The dual-tracer stable isotope method to measure calcium absorption in children on dialysis; a new use for an old technique

Key words: calcium, chronic kidney disease, dialysis, isotope, calcium absorption, fractional absorption, mineral bone disease, children

Dr Nicholas A. Ware MRCPCH

Dr Louise Oni MRCPCH

Professor Kimberly O. O'Brien PhD `

Professor Steven A. Abrams MD

Professor Lesley Rees MD FRCPCH

Corresponding author:

Professor Lesley Rees MD FRCPCH

Consultant Paediatric Nephrologist

Renal Office

Gt Ormond St Hospital for Children NHS Foundation trust

London WC1N3JH

UK

Email: l.rees@ucl.ac.uk

Phone: 0044207 405 9200 ext 8305

Fax : 0044 207 829 8841

In an article on phosphate binder use in CKD, Rees and Shroff stressed the need for studies of calcium balance, in particular in children on dialysis (1). We would like to draw attention to the potential for the new use of an old technique: the dual tracer stable isotope method. This is an established technique which has been used to measure fractional calcium absorption in children of all ages (even premature infants) with many different medical conditions (2). One stable isotope of calcium is given orally and a different one intravenously (IV) 2 hours later. Once the absorbed and IV isotopes are equilibrated, their ratio in blood and urine is independent of

differences in calcium pool size and turnover rates. The percent absorption of calcium can be calculated from the ratio of the oral tracer dose to the IV tracer dose recovered in a 24 hour urine pool post-dosing. In children on dialysis, recovery from dialysate would also be required. Another approach used to estimate fractional calcium absorption is the single timed serum method, which uses a serum sample taken four hours after the oral isotope (and 2 hours after the IV isotope) has been given and does not need urine or dialysate (3). Neither method needs complex metabolic balance studies or faecal collection. Neither has been used in CKD as yet.

Ethical committee permission was obtained from the National Research Ethics Committee, Bloomsbury, to obtain pilot data and to compare use of a single timed serum method in children on dialysis. Informed consent was taken from carers and assent from the children. Firstly we looked at the recovery of isotopes in urine and dialysate using typical doses used previously in this age group: 3 mg of oral ^{44}Ca and 1 mg ^{42}Ca IV, in an 8 year old child on peritoneal dialysis (PD). Selection of isotopes was based on their fractional abundance (^{44}Ca at 2.083% and ^{42}Ca at 0.647%). Isotopic ratios were measured using magnetic sector thermal ionisation mass spectrometry. The full methodology has been previously described and validated (2).

Enrichment of the oral tracer was readily analysed, but the delta percent excess of isotope was lower than anticipated, at 2.42% and 0.63% in the urine and 0.50% and 0.24% in the peritoneal dialysate for ^{42}Ca and ^{44}Ca respectively. For the next 3 patients, aged 5 to 11 years, we studied children on haemodialysis (HD) to compare the timed serum method with the standard 24 hour urine collection technique. The doses were increased to 6 mg ^{44}Ca orally and 1.5 mg ^{42}Ca IV. The mean percent calcium absorption calculated using the 24 hour urine method was 15.6 ± 8.5% (range 5.9-21.9%) and in the 4 hour serum samples was 8.3 ± 7.6% (range 3.0-17.0%). The absorption values obtained using the ratio of oral to IV isotope in serum were all lower than the results obtained in the 24 hour urine pool, on average by 10.6% ($r=0.73$, $p=0.47$) between the serum and urine methods. Other studies have extended this time point to 6 hours (3). Additional studies are warranted to explore this issue as possible alterations in peak absorption times may be evident in those on dialysis.

In this group of prepubertal paediatric patients, a dose of 1.5 mg ^{42}Ca IV resulted in a mean serum enrichment of 5.1 ± 2.0% at 4 hours post oral dosing (range 2.9 - 6.7%), and a mean urine enrichment of 1.8 ± 0.8% in the 24 hour urine pool (range 0.9 -2.5%). Mean serum enrichment from 6 mg oral ^{44}Ca was 0.6 ± 0.6% (range (0.2-1.2%) and remained suboptimal. Mean urine enrichment of the oral ^{44}Ca tracer was 0.4 ± 0.3% (range 0.2 - 0.7%). With these doses the enrichment was satisfactory for the IV dose but by calculation, a minimum dose of 8 mg of ^{44}Ca would be required to provide urine enrichment of >0.5%, or 17 mg for >1% as an estimate in prepubertal children aged 5 to 11 years.

In conclusion, this data on enrichment in serum and urine pools will assist future investigators with dosing estimations depending on the relative standard deviation of their mass spectrometer. The single serum method may remove the difficulties with complete 24 hour urine collections but the optimum timing for the sample needs further investigation. The knowledge gained from this study about the methodology of this technique in dialysis patients will help to open up the way to future research in children, including calcium absorption from calcium containing phosphate binders, and the effect on calcium absorption of different doses of vitamin D, different phases of CKD, and of age and growth.

References

1. Rees L, Shroff R (2015) The demise of calcium-based phosphate binders—is this appropriate for children? Pediatric Nephrology 30:2061-2071
2. Abrams SA (1999) Using stable isotopes to assess mineral absorption and utilization by children. The American Journal of Clinical Nutrition 70:955-964
3. Lee W, McCabe G, Martin B, Weaver C (2011) Simple isotopic method using oral stable or radioactive tracers for estimating fractional calcium absorption in adult women. Osteoporosis International 22:1829-1834