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Correlation Between First Morning Single Void and 24-Hour Urines: The Reliability to Quantify Niacin Status

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Background: The current common practice when using urine as a biomarker for vitamin excretion is to use a 24-hour sample for analysis. Due to the difficulty involved in this process, we attempted to find an alternative solution through the use of a single first morning void. The aim of our study was to investigate if there is a correlation between the first morning single void and the 24-hour collections of urines for the urine metabolite of niacin, N-1-methylnicotinamide (N1MN), and to test the reliability of utilizing a method using first morning single void collections corrected with the concentration of urine creatinine.

Material/Methods: All urine samples were collected from 30 healthy adult volunteers over the age of 18 years: 20 females and 10 males. Samples were collected after discarding the first morning urine and collecting every other urine voided during the next 24 hours including the first morning urine of the day after in 2 separate vessels. We analyzed the concentration of N1MN by high performance liquid chromatography and the concentration of creatinine by a commercial kit by spectrophotometry. The B3 excretion was expressed as the ratio of N1MN to creatinine.

Results: We found a significant correlation between the ratios of first morning single void and 24-hour urines. When comparing males and females, the ratio demonstrated a significant correlation as well.

Conclusions: Our results demonstrated that it is possible to substitute a 24-hour collection with a first morning single void urine for the estimation of N1MN excretion.

MeSH Keywords: **Biological Markers • Niacin • Nutritional Status**

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Background

Vitamins are organic substances not synthesized by the organism in question and are required in small amounts (milligrams or micrograms). They are important cofactors in energy metabolism and other functions, although their own catabolism does not provide energy directly. Being chemically distinct, vitamins are classified into 2 groups in accordance to their solubility, being either soluble in lipids or in water [1]. Vitamin deficiency (hypovitaminosis) can be caused due to lack of ingestion (primary deficiency) or increased catabolism/excretion (secondary deficiency). Vitamin deficiency can provoke different diseases depending on which vitamin is lacking and should therefore be diagnosed and treated as quickly as possible to lower the risk of mortality in vitamin deficient patients.

The importance of niacin in human metabolism is evidenced by the number of reactions involved: more than 500 enzymes need niacin as a cofactor. All energy production in our body, including oxidative phosphorylation and mitochondrial citric acid cycle and glycolysis in the cytosol, are dependent on these enzymes [2]. In addition to its role as a cofactor in redox reactions and as a regulator of the redox state (NAD⁺/NADH) to NAD⁺ serves as substrate for numerous classes of adenosine diphosphate (ADP)-ribosyl transferases involved in cellular processes including transcription, calcium homeostasis, DNA repair and cell death [3]. Nicotinic acid has also been used to regulate abnormalities in lipoprotein metabolism and the treatment of coronary heart disease due to its ability to reduce plasma cholesterol, triglycerides, low-density lipoprotein (LDL), lipoprotein(a), and increase high-density lipoprotein (HDL) [4].

Niacin deficiency leads to a systemic disease known as Pellagra. Pellagra is generally caused by malnutrition (lacking in niacin or tryptophan) in patients, frequently found in alcoholics, and by secondary causes such as prolonged diarrhea, anorexia, cirrhosis, cancer, HIV/AIDS, and certain side effects provoked by medications. The 3 major symptoms associated with Pellagra are dermatitis, diarrhea, and dementia alongside other symptoms such as muscle weakness, loss of appetite, irritation, anxiety, and depression [5].

Although considered essential in humans, niacin can be synthesized in the liver by action of tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) which cause oxidation of the amino acid tryptophan to N-formyl kynurenine. In the acute phase response to inflammation, certain cells, such as macrophages, are capable of producing interleukins that stimulate the action of these enzymes. Inflammation status itself can also increase nutrient consumption and trigger biochemical manifestations of deficiency [6].

Aside from clinical diagnosis that can be performed by analyzing the signs and symptoms of the patient, biochemical diagnosis for some water-soluble vitamins might be complemented by use of urinary excretion as a biomarker for the state of sufficiency or insufficiency [1,7–9]. This analysis is usually done by a collection of urine for a period of 24-hour to obtain an estimate of nutritional status [9]. Although this type of collection has the advantage of being a noninvasive collection of biological material, it can be a difficult and troublesome method for patients, which can result in errors during the 24-hour period ultimately resulting in an inaccurate measure of vitamins in urine. Several errors might occur if the patient does the collection at home, such as storing the vessel incorrectly, possibly forgetting a single or multiple voids, and even abandonment of the collection by the patient [10].

In a hospital environment, other problems, such as an incorrect collection of urine from probes, risk of contamination, and technical errors by the party responsible for the collection, can and often do occur. These errors can result in grave consequences for patients, since it would require another 24-hour period to recollect and analyze the sample, preventing the patient from receiving treatment as soon as possible.

An alternative solution would be to utilize a single urine sample in a fixed time period to substitute for the 24-hour collection and correct the value using the concentration of creatinine encountered [10]. This study aimed to verify if there was a correlation between a 24-hour collection and a first morning single void urine sample for the urine metabolite of niacin, e.g., N-1-methylnicotinamide (N1MN), and to test the authenticity of utilizing this method in future research and clinical cases.

Material and Methods

Study participants

All urine samples (24-hour and first morning single urine samples) were obtained from 30 volunteers over the age of 18 (mean $\pm$ SD=29.96 $\pm$ 12.61 years): 10 males with ages ranging from 23 to 49 years (mean $\pm$ SD=26.5 $\pm$ 7.97 years) and 20 female with ages ranging from 20 to 77 years (mean $\pm$ SD=31.7 $\pm$ 14.26 years), with written informed consent from all individuals. All volunteers were presumed to be in good health. Our study was conducted in accord with the rules for human experimentation in our institution, and approved by our Research Ethics Commission (Protocol # 1.075.063/2015).

The samples were collected after discarding the first morning urine and collecting every other urine voided during the next 24 hours including the first morning urine of the day after. We utilized one vessel for the collection of the 24-hour sample and

Figure 1. Correlations of N1MN/Cr between first morning single void and 24-hr collections. (A) All study participants; (B) Females; (C) Males. N1MN/Cr: N-1-methylnicotinamide/creatinine.

a secondary vessel for the first morning single void. The vessels used for collection were prepared beforehand with outside protection from sources of light to lower the degradation of N1MN, and the addition of HCl 6N to acidify the urine and preserve the samples.

Methods

We analyzed the concentration of N1MN by high-performance liquid chromatography (HPLC) utilizing a Kinetex™ 5 µm C18 100×4.6 mm column. N-methylnicotinamide was used as the internal standard (IS) at a concentration of 200 mg/mL. Compounds were eluted isocratically with a flow rate of 1.5 mL/min using a mobile phase consisting of 10 mM dipotassium hydrogen phosphate, and 5 mM 1-octane sulfonate, (sodium form) in 10% aqueous acetonitrile (v/v) adjusted to pH 7.0 with phosphoric acid. This mobile phase was passed through a 0.45 µm membrane before use. N1MN and N-methylnicotinamide were detected at 264 nm and concentrations were calculated from a calibration curve prepared utilizing N1MN at concentrations of 20 mg/L; 10 mg/L; 5 mg/L; 2.5 mg/L; and 0.5mg/L. Samples were prepared adding 2 mL of urine aliquot and mixing with 1 mL of IS solution, and 7 mL of the mobile phase before being injected for analysis [6].

To normalize the effect of volume voids the concentration of N1MN was expressed as ratio to creatinine. Then, the concentration of creatinine was analyzed spectrophotometrically by Jaffe's method using a commercial kit.

A linear regression was set up to verify the associations. The normality of the data was verified by the Kolmogorov-Smirnov test. Because all data had normal distribution it was calculated the Spearman correlation coefficients.

Results

As seen in Figure 1, the N1MN/Cr ratio of first-morning void correlated significantly with the amount excreted in 24-hour urines ($Y=0.6244X+0.0053$, $r=0.6817$, $P<0.0001$). When the analysis was realized by gender, the N1MN/Cr ratio in females showed a strong correlation as well ($Y=0.5706X+0.0061$, $r=0.6020$, $P=0.0050$), and similar results were found in males, with a significant correlation between first-morning voids and 24-hourr urines ($Y=0.5293X+0.0060$, $r=0.6979$, $P=0.0248$). These results demonstrated that it was possible to utilize a first morning void instead of a 24-hour urine collection for estimation of N1MN excretion.

Discussion

The current common practice when using urine as a biomarker for vitamin excretion is to use a 24-hour sample for analysis. Due to the complications and difficulty involved in this process, we attempted to find an alternative solution in this study through use of a single first morning void. A single first morning void is a much more convenient method to use when estimating vitamin sufficiency, as it is simpler, quicker, and much more cost effective.

In our study, we restricted volunteers over 18 years of age, who were all presumed to be healthy individuals. Even though our results demonstrated a strong correlation between 24-hour urine and first morning voids, these results do not properly

demonstrate vitamin excretion in hospital patients. Several diseases that cause vitamin deficiency (e.g., renal failure, HIV, and bacterial infections) need to be tested to ascertain whether this is a valid method with applicability in hospital environments.

Conclusions

When considering the analysis of N-1-methylnicotinamide in urine, this study found that the usage of a single first morning void instead of a 24-hour collection was a valid collection method to use in healthy individuals. Further studies are needed to determine whether this method demonstrates applicability in hospital patients with varying pathologies.

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