Research and Reviews: Drug Delivery

Progress in the Study of Calcium Formulations

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ABSTRACT

Calcium is an important mineral element in the human body, which affects human life and health. 99% of calcium is found in bones and teeth, and 1% in blood, extracellular fluids and soft tissue cells called mixed calcium pools. Calcium in bones and teeth can maintain the firmness of bones and teeth and prevents various orthopaedic diseases, including osteoporosis, osteopenia, osteomalacia and rickets. Calcium in the blood, extracellular fluid and soft tissue cells can maintain the normal contraction of skeletal muscle and myocardium; maintain the permeability of biological membranes, and secretion and storage of hormones. This review summarizes the physiological function and the absorption mechanism of calcium as well as the factors affecting the calcium absorption. This paper mainly introduces the absorption mechanism of calcium factors affecting calcium absorption, the side effect of the excess of the calcium formulation and the classification of the calcium formulation. Calcium absorption is influenced by hormones, dietary ingredients, personal factors and other factors, of which hormones are the most important factor. The adverse effects of calcium overdose include cardiovascular disease, kidney stones, prostate cancer and adverse gastrointestinal events. So we should choose the right dose for calcium supplement. The classification of the calcium formulation includes inorganic calcium, organic acid calcium, calcium amino acid chelate and collagen peptide chelated calcium. At present, the most common calcium supplement in the market is inorganic calcium and organic acid calcium. Calcium peptides have the advantages of high absorption efficiency, no side effects and high solubility. So it has become a research hotspot.

INTRODUCTION

Calcium, the 20th element in the periodic table, is the most abundant mineral element in the human body. Basically, all the life processes of the body need calcium. Calcium homeostasis is an important factor to maintain life and health. An adult body contains about 1-1.5 kg calcium, more than 99% of which exists in bones and teeth to maintain their firmness, and the existence form of calcium in bones is hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and dicalcium phosphate ($CaHPO_4$) [1]. In bone, calcium provides structural strength, which enables the skeleton to support the weight of the body, and to support the muscles. Therefore, the lack of calcium will lead to a variety of diseases in the department of orthopedics including osteoporosis, osteopenia, osteomalacia, and rickets [2]. Viguetcarrin et al. [3] studied the effect of calcium intake on bone in growing rats. Rats aged 28 days were divided into three groups and were given high calcium (1.2%), adequate calcium (0.5%) and low calcium (0.2%), respectively. Four weeks later, the bone size, bone strength and bone density were observed. The length of femur in adequate calcium group and high calcium

group was similar and significantly higher than that in low calcium group. For the bone strength, the low calcium group can significantly reduce the femoral failure load, while high calcium increased bone strength (failure load+30% p=0.0009; stiffness+67% p=0.0002) compared with adequate calcium group. In addition, with the increase in calcium, bone density and mineralization density increased. The experiment shows that proper calcium intake has a positive effect on bone.

Approximately 1% of the calcium in the human body is distributed in the blood, extracellular fluid, and soft tissue cells, which are called miscible calcium pool, maintain a dynamic balance with bone calcium. The normal reference value of serum calcium is 2.15-2.58 mmol/L [4]. This calcium is an important electrolyte in the body, and many of the fundamental biological functions require calcium participation. Evidence indicates that extracellular calcium concentration directly regulates cell function (such as parathyroid glands, renal tubules.) and directly affects cell membrane potential, thereby affecting the function of all excitatory tissues, especially the nervous system and heart [5]. Calcium can maintain normal contractile function of skeletal muscle and myocardium plays an important role in the permeability of cell membranes and capillaries, regulates the secretion and storage of neurotransmitters and hormones, uptake and utilization of amino acids, the absorption of vitamin B12, etc. In addition, calcium is also involved in the coagulation process [6].

Calcium plays an important role in the human body. Long time calcium deficiency in the elderly is one of the main causes of rickets, osteoporosis, asthma and hypertension, etc. Even in developed countries, many children's calcium intake is only 1/3 to 1/2 of the recommended amount [7]. Postmenopausal women need extra calcium due to hormonal changes that can accelerate bone loss rates and lead to a further reduction in bone mass. In addition, fetal skeletal development increases calcium demand during the pregnancy, so the maternal need extra calcium supplement [8]. Patients with inflammatory bowel or celiac disease should also be supplemented with calcium. **(Table 1)** lists the daily calcium requirements of different age groups. In recent years, with the improvement of living standards, people's awareness of the importance of calcium is gradually increasing, and the demand for calcium preparation is also increasing.

Table 1. Daily Calcium Requirements for Different Age Groups [9,10].

Age	Adequate intake (mg/d)
0-6 months	210
7-12 months	270
1-3 years	500
4-8 years	800
9-18 years	1300
19-50 years	1000
> 50 years	1200
Pregnant and lactating females	1500
Menopausal women	
On hormone therapy	1000
No hormone therapy	1500

Absorption Mechanism and Influencing Factors of Calcium

Calcium is mainly absorbed in the ileum and jejunum, through the active (energy required) and passive (diffusive) transport. According to the study, calcium is absorbed most in the ileum (65%), followed by the jejunum (17%), and finally the duodenum (8%) [11]. There are two pathways of calcium absorption: saturated transcellular pathway and unsaturation paracellular pathway **(Figures 1)**. The transcellular pathway is that the intestinal calcium enters the cell through the apical membrane of the small intestinal epithelial cells, and then enters the extracellular space through the base side membrane, and finally enters the blood or lymph. The transcellular pathway is a kind of active absorption pathway, mainly occur in the proximal small intestine (mainly the duodenum), and are regulated by nutrition and physiology. The paracellular pathway is that the intracellular calcium enters the intercellular space through the tight junctions of small intestinal epithelial cells and then enters the blood. The paracellular pathway is a passive absorption pathway, which is absorbed in the whole intestinal tract [12].When the concentration of Ca^{2+} in the intestinal lumen is high, Ca^{2+} will enter the blood through the paracellular pathway.

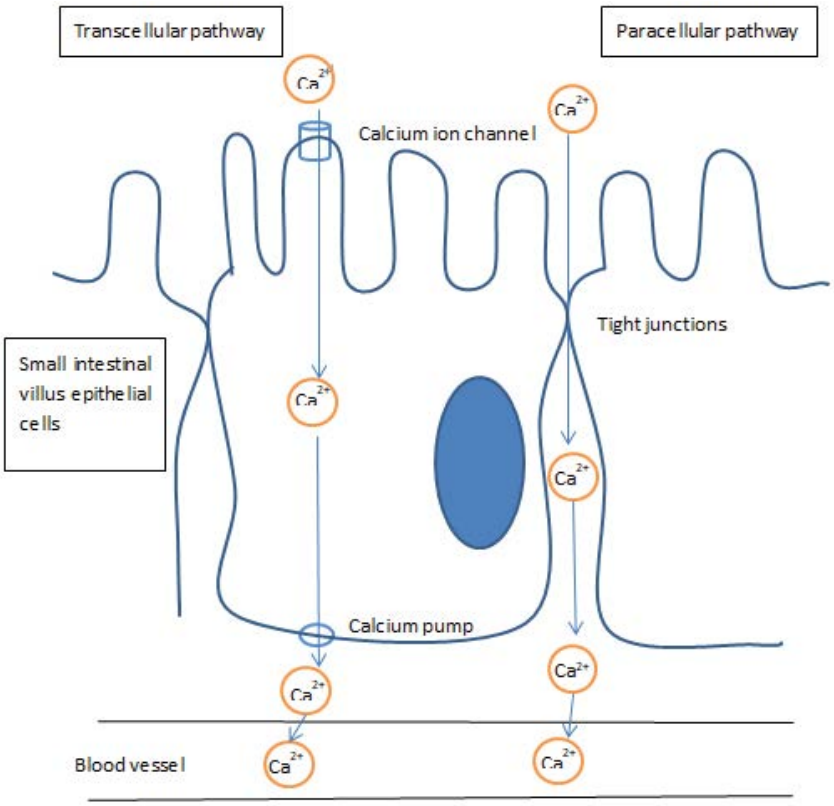

Figure 1. Transcellular Pathway and Paracellular Pathway

Hormone

Calcium balance is regulated by Parathyroid Hormone (PTH), Calcitonin (CT) and vitamin D3, and the target organs of these hormones are gut, kidney, and bone [13]. **(Figures 2 and 3)** illustrate the regulatory effects of PTH, CT and vitamin D3 on serum calcium. PTH can mobilize calcium from bone by enhancing osteoclast activity and promoting proliferation and promote calcium absorption in the proximal tubule of the kidney. High plasma calcium concentration can stimulate the secretion of calcitonin. CT can reduce the calcium concentration by inhibiting osteoclast mediated bone resorption and enhancing renal excretion. 7-dehydrocholesterol in human subcutaneous can be converted into cholecalciferol under the influence of sunlight. Cholecalciferol is delivered to the liver into 25-hydroxy cholecalciferol (calcidiol) which delivers to the kidney under the stimulation of PTH further into 1,25-dihydroxy-cholecalciferol (calcitriol) or be downgraded into 24,25-dihydroxycholecalciferol. 1,25-dihydroxy- cholecalciferol can promote intestinal absorption of calcium and renal calcium reabsorption, decrease bone calcium excretion, and also indirectly feedback regulation of PTH release [14].

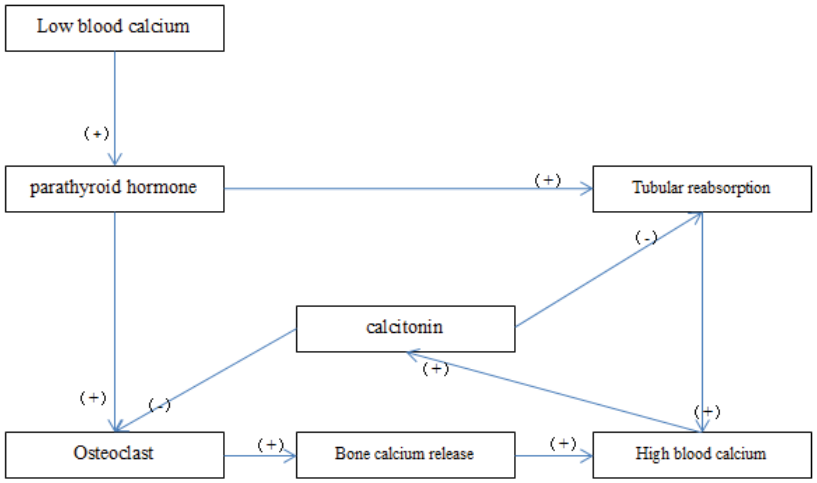

Figure 2. Effect of Parathyroid Hormone and Calcitonin on Serum Calcium

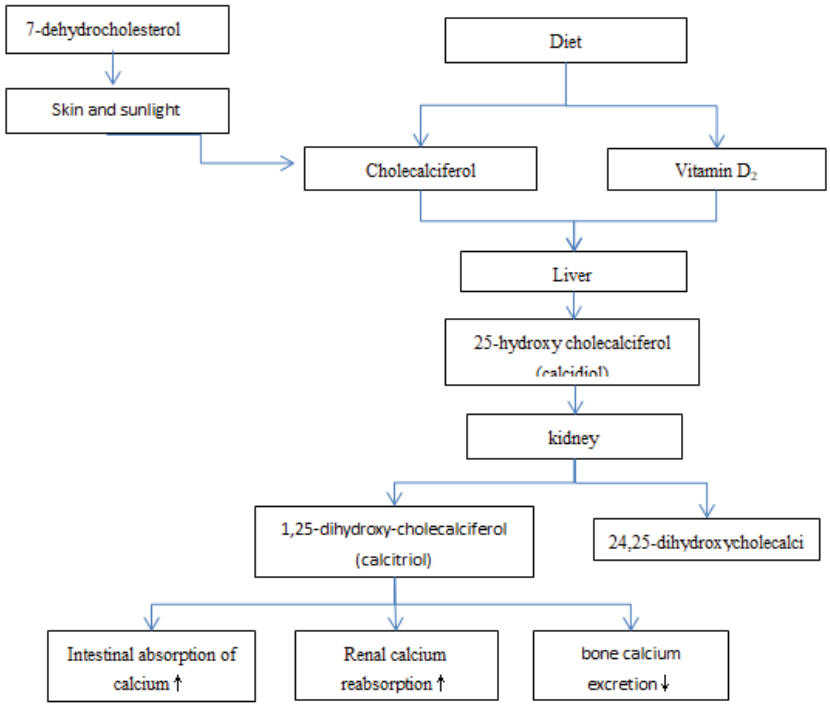

Figure 3. Effect of Vitamin D on Calcium Absorption

Dietary Ingredients

Low dietary calcium content is an important reason to affect the calcium absorption. In addition, sugar, protein, organic acids, minerals and vitamins and other ingredients in the diet will affect the absorption and utilization of calcium. Both hydrolyzed and unhydrolyzed forms of lactose can be used as a "*carrier*" of calcium to promote the absorption and retention of calcium [15]. Phytate and oxalate in the diet are combined with calcium to form insoluble salts that interfere with the absorption of calcium [16]. Excessive intake of fat can lead to excessive free fatty acids in the body. Free fatty acid binding calcium form insoluble calcium soap, also can reduce the absorption of calcium.

Personal Factors

The ability to absorb calcium is not the same for everybody. Wolf et al. [17] explored individual differences in calcium absorption in women by assessing the calcium uptake rate of 142 pre- and postmenopausal women. The results showed that the average calcium absorption value was 35% and range from 17% to 58%. The absorption rate of calcium was positively correlated with the body mass index, dietary fat intake, serum 1,25-dihydroxy vitamin D and parathyroid hormone levels, while it was negatively correlated with total dietary calcium, dietary fiber, drinking and constipation. The experimental results demonstrated that the calcium absorption rate is different between individuals.

Other Factors

Pathological changes such as inflammatory bowel disease affect the absorption of calcium. Unqualified disintegration and dissolution or unreasonable oral method of calcium preparation will affect the calcium absorption rate and utilization. Bad habits such as smoking and drinking can also lead to a decline in calcium absorption. In addition, individual age factors also have a great influence on calcium absorption [18]. The calcium absorption rate can reach 60% in infancy because growth hormone can stimulate calcium absorption, reduced to 15-20% in adulthood. The absorption of calcium in the gastrointestinal tract is decreased in the elderly due to the decrease of physiological function, digestive function and appetite.

The Side Effects of Calcium Supplements

Cardiovascular disease

In recent years, with the continuous progress of the research, people found that excessive use of calcium supplements may increase the risk of myocardial infarction and stroke. Calcium in the blood is a risk factor for cardiovascular disease, a result of 30 years of research involving thousands of participants. Bolland et al. [19] divided 1471 postmenopausal women with an average age of 40 into two groups: 732 were randomized to the calcium group and 739 to the control group. The calcium group took calcium 1 g/day, and the control group received placebo for five years. The results showed that the incidence of cardiovascular events in the calcium group was 40% higher than that in the control group. This experiment indicated that calcium supplementation can increase the incidence of cardiovascular disease in postmenopausal women. Kuanrong Li et al. [20] followed up 23,980 participants aged 35-64, found an increase of 86% in the risk of myocardial infarction in patients taking calcium supplements, and the risk

of only taking calcium supplements was 2.4 times that of those who did not take any supplements. This may be due to vascular calcification caused by the increase of serum calcium after supplementation of calcium [21]. Vascular calcification is a process similar to bone formation, which can increase the stiffness of the blood vessels, leading to a variety of cardiovascular diseases. This is not a simple physical process, which is associated with the formation of matrix vesicles in vascular smooth muscle cells. Matrix vesicles are produced by living vascular smooth muscle cells or apoptotic bodies from dying cells, which are then extruded, resulting in extracellular calcium deposition (Figure 4). Vascular smooth muscle cells exposed to calcium lead to change in gene expression and the expression of osteoblast phenotype [22].

Kidney stones

Excess calcium in the body passes through the kidneys, increasing the risk of kidney stones and renal calcification [23]. Calcium oxalate stones are the most common kidney stones which accounts for approximately 80% of all kidney stones [24]. The formation of calcium oxalate stone is caused by the interaction of calcium and oxalic acid in the gastrointestinal tract, and the excretion of oxalic acid and calcium in urine promoted the formation of the stone.

Research shows that high dietary calcium intake can reduce the formation of kidney stones, while the use of calcium supplements may increase the formation of kidney stones [25]. In 5 years of study, 120 patients with recurrent calcium oxalate stones and hypercalciuria were divided into two groups. Sixty men were given a normal calcium intake (30 mmol per day), and the other 60 were given a low calcium diet (10 mmol per day). The results showed that 12 men had recurrence of kidney stones in group with normal calcium diet while 23 men got recurrence in group with low calcium diet. The risk of recurrence of kidney stones in the normal group was 50% lower than that in the low calcium group. In addition, the low calcium oxalate urinary excretion increased (60 μmol per day), while the normal calcium diet decreased (80 μmol per day). It is believed to be the calcium in the diet combined with oxalic acid to prevent absorption in the intestine. It is concluded that the risk of recurrence of calcium oxalate kidney stones in the normal calcium diet is lower than that in the low calcium diet [26]. However, Rebecca et al. [27] recruited 36,282 postmenopausal women who had participated in Women's Health Initiative (WHI) clinical trial. Participants were randomly assigned to receive 1000 mg of elemental calcium and 400 IU of vitamin D3 daily or placebo. There were 449 women with kidney stones in the calcium plus vitamin D group, while 381 women in the placebo group (hazard ratio, 1.17; 95 percent confidence interval, 1.02 to 1.34). The result found that use of calcium supplements will increase the risk of kidney stones.

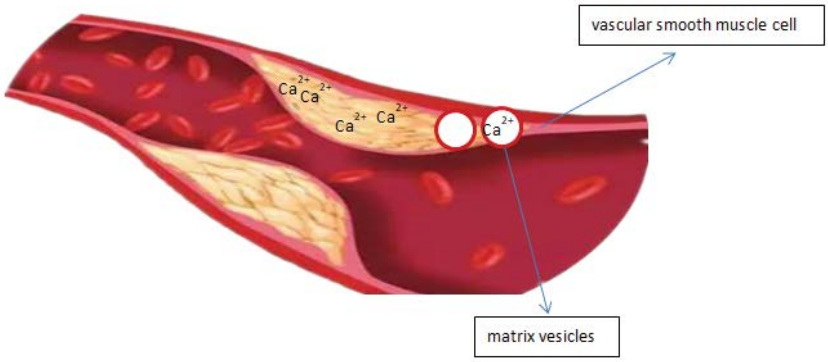

Figure 4: Calcium and Cardiovascular Disease

Prostate cancer

Many studies have shown that calcium increases the risk of prostate cancer in men during the past two decades. This is due to the 1,25-dihydroxy vitamin D3 consistently been shown to inhibit prostate cancer cell growth and development. 1,25-dihydroxy vitamin D3 regulates calcium metabolism in the body, when the serum calcium level is lower than the normal value, 1,25-dihydroxy vitamin D3 acts on the intestine, kidney and bone to increase retention and absorption of calcium until the serum calcium levels are normal. Consequently, dietary calcium intake can suppress circulating 1,25-dihydroxy vitamin D3 levels and increases prostate cancer risk [28]. Chan et al. [29] found that men who consumed>600 mg Ca/d had lower plasma 1,25-dihydroxy vitamin D3 concentrations than did those consuming ≤ 150 mg Ca/d (71 compared with 85 pmol/L (30.06 compared with 35.64 pg/mL); P=0.005), and compared with men consuming ≤ 150 mg Ca/d, men consuming>600 mg Ca/d had a 32% higher risk of prostate cancer.

Adverse gastrointestinal events

The calcium preparation is generally well tolerated. However, some people complain that after taking calcium supplements appear symptoms such as constipation, abdominal distention and other gastrointestinal symptoms. Bolland et al. [30] analyzed 7 randomized controlled trials and found that the incidence of gastrointestinal disorders was higher in the calcium preparation group than in the placebo group. These gastrointestinal disorders include constipation, excessive abdominal cramping, bloating, upper GI events, GI disease, GI symptoms, and severe diarrhoea or abdominal pain (calcium 14.1%, placebo 10.0%). In addition, Lewis et al. [31] found that the proportion of patients admitted to hospital with acute abdominal pain was 3.6% in the placebo

group and 6.8% in the calcium preparation group. These data indicate that calcium supplementation increases the incidence of gastrointestinal adverse events.

Types of Calcium Formulations

According to the development of calcium preparation, it can be divided into three categories: inorganic calcium, organic acid calcium, amino acid chelate calcium and collagen peptide chelated calcium.

Inorganic calcium

The main source of calcium in inorganic calcium preparation is calcium carbonate, calcium bicarbonate, calcium phosphate, calcium phosphate, calcium chloride as well as oyster shell, shell, bone and other natural products.

Calcium carbonate is the most common and the least expensive form of inorganic calcium supplement with high calcium content. Calcium carbonate is less soluble in water so requires a lot of natural stomach acid to free calcium to be absorbed after it is taken, and its bioavailability is poor. So, it can't be taken with food, nor is it suitable for individuals with the hypochlorhydria or achlorhydria [32]. In addition, it has some side effects which are easy to cause gastrointestinal irritation. Meiron et al. [33] compared the solubility and absorption fraction of Amorphous Calcium Carbonate (ACC), Amorphous Calcium Carbonate with Chitosan (ACC-C) and Crystalline Calcium Carbonate (CCC). The three kinds of calcium carbonate were dissolved in dilute phosphoric acid, and the solubility was compared by measuring pH. It is found that the solubility of ACC and CCC is higher than that of ACC-C, and the solubility of ACC is at least 10 times higher than that of CCC. Absorption fraction was determined by 45 Ca labeled calcium carbonate. Calcium serum analysis revealed that calcium absorption from ACC and ACC-C preparations was 40% and 12% higher than that of CCC. Absorbed calcium in the femurs of ACC group was 30% higher than in CCC group, whereas 15% more calcium was absorbed following ACC-C than following CCC. The results showed that the absorption fraction of ACC and ACC-C was better than that of CCC, so the effect of ACC as calcium supplement was preferable to that of CCC.

Sandra et al. [34] studied the effects of calcium phosphate, milk calcium, calcium chloride and calcium carbonate on intestinal resistance to Salmonella in rats. Rats were given low calcium (20 mmol calcium phosphate/kg; negative control group), calcium phosphate, milk calcium, calcium chloride, and calcium carbonate (100 mmol Ca supplement/kg). Two weeks later, animals were orally infected with *S. enteritidis*. The food intake and weight changes, the persistence of Salmonella, and the permeability of the intestine were measured. The results showed that the average food intake of calcium phosphate group was higher than that of low calcium group in the first week after infection, and the weight gain in calcium phosphate group was higher than that in other groups. All calcium supplementation groups reduced Salmonella persistence and intestinal permeability compared to the low calcium group. Therefore, it was concluded that calcium salt can increase the intestinal resistance to Salmonella in rats.

Bone meal and eggshell powder are universal natural calcium preparations. Fish bone tissue dry matter contains 60-70% minerals, mainly calcium phosphate and hydroxyapatite [35]. Bubel et al. [36] used the backbone of COD and salmon as a raw material, after cutting, alkali treating, citric acid treating, odor removing, disinfection materials, tap water washing, drying, grinding to prepare bone powder. Calcium, protein, and fat content of calcium preparations made from cod bone were 27.29%, 14.20%, 0.25%, respectively; while these components in salmon were 24.92%, 10.78%, 0.12%, respectively. Eggshell contains about 39% elemental calcium [37]. The eggshell is ground into powder after being cleaned, sterilized and dried. The main chemical composition of eggshell powder is calcium carbonate [38], but the eggshell calcium is more soluble than calcium carbonate, and the calcium content is as much as that of the milk. Eggshell powder has been shown to have a higher absorptivity and availability than calcium carbonate [39]. Jang et al. [40] compared the effects of water-soluble calcium supplements made from eggshell and oyster shell and the water-soluble calcium supplement produced by imported seaweed powder on the calcium metabolism of growing rats. Methods: 21 female rats were randomly divided into three groups, which were treated with water-soluble calcium supplement prepared from seaweed powder, egg shell and oyster shell. After six weeks, serum calcium in seaweed calcium supplement group was 11.85 mg/dL, was significantly higher than that of eggshell calcium group (11.43 mg/dL) and oyster shell calcium group (11.32 mg/dL), and no significant difference between the three groups of blood alkali phosphatase activity, osteocalcin and urine crosslink levels. There was no significant difference in bone mineral density and bone mineral content in the spine, femur and tibia between the three groups except for the significant increase in bone mineral density and bone mineral content of femur in oyster shell calcium group. The result showed that the effect of eggshell calcium and oyster shell calcium are similar to that of seaweed calcium supplement When short-term calcium supplementation. Natural calcium preparations are generally made by high temperature calcination, and high degree of ionization is easy to be absorbed by the body. Natural calcium preparation is a good biological agent, but its safety has been questioned because it may contain some toxic elements such as lead, aluminum, cadmium and mercury etc [18].

Organic acid calcium

Organic acid calcium mainly refers to calcium citrate, calcium lactate, calcium gluconate, calcium acetate and other organic calcium salts. This kind of preparation has better solubility and less gastrointestinal irritation compared with inorganic calcium **(Table 2)**.

Table 2: Calcium Preparation Comparison Chart

Ca salt	Chemical formula	%Ca	Preparation name	Dosage form	Specifications	Calcium content per unit/mg
Calcium carbonate	$CaCO_3$	40.04	Caltrate	Tablet	1500 mg X 30	600
Calcium phosphate	$Ca_3(PO_4)_2$	38.79	DHU Biochemie 2 Calcium phosphoricum D6	Tablet		250
Calcium hydrogen phosphate	$CaHPO_4$	29.46	Lysine Hydrochloride and Calcium Hydrogen Phosphate Granules	Granule	5 g X 12	147
Calcium citrate	$Ca_3(C_6H_5O_7)_2{\cdot}4H_2O$	21.08	GNC Calcium Citrate	Tablet	1000 mg X 180	53
Calcium lactate	$Ca(C_3H_5O_3)_2{\cdot}5H_2O$	13	BioCalcium - Calcium L-Lactate	Chewable tablet	1100 mg X 120	150
Calcium gluconate	$Ca(C_6H_{11}O_7)_2$	9.32	Calcium Sandoz	Effervescent tablet		500
Calcium L-threonate	$Ca(C_4H_7O_5)_2$	12.92				
Calcium L-aspartate	$C_8H_{12}O_8N_2Ca$	13.17	Byl-calcium	Capsule		66
Calcium glycine	$C_4H_8O_4N_2Ca$	21.3	Albion Chelated Calcium Glycinate	Capsule		38

Calcium citrate was prepared by the reaction of citric acid with the calcium carbonate or sodium citrate and calcium chloride. It contains an acidic group that can release calcium ions under the condition of less gastric acid, making this type of calcium more easily absorbed than calcium carbonate [41] and is especially beneficial for individuals with hypochlorhydria or achlorhydria. Howard et al. [42] chose 25 postmenopausal women for a three stage of the study. Calcium citrate was found to increase more calcium in serum and urine than calcium carbonate, which suggests that the bioavailability of calcium citrate is higher than that of calcium carbonate when taken with meals. The results showed that calcium citrate uptake was better than that of calcium carbonate at lower gastric acid concentration. Barbara et al. [43] prepared calcium citrate tablets and calcium carbonate tablets use eggshells as raw material, and their *in vitro* release rate was investigated. After 30 min of the experiment, from calcium citrate 79% of calcium was released, reaching 100% after 3 h. For calcium carbonate, there were 7% and 60% of calcium was released, respectively. Calcium citrate release of calcium with a half-life of 0.5 h and calcium carbonate is 2.2 h, so the release of calcium citrate is 4 times faster than calcium carbonate. This is related to the solubility of calcium salts. Calcium carbonate is only dissolved in strong acidic medium while calcium citrate is dissolved in neutral and alkaline medium.

Calcium lactate is prepared by the reaction of lactic acid or lactic acid fermentation liquid with calcium carbonate or calcium hydroxide. Calcium lactate has many advantages, such as easy to dissolve, does not stimulate the stomach and high absorption and utilization rate. Furthermore, experiments show that calcium lactate has a therapeutic effect on rectal cancer. Calcium lactate can induce down-regulation of betaine-γ-aminobutyric acid transporter, which can produce the effect of anti-rectal cancer cells [44]. However, the lactic acid in calcium lactate is easy to give the body a sense of fatigue, so it is not suitable for long-term use. Lee et al. [45] studied the effects of calcium lactate and chungkukjang on bone, calcium status, body weight, serum glucose and cholesterol levels in rats. Rats were randomly divided into 4 groups: calcium deficiency group (Ca-De), calcium phosphate group (Ca-P), calcium lactate group (CaL-A), and CaL-A+chuntkukjang group (CaL-AC). After 4 weeks, the body weight gain of Ca-P, CaL-A, CaL-AC and Ca-De was 130.45 g, 112.50 g, 143.40 g and 10.20 g, respectively. The femur weight and serum calcium concentration of Ca-De was lower than those of the other 3 groups. Microscopic observation showed that there were many regular holes in the femur of group Ca-De, while the CA-P group was smaller than the CA-DE group, and there was no hole in the CaL-A and CaL-AC groups. The blood glucose concentration and total cholesterol index of the CaL-AC group were lower than those of the other three groups. These results indicate that the bioavailability of CaL-A is higher than that of Ca-P, and the presence of chuntkukjang is beneficial to calcium metabolism.

Calcium gluconate is made from the reaction of glucose acid with lime or calcium carbonate. Calcium gluconate is the preferred intravenous injection of calcium preparations in the treatment of fatal hypocalcemia, and it can also be used as an oral or intramuscular injection. Patients with gastrectomy due to long-term proton pump inhibitors treatment induced hypoacidity causes calcium malabsorption. This is a part of factors that cause bone mineralization defects and secondary hyperparathyroidism. Krause et al. [46] observed the bone metabolism in patients with gastrectomy by histologically investigate and effects of calcium supplementation on bone integrity of mice with gastric acid deficiency. The results showed that bone mineralization increased in patients with gastrectomy after calcium gluconate treatment, and the treatment effect of calcium homeostasis damaged mice is better than that of calcium carbonate. In addition, an experiment showed that arterial infusion of calcium gluconate can treat the hydrofluoric acid burns of the distal human limbs. Zhang et al. [47] performed arterial infusion therapy with calcium gluconate on 118 patients whose distal limbs were burned by hydrofluoric acid. The visual analogue scale was used to measure the pain of immediately after the infusion, 4 h after the infusion and 2 days after the infusion. The time of wound healing and the proportion of surgical procedures were also evaluated. The results found that 107 patients had pain relief immediately after infusion, and showed that the earlier infusion of calcium gluconate, the shorter the wound healing time, the lower the proportion of patients who need surgery. However, calcium gluconate can be decomposed into glucose in the body. Therefore, diabetes patients cannot use calcium gluconate.

The biggest disadvantage of organic acid calcium preparation is low calcium content. Calcium carbonate in inorganic calcium supplements contains 40% calcium, while calcium citrate in organic acid calcium supplements contains only about 21% calcium. The only drawback associated with the use of organic calcium supplements is the need to take more tablets or capsules to make the dose equivalent to that of inorganic calcium [10]. Therefore, the calcium content of organic calcium can be increased by the preparation of compound preparation. Calcium citrate malate is a compound salt of the Ca ion with citrate and malate ions. Its calcium content is up to 23%. Calcium citrate malate has many advantages other calcium salts do not possess include [48]: higher water solubility, greater absorbability and higher bioavailability in a wide range of ages, more flexible composition (i.e., adjustable molar ratios and mineral content). Calcium citrate malate can play an important role in different stages of life. It has been shown to promote calcium retention and bone formation in children and adolescents. In adults, it effectively promotes bone consolidation and maintenance. Calcium citrate malate also reduces the risk of fracture in older adults and delays the rate of bone loss in older adults. It can promote the health of postmenopausal women. In addition, calcium citrate malate does not increase the risk of kidney stones, and in fact it can prevent the formation of stones.

Calcium amino acid chelate

Calcium amino acid chelate is produced by chemical reaction of amino acid and inorganic calcium salt. It overcomes the inorganic calcium insoluble in water, low utilization rate, easy to cause stones, and also avoids the disadvantages of low calcium content and toxic side effect of the organic calcium preparation. At the same time, calcium amino acid chelate has the advantages of good stability, high absorption rate, high biological potency, anti-stress, low toxicity, no incompatibility with vitamins and other advantages [49,50]. The human body at the same time takes 2 kinds of nutrients when intake of calcium amino acid chelates, and thus has a dual nutritional role. Therefore, the research and development of calcium amino acid chelated has been paid more and more attention by researchers.

Calcium amino acid chelate is a mixture, but also has specific amino acid calcium salts, such as calcium L-threonate, calcium L-aspartate, calcium glycine and Calcium glutamate.

Calcium L-threonate is a new drug for the treatment of osteoporosis. L-Threonic acid is an active metabolite of vitamin C. It is reported that L-Threonic acid can stimulate the uptake of vitamin C and prolong the retention time of vitamin C in T lymphocytes [51]. Vitamin C can stimulate the synthesis of procollagen and collagen. So, L-Threonic acid can play an important role in stimulating bone mineralization by stimulating vitamin C. Therefore, the combination of L-Threonic acid and calcium to form Calcium L-threonate can be used to treat osteoporosis from two aspects. Aguilar et al. [52] studied the absorption, genetic toxicity and reproductive development toxicity of calcium L-threonate. Eight groups of male wistar rats were anesthetized, and then were intestinal injected with calcium acetate, calcium gluconate and L- calcium gluconate, respectively. Blood samples were collected at 15, 30, 60 and 90 min after injection to determine serum calcium concentration. After the administration of 15, 30, 60 and 90 min, passive absorption of calcium in percentage of calcium acetate was 40, 33, 40, and 50. Calcium gluconate was 71, 58, 51 and 51. Middle dose of calcium L-threonate was 82, 74, 56 and 36. The high dose of calcium L-threonate was 93, 78, 56 and 52. These data suggest that the serum calcium concentration after injection of calcium L-threonate is higher than that of other forms of calcium. The results showed that the bioavailability of calcium L-threonate was better. *In vivo* and *in vitro* experiments showed that calcium L-threonate was not genotoxic, which is due to the fact that L-Threonic acid is an endogenous compound. The mice were treated with 6 g/kg body weight/day for their reproductive and developmental toxicity. The results showed that calcium L-threonate had no adverse effects on reproduction and fetal development, and did not cause maternal toxicity.

Luneva et al. [53] were prepared calcium glycine by mixing glycine solution and calcium chloride solution. Calcium glycine was used to treat osteoporosis mice. Fifty-four CBA mice with osteoporosis were divided into 3 groups. The first group as a control group, the second group and the third group daily oral calcium glycine solution and calcium chloride solution. The serum and bone biochemical indexes of the experimental group were compared with those of the control group. The serum calcium concentration increased and the level of bone calcium decreased in the first group and the third group, while bone calcium accumulation and serum calcium increased in the second group, indicating that calcium from the gastrointestinal tract amino acid chelate calcium absorption. The results showed that the presence of glycine in calcium glycine increased the bioavailability of calcium, and made calcium into bone tissue directly. Calcium glycine can be used to treat osteoporosis as a good calcium preparation.

Amino acid chelated calcium into the gastrointestinal tract can be actively absorbed by intestinal mucosa cells in the form of molecules. Molecular into the blood can be sustained release of calcium ions for the body to use. This can avoid hypercalcemia and other adverse symptoms. At the same time, the continuous release of calcium ions can be fully absorbed and utilized by the body.

Collagen peptide chelated calcium

Collagen peptide chelated calcium is a new kind of biological calcium preparation, which has the advantages of high absorp-

tion efficiency, no side effect, convenient use and so on. As a part of the raw material, collagen peptide can be used to assist in the transportation and absorption of calcium. After collagen peptide chelated calcium enters the gastrointestinal tract, two kinds of nutrients can be added at the same time. It has been reported that phosphopeptides derived from casein are bound to minerals, and the complex increases the stability, absorption, and bioavailability of calcium [54].

Collagen peptide is produced by the hydrolysis of collagen, compared with pure collagen, collagen peptide molecular weight is lower and more easily absorbed by the human body [55]. Collagen peptide has a variety of biological activities, such as antioxidant, antibacterial, antihypertensive, anti-tumor. In addition, collagen peptides exhibit considerable ability to bind to divalent metal ions. Collagen peptide and calcium chelate complex can promote the absorption of calcium in the human body, so it can improve the bioavailability of calcium preparation [56]. At present, most studies have shown that most of the collagen peptides are extracted from animal and fish, and the common sources are the horn, hoof, bone and skin, or fish, such as bone and scales [57]. The extraction methods were subdivided into acid method, alkali method, high temperature pyrolysis, fermentation and enzymatic method.

Jin et al. [58] used the ox bone as raw material. Ox bones are protease digestion 3 h and then heated in boiling water for 10 min to inactivate protease, then cooled to room temperature, centrifuged and freeze-dried to obtain collagen peptide. The bone residue was digested by hydrochloric acid for 1 h and then cooled to room temperature. The filtered supernatant was neutralized by calcium hydroxide to obtain soluble calcium. The freeze-dried product was dissolved in distilled water. After adding $CaCl_2$ solutions, the solution was incubated for 30 min. The supernatant was dialysis to obtain collagen peptide chelated calcium. Structural analysis revealed that the collagen peptide chelated calcium is a five membered ring structure. Calcium is the center and strongly bound to amino and carboxyl groups. Guo et al. [59] mixed the clean fish scales with sulfuric acid water solution, heated and stirred for 4 h. The hydrolysate was purified by 1812-type spiral-wound ultrafiltration and 1812-type spiral-wound nanofiltration to obtained collagen peptide. After that, calcium hydroxide was used to adjust the pH value of the collagen peptide aqueous solution to the range of 6.8 to 7.2, and the solution was stirred at room temperature for 24 h. Finally, the 300 u nanofiltration was used to remove the free calcium and the small peptide to obtain the collagen peptide chelated calcium.

Chen et al. [60] isolated calcium binding peptide from protein hydrolysates of tilapia scales and measured its bioavailability in rats. Four groups of rats with calcium deficiency were treated with TSPH-Ca (tilapia scale protein hydrolysate calcium complex), CPP-Ca (Casein Phosphopeptide Calcium Complex), CaCO3 and saline (control) respectively. The body weight, serum phosphorus content and alkaline phosphatase were measured, and no significant difference was found between the three indexes in the four groups. In addition, the apparent calcium absorption rate, serum and bone calcium content, bone mineral density and intensity of TSPH-Ca and CPP-Ca were similar, but they were significantly higher than those in $CaCO_3$ group and control group. Zhe et al. [61] prepared calcium binding peptides (BCP-Ca) from pacific cod bone and measured its bioavailability in rats. Rats were randomly divided into control group (normal diet), model group (low-Ca diet), calcium carbonate group and three experimental groups 50 (BCP-Ca-L group), 100 (BCP-Ca-M group) and 200 (BCP-Ca-H group) mg/kg body wt/day), and the correlation index was measured four weeks later. There was no significant difference in weight gain and organ index between the six groups, indicating that BCP-Ca did not affect normal growth and health. The urinary calcium and fecal calcium in the experimental group were significantly lower than those in the control group and calcium carbonate group, while the apparent absorption rate of calcium was higher than that of the control group and calcium carbonate group, indicating that the effect of increasing calcium intake by BCP-Ca was better than that of calcium carbonate. The apparent calcium absorption rate and calcium retention rate of the three experimental groups were not significantly different, which might be due to higher mineral solubility after chelation of Ca with BCP. The two experiments showed that, peptide chelated calcium could better prevent calcium deficiency and improve the bioavailability of calcium.

Collagen peptide chelated calcium has the advantages of high utilization rate, high solubility and high absorption rate. Collagen peptide is one of the raw materials of calcium chelating peptide, which is extracted from the leftover of animal body. Therefore, the cost is reduced and the resources are fully utilized. At the same time, the collagen peptide chelated calcium can be absorbed by human body without decomposition, which improves the bioavailability. The collagen peptide chelated calcium contains collagen peptide and calcium, which is a kind of comprehensive supplement.

CONCLUSION

Calcium plays an important role in maintaining the physiological function of body, and can effectively prevent and treat osteoporosis, osteopenia, osteomalacia and rickets. Although a large amount of calcium can be obtained from the diet, the dose often fails to meet the needs of the body; therefore it is necessary to take calcium preparations.

Most calcium preparations are poorly soluble and need to be dissolved by hydrochloric acid after oral administration, so the amount of gastric acid secretion directly affects the efficiency of calcium preparations. The elderly and children with poor adaptability cannot fully absorb calcium, resulting in low bioavailability of calcium preparations. Therefore, the elderly and children should choose good water solubility and good taste of calcium salts such as calcium lactate, calcium gluconate, and vitamin D can be added to promote the absorption of calcium in the small intestine. In addition, the absorption rate of calcium supplements can be increased to 10-30% at the same time with meals or after meals than before meals [32]. This is because the increase in the concentration of glucose and glucose polymer after meals can stimulate the absorption of calcium. In addition, taking calcium

can cause transient increase in serum calcium level before, so that the probability of cardiovascular disease and kidney stones increase. The human body needs about 1000 mg of calcium per day, and WHO recommended adults need 800 to 1000 mg calcium per day [1], while children, women, the elderly need 1000 ～ 1500 mg calcium per day [62]. The Ca^{2+} content of calcium preparation is very low. Calcium carbonate supplement contains 40% calcium; calcium citrate contains 21% calcium; calcium lactate contains 13% calcium; calcium gluconate contains only about 9% calcium [10]. The dosage cannot fully meet the needs of the human body, so the calcium intake should be considered with the individual person's diet, age and other factors.

The calcium formulations developed rapidly, have gone through four processes: inorganic calcium, organic acid calcium, amino acid chelated calcium and collagen peptide chelated calcium. At present, inorganic calcium and organic acid calcium have been widely used in clinical practice, especially organic acid calcium, which is widely used in children and the elderly because of its fewer side effects and better effect of calcium supplement. The application time of calcium amino acid chelate in calcium supplement market is short, and the mechanism of action and metabolic pathway *in vivo* need to be further explored. Peptide calcium has become a hot research topic, because of its high absorption efficiency, no side effect and high solubility. However, the chelating rate of peptide calcium is not very high. Furthermore, the content of calcium is relatively low, and the preparation process is complex. Therefore, peptide calcium has a broad space for development.

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