A comparison of radioactive calcium absorption tests with net calcium absorption

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Summary

1. Four different methods of calculating calcium absorption by radioactive calcium procedures have been compared with each other and with net calcium absorption in calcium-balance studies in 100 consecutive studies on 71 patients.

2. All four isotope procedures yielded highly significant correlations with net calcium absorption derived from the balance studies, but there was little to choose between the validity of the double-isotope and single-isotope procedure judged by these criteria.

3. The rate of calcium absorption calculated from one or other isotope procedure correlated better with net calcium absorption than did the fraction of the radioactive calcium absorbed.

4. The measurement of plasma radioactivity 1 h after single-isotope administration, corrected for body weight, proved almost as useful as the more complex procedures but would be expected to underestimate calcium absorption in states of very high bone turnover.

Key words: absorption, calcium.

Introduction

There is no standard way of measuring calcium absorption. A great many different methods exist ranging from intubation with a triple lumen tube [1] to calcium balance procedures and procedures involving the administration of radioactive calcium either as a single isotope [2] or as a double isotope [3]. Variants of the latter have included total-body retention of radioactive calcium after oral administration [4] and forearm counting [5]. In attempting to assess the relative validities of these very different procedures, one is faced with the problem that there is no ultimate reference standard. However, the most direct procedure, which makes the fewest assumptions, is the calcium balance, in which calcium absorption is defined as the difference between dietary intake and faecal output. Although subject to considerable technical error, this is a conventional and well-established procedure which most workers would probably accept as a reasonable reference standard if the balance is performed with sufficient care and one with which other more indirect methods can reasonably be compared.

We have therefore compared various measurements of calcium absorption using radioactive calcium with the results obtained by balance studies performed on the same subjects at the same time. The results are presented in this paper.

Materials and methods

Patients

The study was based on 100 consecutive sets of observations in 71 patients (nine men, 62 women). Of these studies 36 were performed in untreated patients and 64 in patients on a variety of treatments. The details of the diagnostic categories and treatments are shown in Table 1.

Clinical procedure

The balance procedure used has been described in detail elsewhere [6]. It was a 2-week
study with polyethylene glycol as a non-absorbable faecal marker. Patients were equilibrated on a standard diet for the first week and were then given intravenous radioactive calcium (10 μCi of ⁴⁷Ca as calcium chloride) on day 8, after which daily faecal and urinary collections are performed for a further week. The oral radioactive calcium test was performed on day 15. The oral dose of radioactive calcium consisted of 5 μCi of ⁴⁴Ca or ⁴⁷Ca in a solution (2.0 mmol/l) of 20 mg of calcium carrier as the chloride in 250 ml of distilled water administered after an overnight fast at about 09.00 hours. Plasma samples were obtained at 10, 20, 40, 90 and 180 min after the intravenous radioactive calcium and at 15, 30, 45, 60, 90 and 120 min after the oral dose.

Calculations

The following calculations were performed. (1) Net calcium absorption, defined as the difference between dietary calcium intake and faecal calcium output expressed in mmol/day. (2) The initial fractional rate of calcium absorption after radioactive calcium administration (λ). This measurement makes use of the two calcium isotopes administered on days 8 and 15 and is calculated by a standard deconvolution procedure, which has been described in detail elsewhere [7], with 10 min intervals. After calculating the cumulative absorbed radioactive calcium as a function of time, the exponential fall in unabsorbed dose in the first hour after the initial delay is expressed as a fractional rate constant (λ). (3) The fraction of the dose of radioactive calcium absorbed in 2 h (φ); this is derived from the deconvolution procedure and represents the integrated rates of radioactive calcium absorption for the first 2 h after oral administration. (4) The mean fractional rate of radiocalcium absorption (α) is based on single-isotope measurements only [8], where the fractional rates of absorption and clearance are obtained by non-linear curve fitting. (5) The fraction of the dose of radioactive calcium circulating in the plasma and extracellular fluid 1 h after the oral dose (Fₚ); this is derived from the fraction of the dose per litre of plasma 1 h after administration multiplied by 15% of the total-body weight to allow for the extracellular calcium [9].

Results

In the following analysis the initial fractional rate (λ) and the mean fractional rate (α) have been transformed to their square roots to normalize their distribution and yield linear correlation. The correlation matrix embracing all the data is shown in Table 2 and the relationships between net calcium absorption by the balance method on the one hand and the various radioactive calcium measures on the other are illustrated in Figs. 1–4.

The relation between net calcium absorption and the square-root of the initial rate of radioactive calcium absorption (λ) is shown in Fig. 1. There was a highly significant correlation between these two variables (r = 0.74; P < 0.001). The regression equation is: net = 13.5 × λ - 6.6 and the sd about the line is 3.3 mmol/day.
The relation between the fraction of the dose absorbed in 2 h derived from the double-isotope procedure (φ) is shown in Fig. 2. The correlation coefficient was again highly significant (r = 0.63; P < 0.001), though not as high as in Fig. 1. The regression equation is: net = 14.7 × φ − 4.3 and the SD about the line is 3.8 mmol/day.

The relation between net calcium absorption and the square-root of the average fractional rate of absorption from the single-isotope procedure (\( \alpha \)) is shown in Fig. 3. The correlation between them was highly significant (r = 0.76; P < 0.001) and very similar to that between net calcium absorption and \( \lambda \) (Fig. 1). The regression is: net = 17.0 × \( \sqrt{\lambda} \) − 7.3 and the SD about the line is 3.2 mmol/day.

The relation between net calcium absorption and the fraction of the single-isotope dose circulating at 1 h corrected for body weight (\( F_c \)) is shown in Fig. 4. The correlation coefficient was highly significant (r = 0.70; P < 0.001). The regression equation is: net = 32.7 × \( F_c \) − 3.2 and the SD about the line is 3.5 mmol/day.

Since the double-isotope calculation which gives the highest correlation with net calcium absorption is \( r = 0.74 \), we have compared the single-isotope calculations with \( \lambda \) in Figs. 5 and 6. It should be noted that these are not independent measurements, since they have in common the plasma concentrations from the oral dose.

Fig. 5 shows the relationship between \( \sqrt{\lambda} \) and \( \sqrt{\alpha} \); that between \( \sqrt{\lambda} \) and \( F_c \) is shown in Fig. 6. The correlation coefficient was very significant (r = 0.85; P < 0.001), but there was slightly more scatter than in Fig. 5.

Discussion

In the absence of an ultimate standard of calcium absorption, we have attempted to assess the
validity of various radioactive calcium procedures and calculations by comparing them with each other and with net calcium absorption measured by the balance technique in an unselected series of 100 studies in 71 patients with a variety of diagnoses, some untreated and others treated. In this way, we have been able to perform our comparisons over a wide range of absorptive states.

Net absorption has been taken as the reference standard rather than 'true' absorption which requires, in addition to the measurement of dietary and faecal calcium, the estimation of endogenous faecal calcium. This endogenous excretion is small in comparison with the total faecal calcium at these intakes and subject to a relatively large error in its estimation. This 'true' absorption has not been found to be significantly better than the 'net' absorption to the radioactive isotope tests.

Taking the net calcium absorption, measured as accurately as we were able and knowing that it was subject to an error of not less than 0.5 mmol/day, we have shown that there is little to choose between the various radioactive calcium procedures. In particular, the calculation of radioactive calcium absorption rates from analysis of six blood samples after a single-isotope test is at least as good, on this criterion, as the initial rate of absorption calculated from the double-isotope test. Again, and using the same criterion, there is surprisingly little lost by confining the analysis to a single blood sample taken 1 h after administration of the dose and corrected for body weight. However, it is of interest that the measurement of calcium absorption rate ($\lambda$ and $\alpha$) agrees better with net calcium absorption than does the measurement of
the fraction of the dose absorbed (φ), confirming the observations of Reeve et al. [10].

Internal comparison of the different radioactive calcium methods again shows such high correlations between them as to cast considerable doubt on the need for a double-isotope procedure or even multiple blood sampling, after a single isotope, unless high precision is required, although it must be borne in mind that the various procedures share a number of common measurements which must contribute to these very high correlations. These correlations obtained despite the inclusion in the material of patients with high bone turnover due to osteomalacia and hyperparathyroidism. The implication is that the six blood-sample single-isotope test is as good as the double-isotope test in correcting for variations in bone uptake. Even the simplest procedure involving a single blood sample, where no correction for bone uptake is possible, will inevitably tend to underestimate calcium absorption in states of very high bone turnover such as severe hyperparathyroidism and osteomalacia, particularly perhaps after vitamin D treatment of the latter. Examination of our data shows that some of the scatter in Fig. 6 is accounted for in this way; doubtless the same would be true in Paget's disease.

Nonetheless, and these reservations notwithstanding, the single-isotope single blood-sample procedure would appear to be adequate for most clinical purposes. If the object of a calcium-absorption test is to establish whether the patient is a malabsorber, normal absorber or high absorber of calcium, then this simple procedure would appear to be adequate and has the enormous merit of being suitable for multiple observations on the same subject both because of the minimal discomfort to the patient and the minimal technical and medical time that it involves. Where it is essential to measure calcium absorption with high precision, we do not doubt that multiple blood sampling or even double-isotope procedures may be required, but it is our strong impression at the present time that the considerable variation in calcium absorption, not only between but also within individuals, generally makes high-precision measurement a somewhat uneconomic exercise.

References