Absorption Characteristics of Novel Compound Calcium Carbonate Granules: Effects of Gastric Acid Deficiency and Exogenous Weak Acids

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Summary: Calcium carbonates are commonly administered as supplements for conditions of calcium deficiency. We report here pharmacokinetic characteristics of a novel formulation, calcium carbonate compound granules (CCCGs), forming complexes of calcium carbonate and calcium citrate in water. CCCGs were compared to a kind of commonly-used calcium carbonate D_3 preparation (CC) in the market in 5-week-old mice that had been treated with omeprazole, to suppress gastric acid secretion, and in untreated control mice. The results showed that: (1) CCCGs had better water solubility than CC *in vitro*; (2) In control mice, calcium absorption rates after CCCGs administration were comparable to those after CC administration; (3) Inhibition of gastric acid secretion did not affect calcium absorption after CCCGs, but moderately decreased it after CC; (4) The presence of phytic acid or tannin did not affect calcium absorption rates after CCCGs but did for CC; and (5) In normal mice, CCCGs did not inhibit gastric emptying and intestinal propulsion, and did not alter the gastrointestinal hormones. The results suggest that CCCGs may be therapeutically advantageous over more commonly used calcium supplement formulations, particularly for adolescents, because of their stable calcium absorption characteristics and their relatively favorable adverse effect profile.
Key words: calcium carbonate; gastric acid inhibition; phytic acid; tannin; calcium absorption

Calcium is the fifth richest element in the body, accounting for about 1.5%–2% of the human body mass, with more than 99% of the composition stored in the bones and teeth[1]. Calcium not only is a necessary structural element for building human body, but also regulates activities as an important physiological regulator of muscle, nerve, and other tissues with the form of ions[2–4]. The Institute of Medicine (IOM) and the National Institutes of Health (NIH) recommend a daily calcium intake of 1200–1500 mg for women and men older than 65[5, 6]. Generally, in view of the recent report that calcium intake from food in many parts of the world including China and other Pacific rim countries and much of South America, is less than 400–500 mg per day[7]. Individuals with special conditions associated with calcium deficiency, such as infants, adolescents, and the elderly[8], or pregnant, lactating, or postmenopausal women[9], may require calcium supplementation[10]. For example, infants and adolescents[8] need more calcium to meet the requirements of rapid growth and development. Adequate levels of calcium and vitamin D are required to maintain bone density and to prevent fractures associated with osteoporosis[11, 12].

Calcium supplements are mainly classified into inorganic, organic, "natural", and amino acid calcium formulations[13]. Long-term use of many of these calcium supplements often causes gastrointestinal symptoms such as bloating, constipation, gas, or a combination of all three[14]. The absorption of calcium is influenced by variables including levels of vitamin D, intra-gastric and intra-intestinal pH[15, 16], and gastrointestinal motility[17–19]. Largely because of its lower pKa, the citrate salt of calcium usually has fewer and less pronounced side effects than the carbonate salt does. However, most or all available calcium formulations are subjected to restricted absorption by forming calcium complexes with anions and other components of gastric and intestinal contents[20].

Recently, we gained a novel formulation, calcium carbonate compound granules (CCCGs), forming a

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complex of calcium carbonate and citric acid in water. In this report, we compared the effects of CCCGs with those of commonly-used calcium carbonate (CC): (1) on rates of calcium absorption in the normal mice or mice with gastric acid having been inhibited by omeprazole; (2) on rates of calcium absorption in the presence of calcium binding agents phytin acid and tannin in foods; (3) on gastric emptying, intestinal propulsion, and levels of the gastrointestinal hormones motilin (MTL) and gastrin (GAS) in control mice.

1 MATERIALS AND METHODS

1.1 Preparation of CCCGs

The CCCGs were prepared according to a patent described (the patent number is ZL201110139889.8 in China). In brief, β-cyclodextrin was combined with vitamin D_3 and dissolved in ethanol-water with an ethanol binder. Then, calcium carbonate was mixed with binder and acid vesicant to form particles in alkaline media. Finally, the two particles were mixed to form the CCCGs. This kind of novel CCCGs contained calcium carbonate, vitamin D, foaming agent and the binder at the ratio of 1:0.3–0.7:1–2:0.01–0.4. Specifically, the foaming agent is selected from one kind or its arbitrary combination in citric acid, apple acid, the tartaric acid and the binder is polyvidone, or hydroxypropyl methyl cellulose or its combination.

1.2 Experimental Animals

Male 5-week-old C57BL/6 mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (China). All animal experiments were approved by the Institutional Animal Care and Use Committee of Tongji Medical College, Huazhong University of Science and Technology (China). Experimental colonies were maintained at the Experimental Animal Center of Tongji Medical College (Huazhong University of Science and Technology, China) under specific pathogen-free conditions (SPF). The animals were kept in cages at 23±2°C and fed on the standard laboratory diet and tap water throughout the experiments.

The mice were given calcium-free feed (Beijing Vital River Laboratory Animal Technology Co., Ltd., China) for 1 week (n=8 for each group). To suppress gastric acid secretion, omeprazole (20 mg/kg) was orally administered at a dose of 20 mg/kg daily until accomplishing the experiment, and assessing the calcium absorption. In different groups, solutions of phytic acid (0.5%) (Tokyo Chemical Industry, Japan) or tannin (0.8%) (J&K Chemical Ltd., China) were added. CC (Wyeth Pharmaceuticals Ltd., USA), or calcium citrate (Sinopharm Chemical Reagent Co., Ltd., China) were orally administered at the doses indicated in the text. CCCGs were orally administered at one of three doses (mg Ca/kg body weight): 90, 180, and 360.

1.3 Intragastric pH Values

One after administration of omeprazole (20 mg/kg), the mice were sacrificed and the stomach was removed. The interior was then rinsed with 4 mL physiological saline until thoroughly rinsing the contents of stomach. The pH of the rinsed solution was then determined.

1.4 Sample Preparation

Mice were fed on calcium-free diets for 1 week to metabolize the original residual calcium *in vivo*. Omeprazole (20 mg/kg) was then administered to establish a model of low gastric acid secretion. One h later, CCCGs or CC was administered. Urine samples were then collected at 1, 2, 4, 6, 8, 12 and 24 h post-administration, and volume of excretion was recorded (n=8 for each group). After a week of calcium-free feeding, CCCGs, CC, or calcium citrate were administered continuously for 6 days, and fecal samples were collected daily over the final 3 days.

1.5 General Procedure for Analysis of Urine and Fecal Samples

Approximately 0.05 g of the fecal sample (n=8) was weighed into a conical flask. 10 mL of HNO_3 and 0.5 mL of perchloric acid (Sinopharm Chemical Reagent Co., Ltd., China) were then added, and the mixture was heated at 280°C until the solution evaporated completely, producing a white solid. After cooling, the processed fecal samples and unprocessed urine samples were diluted with 0.025 mg/mL strontium chloride solution (Sinopharm Chemical Reagent Co., Ltd., China). Calcium levels were determined by atomic absorption spectroscopy.

1.6 Flame Atomic Absorption Spectrometry (FAAS)

FAAS measurements were obtained with a SpectrAA-240FS atomic absorption spectrometer (Agilent, USA). A hollow cathode lamp was used as the radiation source to determine fecal and urinary calcium levels. The lamp was set at 422.7 nm and 10 mA (slit width: 0.5 nm), and the samples were contained in an air-acetylene mixture (acetylene flow rate: 2.00 L/min; oxidant gas flow rate: 13.50 L/min). BGC-D2 was used in the background correction method.

1.7 Calcium Absorption

These fecal samples were collected and then calcium levels were measured by FAAS: Absorption=[(Calcium intake–Fecal calcium)/Calcium intake]×100%, and the apparent calcium absorption rate was calculated in normal mice or the mice with gastric acid secretion inhibition. Urinary calcium was also measured by FAAS, and the mean urinary calcium excretion rate was calculated by the content of calcium excretion/time×100% within the different time ranges of collecting urine. Relative bioavailability (%) of the CCCGs was estimated as the ratio of total sample calcium levels 24 h after CCCGs administration to

those after CC.

1.8 Gastric Emptying

Gastric emptying time was measured by determining the rate of disappearance from the stomach of nutrient semi-solid paste. The paste was prepared stepwise. First, 10 g carboxymethyl cellulose sodium was dissolved in 250 mL distilled water. Then 16 g milk powder, 8 g sugar, 8 g starch, and 2 g activated carbon powder were added sequentially while stirring. This procedure yielded 300 mL of black semi-solid paste. The paste was stored at 4°C.

Either CCCGs or CC was administered for 2 weeks (n=8 for each group). The mice were then deprived of food but allowed free access to water for 16 h. 0.8 mL of semi-solid paste was measured (m) and orally administered. 20 min later, the mice were sacrificed by cervical dislocation, and the stomach was weighed (m_1), washed of the stomach contents, and weighed again (m_2). The gastric emptying rate was calculated as $100\%–[(m_1–m_2)/m\times100\%]$.

1.9 Intestinal Propulsion

The rate of intestinal propulsion was quantified according to a procedure reported previously[21, 22]. As in the procedure for calculating gastric emptying, the mice were given either CCCGs or CC orally for two weeks (n=8 for each group), and then deprived of food but not water for 16 h. Then 10 mL/kg of 10% activated charcoal powder suspended in 0.5% sodium carboxymethyl cellulose was orally administered. Thirty min later, the mice were sacrificed by cervical dislocation and the small intestine was exposed by laparotomy. Intestinal propulsion was then determined as the ratio of the propelling distance of activated charcoal and the whole length of small intestine as a fraction of the total intestinal length.

1.10 Enzyme-linked Immunosorbent Assay (ELISA)

After 2 weeks of continuous CCCGs or CC administration, the mice were sacrificed and the serum of mice was collected by extracting blood from the eye socket. MTL and GAS in the serum of treated and untreated mice were measured by commercial ELISA kits (Nanjing Jiancheng Bio Co., China) (n=8).

1.11 Statistical Analysis

Data are presented as mean±standard deviation (SD). Statistical analysis was performed in GraphPad Prism 5. Differences between multiple groups were examined for statistical significance using one-way analysis of variance (ANOVA) and statistical comparisons between two groups were assessed using the Student's t test. A calculated probability of less than 0.05 was considered to be significant.

2 RESULTS

2.1 Mouse Model of Gastric Acid Inhibition

Gastric acid increases the rate of calcium absorption of calcium carbonate[15, 16]. By increasing gastric pH in a low-pH environment carbonate salts of calcium retard absorption. We used this formulation in order to minimize the variability of calcium absorption. We established a mouse model of suppressed gastric acid secretion by orally administering omeprazole (20 mg/kg) once a day for consecutive 6 days[23]. The effectiveness of omeprazole was confirmed by an average increase in gastric pH of nearly two orders of magnitude in treated mice, from 3.63±0.39 to 5.65±0.34 (fig. 1).

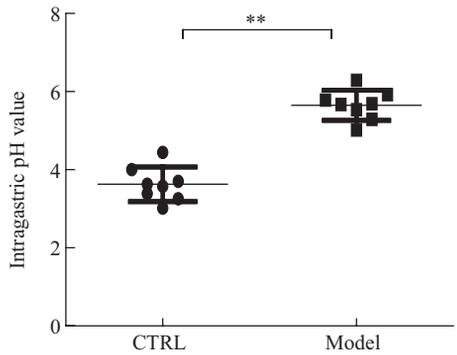

Fig. 1 Intragastric pH of untreated mice (CTRL) and mice that had been treated with omeprazole (20 mg/kg) one h before administration of calcium supplements (Model) (n=8 in each group). Data were represented as mean±SD. $^{**}P$<0.01. The data confirm the effectiveness of omeprazole in suppressing gastric acid secretion. Omeprazole treatment increased intragastric pH by two orders of magnitude, from 3.63±0.39 to 5.65±0.34.

2.2 Rate of Calcium Absorption

In preliminary *in vitro* studies, we found that CCCGs were more water-soluble than CC, when compared in either acidic solutions or water (data not shown). Effects of CC and CCCGs *in vivo*, at the same calcium dose of 180 mg Ca/kg, on calcium absorption in control mice were not different (fig. 2). Notably, suppression of gastric acid production with omeprazole inhibited calcium absorption after CC administration (180 mg Ca/kg), but not after the administration of the same calcium dose of CCCGs. In both experimental groups, calcium absorption rates were the same after administration of CCCGs at 90, 180, or 360 mg Ca/kg. Thus, the calcium absorption of CCCGs was unaffected under the suppression of gastric acid secretion.

2.3 Rate of Urinary Calcium Excretion

The total 24-h urinary calcium excretions after administration of CCCGs and CC in the control mice were not different: 150.7±4.6 and 150.5±2.4 μg, respectively (fig. 3A). The relative bioavailability of CCCGs was 100%. However, the effects of both compounds in acid-suppressed mice were different. In the gastric acid-suppressed mice, peak urinary excretion after administration of CC appeared earlier

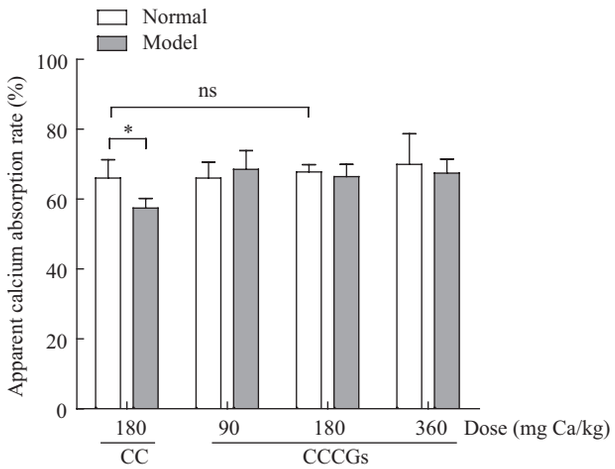

Fig. 2 Effects of CC and CCCGs on calcium absorption in untreated (normal) and omeprazole-treated (model) mice (n=8 in each group). The dose of CC was 180 mg calcium/kg and the three doses of CCCGs were 90, 180, and 360 mg calcium/kg. Data were represented as mean±SD. $^{*}P$<0.05. Inhibition of gastric acid secretion retarded the absorption of calcium from CC but not from the CCCGs.

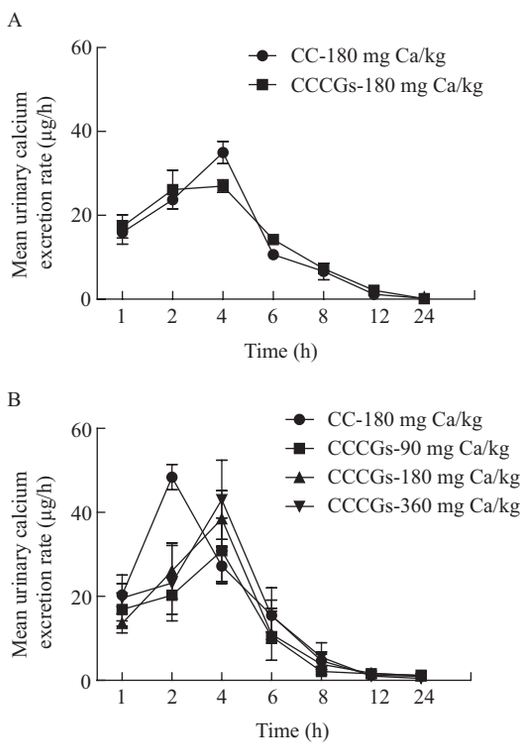

Fig. 3 Comparison of the effects of CCCGs and CC on the rates of urinary calcium excretion
A: Time course of urinary calcium excretion in response to 180 mg calcium/kg CCCGs or CC in untreated control mice (n=8 in each group); B: Time course of urinary calcium excretion in response to CC (180 mg calcium/kg) and CCCGs (90, 180, and 360 mg calcium/kg) in omeprazole-treated mice (n=8 in each group). Peak urinary calcium excretion in mice given CCCGs appeared later, regardless of dose, than that in mice given CC.

than that after CCCGs administration (fig. 3B). The results further supported that CCCGs were more resistant to the variation of gastric pH.

2.4 Effects of Phytic Acid and Tannin on Calcium Absorption

Phytic acid and tannin are common dietary components that can precipitate calcium and inhibit its absorption from the gastrointestinal tract[20, 24]. Here, we tested the effects of phytic acid and tannin administration, as described in Methods, on the subsequent absorption of calcium from CCCGs compared to that of the reference compounds CC and calcium citrate (fig. 4). The presence of either phytic acid or tannin moderately but significantly inhibited the absorption of calcium from CC, but not from either calcium citrate or CCCGs.

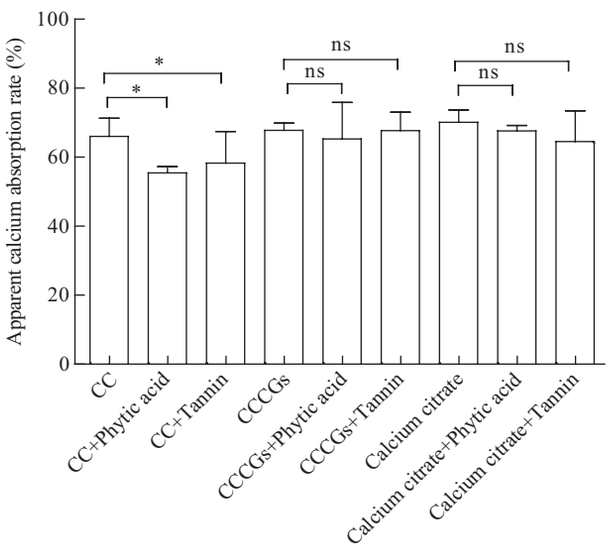

Fig. 4 Effects of the calcium-binding agents phytin acid and tannin on the absorption of calcium from 180 mg calcium/kg CC, calcium citrate, and CCCGs (n=8 in each group). The presence of either phytin acid or tannin inhibited absorption of calcium from CC, but not from calcium citrate or CCCGs. Data were represented as mean±SD. $^{*}P$<0.05; ns: no significant

2.5 Adverse Gastrointestinal Effects

Adverse effects of CCCGs and the reference compound CC on the gastrointestinal tract were determined in normal control mice by focusing on changes in gastric emptying, intestinal propulsion, and serum levels of MTL and GAS. CC slightly but significantly decreased gastric emptying, but CCCGs had no effect on any of the three calcium doses tested (fig. 5A). Similarly, CC, but not CCCGs, inhibited intestinal propulsion (fig. 5B). Neither CC nor CCCGs had any significant effect on levels of MTL or GAS (fig. 5C). Thus, CC, but not CCCGs, decreased gastric emptying and intestinal propulsion, but neither compound altered levels of MTL or GAS.

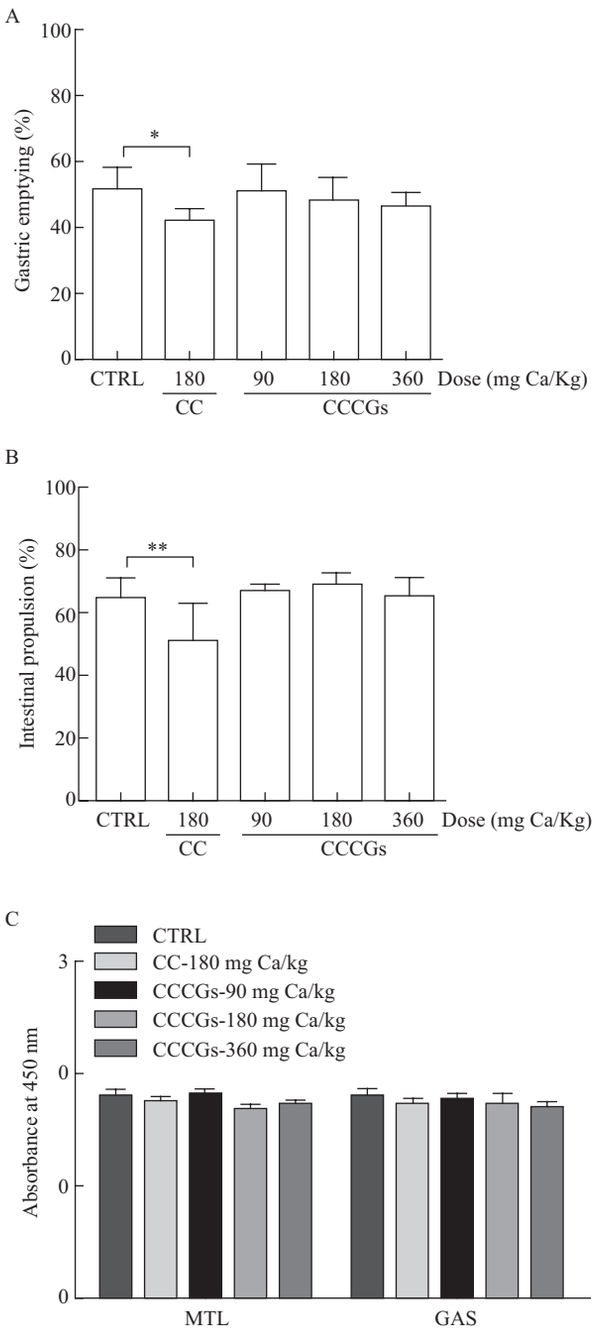

Fig. 5 Effects of CC or CCCGs on gastric emptying (n=8) (A), intestinal propulsion (B), and serum levels of the gastrointestinal hormones MTL and GAS (C)
Data were represented as mean±SD. $^{*}P<0.05$, $^{**}P<0.01$

3 DISCUSSION

Calcium carbonates are widely used as calcium supplements, largely because of their high calcium content and low cost[25]. The rank order of calcium absorption from the three most common calcium salts are calcium citrate > calcium gluconate > calcium carbonate[26]. Limitations of currently-used supplements include adverse gastrointestinal effects, manifested in part by alterations in gastric emptying time and intestinal motility[27–29], and by variable calcium absorption due to fluctuations in gastric pH and the presence of food components that can bind to calcium salts.

For this study, we gained a novel compound of calcium carbonate and calcium citrate to form a granular complex (CCCGs). We then characterized key pharmacokinetic and biological properties of the granules, focusing on solubility, rates of absorption, and actions on gastric and intestinal motility. The effects of CCCGs were compared with those of CC, a commonly used calcium carbonate preparation. The CCCGs granules were formulated to combine citrate and carbonate salts of calcium in an attempt to shield the calcium salts against unwanted effects of gastric acid on calcium absorption, and perhaps to decrease the extent of adverse effects on gastrointestinal motility.

Pharmacokinetic properties of the granules were tested in mice whose gastric acid had been inhibited by omeprazole treatment in an attempt to minimize inhibitory effects of high gastric pH on calcium absorption[20]. Unexpectedly, absorption of calcium after CC administration in this study was actually lower in the omeprazole-treated mice than in the control group (fig. 2). In contrast, calcium absorption after CCCGs administration was not affected by inhibition of gastric acid secretion and the results showed there was no significant difference among different dose groups of CCCGs. *In vitro*, CCCGs were water-soluble in either acidic or water solutions (preliminary observations). Apparently, the unique formulation of the CCCGs may have stabilized calcium absorption under conditions of variable pH *in vivo*. This stabilizing effect seems to be nonspecific. The presence of phytic acid and tannin, two anionic compounds known to precipitate calcium[19, 20], inhibited the absorption of CC but not of CCCGs (fig. 3).

Perhaps the most significant finding of this study was that the CCCGs produced fewer adverse gastrointestinal effects than CC did (fig. 5). CC, as expected, delayed gastric emptying and inhibited intestinal propulsion, but the CCCGs did not. Neither CC nor CCCGs altered serum levels of the gastrointestinal hormones MTL or GAS (fig. 5C), seemingly ruling them out as contributors to the actions of CC on gastrointestinal motility. Instead, the motility-inhibiting actions produced by CC may be attributable at least in part to the dual-salt complex formulation of the CCCGs, resulting in less carbonate per mole of administered calcium.

Presumed benefits of calcium supplementation are somewhat controversial[11]. Concerns have been raised regarding the potential for increasing the risk of adverse cardiovascular events[30]. The prevailing view, however, is that calcium supplements are generally safe when used appropriately[11, 12], and considered to be

important for bone health generally and for conditions of abnormal bone structure or metabolism such as fractures and osteoporosis[13]. The novel calcium formulation described here may be therapeutically advantageous over more commonly used calcium supplement formulations, because of its stable calcium absorption characteristics and its relatively favorable adverse effect profile.

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Conflict of Interest Statement

The authors declare that they have no potential conflicts of interest regarding this work.

REFERENCES

1 Heaney RP, Kopecky S, Maki KC, *et al*. A review of calcium supplements and cardiovascular disease risk. Adv Nutr, 2012,3(6):763-771
2 Tamamura Y, Yamaguchi A. Bone and tooth in calcium and phosphate metabolism. Clin Calcium (Japanese), 2012,22(1):11-17
3 Nakashima T. Bone and Calcium Metabolisms Associated with Dental and Oral-Maxillofacial Diseases. Bone remodeling and alveolar bone homeostasis. Clin Calcium (Japanese), 2015,25(8):1220-1228
4 Hawkins TE, Merrifield CJ, Moss SE, *et al*. Calcium signaling and annexins. Cell Biochem Biophys, 2000,33(3):275-296
5 Ross AC, Manson JE, Abrams SA, *et al*. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab, 2011,96(1):53-58
6 NIH Consensus conference. Optimal calcium intake. NIH Consensus Development Panel on Optimal Calcium Intake. JAMA, 1994,272(24):1942-1948
7 Balk EM, Adam GP, Langberg VN, *et al*. Global dietary calcium intake among adults: a systematic review. Osteoporos Int, 2017,28(12):3315-3324
8 Abrams SA. Calcium absorption in infants and small children: methods of determination and recent findings. Nutrients, 2010,2(4):474-480
9 Sobczak M, Jablonski E. Mineral elements in diet of pregnant and breast-feeding women. Part I. Macro minerals: calcium, magnesium, phosphorus, sodium, potassium, chloride. Przegl Lek, 2007,64(3):165-169
10 Hawkins TE, Merrifield CJ, Moss SE. Calcium and vitamin D supplements improve cholesterol in older women. Harv Womens Health Watchm, 2014,21(10):8
11 Manson JE, Bassuk SS. Calcium supplements: do they help or harm? Menopause, 2014,21(1):106-108
12 Bauer DC. Clinical practice. Calcium supplements and fracture prevention. N Engl J Med, 2013,369(16):1537-1543
13 Huang J, Ou HY, Chiu KC, *et al*. Calcium supplements and fracture prevention. N Engl J Med, 2014,370(4):387
14 Hsia J, Heiss G, Ren H, *et al*. Calcium/vitamin D supplementation and cardiovascular events. Circulation, 2007,115(7):846-854
15 Ye L, Morse LR, Zhang L, *et al*. Osteopetrorickets due to Snx10 deficiency in mice results from both failed osteoclast activity and loss of gastric acid-dependent calcium absorption. PLoS Genet, 2015,11(3):e1005057
16 Kopic S, Geibel JP. Gastric acid, calcium absorption, and their impact on bone health. Physiol Rev, 2013,93(1):189-268
17 Keller J, Schinke T. The role of the gastrointestinal tract in calcium homeostasis and bone remodeling. Osteoporos Int, 2013,24(11):2737-2748
18 Hiligsmann M, Ben Sedrine W, Bruyere O, *et al*. Cost-effectiveness of vitamin D and calcium supplementation in the treatment of elderly women and men with osteoporosis. Eur J Public Health, 2015,25(1):20-25
19 Aloia JF, Dhaliwal R, Shieh A, *et al*. Calcium and vitamin D supplementation in postmenopausal women. J Clin Endocrinol Metab, 2013,98(11):E1702-1709
20 Hanzlik RP, Fowler SC, Fisher DH. Relative bioavailability of calcium from calcium formate, calcium citrate, and calcium carbonate. J Pharmacol Exp Ther, 2005,313(3):1217-1222
21 Satoh K, Kase Y, Yuzurihara M, *et al*. Effect of Dai-kenchu-to (Da-Jian-Zhong-Tang) on the delayed intestinal propulsion induced by chlorpromazine in mice. J Ethnopharmacol, 2003,86(1):37-44
22 Su SF, Yang AM, Yang SB, *et al*. Intracerebroventricular administration of neuronostatin delays gastric emptying and gastrointestinal transit in mice. Peptides, 2012,35(1):31-35
23 Gompertz RH, Man WK, Li SK, *et al*. Acid blockade by omeprazole or ICI 162846 in a chronic duodenal ulcer model. Agents Actions, 1991,33(1-2):161-163
24 Al-Mamary M, Molham AH, Abdulwali AA, *et al*. *In vivo* effects of dietary sorghum tannins on rabbit digestive enzymes and mineral absorption. Nutr Res, 2001,21(10):1393-1401
25 Fausett H, Gayser C Jr, Dash AK, *et al*. Evaluation of quick disintegrating calcium carbonate tablets. AAPS Pharm Sci Tech, 2000,1(3):E20
26 Johnson RN. A study of five calcium supplements: estimation of calcium absorption and sodium content. Eur J Clin Nutr, 1991,45(2):117-119
27 Reid IR, Mason B, Horne A, *et al*. Randomized controlled trial of calcium in healthy older women. Am J Med, 2006,119(9):777-785
28 Salovaara K, Tuppurainen M, Karkkainen M, *et al*. Effect of vitamin D(3) and calcium on fracture risk in 65- to 71-year-old women: a population-based 3-year randomized, controlled trial--the OSTPRE-FPS. J Bone Miner Res, 2010,25(7):1487-1495
29 Reid IR, Bristow SM, Bolland MJ. Calcium supplements: benefits and risks. J Intern Med, 2015,278(4):354-368
30 Schwingshackl L, Hoffmann G, Buijsse B, *et al*. Dietary supplements and risk of cause-specific death, cardiovascular disease, and cancer: a protocol for a systematic review and network meta-analysis of primary prevention trials. Syst Rev, 2015,4:34

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