96.3±0.6	–	46.2±2.5

Means + SEM or median (interval).

and the incidence or progression of diseases including chronic kidney disease (CKD), autosomal dominant polycystic kidney disease, diabetic nephropathy, obesity, metabolic syndrome, and insulin resistance [4, 10–21]. A number of experimental studies have demonstrated the adverse effects of AVP or a low level of hydration in animal models of these disorders [10, 22–26]. A recent double-blind, placebo-controlled clinical trial, using a selective AVP V2 receptor antagonist, tolvaptan, proved to bring significant benefit over a 3-year period in patients with autosomal dominant polycystic kidney disease and well preserved renal function [27].

Because AVP is difficult to measure due to its small mass, very low circulating concentrations, poor stability in vitro, and time-consuming assay, most of the recent studies dealing with this hormone rely on the measurement of copeptin (a peptide that is part of the pre-prohormone containing AVP) in plasma or, more indirectly, on fluid intake or daily urine volume [28, 29]. Urine osmolarity (U_{osm}), the most direct parameter reflecting the action of AVP on distal tubular segments of the kidney, is rarely measured due to technical constraints, and is thus usually not available in epidemiologic studies.

Various surrogates of U_{osm} have been used in clinical studies. They include the specific urine density (UD) or the refraction index that give only an approximate value of the solute content in the urine and are subjected to several biases including distortion in the case of proteinuria and poor precision of readings. Two other surrogates are the urine concentrating index (UCI) based on the handling of creatinine by the kidney [30, 31], and the estimated U_{osm} (eU_{osm}) based on the concentration of the 3 main osmoles present in the urine: sodium, potassium, and urea [31, 32]. To our knowledge, the validity of these 2 surrogates has not been evaluated in large, population-based cohorts with normal or altered renal function. The aim of the present study was to assess the value of eU_{osm} and UCI compared to measured U_{osm} (mU_{osm}) in large population-based and CKD cohorts, and to test the influence of sample type, gender, and age on these markers.

Subjects and Methods

Cohorts

The general characteristics of the subjects belonging to the different cohorts are presented in Table 1.

Generation Scotland:Scottish Family Health Study (GS:SFHS) and Croatia-Korcula

Aberdeen and Glasgow subjects were selected from the Generation Scotland study, a family-based genetic epidemiology study that included 24,000 volunteers from across Scotland, as previously described [33]. Biological samples including morning spot urine were collected during participation from 2006 to 2011 [34]. We also studied subjects from the Croatia-Korcula cohort, a family-based, cross-sectional study from the island of Korcula (Croatia) that initially included 965 subjects, as previously described [35]. Studies of these 3 cohorts included clinical information, biochemical measurements, and lifestyle and health questionnaires. For the present study, subjects from these 3 cohorts were randomly selected for measurement of U_{osm} (n = 554 from GS:SFHS Aberdeen, 2,305 from GS:SFHS Glasgow and 463 from Croatia-Korcula). All participants provided written informed consent. For GS:SFHS, national ethical approval has been obtained from the National Health Service Tayside Research Ethics committee. The Croatia-Korcula study was approved by the Ethical Committee of the Medical School, University of Zagreb.

Swiss Kidney Project on Genes in Hypertension

Swiss Kidney Project on Genes in Hypertension (SKIPOGH) is a family- and population-based cross-sectional multicenter study that examines the genetic determinants of blood pressure. Participants were recruited in 2009–2013 in the cantons of Bern and Geneva, and the city of Lausanne. Detailed methods have been previously described [36, 37]. The study visit was performed in the morning after an overnight fast. Participants were asked to bring urine of the previous 24 h collected separately during day and night periods defined according to each participant's self-reported bedtime and wake-up time. The SKIPOGH study was approved by the Human Research Ethics Committee of Lausanne University Hospital and University of Lausanne (Lausanne, Switzerland), Ethics Committee for the Research on Human Beings of Geneva University Hospitals (Geneva, Switzerland), and Ethics Committee of the Canton of Bern (Bern, Switzerland).

CKD Patients

This study includes 146 outpatients with CKD of diverse etiologies and various levels of renal dysfunction, who were attending the Nephrology Department of Necker Hospital (Paris, France) in 1993 for a bi-annual checkup [19, 38]. All patients provided 24-h urine. Informed consent was obtained for storage of the samples and additional future measurements to enable a more complete understanding of the pathophysiological characteristics related to CKD. On the freshly collected plasma and urine samples, osmolality was measured with a freezing point osmometer (Roebling, Berlin, Germany). Creatinine concentration was measured by the Jaffe colorimetric method and creatinine clearance in mL/1.73 m^2 was used as an estimate of glomerular filtration rate (GFR). Concentration of urinary solutes was measured with a classical automatic multianalyzer.

Measurements in Plasma and Urine Samples

In the 4 population-based cohorts, urine samples were kept frozen at –80°C until U_{osm} and urinary solute concentrations were measured. Sodium, potassium, glucose, creatinine, and urea were measured with a Beckman Coulter Synchron System Assays (Unicell DxC Synchron Clinical System). The CKD-EPI formula was used to calculate eGFR [39]. U_{osm} was measured on 20 μL samples by the freezing point depression technique using an Advanced Osmometer (Norwood, MA, USA). A control (Clinitrol 290) and a set of calibration standards (50, 850, and 2,000 mosm/kg H_2O) were used before running each batch. The intra-assay coefficient of variability was 0.19% and the inter-assay coefficient of variability was 1.32%.

Calculations and Statistical Analyses

Most modern osmometers measure the osmolality of the fluids in milliosmoles per kilogram of water (mosm/kg H_2O)[1]. Osmolarity expresses the concentration of osmotically active molecules in milliosmoles per liter of water (mosm/L). Sweeney and Beuchat [40] described the technical aspects and limitations of osmometry methods and provided detailed considerations about the concepts of osmotic pressure, osmolarity, osmolality, and solute concentrations.

Estimated U_{osm}

The major urinary solutes, accounting for more than 90% of all urinary osmoles, are urea and the 2 cations sodium and potassium along with their accompanying anions. Thus, their cumulated concentrations (in mmol/L) should be close to the actual U_{osm} (in mosm/L). An eU_{osm} can be calculated according to the following formula:

$$eU_{osm} = (U_{Na} + U_K)*2 + U_{urea}$$

where U_{Na}, U_K, and U_{urea} are the urinary concentrations of sodium, potassium, and urea, respectively, in mmol/L. $U_{Na} + U_K$ is multiplied by 2 to account for the accompanying anions. If urea was measured as urea nitrogen, it should be remembered that there are 2 atoms of nitrogen (MW = 14) per molecule of urea. Urea in mmol/L = urea nitrogen in mg/dL × 0.357 (explanation: urea nitrogen in mg/dL multiplied by 10 [conversion of dL to L] and divided by 14 × 2 [mg N per mmol urea]). In case of significant glycosuria, glucose concentration can be added to the formula.

Urine Concentrating Index

Creatinine is freely filtered and is assumed to undergo negligible secretion or reabsorption along the nephron when kidney function is normal. Thus, the concentration of creatinine in urine relative to that in plasma (U_{creat} and P_{creat}, respectively), that is, the ratio of urine-to-plasma creatinine concentrations, is proportional to the fraction of filtered water that has been reabsorbed to concentrate the solutes in the urine. This ratio provides an UCI, a ratio that has no unit:

$$UCI = U_{creat}/P_{creat}$$

Statistical Analyses

The Statistical Package for Social Sciences (SPSS) version 19 (IBM Corporation, New York) and GraphPad Prism 5 were used to carry out the statistical analyses and generate the figures. Results are shown as means ± SEM for normally distributed variables, or as medians and 25–75% interquartile range (IQR) for other variables. The agreement between mU_{osm} and eU_{osm} was assessed by Bland-Altman plots. The Shapiro-Wilk test was used to assess the distribution of mU_{osm} in the SKIPOGH study. Correlations were studied using Pearson's correlation analysis (in case of normality) or Spearman's rho test (for other variables). Wilcoxon signed-rank test was used to compare repeated measures for day and night urine samples of the SKIPOGH subjects. The significance level was set at 5%.

Results

U_{osm} Surrogates in Population-Based Cohorts

Large variations in urine concentration are observed among individuals. The mU_{osm} in different subjects varies from ≈150 to 1,200 mosm/kg H_2O in spot urine of the

[1] The terms osmolarity or osmolality should be preferred to "osmotic pressure" because this physical osmotic force is not a pressure. It was named in this way in the past, when osmolarity was evaluated indirectly as a hydrostatic pressure generated by an unknown fluid opposed to a reference fluid, separated by a semi-permeable membrane. The measurements were expressed in mm height between the levels of the 2 fluids in the 2 compartments.

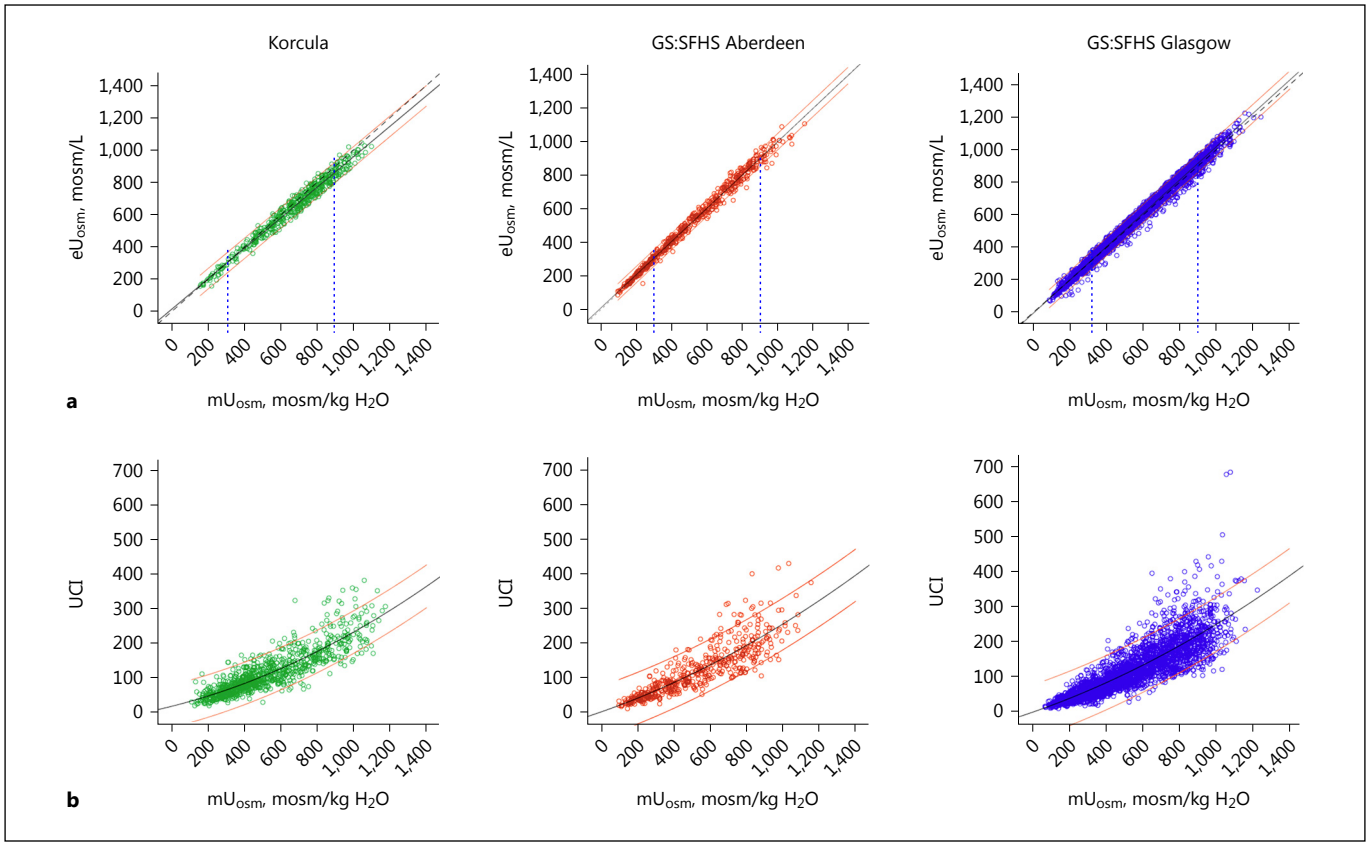

Fig. 1. Comparison of urine osmolality surrogates with measured urine osmolality. **a** Linear correlation between measured osmolality and estimated osmolarity in 3 cohorts. Croatia-Korcula: mU_{osm} = 1.03 eU_{osm} + 3.3 ($p < 0.001$, $r = 0.98$); GS:SFHS Aberdeen: mU_{osm} = 0.99 eU_{osm} – 4.4 ($p < 0.001$, $r = 0.98$); GS:SFHS Glasgow: mU_{osm} = 0.96 eU_{osm} + 11 ($p < 0.001$, $r = 0.99$). The thin vertical lines show mU_{osm} of 300 and 900 mosm/kg H_2O, that is, approximatively 1 time and 3 times the plasma osmolality. **b** Quadratic correlation between UCI and measured urine osmolality in 3 cohorts. Croatia-Korcula: mU_{osm} = 5.04 UCI – 0.009 UCI^2 + 126 ($p < 0.001$, $r = 0.76$); GS:SFHS Aberdeen: mU_{osm} = 5.52 UCI – 0.009 UCI^2 + 28 ($p < 0.001$, $r = 0.90$); GS:SFHS Glasgow: mU_{osm} = 4.89 UCI – 0.006 UCI^2 + 90 ($p < 0.001$, $r = 0.89$). Black lines represent the best-fit curves. Red thin lines represent the 95% CIs. Dotted lines in the top panel represent the medians.

3 population-based cohorts, as well as in day and night urine of the SKIPOGH cohort (Fig. 1a, 2b). A substantial number of subjects (21%) dilute their urine below plasma osmolality whereas others (9%) concentrate their urine up to 3 times more than the level of plasma osmolality (Fig. 2a). These extreme mU_{osm} are not associated with differences in eGFR.

Highly significant linear correlations are observed between mU_{osm} and eU_{osm} in all populations (Croatia-Korcula r = 0.98, GS:SFHS Aberdeen r = 0.98, GS:SFHS Glasgow r = 0.99; Fig. 1a, 2b). The best-fit linear regression lines are almost superimposed with the medians. Bland-Altman plots show a good agreement between eU_{osm} and mU_{osm} in the 3 population-based cohorts (online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000475769). This is reflected in the small bias values (Croatia-Korcula bias = 24, GS:SFHS Aberdeen bias = –6, GS:SFHS Glasgow bias = –23), and relatively narrow precision range (Croatia-Korcula = –44 to 90, GS:SFHS Aberdeen = –54 to 43, GS:SFHS Glasgow = –80 to 34). Plot for the GS:SFHS Aberdeen population is given as an example in Figure 3.

Although the relations between UCI and mU_{osm} are significant, they exhibit a relatively large dispersion of individual values, increasing with increasing osmolality (Fig. 1b, 2c). Nonetheless, as an average, the ratio of UCI to mU_{osm} is fairly constant (0.20, 0.21, and 0.22 for mU_{osm} = 300, 600, and 900 mosm/kg H_2O, respectively).

The possible influence of glycosuria that occurred in some subjects on eU_{osm} was evaluated. Among 3,322

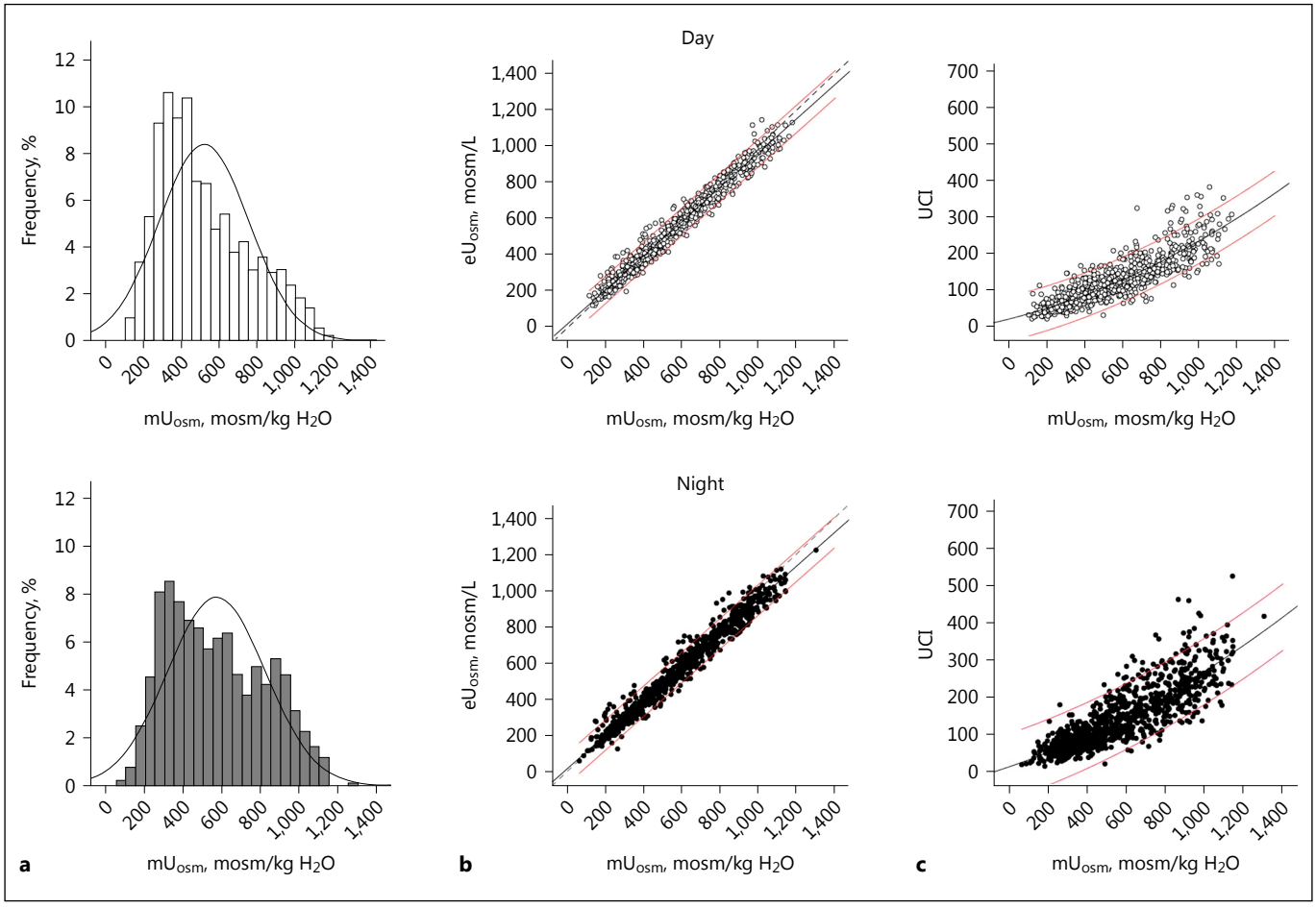

Fig. 2. Daytime and night-time urine in the SKIPOGH population (n = 925). **a** Distribution of mU_{osm} among SKIPOGH subjects. Thin curves represent the normal distribution model. **b** Linear correlation between measured and estimated U_{osm} in daytime and night-time urine. **c** Quadratic correlation between UCI and mU_{osm} in daytime versus night-time urine. Black lines represent the best-fit curves and red thin lines 95% CIs (**b**, **c**). Dotted lines represent the medians (**b**).

subjects of the 3 cohorts in which urinary glucose was available, 58 exhibited glycosuria >1.66 mmol/L [41] (mean ± SEM 11.58 ± 2.28 mmol/L; range 1.7–86.8). Their age and eGFR were 57.0 ± 1.8 years and 86.8 ± 2.4 mL/min/1.73 m^2, respectively. mU_{osm} in these subjects was 642 ± 32 mosm/kg H_2O. eU_{osm}, calculated without or with the addition of urinary glucose, was 624 ± 31 and 635 ± 32 mosm/L, respectively, both within 3% of mU_{osm}.

Urine osmolality (U_{osm}) is known to be higher in men than in women. This was verified in the cohorts of the present study (online suppl. Table 2); men exhibited higher mU_{osm} and eU_{osm} than women although the magnitude of this gender difference differed among the 3 populations. eU_{osm} was very close to mU_{osm} in both genders and the men/women ratio of eU_{osm} was very similar to that for mU_{osm}. For UCI, there was a tendency for more inter-individual variation in women than in men as well as lower men/women ratios which tended to underestimate the gender difference (online suppl. Table 2).

U_{osm} Surrogates in Day and Night Urine

In healthy subjects, urine is usually more concentrated during the night than during the day. We investigated if the relationships between mU_{osm}, eU_{osm}, and UCI are comparable in day and night urine of the 925 subjects of the SKIPOGH study (Fig. 2a). The Shapiro-Wilk test indicates that these variables diverge from a normal distribution (shown by a thin curve). Mean mU_{osm} ± SEM during day and night was, respectively, 520 ± 4 and 572 ± 7 mosm/kg H_2O. Median (IQR) values were 457 (334–

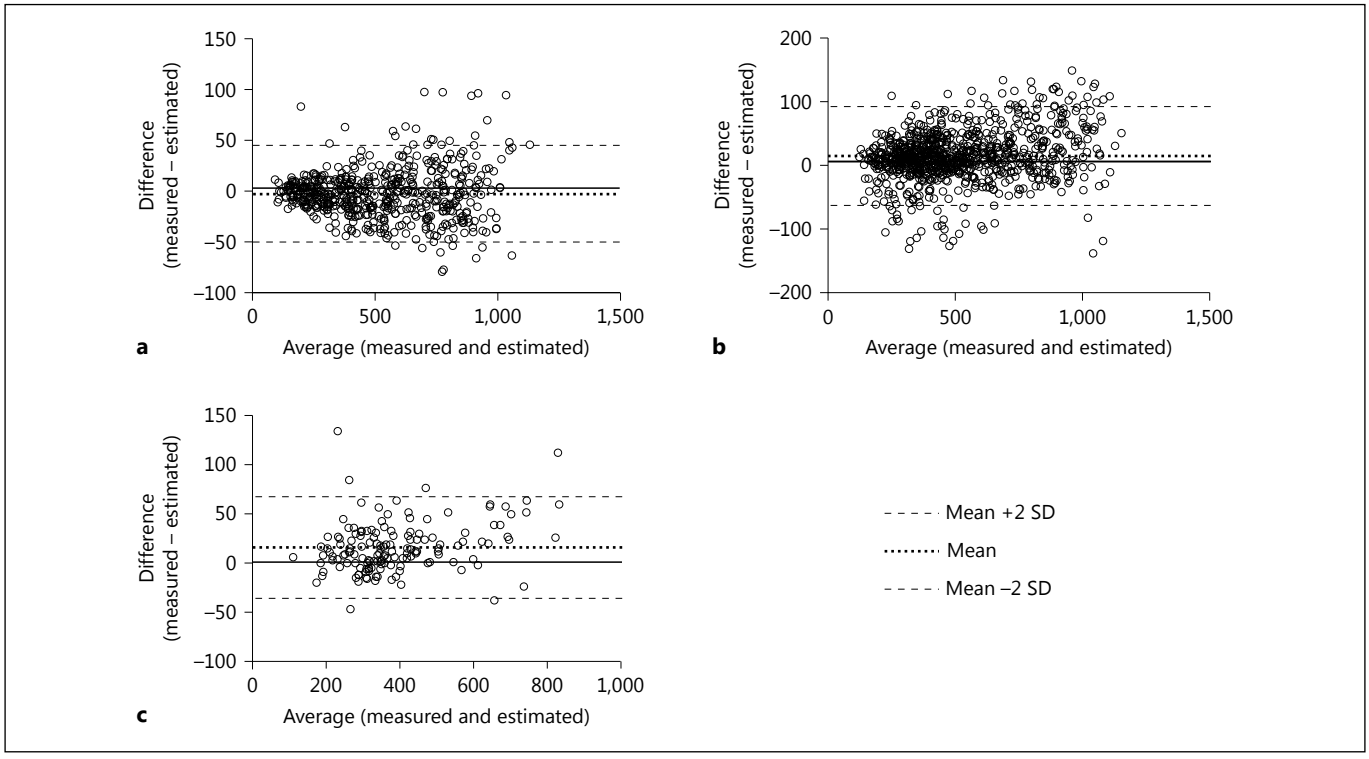

Fig. 3. Bland-Altman plots showing the agreement between estimated and measured U_{osm} in the spot urine samples of GS:SFHS Aberdeen (**a**), the day urine samples of SKIPOGH (**b**) and the 24-h urine of the CKD patients (**c**).

676) and 541 (356–777) mosm/kg H_2O, respectively ($p < 0.001$, Wilcoxon signed-rank test). The histograms of mU_{osm} during day and night do not follow a normal distribution and there is a tendency for a bimodal distribution during the night.

Measured and eU_{osm} values exhibit highly significant linear correlations in both day and night urine (Fig. 2b), as also observed in the spot urine of the other cohorts. Bland-Altman plots show a good agreement between eU_{osm} and mU_{osm} in day and night urine, as reflected by the small bias values (day bias = 9, night bias = 24) and the precision range (day –71 to 89, night –66 to 114; Fig. 3; online suppl. Table 1). The relations between UCI and mU_{osm} are best described by quadratic correlations. Thin red lines show the 95% CIs as in the 3 cohorts shown in Figure 1. UCI vs. mU_{osm} values were more widely dispersed than eU_{osm} vs. mU_{osm} values.

U_{osm} Surrogates in CKD Patients

Table 2 compares the values of eU_{osm} and UCI to those of mU_{osm} in CKD patients, according to their level of renal function. In all CKD classes, eU_{osm} is very close to mU_{osm}. Both variables decline in parallel with declining eGFR. Bland-Altman plot show a relatively good agreement between the 2 methods, as reflected by the small bias value (15) and the precision range (–37 to 67; Fig. 3; online suppl. Table 1). In contrast, UCI declines much more dramatically than mU_{osm}. These differences are due mostly to the progressive rise in plasma creatinine concentration (from 91 ± 5 to 514 ± 34 μmol/L in the 2 extreme classes, a 5.6-fold increase) while urine creatinine concentration declines only 2-fold as a result of a lower total creatinine excretion and a moderately higher 24-h urine volume. In these patients, a spot urine sample was collected in the morning following the 24-h urine collection (Table 2). mU_{osm} in morning urine is 10–20% higher than mU_{osm} in 24-h urine, a difference that seems independent of the level of renal function.

Discussion

The urine concentrating activity of the human kidney was rarely investigated, except in a few conditions such as urolithiasis and diabetes insipidus. Recent ex-

Table 2. Osmolality and its surrogates in 147 CKD patients according to the level of renal function

CKD stage	*n*	Creatinine excretion, mmol/day	Spot mU_{osm}, mosm/kg H_2O	mU_{osm}, mosm/kg H_2O	eU_{osm}, mosm/L	eU_{osm}/mU_{osm}	UCI, U_{creat}/P_{creat}	UCI*100/mU_{osm}[1]
Stage 1 (>90)	13	13.4±1.0	738±61	650±48	608±43	0.94±0.01	146±17	22.1±1.6
Stage 2 (60–89)	29	13.2±0.7	517±32	479±34	458±34	0.95±0.01	59±5	12.3±0.4
Stage 3 (30–59)	54	11.5±0.5	450±14	371±14	358±14	0.97±0.01	34±2	9.2±0.3
Stage 4 (15–29)	32	9.4±0.5	376±13	321±13	311±12	0.98±0.01	17±1	5.2±0.3
Stage 5 (<15)	19	8.1±0.5	318±11	296±12	291±12	0.98±0.02	7±1	2.5±0.2

Means ± SEM.
CKD stages are shown with the limits of eGFR in mL/min/1.73 m^2.
Spot mU_{osm} = mU_{osm} of a morning spot urine sample. All other values concern 24-h urine collection.
[1] For the ratio of UCI/mU_{osm}, UCI was multiplied by 100 to make the reading easier.

perimental and epidemiological findings have renewed the interest in the components of the water balance and in the parameters reflecting this integrative function [1, 4, 9, 18, 22, 24, 28, 29, 32, 42, 43]. It is indeed quite different for the kidney to excrete a daily osmolar load of 900 mosm in 1 L of urine at 900 mosm/L or in 3 L of urine at 300 mosm/L. Increased urine concentration (associated with increased solute-free water reabsorption) results in a lower fractional excretion of several solutes and in a significant hyperfiltration, that is, at least in part, mediated by AVP acting on renal V2 receptors. It has been proposed that this hyperfiltration is mediated by changes in the composition of the tubular fluid at the macula densa, resulting from AVP's action on water, sodium, and urea transport in the collecting duct and the resulting recycling of urea in the medulla (see review in [9]). U_{osm}, the most direct reflect of the urine concentrating activity, is rarely measured in large cohorts because of technical issues (see below). The present study, in a cross-sectional design, describes 2 practical, easily accessible surrogates of U_{osm} and assesses their validity by comparing the results to the actually mU_{osm} in large cohorts of the population and in a group of patients with CKD. We also checked the value of these surrogates in various sample types (spot or 24-h, day and night), and according to gender and renal function.

Our results show that the *estimated* U_{osm}, based on sodium, potassium, and urea concentrations, is an excellent surrogate of the *measured* U_{osm}. In most cases, eU_{osm} is within ±5% of mU_{osm}. This is similarly true in men and women, as well as in urine collected during day or night, and in patients with impaired renal function at any level of glomerular filtration rate. One may wonder how eU_{osm} and mU_{osm} may be so close when the formula used for the calculation of eU_{osm} neglects the minor solutes that should however represent more than 5% of all urinary solutes. This is partly explained by the fact that the units are not the same. eU_{osm} is expressed in mosm/L while mU_{osm} is in mosm/kg H_2O. Because 1 L of water with dissolved solutes weights more than 1 kg, the osmolality is lower than the osmolarity. The 2 measures differ only modestly for solutions within the biological range. For example, a solution containing 140 mmol/L NaCl and 500 mmol/L urea has an *osmolarity* of 780 mosm/L and an *osmolality* of 751 mosm/kg H_2O (i.e. 3.7% lower). This difference partially compensates for the missing solutes and thus contributes to the almost equality of eU_{osm} and mU_{osm}. Another factor is that electrolytes are assumed to be totally dissociated in the eU_{osm} formula. Although the dissociation is high in solutions within the physiological range, it is less than 100%, thus also contributing to modestly overestimate eU_{osm}.

UCI is a less accurate reflection of mU_{osm} than eU_{osm} because creatinine is known to undergo some secretion as well as some reabsorption along the tubule. The net result of these opposite effects depends on the rate of urine flow [44]. Our study shows that individual values are fairly dispersed and the correlations between the 2 variables are not linear. However, when no other approach is available, UCI remains a possible surrogate of urine concentration, provided it is applied to subjects with normal renal function. As clearly demonstrated in the present study, UCI diverges markedly from mU_{osm} in patients with CKD, limiting its use when renal function is impaired and probably also when abnormal handling of creatinine or excessive 24-h intake of creatine are suspected.

A few alternative methods for quantifying urine concentration have been used. UD (or specific gravity) may be evaluated in 7 colored grades with commercially available dipsticks (Labtix 8SG and Multistix 8SG AMES/Bayer Diagnostics) or evaluated by refractometry using a hand-held refractometer (Pen Urine S.G., Atago, Tokyo, Japan) [45]. In the DESIR study (a cohort of the French population), UD was measured with dipsticks in fresh spot morning urine samples from 1,604 subjects, and eU_{osm} was calculated (same formula as here) [8]. Median (IQR) eU_{osm} was 664 (272) mosm/L. UD was well correlated with eU_{osm} ($r = 0.446$, $p < 0.00001$). Another study showed that UD was well correlated with mU_{osm} but the wide dispersion made it "impossible to use UD as a dependable clinical estimate of U_{osm}" [46]. Moreover, UD or specific gravity cannot be used if urine contains proteins or glucose [47].

It is important to note that U_{osm} varies greatly among different subjects, as shown in the 4 populations of the present study and in a few previous reports [31, 42]. In usual conditions, some subjects produce hypo-osmotic urine while others show U_{osm} up to 1,200 mosm/kg H_2O. This wide range of spontaneous U_{osm} is possibly due to large inter-individual variations in the daily solute load [48], fluid intake [42], and thresholds for AVP secretion and/or thirst that are, in part, genetically determined [49]. Both AVP concentration and U_{osm} are known to differ between sexes. Men have higher AVP/copeptin levels [18, 21, 50] and higher U_{osm} than women [31]. This difference is mostly due to the fact that men excrete a larger osmolar load than women with a higher U_{osm} but an approximatively similar 24-h urine volume [31, 51]. Therefore, in studies using these variables, data for the 2 sexes are often presented separately. We verified here the validity of the 2 surrogates in each gender. For both genders, the relation between eU_{osm} and mU_{osm} is highly significant and the regression line between these 2 variables is very close to the identity line. The UCI also reflected this gender difference but tended to underestimate it slightly, possibly because of the known difference in creatinine handling in men and women.

Differences in the usual urine concentration may be associated with the ethnic background. A few studies showed that African Americans tend to concentrate urine about 20% more than Caucasians and have higher AVP levels [30, 52, 53]. To our knowledge, very few studies have evaluated other possible differences in usual urine concentration related to habitat or ethnic background [54–58].

The results of the SKIPOGH study illustrate the fact that urine is usually on the average more concentrated during the night than during the day by about 50–100 mosm/kg H_2O. Few studies have investigated day and night urine separately [59–61]. They showed that the circadian pattern of urine flow rate/urine concentration and/or sodium excretion rate may be disturbed in some subjects. An excessive urine concentration during daytime, limiting sodium and/or water excretion rate, is subsequently compensated at night by the pressure-natriuresis mechanism [59–63]. Accordingly, measurement of U_{osm} in overnight urine samples may not be representative of 24-h urine.

There are several advantages for using surrogates of U_{osm}. Osmometers, based on either freezing point depression or vapor pressure methods, are expensive and rarely equipped with automatic sample changers. Each measurement lasts a minute or 2 (due to the time needed to freeze or heat the sample, respectively), thus allowing some evaporation if samples are loaded in the changer in advance. We tested the automatic changer and observed that mU_{osm} values in the same sample increased after 10 loads. In studies involving a large number of subjects in which individual measurements are practically impossible, values may increase artifactually depending on the timing of the measurements. Moreover, osmolality measurements cannot be coupled with measurements of various solutes performed by automatic analyzers; they thus require separate aliquots and time-consuming manipulations. The excellent correlation between eU_{osm} and mU_{osm}, over the whole range of mU_{osm} values, even in CKD, validate eU_{osm} as an appropriate surrogate of mU_{osm}, especially in large cohorts.

Urine electrolytes are often available in epidemiological studies, but urea, needed for the calculation of eU_{osm}, is less frequently measured. When new measurements are initiated on previously stored samples in order to evaluate the kidney's concentrating activity, authors should consider the respective advantages of measuring either osmolality or urea concentration. Urea is much easier, quicker, and cheaper to measure than osmolality. Moreover, it will also provide data for a significant solute in the urinary concentrating process, and allow an indirect evaluation of protein intake.

This study has some limitations. It concerns exclusively subjects of European descent. The possible influence of sociodemographic factors has not been considered. However, we think it is reasonable to assume that the highly significant correlations between eU_{osm} and mU_{osm}, and

the relatively good relationships of UCI with mU_{osm} are not dependent upon the population under study and may be extended to all populations, as long as the measurements of sodium, potassium, urea, and creatinine concentrations are performed in appropriately equipped laboratory with rigorous methods.

In summary, the present study validates, in large cohorts, the use of an "estimated osmolarity," based on the measurement of sodium, potassium, and urea, as an excellent surrogate of the mU_{osm}. It also shows that the "urine concentration index," based on the ratio of creatinine concentrations in plasma and urine, may be used as a relative index of urine concentration only in subjects with normal renal function because of the disturbed handling of creatinine in CKD. In contrast, eU_{osm} is valid whatever the level of renal function. In future epidemiologic studies addressing the influence of AVP and urinary concentrating activity, the use of the "estimated urine osmolarity" should be recommended when the actual U_{osm} cannot be measured.

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Disclosure Statement

None of the authors have conflicts of interest to declare.

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