Quantification of chromatographic effects of vitamin B supplementation in urine and implications for hydration assessment


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Keneffick RW, Heavens KR, Dennis WE, Caruso EM, Guerriere KI, Charkoudian N, Cheuvront SN. Quantification of chromatographic effects of vitamin B supplementation in urine and implications for hydration assessment. J Appl Physiol 119: 110–115, 2015. First published May 14, 2015; doi:10.1152/japplphysiol.00068.2015.—Changes in body water elicit reflex adjustments at the kidney, thus maintaining fluid volume homeostasis. These renal adjustments change the concentration and color of urine, variables that can, in turn, be used as biomarkers of hydration status. It has been suggested that vitamin supplementation alters urine color; it is unclear whether any such alteration would confound hydration assessment via colorimetric evaluation. We tested the hypothesis that overnight vitamin B2 and/or B12 supplementation alters urine color as a marker of hydration status. Thirty healthy volunteers were monitored during a 3-day euhydrated baseline, confirmed via first morning nude body mass, urine specific gravity, and urine osmolality. Volunteers then randomly received B2 (n = 10), B12 (n = 10), or B2 + B12 (n = 10) at ~200 × recommended dietary allowance. Euhydration was verified on trial days (two of the following: body mass ± 1.0% of the mean of visits 1–3, urine specific gravity < 1.02, urine osmolality < 700 mmol/kg). Vitamin purity and urinary B2 concentration ([B2]) and [B12] were quantified via ultraperformance liquid chromatography. Two independent observers assessed urine color using an eight-point standardized color chart. Following supplementation, urinary [B2] was elevated; however, urine color was not different between nonsupplemented and supplemented trials. For example, in the B2 trial, urinary [B2] increased from 8.6 × 10^(-5) ± 7.7 × 10^(-5) to 5.7 × 10^(-5) ± 5.3 × 10^(-5) nmol/l (P < 0.05), and urine color went from 4 ± 1 to 5 ± 1 (P > 0.05). Both conditions met the euhydrated color classification. We conclude that a large overnight dose of vitamins B2 and B12 does not confound assessment of euhydrated status via urine color.

Urochrome is a breakdown product of hemoglobin that gives urine its characteristic yellow color. Like other solutions, the concentration (and color) of urine varies inversely with its volume. A net body water deficit can lead to a small volume of darkly colored urine, thus providing a simple qualitative assessment of dehydration (2). The reciprocal is also true. A lightly colored urine is often considered the simplest hallmark of euhydration and can be semiquantitatively assessed using a simple and practical standardized color chart (2). However, many factors can alter the relationship between UCol and hydration status. It is often suggested (anecdotally) that vitamin supplementation makes urine a more yellow color (11), thus potentially making urine appear more concentrated that the simple ratio of urochrome to water would otherwise suggest. There are no objective reports that either support or refute this belief, although it is widely held among professionals in science and medicine. The “B vitamins” are collectively implicated to impact UCol, although riboflavin (vitamin B2) and cobalamin (vitamin B12) are the best candidates (10, 12) because of their bright characteristic yellow and red powder forms, respectively. While the belief that vitamin supplementation can alter UCol is commonly held and experimentally demonstrated to a small extent (10, 12), no investigation has sought to determine whether this alteration would produce darker urine in euhydrated volunteers. Such an effect would decrease the usually high specificity (97%) of UCol for euhydration (7).

Thus the purpose of this study was to determine whether oral supplementation with vitamins B2 and/or B12 would darken UCol and confound the potential specificity of UCol for hydration assessment in healthy young men and women. As the B vitamins are not stored within the body and must be replaced each day, acute supplementation was chosen. We hypothesized that volunteers consuming the B2 and/or B12 supplements would exhibit significantly more yellow urine, consistent with a category of dehydration on a commonly used, standardized UCol chart (2), while other measures of urine concentration would remain unchanged. To our knowledge, this is the first investigation that has used simultaneous measurement of UCol and urinary B vitamin concentration to systematically address this question.

METHODS

Volunteers. Twenty-five men and five women (n = 30; age: 26 ± 7 yr; height: 172 ± 9.2 cm; weight: 77.7 ± 15.4 kg; %body fat: 12.6 ± 5.6%) volunteered to participate in this study. Appropriate institutional review boards approved this study. Before participation, each volunteer attended briefings informing them of the purpose of the experiment and possible risks and signed a written, informed consent.

THE KIDNEY IS THE PHYSIOLOGICAL first line of defense against disturbances to fluid volume homeostasis. Renal filtration, reabsorption, and secretion mechanisms provide complex and comprehensive protection of plasma water and other blood constituents, while allowing for appropriate excretion of waste as urine (1, 17). Because of the close relationship between renal urine excretion and physiological maintenance of normal fluid volume status, urinary markers, including volume, specific gravity, osmolality, and color are often used for assessment of hydration status in clinical, athletic, and military settings. Of these, urine color (∇UCol) is often the most expedient.
document. Investigators adhered to policies for protection of human subjects as prescribed in US Army Medical Research and Materiel Command Regulation 70-25 and adhered to DoD Instruction 3216.02 and 32 CFR 219 on the use of volunteers in research. Study restrictions were limited to abstinence from exercise and the consumption of caffeine or alcohol for 24 h before each visit. The use of dietary supplements (including a multivitamin) and any medication (prescription or over the counter) other than an oral contraceptive was also prohibited. Confirmation of adherence to study restrictions was obtained upon arrival at the laboratory.

Study overview. Volunteers were randomly assigned to three different groups: one group was supplemented with vitamin B2 (n = 10); another supplemented with vitamin B12 (n = 10); and a final group supplemented with both vitamin B2 and B12 (n = 10). Volunteers were required to visit the laboratory at 0630 on five separate occasions over the course of 4 wk. The first three visits to the laboratory served to establish a euhydrated baseline for each volunteer, assessed via nude body mass (Bm), urine specific gravity (USG), and urine osmolality (UOSM). The final two visits served as a euhydrated control trial and vitamin supplementation trial (Fig. 1). All visits to the laboratory were approximately 2–7 days apart. To achieve/maintain a euhydrated state, on the day before each of the five laboratory visits, volunteers were provided 2.0 liters of water to consume in addition to ad libitum fluid consumption and habitual dietary practices. Subjects were instructed to consume the water provided between 1800 and 2200, verified by an independent observer. No food or drink was permitted between 2200 and 0600 the next morning, and the first morning urine sample after 0600 was collected.

Vitamin supplementation. On the day before the vitamin supplementation trial, based on group assignment, subjects were provided a single dose of B2, B12, or both supplements. Subjects were instructed to ingest the provided supplement at 2000. The 10-h time between ingestion of the supplement and collection of the first morning urine sample was considered euhydrated on the days of the control trial and the vitamin supplementation trial if any two of the three criteria were met: Bm within <1.0% of the mean of the first three laboratory visits (6); USG <1.02; or UOSM of ±700 mmol/kg (18). The imprecision of the platform scale used to measure Bm (Mettler Toledo, Model WSI-600, Toledo, OH) was checked daily at 25, 70, and 95 kg, and ±0.05 kg was considered acceptable performance.

Urine collection and assessment of UCol. Volunteers reported to the laboratory at 0630 with their first-morning urine void collected after 0600, in a sterile, inert polypropylene cup (Tyco Healthcare Group, Mansfield, MA). The sample was then placed in a photo box next to a UCol chart (Human Hydration) with the polypropylene cup lid removed to determine UCol based on the eight-point numerical scale (2). On this scale, UCol corresponding to numerical values of 1 and 2 signify “well hydrated,” colors within the range of ≥3 and <7 are defined as “euhydrated,” and colors of ≥7 are considered “hypohydrated.” The color of each urine sample was subjectively determined before and after supplementation by two independent observers who did not consult each other regarding assignment of a specific numeric value. Raw agreement between the two independent observers’ assessments of UCol was 88%, where 53 of 60 assessments were in perfect agreement, and 7 were not. Of those 7 not in agreement, the assessed values were always within one UCol value. Further assessment of interrater agreement for determination of UCol was performed via an intraclass correlation (8), which was 0.99. Given the high degree of agreement between the two independent observers, UCol values between raters were averaged for each volunteer.

Following UCol assessment, urine samples were then transported to the laboratory for analysis. A small aliquot was transferred into a 1.5-ml polypropylene cryule vial (Wheaton, Millville, NJ) and briefly vortexed before analysis of USG and UOSM. USG was measured in duplicate for each specimen using a digital refractometer (1110400A TS Meter, AO Reichert Scientific Instruments, Keene, NH). UOSM was measured in triplicate by freezing-point depression (model 210, Fiske Micro-osmometer, Norwood, MA) and calibrated using standards within the dedicated range for urine (850 mmol/kg).

Urinary B2 and B12 vitamin assessment. Frozen urine samples were stored (~80°C) for vitamin analysis at a later time. Samples were thawed at room temperature and then vortexed. Five hundred microliters of sample were diluted with 500 μl of Optima grade acetonitrile.
Vitamin Supplementation Group

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<tr>
<td>Bm, kg</td>
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<tr>
<td>Mean euyhydrated baseline</td>
<td>78.8 ± 10.2 (61.6–98.3)</td>
<td>69.1 ± 9.2 (56.3–83.1)</td>
<td>74.9 ± 7.2 (61.4–82.3)</td>
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<tr>
<td>Euyhydrated control</td>
<td>79.1 ± 10.5 (62.0–99.8)</td>
<td>69.3 ± 9.2 (56.1–84.0)</td>
<td>75.2 ± 7.3 (61.3–82.3)</td>
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<tr>
<td>Vitamin supplementation</td>
<td>79.6 ± 10.7 (61.6–101.1)</td>
<td>69.5 ± 8.9 (56.4–83.0)</td>
<td>75.9 ± 7.0 (61.8–83.3)</td>
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<tr>
<td>USG</td>
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<tr>
<td>Mean euyhydrated baseline</td>
<td>1.017 ± 0.003 (1.013–1.022)</td>
<td>1.016 ± 0.004 (1.012–1.023)</td>
<td>1.015 ± 0.004 (1.006–1.021)</td>
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<tr>
<td>Euyhydrated control</td>
<td>1.017 ± 0.005 (1.008–1.023)</td>
<td>1.012 ± 0.004 (1.004–1.021)</td>
<td>1.014 ± 0.008 (1.002–1.020)</td>
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<td>Vitamin supplementation</td>
<td>1.017 ± 0.008 (1.009–1.018)</td>
<td>1.014 ± 0.005 (1.006–1.020)</td>
<td>1.016 ± 0.001 (1.007–1.024)</td>
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<td>UOSM, mmol/kg</td>
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<tr>
<td>Mean euyhydrated baseline</td>
<td>631 ± 105 (486–830)</td>
<td>574 ± 178 (435–817)</td>
<td>540 ± 155 (248–747)</td>
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<td>Euyhydrated control</td>
<td>590 ± 171 (305–803)</td>
<td>407 ± 180 (136–727)</td>
<td>513 ± 131 (146–716)</td>
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<tr>
<td>Vitamin supplementation</td>
<td>628 ± 168 (322–724)</td>
<td>510 ± 176 (211–708)</td>
<td>583 ± 165 (276–758)</td>
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Values are means ± SD of baseline with range in parentheses. Bm, body mass; USG, urine specific gravity; UOSM, urine osmolality.
knowledge, that has simultaneously assessed UCol (via standardized color chart) and quantified urinary vitamin concentration (via ultraperformance liquid chromatography) to address this question. To ensure that any change in UCol would be the result of vitamin supplementation, we carefully controlled hydration by prescribing fluid intake and establishing a 3-day euhydrated baseline before assessment. Furthermore, we measured first morning USG and UOSM against established first morning criteria (18). Lastly, we supplemented volunteers with B2 and B12 vitamins in compliance with USP standards for weight and disintegration. Importantly, we also confirmed vitamin purity against certified B2 and B12 standards and, most importantly, verified the presence of vitamin B2 and B12 concentration in the urine via ultraperformance liquid chromatography.

We hypothesized that vitamin B2, but not B12, supplementation would result in a UCol more consistent with a category associated with “dehydration” (>7 in the standardized chart) (2), and that other measures of urine concentration would remain unchanged. Contrary to our hypothesis, we found that neither vitamin B2 nor B12 supplementation altered UCol such that it was consistent with a classification of “dehydration” in euhydrated volunteers. While B2 supplementation did significantly increase urinary B2 concentrations, it did not significantly alter UCol classification. Vitamin B12 supplementation did not alter urine B12 concentrations to levels different from control and thus could not have influenced UCol. Given that the mean UCol did show a nonsignificant increase of 1 mean unit in all three trials, one might be tempted to conclude that B2 supplementation could alter hydration assessment (e.g., urine classified as 6 “euhydrated” to 7 “hypohydrated”) in a subset of cases. In this context, we have previously reported that the daily variability in UCol in euhydrated men and women is ±1

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**Fig. 2.** Urinary vitamin concentration (left y-axis) and urine color (UCol) (right y-axis) of vitamin B2 (A), B12 (B), and B2 + B12 (C). Values are means ± SD. *Significantly different from control (P < 0.05).
In the present trial, UCol increased by ≥1 unit in 15 volunteers and stayed the same or decreased by ≥1 unit in 15 volunteers. Because of inherent variability among individuals, two subjects increased UCol by as much as 3 units, and two subjects decreased UCol by 2 units. Thus, as a result of the daily variability in UCol, there appears to be equal chance that UCol might increase, remain unchanged, or decrease. Importantly, since the one category increase in UCol in the B12-only trial could not have been due to B12, the mean increase is almost certainly unrelated to the vitamin supplementation in all three trials.

Vitamin B2 or riboflavin gets its name from the Latin word “flavus,” or yellow, and the oxidized forms of different flavoenzymes are intensely colored yellow, green, or red (3). In particular, vitamin B2 has been associated with a change in UCol that is bright or fluorescent yellow (10) (Fig. 3). When B2 is absorbed in excess, very little is stored in the body tissues, and excess is excreted, primarily in the urine (15). In fact, vitamin B2 accounts for 60–70% of the excreted urinary flavins (15). Despite these particular color characteristics of vitamin B2, in the present study, oral dosages of ~200× the RDA did not result in a significant alteration of UCol as a marker of hydration status. Our main finding was that, in euhydrated men and women, vitamin B2 and B12 supplementation did not alter UCol more than the typical day-to-day variation in UCol (~1 au) (7), and in no case altered a euhydration color rating to become classified as “dehydration” (2). In several volunteers, vitamin B2 (Fig. 3) supplementation did result in a more fluorescent yellow UCol; however, assessment of hydration status via color was not different from matched samples in euhydration control trials. We also found that vitamin B12 supplementation did not result in a substantial increase in UCol concentration within the urine and, as a result, played no role in alteration of UCol. These findings have application to military, sport, and wilderness medicine, where assessment of first morning UCol can be a valuable and field-expedited tool to assess hydration status.

Conclusions. To our knowledge, this is the first investigation to comprehensively and simultaneously assess UCol and quantify urinary vitamin concentration to evaluate whether supplementation with vitamin B2 and B12 alters UCol as a marker of hydration status. Our main finding was that, in euhydration men and women, vitamin B2 and B12 supplementation did not alter UCol more than the typical day-to-day variation in UCol (~1 au) (7), and in no case altered a euhydration color rating to become classified as “dehydration” (2). In several volunteers, vitamin B2 (Fig. 3) supplementation did result in a more fluorescent yellow UCol; however, assessment of hydration status via color was not different from matched samples in euhydration control trials. We also found that vitamin B12 supplementation did not result in a substantial increase in B12 concentration within the urine and, as a result, played no role in alteration of UCol. These findings have application to military, sport, and wilderness medicine, where assessment of first morning UCol can be a valuable and field-expedited tool to assess hydration status.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES