Estimation of Vitamin B1 Excretion in 24-hr Urine by Assay of First-Morning Urine

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Urinary B1 (vitamin B1) excretion is commonly determined in 24-hr urine specimens to obtain an estimate of nutritional status. The aim of our study was to investigate whether B1 in random urine specimens, corrected for the urine creatinine (Cr), can be substituted for B1 in 24-hr urines. Collection of such hour urines is often fraught with errors; an alternative method is described here.

All urine specimens voided over 24 hr were collected from 32 healthy adults as were the first-morning urines from 30 healthy Japanese women. The B1 excretion was expressed as the ratio of B1 to Cr. Although the B1 excretion was expressed

as the B1/Cr ratio, the B1 excretion varied with the urine volume and the time of urine collection. The B1/Cr ratio in random urine specimens not collected at a fixed time may mislead the evaluation of the nutritional status. We found that the B1/Cr ratio in the first-morning urine correlated significantly with the ratio in 24-hr urines ($r = 0.970$, $P < 0.001$) and also with the concentration of total B1 (B1 plus its phosphate esters) in whole blood ($r = 0.733$, $P < 0.001$). We conclude that the B1/Cr ratio in 24-hr urines could be estimated by measuring the ratio in the first-morning urine. *J. Clin. Lab. Anal.* 22:291–294, 2008. © 2008 Wiley-Liss, Inc.

Key words: creatinine correction; first-morning urine; vitamin B1; 24-hr urine; thiamine

INTRODUCTION

Vitamin B1, an essential vitamin, participates in carbohydrate metabolism, in the tricarboxylic acid cycle and the pentose phosphate pathway. Vitamin B1 is determined to assess body nutriture by measuring the blood concentrations of B1 vitamers (i.e., B1 and its phosphate esters) and the urinary excretion of B1 (1). The common practice is to measure the concentration of total B1 (sum of B1 and its phosphate esters) in whole blood and the concentration of B1 in 24-hr urine collections (2). Collections of 24-hr urines are fraught with errors so that an accurate measure of the B1 excretion is difficult to obtain. The goal of this study was to investigate whether random urine specimens collected at the appropriate time of day and corrected for the creatinine (Cr) excretion can be substituted for 24-hr urines in the assay of urinary B1.

MATERIALS AND METHODS

Subjects

All urines collected over 24 hr were obtained from 32 adult Japanese volunteers. Seventeen were women

(22–60 year) and 15 men (23–57 year); all were presumed to be in good health. Written informed consent was obtained from all subjects; our study was in compliance with the rules for human experimentation in our institution. On the first morning when we started the collections, we discarded the first urine. We then saved all urines voided for the next 24 hr plus the first collection from the next morning. At that time, we collected venous blood specimens before breakfast for the analysis of total B1 (B1 plus its phosphate esters) concentration in whole blood. We also measured the first-morning urine in 30 healthy Japanese women (age 19–26 year) having total B1 concentration in whole blood of 28–51 ng/mL. Our reference range for total B1 concentration in whole blood is 28–56 ng/mL.

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Methods

We measured the B1 concentration by a high-performance liquid chromatography (HPLC) method using a gel-permeation column (TSKgel G3000PWXL Tosho Corporation, Tokyo, Japan) (3). A urine aliquot of 0.05 mL was mixed with 0.95 mL of 0.2 mol/L potassium acetate buffer at pH 4.5 and 0.2 mL of the mixture was oxidized with 0.02 mL of 0.3 mol/L cyanogen bromide. The reaction was terminated by the addition of 0.03 mL of 2.0 mol/L sodium hydroxide. We injected the 0.05 mL specimens into the column and eluted isocratically at a flow rate of 1.0 mL/min at 40°C with 90 mmol/L phosphate buffer, pH 8.6, and acetonitrile (ratio of 15:85). An emission wavelength of 430 nm and excitation wavelength of 375 nm were used for the assay (Fig. 1). The urinary B1 concentration was expressed as the ratio to Cr, i.e., the B1/Cr ratio.

For the analysis of the total B1 concentration in blood, we used 0.5 mL whole blood collected with heparin, mixed with 0.5 mol/L potassium acetate buffer (pH 4.5), and deproteinized by the addition of 0.5 mL of 10% trichloroacetic acid. After spin down (3,000 rpm, 10 min), the pH of the supernatant was adjusted to 4.5 with 4.0 mol/L potassium acetate. B1 phosphate esters in the supernatant were hydrolyzed to B1 by Takadiastase B (Sankyo Co. Ltd, Tokyo, Japan). Oxidation and the HPLC procedure were the same as those for the analysis of urine. We used B1 hydrochloride as the calibrant for the analyses of urine and blood.

RESULTS

Within-Day Variance of Urinary B1 Excretion

Our subjects collected 4 to 12 random urine specimens over 24 hr; we had an average of 8 unique collections for each subject. Urinary excretion of B1 expressed in terms of the absolute amounts and the B1/Cr ratio varied according to their voided urine volume and with the

Fig. 1. A typical chromatogram of B1 in urine from healthy subject. B1 was eluted at 8.17 min.

time of urine collection. Within-day variance CV of the B1/Cr ratio ranged between 16 and 66% (mean, 37%). The highest B1 excretion (both absolute amounts and ratio) was commonly observed in first-morning urine.

Comparison With 24-hr Urine

We compared the B1 concentrations in all of the random urine specimens collected over 24 hr to that of 24-hr urines. The amount of B1 in the first-morning voiding divided by the quantities in the 24-hr collections ranged between 4 and 48% (mean, 24%), i.e., the mean excretion was less than one-fourth of the total excretion. The percentage was increased in parallel with the increase in the retention time of the first-morning urine. The amounts of B1 excreted in the first-morning urine correlated significantly with the amounts of B1 excreted in 24-hr urines ($Y = 3.81X + 25.8$, $r = 0.931$, $P < 0.001$; figure not shown). Furthermore, the B1/Cr ratio in the first-morning urine correlated significantly with the B1/Cr ratio in 24-hr urines ($Y = 1.12X - 3.2$, $r = 0.970$, $P < 0.001$, Fig. 2). The B1/Cr ratio in a random urine specimen voided at the second collection or later did not correlate with the same in a 24-hr urine. In addition, when the B1/Cr ratio in the first-morning urine was compared with its hourly excretion (i.e., amount of B1 excreted in the first-morning urine divided by retention time of the first-morning urine), a significant correlation was observed ($Y = 0.045X + 0.51$, $r = 0.948$, $P < 0.001$, Fig. 3).

Comparison With Blood Concentrations

The concentration of total B1 (B1 plus its phosphate esters) in whole blood collected both from the 32 adults

Fig. 2. Comparison of B1/Cr ratio in first-morning urine and B1/Cr ratio in 24-hr urine from 32 healthy adults. Linear regression analysis gave a slope of 1.12, an intercept of -3.2 , and $r = 0.970$, $P < 0.001$.

Fig. 3. Comparison of B1/Cr ratio in first-morning urine and its hourly excretion from 32 healthy adults. Hourly excretion was defined as the amount of B1 excreted in the first-morning urine divided by retention time of the first-morning urine. Linear regression analysis gave a slope of 0.045, an intercept of 0.51, and $r = 0.948$, $P < 0.001$.

Fig. 4. Comparison of total blood B1 concentration in whole blood and B1/Cr ratio in first-morning urine from 62 healthy adults. Linear regression analysis gave a slope of 14.0, an intercept of -338, and $r = 0.733$, $P < 0.001$.

and 30 young women was significantly correlated with the B1/Cr ratio in the first-morning urine ($r = 0.733$, $P < 0.001$, Fig. 4). Of the 32 adults, 4 had a total B1 concentration in whole blood of less than 28 ng/mL. Each point showed variable bias from the regression line. The biases would arise from intra- and interindividual variabilities in daily intake and/or requirement of this nutrient.

The 95th Percentile of the Ratio

When we calculated the B1/Cr ratio in first-morning urine from 58 subjects who had a total B1 concentration in whole blood higher than 28 ng/mL, a 95th percentile defined as the antilog of $[\log(\text{mean}) \pm 2 \times (\log \text{SD})]$ (4) for the B1/Cr ratio in 58 first-morning urines from our subjects was 39–524 µg/g. The ratio for these 58 subjects ranged from 44–626 µg/g. In four subjects having lower total B1 concentration in whole blood as 19, 20, 23, and 26 ng/mL, the B1/Cr ratio in the first-morning urine was 28, 37, 35, and 35 µg/g, respectively.

DISCUSSION

The B1 vitamers generally observed *in vivo* are B1 and its phosphate esters, i.e., B1 monophosphate (TMP), B1 diphosphate (TDP), and B1 triphosphate (TTP). Although B1 and TMP have no physiological activity, they are present in plasma. B1, TMP, TDP, and TTP are found in blood cells and tissues (5). When TDP and TTP exert their physiological functions, they are catabolized to B1 and B1 metabolites and excreted in urine and bile (6). In this study, the B1/Cr ratios in first-morning urine were significantly correlated to the concentrations of

total B1 (B1 plus its phosphate esters) in whole blood. Furthermore, because the amounts of B1 excreted in urine were, in part, regulated by proximal reabsorption (7), it seemed that an assay of the B1/Cr ratios in the first-morning urine might be useful in estimating B1 nutriture.

The current common practice is to use early morning single-void urine specimens for analysis largely owing to the difficulties with 24-hr collections. A correction with the Cr concentration provides a convenient way to estimate 24-hr excretion (8) based on the assumption that corrected values in random urine voided any time yield results consistent with those of 24-hr urines. However, we have found this assumption to be in error. Corrected values did not correlate with the finding in 24-hr urine. We found that for urinary excretion of B1 expressed in terms of both absolute amounts and the B1/Cr ratio, only an assay of first-morning urine agreed with 24-hr urine.

For an evaluation of B1 nutriture, we recommend the use of the B1/Cr ratio rather than the absolute amounts and, if at all possible, an assay of first-morning urine. The absolute amounts excreted in the first-morning urine were 4–42% of the total in 24-hr urine. A determination of the B1/Cr ratio requires only an aliquot of a random urine specimen, with the results between the first-morning urine and 24-hr urine being in a nearly 1:1 correlation. In 1947, Pappageorge and Lewis compared the excretion of B1 in a 1-hr urine specimen collected after the completion of a 24-hr period urine collection with an overnight fast to that in a 24-hr excretion (9). They reported that there was a good correlation with a coefficient of 0.70, and that the

nutritional status of B1 could be assessed by excretion in 1-hr fasting specimen, and the critical level for inadequate B1 intake would be $<4\mu\text{g/hr}$. In their test, subjects were asked to drink one glass of water and to collect urine 1 hr later. Our test does not include drinking water and only requires the collection of an aliquot of first-morning urine as a specimen. Accordingly, it provides a more convenient method of estimating B1 nutritional status in a large number of individuals under their ordinary daily life. The correlation coefficient between the first-morning and 24-hr urine rose considerably higher than 0.70 ($r = 0.970$ in our study) owing to the correction with the Cr concentration. Their values in 1-hr fasting excretion tests were not one twenty-fourth of that in 24-hr urine, whereas our results showed a nearly 1:1 correlation between the first-morning and 24-hr urine. In addition, our values significantly reflected blood B1 levels.

For 24-hr urine, B1 excretion in adult subjects was reported to be $40\text{--}100\mu\text{g}$ ($27\text{--}66\mu\text{g/g}$) in marginal deficiency and $<40\mu\text{g}$ ($<27\mu\text{g/g}$) in deficiency (10); this author defined total B1 concentration in whole blood in marginal deficiency as $21\text{--}27\text{ ng/mL}$. Formerly, O'Neal et al reported that urinary B1 excretion in adult subjects was $27\text{--}65\mu\text{g/g}$ in marginal B1 deficiency, $<27\mu\text{g/g}$ in significant deficiency, and $\geq 66\mu\text{g/g}$ in normal (11); this author, however, did not define whether the specimens were random or 24-hr urine. This led us to conclude that the B1 excretion in 24-hr urine could be estimated by measuring the B1/Cr ratio in the first morning urine and by defining a marginal deficiency concentration as being $<39\mu\text{g/g}$; i.e., the lower limit of the 95th percentile from our study. By substituting $X = 39\mu\text{g/g}$ in the regression equation ($Y = 0.045X + 0.51$) between the B1/Cr ratio in the first-morning urine and its hourly excretion of B1, Y gave $2.3\mu\text{g/hr}$, which was close to the critical level reported by Pappageorge and Lewis (9). Because 24-hr excretions fluctuate from day to day influenced by diet, an analysis of the first-morning urine over several days would yield the most reliable estimate of vitamin B1 nutritional status. We also conclude that, owing to the limited reliability of the 24-hr urine collection, the Cr should always be determined and that the ratio of B1 to

Cr should always be obtained, as this measure must be used to estimate the vitamin B1 nutritional status.

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