Received: 17 October 2026 | Revised: 4 November 2026 | Accepted: 5 November 2026

DOI: 10.14814/phy2.71215

The Physiological Society | american physiological society | PHYSIOLOGICAL REPORTS

LETTER TO THE EDITOR

Response to Chana et al.: Methodological considerations in the assessment of the MX3 hydration testing system

We thank Chana et al. for their interest in our recently published study evaluating the MX3 Hydration Testing System (HTS) in adults at rest and following exercise in the heat (Zhao et al., 2026). We appreciate the opportunity to clarify several methodological considerations and the interpretation of our findings. While we acknowledge certain limitations raised in their correspondence, we believe that several criticisms warrant further clarification in the context of our study objectives, experimental design, and previously reported findings. We address each concern below.

SELECTION OF REFERENCE MEASURES

We agree that urine specific gravity (USG) has limitations as an indicator of hydration status, particularly when assessed using spot urine samples that may be influenced by recent fluid intake. However, our study was designed to evaluate the practical performance of the MX3 HTS relative to commonly used, non-invasive hydration indicators. Specifically, spot USG was used to classify pre-exercise hydration status, whereas exercise-induced body mass loss (BML) was used to assess post-exercise hypohydration. Notably, spot USG has also been used as a comparator for MX3-derived saliva measurements in previous investigations, including studies involving authors of the present correspondence (Atjo et al., 2022; Faidah et al., 2021) and an independent investigation (Pettersson et al., 2026). As acknowledged in our original article, no single measure can fully characterize hydration status, consistent with the recommendations of Francisco et al. (2026), which emphasize the inherent limitations of single hydration indicators, including plasma osmolality. Accordingly, our findings should be interpreted as reflecting the classification performance of the MX3 HTS relative to the selected field-based indicators rather than establishing definitive diagnostic validity.

SALIVA SAMPLING PROCEDURES AND LOW OSMOLALITY VALUES

Saliva sampling procedures can influence osmolality measurements, and standardized collection techniques are essential. However, the assertion that saliva osmolality values below 25 mOsm/kg are physiologically implausible warrants further consideration. Sawinski et al. (1966) reported an osmolality range of 16–88 mOsm/L in 92 fresh, unstimulated saliva samples from healthy young adult males, while Mentes et al. (2019) documented substantial variability in saliva osmolality among community-dwelling older adults, with values extending from approximately 20–300 mOsm/kg based on their reported distribution. Although differences in measurement units, analytical methods, and sampling conditions should be considered, these findings indicate that low saliva osmolality values are not necessarily physiologically implausible. We acknowledge that contamination, air bubbles, or insufficient saliva may affect MX3 measurements; however, low readings alone cannot establish that these factors occurred in our study. Without direct evidence, attributing these observations specifically to sampling errors would be premature.

TEST–RETEST RELIABILITY AND BIOLOGICAL VARIABILITY

Biological variability may contribute to differences in saliva osmolality measurements obtained across separate days, as acknowledged in our original article (Zhao et al., 2026). However, analytical repeatability and between-day reproducibility represent distinct measurement properties. Winter et al. (2024) reported an intraclass correlation coefficient (ICC) of 0.75 for independently collected saliva samples obtained 3–5 min apart, whereas Pettersson et al. (2026) and Winkler et al. (2026) reported ICCs of 0.93 and 0.991, respectively,

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2026 The Author(s). *Physiological Reports* published by Wiley Periodicals LLC on behalf of The Physiological Society and the American Physiological Society.

for repeated measurements of the same collected saliva samples. These findings are not directly comparable with those of our study, which evaluated measurements obtained on separate days under matched experimental conditions. Although physiological variability may have contributed to our findings, between-day reproducibility remains relevant to the intended application of the MX3 HTS for repeated hydration assessments in field settings. Accordingly, our results reflect the combined effects of biological, sampling, and analytical variability rather than instrument precision alone.

LIMITATIONS OF DEHYDRATION ANALYSIS

We acknowledge that the small number of observations meeting the hypohydration criteria limited the precision and generalizability of our classification estimates, as discussed in our original article (Zhao et al., 2026). Of the 40 experimental trials, six met the USG criterion of >1.020, and five met the criterion of ≥2% exercise-induced BML. Accordingly, the sensitivity and specificity estimates should be interpreted cautiously. These exploratory analyses were intended to evaluate MX3 classification performance under the study conditions rather than establish definitive diagnostic thresholds. Pettersson et al. (2026) also reported limited discrimination of MX3-derived saliva osmolality relative to USG in an independent investigation involving 230 paired saliva–urine samples from 118 elite athletes (area under the receiver operating characteristic curve = 0.40–0.49). Although differences in study populations and protocols preclude direct comparisons, their findings provide additional evidence of limited classification performance relative to USG. We agree that larger studies encompassing a broader range of hydration states and appropriate reference measures are needed to obtain more precise estimates of diagnostic performance.

CONCLUSION

We acknowledge that the selection of hydration reference measures, saliva sampling procedures, between-day biological variability, and the limited number of observations meeting the hypohydration criteria warrant consideration when interpreting our findings. Evidence from recent investigations highlights the importance of distinguishing measurement repeatability, analytical agreement, and hydration classification performance when evaluating the MX3 HTS (Pettersson et al., 2026; Winkler et al., 2026). Our findings provide preliminary evidence regarding the performance of MX3-derived saliva osmolality measurements at rest and following exercise in the heat, within the methodological constraints of the study. Further independent investigations incorporating standardized sampling procedures, appropriate reference measures, and a broader range of hydration states are warranted to establish the reliability and validity of this technology across applied settings.

AUTHOR CONTRIBUTIONS

B.P.M.: Conceptualization, supervision, writing—review and editing; X.Z.: Conceptualization, writing—original draft, writing—review and editing; Z.J.M.: Conceptualization, writing—review and editing; R.I.P., S.C.W., and B.M.A.: Writing—review and editing. All authors have read and approved the final version of the manuscript.

FUNDING INFORMATION

The authors have nothing to report.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICS STATEMENT

The authors have nothing to report.

Xiujing Zhao[1,2,3]
Zachary J. McKenna[2,3]
Rosie I. Perez[1,2,3]
Shawn C. Wierick[1,2,3]
Becca M. Allen[3]
Brendon P. McDermott[1,2,3]

[1]*Heat & Hydration Optimization (H2O) Lab, University of Arkansas, Fayetteville, Arkansas, USA*
[2]*Exercise Science Research Center, University of Arkansas, Fayetteville, Arkansas, USA*
[3]*Department of Health, Human Performance & Recreation, University of Arkansas, Fayetteville, Arkansas, USA*

Correspondence
Brendon P. McDermott, Heat & Hydration Optimization (H2O) Lab, University of Arkansas, Fayetteville, Arkansas, USA.
Email: brendonm@uark.edu

ORCID

Xiujing Zhao https://orcid.org/0009-0001-3064-8460
Zachary J. McKenna https://orcid.org/0000-0003-2502-8870
Brendon P. McDermott https://orcid.org/0000-0001-7238-8145

REFERENCES

Atjo, N. M., Soraya, G. V., Natzir, R., Kasyim, H., Rasyid, H., Chana, G., Erlichster, M., Skafidas, E., & Hardjo, M. (2022). Point-of-care saliva Osmolarity testing for the screening of hydration in older adults with hypertension. *Journal of the American Medical Directors Association*, *23*(12), 1984.e9–1984.e14. https://doi.org/10.1016/j.jamda.2022.08.015

Faidah, N., Soraya, G. V., Erlichster, M., Natzir, R., Chana, G., Skafidas, E., Hardjo, M., Ganda, I. J., & Bahar, B. (2021). Detection of voluntary dehydration in paediatric populations using non-invasive point-of-care saliva and urine testing. *Journal of Paediatrics and Child Health*, *57*(6), 813–818. https://doi.org/10.1111/jpc.15325

Francisco, R., Armstrong, L. E., & Silva, A. M. (2026). Recommendations for optimizing research regarding the effects of dehydration on athletic performance. *Sports Medicine*, *56*(1), 23–34. https://doi.org/10.1007/s40279-025-02310-6

Mentes, J. C., DeVost, M. A., & Nandy, K. (2019). Salivary osmolality, function, and hydration habits in community-dwelling older adults. *SAGE Open Nursing*, *5*, 2377960819826253. https://doi.org/10.1177/2377960819826253

Pettersson, S., Tamm, L., Mattsson, S., & Kalén, A. (2026). Salivary osmolality measured by MX3 hydration testing system demonstrates high reliability but limited validity in elite athlete hydration assessment. *PLoS One*, *21*(6), e0349955. https://doi.org/10.1371/journal.pone.0349955

Sawinski, V. J., Goldberg, A. F., & Loiselle, R. J. (1966). Osmolality of Normal human saliva at body temperature. *Clinical Chemistry*, *12*(8), 513–514. https://doi.org/10.1093/clinchem/12.8.513

Winkler, A. M., Zaplatosch, M. E., Smith, C. J., Adams, W. M., Willis, C. F., Bovine, S., McClure, M. R., Santos, G., Chmunevich, Y., Mitchell, S. J., O'Grady, E., & Kinnaird, W. (2026). Validation of the portable MX3 hydration testing system for measuring saliva osmolality. *Frontiers in Nutrition*, *13*, 1882490. https://doi.org/10.3389/fnut.2026.1882490

Winter, I., Burdin, J., & Wilson, P. B. (2024). Reliability and minimal detectable change of the MX3 hydration testing system. *PLoS One*, *19*(11), e0313320. https://doi.org/10.1371/journal.pone.0313320

Zhao, X., McKenna, Z. J., Perez, R. I., Wierick, S. C., Allen, B. M., & McDermott, B. P. (2026). Assessment of the MX3 hydration testing system in adults at rest and following exercise in the heat. *Physiological Reports*, *14*(6), e70757. https://doi.org/10.14814/phy2.70757