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Case Report

Stability of analytes related to clinical chemistry and bone metabolism in blood specimens after delayed processing☆

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Abstract

Objectives: We investigated the stability of 36 analytes related to clinical chemistry in a controlled storage study.

Design and methods: Blood was collected from 11 subjects and was maintained for 45 min, 2.5 h, 5 h, or 24 h after phlebotomy before centrifugation.

Results: Statistically significant changes were observed only for parathyroid hormone, osteocalcin, zinc, pyridoxal 5′-phosphate, and homocysteine.

Conclusions: These studies indicate that many analytes in clinical chemistry are stable for 24 h before centrifugation.

Keywords: Centrifugation; Clinical chemistry; Nutrition assessment; Nutritional status; Space flight

Introduction

As part of a study designed to assess the nutritional status of astronauts during long-duration space flight, in-flight phlebotomy sessions are performed on the International Space Station (ISS). Blood specimens are nominally centrifuged within 45 min of phlebotomy, and then stored at −80 °C. Because of the nature of the space missions, blood processing can unavoidably be delayed beyond the standard recommended 30- to 120-min timeframe. This recommended timeframe is based on data showing that changes in glucose, potassium, and lactate dehydrogenase occurred after 120 min of storage of whole blood at room temperature.

Prompt centrifugation is critical to preserve the integrity of blood samples when measuring certain analytes, including cholesterol, creatinine, potassium, calcium, and chloride [1–4]. Other analytes, such as triglycerides, plasma parathyroid hormone (PTH), sodium, and ferritin [5–7], have been shown to be unaffected by increased storage time (up to 48 h) before centrifugation. Prolonged contact of serum or plasma with red blood cells can result in an exchange of substances between serum and the cells, which can increase or decrease analyte concentration in serum. Hemolysis can result in altered values for similar reasons.

To our knowledge, no studies have been done to investigate the effects of delayed centrifugation on other markers of bone metabolism and clinical chemistry, including bone-specific alkaline phosphatase (BSAP), osteocalcin, and vitamin D.

In this study, we sought to determine the effects of delayed centrifugation on the concentrations of 36 analytes nominally measured during space flight as part of the nutritional status assessment experiment. The results presented here are data from a ground-based study designed to parallel the conditions on the

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ISS that could lead to delayed centrifugation. These data establish information about the integrity of samples that undergo delayed processing up to 24 h after phlebotomy.

Subjects and methods

Subjects

Eleven healthy subjects were recruited for the study. Five were recruited for the initial study and then 6 subjects were added to the study 1 y later. The findings from the initial study with 5 subjects indicated that meaningful results could be obtained by having more subjects but analyzing at fewer time points for some of the tests. The following analytes were measured 45–60 min and 24 h after phlebotomy: 25-hydroxyvitamin D, calcium, bone-specific alkaline phosphatase, retinol-binding protein, ceruloplasmin, cortisol, total antioxidant capacity, zinc, copper, iron, selenium, pyridoxal 5′-phosphate, homocysteine, folate, transferrin receptors, transthyretin, and methylmalonic acid. All others were analyzed after 45–60 min, 2.5 h, 5 h, and 24 h. All subjects fasted for 8 h before the blood collection. The protocol for this study was approved by the Johnson Space Center Committee for the Protection of Human Subjects.

Sample collection and processing

Blood samples (5 tubes per person) were collected into plastic BD Vacutainer® SST™ Plus Blood Collection Tubes (reference #: 367986, 5 mL, BD, Franklin Lakes, NJ) and stored at room temperature, shielded from light. Samples were centrifuged using a swinging bucket rotor at 1850 ×*g* for 15 min at different time points after the blood draw: 45–60 min, 2.5 h, 5 h, and 24 h after blood collection (some analytes were intentionally analyzed only at the first and last time points). The serum was removed and divided into aliquots that were frozen in cryogenic polypropylene vials (Corning, Lowell, MA) at −80 °C for subsequent analysis. Enough aliquots were made so that only one analyte was measured from each aliquot, thus minimizing the number of freeze–thaws for the sample.

Biochemical analyses

For most tests, analyses were performed at the Johnson Space Center. Most analyses were performed by standard commercial techniques, and all have been previously described in detail [8,9].

Statistical analyses

Markers of bone metabolism and clinical chemistry analytes were analyzed using a 1-way repeated-measures analysis of variance, with time as the dependent variable. For some analytes, such as homocysteine, 25-hydroxyvitamin D, and folate, statistical analyses were only performed to compare the 45–60-min and 24-h time points because of the larger sample size ($n=11$ compared to $n=5$ at the 2.5- and 5-h time points). For those 3 analytes with data from 5 subjects at 4 time points, the data are included in the results but statistical analyses were not done. For all analytes, the sample size included in the

Table 1
Serum concentrations of analytes from blood that was centrifuged and processed at different times after phlebotomy [a, b]

	45–60 min	2.5 h	5 h	24 h
25-hydroxyvitamin D, nmol/L	51±28	#	#	49±28
Alanine aminotransferase, U/L	16±7	16±7	15±5	16±6
Albumin, g/L	39±2	39±3	41±3	39±3
Alkaline phosphatase, U/L	57±14	58±14	57±18	56±16
Aspartate aminotransferase, U/L	20±4	19±4	19±3	20±4
Alpha 1 globulin, g/L	2±0	2±1	2±0	2±1
Alpha 2 globulin, g/L	7±1	7±1	7±1	7±1
Beta globulin, g/L	13±2	13±2	13±2	13±2
Bone-specific alkaline phosphatase (BSAP), U/L	25±11	#	#	25±12
Calcium, mmol/L	2.2±0.1	#	#	2.2±0.0
Ceruloplasmin, mg/L	290±100	#	#	300±120
Chloride, mmol/L	107±6	104±2	105±6	102±3
Cholesterol, mmol/L	5.2±0.9	5.3±0.7	5.2±0.9	5.2±0.9
Copper, μmol/L	18±7	#	#	19±7
Cortisol, nmol/L	569±244	#	#	570±222
Creatinine, μmol/L	80±9	75±8	76±10	77±11
Ferritin, pmol/L	148±127	201±184	200±167	207±185
Folate, nmol/L	42±36	#	#	47±38
Gamma globulin, g/L	11±3	11±2	11±2	11±3
Homocysteine, μmol/L	8±2	#	#	12±2**
Iron, μmol/L	19±7	#	#	18±5
Methylmalonic acid, mmol/L	172±38	#	#	181±40
Osteocalcin, nmol/L	2.5±0.7	2.4±0.8	2.3±0.7	1.6±0.5**
Parathyroid hormone (PTH), ng/L	71±27	68±24	73±22	60±19*
Potassium, mmol/L	4.3±0.4	4.2±0.4	4.3±0.4	4.2±0.3
Pyridoxal 5′-phosphate (PLP), nmol/L	97±56	#	#	113±72*
Retinol-binding protein, mg/L	55±14	#	#	55±7
Selenium, μmol/L	2.9±0.3	#	#	2.9±0.4
Sodium, mmol/L	143±7	140±2	142±6	140±3
Total protein, g/L	73±4	72±4	74±6	72±4
Transferrin, g/L	3.24±0.72	3.15±0.77	3.17±0.71	3.21±0.87
Transferrin receptors, mg/L	7±4	#	#	7±3
Transthyretin, g/L	0.28±0.06	#	#	0.27±0.05
Total antioxidant capacity, mmol/L	1.8±0.1	#	#	1.8±0.1
Triglyceride, mmol/L	1.9±1.7	2.0±1.8	1.9±1.7	2.2±1.7
Zinc, μmol/L	16±2	#	#	18±2**

[a] Values are means±SD of all subjects at each time point. $n=11$ for 25-hydroxyvitamin D and bone-specific alkaline phosphatase; $n=9$ for calcium, retinol-binding protein, ceruloplasmin, total antioxidant capacity, homocysteine, methylmalonic acid, transthyretin; $n=8$ for pyridoxal 5′-phosphate, and $n=10$ for all other analytes. The sample size was constant across time points.

[b] 45–60 min, and 2.5, 5, and 24 h are the times following phlebotomy at which blood was centrifuged. Samples remained at ambient temperature in the dark until they were centrifuged and processed. *$P<0.05$, **$P<0.001$. [#]Intentionally not analyzed at this time point because no samples were collected at those time points.