Calcium Bioavailability and Kinetics of Calcium Ascorbate and Calcium Acetate in Rats

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The objective was to investigate the bioavailability and mechanism of calcium absorption of calcium ascorbate (ASC) and calcium acetate (AC). A series of studies was performed in adult Sprague-Dawley male rats. In the first study, each group of rats (n = 10/group) was assigned to one of the five test meals labeled with ^{45}Ca: (i) 25 mg calcium as heated ASC or (ii) unheated ASC, (iii) 25 mg calcium as unheated AC, (iv) 3.6 mg Ca as unheated ASC, or (v) unheated AC. Femur uptake indicated better calcium bioavailability from ASC than AC at both calcium loads. A 5-min heat treatment partly reduced bioavailability of ASC. Kinetic studies were performed to further investigate the mechanism of superior calcium bioavailability from ASC. Two groups of rats (n = 10/group) received oral doses of 25 mg Ca as ASC or AC. Each dose contained 20 μCi ^{45}Ca. Two additional groups of rats (n = 10/group) received an intravenous injection (iv) of 10 μCi ^{45}Ca after receiving an unlabeled oral dose of 25 mg calcium as ASC or AC. Sequential blood samples were collected over 48 hrs. Urine and fecal samples were collected every 12 hrs for 48 hrs and were analyzed for total calcium and ^{45}Ca content. Total calcium and ^{45}Ca from serum, urine, and feces were fitted by a compartment kinetics model with saturable and nonsaturable absorption pathways by WinSAAM (Windows-based Simulation Analysis and Modeling). The difference in calcium bioavailability between the two salts was due to differences in saturable rather than passive intestinal absorption and not to endogenous secretion or calcium deposition rate. The higher bioavailability of calcium ascorbate was due to a longer transit time in the small intestine compared with ASC. Exp Biol Med 229:40–45, 2004

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Vitamin and mineral supplements are widely used to maintain recommended vitamin and mineral intakes. Many types of calcium supplements have been commercially available and calcium bioavailability of several of these preparations has been reported (1). Two salts that appear to have disparate calcium absorption efficiency for which the mechanism of absorption has yet to be studied are calcium ascorbate and calcium acetate.

Calcium ascorbate is used mainly as a buffered vitamin C supplement to overcome the gut irritation side effect of ascorbic acid supplements (2). Calcium ascorbate provides not only ascorbate but also elemental calcium as 9.4% by weight. Presently, high intakes of vitamin C are suggested to prevent certain diseases. If vitamin C is taken as calcium ascorbate, the high intake could also bring in fairly significant amounts of elemental calcium in addition to calcium from other food resources. Calcium absorption from calcium ascorbate was superior to calcium carbonate and calcium chloride using pharmacokinetic analysis of serum calcium after the salts labeled with calcium radioisotope were ingested in rats. Femur uptake of ^{45}Ca was also higher from calcium ascorbate than from calcium carbonate and calcium chloride (3).

Calcium acetate is used mainly as a phosphate binder in renal-compromised patients to reduce absorption of phosphate. Calcium acetate has been shown to be absorbed similarly to calcium citrate, calcium lactate, and calcium carbonate in human subjects (4). Unlike calcium carbonate, which is insoluble in water, calcium acetate is much more soluble, which makes it easier to be delivered to the stomach by gavaging. The gavaging method is commonly used in animal studies to completely deliver radioisotopes or food ingredients.

Calcium is absorbed in the small intestine through two pathways: active or transcellular and passive or paracellular pathways. The aims of our study were to compare calcium bioavailability of calcium ascorbate and calcium acetate and

to further investigate the mechanism of calcium absorption by evaluating the pathways of absorption using kinetic modeling.

Methods

Animals. Male Sprague-Dawley rats (Harlan Inc., Indianapolis, IN) 3 months old, weighing 250–275 g, were randomly divided into groups of 10 rats each. The rats were individually housed in stainless steel cages in an environmentally controlled room (temperature, 20°C; relative humidity, 30%–60%; reversed light:dark cycle, 12:12 hr) at Purdue University (West Lafayette, IN). The rats were fed *ad libitum* pelleted laboratory rat chow adequate in nutrients (Purina Mills, Inc., St Louis, MO) and deionized water. This study was approved by Purdue University Animal Care Committee.

A series of studies was conducted to study the bioavailability of calcium and pathways of absorption from calcium ascorbate and calcium acetate in rats. Solutions of the calcium salts labeled with radioactive calcium containing either 20 or 10 μCi ^{45}Ca /ml were prepared from concentrated ^{45}Ca solution (2 mCi/ml) and delivered directly to the stomach of the animals.

All animals were moved to metabolic cages 12 hrs prior to gavaging. The animals were fasted overnight preceding and food was resumed 3 hrs after the gavage. Urine and fecal samples were collected separately for 48 hrs.

Surgery. Jugular catheters were inserted for blood-sample collection and intravenous injection of radioactive ^{45}Ca. The surgery was performed with the aid of light anesthesia (0.8 mg/kg xylazine and 7.2 mg/kg ketamine). Detailed surgical techniques were as follows: The rats were placed in dorsal recumbency. A 1-cm incision was made over the right jugular area and blunt dissection was used to identify and isolate the jugular vein. A curved hemostat was placed under the vein. A small longitudinal incision was made with small iris scissors and the catheter was positioned into the vein using a plastic introducer. The catheter was made of silastic tubing (0.020 in. i.d., 0.037 in. o.d.; VWR, Chicago, IL) with an enlargement of 1–2 mm at the site of entry into the vein to allow anchoring of the catheter. A 3/0 silk suture was used to ligate around the vein and catheter at both sides of the enlargement. The catheter was filled with heparinized saline (100 units/ml; Pharmacy, Purdue University) to prevent any entry of air into the vein and circulatory system. The catheter was placed 10–15 mm into the jugular toward the heart. The free end of the catheter was tunneled under the skin to exit between the ears. Both skin incisions were closed with 3/0 nylon sutures. The catheter was trimmed to a suitable length, flushed with saline, and then plugged with a stopper.

Postsurgery Recovery. Rats were housed in a warm room (27°C) to maintain their body temperature during the night after surgery. Rats were allowed free access to food and water right after surgery. Two or three days were allowed for recovering, and body weight was monitored during the period. Jugular catheters were flushed with heparin (100 units/ml) once a day to prevent blocking.

Experiments. Three studies were conducted to compare the bioavailability and absorption pathways of calcium from calcium ascorbate and calcium acetate in rats.

Study 1: Femur Uptake. Femur uptake of ^{45}Ca was used to measure the fractional absorption of calcium from calcium acetate and calcium ascorbate. Calcium ascorbate and calcium acetate were obtained from Sigma Inc. (St. Louis, MO). Rats (n = 10/group) were assigned to receive either calcium ascorbate or calcium acetate at a low dose of 3.6 mg calcium or a dose of 25 mg, representing one third of the daily requirement for a rat. One additional group was given 25 mg Ca as calcium ascorbate, which had been heated by boiling for 5 mins. The heating treatment was assumed to degrade ascorbate due to heat-induced oxidation. Each dose contained 9 μCi ^{45}Ca as $CaCl_2$. For each study, 10 additional rats received an intraperitoneal injection (IP) of 9 μCi ^{45}Ca as $CaCl_2$ to mimic 100% absorption. Femurs of the rats were taken 48 hrs after gavage. Fractional absorption was determined by radioactive uptake of ^{45}Ca into the right femur of rats as described previously (5) according to the following equation:

$$\text{Percent absorption} = \frac{(\%\ \text{dose in the femur of experimental group})}{(\%\ \text{dose in the femur of the IP group})} \times 100. \quad (1)$$

The ratio can be used to assess calcium absorption because transport of calcium after crossing the gut subsequent to dissociation of calcium from its ligands and subsequent uptake of ^{45}Ca by femurs would be equal for rats on oral and intraperitoneal doses. Relative differences in bioavailability using this model parallels results of human studies (6).

*Study 2: Calcium Kinetics. **Serum sampling only**.* Two groups of rats (3–6 rats/group) received oral doses of 25 mg calcium as calcium ascorbate or calcium acetate. Each dose contained 20 μCi ^{45}Ca. Another two groups (3–5 rats/group) received an intravenous injection (iv) of 10 μCi ^{45}Ca as calcium chloride to profile calcium serum kinetics while receiving orally 25 mg calcium as calcium ascorbate or calcium acetate.

Blood samples were collected at 0, 5, 10, 15, 20, 30, 45, 60, 80, 100, 120, 175, and 250 mins after dosing and assayed for ^{45}Ca. At each time point, 0.3 ml blood was collected through the jugular catheter, and serum was separated by immediate centrifugation after sampling. After serum was separated, 100 μl serum from each sample was transferred to scintillation counting vials in which 25 μl 3 N KOH and 100 μl H_2O_2 (at a concentration of 30%) were added to bleach the serum. After 30 mins of incubation, 25

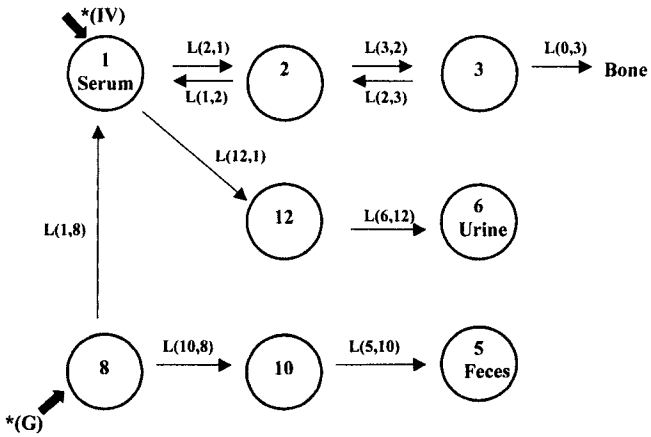

Figure 1. Kinetic model of calcium metabolism in rats. Circles represent compartments. Numbers in circles represent compartment number. Thin arrows represent movement between compartments, and thick arrows represent entry of calcium via oral dosing (*(G)) or intravenous injection (*IV). Calcium absorption pathways were determined with serum samplings only. Compartment 1, serum; Compartments 2, 3, calcium exchange pools in the body; Compartment 5, feces; Compartment 6, urine; Compartment 8, gastrointestinal tract; L(I, J), transfer coefficient (fraction/minute) representing the fraction of compartment J transferring into Compartment I per unit time.

μl 3 *N* HCL was added to neutralize the pH and 15 ml Ecolite (+) was added to each sample before counting by liquid scintillation counter (Beckman 6500). Results were analyzed by compartmental modeling using the WinSAAM (Simulation Analysis and Modeling) program (developed by the National Institutes of Health) in which a curve generated from blood sampling was fitted by a computer-simulated curve. The computer-simulated curve provided transfer rates between different compartments, which can be used to calculate absorption, excretion, and other data.

A complete calcium kinetic model is shown in Figure 1. Because the purpose of Study 2.1 was to compare plasma calcium kinetic profiles from two calcium salts, only part of the model was used to calculate the calcium absorption. Compartments 5, 6, and 12 were not included in the analysis. Calcium absorption was calculated using the following equation (see Fig. 1 for terms):

$$\frac{L(1,8)}{[L(1,8)+L(10,8)]} \times 100. \quad (2)$$

Serum, urine, and fecal sampling. Study 2 was repeated using 10 rats per group to design a more complete model. As in Study 2, in this study, 13 serum samples were obtained at 0, 2, 10, 20, 40, 60, 120, 180, 360, 720, 1440, 2160, and 2880 mins after dosing. Urine and feces were collected every 12 hrs for up to 48 hrs. Femurs of the rats were taken upon sacrifice at 48 hrs after dosing. ^{45}Ca radioactivity was measured in urine, fecal, and femur samples. Fractional calcium absorption from femur uptake was calculated as described in Study 1 and also by tracer balance as:

Table 1. Fractional Absorption of Calcium from Calcium Ascorbate and Calcium Acetate from Femur Uptake (Study 1)[a]

	25-mg calcium load	3.6-mg calcium load
Calcium ascorbate	70 ± 5 A	76 ± 6 A
Boiled (5 mins) calcium ascorbate	60 ± 4 B	ND
Calcium acetate	45 ± 5 C	60 ± 6 B

[a] ND, not determined. Significant difference in group means at $P < 0.05$ within calcium load are denoted by different letters.

$$\% \text{ dose absorption} = \frac{(\text{tracer in oral dose} - \text{tracer in fecal and urine loss})}{\text{tracer in oral dose}} \times 100. \quad (3)$$

^{45}Ca data in serum, urine, and feces were analyzed by the complete calcium compartmental modeling (Fig. 1). This model included the pathways of urinary calcium excretion (Compartment 6), endogenous calcium excretion and fecal calcium loss (Compartment 10). Calcium absorption was calculated as in Eq. 2.

Calcium tracer retention was also calculated as shown here:

$$\frac{(\text{tracer in oral dose} - \text{tracer in urine and feces})}{\text{tracer in oral dose}} \times 100. \quad (4)$$

The available time of calcium salts for absorption was described by the turnover of Compartment 8. Compartment 8 represented the site of absorption. The turnover rate of Compartment 8 was calculated as the reciprocal of the losses from Compartment 8 (Eq. 5).

$$\frac{1}{L(8,8)} = \frac{1}{L(1,8)+L(10,8)} \times 100. \quad (5)$$

Study 3: Calcium Dynamics. Calcium absorption from calcium acetate was determined as described in Study 2 at three calcium loads: 4, 7, and 25 mg. Serum profiles were compared with those from calcium ascorbate tested at seven calcium loads: 1, 3.8, 7, 9, 11.08, 15, and 25 mg as previously reported (7). L(1, 8) was obtained by fitting all data at various calcium loads simultaneously. The function of L(1, 8) contained a linear term and a saturable term of the Michaelis-Menten form:

$$L(1,8) = \frac{V_{max}}{(K_m + load)}, \quad (6)$$

where L(1, 8) is the transfer from the intestinal pool to serum (fraction/min), A is the constant fraction representing paracellular transfer, V_{max} is the V_{max} of the saturable pathway (mg/min), K_m is mg, and *load* is the amount of calcium (mg) in the gut compartment.

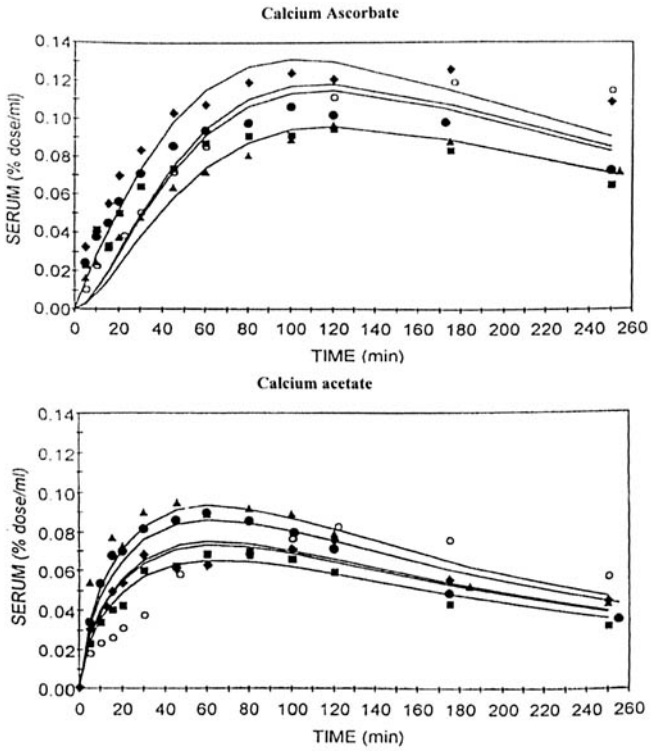

Figure 2. Serum ^{45}Ca profile of calcium ascorbate and calcium acetate at 25-mg calcium load. Different symbols represent serum ^{45}Ca profile of calcium ascorbate (upper panel) and calcium acetate (lower panel) of individual rats.

Results

The results obtained by the femur uptake experiment (Study 1) showed that calcium absorption from calcium ascorbate was significantly higher than from calcium acetate ($P < 0.05$) at both the 3.6- and 25-mg calcium loads (Table 1). Kinetic analysis of calcium absorption at 25-mg calcium load also confirmed the observation (Fig. 2). Five minutes boiling apparently altered the ascorbate salt as the absorption of calcium from the heated calcium ascorbate was lower than from the nonheated salt. However, absorption of calcium from heated calcium ascorbate was still significantly higher than that from calcium acetate (Table 1).

Table 2. ^{45}Ca Retention from Calcium Ascorbate and Calcium Acetate Following Oral Dose at 25-mg Calcium Load (Study 2)[a]

	Calcium ascorbate		Calcium acetate	
Retention (% dose)	63 ± 9	A	18 ± 7	B
Fecal excretion (% dose)	37 ± 9	A	82 ± 7	B
Urinary excretion (% dose)	0.9 ± 0.1	A	0.1 ± 0.1	B

[a] Significant difference at $P < 0.05$ between group means are denoted by different letters.

Table 3. ^{45}Ca Retention from Calcium Ascorbate and Calcium Acetate Following iv Dose (Study 2)

	Calcium ascorbate	Calcium acetate
Retention (% dose)	82 ± 3	79 ± 2
Fecal excretion (% dose)	16 ± 3	19 ± 2
Urinary excretion (% dose)	1.8 ± 0.8	1.6 ± 0.8

No significant difference at $P < 0.05$ was observed between groups for any parameters.

^{45}Ca retention using a metabolic balance method confirmed that calcium ascorbate from the ascorbate salt was absorbed better than from calcium acetate (Table 2). Fecal excretion of orally dosed ^{45}Ca was higher in the calcium acetate group than in the calcium ascorbate group while urinary excretion was the opposite (Table 2). A portion of fecal excretion is derived from endogenous secretion, but use of an iv tracer minimizes error of endogenous secretion ^{45}Ca (Table 3).

Further analysis by kinetic modeling showed that the fractional transfer (L(1, 8)) of both salts representing absorption rate were similar, but the loss to feces (L(10, 8)) was much faster from calcium acetate than calcium ascorbate. Therefore, the turnover rate of Compartment 8, which is the site of absorption, 1/L(8, 8), was much slower for calcium ascorbate than calcium acetate (Table 4). Analysis of the absorption pathway (Study 3) showed that the linear term and V_{max} were similar for both salts, while K_m was threefold higher for calcium acetate (Table 5, Figs. 3 and 4).

Discussion

Absorption of calcium from calcium ascorbate was substantially higher than from calcium acetate using three

Table 4. Kinetic Model Parameters in Calcium Metabolic Study (Study 2)[a]

	Calcium ascorbate	Calcium acetate
L(2, 1)	0.0800 ± 0.0210	0.1110 ± 0.0200
L(1, 2)	0.0220 ± 0.0120	0.0310 ± 0.0090
L(3, 2)	0.0094 ± 0.0046	0.0109 ± 0.0021
L(2, 3)	0.0013 ± 0.0005	0.0015 ± 0.0003
L(0, 3)	0.0006 ± 0.0001	0.0007 ± 0.0001
L(1, 8)	0.0016 ± 0.0003	0.0017 ± 0.0001
L(6, 12)	0.003 ± 0.003	0.01 ± 0.03
L(12, 1)	0.0003 ± 0.0001	0.0003 ± 0.0001
L(10, 8)	0.0007 ± 0.0003	0.0064 ± 0.0068
1/L(8, 8)	419 ± 140	125 ± 43

[a] L(I, J), transfer coefficient (fraction/min) representing the fraction of compartment J transferring into compartment I per unit time; Compartment 1, serum; Compartments 2, 3, calcium exchange pools in the body; Compartment 5, feces; Compartment 6, urine; Compartment 8, gastrointestinal tract. 1/L (8, 8) units are min.

Table 5. Analysis of Absorption Pathway by Kinetic Modeling (Study 3)[a]

	A (min)	V_{max} (mg/min)	K_m (mg)
Ca ascorbate	0.00041 ± 0.00019	0.030 ± 0.004	2.47 ± 0.42
Ca acetate	0.00049 ± 0.00052	0.036 ± 0.018	7.35 ± 3.55

[a] A, Constant fraction representing paracellular transfer; V_{max}, V_{max} of saturable pathway (mg/min); K_m, mg.

methods: the femur uptake method, calcium tracer retention, and kinetic analysis. The 25% higher calcium absorption from ascorbate compared with acetate was reduced by preheating the salt. The heating treatment indicated that the higher absorption of calcium ascorbate is related to the anion because the heating treatment would only oxidize the ascorbate, not the calcium. The 5-min boiling procedure may not have been sufficient to oxidize all the ascorbate, as the calcium from the heated calcium ascorbate was still absorbed better than calcium from calcium acetate by 15%. The results from ^{45}Ca retention from the oral and intravenous doses further indicated that the difference in calcium bioavailability between the two salts was due to intestinal absorption, not endogenous secretion or calcium deposition rate. This observation is supported by a recent study (3). In that study, calcium retention from calcium ascorbate was twice as high as calcium carbonate and calcium chloride.

Generally, bioavailability of calcium from calcium supplements depends on intrinsic and extrinsic factors. Intrinsic factors include the state of gastric acid secretion and calcium and vitamin D status, which affect the solubility of calcium salts and the efficiency of calcium absorption. These would be similar in inbred rats. Extrinsic factors represent chemical composition of calcium supplements and interaction of calcium supplements with food (8). Both salts are very soluble in water (solubility of calcium acetate is 43.6 g/100 g water, calcium ascorbate NA), but solubility of a calcium source has very little influence on its absorbability (9); and absorbability of calcium from food sources is determined mainly by other food components (9). Diets in this study were the same for both testing groups. The behavior of the anion may explain the difference in bioavailability of the two salts. Ascorbate forms a stable soluble complex with Ca^{2+} that may prevent precipitation of calcium by phosphate in the intestine (10, 11). Calcium acetate, in contrast with calcium ascorbate, is a very effective phosphate binder. It binds twice as much phosphate as calcium carbonate does, which reduces its calcium bioavailability (12, 13). However, this does not reduce its bioavailability to below that observed for other salts, as calcium absorption from calcium acetate and calcium carbonate are similar (4).

Kinetic analysis of calcium absorption at varied calcium loads demonstrated that the paracellular absorption, a non-saturable, energy-independent pathway, was similar for calcium ascorbate and calcium acetate because all the paracellular transfer rates in serum were similar for the two salts (Table 4). The saturable pathway, which is an energy-dependant, transcellular pathway, however, was different between two salts because K_m for calcium acetate was higher than calcium ascorbate. The lower level of total absorption from calcium acetate is probably because it was available at the absorption site for a shorter period of time than calcium ascorbate. This suggests that calcium may form a soluble complex with ascorbate, which may prolong its transit time through the small intestine. This possibility is supported by the results from kinetic analysis that the turnover of the gut compartment is slower for calcium ascorbate than calcium acetate (Table 4). Furthermore, faster turnover by calcium acetate than calcium ascorbate could also be due to the pH gradient down the intestine. Calcium

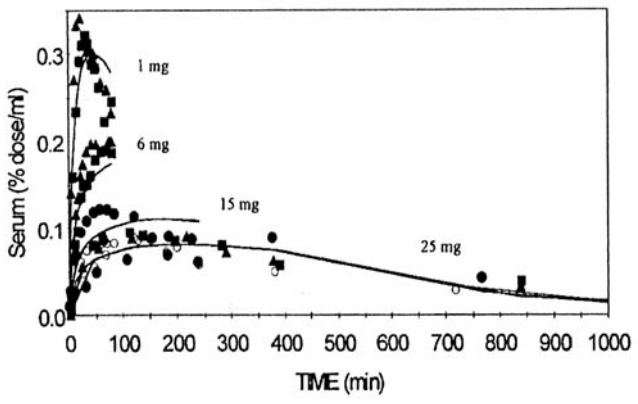

Figure 3. Appearance of calcium tracer in serum following oral administration with loads of 1 to 25 mg of calcium ascorbate.

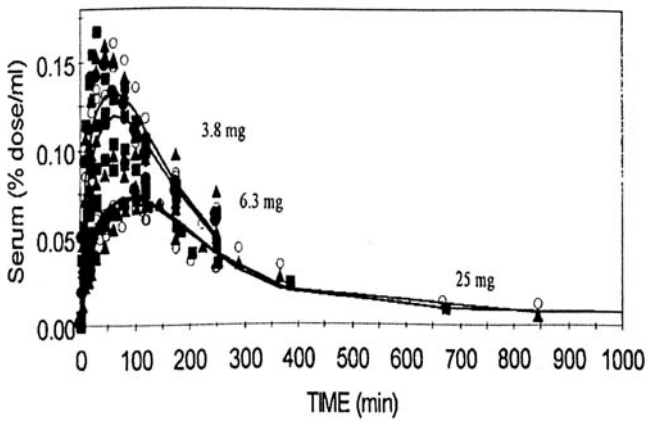

Figure 4. Appearance of calcium tracer in serum following oral administration with loads of 1 to 25 mg of calcium acetate.

acetate is less soluble than calcium ascorbate at neutral or higher pH (12). Therefore, as it moves down the intestine, the pH increases, which makes calcium acetate less available for absorption. An enhanced calcium absorption by ascorbate may explain a recent observation in postmenopausal women that vitamin C supplementation had a beneficial effect on bone mineral density (14).

In conclusion, calcium absorption from calcium ascorbate is higher than that from calcium acetate. Therefore, in addition to being a good source of vitamin C, calcium ascorbate also provides, at 9.4% by weight, highly bioavailable calcium. The calcium ascorbate can withstand boiling heat treatment for at least 5 mins without losing much of its calcium bioavailability.

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