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Absorption of Calcium Fumarate Salts Is Equivalent to Other Calcium Salts When Measured in the Rat Model

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Calcium absorption from fumarate salts (calcium fumarate and calcium malate fumarate), which have recently been considered for use as sources for food and beverage enrichment, was compared to that from calcium citrate malate, calcium citrate, and calcium carbonate. Salts were instrinsically labeled with ^{45}Ca and orally administered to Sprague−Dawley rats. Fractional absorption of calcium from each salt was determined using the femur uptake model. Fractional absorption from the five salts (0.30−0.27) was not significantly different ($p > 0.05$). Thus, when measured in the rat model, calcium from calcium fumarate and calcium malate fumarate is absorbed equally well as compared to other salts, which are common calcium sources in many foods, beverages, and supplements.

KEYWORDS: Calcium salts; absorption; bioavailability; rats

INTRODUCTION

Calcium salts, which are effective and appropriate for use as food additives or supplements, must be bioavailable and compatible with the food system. Calcium absorption has been reported to be superior for the highly soluble salt, calcium citrate malate (CCM), in some human studies (*1*, *2*). However, when a rat model was used, no significant difference was observed between CCM and calcium carbonate ($CaCO_3$) (*3*). Calcium solubility does not seem to be the major responsible factor for calcium bioavailability. Heaney et al. (*4*) found no difference in calcium absorption from seven salts over a range of solubilities in humans. Similarly, Hansen et al. (*5*) reported that calcium absorption was not determined only by the solubility of the calcium salt administered in therapeutical doses to healthy volunteers.

In response to increasing concern that calcium intakes are inadequate for protection against osteoporosis, food companies are considering new calcium salts for food and beverage enrichment that have cost, flavor, stability, and bioavailability advantages. The aim of this study was to use a rapid screening rat model (*6*) to compare calcium absorption from calcium fumarate (CF) and calcium malate fumarate (CMF), two new candidate salts for Ca enrichment, with CCM, $CaCO_3$, and calcium citrate (CC).

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Table 1. Ingredients Used to Synthesize 30 Doses of Each Salt

	salt				
ingredient	CCM	CF	CMF	CC	$CaCO_3$
citric acid (g)	1.2			2.4	
malic acid (g)	1.26		1.2		
$CaCO_3$ (g)	1.875			1.875	
fumaric acid (g)		2.24	1.16		
$Ca(OH)_2$ (g)		1.39	1.39		
$CaCl_2$ (g)					3.45
Na_2CO_3 (g)					2.49
H_2O (mL + rinse)	6.25	10.85	11.78	6	8
^{45}Ca (stock solution)[a] (mL)	1.35	1.35	1.35	1.35	1.50
solubility (g salt/100 mL H_2O)	1.10	2.11	2.0	0.85	0.0014

[a] Stock solution: 10 mL of $^{45}CaCl_2$ in water (0.2 mCi/mL).

MATERIALS AND METHODS

Preparation of Intrinsically Labeled Salts and Doses. To ensure accurate interpretation of femur uptake data, the salts to be tested were synthesized to incorporate the ^{45}Ca label instrinsically. Amounts of the ingredients for each salt were calculated to provide 30 doses for gavage containing 25 mg of calcium and 9 μCi of ^{45}Ca in each dose. The composition and solubilities of the salts are shown in **Table 1**. The ingredients were mixed using a magnetic stir bar, and the $^{45}CaCl_2$ was added slowly to the mixture during the synthesis process. They were stirred for about 2 h and were freeze-dried for 72 h.

After they were dried, salts were weighed to provide 25 mg Ca per dose and then mixed with 2 mL of 3% (w/v) pregelatinized starch (AMAIZO-Instant 721-AE starch, American Maize-Products Co., Hammond, IN) to preserve a consistent salt suspension. Each radio-

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Table 2. Fractional Absorption of ^{45}Ca from Five the Salts (mean values ± Sem)

salt	N[a]	fractional absorption (%) ± SEM[b]
CCM	15	28.06 ± 1.58
CC	10	28.69 ± 2.25
CF	13	30.09 ± 1.02
CMF	14	29.13 ± 1.65
$CaCO_3$	11	27.42 ± 3.09

[a] N = number of rats. [b] Means were not different.

labeled dose was administered directly to the stomach of each rat. To ensure administration of the total dose, the vials containing the salts were rinsed with 1 mL of double deionized water. The rinse was then gavaged immediately after the first gavage. To quantitate any further residual ^{45}Ca, 1 mL of double deionized water was added to the vials, followed by about 15 mL of scintillation cocktail (EcoLite, ICN Biomedicals). All remains were counted in a scintillation counter (Beckman LS 6500). To mimic 100% absorption, one group (IP) received an intraperitoneal injection of 0.3 mL of normal saline (0.9% NaCl) containing 9 μCi ^{45}Ca as $^{45}CaCl_2$.

Experimental Design. Adult male Sprague−Dawley rats (230−244 g, Harlan Industries, Indianapolis, IN) were placed in individual stainless steel cages upon arrival and adjusted to a reverse day−night light cycle. They were randomly divided into six groups of 15. Rats received a control AIN93G diet (*7*) and deionized water ad libitum for 5 days. To ensure consistent gastrointestinal conditions, rats were fasted for 15 h prior to administration of the salt doses. Three hours postdose, they were returned to the AIN93 control diet. When gavage was not completed successfully, rats were excluded from the study.

Forty-eight hours after gavage, the animals were weighed and sacrificed by exposure to CO_2. Their right femur was removed, placed in a 25 mL volumetric flask with 3 mL of concentrated nitric acid, and allowed to dissolve overnight. The volume was completed with double deionized water, and a 1 mL aliquot was mixed with 15 mL of scintillation cocktail (EcoLite, ICN Biomedicals) and counted in a scintillation counter (Beckman LS 6500) for three 1 min periods. One dose of each salt and IP solution were prepared for counting in a similar fashion using appropriate dilutions.

The fractional absorption was calculated as follows: (i) actual ^{45}Ca dose = {[(dose − blank) × dilution factor] − [(residual − blank) × dilution factor]}; (ii) actual bone ^{45}Ca retention = (bone ^{45}Ca retention − blank) × dilution factor; (iii) ^{45}Ca absorption of the salt = actual bone ^{45}Ca retention/actual ^{45}Ca dose; (iv) fractional absorption = ^{45}Ca absorption of the salt/average ^{45}Ca absorption of IP group.

The values of fractional absorption were analyzed statistically by one way analysis of variance, and the mean values were compared by multiple *t*-test ($p < 0.05$), using Statistical Analysis System, version 6 (SAS Institute, Cary, NC).

RESULTS

Fractional absorption of ^{45} Ca from all of the salts was about 30% as shown in **Table 2**. There were no statistical differences among salts ($p < 0.05$).

DISCUSSION

Several studies using the femur uptake model in rats have verified 25−30% absorption of Ca from several salts, which have been instrinsically labeled (*6*, *9*). Known solubility of calcium salts did not influence absorbability in the rat. Similar results were found when calcium salts of different solubilities were tested in humans (*1*, *8*) and in the rat (*10*). No difference was observed when CCM was compared with $CaCO_3$ in a rat model (*1*), although calcium absorption from CCM was superior to $CaCO_3$ in adults (*1*) and children (*11*).

The bioavailability of calcium from calcium salts appears to be influenced by gastric acid secretion and by simultaneous ingestion of food (*12*), rather than primarily by its solubility (*4*). Acid dissolution in the gastrointestinal tract may be responsible for the similar absorption of calcium from salts with widely different water solubilities (*10*).

These results suggest that CF and CMF are good candidates for calcium fortification of foods and beverages. In the rat model, they have equivalent calcium bioavailability to more traditional salts used for this purpose.

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