Estimation of True Calcium Absorption in Men, Robert P. Heaney,† Mary Susan Dowell,‡ and Randi L. Wolf§
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When both calcium intake and calcium absorption are measured under controlled conditions, variation in absorption efficiency explains more of the interindividual differences in balance than does actual calcium intake (1). Small wonder, therefore, that interest in measuring absorption has remained high for nearly 40 years.

True calcium absorption is defined as the quantitative, unidirectional flux of calcium from intestinal lumen into the blood. It is most accurately measured by a dual-tracer method, with one tracer labeling the oral calcium load and the other labeling the miscible calcium pool into which the absorbed calcium is introduced. This approach was first developed into a practicable human test by deGrazia et al. (2). As described, it is usually time-consuming and expensive. To reduce these barriers for widespread use, Heaney and Recker (3, 4) developed a single-tracer variant for women, requiring only a single blood sample, and calibrated it against a simultaneously performed double-tracer method. The single-tracer method has been used efficiently in thousands of women (5). However, because the calibration is empirical and based on body-size variables, it is not directly suitable for use in men who, with a typically higher proportion of fat-free mass than women, would be expected to distribute absorbed tracer in a larger mass of calcium.

To fill this methodologic gap, we performed a small set of parallel measurements in adult men, using the female-based algorithm together with a modified double-tracer approach.

Participants in the study were 30 Caucasian men (age range, 20–60 years; weight range, 63.5–104 kg; height range, 1.67–1.93 m). All participants were free of known diseases affecting bone remodeling or calcium homeostasis, and tests were not performed if the individual had experienced any gastrointestinal disturbance in the preceding 5 days. Each gave informed consent after the procedures of the study were explained. Both the project and the consent were approved by the Creighton Institutional Review Board. Each volunteer was tested twice. We performed the first test for several unrelated projects, using the standard, single-dose protocol, giving a 45Ca-labeled oral load and obtaining the usual 5-h serum sample for measurement of serum calcium specific activity. The volunteers abstained from all food after the test breakfast until the 5-h blood sample was drawn. The test calcium load (depending on the individual projects) was 300 mg in 25 individuals and 500 mg in 5. Sources were calcium-fortified orange juice in 20 volunteers, skim milk in 5, and precipitated calcium carbonate in 5. The second test, performed 6.2 (± 3) days later, used an intravenous (i.v.) dose of high-specific activity 45Ca, given 2 h after an identical test breakfast that contained the same oral calcium source and the same calcium load as on the first test. With the second test, serum was obtained 3 h after the i.v. dose for measurement of serum calcium specific activity. This timing reproduces accurately the dosing scheme of a simultaneous double-tracer experiment in which, as originally described (2), the i.v. tracer is given 2 h after the oral tracer. (The 2-h lag introduces the i.v. tracer at the approximate midpoint of absorption of the oral tracer.) Because the same tracer was used to label both the oral load and then subsequently the miscible calcium pool, a baseline serum sample was obtained at the second test to determine the concentration of residual tracer from the earlier oral test. (Mean correction was −9.6% of the total counts in the 3-h blood sample, with the maximum being −25% for the shortest interval and the minimum being −3.1% for the longest.)

Absorption fraction was calculated in two ways. The first consisted of the female-derived algorithm:

\[
\text{AbsFx} = (\text{SA}_5^{0.9237}) \times [0.3537 \times (\text{Ht}^{0.52847}) \times (\text{Wt}^{0.37213})]
\]

in which AbsFx, SA, Ht, and Wt represent absorption fraction, 5-h serum calcium specific activity (fraction of oral tracer dose/g calcium), height (m), and weight (kg), respectively.


8. Herruer MH, van Kooten EAW, Slutter HE, Zuijderhoudt FM. Influence of

uramia on the determination of blood glycohaemoglobin by HPLC, electrophoresis and affinity chromatography in diabetic and non-diabetic patients.


haemoglobin and fructosamine in non-diabetic subjects with chronic renal failure.


respectively. The factors in brackets serve, effectively, to adjust the measured serum tracer concentration for the ‘volume’ of distribution of the tracer at 5 h.

The second method used both tracers, calculating the quotient of the 5-h calcium specific activity after the oral tracer dose and the net 3-h activity after the i.v. tracer. The results with the two methods were then regressed on one another by both standard Pearson and orthogonal regression analyses (6). Additionally, the mean ratio of the within-individual values for the two methods was tested against a null hypothesis value of 1.0. The statistical software was Crunch 4.01 (Crunch Software) and SPSS (SPSS, Inc.) for Windows (Release 10).

The results of the paired measurements are presented graphically in Fig. 1 and in tabular form, individual-by-individual, in the data supplement available at Clinical Chemistry Online (http://www.clinchem.org/content/vol48/issue5). The similarity of the values obtained by the two methods was extremely close ($r^2 = 0.904$). However, the slope of the double-tracer value on the female-derived value was greater than unity, with a point estimate of 1.085. This quotient of the two test values was, of necessity, also 1.085, with a SE of 0.016. This value differs significantly from the null hypothesis value of 1.0 ($P < 0.001$; 95% confidence interval, 1.054–1.117). This quotient was constant across the full range of absorption values. The set of quotients was also independent of both height and weight. Alternatively, when the data were evaluated by orthogonal regression, the equation became $y = 1.149x - 0.0173$. This slope was significantly different ($P < 0.05$) from 1.0 (the value under $H_0$, the null hypothesis).

Because the intercept with the standard Pearson regression was virtually zero (and not significantly different from zero), it is simpler to represent the relationship as $y = 1.087x$ (the equation when the line is forced through the origin), where $y$ is the true absorption in men, and $x$ is the calculated value with the parameters derived from women. Using this equation, we obtained the best estimate of absorption fraction in men by multiplying the value derived from the female algorithm by 1.087. Alternatively, the factor could be introduced directly into Eq. 1, which then becomes:

$$\text{AbsFx} = (\text{SA}_5^{0.92373}) \times [\text{BSC} \times (\text{Ht}^{0.52847}) \times (\text{Wt}^{0.37215})]$$

in which BSC presents body-size coefficient, with a value of 0.3537 in women and 0.3845 in men.

This work provides a firm basis for estimating the true calcium absorption fraction in men from nutritionally meaningful, labeled loads. The coefficient of determination from the regression of the two methods on one another was essentially the same as reported previously for the same regression in a much greater number of tests in women (3). Although, in that earlier study, the 5-h sampling time was selected empirically, it makes good intuitive sense because absorption is virtually complete by 5 h (7); in addition, pool turnover is typically small relative to pool size, and hence early time points reflect mainly dilution.

The adjustment factor we found (1.087) is somewhat less than might have been predicted from body-water differences between men and women, where it is generally considered that, per unit of weight, men have ~15% more water than women (8). However, calculations of calcium absorption after an oral tracer based on body water have never seemed very plausible because it is known from tracer distributional studies that, by 3 h after an i.v. injection, tracer is distributed in a mass of calcium that is more than twice the amount of calcium contained in the entire extracellular fluid volume (9, 10). This point is shown clearly in Eq. 2 when one substitutes arbitrary height and weight values. Thus, for a height of 1.65 m and a weight of 75 kg, the apparent volume of distribution at 5 h in women is 2.30 g and 2.50 g in men.

Thirteen years ago, Favus (11) editorialized that enough had been learned about calcium absorption measurement to warrant making a calcium-tracer-based absorption test routinely available. To date, that challenge has not been accepted by any major laboratory. Instead, several strontium-based tests have been used (12, 13). Although they exhibit pragmatic utility in detecting large departures from normal, these strontium-based tests cannot provide the desired quantification of mass transfer of calcium from gut to blood. Moreover, they resist empirical calibration because the active transport process in both kidney and gut discriminates against strontium ions relative to calcium (14, 15). These relationships tend to be nonlinear. A calcium isotope obviates this difficulty.

The tests described here used $^{45}$Ca, but they could have been performed just as well with a stable calcium isotope. $^{45}$Ca is easier and less expensive to measure. The test we describe can be performed with 3–7 µCi (0.11–0.26 MBq) of $^{45}$Ca, which, at typically incomplete absorption fractions (~30%), produce a trivial increase in total body radiation (less, for example, than that experienced from cosmic ray sources during a vacation in the mountains or a transcontinental airplane trip). Hence, there would seem

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**Fig. 1.** Plot of the values obtained with the double-tracer method against values according to the single-tracer method using the female algorithm.

The equation is for the regression line passing through the origin. The dashed line represents the line of identity. (Used with permission from Robert P. Heaney.)
to be no substantive objection to implementing the suggestion by Favus (11) with $^{45}$Ca as the tracer.

However, whether a stable or a radioactive tracer is used, there is now a suitable algorithm for both men and women, requiring only a single serum sample and providing results within 1 day.

This study was supported in part by an agreement with the University of Pittsburgh, Graduate School of Public Health, by contracts with Roots, Inc. and DepoMed, Inc., by a grant from Health Future Foundation, and by Creighton University funds.

References


Sensitive Immunoluminometric Assay for the Detection of Procalcitonin, Nils G. Morgenthaler,* Joachim Struck, Christina Fischer-Schulz, and Andreas Bergmann (Research Department, B·R·A·H·M·S AG, Biotechnology Centre, D-16761 Hennigsdorf/Berlin, Germany; * author for correspondence: fax 43-3302-883-451, e-mail n.morgenthaler@brahms.de)

Procalcitonin (PCT) and other calcitonin precursors are detectable in various conditions leading to systemic inflammatory response syndrome. Among them are pancreatitis (1, 2), burns (3), polytrauma (4), and most importantly, bacterial infection (5). PCT reflects the severity of bacterial infection and has been used as a marker for the diagnosis and therapeutic monitoring of sepsis, severe sepsis, and septic shock of bacterial origin (6–10). The usual two-sided chemiluminescence assay [immunoluminometric assay (ILMA)] for PCT has a functional assay sensitivity (FAS) of 300 ng/L. This FAS is sufficient for the monitoring of septic patients in intensive care units, but the usefulness of the present ILMA in the usual hospital or outpatient setting is limited. Furthermore, except for an initial report on PCT and other calcitonin precursors in a few controls (8), it has not been possible to define the range of PCT in healthy individuals or to determine whether increased PCT exerts a pathophysiologic role (11–13).

We developed a new PCT assay with a >30-fold lower FAS compared with the established ILMA and measured PCT values in 500 healthy controls.

Samples were obtained from healthy blood donors (age range, 18–62 years; 241 males, 259 females) with no history of acute or chronic disease and with no symptoms of the common cold for the last 7 days. Written consent was obtained from all donors.

For the PCT assay, tubes were coated with a monoclonal antibody specific for the kalcitomin part of PCT. This antibody binds to amino acids 102–111 of PCT (ERDH-PHV5M). Coating of the antibody was done for 20 h on polystyrene tubes (2.0 µg/tube) in 0.3 mL of buffer (10 mmol/L Tris-HCl pH 7.8, 10 mmol/L NaCl). Tubes were blocked with 10 mmol/L sodium phosphate buffer containing 30 q/L Karion FP, 5 g/L protease-free bovine serum albumin (Sigma), pH 6.8, and lyophilized. A polyclonal sheep antibody specific for the calcitonin part of PCT was used as tracer. This antibody was raised to peptide 69–79 (GTTYQDLNKFH) of PCT and was affinity-purified on a calcium-sulfolate column and subsequently labeled with acridinium ester as follows: 100 µg of antibody in 20 mmol/L sodium phosphate buffer, pH 8.0, was incubated for 20 min at room temperature with 10 µl of acridinium ester (1 g/L in acetonitrile; Hoechst AG). Labeled antibody was purified by HPLC using a Knauer hydroxyapatite column (buffer gradient, 1–500 mmol/L potassium phosphate, pH 6.8; flow rate, 0.8 mL/min).

PCT was measured in a coated-tube assay in which 100 µL of a patient sample or calibrator was added in duplicate to each antibody-coated tube and incubated for 30 min at room temperature; 200 µL of tracer containing acridinium ester-labeled anti-PCT antibody was then added, followed by a 2-h incubation at room temperature. Tubes were washed five times with 2 mL of standard LUMTest® washing buffer (B·R·A·H·M·S AG), and detection was performed in a luminometer (detection time per sample, 1 s). This assay system was named B·R·A·H·M·S ProCa-S® to distinguish it from the similar LUMTest PCT® (B·R·A·H·M·S AG). Relative light units for the chemiluminescence assay were expressed in ng/L PCT as calculated from a calibration curve that was included in every analytical run.