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Effect of anions or foods on absolute bioavailability of calcium from calcium salts in mice by pharmacokinetics

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Abstract: We studied the absolute bioavailability of calcium from calcium L-lactate in mice using pharmacokinetics, and reviewed the absolute bioavailability of calcium from three other calcium salts in mice previously studied: calcium chloride, calcium acetate, and calcium ascorbate. The results showed that calcium metabolism is linear between intravenous administration of 15 mg/kg and 30 mg/kg, and is not affected by anions. Results after oral calcium administration of 150 mg/kg showed that the intestinal absorption process was significantly different among the four calcium salts. The rank of absolute bioavailability of calcium was calcium ascorbate > calcium L-lactate ≥ calcium acetate > calcium chloride. The mean residence time (MRT_{ab}) of calcium from calcium ascorbate (32.2 minutes) in the intestinal tract was much longer than that from calcium L-lactate (9.5 minutes), calcium acetate (15.0 minutes) and calcium chloride (13.6 minutes). Furthermore, the foods di-D-fructo-furanose-1,2′:2,3′-dianhydride, sudachi (*Citrus sudachi*) juice, and moromi-su (a Japanese vinegar) increased the absolute bioavailability of calcium from calcium chloride by 2.46-fold, 2.86-fold, and 1.23-fold, respectively, and prolonged MRT_{ab} by 48.5 minutes, 43.1 minutes, and 44.9 minutes, respectively. In conclusion, the prolonged MRT_{ab} of calcium in the intestinal tract by anion or food might cause the increased absorbability of calcium.

Keywords: absolute bioavailability of calcium, pharmacokinetics, calcium chloride, calcium L-lactate, DFA III, sudachi juice

Introduction

Calcium is an essential mineral, acting primarily as a component in bones and teeth, as well as playing various physiological roles in cells, even at low levels.[1,2] It has been shown that a deficit in calcium causes various diseases, including osteoporosis, hypocalcemia, hypertension, hypercholesterolemia, and cancer.[3,4] The intestinal absorption of calcium takes place through both active and passive transport from the gut lumen after food intake in humans and other animals.[1] Active transport occurs transcellularly with saturable kinetics and involves the binding of calcium ions by a vitamin D-dependent calcium binding protein in the intestinal mucosa. By contrast, passive transport occurs paracellularly with nonsaturable kinetics, and a constant fraction of calcium is absorbed at high loads. The calcium absorbability from the diet or foods usually has been measured using traditional mass balance techniques involving tracer, urine increment techniques.[5] The quantity and retention of calcium are defined by levels in the blood, urine, or body compartments (particularly bone) after multiple administrations or ingestions over several days. These levels are defined

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as absorption, fractional absorption, or nutrient bioavailability of calcium, using the following formulas:[6]

$$\text{Apparent absorption (\%)} = ([\text{Intake} - \text{Fecal excretion}]/[\text{Intake}]) \times 100 \quad (1)$$

and

$$\text{Apparent retention (\%)} = ([\text{Intake} - \text{Fecal excretion} - \text{Urinary excretion}]/[\text{Intake}]) \times 100. \quad (2)$$

Calcium absorption has been measured in previous studies using mass balance techniques from an oral calcium source intrinsically labeled with a suitable calcium isotope (Table 1).[6–10] The studies showed that calcium absorbability is as low as 20%–40% after calcium salt administration,[6–11] and many modern diets do not provide the recommended levels of calcium (400–1,200 mg/day).[4,8,12] Therefore, calcium supplements are recommended for the prevention of calcium-related diseases, and various calcium salts, including calcium carbonate and calcium lactate, have been examined as calcium supplement sources.[13,14]

Table 1 Calcium absorbability from calcium salts measured by mass balance method

Calcium salts	Absorbability (%)	Animals, condition	References
Humans			
Calcium carbonate	23.5 ± 12.3	Fast	Heaney[7]
	39 ± 7	Human	Martin[9,10]
	29.6 ± 5.4	Diet	Patrick[8]
	23.5 ± 12.3	Fast	Patrick[8]
	39 ± 3	Human	Sheikh[6]
	14.7 ± 6.4	Human	Uenishi[11]
Calcium citrate	24 ± 4.9	Fast	Heaney[7]
	24.2 ± 4.9	Fast	Patrick[8]
	30 ± 3	Human	Sheikh[6]
Calcium citrate malate	36.3 ± 7.6	Diet	Patrick[8]
	36.3 ± 7.6	Diet	Heaney[7]
Calcium sulfate	41 ± 7	Human	Martin[9,10]
Calcium lactate	47 ± 8	Human	Martin[9,10]
	32 ± 4	Human	Sheikh[6]
Calcium oxalate	10.2 ± 4.0	Diet	Patrick[8]
Tricalcium phosphate	25.2 ± 13.0	Diet	Patrick[8]
Calcium acetate	32 ± 4	Human	Sheikh[6]
Calcium gluconate	27 ± 3	Human	Sheikh[6]
Rats			
Calcium carbonate	27.42 ± 3.09	Rat	Weaver[9,10]
Calcium citrate	28.69 ± 2.25	Rat	Weaver[9,10]
Calcium citrate malate	28.06 ± 1.58	Rat	Weaver[9,10]
Calcium fumalate	30.09 ± 1.02	Rat	Weaver[9,10]
Calcium malate fumalate	29.13 ± 1.65	Rat	Weaver[9,10]

Actual calcium absorption is influenced by both dietary and nondietary factors, such as salts, other foods, or food constituents.[7,15–17] Heaney et al[7] showed that even under controlled, chemically defined conditions, absorbability of calcium from food sources is determined mainly by other food components. Wasserman,[15] as well as Buchowski and Miller[16] showed that lactose increases the bioavailability of calcium from a variety of sources, but the magnitude of the effect varies between these sources. Suzuki et al[18] and Tomita et al[19] showed that difructose anhydride III (DFA III; di-D-fructo-furanose-1,2′:2,3′-dianhydride) enhances the absorption and retention of calcium. Such nondigestible diets also have been used as calcium supplement sources. Furthermore, Nii et al[20] showed that sudachi (*Citrus sudachi*) juice enhances intestinal absorption of calcium from small fishes. Kishi et al[21] showed that dietary vinegar enhances the intestinal absorption of calcium in ovariectomized (OVX) rats.[21]

In current medical care, calcium is used as a medicinal element to cure calcium-deficient cases by enteral nutrition or continuous intravenous (IV) infusion. This therapy requires predicted suitable doses using reproducible and precise absorbability of calcium. However, as shown in Table 1, there is no reproducible and precise absorbability of calcium for current drug therapy. All the data are measured using mass balance techniques, in which it is hard to remove the effects of various foods because of the long period of measurement, for example 2 days or more. Pharmacokinetics might be able to determine a more precise bioavailability of calcium after calcium administration using serum concentrations of calcium for several hours. The area under the plasma concentration-time curve (AUC) is a primary function in pharmacokinetics in which the AUC is calculated using the trapezoidal rule from the plasma concentrations measured periodically after dosing. Even though the AUC itself is a relative measure for the extent of absorbability, in pharmacokinetic studies of drugs the absorbability is usually defined using the absolute (F_{abs}) or relative (F_{rel}) bioavailability. F_{abs} was defined as AUC_{oral} after an oral dose of D_{oral}, and was normalized with AUC_{IV} after an IV dose of D_{IV} as follows:[22]

$$F_{abs} = (AUC_{oral}/D_{oral})/(AUC_{IV}/D_{IV}). \quad (3)$$

F_{rel} is the quantity indicating the equivalency between drugs A and B as follows:

$$F_{rel} = (AUC_B/D_B)/(AUC_A/D_A) \quad (4)$$

where a certain drug (B), dosed D_B, is compared with a standard drug (A), dosed D_A, and usually the standard

drug (A) has been established by pharmacokinetics. Then, the absolute bioavailability is usually defined as drug absorbability.

However, many studies of calcium have shown a relative measure for the extent of calcium absorbability,[23–25] even though Tsugawa et al,[26] Cai et al,[27] and Hanzlik et al[12] indicated the importance of absolute bioavailability. Tsugawa et al[26] showed that the calcium absorbability from calcium ascorbate is almost comparable to, or higher than, that from calcium chloride, and is significantly higher than that from calcium carbonate. Cai et al[27] showed that the higher bioavailability of calcium ascorbate was due to a longer transit time in the small intestine compared with calcium ascorbate. Hanzlik et al[12] showed that calcium formate is clearly superior to calcium carbonate and calcium citrate in the ability to deliver calcium to the blood stream after oral administration in humans. Thus, no one has examined comparably the absorbability among calcium salts using the absolute bioavailability of calcium by modern pharmacokinetics, and our recent report is the first study to examine supplement sources using the absolute bioavailabilities of calcium from three calcium salts: calcium chloride, calcium acetate, and calcium ascorbate, which are very soluble in water.[22,28]

As shown in Table 2, the absolute bioavailability of calcium from calcium ascorbate and calcium acetate was 2.6-fold and 1.5-fold, respectively, greater than that of calcium chloride; the calcium absorbability from calcium ascorbate via the intestinal track is significantly higher than that of calcium chloride and calcium acetate.[22] Furthermore, as shown in Table 3, Ueda et al[28] studied the effects of Hachimi-jio-gan extract on intestinal calcium absorption using pharmacokinetic calculations in an osteoporosis animal model of OVX and sham-operated (SHAM) mice. Hachimi-jio-gan is used clinically and has been shown to be effective in preventing bone loss in OVX rats.[29] Hachimi-jio-gan enhanced the absolute bioavailability of calcium from calcium chloride (5.7%) in OVX (20.2%) and SHAM (19.9%) mice. Hachimi-jio-gan extract potentially improved the intestinal calcium absorption by 1.96-fold and 1.86-fold in OVX and SHAM mice, respectively. Hachimi-jio-gan extract further suppressed the potent stimulation of a receptor activator of the NF-κB ligand-induced osteoclast differentiation in RAW264.7 cells.

In this study, we measured the absolute bioavailability of calcium from calcium L-lactate, and examined the effect of the foods DFA III, sudachi juice, and moromi-su (a type of vinegar and healthy food made from fermenting mash in the production of sake, Japanese liquor) on the absorbability of calcium from calcium chloride. Furthermore, we reviewed, comparatively, the absorbability from three other soluble calcium salts – calcium chloride, calcium acetate, and calcium ascorbate – demonstrating the usefulness of pharmacokinetics in nutrition.[22,28]

Table 2 Pharmacokinetic parameters of calcium in mice after IV or oral administration of calcium chloride, calcium acetate, or calcium ascorbate

Salts	Dose (mg/kg)	AUC_{IV} (μg/mL)	MRT_{IV} (minutes)	CL_{IV} (mL/minute/kg)	V_{dss} (mL/kg)
IV administration					
$CaCl_2$	15	1484.5 ± 41.0	29.3 ± 1.3	10.1 ± 0.3	296.5 ± 8.5
	30	2870.6 ± 90.8	33.0 ± 1.1	10.5 ± 0.3	345.1 ± 15.8
$CaAc_2$	15	1507.9 ± 128.4	29.1 ± 2.0	10.0 ± 0.9	289.9 ± 7.4
	30	2637.2 ± 121.6	30.0 ± 2.4	11.4 ± 0.5	340.9 ± 24.7
$CaAs_2$	15	1193.9 ± 101.7	30.4 ± 1.0	12.6 ± 1.1	383.6 ± 20.9
	30	2711.3 ± 154.2	32.5 ± 1.2	11.0 ± 0.6	359.2 ± 34.7
$CaLc_2$	15	1396.2 ± 104.4	28.4 ± 2.0	10.8 ± 0.8	305.8 ± 29.3
	30	3139.8 ± 123.1	31.9 ± 2.1	9.6 ± 0.4	304.2 ± 10.4
Means			30.6 ± 1.7	10.8 ± 1.0	328.2 ± 33.9

Salts	Dose (mg/kg)	T_{max} (minute)	C_{max} (μg/mL)	AUC_{oral} (μg/mL · minute)	MRT_{oral} (minutes)	F_{abs} (%)
Oral administration						
$CaCl_2$	150	30	94.5	813.0 ± 187.6	46.6 ± 1.8	5.7 ± 1.3
$CaAc_2$	150	45	103.6	1137.4 ± 225.1	45.0 ± 2.6	8.6 ± 1.7
$CaAs_2$	150	15	100.8	2007.6 ± 159.9	64.7 ± 3.8	14.8 ± 1.2
$CaLc_2$	150	30	98.2	1394.6 ± 225.3	41.4 ± 2.8	8.9 ± 1.4

Note: Each value represents the mean ± standard deviation (n = 4).

Abbreviations: IV, intravenous; AUC, area under the curve; MRT, mean residence time; CL, plasma clearance; V_{dss}, volume of distribution; $CaCl_2$, calcium chloride; $CaAc_2$, calcium acetate; $CaAs_2$, calcium ascorbate; $CaLc_2$, calcium L-lactate; T_{max}, time to reach the maximum plasma concentration; C_{max}, maximum plasma concentration; F_{abs}, absolute bioavailability; n, number.

Table 3 Pharmacokinetic parameters of calcium in female mice after IV or oral administration of calcium

Mice	AUC_{IV} (μg/mL·minute)	MRT_{IV} (minutes)	CL_{IV} (mL/minute/kg)	V_{dss} (mL/kg)	
IV administration (dose: 30 mg/kg)*					
SHAM	2101.0 ± 14.3	27.1 ± 3.6	14.3 ± 0.9	386.0 ± 23.8	
OVX	2097.0 ± 10.5	26.5 ± 1.9	14.3 ± 0.7	379.0 ± 34.0	
Mice	**T_{max} (minutes)**	**C_{max} (μg/mL)**	**AUC_{oral} (μg/mL·minute)**	**MRT_{oral} (minutes)**	**F_{abs} (%)**
Oral administration (dose: 150 mg/kg)*					
SHAM	30	93.0 ± 2.5	1121.0 ± 22.8	57.2 ± 4.2	10.7 ± 2.8
OVX	30	94.0 ± 1.9	1086.0 ± 20.1	45.3 ± 2.9	10.3 ± 2.1
SHAM + HJ	30	104.0 ± 3.8	2091.0 ± 80.6	76.8 ± 2.4	19.9 ± 3.1
OVX + HJ	30	104.0 ± 2.5	2120.0 ± 71.7	75.4 ± 5.6	20.2 ± 1.7

Notes: Each value represents the mean ± standard deviation (n = 5). *Dose refers to a quantity of calcium in the $CaCl_2$ solution; the pharmacokinetic parameters of calcium in male mice (ddY strain) were obtained from Ueda and Taira.[22]

Abbreviations: IV, intravenous; AUC, area under the curve; MRT, mean residence time; CL, plasma concentration; V_{dss}, volume of distribution; SHAM, sham-operated mice; OVX, ovariectomized rats; T_{max}, time to reach maximum plasma concentration; C_{max}, maximum plasma concentration; F_{abs}, absolute bioavailability; HJ, Hachimi-jio-gan extract.

Materials and methods

Chemicals

DFA III was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Calcium L-lactate $5H_2O$ was purchased from Sigma-Aldrich (St Louis, MO, USA). Sudachi juice was prepared by squeezing the juice from fruits of an orange, sudachi, and separating supernatant by centrifugation at 100,000× g for 30 minutes. The moromi-su was purchased from a market store. Other reagents were purchased from commercial sources and were of the highest grade available.

Animals and pharmacokinetic procedures

Seven-week-old male ddY mice, weighing 20–30 g, were obtained from SLC Co, Ltd (Shizuoka, Japan). Animals had free access to food (commercial diet, MF pellets; Oriental Yeast Co. Ltd., Tokyo, Japan) and water during the experimental period. Mice were allowed to recover from anesthesia. Solutions of calcium (1% w/v) were prepared for each calcium salt, and either 15 mg or 30 mg calcium/kg of body weight was administered intravenously to the tail vein. The plasma calcium concentrations were measured spectroscopically, as described previously.[22] For oral administration, 150 mg calcium/kg of body weight was delivered to the duodenum, and the plasma calcium concentrations were measured. Ten mL/kg of 10% (v/v) of sudachi juice or moromi-su, or 1% (w/v) aqueous solution of DFA III, was administered orally, following oral administration of 150 mg of calcium per kg from calcium chloride, and blood samples were collected. The plasma calcium concentrations were determined. All protocols conformed to the guide for the institutional care and use of animals of Tokushima Bunri University, Tokushima, Japan.

Pharmacokinetic calculation

The pharmacokinetic parameters were calculated, as described previously.[22] Briefly, the parameters for intestinal absorption, distribution, metabolism, and elimination of calcium – the area under the calcium concentration in the blood-time curve (AUC_{IV}); mean residence time (MRT_{IV}) after IV administration; plasma clearance (CL_{IV}); and apparent volume of distribution (V_{dss}) at steady-state after IV administration; and AUC_{oral}, maximum plasma concentration (C_{max}), and MRT_{oral} after oral administration – were calculated using the time course of serum calcium concentrations by an iterative nonlinear least-squares method using the MOMENT software program, described by Yamaoka et al.[30,31] $AUC^{0-\infty}$ was calculated on the basis of the trapezoidal rule. When MRT_{oral} in all compartments was calculated after oral administration, the MRT_{ab} in the absorption track as follows:[22,30]

$$MRT_{ab} = MRT_{oral} - MRT_{iv}. \quad (5)$$

Statistical analysis

Data are presented as the mean value ± standard deviation. A parameter was considered to be significantly different when the *P*-values were <0.05 using Student's *t*-test.

Results

The absolute bioavailability of calcium from calcium L-lactate

We studied the pharmacokinetic parameters using time courses of plasma concentrations of calcium in male ddY mice after IV or oral administration of calcium from calcium L-lactate.

The plasma concentrations of calcium from calcium L-lactate in mice for 2 hours after IV administration of 15 mg/kg or 30 mg/kg of calcium were measured as shown in Figure 1A, and the pharmacokinetic parameters of calcium were calculated as summarized in Table 2. The results showed that the pharmacokinetic process is nearly linear owing to a first-order reaction, because mean AUC values increased 2.25-fold, compared with administrations of 15 mg and 30 mg ($P < 0.05$). Furthermore, MRT, CL, and V_{dss} did not significantly differ between the two administered doses. The pharmacokinetic behavior of calcium from calcium L-lactate in male mice following oral administration of calcium using time courses of plasma concentrations of calcium (Figure 1B) in mice that were orally administered a dose of 150 mg/kg of calcium, as well as the pharmacokinetic parameters of calcium are summarized in Table 2. The results showed that the blood concentration of calcium reached the C_{max} of 98.2 μg/mL at the time to reach the maximum plasma concentration (T_{max}) of 30 minutes. The absolute bioavailability value of calcium L-lactate was 8.9%.

Effects of three foods – DFA III, sudachi juice, and moromi-su – on the absolute bioavailability of calcium from calcium chloride

To examine the enhancing effects of three foods – DFA III, sudachi juice, and moromi-su – on the absolute bioavailability of calcium, 10 mL/kg of 10% (v/v) of sudachi juice or moromi-su, or 1% (w/v) aqueous solution of DFA III, was administered orally, following oral administration of 150 mg of calcium per kg from calcium chloride, and plasma calcium concentrations were measured as shown in Figure 2. The pharmacokinetic parameters were calculated as summarized in Table 4. The result showed that the foods enhance the absorbability of calcium from calcium chloride after oral administration. That is, the foods increased the absolute bioavailability (5.7% ± 1.3%) of calcium from calcium chloride by 14.0% ± 2.1%, 16.3% ± 2.8%, and 7.0% ± 3.1%, respectively.

Discussion

In this study, we examined the pharmacokinetic characterization of calcium from calcium L-lactate, and reviewed the other three calcium salts previously studied – calcium chloride, calcium acetate, and calcium ascorbate – after IV or oral administration in mice.[22] The results for those four calcium salts showed that the corresponding pharmacokinetic parameters (the AUC values) increased 2.04-fold (mean) compared with administrations of 15 mg and 30 mg ($P < 0.05$); in addition, MRT, CL, and V_{dss} did not differ significantly between the two administered doses, with mean values of 30.6 ± 1.7 minutes, 10.8 ± 1.0 mL/minute/kg, and 328.2 ± 33.9 mL/kg, respectively. This might indicate that calcium metabolism in animals is linear between those doses, and is not physiologically affected by anions ($P < 0.05$). However, the results after oral calcium administration of 150 mg/kg of body weight showed that the intestinal absorption process was significantly different among the four calcium salts. That is, the absolute bioavailabilities of calcium from calcium chloride, calcium acetate, calcium ascorbate, and calcium L-lactate were 5.7%, 8.6%, 14.8%, and 8.9%, respectively. The rank of the absolute bioavailability of calcium was calcium ascorbate > calcium

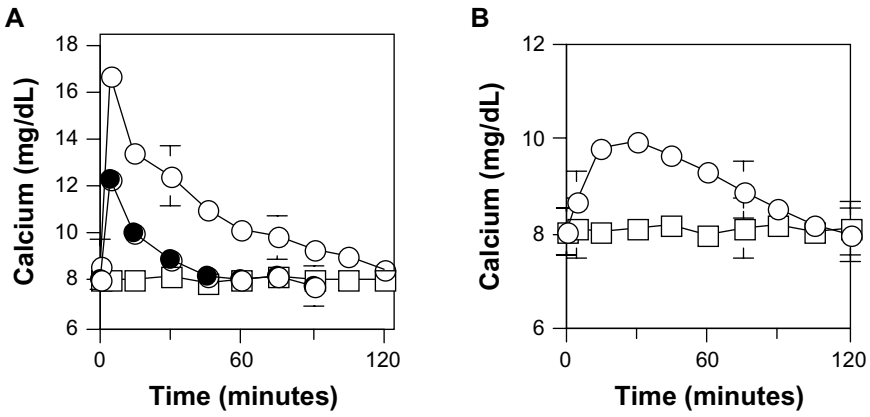

Figure 1 Time course of plasma calcium concentrations after intravenous calcium administration of 15 mg/kg or 30 mg/kg of body weight of calcium L-lactate.
Notes: (**A**) Open circles refer to plasma calcium concentrations after administration of 30 mg/kg of body weight, closed circles refer to calcium administration of 15 mg/kg of body weight, and open squares are the plasma calcium concentrations of the control mice. (**B**) In total, 150 mg/kg of body weight of calcium L-lactate in 1% solution was orally delivered to the duodenum and blood was collected. Open circles refer to plasma calcium concentrations after oral administration of one of the three calcium salts, and open squares are the plasma calcium concentrations of the control mice. Data points represent the mean ± standard deviation (n = 4).
Abbreviation: n, number.

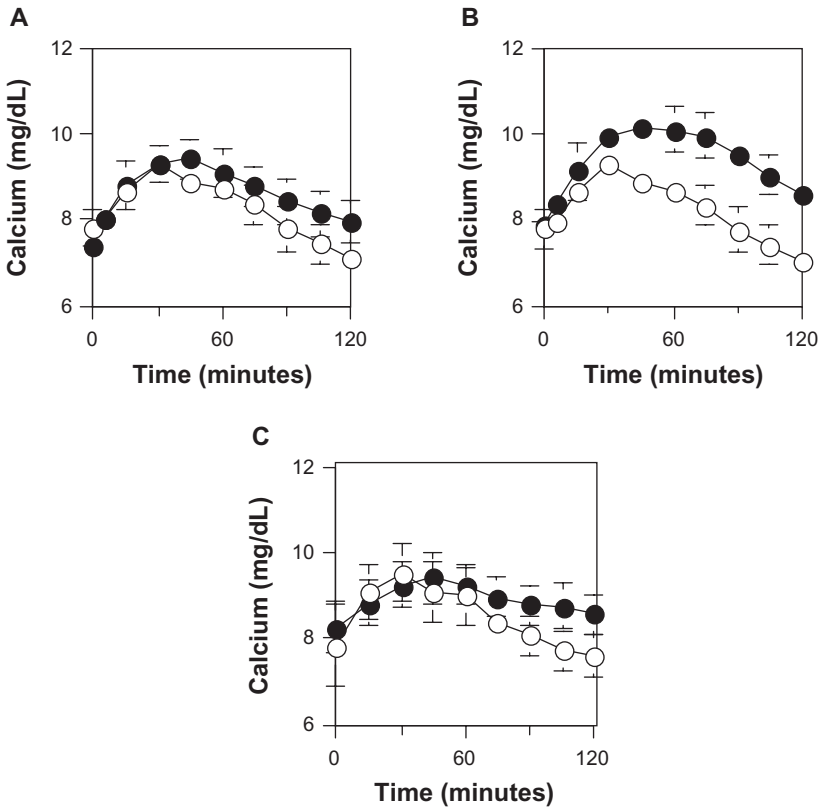

Figure 2 Effect of the foods DFA III, sudachi juice, and moromi-su on the absolute bioavailability of calcium of 150 mg/kg from $CaCl_2$.
Notes: (**A**) $CaCl_2$ + DFA III; (**B**) $CaCl_2$ + sudachi juice; and (**C**) $CaCl_2$ + moromi-su. $CaCl_2$ + food means that food was orally administered, following oral administration of 150 mg of calcium per kg from $CaCl_2$, and blood samples were collected. Closed circles refer to plasma calcium concentrations after oral administration of food and calcium chloride, and open circles are the plasma calcium concentrations after oral administration of $CaCl_2$. Data points represent the mean ± standard deviation (n = 4).
Abbreviations: DFA III, di-D-fructo-furanose-1,2′:2,3′-dianhydride; $CaCl_2$, calcium chloride; n, number.

L-lactate ≥ calcium acetate > calcium chloride, and the rank was consistent with that of the AUC. Thus, the rank also was consistent with the findings of Tsugawa et al[26] and Cai et al.[27] Furthermore, the specific magnitude of the greater MRT_{ab} of calcium from calcium ascorbate (32.2 minutes) might result in the greater absolute bioavailability of calcium compared with calcium L-lactate (9.5 minutes), calcium acetate (15.0 minutes), and calcium chloride (13.6 minutes). That is, calcium from calcium ascorbate might cross the gut membrane for a longer period of time.

Furthermore, effects of the foods DFA III and sudachi juice significantly increased the absorbability of calcium from calcium chloride 2.46-fold and 2.86-fold, respectively, but moromi-su was less effective (a 1.23-fold increase) compared with other foods. In addition, the foods prolonged MRT_{ab} of calcium from calcium chloride in the intestinal tract by 48.5 minutes, 43.1 minutes, and 44.9 minutes, respectively. Thus, DFA III and sudachi juice might be recommended as a supplementary food for promoting the effect of calcium absorption.

In conclusion, the pharmacokinetic calculations showed that calcium metabolism in animals is linear between doses at 15 mg/kg and 30 mg/kg, and is not physiologically affected by anions. However, the intestinal absorption process was

Table 4 Effect of foods on pharmacokinetic parameters of calcium in male mice after oral administration of calcium chloride

Foods	T_{max} (minutes)	C_{max} (μg/mL)	AUC_{oral} (μg/mL·minute)	MRT_{oral} (minute)	F_{abs} (%)
Oral administration (dose: 150 mg/kg)					
DFA III	45	94.1 ± 15.3	2013.5 ± 20.1	81.5 ± 7.9	14.0 ± 2.1
Sudachi juice	45	101.3 ± 21.1	2343.2 ± 22.8	76.2 ± 4.2	16.3 ± 2.8
Moromi-su	45	115.5 ± 32.8	997.3 ± 80.6	78.3 ± 8.4	7.0 ± 3.1

Note: Each value represents the mean ± standard deviation (n = 4).
Abbreviations: T_{max}, time to reach maximum plasma concentration; C_{max}, maximum plasma concentration; AUC, area under the curve; MRT, mean residence time; F_{abs}, absolute bioavailability; DFA III, di-D-fructo-furanose-1,2′:2,3′-dianhydride; n, number.

significantly different among the four calcium salts after oral calcium administration, and the greater MRT_{ab} of calcium in the intestinal tract might cause higher absorbability. Food also might increase MRT_{ab} of calcium in the intestinal tract.

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Disclosure

The authors report no conflicts of interest in this work.

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