Urea and Minerals Monitoring in Space Missions by Spot Samples of Saliva and Urine

Giancarlo Bilancio; Pierpaolo Cavallo; Cinzia Lombardi; Ermanno Guarino; Vincenzo Cozza; Francesco Giordano; Massimo Cirillo

BACKGROUND: Microgravity induces redistribution of body fluids and reductions in muscle and bone mass. These effects correlate with changes in lab test results, including urea and bone minerals. Difficulties with collecting blood and urine during space missions limit the available data. This pilot study investigated metabolic changes during a space mission using untimed spot samples of urine and saliva. Untimed spot urine was used for urinalysis with data normalization per creatinine concentration. Saliva was proven useful as an index of serum urea and phosphorus.

METHODS: Two astronauts collected urine and saliva samples 75 ± 5 d before launch (baseline) and 3–5 times during a 6-mo space mission. Samples were collected 3 h after morning breakfast. Urine was collected using a standard NASA device. Saliva was collected using a Salivette™ synthetic swab. Samples were kept frozen using automated biochemistry until lab work-up. Anthropometric data were collected at baseline and after the mission.

RESULTS: For astronauts 1 and 2, respectively, total bone mineral density decreased (−1.4% and −0.9%). In-flight changes were as follows: transiently decreased urine urea/creatinine ratio (−32% and −24%), transiently decreased urine phosphorus/creatinine ratio (−52% and −30%), increased urine calcium/creatinine ratio (up to +116% and +27%), and transient increases in saliva urea (up to +48% and +195%) and phosphorus (up to +29% and +46%). The astronaut with greater changes in urine minerals had greater reduction in bone mineral density.

DISCUSSION: The results support the hypothesis that untimed samples of urine and saliva are useful for investigation of metabolic changes during space missions. Changes in urine and saliva minerals suggested down-regulation of parathyroid gland activity during the space mission.

KEYWORDS: urine, saliva, urea, phosphorus, calcium, space mission.

Bilancio G, Cavallo P, Lombardi C, Guarino E, Cozza V, Giordano F, Cirillo M. *Urea and minerals monitoring in space missions by spot samples of saliva and urine.* Aerosp Med Hum Perform. 2019; 90(1):43–47.

Spaceflight and microgravity induce several physical changes, including a reduction in bone mass and/or in bone mineralization, a reduction in skeletal muscle mass, and a reduction in the body fluids with redistribution from the caudad to the cephalad body segments.[12,14,18] Some of these changes can be partially mimicked by bed rest or other terrestrial microgravity models.[8,12] Secondary to or in association with the physical changes, there are changes in lab test results that reflect changes in bone metabolism, protein catabolism, and hydration status.[8,12] Increases in urine calcium, serum phosphorus, and serum urea were consistent changes that accompanied the reductions in bone mineralization, muscle mass, and plasma volume in the course of collaborative 35-d bed rest experiments.[4,5,15] Demonstrating changes in lab test results and defining the time-course of these changes is difficult during space missions because of obvious limitations in collecting, storing, and transporting biological samples. These limitations could be reduced using untimed spot samples of urine and saliva. The use of untimed spot urine samples has progressively replaced the use of timed urine collection in medical practice on the basis of the evidence that the urinary excretion of a given analyte can be assessed in untimed urine samples as the ratio between the concentration of the analyte and the concentration of creatinine.[7,13,16] Significant correlations of saliva with serum levels were reported for small molecules including urea and

From the Dept. "Scuola Medica Salernitana", University of Salerno, Baronissi (SA), Italy.

This manuscript was received for review in June 2018. It was accepted in September 2018.

Address correspondence to: Prof. Massimo Cirillo, Dept. "Scuola Medica Salernitana", Via Salvador Allende 43, 84081 Baronissi (SA), Italy; mcirillo@unisa.it.

DOI: https://doi.org/10.3357/AMHP.5200.2019

Table I. Selected Individual Values of Anthropometrical Indices: Preflight Data (Baseline) and Postflight Changes Over Baseline (Change).

			CHANGE OVER BASELINE	
	ASTRONAUT	BASELINE	ABSOLUTE	%
Lean body mass at DXA*, kg	1	64.4	+2.2	+3.42%
	2	41.3	−1.7	−4.11%
Leg muscle area at pQCT†, cm^2	1	72.8	−3.0	−4.12%
	2	65.3	−14.0	−21.44%
Total body BMD‡ at DXA*, g/cm^2	1	1.42	−0.02	−1.41%
	2	1.14	−0.01	−0.88%
Cranial BMD‡ at DXA*, g/cm^2	1	2.50	−0.01	−0.40%
	2	2.39	−0.05	−2.09%
Calcaneal BMD‡ at DXA*, g/cm^2	1	0.80	−0.03	−3.75%
	2	0.62	−0.03	−4.84%
Leg total BMD‡ at pQCT†, mg/cm^3	1	419	−7	−2.93%
	2	313	−23	−7.34%
Leg cortical BMD‡ at pQCT†, mg/cm^3	1	931	0	0.00%
	2	910	−3	−0.33%
Leg trabecular BMD‡ at pQCT†, mg/cm^3	1	269	−3	−1.11%
	2	194	−19	−9.79%

* DXA = dual-energy X-ray absorptiometry; †pQCT = peripheral quantitative computed tomography; ‡BMD = bone mineral density.

phosphorus,[11] even after freezing/thawing.[3] Therefore, in collaboration with the Italian Space Agency (Agenzia Spaziale Italiana) and the National Aeronautics and Space Administration (NASA), the present study was designed as a pilot investigation of urea and minerals in untimed spot samples of urine and saliva during a 6-mo space mission.[6]

METHODS

This observational study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the local institutional ethical committee (no. 42/2013) and by the institutional review boards of NASA (Protocol number Pro0506; study title: "Bone-Muscle Check," formerly "Check Saliva"; NASA IRB protocol number CR00000634) and the European Space Agency (Medical Board Meeting #2015-06). The participants were one male and one female astronaut, ages 52 and 37 yr, one from NASA and one from the European Space Agency, and each signed an informed consent agreement.

The study design consisted of three phases: preflight (baseline), in flight, and postflight. The preflight baseline phase took place at the Clinical Lab at NASA-Johnson Space Center (JSC), Houston, TX. It was performed 75 ± 5 d before launch and consisted of collections of saliva and urine samples and of measurements of anthropometrical indices. The in-flight phase took place on board the International Space Station (ISS) and consisted only of collections of saliva and urine samples at 3–5 timepoints during the 6-mo mission starting from day 15 ± 5 after launch. The postflight phase took place at JSC and consisted of measurements of anthropometrical indices 7 ± 1 d after return to Earth.

Baseline and in-flight untimed spot samples of saliva and urine were collected about 3 h after the completion of breakfast, without stimulation of salivation, and with the use of a synthetic swab (Salivette, Sarstedt, Denmark) placed for 2 min in the vestibulum oris. Stimulated salivation was not used because of the limited time available during the space mission and restrictions of the astronauts' schedules. The swab was used to overcome difficulties in saliva collection during microgravity. Right after completion of saliva collection, urine collection was performed using the standard NASA hardware. Samples of saliva and urine were rapidly frozen after collection either at JSC or onboard the ISS. Frozen samples of saliva and urine collected onboard the ISS were transported to Earth by a SpaceX Dragon capsule and successively to JSC, and finally to the authors' lab in Italy together with the frozen samples collected at the JSC.

After the set-up of the procedures for measurements of urea and phosphorus in saliva,[3] frozen samples of urine and saliva were thawed at 2–4°C, centrifuged for 5 min at 3000 g, and processed using automated biochemistry and commercially available kits (Abbott, Santa Clara, CA) for measurements of creatinine, urea, phosphorus, and calcium in urine, and for measurements of urea and phosphorus in saliva. Urine excretion of analytes for analysis, i.e., urea, phosphorus, and calcium, was assessed as the urine ratio between the analyte concentration and the creatinine concentration given that urine creatinine excretion tends to be constant over time and can be used to reduce the confounding of urine concentration.[7,13,16]

Preflight and postflight anthropometrical indices were derived from dual-energy X-ray absorptiometry and of peripheral quantitative computed tomography (pQCT). Dual-energy X-ray absorptiometry and pQCT were performed at the JSC Clinical Lab using Hologic Discovery (Hologic, Marlborough, MA) and XCT 3000 (Stratec Medizintechnik, Pforzheim, Germany), respectively.[18]

Results were expressed as percent (%) change over baseline to facilitate quantitative comparisons among different variables. The presentation of results did not include statistical analyses given the low number of participants in the study.

RESULTS

Table I reports baseline and postflight changes in selected anthropometrical indices. Leg muscle area at pQCT decreased in both astronauts, but the percent decrease was 5.20 times greater in the astronaut who also experienced reduction in lean body mass. A decrease in bone mineral density was found in both astronauts and was greater in lower body segments (calcaneal scan) as compared to upper body segments (cranial scan). Demineralization

Table II. Individual Baseline Preflight Values of Urine and Saliva Lab Tests.

	ASTRONAUT	
	1	2
Urine creatinine, mmol · L^{-1}	16.98	5.05
Urine urea, mmol · L^{-1}	445	138
Urine urea/creatinine ratio, mmol · $mmol^{-1}$	26.2	27.3
Urine phosphorus, mmol · L^{-1}	9.46	2.84
Urine phosphorus/creatinine ratio, mmol · $mmol^{-1}$	0.557	0.562
Urine calcium, mmol	3.68	0.981
Urine calcium/creatinine ratio, mmol · $mmol^{-1}$	0.217	0.194
Saliva urea, mmol · L^{-1}	33.0	25.1
Saliva phosphorus, mmol · L^{-1}	15.9	14.0

Multiplier for conversion from mmol · L^{-1} to mg · L^{-1}:
for creatinine = 113.12
for urea = 60.06
for phosphorus = 30.97
for calcium 40.08
Multiplier for conversion from mmol · $mmol^{-1}$ to mg · g^{-1}:
for urea/creatinine ratio = 531
for phosphorus/creatinine ratio = 274
for calcium/creatinine ratio = 353.

in the trabecular bone, more so than the cortical bone, contributed to the reduction in leg total bone mineral density at pQCT.

Table II reports baseline data in urine and saliva markers. Urine data differed substantially between the astronauts due to the expression of urine concentration: they were different when expressed as absolute units, but similar when expressed as a ratio to creatinine concentration.

Data from Untimed Spot Urine

A total of 10 untimed spot samples of urine and saliva were obtained: 4 samples in astronaut 1 (baseline and days 26, 66, and 128 of the mission; 6 samples in astronaut 2: baseline and days 16, 30, 58, 119, and 160 of the mission). **Fig. 1** shows the time course of % changes in urine excretion of urea, phosphorus, and calcium expressed as ratio to creatinine concentration. In both astronauts, urine urea excretion decreased by approximately 25–35% at the first time point of the mission (day 16 and 26 of the mission), but, after that, reapproached baseline and fluctuated around ±10% of baseline up to the end of the mission. Changes in urine phosphorus excretion were a transient decrease with a nadir around day 60 of the mission in both astronauts. The lack of data after day 128 for astronaut 1 made it impossible to assess whether urine phosphorus returned to or exceeded baseline by the end of the mission as in astronaut 2. Urine calcium excretion increased in both astronauts, but the trajectory of the urine calcium/creatinine ratio differed between the astronauts. In astronaut 1, the increase in urine calcium excretion was progressive and mild, approaching +30% by day 128. In astronaut 2, the increase was fast and much greater, exceeding +100% at days 30 and 58.

Data from Untimed Spot Saliva

Fig. 2 shows the time course of percent changes in saliva concentrations of urea and phosphorus. Saliva calcium data were not reported because 7 of the 10 saliva samples had calcium concentrations below the detection limit of the present methods (0.3 mmol · L^{-1}). There was a transient increase in saliva urea in both astronauts, but the magnitude and the duration of the increase differed substantially between the astronauts. In astronaut 1 the increase in saliva urea was great, exceeding +100% of baseline at days 66 and 128. In astronaut 2 the increase in saliva urea was mild and transient, peaking close to +50% of baseline at day 30, but returning to baseline thereafter. The trajectories of the changes in saliva phosphorus differed between the astronauts. In astronaut 1, there was a transient decrease at day 28 that was followed by an increase over baseline at days 66 and 128 (approximately +30–40%). In astronaut 2, the changes in saliva phosphorus were a progressive increase up to day 30 (approximately +65%) followed by minor fluctuations above baseline until the end of the mission.

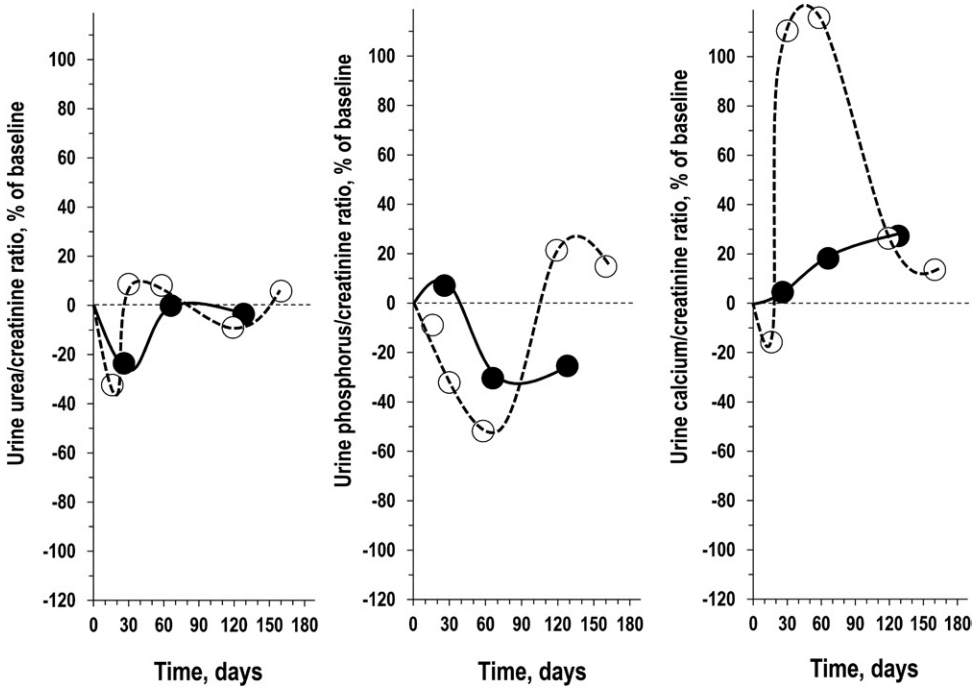

Fig. 1. Changes in urine urea/creatinine ratio (left panel), urine phosphorus/creatinine ratio (center panel), and urine calcium/creatinine ratio (right panel) during a space mission in astronaut 1 (black circles and straight line) and astronaut 2 (white circles and dotted line). Data are presented as % change over baseline. Absolute values at baseline are in Table II. Absolute values during the space mission can be calculated as % change in time absolute values at baseline.

Changes in Saliva Analytes Compared to Changes in Urine Analytes

Time-matched analyses of changes in saliva and changes in urine indicated that the increase in saliva urea correlated with a decrease in urine urea in astronaut

2 but not in astronaut 1, in whom a large increase in saliva urea persisted even in the presence of normal urine urea excretion. Vice versa, increases in saliva phosphorus tended to correlate with decreases in urine phosphorus in both astronauts. Due to lack of reliable data for saliva calcium, time-matched analyses for calcium were not recorded.

DISCUSSION

Based on a review of the literature, the authors believe this is the first study to report data of concentrations of urea, phosphorus, and calcium in untimed spot samples of urine and saliva in astronauts during a long-term space mission. In both astronauts the set of urine data indicated a trend toward a transient decrease of urine urea excretion in the first month of the mission, a trend toward a decrease of urine phosphorus excretion, and a trend for an increase in urine calcium excretion lasting at least up to the second month of the mission. In both astronauts the set of saliva data indicated a trend toward transient increases both in saliva urea and in saliva phosphorus. Time course analyses of these changes indicated that the increases in saliva urea could be due to temporary decreases in urine urea excretion, whereas the transient increases in saliva phosphorus correlated consistently with decreases in urine phosphorus excretion.

This pilot study had obvious limitations due to the low number of astronauts and samples. For urine data, biases and limitations should have been minor, if any, given that the study protocol consisted of procedures which are common in general medicine and include the use of untimed spot urine and of data normalization for creatinine concentration.[7,13,16] Another limitation could have been biases due to freezing, transporting, and thawing saliva samples from the ISS to the authors' lab. The freezing/thawing procedures could have caused an underestimation of saliva concentrations of about 12% for phosphorus and of about 6% for urea.[3] Saliva collected early morning after an overnight fast has higher concentrations of urea and phosphorus because saliva dilution is lower in the early morning hours.[3] It is unknown whether midmorning post-breakfast values are a more reliable index of average daily saliva concentrations in comparison to early morning fasting values. The time for saliva collection was the only possible solution, given the restraints of the astronauts' schedules at baseline and during the space mission.

The lack of previous data concerning saliva urea and saliva phosphorus in astronauts prevented a comparison to previous studies for these parameters. For other data, the comparison to previous studies should be done with caution because all biological samples of this study were collected midmorning 3 h after breakfast, a difference that could be relevant considering the circadian rhythms of most biochemical variables. Given that saliva and serum levels are correlated for urea and phosphorus,[3] the findings of increases in saliva urea and saliva phosphorus were consistent with the transient increases found in serum levels of urea[4,10] and phosphorus[5,12,14] during space missions and bed rest studies. The time-course of increase for calcium in urine was consistent with previous observations during space missions and bed rest studies.[5,12,14]

The results of the present study support the idea that untimed spot samples of urine and saliva can be useful in the noninvasive monitoring of metabolic changes during space missions. Regarding urea, the present observation of decreased urine urea excretion in both astronauts together with increased saliva urea pointed to a decrease in renal excretion of urea during the first month of the mission. Different labs reported that saliva urea can be used as a reliable index of serum urea given the consistent and strong correlation of serum urea with saliva urea.[3,11] Therefore, the first month of the mission should have been characterized by a decrease in renal urea excretion, at least in the midmorning, that in turn led to secondary increases in serum urea and saliva urea. The

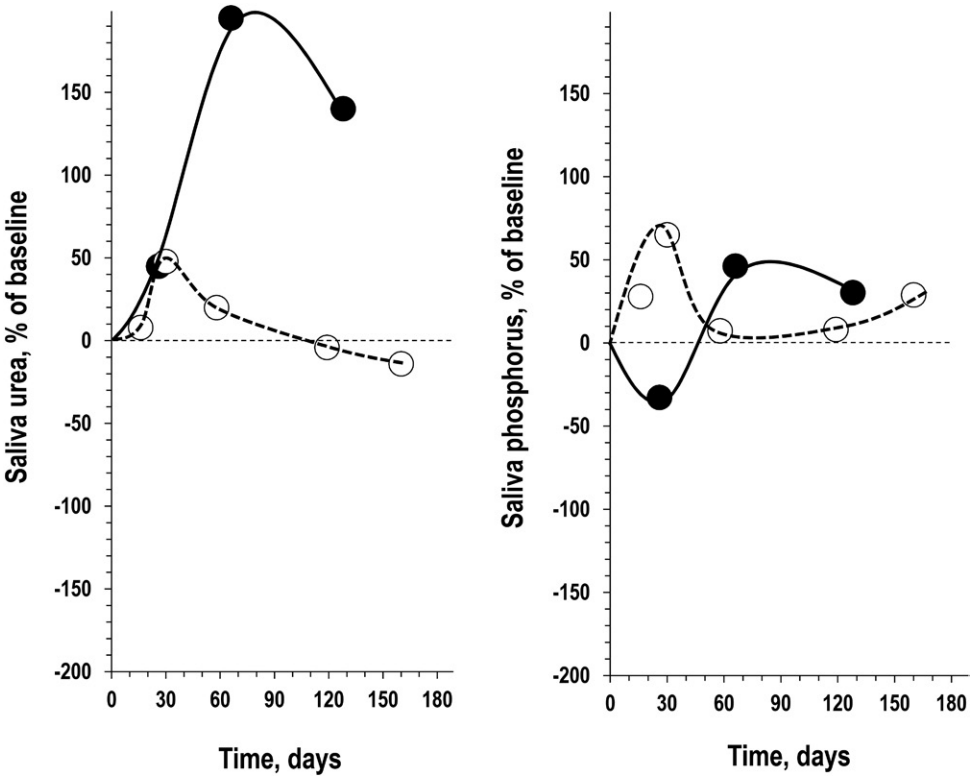

Fig. 2. Changes in saliva urea (left panel), saliva phosphorus during space mission in astronaut 1 (closed circles and straight line) and astronaut 2 (open circle and dotted line). Data are presented as % change over baseline. Absolute values at baseline are in Table II. Absolute values during space mission can be calculated as % change time absolute value at baseline.

alternative interpretation that a decrease in urine urea excretion reflected a decrease in urea generation is unlikely because lower urea generation does not correlate with increases in serum urea and saliva urea in the absence of reduced renal urea excretion. The persistence of a large increase in saliva urea in astronaut 1 must be due to other mechanism(s), given that the urine urea excretion of astronaut 1 returned to baseline after the first month of the mission. A reasonable hypothesis is that low hydration could have a major role in the late changes of saliva urea in astronaut 1 because hydration level affects serum urea.[1]

Regarding bone minerals, the changes in urine excretion for both astronauts appeared to be opposite, with a decrease for phosphorus and an increase for calcium. The greatest changes in the urine excretion both of phosphorus and calcium were observed in astronaut 2, who also had the greatest post-mission loss in bone mineralization in the leg and the calcaneus. Thus, it can be speculated that changes in the urine levels of phosphorus and calcium could be used to monitor the risk of bone demineralization during the mission. To our knowledge, the sustained changes in urine excretion of phosphorus and calcium during the mission could only be explained by a persistent reduction in the secretion of parathyroid hormone, which is capable of inducing increases in urine phosphorus excretion simultaneously with decreases in urine calcium excretion through opposite effects at the kidney tubule.[9] Thus, the set of urine data showed a possible reduction in parathyroid hormone secretion in both astronauts. Coupling between the decreases in urine phosphorus excretion and the increases in saliva phosphorus further supported a reduction in parathyroid hormone secretion. In fact, a reduction in parathyroid hormone secretion is known to increase serum phosphorus[9] and high serum phosphorus is known to correlate with high saliva phosphorus.[3] Moreover, data from other missions and from bed rest studies are in accordance with the hypothesis of a decrease in parathyroid hormone secretion during spaceflight.[2,12,14] Theoretically, a decrease in parathyroid secretion could also have a role in the mission-induced loss of bone mass, considering the favorable effects of the hormone on bone mass in cases of immobilization and/or unloading.[17]

In conclusion, this pilot study reported a small set of new data about the use of untimed spot samples of urine and saliva for defining the time course and the magnitude of some metabolic changes during space missions. The results indicate that spot samples of urine and saliva exhibited changes in the levels of urea, phosphorus, and calcium that were consistent with the available knowledge about metabolic changes in microgravity. Altogether, the changes in urine phosphorus, saliva phosphorus, and urine calcium during the mission suggested a down-regulation of parathyroid gland activity that could mark and perhaps contribute to bone mass reduction.

ACKNOWLEDGMENTS

The study was made possible thanks to a grant of the Italian Space Agency (Agenzia Spaziale Italiana, contract no. 2013–040–I.0).

The authors have no conflicts of interest to declare.

Authors and affiliations: Giancarlo Bilancio, M.D., Ermanno Guarino, M.D., Vincenzo Cozza, M.D., Francesco Giordano, M.D., and Massimo Cirillo, M.D., Dept. "Scuola Medica Salernitana", University of Salerno; Giancarlo Bilancio, M.D., and Massimo Cirillo, M.D., Nephrology Clinic, University Hospital, Salerno; Pierpaolo Cavallo, M.D., Dept. of Physics, University of Salerno; and Cinzia Lombardi, Ph.D., Rummo Hospital, Benevento, Italy.

REFERENCES

1. Armstrong LE, Kavouras SA, Walsh NP, Roberts WO. Diagnosing dehydration? Blend evidence with clinical observations. Curr Opin Clin Nutr Metab Care. 2016; 19(6):434–438.
2. Arnaud SB, Navidi M, Deftos L, Thierry-Palmer M, Dotsenko R, et al. The calcium endocrine system of adolescent rhesus monkeys and controls before and after spaceflight. Am J Physiol Endocrinol Metab. 2002; 282(3):E514–E521.
3. Bilancio G, Cavallo P, Lombardi C, Guarino E, Cozza V, et al. Salivary levels of phosphorus and urea as indices of their plasma levels in nephropathic patients. J Clin Lab Anal. 2018; 32(7):e22449.
4. Bilancio G, Lombardi C, Pisot R, De Santo NG, Cavallo P, Cirillo M. Effects of bed-rest on urea and creatinine: correlation with changes in fat-free mass. PLoS One. 2014; 9(9):e108805.
5. Bilancio G, Lombardi C, Pisot R, Mekjavic IB, De Santo NG, et al. Effects of prolonged immobilization on sequential changes in mineral and bone disease parameters. Am J Kidney Dis. 2013; 61(5):845–847.
6. Cirillo M, Bilancio G. Salivary markers of metabolic changes during space missions (check-saliva) - 11.22.16. [Accessed on August 30, 2018.] Available from: https://www.nasa.gov/mission_pages/station/research/experiments/312.html.
7. Cirillo M, Mellone M, De Santo NG. Can overnight urine replace 24-hour urine collection to measure urinary calcium in epidemiologic studies? Miner Electrolyte Metab. 1993; 19:385–388.
8. Coker RH, Wolfe RR. Bedrest and sarcopenia. Curr Opin Clin Nutr Metab Care. 2012; 15(1):7–11.
9. Felsenfeld AJ, Rodríguez M, Aguillera-Terero E. Dynamics of parathyroid hormone secretion in health and secondary hyperparathyroidism. Clin J Am Soc Nephrol. 2007; 2(6):1283–1305.
10. Grigoriev AI, Noskov VB, Popova IA, Vetrova EG, Markin AA, Strongonova LB. [Effects of a long-term space flight on human biochemical status.] Klin Lab Diagn. 1994; 1:19–22 [in Russian].
11. Lasisi TJ, Raji YR, Salako BL. Salivary creatinine and urea analysis in patients with chronic kidney disease: a case control study. BMC Nephrol. 2016; 17(1):10.
12. LeBlanc AD, Spector ER, Evans HJ, Sibonga JD. Skeletal responses to space flight and the bed rest analog: a review. J Musculoskelet Neuronal Interact. 2007; 7:33–47.
13. Ndzengue A, Ramaiah M, Wallick A, Balasubramony S, Kovesdy C. Use of spot urine urea/creatinine ratio to examine the association of dietary protein intake with kidney function in patients with CKD [Abstract #239]. Am J Kidney Dis. 2016; 67(5):A78.
14. Rambaut PC, Johnston RS. Prolonged weightlessness and calcium loss in man. Acta Astronaut. 1979; 6(9):1113–1122.
15. Rittweger J, Simunic B, Bilancio G, De Santo NG, Cirillo M, et al. Bone loss in the lower leg during 35 days of bed rest is predominantly from the cortical compartment. Bone. 2009; 44(4):612–618.
16. Robinson-Cohen C, Ix JH, Smits G, Persky M, Chertow GM, et al. Estimation of 24-hour urine phosphate excretion from spot urine collection: development of a predictive equation. J Ren Nutr. 2014; 24(3):194–199.
17. Sakai A. Anabolic action of PTH under the loading/unloading condition. Clin Calcium. 2012; 22:365–371.
18. Sibonga JD, Spector ER, Johnston SL, Tarver WJ. Evaluating bone loss in ISS astronauts. Aerosp Med Hum Perform. 2015; 86(12, Suppl.): A38–A44.