

〈Review Article〉

Urinalysis for the assessment of nutrition and oxidative stress

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Summary In addition to blood analysis, urine specimens can potentially be used for the assessment of nutrition and oxidative stress, both of which have immediate effects on health. We aimed to identify urinary biomarkers in 24-h urine, first- and second-morning urine, and random urine specimens. Excluding 24-h urine collection, creatinine concentrations in the urine specimens were used to correct for urinary voided volume. In this review, we describe urinalysis for vitamins, minerals, acetoacetic acid, and nitrogen compounds (i.e., low-molecular-weight proteins and amino acids) as nutrient markers, and dehydroascorbic acid and biopyrrin as oxidative stress markers. Markers composed of larger molecules unable to pass through the glomerulus and hydrophobic compounds (i.e., lipids) require further investigation.

Key words: Urinalysis, Nutrition, Oxidative stress, First- and second-morning urine, Phosphoethanolamine

1. Introduction

Although urine is a noninvasive specimen, analysis of urine compounds reflects the presence of kidney and urinary tract injuries as well as systemic metabolism, because urine is produced from the bloodstream via glomerular filtration. By way of example, excretion of macromolecule compounds such as albumin increases in patients with nephrotic syndrome, and detection of acetoacetic acid in urine implies increased ketone body levels in blood both in patients (diabetes) and healthy subjects (starvation)¹. Currently, several dozen urinary chemical compounds (e.g., low-molecular-weight proteins²,

amino acids³, cell-free nucleic acids⁴, nitrogen compounds⁵, carbohydrates⁶, minerals⁷, and vitamins⁸, including their metabolic products) can be assayed in clinical laboratories⁹. Among these compounds, proteins, amino acids, carbohydrates, minerals and vitamins are nutrients, and certain nitrogen compounds and hormones are proven markers of oxidative stress¹⁰. Because poor nutrition and excess stress can have a negative impact on health, we aim to detect early signs of worsening health status. In this review, we describe urinalysis methods for the assessment of nutrition and oxidative stress, based on investigations in our laboratory and a review of the literature.

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Received for Publication November 30, 2016

Accepted for Publication October 17, 2016

2. Assessment of vitamin B1 nutriture using first-morning urine

Vitamin B1 (thiamine) is required for the proper use of carbohydrates. Because vitamin B1 is a water-soluble B vitamin, it is not stored in the body and thus readily declines to deficiency levels. Symptoms of long-term vitamin B1 deficiency (beriberi) affect the heart and nerves, while the early symptom of deficiency is known as malaise (i.e., feeling of discomfort, fatigue, illness, or lack of well-being). To diagnose vitamin B1 deficiency, vitamin B1 concentrations are typically assayed in whole blood, since vitamin B1 concentrations in erythrocytes reflect the amount stored in the body¹¹.

Instead of whole blood analysis, which requires invasive blood collection, we assessed vitamin B1 nutritional status using first-morning urine and 24-h urine specimens. Initially, whole blood concentrations of vitamin B1 in 62 healthy subjects were compared with their 24-h urine specimens¹². Herein, the 24-h collection started in the morning on the first day, with the first urine discarded. Afterward, all urine was collected in a container for the next 24 h, including the first-morning urine on the next day. Blood specimens were collected prior to breakfast and analyzed for vitamin B1 concentration in whole blood.

This experiment indicated that the amounts of vitamin B1 excreted in the first-morning urine (X) correlated with the amounts of vitamin B1 in 24-h urine (Y: $Y = 3.81X + 25.8$, $r = 0.931$, $P < 0.001$). Vitamin B1 excreted in the first-morning urine was 24% on average of the quantities in the 24-h collections. When urinary excretion of vitamin B1 (B1) was corrected for creatinine (Cr) excretion (as the ratio to Cr; B1/Cr ratio), the B1/Cr ratio in the first-morning urine correlated significantly with the B1/Cr ratio in 24-h urine ($Y = 1.12X - 3.2$, $r = 0.970$, $P < 0.001$; Fig. 1). However, the B1/Cr ratio in random urine specimens voided at the second collection or later did not correlate with the 24-h urine values. Moreover, the B1/Cr ratio in the first-morning urine correlated significantly with the vitamin B1 concentration in whole blood ($r = 0.733$, $P < 0.001$; Fig. 2).

Papageorge and Lewis¹³ reported on the second-morning urinalysis (i.e., 1-h fasting excretion test). After voiding the first-morning urine, subjects were asked to drink one glass of water. They were also asked to rest in bed for one hour without taking breakfast, and then to collect second-morning urine. This suggested that the values (vitamin B1/Cr ratio) in the 1-h fasting excretion corresponded to systemic basal metabolism requiring vitamin B1.

We conclude that the nutritional status of vitamin B1 (i.e., sufficiency or deficiency) can be estimated by measuring first-morning urine, and basal metabolism requiring vitamin B1 can be estimated by measuring second-morning urine.

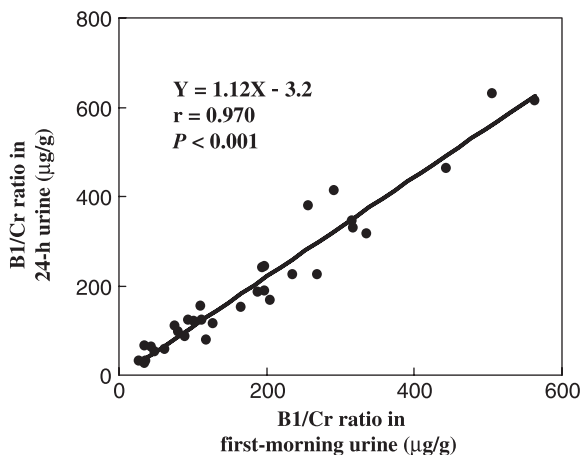


Fig. 1 Comparison of B1/Cr ratio in first-morning urine and B1/Cr ratio in 24-h urine from 32 healthy adults (from Ref. [12]).

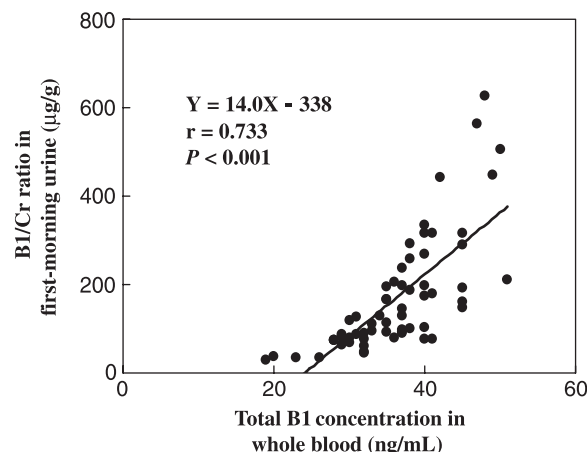


Fig. 2 Comparison of total B1 concentration in whole blood and B1/Cr ratio in first-morning urine from 62 healthy adults (from Ref. [12]).

3. Dehydroascorbic acid in urine as a marker of oxidative stress

Vitamin C (ascorbic acid) is a water-soluble vitamin that has anti-scorbutic and anti-oxidant activities¹⁴. Vitamin C quenches a variety of reactive oxygen and reactive nitrogen species in the aqueous environment of the body, thereby ameliorating oxidative stress. Ascorbic acid is converted to its oxidized form dehydroascorbic acid when acting as an antioxidant. Because dehydroascorbic acid does not exert antioxidant activity, it is reduced by glutathione¹⁵ and NADH (or NADPH) back to ascorbic acid¹⁶. Although dehydroascorbic acid concentrations in plasma were as low as 1.0 mg/L, we determined that the concentration in random urine specimens ranged from 0.9 mg/L to 13.8 mg/L¹⁷. This suggested that the dehydroascorbic acid concentration in urine was a better indicator of oxidative stress levels than plasma concentrations. Kubin et al.¹⁸ reported that urinary excretion of dehydroascorbic acid was increased in patients undergoing total hip joint endoprosthesis surgery. Surgical stress would increase the rate of ascorbic acid oxidation and urinary excretion of dehydroascorbic acid as a consequence of the enhanced formation of free radicals. Moreover, Koshiishi et al.¹⁹ reported that dehydroascorbic acid arose from ascorbic acid through oxidation stress or disorders where oxidative stress is increased. In addition to the oxidative stress arising during surgery and in diseased patients, plasma concentration of dehydroascorbic acid (mean \pm SD) was reported to be increased in smokers (0.14 ± 0.40 mg/L, $n = 82$) compared to non-smokers (0.02 ± 0.41 mg/L, $n = 124$)²⁰. Thus, we expect that urinary excretion of dehydroascorbic acid will be increased in smokers. However, knowledge about which specimens (i.e., the first-, second-morning urine, 24-h urine, or random urine) are optimal for diagnosing exposure to stress is currently lacking. In future studies, we plan to investigate the diurnal variation in dehydroascorbic acid excretion in response to various stresses encountered in daily life. The information acquired in these studies will aid the recognition of

stress overload and the preventive reduction in its harmful effects.

4. Urinalysis for assessment of mineral nutrients

Currently, excretion of sodium chloride is measured in 24-h urine for patients with hypertension, while second-morning urine analysis for sodium and potassium is reported to be a reliable means of assessing sodium and potassium intake²¹. Urinary excretion of calcium, phosphate, and iodine are reported to correlate with the intake of these minerals²². However, calcium deficiency can not be diagnosed by urinalysis, and urinary phosphate rises during renal failure. Moreover, iodine concentrations corrected by creatinine can be misleading because of large inter- and intra-individual variations in urinary creatinine excretion²².

5. Biopyrrin as a marker of physiological and psychological stress

For many years, bilirubin was generally regarded as a potentially toxic waste product formed during heme catabolism. Bilirubin (i.e., unconjugated bilirubin) is a highly neurotoxic substance that may become elevated in the serum, a condition known as hyperbilirubinemia. However, more recent evidence suggests that bilirubin is a physiological antioxidant that may scavenge oxygen and peroxy radicals as efficiently as α -tocopherol (vitamin E)^{23, 24}. Vitamin C, as described above, and uric acid work in the aqueous environment of the body, whereas bilirubin and α -tocopherol work in the hydrophobic environment, thereby quenching oxygen radicals in the lipophilic compartment. Previously, uric acid has also been considered a troublesome material that caused urinary calculus. However, it has been revealed that uric acid provides strong antioxidant defense in humans²⁵.

During oxidative injury (i.e., surgical stress) or oxidative stress (i.e., physiological and psychological stress), hepatic mRNA expression of hemeoxygenase-1 is rapidly induced. Hemeoxygenase-1 cleaves the heme ring to biliverdin, which is subsequently converted to bilirubin

by the cytosolic enzyme biliverdin reductase. Circulating bilirubin then acts as a suicide antioxidant (i.e., a terminal antioxidant) and is decomposed to the tripyrrole moiety of bilirubin (biopyrrin)²⁶ (Fig. 3).

We collected 24-h urine specimens from 11 Japanese adults and assayed the urinary excretion of biopyrrin and cortisol²⁷. Upon exposure to stress, the hypothalamus stimulates the adrenal gland to release a surge of hormones, including adrenaline and cortisol, the primary stress hormone. Adrenaline increases the heart rate, elevates blood pressure and boosts energy supplies. Cortisol increases glucose in the bloodstream and enhances the use of glucose by the brain. As with vitamin B12 excretion, the concentrations of biopyrrin and cortisol in the first-morning urine, in terms of both absolute amounts and the ratios to creatinine, were significantly correlated to those in the 24-h urine specimens. Herein, correlation (r) between concentrations of biopyrrin in the first-morning urine in terms of absolute amounts and the ratios to creatinine was respectively 0.765 and 0.917 ($P < 0.001$), and the r between concentrations of cortisol in the first-morning urine in terms of absolute amounts and the ratios to creatinine was respectively 0.700 and 0.706 ($P < 0.001$)²⁷. In contrast, concentrations in random urine specimens (second or later voiding, corrected for creatinine) did not correlate with the concentration in 24-h urine specimens ($P > 0.05$). The amounts of biopyrrin excreted in 24-h urine specimens were significantly correlated to those of cortisol. The difference was that the excretion of cortisol was at its maximum level in the first-morning urine sample and a nadir during night in accordance with the circadian rhythm. Conversely, the maximum excretion of biopyrrin was not limited to the morning. We interpreted this to mean that biopyrrin excretion was specific in response to stress compared cortisol, and was not influenced by blood glucose levels.

Furthermore, we investigated biopyrrin excretion in the first-morning urine in comparison with 8-hydroxy-2'-deoxyguanosine (8-OHdG) in 63 psychiatric patients. Informed consent was obtained from all patients and their families, and the study

protocol was approved by the Protection of Human Subjects Committee of Aizunishi Hospital and was in accordance with the Helsinki Declaration. 8-OHdG is an oxidized derivative of the base guanine. Guanine is oxidized more readily than adenine, the other purine-derivative base in DNA, and has therefore been widely used as a biomarker for oxidative stress²⁸. During DNA repair processes, 8-OHdG diffuses out of the nucleus of cells, and is then filtered out of the blood and excreted into the urine. In this study, we found a poor relationship between excretion of biopyrrin and 8-OHdG in the first-morning urine ($r = 0.101$, $P > 0.05$; Fig. 4), although both are known as markers of oxidative stress. We think that the formation of biopyrrin is accelerated by the psychological consequences of physical or emotional stress in addition to oxidative

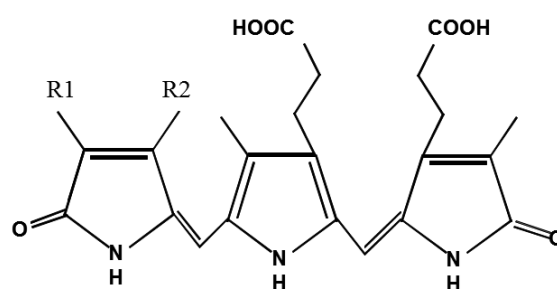


Fig. 3 Structure of biopyrrin.
Biopyrrin-a: R1 (-CH₃), R2 (-CH=CH₂)
Biopyrrin-b: R1 (-CH=CH₂), R2 (-CH₃)

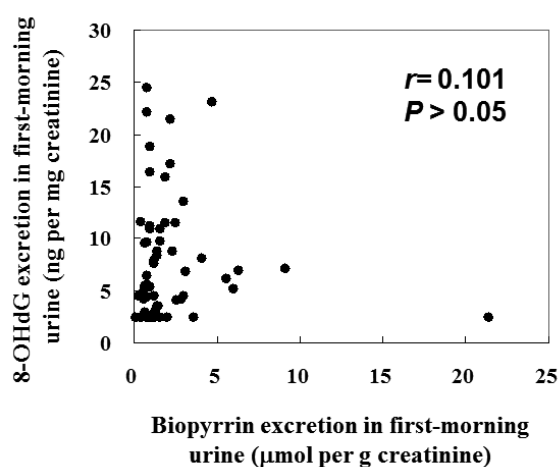


Fig. 4 Comparison of excretions of biopyrrin and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in first-morning urine from 63 psychiatric patients.

stress, however the effects on 8-OHdG will not be the same. Of the 63 patients, eight patients exhibited higher biopyrrin excretion than the reference limit (0.5-3.3 μmol per g creatinine)²⁹. Among them, only one patient showed elevated 8-OHdG (> 12 ng per mg creatinine).

6. Phosphoethanolamine as a urinary marker of depression

Phosphoethanolamine (PEA) is known to be an intermediate of phospholipid metabolism and a component of the fundamental skeleton of the glycosylphosphatidylinositol (GPI) anchor molecule^{30, 31}. Urinary PEA is increased in patients with hereditary metabolic disease³², and hypophosphatasia^{33, 34}, which is due to a mutation in the *ALPL* gene encoding tissue-nonspecific alkaline phosphatase. The enzyme cleaves extracellular substrates, i.e., PEA, pyridoxal-5'-phosphate and inorganic pyrophosphates. Plasma PEA is decreased in patients with depression³⁵, and the excretion of biopyrri, as described above, is increased by psychological stress. In future studies, we are interested in further characterizing the urinary excretion of PEA or its metabolic products, including ethanolamine, in patients with depression.

7. Assessment of carbohydrate nutriture by acetoacetic acid in the urine

Ketone bodies (three water-soluble molecules: acetoacetic acid, 3-hydroxybutyric acid, and acetone) are produced by the liver from fatty acids during periods of low food intake (fasting), carbohydrate restrictive diets, or starvation¹. During glucose deficiency, fatty acids are converted by β -oxidation in liver mitochondria into acetyl-CoA. The resulting acetyl-CoA is converted into ketone bodies, which then enter the blood circulation. The ketone bodies are readily absorbed by extra-hepatic tissues (e.g., brain, myocardium, and skeletal muscle) and converted into acetyl-CoA, which then enters the citric acid cycle and is oxidized in the mitochondria for energy. However, only acetoacetic acid can appear in urine. The renal threshold of 3-hydroxybutyric acid is too high for it to be excreted in urine,

whereas acetone is expired by the lungs. Therefore, ketonuria is a marker of prolonged fasting and shortages of dietary carbohydrates. Conversely, glycosuria (or glucosuria) is observed when plasma glucose levels exceed the renal threshold for glucose (ca. 180 mg/dL), even in healthy subjects.

8. Urinalysis for the assessment of protein nutriture

As a result of our bibliographic search, we describe five urinary markers for assessing protein nutriture, including nitrogen compounds and proteins themselves. These nutritional markers evaluate dietary protein adequacy and assimilation, as well as metabolic imbalance and specific inborn errors of amino acid metabolism (i.e., phenylketonuria, homocystinuria, and maple syrup urine disease).

8.1. Urine urea nitrogen (molar mass: 60.06)

Urea nitrogen is a waste product made from ammonia (a toxic chemical), which is a byproduct of dietary protein metabolism in the liver. The amounts of urea nitrogen in urine indicate the amount of protein intake and kidney function. 24-h urinary urea nitrogen excretion is used as a marker for the validation of dietary protein intake³⁶.

Nitrogen balance is a measure of nitrogen input minus nitrogen output, and is calculated as follows: Nitrogen Balance = Nitrogen intake - Nitrogen loss (in the urine). "Nitrogen Balance" indicates the balance between protein anabolism (protein synthesis) and protein catabolism (protein breakdown).

8.2. Urine creatinine (molar mass: 113.12)

Creatinine is a breakdown product of creatine phosphate in muscle. Once formed, creatinine is a chemical waste product that is normally filtered by the kidneys into the urine. Creatinine is usually produced at a fairly constant rate by the body, depending on fat-free muscle mass. Thus, urinary creatinine excretion rate (CER) is an established marker of muscle mass, and that correlates well with arm muscle circumference (AMC).

8.3. Urinary hydroxyproline (molar mass: 131.13)

Hydroxyproline is a product of collagen metabolism that is excreted in the urine. With protein-energy malnutrition, urinary excretion of hydroxyproline is decreased. Analysis of 24-h urine, first- and second-morning urine, and random urine indicated that second-morning urine is influenced less by dietary hydroxyproline content than first-morning urine³⁷. Furthermore, urinary hydroxyproline is used as a marker of bone resorption³⁸.

8.4. Urinary 3-methylhistidine (molar mass: 169.18)

3-Methylhistidine is a product of peptide bond synthesis and methylation of actin and myosin. During muscle protein turnover, 3-methylhistidine is excreted in the urine. Urinary excretion of 3-methylhistidine has been indicated as a marker of protein-energy malnutrition status. Excretion increases with protein and energy treatment. Therefore, the urinary 3-methylhistidine concentration in 24-h urine or second-morning urine has been used to monitor changes in muscle protein catabolism³⁹.

8.5. Aminoaciduria

Phenylketonuria (PKU) is caused by the absence or marked reductions in phenylalanine hydroxylase (PAH) enzyme activity. Phenylketone (phenylpyruvate), phenyllactate and phenylacetate are excreted in urine⁴⁰.

Homocystinuria (HCU) is caused by cystathionine β -synthase (CBS) deficiency, the enzyme related to methionine metabolism, leading to an abnormal accumulation of homocysteine in the blood and higher levels excreted in the urine.

Maple syrup urine disease (MSUD) is caused by a deficiency in the branched-chain alpha-keto acid dehydrogenase complex, which is required to breakdown branched-chain amino acids (leucine, isoleucine, and valine). Excretion of alpha-ketoacids (isoleucyl ketoacid) gives the urine its distinctive maple syrup-like odor.

9. Conclusion

We investigated urinalysis for the assessment of nutrition and oxidative stress. These analyses commonly use 24-h urine specimens, however 24-h collections are fraught with error. The first-morning urine voided after a night's sleep reflects the concentrations observed in 24-h urine specimens. Urinary concentrations in 24-h urine and first-morning urine vary according to the conditions of the previous day (i.e., diet, exercise, drinking, sweating, and the different kinds of stress), whereas the second-morning urine (i.e., 1-h fasting excretion test) corresponds to basal metabolism of the current day.

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