Original Article

Urine color for assessment of dehydration among college men students in Hebei, China – a cross-sectional study

Na Zhang MM[1], Songming Du PhD[2], Mengqi Zheng MB[1], Zhenchuang Tang PhD[1], Ruixia Yan MB[3], Yitang Zhu MB[4], Guansheng Ma PhD[1,5]

[1]*National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention, Xi Cheng District, Beijing, China*
[2]*Chinese Nutrition Society, Xi Cheng District, Beijing, China*
[3]*Department of Health Management and Service, Cangzhou Medical College, Higher Education District, Cangzhou, China*
[4]*Clinical laboratory, Cangzhou Central Hospital, Xinhua District, Cangzhou, China*
[5]*Beijing Key Laboratory of Toxicological Research and Risk Assessment for Food Safety, Department of Nutrition and Food Hygiene, School of Public Health, Peking University, Hai Dian District, Beijing, China*

Background and Objectives: To examine the association between quantified urine color and urine osmolality, and its validity in distinguishing hydration status among college men in Hebei, China. **Methods and Study Design:** Sixty-eight college men aged 18~25 years completed a cross-sectional study. All participants were asked to complete a 24-h fluid intake record to estimate fluid intake from beverages after anthropometric measurements. The foods eaten by participants were weighed to assess fluid intake from foods. All urine samples for the day were collected by participants to determine urine osmolality and urine color by chromatogram spectrophotometry (in accord with the Commission Internationale de l'Eclarige (CIE) notation L*a*b*). **Results:** A total 413 urine samples from 68 participants were collected and 151 (36.6%) samples indicated dehydration according to urine osmolality. The dehydrated group versus hydrated group had a smaller urine color L* (94.88 vs 98.06) and a* (-2.39 vs -1.91), bigger b* (30.41 vs 15.15), and higher osmolality (958 mOsm/kg vs 486 mOsm/kg). Urine color and osmolality were closely correlated, especially for b* (0.86, $p<0.0001$). The percentage variance in urine osmolality (R^2) explained by a partial least squares (PLS) model was 79%. Urine color b* contributed most substantially to the PLS model, with variable importance for projection of 1.35. The cutoff for b* for adequate hydration was 17.78 (area under the curve=0.899). **Conclusions:** Differences in urine color between dehydrated and hydrated status related to urine osmolality. Urine color quantification is a reliable method to assess hydration status among young Chinese men.

Key Words: urine, urine color, urine osmolality, dehydration, fluid intake

INTRODUCTION

Water is the main constituent of human body and is indispensable for human survival. Water plays a variety of important physiological functions, including participating in body metabolism, maintaining electrolyte balance, modulating normal osmotic pressure, regulating body temperature and so on.

Dehydration occurs when fluid intake is insufficient to replace free water output. It has been reported that dehydration has adverse effect on health. Ganio et al (2011) showed that the mild dehydration induced by combination of exercise and diuretics impaired vigilance and working memory, and increased tension/anxiety and fatigue;[1] Armstrong et al (2012) also reported that dehydration in women can increase perception of task difficulty and degraded mood;[2] Montain et al (2012) reported that moderate dehydration reduces muscle endurance.[3] Sawka et al (2012) found that dehydration and high skin temperature impairs aerobic performance.[4] Dai et al (2013) reported that fluid drinking showed a protective effect against kidney stones in men.[5] Strippoli et al (2011) found that higher fluid intakes appeared to protect against chronic kidney diseases.[6] Sorensen et al (2012) showed that the risk of incident kidney stones was decreased with higher water intake.[7] Dmitrieva et al (2014) showed that dehydration and elevated sodium stimulate inflammatory signaling in endothelial cells and promote atherosclerosis.[8] Thus, dehydration has adverse effects on health, which should be addressed.

The biological indicators of dehydrated status include serum osmolality, urine volume, urine osmolality, urine

Corresponding Author: Dr Guansheng Ma, National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention, Xi Cheng District, Beijing, 100050, China.
Tel: +86-10-8280-5266; Fax: +86-10-8280-5266
Email: mags@bjmu.edu.cn
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specific gravity, and urine color. Serum osmolality is suggested as a good marker for acute dehydrated status and urine osmolality is biologically significant for evaluating mild dehydration.[9,10] However, determination of blood and urine biological indicators needs to be done by professional or accredited technical organizations, and the cost is relatively high. Urine volume is also a good indicator for dehydration assessment, however, the collection and measurement of urine volume is relatively troublesome. Dehydrated status may also reflect total fluid intake. But, the 24-h fluid intake record questionnaire and food duplicate portion method, which is used to determine fluid intake from beverages and food, are demanding. Urine color, as a simple indicator, has been suggested for dehydration assessment by Armstrong et al.[11] Mckenzie et al also suggested that urine color was a valid marker of urine concentration and was useful to identify dehydration after exercise in the heat.[12,13] Thus, it would be useful to define the relationship between urine color and urine osmolality and know its accuracy. Most available studies about urine color are qualitative with the classical eight-point urine color chart. Urine color quantification has not been pursued in China.

The objectives of this study are, firstly, to analyze the differences of quantitative urine color between hydration and dehydrated status classified by urine osmolality; secondly, to examine the association between urine color and urine osmolality; thirdly, to observe the discriminant validity of urine color for hydration assessment.

METHODS

Participants

Male participants were recruited from freshman and sophomore years in one college in Cangzhou, Hebei province of China.

Inclusion criteria: aged between 18 and 25 years; being in healthy status.

Exclusion criteria: aged <18 years or >25 years; smoking, habitual high alcohol (>20g/day) consumption or intensive physical exercise, or with the diseases of cognitive disorder, diabetes, gastrointestinal tract disease, oral disease, kidney disease or other chronic diseases and metabolic diseases.

Ethics

The study protocol and instruments were reviewed and approved by the Ethical Review Committee of the Chinese Nutrition Society (ethical approval number: CNS-2015-001) and was conducted according to the guidelines of the Declaration of Helsinki. Prior to the study, all participants read and signed an informed consent form.

Study procedure

On the study day, height, weight, waist circumference, protein and percent body fat mass of participants were measured at 8:00 AM. All participants were asked to complete a self-administrative 24-h fluid intake record questionnaire after training, which was used to evaluate fluid intake from beverages. The foods eaten by participants were weighed to calculate fluid intake from foods. 24-h urine was collected beginning with the second void of the day and ending with the first void of the next following morning. All urine samples during the day were collected by participants to determine each time the urine osmolality and urine color. Temperature and humidity were recorded at 10:00 AM, 2:00 PM and 8:00 PM.

Definition of dehydration

Dehydrated status: urine osmolality >800 mOsm/kg.
Hydrated status: urine osmolality ≤800 mOsm/kg.

Multivariable partial least squares (PLS) model: It was performed to identify key predictors in modeling urine osmolality with urine color.

The three-dimensional CIE L*a*b*: It is in accordance with accepted color perception theory based on the three separate color receptors (red, green and blue) in the eye.[15] The three three-dimensional CIE L*a*b* represent the lightness of the color (L* = 0 indicates darkest black and L* = 100 indicates brightest white), its position between red/magenta and green (a*, negative values indicate green while positive values indicate red) and its position between yellow and blue (b*, negative values indicate blue and positive values indicate yellow). The asterisk (*) are part of the full name.[16]

Anthropometric measurements

Heights and Weights of participants were measured while wearing light clothing and without footwear by trained investigators following standardized procedures. Height was measured to the nearest 0.1 cm and weight was measured to the nearest 0.1 kg with height-weight meter (HDM-300; Huaju; Zhejiang, China).

The waist circumference was measured to the nearest 0.1 cm at the midpoint between the bottom of the rib cage and the top of the iliac crest at the end of exhalation with the participant standing without clothing covering the waist area by trained investigators using a MyoTape waistline measurer.

Protein and percent body fat mass (%) were measured by trained investigators with a body composition analyzer (Inbody 720; Inbody, Seoul, Korea).

BMI = weight (kg) / height squared (m)]

Body surface area (m^2) = weight (kg) 0.425 × height (cm) 0.725 × 0.007184.

Assessment of fluid intake

Daily total drinks were recorded using a 24-h fluid intake record questionnaire. Type, source, place, time, and amount of fluid intake after specified training were noted. The sources of drinking fluid included water, added sugar and other nutritive sweeteners (SSBs), milk and milk products, alcohol, soybean milk, and tea.

All foods consumed by the participants were weighed to calculate fluid intake from foods using the duplicate portion method. Fluid amounts from foods were measured according to the national standard of GB 5009.3-2010, except for fruits which were assessed according to the China Food Composition Tables.

Daily total fluid intake (mL) = Daily total drinking fluid (mL) + Daily fluid intake from foods (mL).

Assessment of urine

Urine was collected at each time separately in disposable flexible packaging plastic bag by the participants. It was

stored at + 4℃ until assessment. Volume was measured to the nearest 0.1 kg with a desktop electronic scale (YP20001; SPC; Shanghai, China).

'Number of void' indicated the urination frequency to that point of the day. Urine osmolality was determined as osmotic pressure with a molar concentration meter (SMC 30C; Tianhe; Tianjin, China) using the freezing point method.

Urine color: Urine color was measured by trained investigators following standardized procedures using chromatogram spectrophotometer (CR-5, Konica Minolta; Japan). Urine color was displayed in accord with the Commission Internationale de l'Eclarige (CIE) method L*a*b*.[14] The urine color distribution map was drawn using Spectra Magic NX software (Konica Minolta Photo Imaging LTD.; HK).

Temperature and humidity of environment

Temperature and humidity were measured using temperature hygrometer (WSB-1-H2, Exasace; Zhengzhou, China).

Statistical analyses

SAS 9.2 (SAS Institute Inc, Cary, NC, USA) was used for statistical analyses. Quantitative parameters for participants were presented as mean±SD, numeration data were presented as n (percentage). Pearson's correlation coefficients were performed to determine the strength of the relationship between urine color and urine osmolality. Partial least square regression (PLS) model was performed between urine osmolality and urine color. A binary variable (0: hydration; 1: dehydration) was constructed based on urine osmolality to indicate hydrated status. Logistic regression of urine color b* against this binary outcome was performed, and a ROC analysis was used to determine the cutoff value of b* for keeping hydrated status without adjustment made to favor either sensitivity or specificity. Significance level was set at 0.05 (p<0.05, 2-tailed).

RESULTS

Characteristics of participants and environment

Some 68 participants were recruited and all completed the study. The characteristics of the participants were shown in Table 1.

The average temperature was of 13.1℃ at room and 6.9℃ outside, while the average humidity was 31% at room and 35% outside.

Hydrated status

Hydrated status: A total of 413 urine samples of 68 participants in the day were collected and detected. 151 urine samples indicated dehydration, which accounted for a proportion of 36.6%. Nearly 17.6% of participants were in dehydrated status with the frequency of equal or more than 75% during 24 hours. Among these, 8 participants (11.8%) were in dehydrated status with the frequency of completely 100% during 24 hours. And 32.4% of participants were in dehydrated status with the frequency of equal or more than 50%. About 66.2% were in dehydrated status with the frequency of equal or more than 25%. Only 12 participants (17.6%) never appeared dehydrated status during 24 hours.

Table 1. Participants characteristics

	Participants (n=68)
Anthropometric measurements	
Age (year)	19.9±1.1
Height (cm)	174.0±5.2
Weight (kg)	67.0±10.8
BMI (kg/m^2)	22.4±3.6
Waist circumference (cm)	79.2±9.0
Body surface area (m^2)	1.8±0.1
Protein (kg)	10.7±1.2
Percent body fat mass (%)	20.1±6.8
Assessment of fluid intake	
Daily total fluid intake (mL)	2569±659
Percent meet water AI in China (%)	18 (28.1)
Daily fluid intake from foods (mL)	1221±272
Daily total drinking fluid (mL)	1349±602
Percent meet water AI in China (%)	24 (37.5)
Assessment of urine	
24-h urine volume (mL)	1402±571
Number of void	6±2

All values were shown as means±SD. Except: Percentages were shown as n (percentage).

The differences of urine color and urine osmolality between dehydrated and hydrated status

Urine color: L* for dehydration was significantly smaller than that of the hydrated group (94.9 vs 98.1); a* for dehydration was significantly smaller than that for the hydrated group (-2.39 vs -1.91); b* for dehydrated status was significantly bigger than that of the hydrated group (30.4 vs 15.2). Urine osmolality with dehydration was significantly more than that of the hydrated group (958 mOsm/kg vs 486 mOsm/kg) (Table 2).

Correlations between urine color and urine osmolality

Strong correlation were found between urine color b* and urine osmolality (r=0.86, p<0.0001). There also existed correlation between L* and urine osmolality (r=-0.56, p<0.0001), a* and urine osmolality (r=-0.35, p<0.0001).

PLS model of the relationship between urine color and urine osmolality

A PLS model of the relationship between urine osmolality and urine color (L*, a* and b*) was developed. The percentage of variance in urine osmolality (R^2) explained by the PLS model was 79% (Figure 1), with a root mean square error of 129 mOsm/kg. In the PLS model, b* were identified as possible key predictors of urine osmolality. Urine color parameters b* contributed most evidently to the PLS model, with a variable importance for projection of 1.35. The variable importance for projection of urine color parameters L* and a* were 0.90 and 0.59, respectively.

Urine color for assessing dehydrated status

The urine color b* for assessing dehydrated status was 17.78 (area under the curve=0.899) with good sensitivity (97.4%) and specificity (65.6%) (Figure 2).

Color distribution map

The color distribution map of 413 urine samples showed

Table 2. Urine color and osmolality between dehydrated and hydrated status

	All urine samples (n=413)	Dehydration (n=151)	Hydration (n=262)	*t*	*p*
Urine color					
L	97.0±3.2	94.9±3.8	98.1±2.1	11.0	<0.0001*
A	-2.1±1.2	-2.4±1.5	-1.9±0.9	4.1	<0.0001*
b	20.7±11.1	30.4±7.8	15.2±8.6	-18.0	<0.0001*
Osmolality (mOsm/kg)	659±283	958±102	486±198	-27.2	<0.0001*

All values were shown as means±SD.
*There was statistical significantly difference between hydration and dehydrated status, $p<0.05$.

relatively good discriminant validity for different hydrated status (Figure 3).

DISCUSSION

The balance between water outputs and water inputs defines hydrated status.[17] There are 3 sources of water: fluid-drinking, water contained in food and metabolic water. Water outputs include: urine from the kidney and urinary system, sweat from the skin surface, breath from the respiratory system, and feces from the digestive system. Being in optimal hydrated status means maintaining water balance.[15] Dehydration occurs when water input is insufficient to replace water output. Adequate hydration is important for health. Urine osmolality provides a criterion for hydration status. Dehydration is generally defined as urine osmolality greater than 800 mOsm/kg.[18] In our study, hydration was monitored throughout the day and it was found that only 12 of the 68 participants (17.6%) never appeared dehydrated.

In many studies, urine color is also used as an indicator for assessing dehydration status. To validate urine color for assessment of dehydration, urine color was given a numerical value in our study. There were differences in urine color between dehydrated and hydrated groups. In addition, it was found that the quantitated urine color strongly correlated with osmolality. The percentage variance in urine osmolality (R^2) explained by the PLS model was 79%. In similar more qualitative studies, Kavouras et al (2015) found that urine color also displayed a positive relationship with urine osmolality (R^2: 0.45, $p<0.001$).[19] Mckenzie et al (2015) have also reported that 24-h urine color is significantly correlated with 24-h urine osmolality (r=0.61-0.84). In another study, Mentes et al (2006) reported significant associations between average urine color and average urine specific gravity in nursing home residents.[20] Yet again, Eberman et al (2009) found a correlation between urine osmolality and urine color (r=0.540).[21] Compared with studies of qualitative urine color, quantification of urine color provides a stronger relationship with urine osmolality. Therefore, quantification of urine color appears to be a reliable method to assess hydration status. However, Kovacs et al (1999) found that urine color was a poor indicator of hydration status for trained healthy men after 6 hours exercise.12 In our study, there was a linear relationship between urine osmolality and urine color with a relatively high R^2, which indicated that the degree of fit with the PLS model was relatively good.

Among the three parameters of urine color, b*contributed most evidently to the PLS model, with a variable importance for projection of 1.35. The differences in color depends on the collaborative changes of the three-dimensional CIE L*a*b*. L* represents lumi nosity; a* represents the scope from red to green; b* represents the scope from yellow to blue. In theory, compared with L* and a*, urine color should be easier to distinguish with b*, which would mean that b* is the more meaningful when urine color is used to assess dehydration status. Our

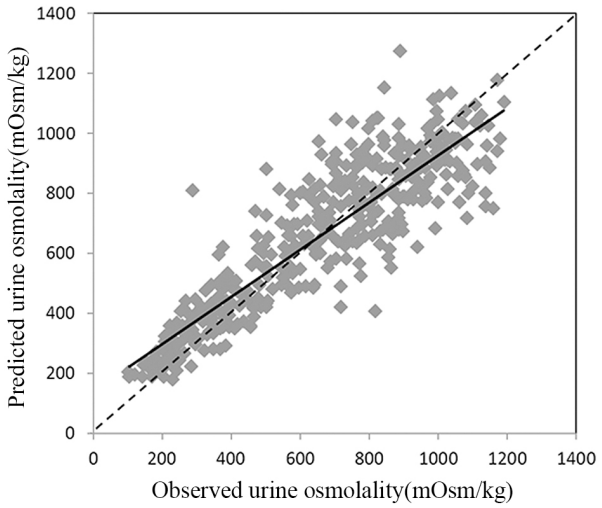

Figure 1. PLS model of the relationship between urine color and urine osmolality. Solid line represents the line of agreement, while dash line represents the line of best agreement.

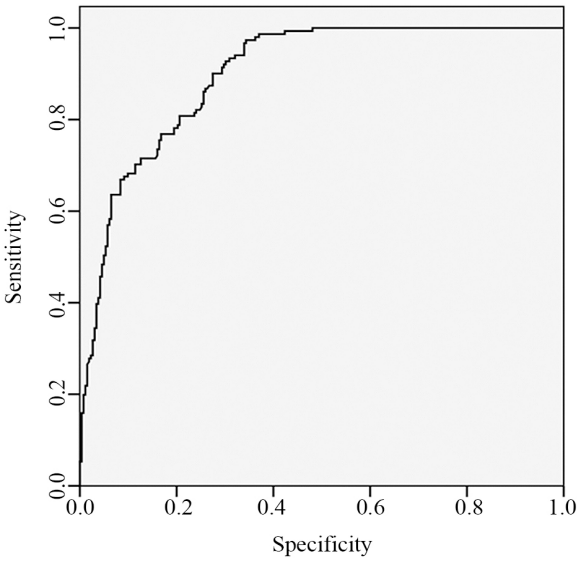

Figure 2. ROC analysis curve of urine color b* for assessing hydrated status.

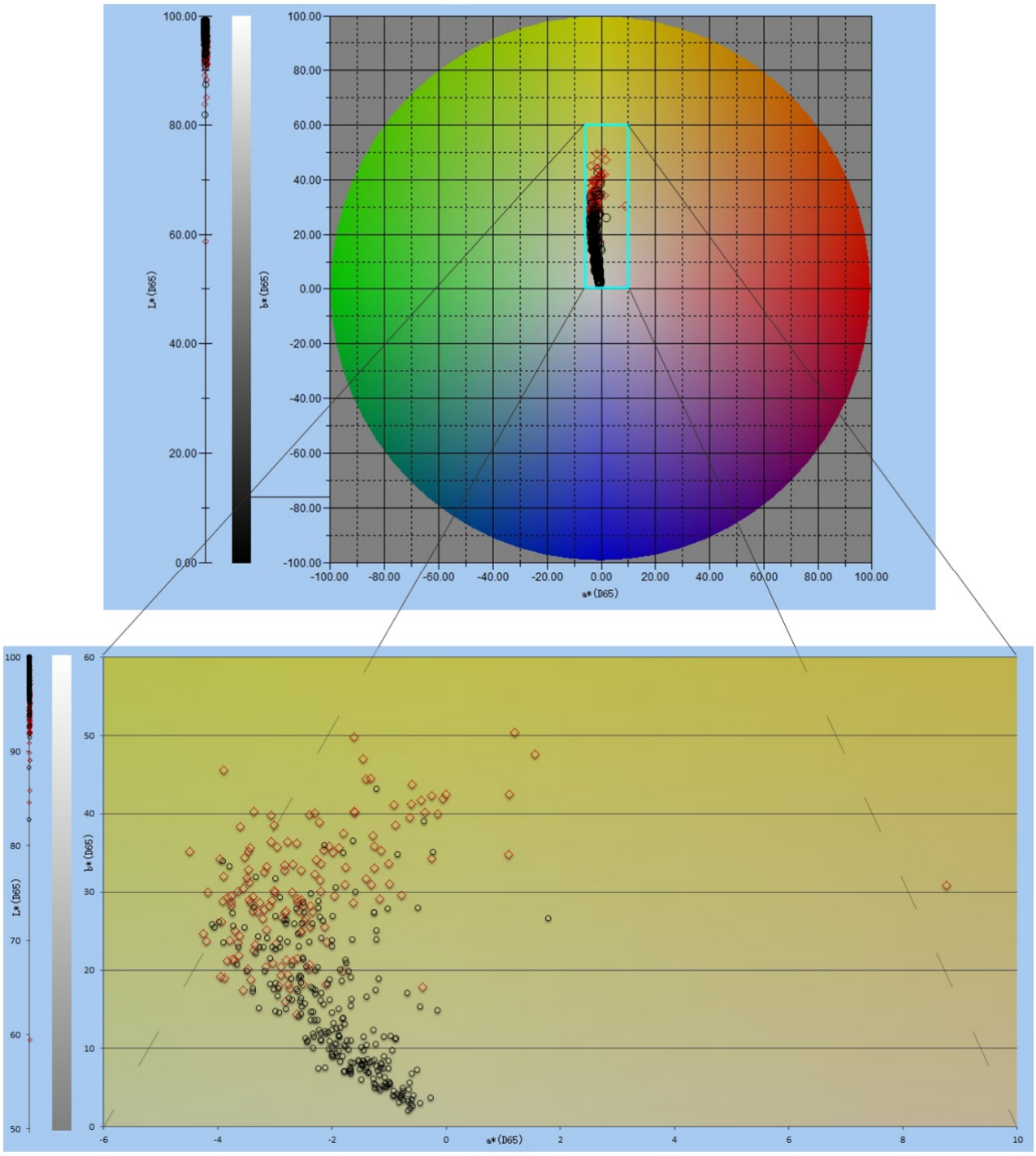

Figure 3. The color distribution map of 413 samples. Red diamonds represent being in dehydrated status, while black circles represent being in hydrated status

study accords with the current color theory.

Furthermore, the urine color b* for assessing dehydrated status was 17.78 (area under the curve=0.899) with good sensitivity (97.4%) and specificity (65.6%). Similarly, Adams et al (2015) found that the overall accuracy of the self-assessment of 1-8 color urine color scale was 65% (area under the curve) based on the ROC analysis.[22] Kavouras et al (2015) found that urine color has good overall classification ability first thing in the morning, before lunch and with 24-h urine sampling (area under the curve 85-92%), with good sensitivity (92-98%) and specificity (55-68%) for detecting dehydrated status.19 Quantification of urine color is also more reliable in assessing hydrated status than is subjective qualitative urine color. To directly observe the ability of urine color for assessing hydrated status, the color distribution map of 413 urine samples was drawn, which showed relatively good discriminant validity for various hydration states.

Our study has some strengths and weaknesses. It provided an opportunity to study the relationship of urine color and urine osmolality, and its accuracy, in regard to dehydration in China. This cross-sectional survey was carried out with in free living conditions, relevant to actual life situations. Participants were monitored throughout the day, taking into account diurnal variation. However, the findings require replication. There may be

differences in gender and age which were not studied. In addition, a larger sample size would have provided greater accuracy and sensitivity. More urine and blood hydration-related biomarkers could be further analyzed in PLS model. It would have been better to have observed hydration status on several and consecutive days.

In conclusion, there were differences in urine color in relation to dehydration and hydration status. Urine color and urine osmolality were related in accordance with hydration status. The urine color parameter b*contributed most convincingly to the PLS model. The color distribution map of urine samples showed a relatively good degree of differentiation for different hydration states. The urine color b* for assessing dehydration status was 17.78. Quantification of urine color appears to be a reliable method to assess hydrated status among Chinese.

AUTHOR DISCLOSURES

The authors declare no conflict of interest.

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