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Using natural, stable calcium isotopes of human blood to detect and monitor changes in bone mineral balance

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ABSTRACT

We are exploring variations in the Ca isotope composition of blood and urine as a new tool for early diagnosis and monitoring of changes in bone mineral balance for patients suffering from metabolic bone disease, cancers that originate in or metastasize to bone, and for astronauts who spend time in low gravity environments. Blood samples are often collected instead of, or in addition to, urine in clinical settings, so it is useful to know if variations in the Ca isotope composition of blood carry the same information as variations in urine. We found that the Ca isotope composition of blood shifts in the same direction and to the same magnitude (~2 parts per ten thousand — pptt) as that of urine in response to skeletal unloading during bed rest. However, the Ca isotope composition of blood is lighter than that of urine by 12 ± 2 pptt. This offset between blood and urine may result from Ca isotope fractionation occurring in the kidneys. This is the first study to confirm the suspected offset between the Ca isotope composition of blood and urine in humans, to directly quantify its magnitude, and to establish that either blood or urine can be used to detect and quantify bone loss.

Introduction

Measuring the natural Ca isotope composition of urine is a promising tool to detect and quantify changes in bone metabolism, based on findings from bed rest studies in which bone loss was induced by skeletal unloading. Urinary isotope data from a 17-week bed rest study indicated bone loss that was of an extent consistent with findings from dual-energy X-ray absorptiometry (DXA) scans of the same subjects [1]. Another bed rest study that lasted only 30 days, with more frequent sampling, showed that Ca isotopes began to shift within 10 days, presumably due to a shift in bone mineral balance to net bone loss. This interpretation agrees with an increase in N-telopeptide (NTx), a biochemical marker of bone resorption, beginning on day 15 [2]. These findings also fit with theoretical models that predict that variations in Ca isotopes should track changes in bone mineral balance [1–3].

The basis of this technique is that each of the six naturally occurring Ca isotopes reacts at a slightly different rate during phase changes or chemical reactions depending on the small differences in their masses ("mass-dependent isotope fractionation"). In particular, lighter Ca isotopes are preferentially incorporated into bone during its formation, depleting these isotopes in blood and other soft tissue. In contrast, there is no isotope preference during resorption of bone, which is a bulk dissolution process. As a result, the Ca isotope composition of blood shifts toward heavier values when bone mineral balance (BMB) is positive (i.e., bone formation exceeds resorption) and toward lighter values when BMB is negative [1–4].

Natural Ca isotope variations complement biochemical markers and radiological techniques in several important ways:

- Molecular biomarkers such as bone-specific alkaline phosphatase (BSAP) and NTx reflect bone resorption or bone formation, respectively [5,6]. These markers are useful for measuring bone remodeling activity, but cannot be combined into a single measurement of net BMB. In contrast, modeling of Ca isotopes indicates that they inherently monitor net BMB directly, and are insensitive to changes in bone remodeling rate [1–4].
- Ca isotopes can detect a shift to negative BMB very soon after it happens, weeks to months earlier than radiologically detectable changes in bone mineral density occur [1,2].
- Unlike other biomedical methods that use administered Ca isotopes [7–10], the technique of using natural Ca isotope variations does not employ radioactive or stable Ca isotope tracers. Instead we track changes in natural Ca isotope composition, which are detectable by high-precision mass spectrometry.

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Table 1
Ca isotope composition measured in blood from each subject organized by day of the bed rest study. Day −12 is 12 days before bed rest began. Values are reported relative to an ICP1 standard, $\delta^{44/42}$Ca (parts per ten thousand) ±2σ; n sample. Baseline is the average of days −12 and 1 through 4 with a ±1σ standard deviation.

Day	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Subject 9	Subject 10	Subject 11	Subject 12
Baseline	−9.4 ± 1.5	−7.9 ± 1.6	−8.2 ± 1.9	−10.2 ± 1.6	−10.3 ± 1.8	−7.9 ± 1.5	−8.0 ± 0.6	−7.7 ± 2.4	−11.7 ± 0.9	−10.9 ± 0.8	−9.9 ± 1.0	−10.6 ± 0.1
−12	−8.6 ± 1.9; 6	−8.8 ± 1.1; 4	−10.1 ± 1.0; 3	−9.8 ± 1.9; 4	−10.6 ± 1.1; 3	−6.3 ± 3.8; 6	−7.4 ± 1.6; 6	−10.1 ± 1.3; 3	−11.8 ± 2.3; 3	−11.4 ± 2.5; 3	−9.9 ± 1.1; 3	−10.6 ± 1.4; 3
1	−11.2 ± 1.1; 5	−8.9 ± 1.7; 4	−7.6 ± 1.8; 5	−10.8 ± 1.6; 6	−11.9 ± 1.1; 4	−8.1 ± 1.4; 3	−8.4 ± 1.6; 6	−5.3 ± 1.4; 3	−10.8 ± 1.7; 6	−10.0 ± 1.5; 4	[a]−11.3 ± 2.0; 4	
											[a]−11.4 ± 4.4; 3	
											[a]−9.4 ± 0.9; 4	
											[a]−8.7 ± 2.4; 4	
2				[a]−11.6 ± 1.7; 4								
				[a]−7.1 ± 2.8; 3								
				[a]−10.3 ± 1.3; 4								
				[a]−9.7 ± 1.4; 3								
3			[a]−6.4 ± 2.2; 3									
			[a]−9.4 ± 2.1; 4									
			[a]−5.8 ± 3.7; 4									
4	−8.5 ± 1.3; 6	−6.0 ± 1.9; 5	−10.1 ± 1.4; 4	−12.1 ± 2.1; 4	[a]−10.9 ± 1.4; 4	−9.2 ± 1.9; 3	−8.3 ± 1.2; 3	−7.6 ± 1.6; 4	−12.5 ± 1.3; 4	−11.4 ± 1.9; 4	−10.0 ± 1.0; 4	−10.5 ± 1.1; 6
					[a]−7.7 ± 2.5; 4							
6			−7.3 ± 1.4; 4								−7.6 ± 1.3; 3	
7				−6.7 ± 1.9; 3	[a]−12.9 ± 0.7; 4					−8.4 ± 1.9; 4		
					[a]−10.7 ± 2.4; 4							
					[a]−11.9 ± 1.3; 3							
					[a]10.0 ± 1.5; 4							
8	−10.4 ± 1.5; 6	−8.5 ± 1.9; 4	−8.2 ± 1.5; 4	−9.7 ± 1.7; 4	−11.8 ± 1.1; 4	−8.5 ± 1.6; 3	−10.5 ± 1.2; 4	−8.6 ± 1.9; 4	−10.5 ± 1.3; 4	−10.1 ± 2.4; 4	−12.4 ± 0.5; 4	
29	−12.6 ± 0.7; 4	−13.7 ± 5.5; 5	−9.7 ± 1.7; 5	−11.8 ± 1.5; 4	−13.5 ± 1.1; 4	−9.8 ± 1.3; 4	−12.0 ± 1.2; 3	−9.1 ± 1.5; 3	−13.2 ± 1.8; 3	−11.8 ± 0.9; 3	−12.0 ± 0.7; 4	−10.7 ± 1.0; 3
31												[a]−12.6 ± 2.1; 5
												[a]−14.5 ± 2.0; 1
												[a]−12.5 ± 0.6; 3
34								[a]−9.7 ± 1.8; 3				−8.0 ± 2.1; 4
								[a]−10.0 ± 2.0; 3				
								[a]−7.5 ± 1.5; 3				
37									[a]−9.5 ± 1.8; 3			
									[a]−14.1 ± 2.0; 4			
									[a]−9.1 ± 0.9; 3			

[a] These measurements are from separate blood samples drawn at different times on the same day.