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Urine color expressed in CIE L*a*b* colorspace during rapid changes in hydration status

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A R T I C L E I N F O

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A B S T R A C T

Background: To investigate how rapid changes in hydration affect urine color expressed in CIE L*a*b* colorspace.
Methods: This study was a two-day crossover design where subjects (N = 30) came in one visit dehydrated, after a 15 h overnight fluid deprivation, and rapidly rehydrated by drinking at least 1000 mL of water in 2 h. On the other visit subjects reported euhydrated and then rapidly dehydrated 2% by walking (3 mph) in a heat chamber (100°F, 50% humidity) for 2 h. Urine samples on both days were collected pre- and post-dehydration/rehydration. Urine osmolality, urine specific gravity, subjective urine color and objective urine color expressed in CIE L*a*b* colorspace were measured.
Results: In the dehydration trial participants experienced a significant weight loss of approximately 2% of their starting, euhydrated body weight. The CIE urine color L*-value significantly decreased (−2.3 units) while the b*-value significantly increased (16 units). Subjective urine color significantly increased (1 unit). Urine osmolality increased (25 mmol/kg) and urine specific gravity increased (0.002 g/mL) between the pre- and post-dehydration conditions, however, neither of these changes were statistically significant. In the rehydration trial participants had a significant 1.5% increase in body weight after the ingestion of water. Significant increases were observed in the CIE urine color L*-value (7 units) and a*-value (1.1 units), while the b*-value significantly decreased (−24 units). Subjective urine color significantly decreased (−3 units). Urine osmolality (−600 mmol/kg) and urine specific gravity (−0.018 g/mL) significantly decreased between the pre- and post-rehydration conditions.
Conclusions: Traditional markers of hydration, including urine osmolality and urine specific gravity, did not significantly change in the acute dehydration trial, suggesting that these values may not be responsive to rapid changes in hydration status. However, the CIE L*- and b*-values of urine color significantly decreased in the rapid dehydration trial and significantly increased in the rapid rehydration trial. Thus, the results of the current study suggest that urine color L*- and b*-values expressed in the CIE L*a*b* colorspace were more responsive to changes in hydration status during rapid dehydration than traditional indices of urine concentration and thus may be better markers under such conditions.

1. Background

Traditionally, hydration status has been measured via urine osmolality (U_{OSM}) and urine specific gravity (USG). In addition, subjective urine color (U_{col}) has also emerged as a marker of hydration status (Armstrong et al., 1994; Kavouras, 2002). More recently, a new objective measure of urine color using spectrophotometry has been examined for its ability to predict hydration status (Belasco et al., 2020; Edwards et al., 2020; Zhang et al., 2017).

A substantial amount of hydration research has used U_{OSM} and USG (Armstrong et al., 2013; Oppliger et al., 2005), but few of these studies have looked at how these measures respond to rapid changes in hydration status over time.

Until recently, urine color has traditionally been measured subjectively using a color match scale. Specifically, Armstrong et al. (1994) created a U_{col} chart in which the sample is subjectively matched to one of eight colors that corresponds to hydration status (Armstrong et al., 1994). Recently an objective method has been used to analyze color

Abbreviations: USG, Urine Specific Gravity; U_{OSM}, Urine Osmolality; U_{col}, Subjective Urine Color.

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using CIE L*a*b* colorspace (Belasco et al., 2020; Zhang et al., 2017). Both color analyzing methods have shown strong correlations ($r > 0.7$) to USG and U_{OSM} (Belasco et al., 2020; Edwards et al., 2020; Zhang et al., 2017; Armstrong et al., 2012; Kavouras et al., 2015; McKenzie et al., 2015).

With the use of the CIE L*a*b* colorspace, Zhang et al. (2017) found a strong correlation between U_{OSM} and the b*-value ($r = 0.86$, $p < 0.0001$). They found that the b*-value showed good sensitivity (97.4%) and specificity (65.6%) for assessing those who were considered dehydrated based on their U_{OSM} (Zhang et al., 2017). These findings have been supported by the recent results of Belasco et al. (2020) who reported a significant 0.71 correlation between the b*-value and urine osmolality during various hydration states. Both of these studies examined the ability of objective urine color to assess hydration in comparison to U_{OSM} and USG, however they did not systematically control hydration status as they only took random spot urine samples throughout the day (Belasco et al., 2020; Zhang et al., 2017).

Many hydration studies have been done in the past using a variety of different methods, but few (Armstrong et al., 1998) have observed the ability of these measurements to capture rapid changes in hydration status. The purpose of the current study was to investigate how rapid dehydration and rehydration affect the different methods of hydration assessment including objective urine color expressed in the CIE L*a*b* colorspace.

2. Materials and Methods

The subjects for this study were 30 healthy volunteers (15 males, 15 females) with a mean ± SD age of 26.4 ± 7.8 years. All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the San Diego State University IRB. Prior to data collection all subjects read and signed an informed consent approved by the San Diego State University IRB (#HS-2018-0146). This study was a two-day crossover design where the participants reported to the lab under two different hydration conditions. The order of the two trials was randomized. At the start and end of each trial the subjects provided a urine sample and were weighed semi-nude to the nearest 0.1 kg.

2.1. Dehydration trial

Subjects reported to the lab euhydrated which was confirmed via U_{OSM} (<600 mmol/kg). After initial measurements were taken subjects then walked on a treadmill at 3 mph for 2-h in a heat chamber set at 100°F and 50% humidity. Subjects were not allowed to ingest water during the trial. All subjects were able to complete the 2-hr dehydration trial in the heat.

2.2. Rehydration trial

Subjects reported to the lab dehydrated after a 15-h period of overnight fluid deprivation. They were also required to not exercise during the fluid deprivation period. The initial measurements were taken and then subjects were instructed to drink as much water as they desired, with a minimum requirement of consuming 1000 mL, over the next 2-h. At the end of the 2-h rehydration period the final measurements were taken.

2.3. Urine samples

The pre- and post-urine samples for each trial were used to measure urine osmolality (U_{OSM}) with a Wescor (Logan, UT) model 5500 vapor pressure osmometer and urine specific gravity (USG) with a clinical refractometer. All urine samples were maximal void attempts with the subjects emptying their bladder completely as possible. Subjective urine color (U_{col}) was also determined using the Armstrong 8-point scale by the same investigator under standardize fluorescent light which had an intensity of 1200 lux (Wardenaar et al., 2022). The urine samples were also measured on a HunterLab Vista spectrophotometer and the urine color quantified in CIE L*a*b* colorspace. The L*-value measures the lightness of the sample, '0' indicating the darkest and '100' indicating the lightest a sample could be. The a*-value measures how much red versus green is in the sample, positive values indicate more red and negative values indicate more green. The b*-value measures how much yellow versus blue is in the sample, the positive values indicate more yellow and negative values indicate more blue. On both the a*- and b*-value axes a value of '0' indicates an absence of both colors represented on that specific axis. A 3D graphic depiction of the CIE L*a*b* colorspace is shown in Fig. 1.

2.4. Data analysis

Relationships between pre- and post-data were determined using paired t-tests and multiple regression using an online statistical program (www.vassarstats.net). Data sets were tested for normal distribution using Kolmogorov-Smirnov D tests. Significance was set at a $p < 0.05$ level.

3. Results

The mean ± SD anthropometric characteristics of the subjects are shown in Table 1. There was no significant difference in the responses seen between the men and the women during either the dehydration or rehydration trial, thus the data was pooled.

3.1. Dehydration trial

Table 2 presents the mean pre-to post-measures during the dehydration trial. Participants experienced a significant weight loss of approximately 2% (range = 1.3–3.0%) of their starting body weight. The L*-value significantly decreased from 96.39 ± 4.23 to 94.01 ± 7.57 (−2.38 units) while the b*-value significantly increased from 14.21 ± 9.70 to 20.01 ± 12.42 (16 units, see Fig. 2). Subjective U_{col} significantly increased from 2.83 ± 1.23 to 3.73 ± 1.57 (1 unit). U_{OSM} increased from 413 ± 274 to 439 ± 277 (25 mmol/kg) and USG increased from 1.012 ± 0.008 to 1.014 ± 0.009 (0.002 g/mL) between the pre- and post-dehydration samples, however neither of these changes were statistically significant.

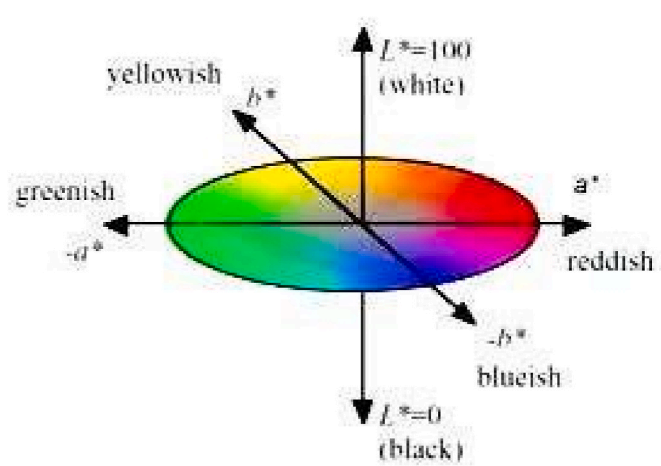

Fig. 1. 3D depiction of CIE L*a*b* color space. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Participants overall characteristics. N = 30.

Characteristic	Mean ± SD
Age (year)	26.4 ± 7.8
Height (cm)	171.7 ± 7.9
Weight (kg)	69.5 ± 9.0
BMI (kg/m^2)	23.3 ± 3.3

Table 2
Mean ± SD of all variables, measured pre- and post-dehydration.

	Pre-Dehydration Trial	Post-Dehydration Trial
Body weight (kg)	69.94 ± 9.06	68.56 ± 9.07*
Subjective Urine Color (units)	2.83 ± 1.23	3.73 ± 1.57*
L*-value (units)	96.39 ± 4.23	94.01 ± 7.57*
a*-value (units)	−1.98 ± 1.27	−2.22 ± 1.36
b*-value (units)	14.21 ± 9.70	20.01 ± 12.42*
Urine Specific Gravity (g/mL)	1.012 ± 0.008	1.014 ± 0.009
Urine Osmolality (mmol/kg)	413 ± 274	439 ± 277

*Indicates significant ($p < 0.05$) difference between the pre- and post-measures.

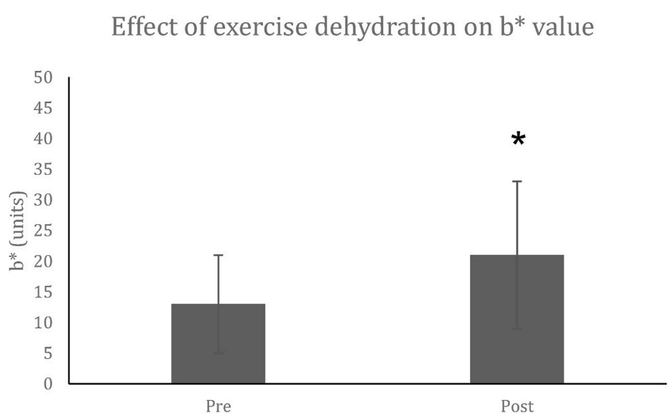

Fig. 2. Mean ± SD b* value pre- and post-rehydration. *$p < 0.05$.

3.2. Rehydration trial

Table 3 presents the mean pre-to post-measures during the rehydration trial. Participants had a significant 1.5% (range = 0.7–3.5%) increase in body weight from 69.17 ± 9.08 to 70.27 ± 9.06 kg after the ingestion of water. The L*-value significantly increased from 91.68 ± 4.85 to 98.52 ± 1.03 (7 units) and the a*-value significantly increased from −2.27 ± 1.37 to −1.18 ± 0.78 (1.1 units), while the b*-value significantly decreased from 30.55 ± 9.49 to 6.54 ± 4.80 (−24 units, see Fig. 3). Subjective U_{col} significantly decreased from 5.10 ± 0.995 to 1.90 ± 0.61 (−3 units). U_{OSM} significantly decreased from 818 ± 162 to 201 ± 106 (−617 mmol/kg) and USG significantly decreased from 1.024 ± 0.004 to 1.005 ± 0.003 (−0.019g/mL) between the pre- and post-rehydration samples.

Table 3
Mean ± SD of all variables, measured pre- and post-rehydration.

	Pre-Rehydration Trial	Post-Rehydration Trial
Body weight (kg)	69.17 ± 9.08	70.27 ± 9.06*
Subjective Urine Color (units)	5.10 ± 0.995	1.90 ± 0.61*
L*-value (units)	91.68 ± 4.85	98.52 ± 1.03*
a*-value (units)	−2.27 ± 1.37	−1.18 ± 0.78*
b*-value (units)	30.55 ± 9.49	6.54 ± 4.80*
Urine Specific Gravity (g/mL)	1.024 ± 0.004	1.005 ± 0.003*
Urine Osmolality (mmol/kg)	818 ± 162	201 ± 106*

*Indicates significant ($p < 0.05$) difference between the pre- and post-measures.

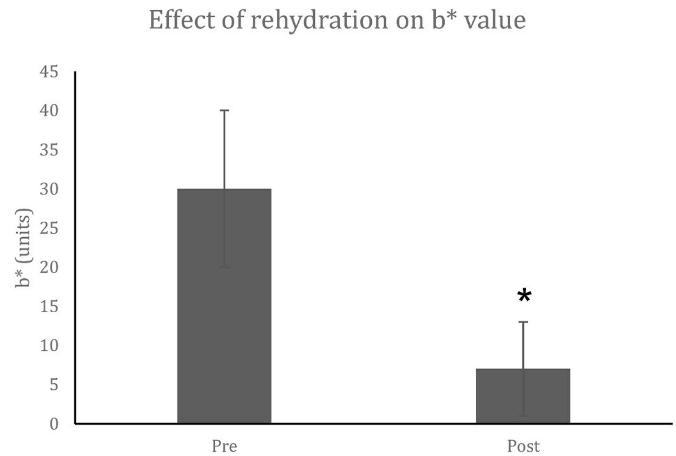

Fig. 3. Mean ± SD b* value pre- and post-dehydration. *$p < 0.05$.

3.3. Statistical analysis

During the individual dehydration and rehydration trials the b*-value was significantly correlated with U_{OSM} (r = 0.828 and 0.861, respectively). When pooling the data across both trials the b*-value likewise had a strong correlation with U_{OSM} (r = 0.846). To further understand the relationship between the CIE L*a*b* variables and U_{OSM} standardized beta coefficient values were calculated. The significant predictors of U_{OSM} were found to be the b*-value (Beta = 0.7204, $p < 0.01$) and the a*-value (Beta = −0.01889, $p < 0.01$). The L*-value was not shown to be a significant predictor of U_{OSM} (Beta = −0.0701, $p < 0.37$). The beta coefficients determined that the b*-value was the most significant predictor, followed by the a*-value, and then the L*-value. A hierarchy multiple regression model was performed to determine the CIE L*a*b* variables ability to predict U_{OSM}. This model was determined to be significant in its prediction of U_{OSM} ($F_{(3,116)} = 107.68$, $p < 0.0001$, adjusted $R^2 = 0.729$). The adjusted R^2 shows that more than 72% of the variance in U_{OSM} is because of the CIE L*a*b* predictor variables.

Likewise, during the individual dehydration and rehydration trials the b*-value was significantly correlated with USP (r = 0.874 and 0.545, respectively). When pooling the data across both trials the b*-value also had a strong correlation with USG (r = - 0.897). To further understand the relationship between the CIE L*a*b* variables and USG, standardized beta coefficient values were calculated. Significant predictors of USG were found to be the L*-value (Beta = −0.1511, $p < 0.02$), the a*-value (Beta = −0.1782, $p < 0.003$), and the b*-value (Beta = 0.7284, $p < 0.01$). The beta coefficients determined that the b*-value was the most significant predictor, followed by the a*-value, and then the L*-value. A hierarchy multiple regression model was performed to determine the CIE L*a*b* variables ability to predict USG. This model was determined to be significant in its prediction of USG ($F_{(3,\ 116)} = 110.19$, $p < 0.001$, adjusted $R^2 = 0.816$). The adjusted R^2 shows that more than 81% of the variance in USG is because of the CIE L*a*b* predictor variables.

4. Discussion

In the dehydration trial participants lost on average 2% of their body weight after walking in the heat chamber for 2-h without ingesting any fluids. Whereas in the rehydration trial participants gained approximately 1.5% of their body weight after consuming water for 2-hr. It was hypothesized that all of the hydration indices would significantly respond in the appropriate direction during each trial. However, while it was observed in the rapid rehydration trial (Table 3) that all variables responded as expected, the same could not be said for the rapid dehydration trial (Table 2). In that trial, subjective and objective urine color indices responded as expected, but Uosm and USG were slow to respond, suggesting that urine concentration indices do not reflect rapid changes in hydration status. Thus, the results of the current study illustrate that the b*-value of CIE L*a*b* colorspace is not only a good indicator of hydration, but it is also better able to respond to rapid changes in hydration than other established measurements.

During the dehydration trial U_{OSM} and USG both experienced changes in the expected positive direction, however, these changes were of a much smaller magnitude than expected and thus did not reach significance. Specifically, mean U_{OSM} at the start of the dehydration trial was 413 $\pm$ 274 mmol/kg and only increased to 439 $\pm$ 277 mmol/kg (Table 2) following rapid dehydration. With such a rapid and large change in hydration status, as evidenced by the significant change in body weight, to have such a small, and nonsignificant, change in U_{OSM} was unexpected. Similarly, USG had a small change with dehydration as the mean was 1.012 $\pm$ 0.008 g/mL at the start of the trial and by the end only increased to 1.014 $\pm$ 0.009 g/mL ($p > 0.05$). Based on such data it appears that during rapid dehydration following exercise in the heat, U_{OSM} and USG are not able to accurately capture the change in hydration status. Such a conclusion agrees with the past findings of Popowski et al. (2001) who reported that U_{OSM} and USG were slow to respond during acute dehydration. CIE L*a*b* colorspace values were more responsive to changes in hydration status during rapid dehydration and thus may be better markers under such conditions.

Other measurements during the rapid dehydration trial had significant responses as was expected. The subjective U_{col} and the b*-value both significantly increased while the L*-value significantly decreased (Table 2). These measures, which had faster reactions than U_{OSM} and USG to the rapid dehydration condition, suggest that various indices of urine color may be a better indicators of hydration status during rapidly changing conditions.

During the rehydration trial U_{OSM} and USG significantly decreased between pre- and post-trial measurements. Participants initially had a mean U_{OSM} of 818 $\pm$ 162 mmol/kg and after ingesting 1000 mL or more of water it significantly decreased to 201 $\pm$ 106 mmol/kg (Table 3). The mean USG was 1.024 $\pm$ 0.004 g/mL which also significantly decreased to 1.005 $\pm$ 0.003 g/mL (Table 3). Both of these changes illustrate that during rapid fluid intake these measurements of urine concentration are able to capture the fluctuations in hydration status. U_{col} and all variables of CIE L*a*b* colorspace had significant responses to rehydration as well (Table 3). Thus, it appears that during rapid rehydration most measurements of hydration are able to respond to changes in hydration status. However, the above points need to be tempered by the fact that it remains questionable as to how much whole-body hydration is actually improved during rapid (i.e., 2-h) oral rehydration with water (Armstrong et al., 1998).

Dehydration and rehydration would appear to effect urine color in a myriad of ways that are not completely understood. However, renal water handling clearly plays a significant role by diluting or concentrating urobilin in the resulting urine (Foot and Fraser, 2006). Dehydration, either from acute exercise or water deprivation, leads to profound urine concentration due to a significant increase in plasma arginine vasopressin from the posterior pituitary as a result of increases in plasma osmolality. The increased arginine vasopressin, after binding to V2 receptors in the basolateral membrane, significantly increases facilitated water reabsorption from the collecting duct, thus decreasing urine production and increasing urine concentration and color. Conversely, rehydration can rapidly decrease plasma arginine vasopressin leading to a profound diuresis with highly diluted (i.e., colorless) urine (Foot and Fraser, 2006; Stachenfeld et al., 1996).

Limitations of the current study include that medications and nutrition were not controlled which can affect both urine color and hydration (Ellis et al., 2016; Wardenaar et al., 2012). The study population was relatively young and healthy, due to the IRB requirements to participate in prolonged exercise in the heat, thus these findings require replication with a broader population. Also, during the acute dehydration trial, the subjects were not allowed to consume any fluid during the 2-h of exercise. This is not the typical situation during real life dehydration as usually some degree of voluntary rehydration occurs, thus attenuating the loss of body fluids. Lastly, only 30 subjects participated in the study and therefore a larger sample size would be warranted in future trials.

4.1. Conclusion

In conclusion, all of the different measures of hydration measured in the current study were able to capture the changes during rapid rehydration. However, U_{OSM} and USG, were inadequate in capturing the changes in hydration status during rapid dehydration. Interestingly, both subjective and objective measures of urine color were more responsive to the rapid changes during rapid dehydration. The findings of this study suggest that the CIE L*a*b* colorspace was more responsive to changes in hydration status during rapid dehydration than traditional indices and thus may be better markers under such conditions.

Funding

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Ethics approval and consent to participate

All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the San Diego State University IRB. Prior to data collection all subjects read and signed an informed consent approved by the San Diego State University IRB.

Clinical Trial Registration: NCT05174793.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the Mendeley Data repository, at https://data.mendeley.com/datasets/fkbjjf95ws/1.

Authors' contributions

M.B. and T.E. designed the protocol, collected, and analyzed the data, and were major contributors in writing the manuscript. Both authors read and reviewed the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Armstrong, L.E., Maresh, C., Castellani, J., Bergeron, M., Kenefick, R., LaGasse, K., Riehi, D., 1994. Urinary indices of hydration status. Int. J. Sport Nutr. 4 (3), 265–279. https://doi.org/10.1123/ijsn.4.3.265.

Armstrong, L., Soto, J., Hacker, F., Casa, D., Kavouras, S., Maresh, C., 1998. Urinary indices during dehydration, exercise, and rehydration. Int. J. Sport Nutr. 8, 345–355. https://doi.org/10.1123/ijsn.8.4.345.

Armstrong, L.E., Johnson, E.C., Munoz, C.X., Swokla, B., Bellego, L.L., Jimenez, L., Casa, D.J., Maresh, C.M., 2012. Hydration biomarkers and dietary fluid consumption of women. J. Acad. Nutr. Diet. 112 (7), 1056–1061. https://doi.org/10.1016/j.jand.2012.03.036.

Armstrong, L., Johnson, E., McKenzie, A., Munoz, C., 2013. Interpreting common hydration biomarkers on the basis of solute and water excretion. Eur. J. Clin. Nutr. 67, 249–253. https://doi.org/10.1038/ejcn.2012.214.

Belasco, R., Edwards, T., Munoz, A.J., Rayo, V., Buono, M.J., 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.

Edwards, T., Belasco, R., Munoz, A.J., Rayo, V., Buono, M.J., 2020. Subjective vs. objective urine color: effect of hydration status. Adv. Appl. Physiol. 5 (2), 19–23. https://doi.org/10.11648/j.aap.20200502.12.

Ellis, L., Yates, B., McKenzie, A., Munoz, C., Casa, D., Armstrong, L., 2016. Effect of three oral nutritional supplements on human hydration indices. Int. J. Sport Nutr. Exerc. Metabol. 26, 356–362. https://doi.org/10.1123/ijsnem.2015-0244.

Foot, C., Fraser, 2006. J. Uroscopic rainbow: modern matula medicine. PGM (Postgrad. Med.) 82, 126–129. https://doi.org/10.1136/pgmj.2005.037598.

Kavouras, S.A., 2002. Assessing hydration status. Curr. Opin. Clin. Nutr. Metab. Care 5 (5), 519–524. https://doi.org/10.1097/00075197-200209000-00010.

Kavouras, S.A., Johnson, E.C., Bougatsas, D., Arnaoutis, G., Panagiotakos, D.B., Perrier, E., Klein, A., 2015. Validation of a urine color scale for assessment of urine osmolality in healthy children. Eur. J. Nutr. 55 (3), 907–915. https://doi.org/10.1007/s00394-015-0905-2.

McKenzie, A.L., Munoz, C.X., Armstrong, L.E., 2015. Accuracy of urine color to detect equal to or greater than 2% body mass loss in men. J. Athl. Train. 50, 1306–1309. https://doi.org/10.4085/1062-6050-51.1.03.

Oppliger, R., Magnes, S., Popowski, L., Gisolfi, G., 2005. Accuracy of urine specific gravity and osmolality as indicators of hydration status. Int. J. Sport Nutr. Exerc. Metabol. 15, 236–251. https://doi.org/10.1123/ijsnem.15.3.236.

Popowski, L., Oppliger, R., Lambert, G., Johnson, R., Johnson, A., Gisolfi, C., 2001. Blood and urine measures of hydration status during progressive acute dehydration. Med. Sci. Sports Exerc. 33, 747–753. https://doi.org/10.1097/00005768-200105000-00011.

Stachenfeld, N., Gleim, G., Zabetakis, P., Nicholas, J., 1996. Fluid balance and renal response following dehydrating exercise in well trained men and women. Eur. J. Appl. Physiol. 72, 446–477. https://doi.org/10.1007/BF00242277.

Wardenaar, F., Thompsett, D., Vento, K., Pesek, K., Bacalzo, D., 2012. Athletes' self-assessment of urine color using two color charts to determine urine concentration. Int. J. Environ. Res. Publ. Health 18, 4126. https://doi.org/10.3390/ijerph18084126.

Wardenaar, F., Armistead, S., Boeckman, K., Butterick, B., Youssefi, D., Thompsett, D., Vento, K., 2022. Validity of urine color scoring using different light conditions and scoring techniques to assess urine concentration. J. Athl. Train. 57, 191–198. https://doi.org/10.4085/1062-6050-0389.21.

Zhang, N., Du, S., Zheng, M., Tang, Z., Yan, R., Zhu, Y., Ma, G., 2017. Urine color for assessment of dehydration among college men students in Hebei, China - a cross-sectional study. Asia Pac. J. Clin. Nutr. 26 (5), 788–793. https://doi.org/10.6133/apjcn.052017.09.