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Calcium Bioavailability From a Calcium-Rich Mineral Water, With Some Observations on Method

Lucia Bacciottini, PhD, Annalisa Tanini, PhD,* Alberto Falchetti, MD,* Laura Masi, MD, PhD,* Francesco Franceschelli, PhD,* Barbara Pampaloni, PhD,* Gianluca Giorgi, PhD,† and Maria Luisa Brandi, MD, PhD**

Goals: The study was designed to determine whether high-calcium mineral water is an efficient additional source of dietary calcium, optimizing a method for calcium determination never used for mineral waters.

Background: It is generally agreed that an adequate calcium intake is necessary for the acquisition of an ideal peak bone mass and for the maintenance of the bone mineral density in adults, in postmenopausal women, and in the elderly. Mineral waters are calorie free, and some, with high calcium levels, might be significant sources of calcium.

Study: The availability of the calcium contained in a high-calcium mineral water was measured in 27 healthy subjects. In 8 subjects the calcium availability of the water was compared with the calcium availability ingested with milk at the same calcium load. Milk and water were labeled extrinsically with 30 mg ^{44}Ca. Fractional absorption from the oral dose was determined from plasma samples using ICP-MS technique.

Results: At an ingested calcium load of 3.18 mmol, percentage of absorption for water averaged 22.53 ± 2.53 (mean ± SD) for men, 22.57 ± 2.10 (mean ± SD) for premenopausal women and 21.62 ± 3.12 (mean ± SD) for postmenopausal women. Percentage absorption from milk was 23.15 ± 4.06 (mean ± SD).

Discussion: The calcium from the mineral water is thus highly bioavailable, at least as bioavailable as milk calcium, and ICP-MS appears to represent a reliable and reproducible method for calcium absorption from alimentary sources.

Key Words: calcium absorption, calcium bioavailability, mineral water, stable isotope^{44}Ca

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Recommended dietary allowance for calcium is between 1200 and 1500 mg/d for adolescent, pregnant and nursing women, and people aged >65 years.[1,2] However, an adequate calcium intake is now considered to be beneficial to bone mass at all stages of life. With decreasing energy expenditure in all industrialized nations, total food intake has declined since the end of World War II and dietary intake of calcium is generally much lower than the DRIs. To attain the optimal calcium intake it has been suggested that the frequency of consumption of dairy products and calcium-rich vegetables be increased. However, increasing dairy products in the diet increases the intake of saturation fat and calories. Although this drawback can be averted by using low-fat dairy products, these are not well tolerated or appreciated by all people. For all these reasons calcium supplements are frequently prescribed and administered daily, with a limited compliance due to gastrointestinal intolerance. Other low-energy calcium sources, not mentioned in the National Institutes of Health (NIH) Consensus Statement from the Health Consensus Conference on Optimal Calcium Intake (1994), are high-calcium mineral waters. If one agrees with the NIH Statement that "absorption of calcium supplements is most efficient at individual doses of 500 mg or less and when taken between meals," drinking calcium-rich mineral water several times a day could be recommended, providing both supplemental calcium and adequate hydration.

Even if limited information exists on bioavailability of high-calcium from mineral waters, some observations support the fact that it is superimposable to that from milk.[3,4] Moreover, in a recent study oral intake of water containing a moderate dose of calcium (172 mg) acutely inhibited parathyroid hormone secretion and bone resorption.[5]

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From the *Department of Internal Medicine, University of Florence, Viale Pieraccini 6, 50139 Florence and the †Interdepartmental Centre of Analysis and Structural Determinations, University of Siena, Via A. Moro, 53100 Siena, Italy.

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Reprints: Maria Luisa Brandi, MD, Department of Internal Medicine, University of Florence, Viale Pieraccini 6 50139 Florence, Italy (e-mail: m.brandi@dmi.unifi.it).

Water composition varies widely according to geographical origin, but only some waters have a high-calcium content (Table 1).[6] Some mineral waters with high levels of calcium are commercially available in Western Europe. These exist in 2 predominant categories, depending on the main accompanying anion, sulfate and bicarbonate rich waters,[4] and may provide a significant amount of additional calcium. We chose to investigate the bioavailability of calcium in a mineral water, widely consumed in Italy, containing calcium in the range of 202 mg/L and, therefore, one-fifth of recommended daily intakes for adults aged >25 years.

MATERIALS AND METHODS

Materials and Reagents

Enriched Ca isotope (^{44}Ca) was obtained by Chemical Research 2000 (Rome, Italy). HNO_3 was obtained from Merck (Darmstadt, Germany). All other chemicals were of the highest quality available. Distilled water was used throughout. Calcium assay on the AEROSET ™ System was purchased from Abbott Laboratories (Abbot Park, Illinois).

The composition of the Uliveto mineral water (Vicopisano, Italy) was as follows: calcium, 202 mg/L; magnesium, 29.8 mg/L; sodium, 113.7 mg/L; potassium, 11.6 mg/L; sulfate, 151 mg/L; bicarbonate, 683.2 mg/L; and chloride, 121.4 mg/L. Commercial skimmed milk, containing 120 mg Ca/100 mL, was used.

Quantitative analyses of the different stable isotopes of calcium in plasma were carried out by an inductively coupled plasma mass spectrometer Hewlett Packard 4500 operating in a cool plasma mode with a forward power of 650W and a reflected power of about 1W.

Subjects

Subjects with extremes of weight for height were excluded, as were those with disorders and medications known to alter intestinal absorptive function. Eight adult male volunteers (aged 32–49 years, weight 74.7 ± 6.9 Kg), 8 adult premenopause women (aged 30–43 years, weight 70 ± 7.5 Kg), and 8 post-menopause women (aged 56–60, weight 68.4 ± 6.9 Kg) were recruited from healthy community volunteers. Table 2 describes profiles of subjects.

All subjects gave informed consent to the procedures, which were approved by the local Ethical Committee (University Institutional Review Board).

TABLE 1. Some European Mineral Waters with Their Calcium

Brand Name	Calium (mg/L)	Country
St. Augustinus	585	Germany
Schillerquelle Ensingen	585	Germany
Vittel Hepar	575	France
Adelborner	569	Switzerland
St. Margareten	566	Germany
Eptinger	555	Switzerland
Contrex	486	France
Ferrarelle	368	Italy
Sangemini	322	Italy
Vittel Grande	202	France
Uliveto	202	Italy
SanPellegrino	208	Italy

Preparation of the Stable Isotope ^{44}Ca

Enriched isotope ^{44}Ca was obtained from Chemical Research 2000 (Rome, Italy) as calcium carbonate (96.4 atom %). The ^{44}Ca solution was prepared by dissolving the calcium carbonate in concentrated hydrochloric acid (2.5 g $CaCO_3$ plus 5 mL HCl), adjusting the pH to 6.0 with 1-M sodium hydroxide and making the solution up to a final volume of 100 mL. Three-milliliter portions were dispersed into ampoules and sterilized.

TABLE 2. Subject General Characteristics

ID*	Test type	Age	MS†	Sex
A1	W	45		M
A2	W/M	39		M
A3	W	43		M
A4	W	32		M
A5	W	49		M
A6	W/M	42		M
A7	W/M	48		M
A8	W	36		M
A9	W	43		M
A10	W	34	pre	F
A11	W/M	38	pre	F
A12	W	43	pre	F
A13	W	32	pre	F
A14	W/M	35	pre	F
A15	W/M	41	pre	F
A16	W	30	pre	F
A17	W	34	pre	F
A18	W	39	pre	F
A19	W	56	post	F
A20	W	63	post	F
A21	W	61	post	F
A22	W	60	post	F
A23	W	59	post	F
A24	W/M	63	post	F
A25	W	66	post	F
A26	W/M	60	post	F
A27	W	58	post	F

*Patient Identification
†Menopausal Status
W, Uliveto Mineral Water; M, Milk.

The exact quantity of isotope given to each subject was measured by weighing the dispensed volume (approximately 3 mL/dose).

Protocol

The test material was Uliveto mineral water or milk externally labeled with 30 mg of ^{44}Ca on the afternoon before the test as described elsewhere.[7]* The labeled calcium load was ingested after an overnight fast and with abstinence from alcohol for the previous 24 hours. The load was taken in the middle of a light breakfast consisting of 2 pieces of toasted Italian bread with butter, together with coffee (decaffeinated or regular) or tea. To provide 100 mg of Ca (2.5 mmol) the quantity of Uliveto water to be ingested was 490 mL and that of milk was 83 mL. The containers were rinsed with deionized water and the rinsing consumed as well, to assure full transfer of the intended dose into subject. The total dose of calcium administered orally was 130 mg calcium (100 mg calcium enriched with 30 mg ^{44}Ca). A single blood sample was taken exactly 5 hours after ingestion of labeled liquid. The 2 tests were separate by 2-months interval. These samples were analyzed for isotopic tracer content. Venous blood was taken from all subjects before ingestion of the tracer, and several pools were prepared. The samples were used to calculate natural $^{44}Ca/^{43}Ca$ ratio. Total plasma calcium was also measured.

Absorption was measured with a single-isotope method,[8] using inductively coupled plasma mass spectrometry (ICP-MS) to measure tracer level of stable isotope of calcium in plasma.[9]

Plasma Sampling

Blood was collected in heparinized tubes, centrifuged (1500 rpm for 15 minutes at 4°C), and trichloroacetic acid (TCA) (35 g/L) added at ratio of 3 parts: 1 part to the plasma fraction. The mixture was allowed to stand for 15 minutes at 4°C, centrifuged (2000 rpm for 30 minutes at 4°C), 1-mL deproteinized supernatant fraction removed, evaporated to dryness under vacuum and redissolved with 100 μL 2M HNO_3.

Analytic Methods

The levels of calcium in plasma samples were determined according to Aeroset System (Abbott Laboratories, Abbot Park, Illinois). Briefly, arsenazo-III dye reacted with calcium in an acid solution to form a blue-purple complex. The color developed was measured at 660 nm and it was proportional to the calcium concentration in the sample.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

The isotopes ratios were measured using inductively coupled plasma mass spectrometry according to Patterson et al.[9]

The ICP-MS instrument employed a plasma (ICP) as the ionization source and a mass spectrometer (MS) analyzer to detect the ions produced. Briefly, the technique consisted of the injection of a nebulized mist from a liquid into the center of an high energy argon plasma that was produced by collisions between the Ar atoms caused by an intense RF field. The sample aerosol was decomposed in the plasma to form analyte atoms that were ionized. The ions produced were extracted from the plasma into the high vacuum mass spectrometer region. The analyte ions were then focused into a quadrupole mass analyzer, which separated the ions based on their mass/charge ratio.

Calculation of Ca Absorption

Amount of tracer present in the plasma of a subject was calculated using the following equation:

$$\text{Amount of tracer} = \frac{\text{mes}\,\frac{^{44}Ca}{^{43}Ca} - \text{na}\,\frac{^{44}Ca}{^{43}Ca}}{\text{na}\,\frac{^{44}Ca}{^{43}Ca}} \times (\text{total Ca}) \times \text{na}\ ^{44}Ca$$

The measured (mes) ratio of ^{44}Ca to ^{43}Ca was corrected for the natural abundance (na) ratio of ^{44}Ca to ^{43}Ca and then divided by the natural abundance ratio, which gives the enrichment of the tracer in the sample. The amount of the tracer was calculated by multiplying the enrichment ratio of the tracer, total calcium of the sample, and natural abundant ratio of the tracer (na).

Total Ca was obtained multiplying calcium concentration per liter of plasma and distribution volume of extracellular calcium, determined multiplying by 0.15 the total body weight.[10]

Calcium absorption was determined from the differences between the quantities of tracer ingested and that evaluated in plasma. The calcium absorption was expressed as a percentage, using the following equation:

$$\%\ \text{Ca absorption} = \frac{\text{amount of tracer}}{\text{dose}} \times 100$$

Dose represented the quantity of tracer administered to each subject.

Statistical Analysis

(Mann-Whitney *U* test, from Statistica program, version 6.0) Paired *t* tests were used to compare percentage Ca absorption from mineral water between groups. Difference between sources (water and milk) were also evaluated by the paired *t* test (Mann-Whitney *U* test).

RESULTS

Preliminarily blank plasma samples were studied. $^{44}Ca/^{43}Ca$ Isotope ratios were in full agreement with the natural value, thus excluding the presence of interferences, such as bicharged ions and oxides. This suggested that the method had little or absent systematic bias. Calibration curves obtained from standard solutions containing different amounts of Ca were used to verify the linearity, accuracy, precision and reliability of the experiments. ^{45}Sc has been added to each sample and used as an internal standard. The plasma samples were bracketed 5 × 5 by unenriched samples containing known amount of calcium.

The normal RDS of the mean of the ratio attainable for multiple measurements of plasma samples using the present instrumentation has been determined to be less than 0.5%.

Mean calcium absorption at this calcium load for Uliveto water was 22.53 ± 2.53 (mean ± SD) in men (Table 3), 22.57 ± 2.10 (mean ± SD) in premenopausal women (Table 4) and 21.62 ± 3.12 (mean ± SD) in postmenopausal women (Table 5). Values of calcium absorption did not differ significantly ($P > 0.05$) for the 3 groups analyzed.

In 8 subjects of the group (3 men, 3 premenopausal women, and 2 postmenopausal women) percent absorption at the same calcium load for milk calcium was evaluated and was demonstrated to be 23.15 ± 4.06 (mean ± SD) (Table 6). Mean calcium absorption from milk was not significantly different ($P > 0.05$) from that of Uliveto mineral water.

DISCUSSION

A reliable estimate of calcium absorption may be obtained using combined oral and IV tracers, as first developed by DeGrazia et al[11] with ^{45}Ca and ^{47}Ca. Through this methodology, fractional intestinal Ca absorption can be determined from the ratio of the specific activities of simultaneously administered oral ^{47}Ca and IV administered ^{45}Ca measured in a plasma or urine samples. However, it was early recognized that these isotopes were not appropriate for studies in children and pregnant women, and, more recently, public perception of the risk associated with the use of radioactive tracers further limited their use. Because of increasing awareness of hazards of

TABLE 3. Analytic Data of Uliveto Water Bioavailability in Men

ID*	WT†	PCa‡	% abs§	Δ¶
A1	79.00	2.47	21.11	23,610
A2	63.00	2.25	24.17	37,200
A3	80.00	2.43	19.74	22,167
A4	80.00	2.37	15.59	17,950
A5	78.00	2.47	13.58	15,380
A6	63.00	2.25	24.52	37,750
A7	74.00	2.37	20.49	25,500
A8	80.00	2.20	24.16	29,950
A9	75.00	2.30	16.13	20,410
Mean ± SD‖	74.67 ± 6.96	2.35 ± 0.10	19.94 ± 4.07	

*Patient Identification
†Body weight (Kg)
‡Plasma calcium concentration (mmol/L)
§Percentage of calcium absorption
¶Enrichment of the tracer ^{44}Ca in the samples
‖Mean of values of WT, pCa and % abs ± Standard deviation

TABLE 4. Analytic Data of Uliveto Water Bioavailability in Premenopausal Women

ID*	WT†	PCa‡	% abs§	Δ¶
A10	70.00	2.37	21.52	28,300
A11	60.00	2.47	12.54	20,210
A12	60.00	2.47	20.75	30,550
A13	75.00	2.20	18.03	23,840
A14	77.00	2.47	11.09	18,900
A15	80.00	2.20	20.21	20,210
A16	63.00	2.25	22.25	34,260
A17	76.00	2.15	21.47	28,680
A18	70.00	2.20	25.62	36,300
Mean ± SD‖	70.11 ± 7.57	2.31 ± 0.14	19.28 ± 4.69	

*Patient Identification
†Body weight (Kg)
‡Plasma calcium concentration (mmol/L)
§Percentage of calcium absorption
¶Enrichment of the tracer ^{44}Ca in the samples
‖Mean of values of WT, pCa and % abs ± Standard deviation

TABLE 5. Analytic Data of Uliveto Water Bioavailability in Postmenopausal Women

ID*	WT†	pCa‡	% abs§	Δ¶
A19	56.00	2.25	21.37	37,000
A20	76.00	2.25	15.93	20,333
A21	74.00	2.40	19.83	24,370
A22	72.00	2.47	16.46	20,200
A23	65.00	2.42	21.21	29,420
A24	68.00	2.47	26.46	34,370
A25	75.00	2.42	19.92	23,940
A26	70.00	2.37	26.20	26,200
A27	60.00	2.47	10.97	13,850
Mean ± SD‖	68.44 ± 6.93	2.39 ± 0.09	19.82 ± 4.92	

*Patient Identification
†Body weight (Kg)
‡Plasma calcium concentration (mmol/L)
§Percentage of calcium absorption
¶Enrichment of the tracer ^{44}Ca in the samples
‖Mean of values of WT, pCa and % abs ± Standard deviation

TABLE 6. Analytic Data of Milk Bioavailability

ID*	WT†	PCa‡	% abs§	Δ‖
A2	60.00	2.37	30.13	34,670
A6	65.00	2.47	25.70	42,830
A7	72.00	2.43	21.15	26,370
A15	83.00	2.15	14.45	17,669
A11	62.00	2.43	26.88	30,180
A14	78.00	2.25	10.27	17,810
A24	69.00	2.15	15.99	21,350
A26	69.00	2.40	18.48	24,350
Mean ± SD‖	69.75 ± 7.81	2.33 ± 0.13	20.38 ± 6.83	

*Patient Identification
†Body weight (Kg)
‡Plasma calcium concentration (mmol/L)
§Percentage of calcium absorption
¶Enrichment of the tracer ^{44}Ca in the samples
‖Mean of values of WT, pCa and % abs ± Standard deviation

ionizing radiation, efforts have been made to use stable isotopes instead of radioisotopes wherever possible. Advantages of stable isotopes are that they do not carry such a perceived risk and that they pose no problems of disposal and can be stored for indefinite times without degradation. In this study, using a single-label stable-isotope technique,[8] calcium absorption from a calcium-rich mineral water was measured in humans and compared with calcium absorption from milk. This technique allows simple and accurate estimation of the fractional absorption of calcium. The amounts of isotope administration are an important consideration of the experimental design because, unlike radioisotopes, stable isotopes are not strictly tracers. Because they are naturally present in all tissues and foods, the dose administered must be large enough to cause measurable enrichment in the samples to be analyzed. Regarding the oral dose, until more information is available, it must be assumed that the added isotope behaves in a similar fashion to the calcium contained in the test source.[7]

If stable isotopes are used as tracers, their concentration must be determined with high precision since tracer levels are typically only a few percent above the endogenous isotopic levels. The measurement of isotopic composition and isotope ratios in biologic samples requires sensitive, precise, and accurate analytical techniques.

Smith[12] was the first to use FAB mass spectrometry to measure calcium isotopes, and he used this methodology for measuring fractional true absorption of calcium by the double isotope technique.[13] In the absence of a dedicated inorganic mass spectrometer, FAB offers an alternative method of measuring inorganic stable isotopes, although it is less sensitive and generally less accurate than thermal ionization.[14] Yergey et al[14] used an oral dose of ^{44}Ca coupled with an intravenous dose of ^{42}Ca to measure fractional Ca absorption from a commercial milk formula, employing thermal-ionization mass spectrometry to quantify the isotope enrichment in urine samples. FAB mass spectrometry, however, have the advantage of requiring less rigorous sample preparation.[15]

In this study we have preliminarily used Liquid Secondary Ion Mass Spectrometry for quantifying Ca isotopes but the method was revealed unsuccessful, owing to the background interferences coming from the Caesium pellet. Our choice moved to use inductively coupled plasma mass spectrometry to quantify the stable isotopic ^{44}Ca tracer concentration of calcium in blood. This approach based on the use of a widely available instrument, a quadrupole inductively coupled mass spectrometer operated in a low power mode, was shown to be a useful and powerful tool for the analysis of the present plasma samples.

An ICP-MS instrument could simultaneously measure most elements in the periodic table and determine analyte concentration down to the sub nanogram-per-liter (ng/L) or part-per trillion (ppt) level. It could perform qualitative, semi quantitative, and quantitative analysis, and because it uses a mass analyzer, it could also measure isotopic ratios.

Even if the use of ICP-MS was limited in the past in the analysis of calcium,[16] the recent improvements in instrumentation have opened new perspectives in this field.[9] In the present case the features of ICP-MS have been successfully used to quantify the 2 calcium isotopes (ie, ^{44}Ca and ^{43}Ca) in plasma samples.

Dual isotopic techniques are cumbersome and expensive and, therefore, difficult to apply to large populations. The results of the studies in adult females by Heaney and Recker[8] indicated that a single blood sample obtained 5 hours after an oral load of calcium, adjusted for height and weight, provided the best estimate of calcium absorption. A study of Miller et al[17] indicated that the level of oral tracer in serum taken 150 minutes post-ingestion is significantly correlated with calcium absorption in children, as determined by the tracer levels in the urine. Marshall and Nordin[18] also compared a number of methods for estimating calcium absorption and concluded that the dual isotope technique was not more precise than single isotope methods. For all these reasons we used the single isotope method and a single 5-hour sample of blood for estimating calcium absorption.

This work establishes that calcium present in the Uliveto water is highly bioavailable as the calcium of milk. Mean absorption for both calcium sources was the same in premenopausal and postmenopausal women. No difference was found between the group of men and that of women. Estrogen status and menopause, that generally exercise some influence on calcium absorbability,[19] had not particular influence on Ca-absorbability from Uliveto water according to Heaney and Dowell.[3] In our study the mean absorption fractions were apparently lower than those reported in other studies.[3] This result could be partially explained according to: (1) different meth-

ods of Ca absorption evaluation; (2) different protocols (use of stable isotope vs. radioactive tracers, single isotope vs. dual isotopic technique, 5 hours plasma sample vs. different time plasma sample, etc); (3) restricted number of subjects with different and uncontrolled diet. Moreover, in our study the Ca amounts present in the breakfast were not strictly controlled.

It should be emphasized that the absorption fractions found in this study (close to 22% for Uliveto water) apply specifically to the calcium amount load employed. In fact, it has been shown that, for a variety of calcium sources, absorption fraction shows an inverse function of the logarithm of the load.[20] Thus, if a source as Uliveto water is consumed apart of a meal containing other appreciable calcium sources, its absorption will probably be higher. On the basis of this last consideration we can consistently emphasize the role of this kind of water as a daily supplement of Ca. Italian national healthy dietary schedules[21] recommend to drink about 2 L of water throughout the day. In the case of Uliveto water 2 L supplies 400 mg of Ca that is 1/3 (for adult postmenopausal women) or more (for adult premenopausal women and men) of the total recommended daily intake. Therefore the use of a such calcium rich mineral water integrated with other calcium rich non dairy-foods (almonds, nuts, and cabbage) along with a mild calcium supplementation can fulfil the calcium requirements of lactose intolerant and overweight subjects

In conclusion, the results of this study are in agreement with previously published findings, supporting that high-calcium mineral waters represent an important dietary source of calcium and should be recognized as good low-calorie nutritional calcium supplements. It is hoped in early future to reach a common agreement on the methods to evaluate Ca absorption from alimentary sources, making possible to create a homogeneous standard protocol to be applied to such studies.

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