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Plasma copeptin and 24-hour urine hydration indices in hospital pharmacy professionals: a pilot study

Copeptina plasmática e índices urinarios de hidratación de 24 horas en profesionales de farmacia hospitalaria: estudio piloto

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ABSTRACT

Introduction: Hydration is critical to physiological homeostasis, influencing renal function and endocrine regulation (e.g., antidiuretic hormone [ADH]/copeptin), though no consensus exists on the optimal assessment method. This pilot study characterized the hydration status of hospital pharmacy professionals through an exploratory comparison between plasma copeptin (Pcop) classification and 24-h urine biomarkers (U24h).

Methods: This pilot study evaluated 22 hospital pharmacy professionals (aged 18–65 years) recruited from the pharmacy services of a university hospital. Hydration status was assessed using 24-h urine (volume, osmolality, and calculated free water reserve [FWR]) and fasting plasma copeptin (enzyme-linked immunosorbent assay [ELISA]). Participants were categorized into four hydration states—optimal, suboptimal, underhydrated, and hypohydrated—based on the thresholds proposed by Perrier et al. (2021). To evaluate the within-subject response, a subset (n=5) identified as underhydrated underwent a 7-day hydration intervention (additional 2 L/day).

Results: According to 24-h urinary indices, 40.9% (n=9) of the participants were classified as hypohydrated (and/or at risk). Plasma copeptin concentrations did not differ between urinary hydration groups (9.3 ± 3.0 *vs.* 8.1 ± 3.8 pmol/L; p= 0.425, Cohen's *d* = 0.353), and categories based on proposed copeptin thresholds showed marked discordance with the urinary classification. In the intervention subgroup (n = 5), 7-day supplementary water intake (2 L/day) did not produce statistically significant changes in plasma copeptin (8.4 ± 2.6 to 9.7 ± 3.1 pmol/L; p = 0.118) or plasma osmolality (291.4 ± 4.1 to 290.4 ± 4.0 mOsm/kg; p = 0.697).

Conclusions: In this volunteer sample, 9 of 22 hospital pharmacy professionals had 24-h urine osmolality above 500 mOsm/kg. Copeptin measured by research ELISA showed marked discordance with 24-h urinary indices and did not decrease after short-term water supplementation in a small subgroup. These exploratory findings do not establish diagnostic value for copeptin and indicate that the assay must be validated before use in larger studies. Larger cohorts and extended intervention periods are warranted to fully elucidate the long-term health and occupational implications of chronic underhydration.

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Keywords: hydration status; copeptin; 24-hour urine; osmolality

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RESUMEN

Introducción: La hidratación es fundamental en el mantenimiento de la homeostasis fisiológica, influyendo en la función renal y endocrina. Sin embargo, no existe consenso sobre el método de evaluación del estado de hidratación. Este estudio piloto tuvo como objetivo caracterizar el estado de hidratación de profesionales de farmacia hospitalaria y realizar una comparación exploratoria entre la clasificación basada en la copeptina plasmática (Pcop) y los biomarcadores urinarios de 24 horas (U24h).

Metodología: Este estudio piloto evaluó a 22 profesionales de farmacia hospitalaria (de 18 a 65 años) reclutados de los servicios de farmacia de un hospital universitario. El estado de hidratación se evaluó mediante orina de 24 horas (volumen, osmolalidad y reserva de agua libre calculada [FWR]) y copeptina plasmática en ayunas (ensayo por inmunoabsorción ligado a enzimas [ELISA]). Los participantes se categorizaron en estados de hidratación según los umbrales propuestos por Perrier et al. (2021). Para evaluar la respuesta intrasujeto, un subgrupo (n=5) identificado como subhidratado se sometió a una intervención de hidratación de 7 días (2L/día adicionales).

Resultados: Según los índices urinarios de 24 horas, el 40,9% (n=9) de los participantes se clasificó como hipohidratados. Las concentraciones de copeptina plasmática no difirieron entre los grupos urinarios de hidratación (9,3 ± 3,0 frente a 8,1 ± 3,8 pmol/L; p = 0,425; *d* de Cohen = 0,353), y las categorías basadas en los umbrales propuestos de copeptina mostraron una marcada discordancia con la clasificación urinaria. En el subgrupo de intervención (n=5), el aporte suplementario de agua no produjo cambios significativos en la copeptina plasmática (8,4 ± 2,6 a 9,7 ± 3,1 pmol/L; p = 0,118) ni en la osmolalidad plasmática (291,4 ± 4,1 a 290,4 ± 4,0 mOsm/kg; p = 0,697).

Conclusiones: En esta muestra de voluntarios, 9 de los 22 profesionales de farmacia hospitalaria presentaron una osmolalidad en orina de 24 horas superior a 500 mOsm/kg. La copeptina, determinada mediante un ELISA de investigación, mostró una marcada discordancia con los índices urinarios de 24 horas y no disminuyó tras la suplementación hídrica a corto plazo en un subgrupo reducido. Estos hallazgos exploratorios no establecen un valor diagnóstico para la copeptina e indican que el ensayo debe validarse antes de

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utilizarse en estudios más amplios. Se necesitan cohortes más amplias y periodos de intervención más prolongados para esclarecer las implicaciones a largo plazo de la subhidratación crónica para la salud y el ámbito laboral.

Financiación: Los autores declaran que no ha existido financiación para la realización de este estudio.

Palabras clave: estado de hidratación; copeptina; orina de 24 horas; osmolalidad.

Although this exploratory pilot study was not prospectively registered in public trial registries such as ClinicalTrials.gov or PROSPERO, the clinical protocol was thoroughly reviewed and approved by the Institutional Review Board (Ethics Committee of the São João Local Health Unit, CES 163/2022).

HIGHLIGHTS

- In this sample of hospital pharmacy professionals 40.9% (n = 9/22) were classified as hypohydrated or at risk by 24-h urine parameters.
- Occupational routines such as cleanroom duties and personal protective equipment (PPE) use warrant further study as potential contributors to fluid intake barriers during hospital shifts.
- Marked discrepancies were observed between 24-h urinary indices and copeptin categories, highlighting the complexity of assessing hydration status in hospital workers.

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INTRODUCTION

Water is essential to human physiology, and adequate hydration is decisive for health (1). As the main constituent of cells, tissues, and organs, water is involved in thermoregulation, nutrient transport, and solute balance, with daily variations of approximately 1% being compensated within a 24-h window through neuroendocrine mechanisms (2). Body water balance is maintained via hypothalamic thirst regulation, antidiuretic hormone (ADH) secretion, renal excretion or retention capacity, and insensible, respiratory, fecal, and sweat losses (3).

Mild acute hypohydration (as little as 2 % body weight loss) induced by heat or exercise has been associated with modest decrements in cognitive functions and increased fatigue, though findings remain heterogeneous, with some studies reporting neutral effects (4). In high-demand hospital settings, El-Sharkawy et al. (2016) reported fewer working memory errors among euhydrated health professionals versus hypohydrated peers, though overall cognitive effects were modest (5). Adequate hydration also benefits renal health by increasing urine volume and decreasing urinary osmolality lowering the risk of urolithiasis and urinary tract infection (6), and mitigates hyperosmolar stimulation of ADH secretion, reducing long-term endocrine strain (7).

Direct ADH quantification is complex; most circulating hormone (90%) is platelet-bound, underestimating secretion (8) and its half-life is short and the molecule is highly unstable (9). ADH and copeptin are derived from the same precursor, preprovasopressin, cleaved during axonal transport to the posterior pituitary (10). Because copeptin is produced in equimolar amounts with ADH, its plasma level reliably reflects ADH release and serves as a stable and easily measurable surrogate (11). However, it also responds to non-osmotic factors, such as physical exertion, clinical stress, or mild shifts in blood volume, which must be considered.

Increased water intake and higher urine volume are associated with lower plasma copeptin levels (12). Copeptin has been proposed as a biomarker of cardiovascular risk, renal function,

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prognosis in acute disease and sepsis (13, 14). It can also assist in the differential diagnosis of diabetes insipidus (13).

A recurring challenge in hydration research is the lack of a universal gold-standard marker. Blood-based and urine-based indices evaluate fundamentally different physiological constructs: plasma osmolality tracks the tightly defended internal osmotic state of body fluids, whereas 24-h urinary markers (osmolality and free water reserve, FWR) reflect recent fluid intake and integrated renal water-handling over the day (15, 16). Urine osmolality > 500 mOsm/kg and negative FWR values identify concentrated urine and active antidiuretic water-conservation effort, rather than acute clinical dehydration (17-19). Because plasma tonicity is defended homeostatically while urine concentrates to buffer low intake, discrepancies between classification methods are biologically expected.

In Portugal, hydration research has so far focused on athletes, children, adolescents, and the elderly (20, 21); No Portuguese study has assessed hydration in hospital pharmacy staff, and none has combined copeptin with 24-h urinary indices in this setting – a relevant gap, as these professionals perform physically and mentally demanding tasks that require optimal hydration for safety and performance. In hospital pharmaceutical services, staff work in aseptic, pressure-controlled cleanrooms where gowning protocols and personal protective equipment (PPE) may restrict fluid access during shifts. This pilot study aimed to characterize hydration status in hospital pharmacy professionals using 24-h urinary parameters (volume, osmolality, and FWR). As secondary aims, we compared these classifications with fasting plasma copeptin, explored biomarker correlations, and evaluated within-subject changes after a 7-day hydration protocol in an underhydrated subset.

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METHODOLOY

Study Design and Participants

This exploratory pilot study combined a cross-sectional baseline assessment with preliminary longitudinal hydration intervention conducted between May and July 2023 among hospital pharmacy professionals in the pharmaceutical services of Central University Hospital. All 116 employees were invited via email; 27.6% (n = 32) expressed interest and were informed of study objectives, procedures, voluntary participation, confidentiality, and data protection (Declaration of Helsinki (22); General Data Protection Regulation [GDPR])(23). Inclusion criteria were active staff aged 18–65 years. Exclusion criteria included medications affecting hydration status (antihypertensives, diuretics, renin–angiotensin system agents), recent acute illness (fever, diarrhea, or vomiting in previous 72h), chronic illness (chronic kidney disease, diabetes mellitus), pregnancy, breastfeeding, or exercise during the collection period. The final sample included 22 volunteers, all with higher education in the pharmaceutical field. This 19% participation rate likely reflects the significant logistical challenges of conducting research in a high-pressure clinical environment, particularly the burden of strict 24-h urine collection during active working shifts. Given the exploratory pilot nature of this study within high-demand clinical setting, no a priori statistical power calculation was performed; sample size was dictated by voluntary participation, staff acceptance of the 24-h urine collection, shift compliance, and laboratory constraints. Only participants completing all baseline procedures were included in the final analysis.

Ethics Approval

The study protocol was reviewed and approved by the Ethics Committee of the São João Local Health Unit (CES 163/2022). Written informed consent was obtained from all participants prior to enrollment.

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Data Collection Tools

Sociodemographic, anthropometric, clinical, and occupational information was collected using a structured questionnaire developed by the research team. The instrument covered sex, age, education level, and work shift duration and sector at the time of sample collection. Clinical data were obtained from participants electronic health records (SClínico®) and verified through self-report to ensure compliance with the inclusion and exclusion criteria.

Urine Sampling and Analysis

Participants collected a 24-h urine sample during a routine working day, using acid-free containers. At delivery, written and oral instruction covered collection procedures, and reinforced refraining from exercise during the collection window. Containers were kept refrigerated (4 - 8 °C) until laboratory delivery; upon receipt, total volume was measured, and aliquots were prepared. Urine samples were analyzed for creatinine (mg/day) and osmolality (mOsm/kg). Osmolality was assessed using an osmometer (Model 3320, Advanced Instruments, Inc.®). The creatinine coefficient was used to verify the completeness of urine collection (24). Hydration status was determined based on 24-h urine osmolality and FWR. Participants were classified as at risk of hypohydration when 24-h urine osmolality exceeded 500 mOsm/kg, a threshold indicating insufficient water intake to compensate for obligatory water losses, indicative of a less-than-optimal hydration status (19). The FWR (mL/24h) was calculated as described by Manz (25) by first determining the renal solutes excretion (mOsm/d); (urine osmolality × urine volume). The obligatory urine volume was then estimated as solute load (mOsm/day) / (830–3.4 × (age–20)). The FWR was obtained by subtracting the obligatory urine volume from the actual urine volume. Positive FWR values indicated euhydration, whereas negative values indicated hypohydration or risk thereof. Participants were categorized as euhydrated or hypohydrated or at risk (including those with 24-h urine osmolality > 500 mOsm/kg).

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Blood Sampling and Laboratory Analysis

Following the 24-h urine collection, fasting (overnight) blood samples were drawn (08:00 - 09:00 AM) for baseline biochemical profile. Plasma osmolality (mOsm/kg) was measured using the same osmometer model (Advanced Instruments 3320, Inc.®). Samples for copeptin analysis were centrifuged within 30 minutes and stored at −80 °C until analysis. Plasma copeptin (Pcop, pmol/L) concentrations were determined by enzyme-linked immunosorbent assay (ELISA; Human Copeptin ELISA Kit, MBS No. 2603322, MyBioSource Inc ®). Hydration status based on plasma copeptin was explored using the thresholds proposed by Perrier et al. (2021). For men, the thresholds were as follows: Optimal hydration ≤ 4 pmol/L, Suboptimal hydration]4;8[pmol/L, Underhydration [8;12[pmol/L, and Hypohydration ≥ 12 pmol/L. For women, the thresholds were: Optimal hydration ≤ 2 pmol/L, Suboptimal hydration]2;6[pmol/L, Underhydration [6;8[pmol/L, and Hypohydration ≥ 8 pmol/L (13). To minimize measurement bias, all samples were processed in duplicate using a single assay kit batch, and laboratory analysts performing the ELISA were blinded to participants' urinary hydration classification.

Hydration Intervention and follow-up blood sampling

Participants categorized as hypohydrated by urinary criteria (FWR < 0 mL/24h and/or osmolality > 500 mOsm/kg) were invited to increase their daily water intake by 2 L for seven consecutive days above habitual intake, following a protocol adapted from Lemetais et al. (2018) (26). Each participant received 15 L of bottled natural mineral water for this purpose. On day 8, fasting blood samples were collected and processed to reassess plasma osmolality and copeptin, allowing for a direct comparison of the dynamic response of the biomarkers to the water load stimulus.

Statistical Analysis

Statistical analyses were performed using IBM® SPSS Statistics software (version 27.0; IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation

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(SD) or median (interquartile range, IQR) as appropriate. Normality was assessed using the Shapiro–Wilk test. Between-group comparisons were made using the t-test or Mann–Whitney test for continuous variables and the chi-square or Fisher's exact test for categorical variables. Agreement between urinary and copeptin-based classifications was assessed with Cohen's kappa, considering copeptin underhydration or hypohydration as concordant with 24-h urine osmolality > 500 mOsm/kg. Pre–post differences were tested with Student's t-test. Associations between continuous variables were assessed with Pearson's or Spearman's coefficients, as appropriate. Effect sizes are reported as Cohen's d. Statistical significance was set at $P < 0.05$.

This manuscript's reporting was verified against the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist (von Elm et al., 2007), included as Supplementary Material.

RESULTS

Sample Characterization

Of the 116 employees invited, 32 (27.6%) expressed interest and 22 (19.0%) were included; 10 were excluded after screening (pregnancy, breastfeeding, interfering medication, type 1 diabetes); all 22 urine collections were complete, and 5 of the 9 eligible participants (55.6%) accepted the intervention. A total of 22 hospital pharmacy professionals participated in this study, including 18 women (81.8%) and four men (18.2%), all of whom held higher education degrees. Regarding the work schedule during the assessment period, the majority of the volunteers (n = 21) performed a standard 7-h day shift, while one participant (n = 1) performed a 12-h night shift. Additionally, 13 participants (59.1%) reported regular use of PPE during the shift in controlled isolated pressure rooms. The sociodemographic and anthropometric, characteristics of the sample are shown in Table 1. No significant differences were observed in body mass index (BMI) between sexes ($p = 0.865$). However, men were significantly taller ($p < 0.001$) and heavier ($p = 0.041$) than women.

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Table 1. Sociodemographic and anthropometric characteristics

	Men (n = 4) Mean (SD)	Women (n =18) Mean (SD)	Total sample (n = 22) Mean (SD)	p
Age (years)	34.8 (8.2)	38.3 (12.0)	37.6 (11.3)	0.594*
Height (cm)	180.8 (3.4)	161.8 (5.9)	165.2 (9.3)	**< 0.001****
Weight (kg)	81.5 (12.4)	65.9 (12.6)	68.7 (13.7)	**0.041***
BMI (kg/m²)	25.0 (4.4)	25.3 (5.3)	25.2 (5.1)	0.865*

a Values are expressed as mean (standard deviation). *p-value according to the Mann–Whitney test.
***p*-value according to Student's t-test. Abbreviations: BMI, body mass index

Hydration Status Based on 24-hour Urine Parameters

Hydration status was assessed by calculating the free water reserve and osmolality values from 24-h urine samples. Figure 1 shows the relationship between these two parameters. It was found that 18.2% of participants (n = 4, three women and one man) had negative FWR values, and 40.9% (n = 9; 95% confidence interval, CI 23.3 - 61.3%) had urinary osmolality > 500 mOsm/kg (with the four individuals with FWR < 0 included in this group). Thus, 40.9% were in a state of hypohydration and/or at risk of hypohydration, while the remaining 59.1% of participants were euhydrated. The classification of hydration status and its association with age, height, weight, BMI, urine volume, and osmolality are presented in Table 2. Hydration classification based on urinary parameters did not differ significantly by sex (p = 0.264).

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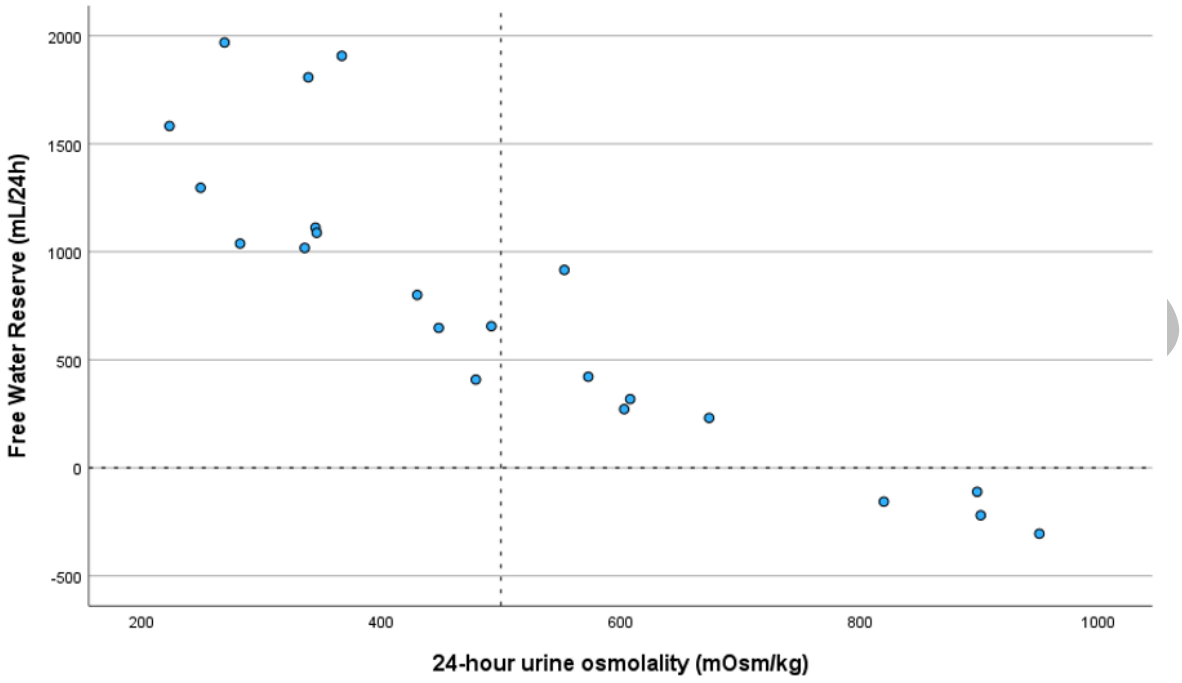


Figure 1. Relationship between 24-h urine osmolality and Free Water Reserve (FWR). Individual participant data are plotted showing 24-h urine osmolality (mOsm/kg) against FWR (mL/24 h). The vertical dashed line indicates the 500 mOsm/kg threshold; participants with values above this limit were classified as being at risk of hypohydration (Perrier et al., 2015). The horizontal line at 0 mL/24 h separates participants into euhydrated (positive FWR) and hypohydrated or at-risk (negative FWR) states, based on the calculation of obligatory urine volume (Manz et al., 2002). Participants with negative FWR (n = 4) form a more extreme subgroup within those with 24-h urine osmolality > 500 mOsm/kg (n = 9).

Table 2. Relationship between hydration status, 24-hour urine (U24h), free water reserve (FWR), age, and anthropometry

	Total sample (n = 22) Mean (SD)	Hypohydration [b] (n = 9) Mean (SD)	Euhydration (n = 13) Mean (SD)	p
Age (years)	37.6 (11.3)	40.1 (9.6)	35.9 (12.4)	0.348*
Height (cm)	165.2 (9.3)	167.7 (12.0)	163.5 (6.9)	0.616**
Weight (kg)	68.7 (13.7)	74.8 (11.1)	64.5 (14.2)	**0.007***
BMI (kg/m²)	25.2 (5.1)	27.0 (6.0)	24.0 (4.1)	0.102*
U24h volume (L)	1.84 (0.77)	1.45 (0.69)	2.11 (0.72)	_
U24h osmolality (mOsm/kg)	508 (224)	731 (160)	354 (87)	_
FWR (mL/24h)	759 (687)	151 (390)	1179 (506)	_

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a Values are expressed as mean (standard deviation). b Hypohydration categorized as U24h osm. >500 and/or FWR <0 *p-value according to the Mann–Whitney test. **p-value according to Student's t-test. Abbreviations: U24h osm, 24-hour urine osmolality; FWR, free water reserve

Hydration Status Based on Plasma Copeptin

Hydration status was further explored using plasma copeptin concentration according to the thresholds proposed by Perrier et al. (2021). These categories may depend on assay calibration. Based on these proposed thresholds, 9.1% (n = 2) of the participants were optimally hydrated, 18.2% (n = 4) were suboptimally hydrated, 13.6% (n = 3) were underhydrated, and 59.1% (n = 13) were hypohydrated, all participants in the hypohydration category were women. Plasma osmolality and copeptin values, together with urinary osmolality, urine volume, and FWR across these categories are summarized in (Supplementary material, Table S1).

Relationship Between Urinary and Plasma Parameters

The relationship between hydration status classified by urinary parameters and plasma variables (osmolality and copeptin) was further explored. Participants classified as hypohydrated presented lower 24-h urine volume (1.45± 0.69 *vs* 2.11 ± 0.72 L) and higher urine osmolality (731± 160 *vs.* 354 ± 87 mOsm/kg) than euhydrated participants (Supplementary Material, Table S2), with no significant differences in plasma osmolality (p = 0.673) or plasma copeptin (p = 0.425; Student's t-test; Supplementary Table S2). Correlations between urinary and plasma parameters (Supplementary Material, Table S3) showed no significant associations between plasma osmolality or copeptin and urine osmolality, urine volume, or FWR (all p > 0.05). Cross-classification of both methods (Supplementary Material, Table S4) showed poor agreement (59.1%; κ = 0.24, 95% CI −0.14 to 0.62; Fisher's exact test p = 0.046), without a consistent pattern across categories. Among the 13 participants classified as euhydrated by 24-h urinary parameters, 8 (61.5%) had copeptin concentrations in the hypohydration category.

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Hydration intervention

Of the nine volunteers initially classified as hypohydrated (FWR<0 and/or urinary osmolality > 500 mOsm/kg), five agreed to increase their daily water intake by 2 L for seven consecutive days. Plasma osmolality and copeptin levels were reassessed at the end of this period (Table 3). Plasma copeptin increased from 8.4 ± 2.6 to 9.7 ± 3.1 pmol/L, p = 0.118, while plasma osmolality remained stable (291.4 ± 4.1 *vs.* 290.4 ± 4.0 mOsm/kg, p = 0.697). Individual participant data and paired differences are detailed in Supplementary Material (Table S5). Given the small sample size (n=5) and absence of a control group, these preliminary intervention results remain inconclusive.

Table 3. Comparison of plasma osmolality (Posm) and copeptin (Pcop) values at baseline and post-hydration (n = 5).

	1st assessment (n = 5) Mean (SD)	2nd assessment (n = 5) Mean (SD)	p
Pcop (pmol/L)	8.4 (2.6)	9.7 (3.1)	0.118
Posm (mOsm/kg)	291.4 (4.1)	290.4 (4.0)	0.697

*p-value according to the student's t-test.

Abbreviations: Posm, plasma osmolality; Pcop, plasma copeptin.

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DISCUSSION

This study involved a convenience sample of hospital pharmacy professionals working in the pharmaceutical services of the São João Local Health Unit, to our knowledge, this was the first study in Portuguese hospital pharmacy staff to combine these markers. Urinary indices are considered most appropriate for population assessment; we therefore combined two urine-based criteria: urine osmolality > 500 mOsm/kg according to European Food Safety Authority (EFSA) (18) and Perrier (19), and negative FWR values (17).

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As no comparable Portuguese adult data exist, we referred to a German population study (Manz et al. 2012; n = 1528) reporting 40% of men and 19% of women hypohydrated and/or at risk by FWR. Sex-specific analysis was not performed, as hydration classification did not depend significantly on sex, and only four individuals were male. In our study, 40.9% of the sample was classified as hypohydrated and/or at risk of hypohydration based on the FWR and U24h osmolality. Our sample size (n = 22) is a limitation, and no statistically significant correlation was observed between plasma copeptin and 24-h urinary parameters. Participation was low; professionals who declined to take part reported that a 24-h urine collection was difficult to carry out on a normal working day, although reasons were not systematically recorded. The additional fasting blood draw the following morning may also have contributed to the burden. Selection bias is therefore possible, although its direction is unknown.

As expected, hypohydrated participants showed lower urine volume and higher urine osmolality, consistent with active renal water conservation mechanisms as described by Perrier (2015).

El-Sharkawy et al. (2016) reported that 36% of nurses and physicians were dehydrated at the start of their shift, rising to 45% by shift end using a cutoff of urine osmolality >800 mOsm/kg. Although direct numerical comparisons are limited by differences in cut-off criteria and study design, our observed proportion of 40.9% (U24h osm. > 500 mOsm/kg and/or FWR < 0) suggests that fluid conservation is common. Occupational barriers-such as PPE use and cleanroom gowning-represent plausible hypotheses that may limit ad libitum fluid intake in hospital settings, warranting formal evaluation.

Using the thresholds proposed by Perrier et al. (2021), 13.6% of the volunteers were categorized as underhydrated and 59.1% as hypohydrated. However, these thresholds were derived with the BRAHMS KRYPTOR platform, and their transferability to a research ELISA cannot be assumed. Copeptin was not significantly associated with U24h osmolality, whereas Lemetais (2018) found this association significant in 82 healthy volunteers (p=0.002)(26).

Optimal hydration based on copeptin reflects sufficient water intake to prevent excessive ADH secretion and ensure abundant dilute urine excretion (12). Underhydration describes a state

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of inadequate, though not thirst-provoking, water intake, without total body water deficit or increased plasma osmolality, but with increased urinary osmolality and ADH (inferred via Pcop), reflecting an active homeostatic water-balance mechanism (27). We observed this pattern in our sample: plasma osmolality was similar across hydration categories, whereas the hypohydration category showed higher copeptin and urine osmolality values, consistent with the literature (12).

Among the 13 participants classified as euhydrated by urinary parameters, eight had copeptin values above the proposed thresholds, demonstrating discordance between the methods. Technical and design factors are the most likely explanations. First, the research ELISA may be calibrated differently from the platform on which the thresholds were derived. Second, non-osmotic stimuli at sampling (stress, venipuncture, posture, nausea) can raise copeptin independently of hydration. Only after these factors are excluded could a physiological explanation be considered.

High copeptin values are positively and independently associated with the incidence of type 2 diabetes mellitus, metabolic syndrome, progression of kidney disease, and cardiovascular disease (28, 29). Increasing water intake has been shown to affect copeptin levels: in our intervention subgroup (Section 3.5), no significant copeptin change was observed. Enhörning et al. (2019) studying 39 healthy participants, found that increasing fluid intake by 3 L for one week resulted in a 15% reduction in copeptin ($p = 0.003$) (30). In another study by Lemetais et al. (2018), 82 participants were divided by usual water intake relative to EFSA recommendations (group A: 50–80%; group B: 80–120%; group C: 121–200%); a 6-week intervention raising groups A and B to group C levels produced a significant reduction in copeptin values ($p = 0.012$)(26). In contrast, in our exploratory subset ($n = 5$), a 7-day additional fluid intake (+2 L/day) did not produce statistically significant change in plasma copeptin or osmolality. This analysis is further limited by the absence of verified fluid logs. Other possible explanations include the short intervention period, constrained by sample stability and the single kit batch, the 2 L/day volume, agreed with participants as feasible during routine shifts, and higher summer ambient temperatures. In summary, while 24-h urinary indices captured renal solute handling, plasma copeptin reflected neuroendocrine

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tone, and their cross-classification showed notable discordance underscoring the complexity of assessing hydration in hospital pharmacy staff.

Limitations

The small sample size and unequal sex distribution limit statistical power and generalizability, constraints primarily dictated by the manufacturer's strict 3-month sample stability period and use of a single assay kit batch to avoid inter-assay variability. Additionally, processing all samples in duplicate—to ensure analytical reliability during this method's initial implementation in our laboratory—further restricted participant numbers. Furthermore, the cross-sectional nature of the baseline assessment precludes inferring causality between specific hospital tasks and hydration status. Finally, because data collection occurred between May and July, elevated ambient summer temperatures may have increased insensible sweat losses, acting as a potential confounder on urinary osmolality. Another analytical limitation concerns the copeptin assay. The thresholds applied were derived from studies using the automated BRAHMS KRYPTOR platform, which was not locally available. As this unfunded academic project relied on a single research ELISA kit, manual assay variability must be acknowledged, and direct threshold transferability cannot be assumed, potentially contributing to classification discrepancies. We noted that while dietary history, caffeine/alcohol records, and work-area environmental data (temperature, humidity, pressure) were recorded, they could not be modeled in the primary text due to word count limits and showed no statistically significant relationship with copeptin or osmolality.

Perspectives and Future Directions

This work highlights the complexity of assessing hydration status through multiple parameters and methodological variability. These preliminary findings provide a foundation for expanding research into other hospital settings and professional categories, particularly high-demand environments such as operating theaters, where occupational stressors and restricted fluid access may further affect water balance, staff well-being, and clinical performance.

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CONCLUSIONS

Inadequate hydration was frequent in this volunteer sample of pharmacy professionals, 9 of 22 participants had 24-h urine osmolality above 500 mOsm/kg, indicating fluid intake below the optimal level. Fasting plasma copeptin, measured by ELISA, showed discordance with 24-h urinary indices; its potential role as a hydration biomarker requires validation before use in larger studies. From a practical standpoint, structured hydration breaks and improved fluid accessibility in hospital settings warrant evaluation in future studies, and larger multicenter studies are needed to confirm these preliminary findings and clarify the clinical relevance of biomarker discordance.

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AUTHORS' CONTRIBUTIONS

J.B. contributed to the conceptualization, data collection, statistical analysis, and manuscript drafting. F.C. and P.S. performed study supervision and methodological review. R.P., S.M., R.F., P.N., B.O., and F.C. contributed to data interpretation and critical revision. All authors critically reviewed previous versions and approved the final manuscript.

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CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest. The authors state that there are no conflicts of interest when writing the manuscript.

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DATA AVAILABILITY

Provision of data on request to the corresponding author.

DECLARATION OF ARTIFICIAL INTELLIGENCE USE

The authors declare that no artificial intelligence tools were used during the preparation, writing, or development of this manuscript.

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