Ca isotope fractionation during bone formation

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Naturally-occurring calcium (Ca) isotopes in human blood and urine reflect net bone mineral balance (BMB). They have been used to monitor cancer progression in multiple myeloma, and the extent of bone loss in astronauts and bed rest patients. However, no direct measurements of isotopic fractionation between actively forming bone and blood has been reported.

Precipitated calcium phases are isotopically lighter than co-existing solution. In contrast, dissolution does not fractionate, so soft tissue $\delta^{44/42}$Ca changes represent the balance between bone formation and resorption. When the formation rate exceeds resorption rate, blood becomes isotopically heavier due to preferential incorporation of isotopically light calcium in bone. This technique originated from the observed $\delta^{44/42}$Ca isotopic offset of ~0.70 ±0.05‰ between animals' diet and bone [1]. Heuser and Eisenhauer [2] estimated the offset between diet and bone in humans as ~-0.65‰ ($\delta^{44/42}$Ca), but measured an offset of -0.3‰ between blood and bone in minipigs [3]. Observations of diet-bone differences can not distinguish between a) isotopic fractionation during absorption during digestion, b) fractionation between different Ca-containing proteins and free Ca in blood and c) fractionation during bone formation.

To address this challenge, murine preosteoblasts (cell line MC3T3-E1) were cultured and passaged three times. Safranin staining confirmed Ca mineralization and mitosis, indicating the cells were actively producing mineralized matrix. We measured the $\delta^{44/42}$Ca value of the cells, media, protein fraction >60 kDa, and the Ca-free isotonic solution used to rinse the cells. The osteoblasts are significantly isotopically lighter than the media, while the protein fraction is only slightly isotopically lighter than the media. This suggests that a primary control on isotope fractionation in bone is the mineralization, not digestion or fractionation between free and protein-bound calcium.

[1] Skulan and DePaolo (1999) *PNAS* **96**:**24**, 13709-13714. [2] Heuser and Eisenhauer (2010) *Bone* **46**, 889-896. [3] Heuser *et al* (2007) *GCA* **71** (Suppl. 1):A402.