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Storing urine samples with moisture preserves urine hydration marker stability up to 21 days

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Abstract

Introduction To assess hydration status, hydration markers [urine color, osmolality, and urine-specific gravity (USG)] are used. Urine color, osmolality, and USG have shown to be stable for 7, 7, and 3 days, respectively, at 4 °C. However, refrigeration could produce a dry environment which enhances evaporation and potentially affects urine hydration markers.

Purpose To examine the effect of duration and moisture on urine markers with refrigeration.

Methods 24 participants provided urine samples between 9 and 10 AM. Urine color, osmolality, and USG were analyzed within 2 h (baseline). Then, each urine sample was divided into two urine cups and placed in a storage container with (moisture condition) and without (no moisture condition) water bath at 3 °C. Hydration markers were analyzed at day 1(D1), D2, D7, D10, D14, and D21. A two-way ANOVA (time x condition) and repeated-measures ANOVA on time were performed to examine differences.

Results No significant ($p > 0.05$) condition x time effect was observed for urine color ($p = 0.363$), urine osmolality ($p = 0.358$), and USG ($p = 0.248$). When urine samples were stored in moisture condition, urine color ($p = 0.126$) and osmolality ($p = 0.053$) were stable until D21, while USG was stable until D2 ($p = 0.394$).

Conclusion When assessing hydration status, it appears that the urine color and osmolality were stable for 21 days, while USG was stable for 2 days when stored with moisture at 3 °C. Our results provide guidelines for practitioners regarding urine storage duration and conditions when urine cannot be analyzed immediately.

Keywords Urine color · Urine osmolality · Urine specific gravity · Urine stability

Introduction

Hydration markers, such as urine color, urine osmolality, and urine specific gravity (USG), are valid measurements for hydration status [1, 2]; thus, urine samples are routinely collected by practitioners to assess one's hydration [2, 3]. In addition, it has been recommended that urine samples should be analyzed within 2 h of collection to ensure the integrity of the sample quality [4]. However, in some situations, analyzing the urine sample within the first 2 h might not be feasible in field research due to a large number of sample sizes and accessibility of devices that require samples to be stored for an extended period before analysis.

Previous studies have demonstrated that urine osmolality is viable at 1.5 [5], 2 [6, 7], 3 [7], and 7 days [8] when the urine samples were stored at 4–7 °C with no moisture, whereas urine color and USG assessment using stored urine appeared to have mixed results [7, 8]. When urine samples were stored at 7 °C with no moisture, USG measurement was stable for 7 days [8], whereas, another study demonstrated that it was not stable for 1 day [7]. Similarly, urine color assessment was stable for 3 days [7], whereas, others demonstrated that it was not stable for 1 day [8]. The inconsistent results observed with urine color and USG could be attributed to evaporation in the refrigerators.

USG measurements work on the principle of refraction, where light passes through the urine and is refracted through the solutes present in the urine [9]. Therefore, when evaporation occurs, there is a higher concentration

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of solutes than solvents, potentially affecting USG measurements. Similarly, urine osmolality is used to measure solute concentration in the sample via freezing point [9]. Again, if evaporation occurs, it increases solute concentration which can affect urine osmolality readings. Lastly, urine color is controlled by the concentration of the urochrome [10], which increases with dehydration resulting in a darker and more concentrated urine [11]. Hydrated urine samples are lighter and less concentrated [1], meaning there is more water than solutes; hence, evaporation might not affect hydrated samples compared to a dehydrated sample. However, urine is more concentrated in dehydrated urine samples, and evaporation could affect these samples' assessments due to the increased solute content.

A dry environment (e.g., refrigerators) creates a gradient difference in humidity [12], promoting water evaporation from the stored urine samples. This could affect the concentration of the urine samples and, thus, affect hydration status assessment. To our knowledge, no research has assessed the role of moisture in the storage environment on hydration markers. Furthermore, past studies have demonstrated that storing urine specimens at a temperature between 4 and 7 °C can maintain markers of hydration viable for one week [5–8]. The ability to store the urine longer than a week could allow researchers to perform multiple urine analyses within a given day, saving researchers time and can be beneficial for field-based research that doesn't allow for immediate analysis. Therefore, this study aimed to determine the effect of duration and moisture on urine color, osmolality, and USG with refrigeration.

Table 1 Demographic data for all participants

Demographic data	Mean ± SD
Age (years)	25 ± 5
Height (cm)	169.8 ± 8.0
Weight (kg)	72.1 ± 13.4

Methods

Participants

A priori power analysis (G*Power, Germany) was used to determine appropriate sample sizes. Based on an effect size of 0.21, a sample size of at least 24 participants was needed for a power of 0.80 and $\alpha = 0.05$. Twenty-four healthy men and women participated in this observational study (Table 1). All participants completed a medical history form to determine eligibility. All participants were included in the study if they were willing to provide a urine sample between 9 and 10 AM and were excluded if they had a history of renal diseases, hypertension, or Type 1 and Type 2 Diabetes. All participants were eligible and provided written informed consent. This study was approved by the Texas Tech University Institutional Review Board and adhered to the Declaration of Helsinki.

Study design

Participants arrived in the morning, and height and weight were measured, followed by providing the researcher with a urine sample between 9 and 10 AM. Once all samples were obtained from the participants, all the urine samples were analyzed for urine color, osmolality, and USG within the first 2 h (baseline). Following the initial hydration markers assessment, each participant's urine sample was evenly divided into two separate cups and capped. Two urine samples from each participant were stored either in a container with 1000 ml water (moisture) or in a container with no water (no moisture), and the container was sealed shut (Fig. 1). The refrigerator (VWR, Radnor, Pa, USA) was set to 4 °C, with an average temperature of 3.2 °C throughout the course of the study. Vortexed urine samples were measured for urine osmolality, color, and USG on days (D)1, D2, D7, D10, D14, and D21. Urine osmolality was analyzed using Advanced Instrument Osmometer (Norwood,

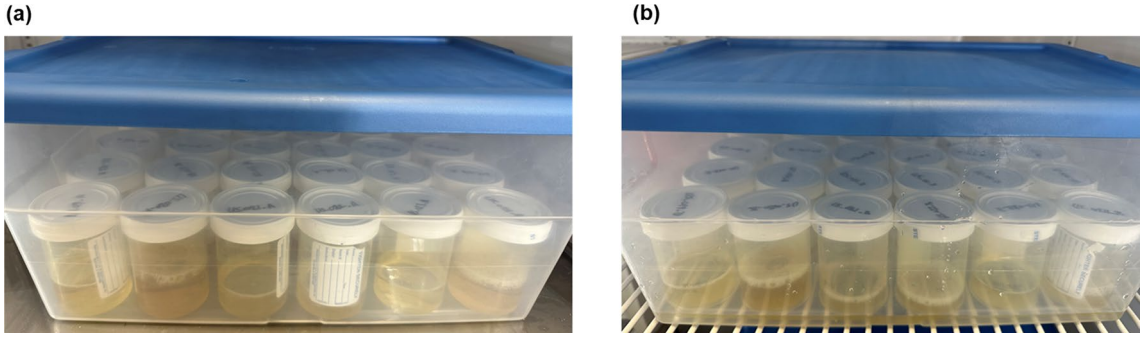

Fig. 1 Sample storage conditions with **a** No moisture and **b** Moisture

Ma, USA) in duplicate. USG was analyzed using an optical refractometer (ATAGO, Tokyo, Japan), and urine color was assessed by the same researcher for all-time points using the 8-point urine color chart [1].

Statistical analyses

SPSS (I.B.M. version 29; Armonk, NY: I.B.M. Corp) was used for all statistical analyses. Urine osmolality, urine color, and USG were analyzed using a two-way ANOVA (time × condition) with repeated measures on condition and time. In addition, urine osmolality, color, and USG were analyzed for time using repeated measures ANOVA for each condition. LSD post hoc tests were used for pairwise comparisons. Effect sizes were calculated between baseline and all other days for both conditions (Table 2). The statistical significance was set at $p < 0.05$. Data are reported as mean ± SE.

Results

Urine osmolality

For urine osmolality, there was no significant condition x time effect ($p = 0.358$; Fig. 2a). No significant time effect was observed in the moisture condition ($p = 0.053$; Fig. 2b), while a significant time effect ($p = 0.013$) was observed for the no moisture condition (Fig. 2c). In the no moisture condition, baseline was significantly greater than D1 and D2, while no differences were observed with D7, D10, D14, and D21.

Urine color

For urine color, there was no significant condition x time effect ($p = 0.363$; Fig. 3a). However, a significant time effect was observed in the moisture condition ($p = 0.044$; Fig. 3b) and no moisture condition ($p = 0.005$; Fig. 3c). No significant differences were observed between baseline and all days in the moisture condition. However, in the no moisture condition, baseline was significantly greater than D7, D14, and D21, while no differences were observed within D1, D2, and D10.

USG

For USG, there was no significant condition x time effect ($p = 0.248$; Fig. 4a). A significant time effect was observed in the moisture condition ($p = 0.0001$; Fig. 4b) and no moisture condition ($p = 0.005$; Fig. 4c). In the moisture condition, baseline was significantly greater than D7, D10, and D21, while no differences were observed on D1, D2, and D14. In the no moisture condition, baseline was significantly greater than D1, D7, D10, D14, and D21, while no differences were observed with D2.

Discussion

The novel finding of this study is that urine samples can be stored at 3 °C with moisture for up to 21 days when analyzed for urine osmolality and color. USG, on the other hand, can be stored at 3 °C for up to 2 days when the samples are being stored with moisture. Previous studies have stored urine samples similar to our no moisture condition [7, 8] and have demonstrated that storing urine in a refrigerator (4–7 °C) can maintain the stability of urine color for up to 3 days [7], urine osmolality and USG for up to 7 days [5–8]; this study further adds that urine can be stable for urine color and osmolality when stored with moisture for up to 21 days.

Urine osmolality has been shown to be stable for up to 7 days when stored at a temperature ranging between 4 and 7 °C [5–8]. Similarly, our results demonstrated that the importance of storing urine in moisture can increase the stability of urine osmolality from 7 to 21 days, further adding to the urine storage literature when considering urine osmolality. Results in our no moisture condition were mixed as it showed that urine osmolality significantly differed from Day 1 and Day 2, but not all other time points. Although the first 2 days were statistically different from the baseline, these time points had small effect sizes (d = 0.01), and mean differences were within 3 mOsm/kg when compared to baseline, which could be ascribed to the machine

Table 2 Effect sizes from all days compared to baseline

Days	Urine osmolality		Urine color		USG	
	Moisture	No moisture	Moisture	No moisture	Moisture	No moisture
Day 1	0.01	0.01	0.06	0.09	0.08	0.32
Day 2	0.01	0.01	0.00	0.17	0.03	0.19
Day 7	0.01	0.00	0.16	0.43	0.85	0.83
Day 10	0.00	0.00	0.16	0.27	0.75	0.90
Day 14	0.00	0.00	0.25	0.36	0.41	0.21
Day 21	0.01	0.00	0.30	0.36	0.25	0.25

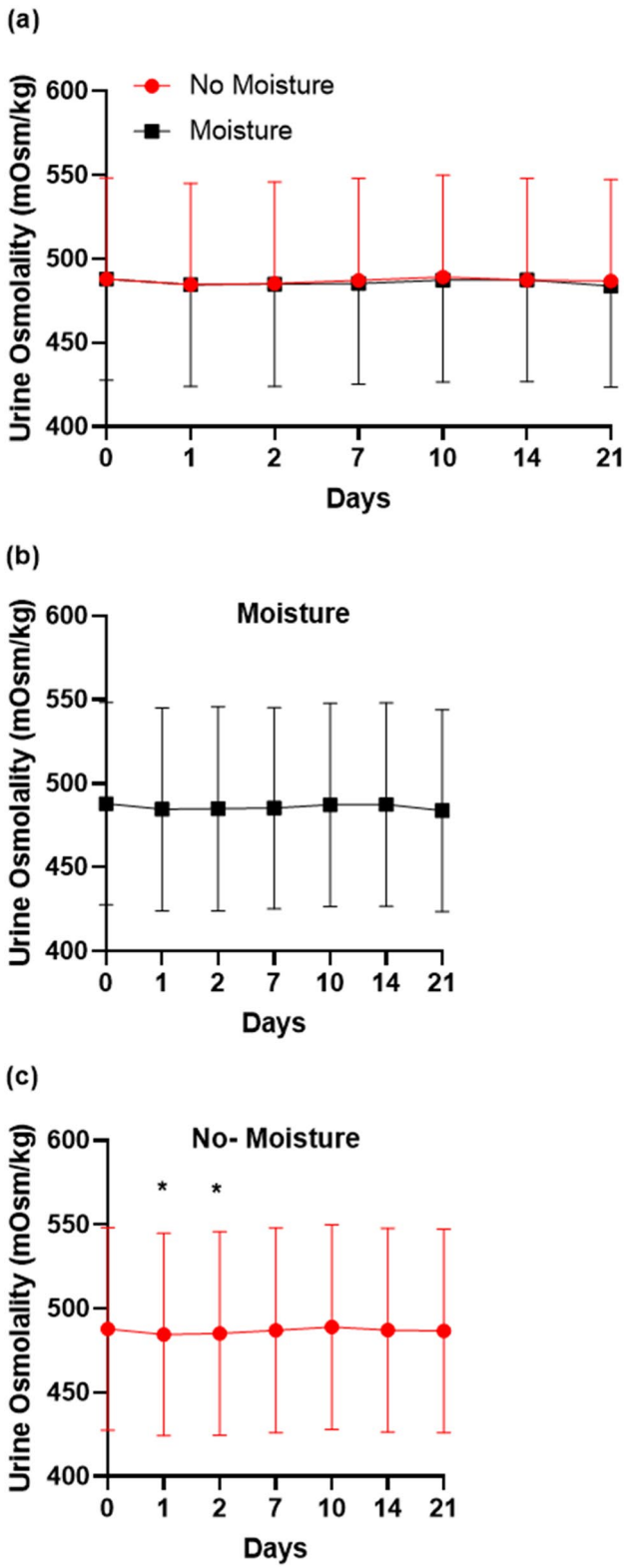

Fig. 2 Markers of hydration analyses for Urine Osmolality. **a** No significant condition x time effect was observed for Urine Osmolality. **b** No significant time effect was observed for urine osmolality in the moisture condition. **c** A significant time effect was observed for urine osmolality in the no moisture condition. Values are mean ± S.E. * $P < 0.05$ vs. Day 0 (Baseline)

operational error [13]. Since all other days were not different from the baseline, it may be concluded that the no moisture condition can also keep urine osmolality stable till 21 days. This would further add to the literature that urine osmolality can be stable up to 21 days more than the previously known 7 days [8], as this study did not add moisture to their samples.

Urine color and USG have demonstrated varied results based on previous literature [7, 8]. Our study found that urine color assessment was stable until Day 21 in the moisture condition, while it was only stable until Day 2 in the no moisture condition. Like our no moisture condition, urine color was stable up to Day 3 at 7 °C with no moisture [7]. In contrast, another study revealed that urine color was not stable on Days 1, 2, and 7 at 7 °C without moisture [8]. USG was stable up to Day 2 only in the moisture condition and not in the no moisture condition. When samples were stored at 7 °C with no moisture, the USG assessment was stable till Day 7 [8], while it was not stable on Day 1 [7]. Differences observed between urine color and USG in our study and prior research [7, 8] could be due to the temperatures the samples were stored (3 °C vs. 7 °C), the type of urine collected (Spot urine vs. 24-h urine), and how the urine was stored (moisture vs. no moisture). However, the possible reason leading to the inconsistency is still not fully understood. It is noteworthy to mention that the USG assessment displayed some erroneous data. Notably, in the moisture condition, Day 14 was not significantly different from the baseline; however, Days 7, 10, and 21 were significantly different from the baseline. Similarly, in the no moisture condition, Day 2 was not significantly different from baseline; however, it was significantly different from Days 1, 7, 10, and 21. These results could be explained due to the large standard errors observed on Day 14 and Day 2 in the moisture and no moisture conditions, respectively. The large standard errors could be attributed to the variance in hydration levels of the participants. To avoid these errors, future studies should aim to standardize the hydration levels of their participants.

In the absence of moisture, refrigerators create a dry environment that can facilitate evaporation; however, this potential confounding variable has not been considered. To address this potential problem, our study stored the sample in a water bath to account for the humidity gradient between the urine sample and the internal environment of the refrigerator. Our results demonstrate that storing urine samples in a water bath at 3 °C prevents the compromise of the sample integrity for urine osmolality and color assessment for up to 21 days compared to no moisture. Additionally, USG was

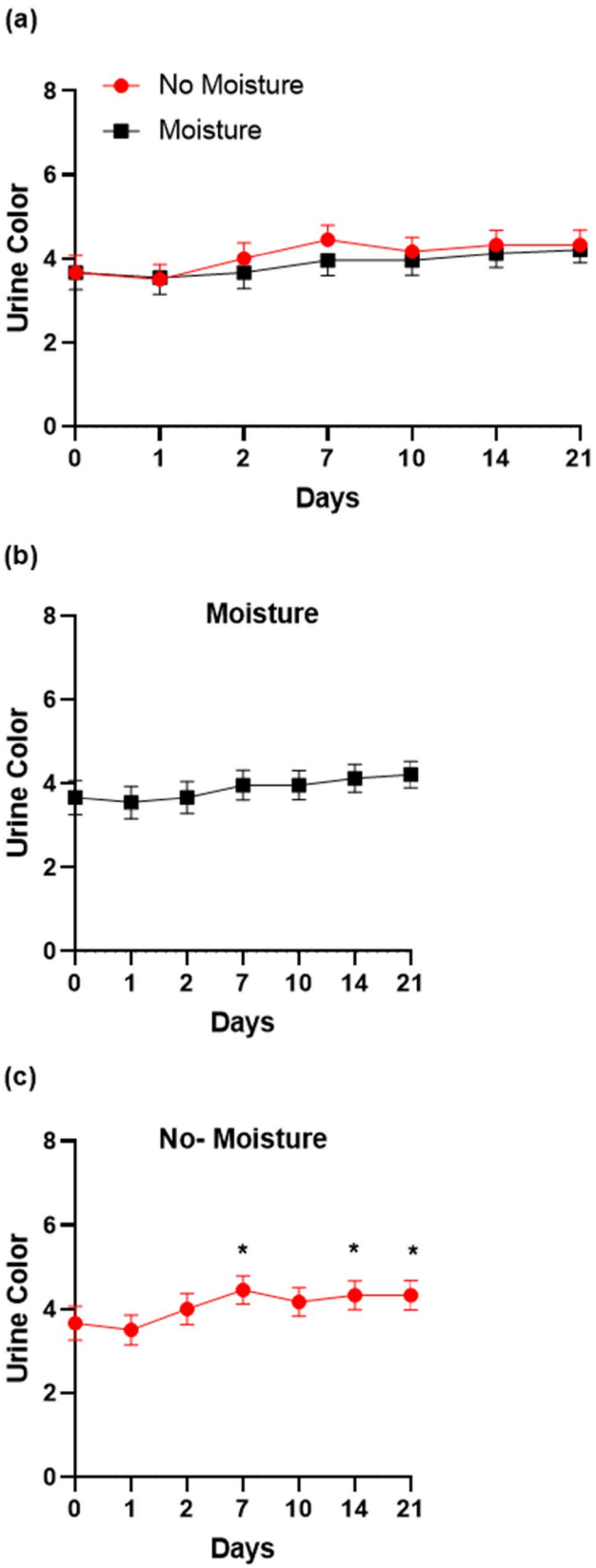

Fig. 3 Markers of hydration analyses for Urine Color. **a** No significant condition x time effect was observed for Urine Color. **b** A significant time effect was observed for Urine Osmolality in the moisture condition. **c** A significant time effect was observed for urine osmolality in the no moisture condition. Values are mean ± S.E. * $P < 0.05$ vs. Day 0 (Baseline)

only stable up to Day 2 and faired better than the no moisture condition, as it was not stable on Day 1. Similarly, previous research has demonstrated that USG is not stable 1 day after refrigeration with no moisture [7]. Thus, although speculative, the moisture added by the water in the containers may have helped reduce the evaporation of the urine and thus maintained the solvent-to-solute ratio in our urine samples, which helped keep the USG stable compared to the no moisture condition.

As with any study, there are limitations to this study. One limitation of this study was that the hydration status of the participants wasn't controlled, which could have affected differences observed in USG. Additionally, urine samples were measured at various time points at refrigerated temperatures, which could have affected these hydration markers. Future studies should aim to control for their participants' hydration status and wait until samples are at room temperature to begin the assessment.

Conclusion

In conclusion, this paper is the first to illustrate the importance of storing urine in the refrigerator with a water bath to prevent loss of moisture, especially if the storage duration exceeds 7 days. Urine osmolality and color are stable until Day 21, while USG is stable only for up to 2 days when stored with moisture at 3 °C. Adding moisture to a container storing the urine samples can further urine shelf life in the refrigerator compared to no moisture. The ability to store urine samples for 21 days could benefit researchers who perform various analyses within a given day and don't have time to run urine analyses. These findings will allow researchers to perform multiple urine analyses within a given day, saving researchers time, and can be beneficial for field-based research that doesn't allow for immediate analysis. If immediate analysis for hydration markers cannot be performed, urine can be stored at 3 °C for up to 21 days with moisture.

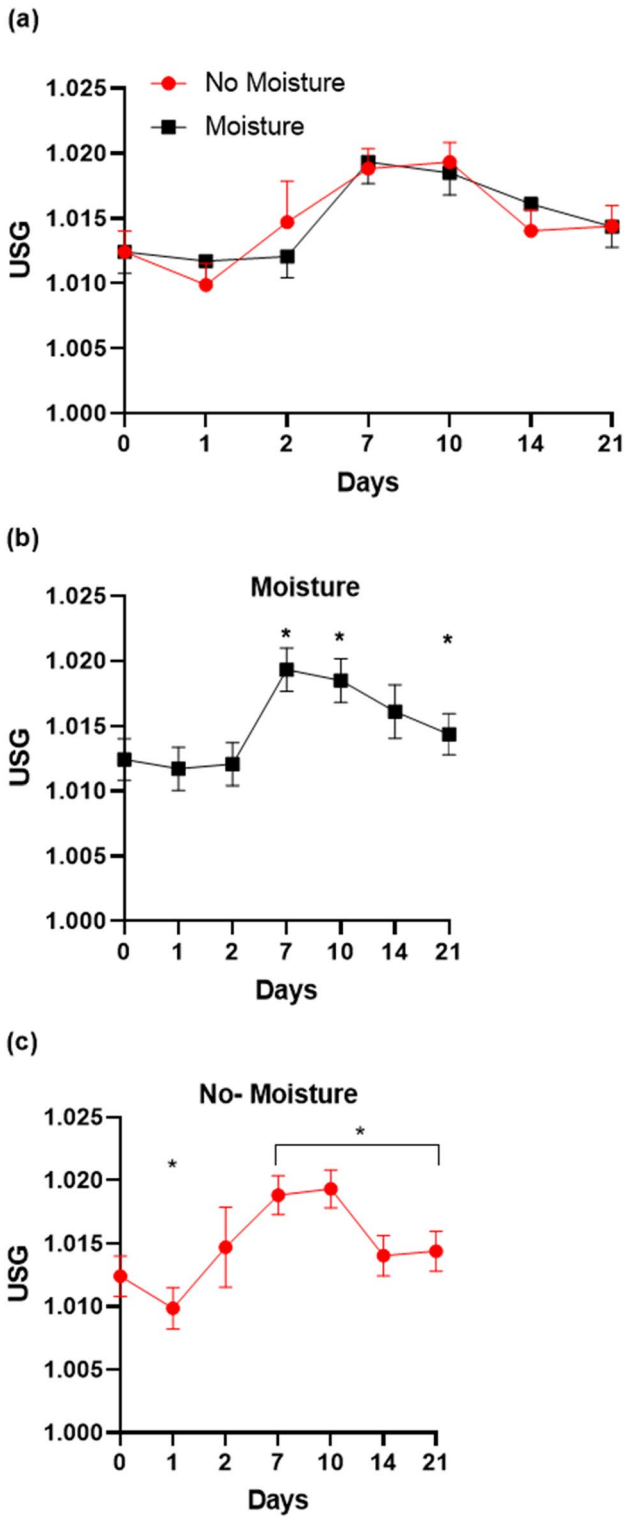

Fig. 4 Markers of hydration analyses for USG. **a** No significant condition x time effect was observed for USG. **b** A significant time effect was observed for USG in the moisture condition. **c** A significant time effect was observed for USG in the no moisture condition. Values are mean ± S.E. * $P < 0.05$ vs. Day 0 (Baseline)

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Author contributions NCJ, H-YL, and YS: conceived and designed the research. NCJ, MK, and YS: conducted data collection. NCJ, CRA, MSK, RAD, H-YL, and YS: performed sample and data analysis. NCJ, H-YL, and YS: wrote the manuscript. NCJ, CRA, MSK, RAD, H-YL, and YS: reviewed the draft. All authors read and approved the manuscript.

Data availability All data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors did not receive support from any organization for the submitted work. The authors have no relevant financial or non-financial interests to disclose. No conflicts of interest, financial or otherwise, are declared by the authors. Additionally, the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

References

1. Armstrong LE, Maresh CM, Castellani JW et al (1994) Urinary indices of hydration status. Int J Sport Nutr 4:265–279. https://doi.org/10.1123/ijsn.4.3.265
2. Kavouras SA (2002) Assessing hydration status. Curr Opin Clin Nutr Metab Care 5:519–524
3. Armstrong LE (2007) Assessing hydration status: the elusive gold standard. J Am Coll Nutr 26:575S-584S. https://doi.org/10.1080/07315724.2007.10719661
4. Delanghe J, Speeckaert M (2014) Preanalytical requirements of urinalysis. Biochem Med (Zagreb) 24:89–104. https://doi.org/10.11613/BM.2014.011
5. Bezuidenhout K, Rensburg MA, Hudson CL et al (2016) The influence of storage time and temperature on the measurement of serum, plasma and urine osmolality. Ann Clin Biochem 53:452–458. https://doi.org/10.1177/0004563215602028
6. Adams HM, Eberman LE, Yeargin SW et al (2015) Effects of agitation and storage temperature on measurements of hydration status. Asian J Sports Med. https://doi.org/10.5812/asjsm.24041
7. Adams WM, Adams JD, Karras EM, Rysanek E (2019) Validity of temperature, duration, and vessel seal on 24-hour urinary hydration markers. PLOS One 14:e0220724. https://doi.org/10.1371/journal.pone.0220724
8. Adams JD, Kavouras SA, Johnson EC et al (2017) The effect of storing temperature and duration on urinary hydration markers. Int J Sport Nutr Exerc Metab 27:18–24. https://doi.org/10.1123/ijsnem.2016-0098
9. Chadha V, Alon US, Garg U (2001) Measurement of urinary concentration: a critical appraisal of methodologies. Pediatr Nephrol 16:374–382. https://doi.org/10.1007/s004670000551
10. Ehrig F, Waller S, Misra M, Twardowski ZJ (1999) A case of "green urine." Nephrol Dial Transplant 14:190–192. https://doi.org/10.1093/ndt/14.1.190
11. Belasco R, Edwards T, Munoz AJ et al (2020) The effect of hydration on urine color objectively evaluated in CIE L*a*b* color space. Front Nutr 7:576974. https://doi.org/10.3389/fnut.2020.576974
12. Sayl KN, Muhammad NS, Yaseen ZM, El-shafie A (2016) Estimation the physical variables of rainwater harvesting system using integrated GIS-based remote sensing approach.

Water Resour Manag 30:3299–3313. https://doi.org/10.1007/s11269-016-1350-6
13. OsmoPRO Multi-Sample Micro-Osmometer User's Guide. Advanced Instruments, Norwood, MA

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