A Simple Single Serum Method to Measure Fractional Calcium Absorption using Dual Stable Isotopes

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Key words
- fractional calcium absorption
- stable isotopes
- ^{44}Ca
- ^{42}Ca

Abstract

The dual stable isotope method with a timed 24-h urine collection is the gold standard approach to measure fractional calcium absorption. However, the need to collect urine for 24 h makes this technique time-consuming and laborious. Our study sought to determine whether a dual isotope method using a single serum sample obtained 4 h after administration of the initial isotope provides a useful approach to measure fractional calcium absorption. Following a metabolic diet with a fixed calcium intake of 30 mmol/day for 10 days, nineteen healthy subjects age 54–74 were given a test meal with an oral isotope (^{44}Ca) followed 2 h later by an intravenous isotope (^{42}Ca). Once the oral isotope was administered, urine was collected for 24 h, and a serum sample was obtained after 4 h. The ratio of the oral to intravenous isotopes was measured in the urine and serum by mass spectroscopy. Fractional calcium absorption was 16.2 ± 7.7 % by the 4-h single serum method versus 18.5 ± 7.5 % by the 24-h urine method. There was a small mean difference between the urine and serum methods of 2.33 % with a confidence interval −3.97 to 8.60 %. The two methods showed a strong linear association ($r = 0.912$, $p < 0.001$). Use of dual stable isotopes with a 4-h single serum method gives fractional calcium absorption values that are 12.5 % lower than with the 24-h urine method; however, it rank orders subjects accurately thus making it a useful alternative method in clinical research applications.

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Introduction

Fractional calcium absorption is the percent of calcium absorbed in the intestine from an oral calcium load. A number of isotopic methods have been developed that can measure fractional calcium absorption employing radioisotopes, stable isotopes or a combination of the two. Of the currently available methods, the dual stable isotope method with a timed 24-h urine collection is considered the most accurate and reliable [Griffin et al., 2005; Heaney, 2001]. Furthermore, it does not involve ionizing radiation. This technique consists of the administration of two different calcium isotopes – one oral and a second intravenous isotope. Following their administration, urine is collected over 24 h. The relative fraction of the oral compared with the intravenous isotope dose in the urine is determined and it represents the fraction of the oral isotope that was absorbed [Abrams, 1999]. The inconvenience of this method is the need to collect urine for 24 h, which is time-consuming and laborious to perform.

Our study sought to determine whether a dual isotope method using a single serum sample obtained 4 h after administration of the initial isotope provides a viable alternative approach to estimate fractional calcium absorption. The 4-h single serum method was performed concurrently with the established 24-h urine method in the same nineteen subjects on fixed calcium intakes and results were compared.

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Materials and Methods

Study design and subjects

Men and women age 50 and older were recruited through direct mailings and advertisements. Women were menopausal for at least 6 months. Exclusion criteria, described in more detail elsewhere [Ceglia et al., 2009b], included use of oral glucocorticoids, gonadal hormones, osteoporosis medications, and thiazide diuretics, medical conditions such as kidney stones and hyperparathyroidism, creatinine clearance <50 ml/min/1.73 X m^2 of body surface area, 24-hour urinary calcium excretion (UCa) >7.5 mmol/day, abnormal serum calcium, elevated alkaline phosphatase, and serum 25-hydroxyvitamin D [25(OH)D] level ≤40 nmol/L. Twenty subjects were enrolled in a randomized intervention study as described previously [Ceglia et al., 2009a; Ceglia et al., 2009b]. One placebo subject was not included in the analysis because the serum sample was mislabeled. Subjects were placed on a 10-day metabolic diet containing a fixed calcium intake of 30 mmol/day. The daily dose and schedule of calcium intake was constant, because a change in calcium intake during the 24 h after isotope administration could alter fractional calcium absorption assessed by the 24-h urine method. On day 10 of the diet, fractional calcium absorption was measured by the 24-h urine and 4-h single serum methods. The Tufts Medical Center-Tufts University Health Sciences Campus Institutional Review Board approved the study, and written informed consent was obtained from each subject.

Diet and supplements

The Metabolic Research Unit provided a metabolic diet containing 15 mmol/day of dietary calcium. Protein intake was 1.5 g/kg/day in nine subjects and 0.5 g/kg/day in ten subjects (as part of a different objective, as previously described [Ceglia et al., 2009a; Ceglia et al., 2009b]). Other diet contents have been described previously [Ceglia et al., 2009a; Ceglia et al., 2009b]. Subjects took a tablet containing 15 mmol of calcium (as tricalcium phosphate) and 125 IU of vitamin D_3 (Posture D, US Rhodia, Cranbury, NJ) and a multivitamin (CVS brand) containing 400 IU of vitamin D_3 with the evening meal.

Biochemical measurements

Blood was drawn after a 12-h overnight fast between 7 and 10 am. All samples from individual subjects were batched for analyses. Urine was collected in an empty collection bottle for 24 h and measured for calcium in the presence of 35% HCl solution (15 µl for 1 ml of urine). We measured urinary calcium excretion, serum calcium, serum 25-hydroxyvitamin D level, and intact PTH as described previously [Ceglia et al., 2009a; Ceglia et al., 2009b].

4-h Single Serum Method

Subjects arrived at the center in the morning after an overnight fast, had a peripheral intravenous catheter placed, were asked to empty their bladder, and were given breakfast at 8 am. Toward the end of breakfast, subjects were given ^{44}Ca (0.375 mmol for subjects <80 kg; 0.575 mmol for those ≥80 kg) that had been pre-mixed in 240 ml of calcium-fortified Minute Maid orange juice (8.5 mmol calcium as phosphate and lactate). Two hours after breakfast (10 am), ^{42}Ca (0.025–0.038 mmol for subjects <80 kg and 0.0575 mmol for those ≥80 kg) was infused intravenously over 2 min. Four hours after administration of the oral isotope (12 noon), a blood sample was drawn. Blood was centrifuged after clotting and a 0.5 ml aliquot of serum was removed and frozen for later analysis. Fractional calcium absorption was calculated as the ratio of the ^{44}Ca dose in serum 4 h after administration to the ^{42}Ca dose 2 h after administration.

24-h Urine Method

This method was carried out on each subject concurrently with the 4-h single serum method. A 24-h urine collection began immediately after the oral isotope was administered with breakfast and was completed at 8 am the next day. A 5–10 ml aliquot of urine was removed from the pooled 24-h collection and frozen for later analysis. Fractional calcium absorption was determined as the ratio of the cumulative ^{44}Ca recovery to the cumulative ^{42}Ca recovery in the urine.

Stable Isotopes

The stable isotopes were purchased from Trace Sciences International Corp. (Richmond Hill, ON, Canada) and were supplied as the carbonate salt. The isotopic enrichments for these isotopes were >95%. Isotopes were prepared by the Tufts Medical Center Research Pharmacy and were tested for sterility and pyrogenicity prior to use. Urine and serum samples were prepared and analyzed by a magnetic sector inductively coupled plasma mass spectrometer (ICP-MS, Bremen, Germany) as previously described [Chen et al., 2003]. The precision of this method is <1%.

Statistical Analysis

Point estimates are reported as mean ± SD. Pearson correlation coefficients were used to describe the association between the two methods. Two-sided p values less than 0.05 were considered to indicate statistical significance. Statistical analyses were conducted with SPSS v. 15.0 (SPSS Inc., Chicago, IL).

Results

Clinical characteristics of the nineteen subjects are shown in **Table 1**. The study included 6 men and 13 women.

Fractional calcium absorption was 16.2 ± 7.5% with the 4-h single serum method versus 18.5 ± 7.5% with the 24-h urine method. The two methods showed a strong linear association (**Fig. 1**; r = 0.912, p < 0.001), thus indicating similar ranking of the subjects' fractional calcium absorption. The mean difference between methods (urine-serum) was 2.33% with 95% confidence interval −3.97 to 8.60%.

Discussion

In healthy older adults studied on a fixed calcium intake of 30 mmol/day, use of dual isotopes with a 4-h single serum method provides a simple and rapid approach to estimate fractional calcium absorption. The correlation coefficient between the serum method and the gold standard 24-h urine method was 0.912, fitting 83% of the variability in fractional calcium absorption in the 24-h urine method. The main advantages of the 4-h single serum method are that it can be performed over a 4 rather than 24 h period, the serum needed for analysis is minimal (0.5 ml), and subjects can avoid the inconvenience of collecting urine for 24 h.

Table 1 Baseline characteristics (individual values and mean ± SD) of the nineteen subjects.

Subject	Age (yr)	Sex (M/F)	Body Weight (Kg)	Serum Calcium (mmol/L)	PTH[a] (pmol/L)	25(OH)D (nmol/L)	UCa (mmol)
1	54	F	79.7	2.27	4.88	40	5.96
2	54	F	58.9	2.32	6.26	85	2.37
3	54	M	84.0	2.22	5.84	45	4.54
4	55	F	68.3	2.40	ND	53	3.54
5	56	F	60.3	2.30	3.71	75	1.80
6	57	F	53.1	2.35	3.82	60	2.79
7	58	F	63.8	2.35	3.82	83	1.80
8	59	F	64.2	2.22	ND	50	4.72
9	59	F	65.1	2.25	4.99	60	4.62
10	59	F	67.4	2.22	6.68	80	2.42
11	60	M	88.8	2.22	6.05	65	2.02
12	61	F	67.2	2.22	4.35	40	2.30
13	62	M	81.1	2.30	3.29	50	7.04
14	64	M	64.0	2.22	5.52	55	1.20
15	67	M	74.3	2.32	5.09	45	3.24
16	69	F	58.7	2.27	5.62	65	2.35
17	69	M	83.1	2.32	4.14	40	2.15
18	71	F	61.1	2.40	4.35	90	0.37
19	82	F	60.8	2.27	ND	70	1.01
mean ± SD	62 ± 7		68.6 ± 10.2	2.28 ± 0.06	4.90 ± 1.02	60.5 ± 16.2	2.96 ± 1.73

Reference ranges for the above biochemical measurements are: serum calcium 2.08–2.55 mmol/L, PTH 1.06–7.32 pmol/L, 25(OH)D levels 23–95 nmol/L, and uca excretion 2.5–7.5 mmol/24 h

ND = not performed

[a] PTH levels were drawn on 16 subjects approximately 5 months prior to the start of the study

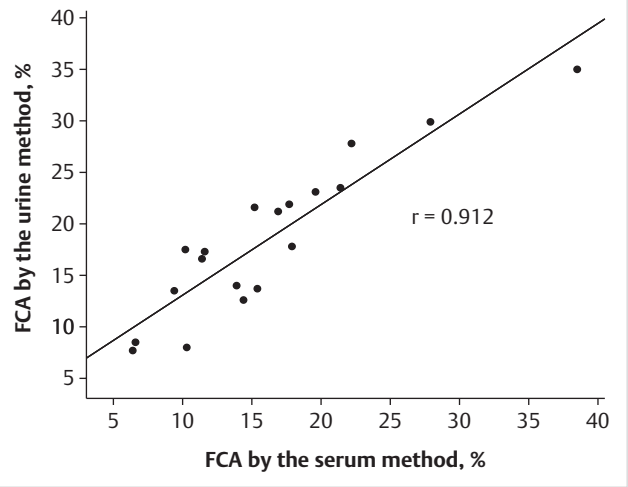

Fig. 1 Relationship between fractional calcium absorption (FCA) measured by the 4-h single serum method and FCA measured by the 24-h urine method.

There was a mean 12.5% bias toward lower fractional calcium absorption values from the 4-h single serum method compared with the 24-h urine method. This difference could reflect a longer time needed for delayed absorption to appear in the serum, particularly in our older population [Heaney et al., 1985; Yergey et al., 1994]. Nevertheless, the serum method is able to rank order subjects' fractional calcium absorption similarly to the urine method.

The strengths of our study included the fact that the two methods were performed at the same time and in a carefully timed protocol. Furthermore, by placing subjects on a metabolic diet with a fixed calcium intake, we did not allow potential alterations in diet to affect the 24-h urine fractional calcium absorption results. Limitations of the study included the modest sample size and the fact that we studied fractional calcium absorption at a single calcium intake of 30 mmol/day, rather than a range of intakes. We would anticipate that serum fractional calcium absorption values would be valid at other intakes; however, further investigation is needed to confirm this. In addition, this study was performed in healthy older adults; thus, whether this method would be useful for individuals with altered mineral metabolism cannot be determined from this study.

In conclusion, the 4-h single serum method appears to be a practical and convenient approach to estimate calcium absorption in older adults. It can be useful in rank ordering fractional calcium absorption values which is important and adequate for most clinical research applications. However, when it is essential to measure calcium absorption with high accuracy, the 24-h urine method may be required.

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