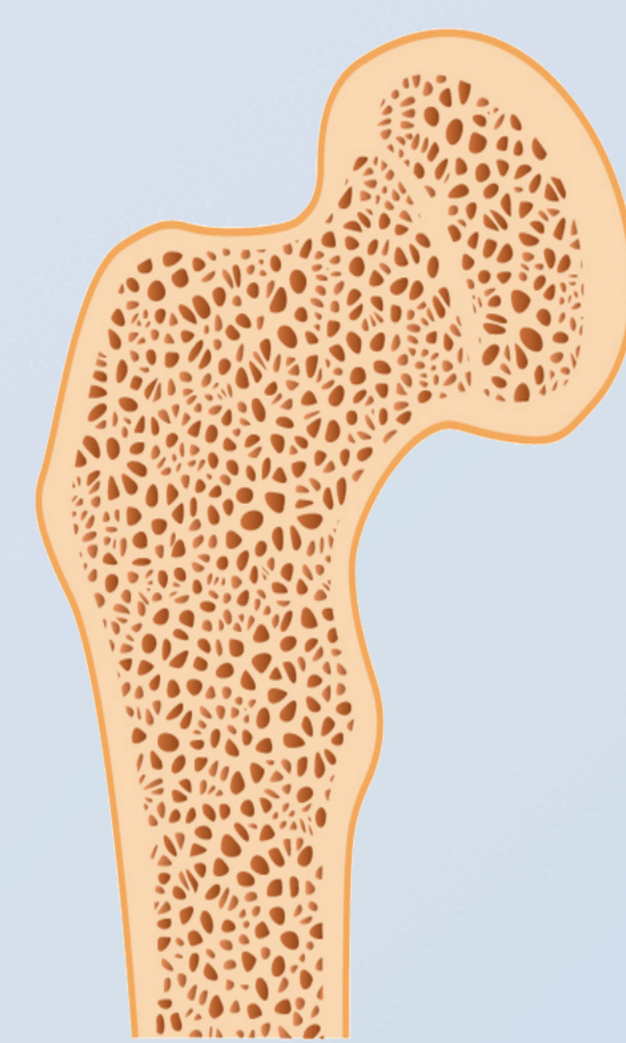


## Ca isotope ratios and bone metabolism

- Changes in urinary composition of naturally-occurring Ca isotopes are representative of corresponding changes in soft tissue
- Lighter isotopes are preferentially incorporated into bone during formation
- Isotopes are released nonselectively from bone during resorption
- An increase in the relative abundance of heavy isotopes in urine is indicative of positive bone mineral balance (BMB) in that bone formation exceeds resorption
- Thus, during loss of bone density, BMB is negative and isotopic composition of urine is lighter

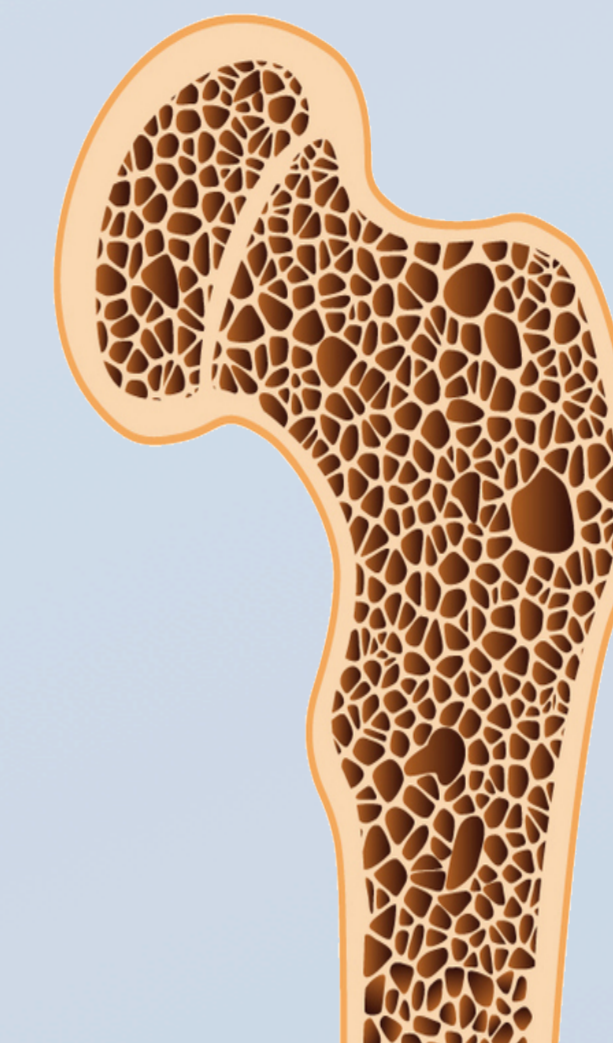


Normal bone

## Over 25% of all individuals experience an osteoporotic fracture in their lifetime.

These fractures increase mortality and often permanently impede quality of life. However, with proper diagnosis and treatment, at least **50% of these fractures can be avoided.**

This study explores a novel approach to discriminate shifts in bone metabolism before significant deterioration has occurred.

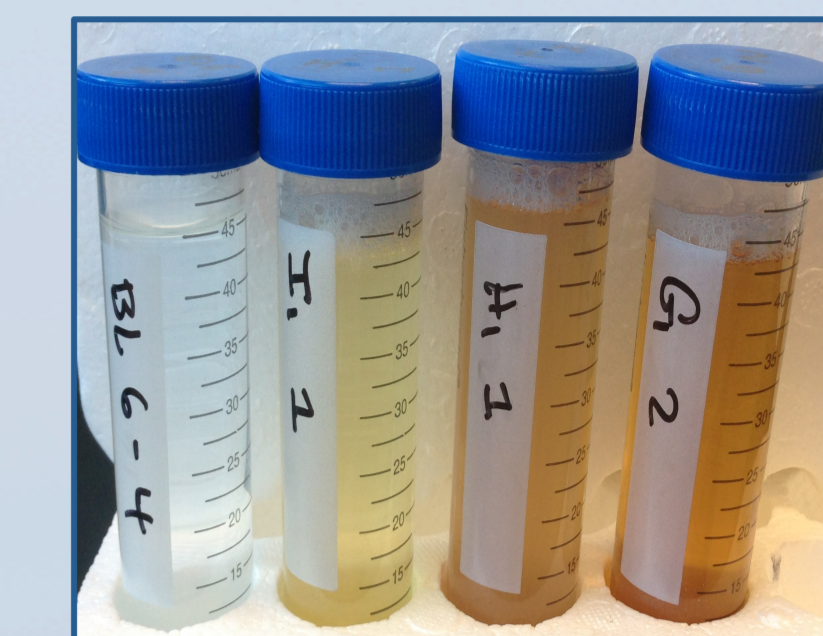


Osteoporosis

## Procedure

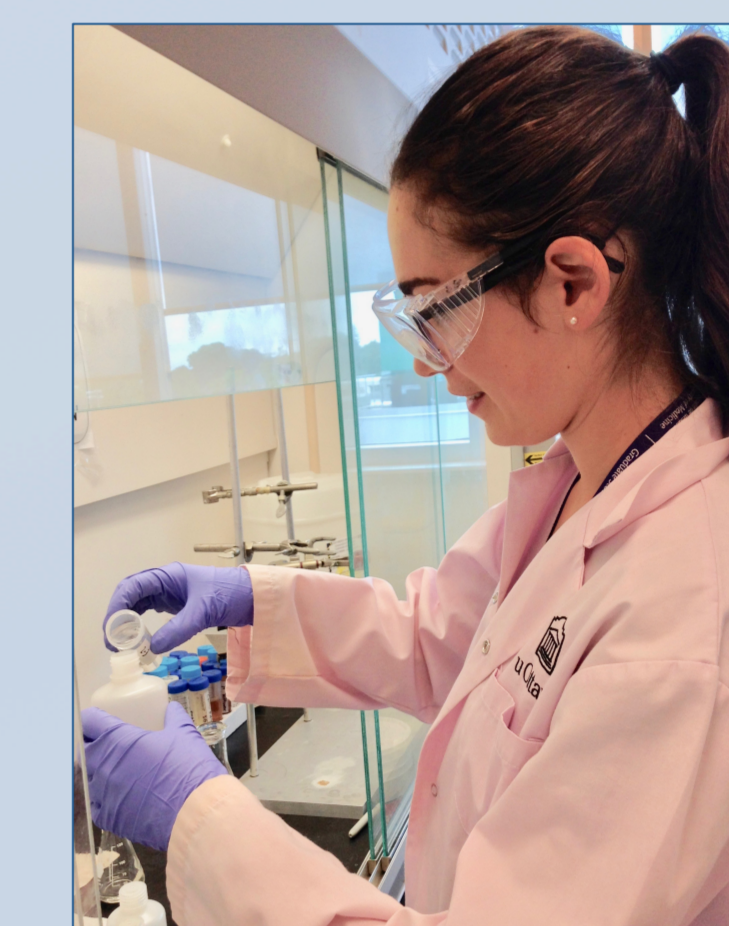
### Precipitation

$\text{CaC}_2\text{O}_4$  was isolated from urine samples. Hot ammonium oxalate, sodium acetate, and ammonium hydroxide facilitated precipitation.



### Cation exchange chromatography

$\text{K}^+$  was removed to purify  $\text{Ca}^{2+}$ . The mono- and divalent cations eluted at different concentrations of  $\text{HNO}_3$ .



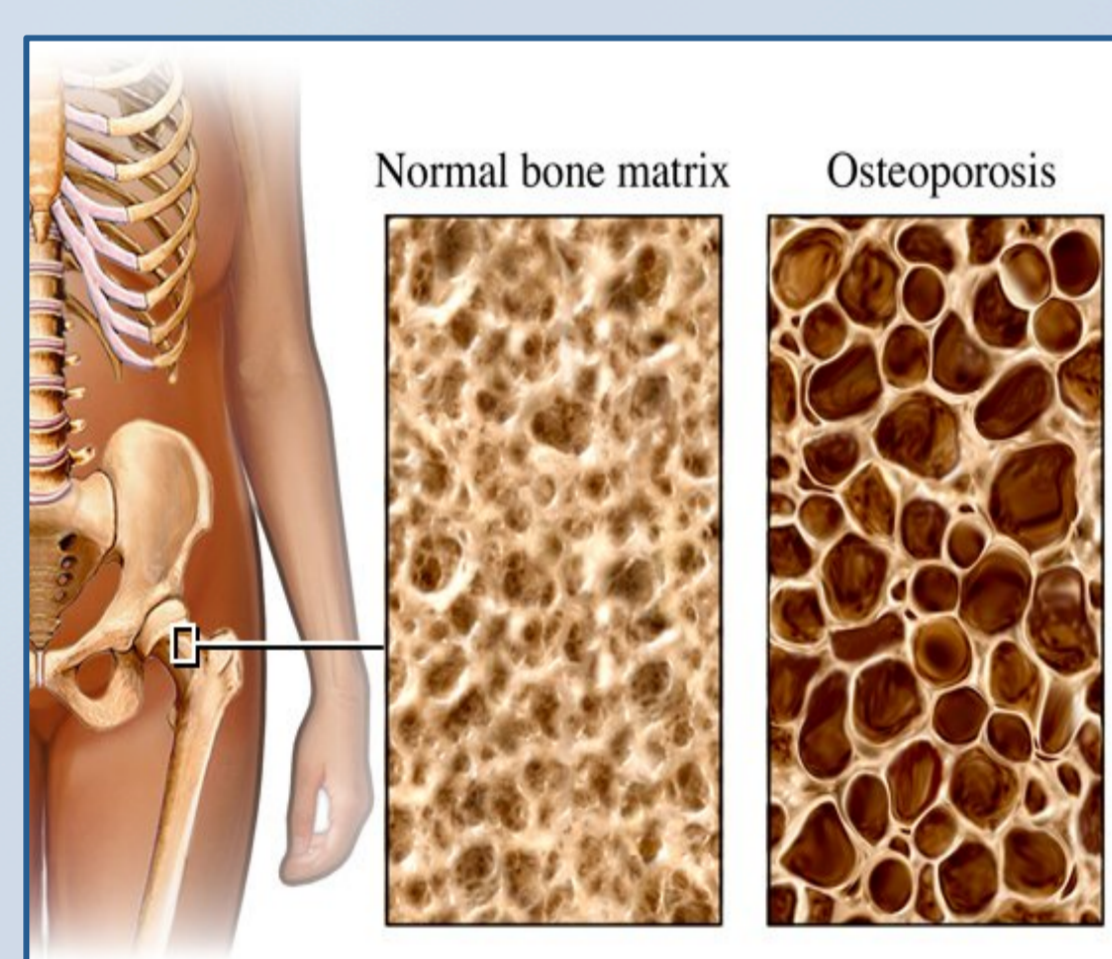
### ICP-OES/ICP-MS

Ca yield and Sr content were measured.

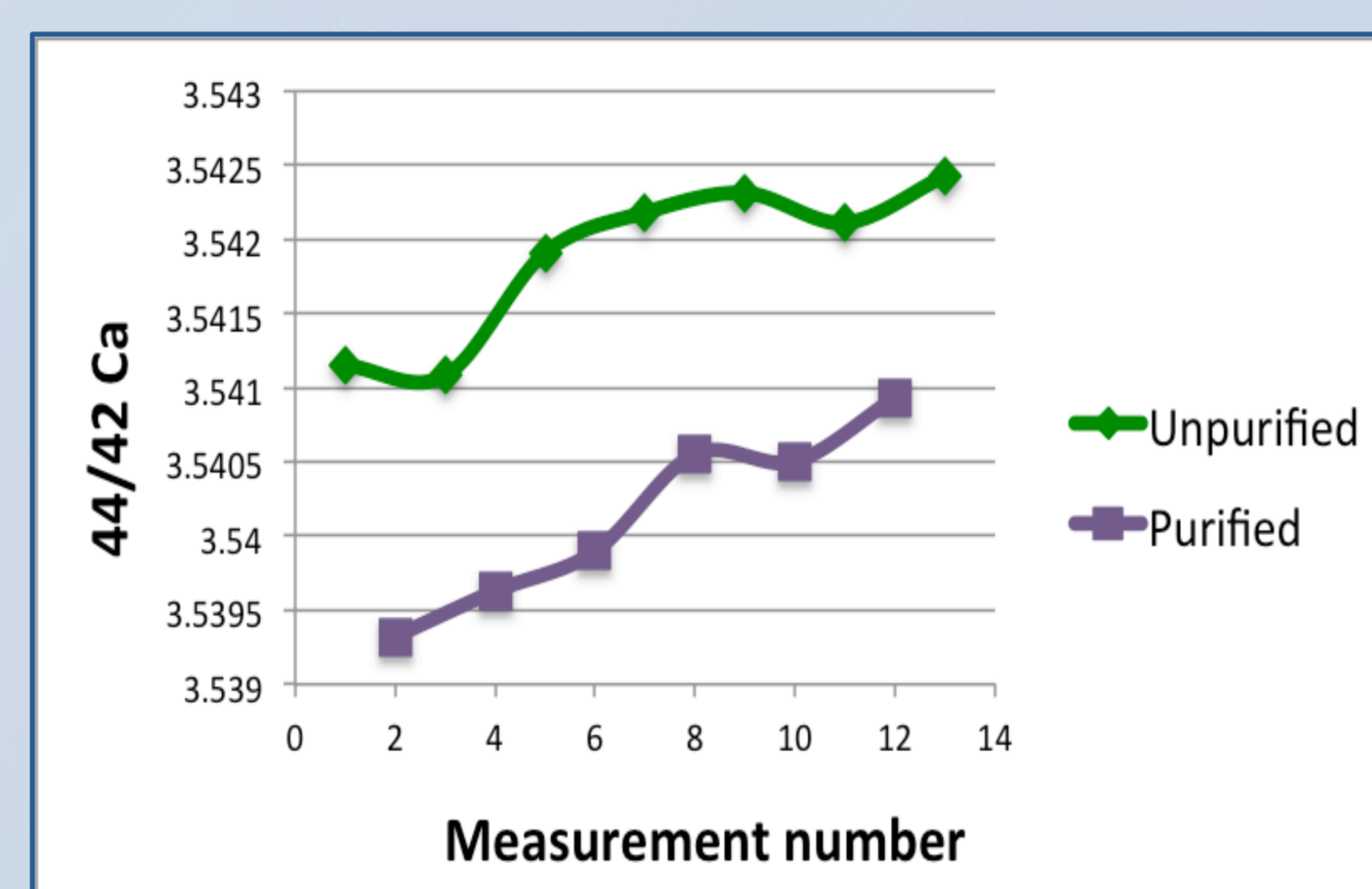
**MC-ICP-MS**  
Ratios of stable Ca isotopes were measured.

## Medical applications

- Osteoporosis is a disease characterized by deterioration of bone tissue resulting in fragile, low-density bone.
- Currently, there is no reliable method for detecting small-scale changes in BMB.
- Measuring urinary isotopes is noninvasive and can identify shifts in bone metabolism before bone density has discernibly declined.
- Earlier detection will facilitate medical intervention before irreversible osteoporotic damage and fractures occur.
- In addition to expediting diagnosis, these measurements will allow for rapid evaluation of treatment efficacy and disease progression.

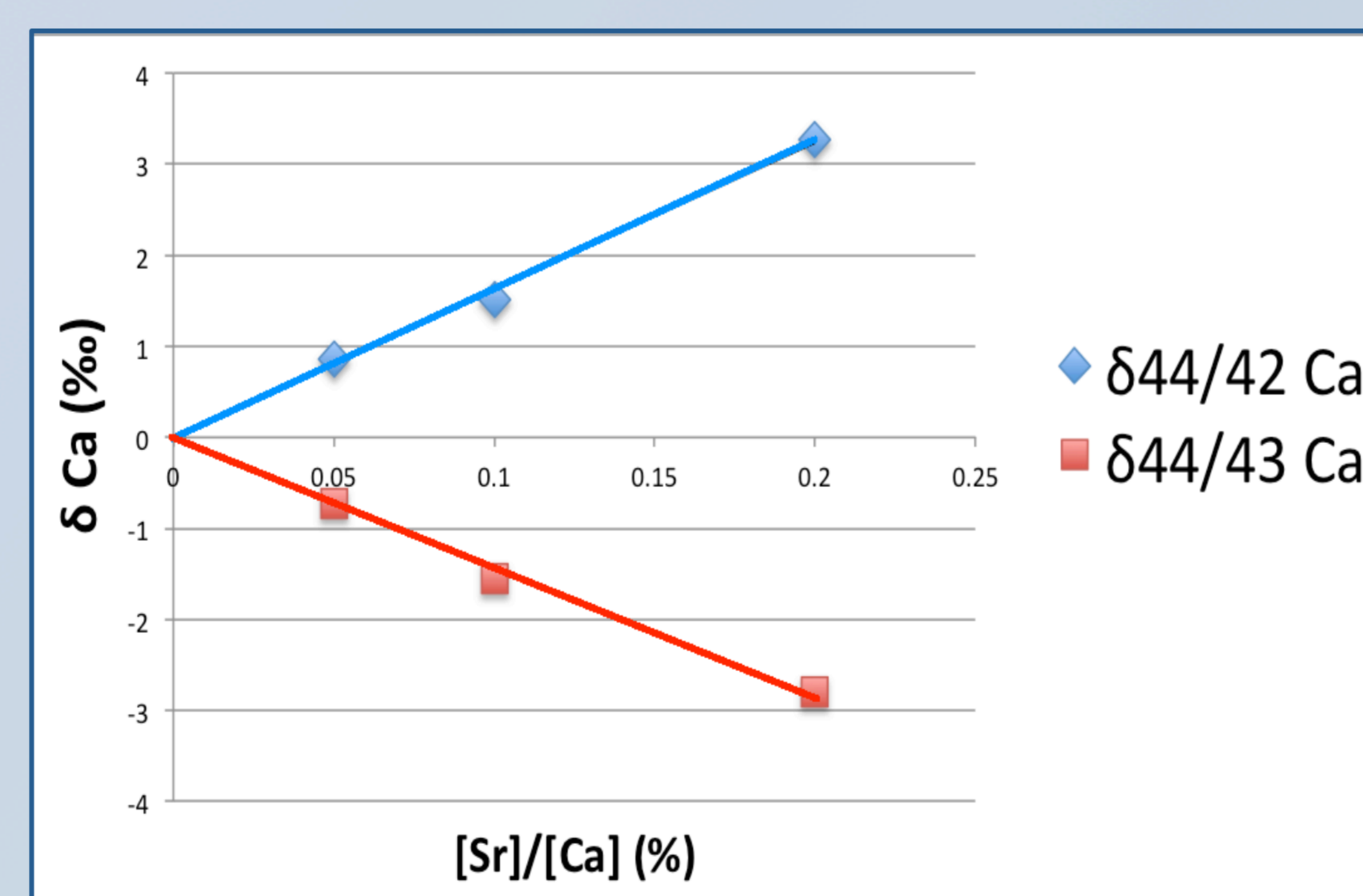


## Results



### Figure 1. Fractionation occurs during calcium purification.

Purified and unpurified samples of NIST 915b Ca reference material with equal initial isotopic compositions were measured for comparison. Note that these ratios are absolute measurements and therefore the positive trend is attributable to machine drift.



### Figure 2. Correlation between strontium concentration and $\delta\text{Ca}$ values measured using MC-ICP-MS.

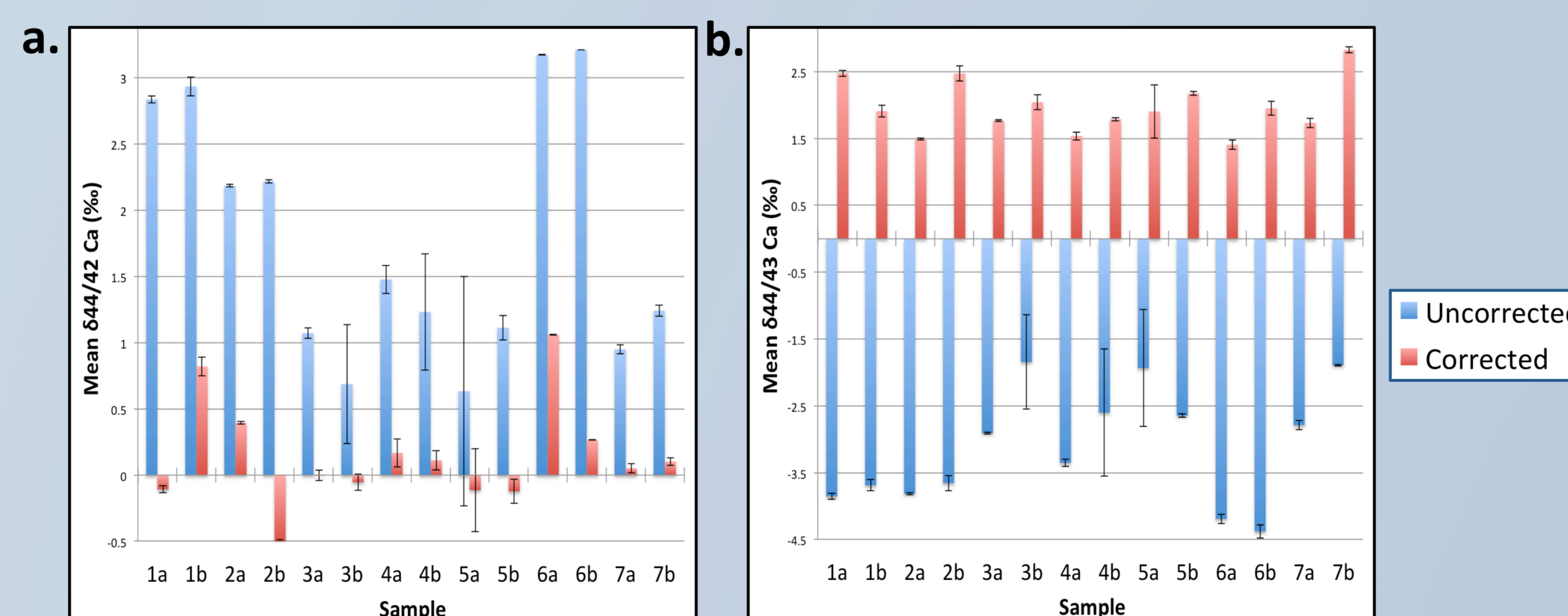
Solutions of equal concentrations of NIST 915b were spiked with varying amounts of Sr. All  $\delta\text{Ca}$  values are standardized to NIST 915b.

## Conclusions and next steps

- A small amount of fractionation occurs during Ca purification (Figure 1), which can be corrected for mathematically.
- MC-ICP-MS measurements of isotopically identical Ca samples correlate with increasing Sr concentration (Figure 2), indicating that  $^{88}\text{Sr}^{2+}$  cations interfere with  $^{44}\text{Ca}^+$  measurement.
- Higher variance between measurements of replicate samples (e.g. 1a vs 1b) after mathematical correction (Figure 3) indicates that the linear method of correcting for Sr interference is not sufficient, especially for  $\delta^{44}/^{42}\text{Ca}$ .
- Future samples will be eluted through a specially designed column to remove Sr before measurement, which will eliminate the need for this correction and improve data reliability.
- No conclusions can be drawn regarding the effects of individual or lifestyle factors on Ca composition until the variability of measurement is reduced.

## Goals of investigation

- Design a methodology for the isolation of urinary Ca with minimal loss of yield.
- Evaluate the reproducibility of multicollector mass spectrometry (MC-ICP-MS) measurements, as well as the extent of predicted  $^{88}\text{Sr}^{2+}$  interference.
- Determine the magnitude of procedural fractionation and correct for its effects.
- Explore the impacts of age, diet, and lifestyle variables on urinary Ca composition.



**Figure 3. (a)  $\delta^{44}/^{42}\text{Ca}$  and (b)  $\delta^{44}/^{43}\text{Ca}$  Ca values of purified samples before and after linear corrections for fractionation during purification and Sr interference.** Samples are depicted in order of their measurement on MC-ICP-MS. Samples with identical numbers refer to Ca obtained from the same initial urine sample but purified independently. Uncertainties are given to 1 standard deviation,  $n = 2$  or 3 measurements. All  $\delta\text{Ca}$  values are standardized to NIST 915b.

## References and acknowledgments

- Miller, J. J., Hui, S. K., Jackson, G. S., Clark, S. P., Einstein, J., Weaver, C. M., & Bhattacharyya, M. H. (2013). Calcium isolation from large-volume human urine samples for 41 Ca analysis by accelerator mass spectrometry. *Applied Radiation and Isotopes*, 78, 57–61.
- Morgan, J. L. L., Skulan, J. L., Gordon, G. W., Romaniello, S. J., Smith, S. M., & Anbar, A. D. (2012). Rapidly assessing changes in bone mineral balance using natural stable calcium isotopes. *Proceedings of the National Academy of Sciences of the United States of America*, 109(25), 9989–94.

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