First Void Urinary Calcium for Tracking Bone Loss and Kidney Stone Risk in Space

Semran Thamer; Jay C. Buckey

INTRODUCTION: Microgravity exposure unloads the skeleton. This increases urinary calcium excretion, which reflects both increased bone loss and kidney stone formation risk. We studied the probability that first morning void (FMV) urinary calcium (Uca) measurements would capture the highest Uca concentration in a day.

METHODS: For 8 wk, three men and three women collected void-by-void 24-h urine samples weekly. Uca concentration was analyzed using a calcein-based system. Uca concentrations were ranked among all samples from each person. FMV and non-FMV (nFMV) Uca concentrations were compared with a Mann Whitney *U*-test. The probability that an FMV would capture the highest Uca concentration in a day was assessed.

RESULTS: Among 377 voids collected, 46 were FMV and 331 were nFMV. Among all samples, the Uca concentration for FMV was significantly higher than nFMV ($P < 0.0001$). Out of the 46 FMVs, 24 were highest in Uca concentration for the corresponding 24-h period, giving a 52.2% probability that any given FMV would capture the highest Uca concentration in a day. The probability of measuring the highest Uca concentration from at least 1 d increased to 77.1%, and 89.1% when two or three FMVs were collected respectively.

DISCUSSION: Acquiring 2–3 repeated FMVs provides a high likelihood of capturing the highest Uca from a day. This suggests repeated first morning void Uca concentrations could assess the risk of bone loss and kidney stone formation, which may provide ability for real-time implementation of countermeasure programs to prevent bone and renal complications in prolonged spaceflight.

KEYWORDS: urinary calcium, bone loss, kidney stone risk.

Thamer S, Buckey JC. *First void urinary calcium for tracking bone loss and kidney stone risk in space*. Aerosp Med Hum Perform. 2022; 93(7):546–550.

Prolonged microgravity exposure leads to loss of muscle and bone mass.[4] Biomechanical unloading of the skeleton in weightlessness results in increased bone resorption, which alters metabolic calcium homeostasis.[18] Calcium lost from bone resorption decreases bone strength and increases the risk of developing kidney stones. This effect of microgravity on calcium homeostasis occurs rapidly and persists throughout a spaceflight.[29]

A negative calcium balance and increased urinary calcium excretion in space have been well documented since they were first studied during the Gemini and Apollo missions in the 1960s and early 1970s.[11,13] This observation has also been extensively supported by ground-based analogs, primarily with bed rest studies that have shown qualitatively similar increases in urinary calcium excretion, although to a lesser degree than that observed in spaceflight.[21,23] The observed increase in calcium excretion has been correlated with actual changes in bone density measured by densitometry using data from the Skylab and Mir missions.[16,30] Calcium loss from altered bone metabolism is excreted in urine where it can supersaturate and lead to renal stone formation.[10] More than half a century of research has now identified bone resorption as a key factor of space-induced bone loss, with secondary effects on calcium metabolism, which leads to an increased risk of renal stones.[24]

While several physical, nutritional, and pharmacological measures can counteract the risks of bone loss and kidney stone

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This manuscript was received for review in July 2021. It was accepted for publication in April 2022.

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DOI: https://doi.org/10.3357/AMHP.5979.2022

formation in space, no simple and reliable in-flight methods exist to monitor the effectiveness of these measures. Countermeasure effectiveness is currently evaluated postflight, which does not allow for the early identification of spaceflight-induced homeostatic calcium imbalances and limits the ability to personalize countermeasures based on an individual's in-flight risk.[19,24] An in-flight method to assess bone loss and kidney stone risk in space reliably may help mitigate these risks and provide real-time feedback about an individual's response to countermeasures.

Several techniques to assess bone loss and calcium metabolism exist. Bone densitometry is among the most widely used techniques to assess bone health in clinical practice and has been shown to be useful in research assessing weightlessness-induced changes specific to bone.[2,17] But it can only detect large changes in bone mineral density, which require several months to occur.[20] Furthermore, radiographic equipment is not available on most spacecraft and densitometry provides no information about calcium excretion. Instead, novel techniques to assess changes in bone metabolism rapidly and quantitively are needed for evaluating this in space.

Several biochemical markers derived from collagen breakdown have been proposed as reliable measures of bone resorption. These include deoxypyridinoline, pyrinoline, carboxyterminal telopeptide of type 1 collagen, and urine amino-terminal crosslinked telopeptides of collagen 1.[9,21] Research suggests that measuring these markers in space may be useful in determining rapid alterations in bone metabolism in microgravity. These tests, however, require some sample handling and reagents and so are not currently suitable for space applications, where time and mass are very limited. Also, their utility is limited in assessing kidney stone risk, to which bone resorption is inextricably linked.[21]

A simple approach to measure both bone resorption and renal stone formation is by measuring changes in urinary calcium. Previous research has shown that biochemical markers of bone resorption like deoxypyridinoline and pyrinoline correlate with increased urinary calcium excretion.[4,5,21] Additionally, urinary calcium excretion has been shown to be inversely correlated with femoral neck and spine bone mineral densities, suggesting a relationship between the two.[1,6,15] Urinary calcium excretion has been used in many studies evaluating bone loss in bed rest and the use of bone loss countermeasures.[7,22,26–28] Bedrest studies have shown that 24-h calcium excretion measurements reliably predict bone loss, but measuring 24-h excretion requires both urine volume and urine calcium determinations, which are challenging to measure in space.[14] Unlike 24-h urine calcium excretion, urinary calcium concentration in a single void could likely be measured easily using simple and inexpensive equipment.

The challenge of using urinary calcium concentrations from single voids is that urinary calcium concentration in individual voids can vary substantially during the day depending on hydration, activity, and diet. Research has shown that random spot urine collections do not correlate well with 24-h urinary calcium excretion and may underestimate calcium loss in urine.[8] First morning voids, however, often reflect urinary calcium excretion during the night when an individual is not eating or drinking. These voids have a tendency to have some of the highest urinary calcium measurements during the day and the urinary concentration in these voids correlates with 24-h urinary calcium excretion.[12] The ratio of calcium to citrate concentrations also tends to be highest in the early morning, which is a high-risk time of day for kidney stone formation.[3] Therefore, determining urinary calcium concentration in the first morning void may be a promising method for determining the risk of bone loss and kidney stone formation and may inform countermeasure programs when urinary calcium is substantially increased or decreased in flight.

To use first morning voids effectively, however, requires some knowledge on how often these voids have the highest urinary concentration in a given day and how many repeated first morning voids would be needed over several days to get a reliable estimate of the highest urinary calcium concentration. In this research, we studied first morning urine samples to assess: 1) the probability that an individual first morning void would capture the highest urinary calcium concentration in a 24-h period; and 2) how many repeated first morning voids would be needed to capture the highest urinary calcium concentration from an individual. These data are important for using first morning voids as a practical indicator for measuring bone loss and kidney stone risk in space.

METHODS

This study was reviewed and approved by the Dartmouth College Committee for the Protection of Human Subjects and informed consent was obtained from each subject. Six subjects, three men and three women, ages 30–56, were enrolled in this study. Subjects who reported any history of derangements in calcium metabolism or if they were taking any drugs that could influence calcium excretion were excluded.

The data collection procedures have been described elsewhere but are summarized here.[14] All subjects collected void-by-void urine samples over a 24-h period once a week for 8 wk. The collection began with the second void of the first day and concluded with the first void the following morning. The collection period typically began on Thursdays and concluded the following Friday morning. All samples were analyzed the same day. If a subject was unable to provide urine on the usual Thursday/Friday, Sunday/Monday was reserved as an alternative collection period. All subjects were instructed to continue with their usual dietary habits and routines. This allowed for the assessment of the normal variability of human calcium excretion without dietary control, as would be the case before and during spaceflight. During weeks 7–8, subjects consumed calcium carbonate 500 mg and Vitamin D 400 IU to assess how an acute change in the dietary intake of calcium might affect the urinary calcium excretion.

Each urine sample was collected into a separate 1-L Nalgene container with the date and time recorded on printed labels. Samples were refrigerated until they were delivered to Dartmouth-Hitchcock Medical Center for analysis. Calcium content in the samples was assessed using a calcein-based

Table I. Mean 24-h Urinary Calcium Excretion for Each Subject.

	GENDER	MEAN 24-h Uca (mg · d^{-1})	MINIMUM 24-h Uca (mg · d^{-1})	MAXIMUM 24-h Uca (mg · d^{-1})
Subject 1	M	280.7	159.3	379.7
Subject 2	M	144.8	95.2	214.8
Subject 3	M	235.2	156.4	335.5
Subject 4	F	209.3	118.7	295.9
Subject 5	F	181.6	137.8	257.7
Subject 6	F	249.9	122.8	361.4

Uca: urinary calcium.

prototype urinary analysis system developed by Creare LLC. This system used a fluorescent indicator method where predetermined concentrations of calcein were mixed with urine.[9] The samples were passed through an optical excitation cell and optical measurements were made by exciting the mixture of urine and calcein at a wavelength of 480 nm. The strength of the resultant fluorescent signal was proportional to the concentration of dissolved ionized calcium in the urine. All data were expressed as a concentration in mg · L^{-1}. Urine calcium concentration was not normalized for grams of creatinine because obtaining creatine measurements in space would not be feasible with this system.

A first morning void was defined as the first void collected in the morning at the end of the 24-h collection period which began the previous morning. Urinary calcium concentrations were ranked among all samples from each person and an average rank for first morning voids and nonfirst morning voids (all other samples) were calculated. All voids in a day for each subject were ranked intraindividually, per subject rather than between subjects, to isolate possible confounders like gender, interindividual dietary variability, and baseline calcium excretion. A Mann Whitney *U*-test was conducted to compare urinary calcium concentrations between all first morning voids and nonfirst morning voids. The probability that first morning voids would capture the highest urinary calcium concentration in a 24-h collection period was also analyzed for a single sample as well as for two and three samples by calculating the frequency with which a first morning void and nonfirst morning void was the highest ranked (highest calcium concentration in the 24-h collection period). We assumed that the result of each first morning void collection was an independent event with a binomial outcome (highest urinary calcium concentration of the day or not the highest); the probability of capturing the highest urinary calcium for multiple collections was calculated by using $1 - (1 - p)^n$ where p represents the probability of capturing the highest urinary calcium concentration in a day and n represents the number of measurements. All analyses were conducted using Excel and Matlab 2020a (Mathworks, Natick, MA, USA).

RESULTS

The mean urinary calcium concentration was 92.4 mg · L^{-1} (SD = 80.1) for all 377 voids collected; 128.8 mg · L^{-1} (SD = 83.9) for all 46 first morning voids and 87.3 (SD = 78.4) for the 331 nonfirst morning voids. The oral calcium and Vitamin D supplementation during weeks 7–8 did not increase urinary calcium excretion levels significantly. **Table I** shows the mean 24-h urinary calcium excretion measurements for each subject. Urinary calcium concentrations among first morning voids were significantly higher than nonfirst morning voids ($z = -6.36$, $P < 0.00001$). The average rank for all first morning voids was 2.08 (SD = 1.50) and 5.28 (SD = 2.83) for all nonfirst morning voids, where 1.0 represents the highest urinary calcium concentration captured during the 24-h collection period. Of the 46 first morning voids collected, 24 were ranked highest in urinary calcium concentration for the corresponding 24-h collection period (**Table II**). The probability that any given first morning void would represent the highest urinary calcium concentration in a 24-h period was 52.2%. The probability increased to 77.1% and 89.1% when two or three first morning voids were collected, respectively.

DISCUSSION

To the best of our knowledge, this is the first study evaluating how first morning voids could be used as a potential indicator of bone loss and kidney stone risk. The probability that a first morning void captured the highest concentration of urinary calcium in a day was high and increased substantially when multiple first morning voids were collected. When three first morning voids are collected, the probability that one of the collections would capture the highest urinary calcium concentration from a day was almost 90%, which is likely sufficient for operational use.

Our previous research has shown that first void urinary calcium significantly correlates with 24-h urinary calcium concentration and may, therefore, be a useful method for estimating

Table II. First Morning Voids Ranked by Urinary Calcium Concentration for Each Subject by Week (1 is Highest Rank Among Total Number of Voids Collected During a 24-h Period).

	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	WEEK 7	WEEK 8	MEAN FMV RANK
Subject 1	1 out of 10	7 out of 11	1 out of 8	6 out of 9	2 out of 11	1 out of 9	1 out of 12	1 out of 7	2.5/77
Subject 2	3 out of 9	1 out of 8	1 out of 8	1 out of 9	4 out of 8	2 out of 8	3 out of 9	1 out of 6	2.0/65
Subject 3	1 out of 8	1 out of 8	6 out of 8	2 out of 5	1 out of 7	1 out of 7	3 out of 8	2 out of 5	2.1/56
Subject 4	3 out of 9	2 out of 10	1 out of 7	1 out of 8	2 out of 7	3 out of 7	3 out of 6	1 out of 5	2.0/60
Subject 5	1 out of 11	2.0 out of 14	1 out of 13	5 out of 11	2 out of 9	1 out of 10	1 out of 12	1 out of 10	1.8/90
Subject 6	1 out of 4	1 out of 4	1 out of 3	2 out of 4	2 out of 4	4 out of 4	3 out of 4	3 out of 3	2.1/30

FMV: first morning void.

24-h urinary calcium excretion.[14] The advantages of using first morning voids rather than 24-h urine collections are that determining first void urinary calcium does not require measuring urine volumes or collecting urine over a 24-h period, which is not practical in spaceflight. A first morning void calcium measurement, however, may be achievable using simple and inexpensive equipment. This study did not attempt to control either dietary intake of calcium or fluid intake and so reflect the variability in urinary calcium measurements that might be expected in "real-world" conditions. Nevertheless, the natural variability of urinary calcium may be smaller in the controlled environment of space, where the dietary choices are limited, suggesting that the results of this study may have under-estimated the utility of measuring several first morning voids to capture the highest urinary calcium concentration in a day.

The risk of bone loss and kidney stone formation are critical barriers that need to be overcome for successful long duration spaceflight. Bone loss and kidney stone formation in space can potentially pose a high risk to the health of crewmembers in prolonged spaceflight and could reduce the success of future missions. Exposure to microgravity has been shown to decrease bone mass at a rate approximately 10 times that of osteoporosis.[17] While the exact etiology of bone loss in space has not been fully established, it is likely related to increased bone resorption and possibly decreased bone formation associated with reduced loading stimuli.[25] The resulting loss of bone density poses a significant long-term risk to astronauts exposed to prolonged spaceflight.

While kidney stones are commonly treated successfully on Earth, their formation during spaceflight can be problematic. Due to altered bone metabolism and unbalanced bone resorption in space, urine becomes supersaturated because of increased urinary excretion of calcium. In turn, the risk of kidney stone formation is intimately linked to hypercalciuria associated with imbalanced bone remodeling in space. Previous studies have shown that negative calcium balance observed during Skylab and Mir missions were attributed to increased urinary calcium excretion, which is the major contributor to the increased risk of kidney stone formation observed in spaceflight. Serum concentrations of total and ionized calcium had little to no change.[20] Thus, using urinary calcium measures may be a reliable method of evaluating these risks.

Preventing kidney stones and bone loss is much more efficacious and feasible than trying to recover lost bone after a mission or treating a kidney stone is space. Currently, the countermeasures to decrease and address the risk of bone loss and kidney stone formation are not individualized. Interindividual variability in the skeletal response to microgravity exists and a set countermeasure program may be more than needed for some and ineffective for others. Instead of assessing the effectiveness of countermeasures postflight, simple urinary calcium measurements may allow this to be done in flight in real time.

This study has some limitations. The number of individuals studied was small and these individuals may not represent the population of astronauts accurately. Further study is needed to confirm these results on a larger number of people. We also assumed that the probability of the first morning void being the highest during the day was similar between individuals. There may be individuals where this is not true. Dietary intake and hydration status, which can affect urinary calcium excretion, were not controlled in this study. While this was intentional to show the natural variability of urinary calcium excretion, more research on the effects of diet and hydration on urinary calcium excretion in first morning voids is needed. Nevertheless, this study demonstrates that first morning voids often have the highest urinary calcium values during the day and deserve further study as a monitoring technique.

Monitoring multiple first morning void urinary calcium concentrations over time may be an effective and reliable method for assessing the in-flight risk of bone loss and kidney stone formation. Measuring first morning void urinary calcium concentration may assess these risks in real time and enable individualized countermeasure programs as needed while also allowing for the assessment of the efficacy of these countermeasures. Further research comparing the efficacy of first morning voids and 24-h calcium measures may provide more further information about the utility of this proposed method.

ACKNOWLEDGMENTS

We thank Donna Alvarenga for the data collection done on this project. Dave Kynor, Darin Knaus, and their team at Creare, LLC, developed the device used for the analysis. We appreciate the time and effort of all the individuals involved in the study.

Financial Disclosure Statement: The authors have no competing interests to declare.

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