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Combinatory Nutrition Supplementation: A Case of Synergy for Increasing Calcium Bioavailability

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***Abstract*—**This paper presents an overview of how calcium interacts with the various essential nutrients within an environment of cellular and hormonal interactions for the purpose of increasing bioavailability to the human body. One example of such interactions can be illustrated with calcium homeostasis. This paper gives an in-depth discussion on the possible interactive permutations with various nutrients and factors leading to the promotion of calcium bioavailability to the body. The review hopes to provide further insights into how calcium supplement formulations can be improved to better influence its bioavailability in the human body.

***Keywords*—**Bioavailability, environment of cellular and hormonal interactions, combinative nutrition, nutrient synergy.

I. Introduction

CALCIUM is an important micronutrient required for the maintenance of a healthy skeletal system. Its bioavailability is affected by interactions with other components of the human diet. In general, nutrient bioavailability usually includes two main components, i.e. absorption and utilization. During absorption, calcium is taken up from the small intestines into the body. The absorbed nutrients are utilized via transport within the body and cell metabolic activities, and converted to more biologically active forms. The bioavailability level of calcium depends on the absorption capacity of the body, distribution and elimination of that nutrient [1]. Other nutrients can also affect the bioavailability of calcium via different mechanisms such as during absorption, transport within the body and excretion from the human body.

II. Importance of Calcium Balance

Among the body's minerals, calcium is the most abundant, comprising 1.5–2.0% of our body weight. About 1000–1200 g calcium is found in the skeleton, and about 1 g/d is needed to maintain this level. Calcium is important for bone and teeth formation [2].

A balanced serum calcium level is essential. Normal serum calcium level is around 10 mg/100 ml of blood. When insufficient dietary calcium is ingested, the parathyroid glands are activated to release parathyroid hormone (PTH), which will cause calcium to be drawn out of the bones and stimulate intestinal calcium absorption. As most of our body's calcium is in the bones, the blood and cellular concentrations of this mineral are maintained first. Calcium is added to and removed from bones to sustain calcium balance [2].

The requirement for calcium is also related to the size of the calcium reserve. Calcium functions as a threshold nutrient due to the limitations of the calcium reserve [3]. Calcium intake is important to sustain healthy bones, consisting of calcium phosphate and a protein matrix. In the long term, a deficiency in calcium intake eventually depletes bone stores, rendering the bones weak and prone to fracture.

III. Calcium Bioavailability via Absorption

The intestine is responsible for dietary calcium absorption into the body. Between 25 and 35% of the ingested calcium is absorbed by the intestine [4] via the paracellular and transcellular pathways. Calcium absorption paracellularly occurs throughout the entire intestine [5] while transcellular absorption requires calcium transport proteins to be expressed on the intestine cell's apical and basolateral membranes, requiring energy in the form of ATP. Transcellular calcium absorption occurs mainly in the duodenum and the jejunum [6]. Regulation of this calcium absorption pathway occurs via the active metabolite of vitamin D [1,25$(OH)_2$-vitamin D], which stimulates transcellular calcium uptake to sustain calcium serum levels in balance. Calcium intake, however, would still be insufficient because of intermediary lower absorption and excretory loss.

A. Fructooligosaccharides and Calcium Absorption

Carbohydrates which are resistant to degradation by human enzymes include certain short-chain carbohydrates such as fructooligosaccharides [7]. Research has shown that inulin-type fructans can boost intestinal calcium absorption [8]-[10]. Fermentation of these nondigestible carbohydrates produces short chain fatty acids (SCFA) and other acids that lower pH levels in the large intestine, leading to a rise in calcium solubility in the intestinal lumen, increasing calcium bioavailability [11], [12]. SCFA also increase calcium absorption via a cation exchange mechanism [13]. Inulin-type fructans also improve transcellular active calcium transport by altering vitamin D receptor activity and increasing calbindin D_{9K}, one of the important calcium transport proteins in the intestine. Findings indicate that inulin-type fructans can increase calcium bioavailability [2].

Non-digestible oligosaccharides have been shown to increase the absorption of minerals and trace elements. Fructooligosaccharides stimulated mineral absorption and

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bone mineral deposition when combined with probiotic lactobacilli. Inulin also suppresses bone resorption [14]. Inulin-type fructans may improve mineral absorption by improving gut health. Inulin-type fructans could thus improve calcium bioavailability and contribute to bone health [15].

IV. Calcium Bioavailability via Excretion

The kidneys play a key role in calcium homeostasis. Gastrointestinal absorption is balanced by renal excretion. When body stores of calcium decrease, gastrointestinal absorption, bone resorption, and renal tubular reabsorption of the ion increase to counter-balance. Renal regulation of calcium ions, an important determinant of serum calcium levels, is affected by a host of hormonal factors. Physiologically, the overall body balance of calcium is maintained by adjusting urinary excretion to balance the dietary intake [16].

V. Regulators for Calcium Homeostasis

Normal serum calcium level is regulated by the combined effort of vitamin D, PTH and calcitonin. All three hormones can influence serum calcium levels by acting on the intestine, the kidney, or bone. Calcitriol or 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2$-vitamin D), the active vitamin D metabolite, primarily modulates the intestinal calcium absorption. Apart from hormones to regulate calcium metabolism, calcium sensing receptor (CaSR) is also a factor for determining current levels of serum calcium. Calcitropic hormones exert their activities via CaSR [17].

A. Vitamin D Molecular Mechanism of Action on Calcium Transport Protein Genes

Vitamin D is one of the key regulators of calcium homeostasis. Our body has two sources of vitamin D, a dietary source (vitamin D_2 and vitamin D_3) and an endogenous source, dependent on ultraviolet (UV) light catalyzed synthesis in the skin (vitamin D_3). $1,25(OH)_2$-vitamin D exerts its main effects on cells via gene regulation, with the active vitamin D metabolite binding to its intracellular vitamin D receptor (VDR) [18], a nuclear receptor. Upon $1,25(OH)_2$-vitamin D binding to the receptor, heterodimerization with the retinoid X receptor (RXR) [19] occurs. This complex, as a transcription factor, then interacts with a vitamin D response element (VDRE) in the 5' promoter region of the regulated gene, affecting transcription [17].

B. Effect of Vitamin D on Calcium Absorption

The intestine is one of the main sites of $1,25(OH)_2$-vitamin D. $1,25(OH)_2$-vitamin D upregulates the expression of intestinal calcium transport proteins which mediate transcellular calcium absorption. The $1,25(OH)_2$-vitamin D may modulate calcium uptake through the paracellular route. Hence, $1,25(OH)_2$-vitamin D directly elevates serum calcium levels. The importance of $1,25(OH)_2$-vitamin D for intestinal calcium absorption is seen in $1,25(OH)_2$-vitamin D-deficient patients [21]. $1,25(OH)_2$-vitamin D and VDR are important in regulating intestinal calcium absorption [17].

$1,25(OH)_2$-vitamin D has direct influences on bone formation, as evidenced from the presence of VDR in certain bone cells [21]. The bone-forming osteoblasts initiate the majority of the direct effects of $1,25(OH)_2$-vitamin D such as the differentiation of mesenchymal stem cells into new osteoblasts and regulation of the formation of certain proteins, such as osteocalcin, alkaline phosphatase, collagen I and receptor activator of nuclear factor kappa B ligand (RANKL) within osteoblasts. Effects of $1,25(OH)_2$-vitamin D on bone mineralization have also been reported. For example, an increase in the mineralization of extracellular matrix was observed following concomitant $1,25(OH)_2$-vitamin D and vitamin K exposure [22].

The kidney is the major site of $1,25(OH)_2$-vitamin D synthesis and vitamin D target site. The kidney acts as a key regulator of calcium homeostasis by determining the amount of reabsorbed calcium, with the majority of filtered calcium reabsorbed in the proximal tubule through the paracellular space. Further regulation of calcium absorption occurs in the distal tubule where the epithelial cells express calcium transport proteins most of which are upregulated by $1,25(OH)_2$-vitamin D [23], [24].

C. PTH and Its Effects on Calcium Balance in the Body

The parathyroid gland is responsible for synthesizing PTH, which is essential for calcium homeostasis. Serum PTH concentration decreases as the serum calcium concentration increases. The biological actions of PTH include: (a) stimulation of bone resorption by osteoclasts and release of calcium and phosphate from bone, (b) activating calcium reabsorption in the kidney, and (c) promoting renal production of $1,25(OH)_2$-vitamin D to raise intestinal absorption of calcium [25]. PTH exerts its activities via activation of PTH receptor type 1 (PTH1R), a G protein–coupled receptor (GPCR) found in kidney and bone cells. Binding of PTH to PTH1R causes activation of at least two G proteins, Gαq/11 and Gαs. Gαq/11 initiates calcium release within cells via stimulation of phospholipase C (PLC) and raises inositol trisphosphate (IP_3), while Gαs activates adenylyl cyclase leading to increase in cAMP. The former pathway activates protein kinase C (PKC). The later pathway will activate protein kinase A (PKA). PKA is required for transcriptional upregulation of CYP27B1, which is the mitochondrial enzyme responsible for the synthesis of $1,25(OH)_2$-vitamin D from 25(OH)-vitamin D in the kidney [17]. Both PKA and PKC cause a rise in RANKL and macrophage colony-stimulating factor (CSF), as well as a fall in the decoy receptor osteoprotegerin. Together these factors stimulate the recruitment of osteoclast precursors to form osteoclasts in a controlled manner [26].

In the kidney, PTH increases calcium absorption, and induces the synthesis of $1,25(OH)_2$-vitamin D [27]. This in turn increases intestinal and renal calcium uptake to counteract low serum calcium levels. The PTH-stimulated increase in $1,25(OH)_2$-vitamin D levels is achieved on a transcriptional level via upregulating the transcription of CYP27B1, the mitochondrial enzyme responsible for the conversion from

25(OH)-vitamin D to 1,25$(OH)_2$-vitamin D. Transcriptional upregulation occurs via PTH binding to PTH1R, leading to increases in cAMP and activation of PKA. PTH can directly upregulate the renal calcium reabsorption in distal segments of the nephron which express calcium transport proteins that mediate the process of active transcellular calcium absorption in the kidney. PTH can regulate all of these protein levels on a transcriptional level independent of vitamin D [28].

PTH causes bone resorption mainly via osteoclast activation. This effect is carried out through osteoblast signaling. The PTH-induced communication between osteoblasts and osteoclasts is mainly mediated by receptor activator of nuclear factor kappa B (RANK), osteoprotegrin (OPG), and RANK ligand (RANKL). Both RANKL and OPG are expressed by osteoblasts and exert differing actions on osteoclasts. RANKL promotes bone resorption by binding to RANK on osteoclasts while OPG inhibits RANKL interaction with RANK by directly binding to RANKL, thereby suppressing osteoclast activation. Exposure to PTH affects RANK-RANKL signaling by downregulating antiresorptive OPG, while simultaneously activating production of RANKL by osteoblasts [29], [30]. The enhanced RANK-RANKL signaling induces osteoclast formation, which leads to increased bone resorption and serum calcium levels [17].

D.Indirect Effects of Magnesium on Calcium via PTH

Magnesium (Mg) being a divalent cation like calcium is able to bind to CaSR in the parathyroid gland, which will cause a downstream cascade to inhibit PTH secretion [31]. Mg also regulates parathyroid glands function through upregulation of the key cellular receptors CaR, VDR and fibroblast growth factor 23 (FGF23)/Klotho system [32].

E. Calcitonin and Its Effects on Calcium Homeostasis

The parafollicular cells of the thyroid gland are responsible for synthesizing and secreting the amino acid peptide calcitonin. The serum calcium level is the most important regulator of calcitonin secretion; increases in ionized calcium produces an increase in calcitonin secretion [25]. The main biological effects of this hormone are expressed via the hormone binding to its receptor which is a seven-transmembrane domain GPCR expressed in two main target tissues; osteoclasts and the kidney. An increase of intracellular cAMP levels via Gs-dependent activation of adenylate cyclase is caused by the activation of the calcitonin receptor.

Calcitonin reduces serum calcium levels mainly by directly inhibiting osteoclast activity, suppressing bone resorption via receptor activation, on the surface of osteoclasts [33]. This inhibits the formation of luminally acidified resorptive pits in the osteoclasts that assimilate bone via these pits [34]. Activity and mobility of osteoclasts is significantly lowered, resulting in osteoclasts entering a state of dormancy [35]. Renal calcium handling is also influenced by calcitonin. In humans, calcitonin increases calcium excretion via urine and thereby jointly acts with its inhibitory action on osteoclasts to lower serum calcium levels. Calcitonin indirectly affects calcium homeostasis via decreasing the expression of CYP27B1, the renal enzyme responsible for the synthesis of 1,25$(OH)_2$-vitamin D [36].

F. Negative Feedback of Extracellular Calcium on Parathyroid via CaSR

The CaSR is a GPCR primarily expressed in the parathyroid gland and renal tubules of the kidney. This receptor senses extracellular calcium levels and it is important for calcium homeostasis in the body [37].

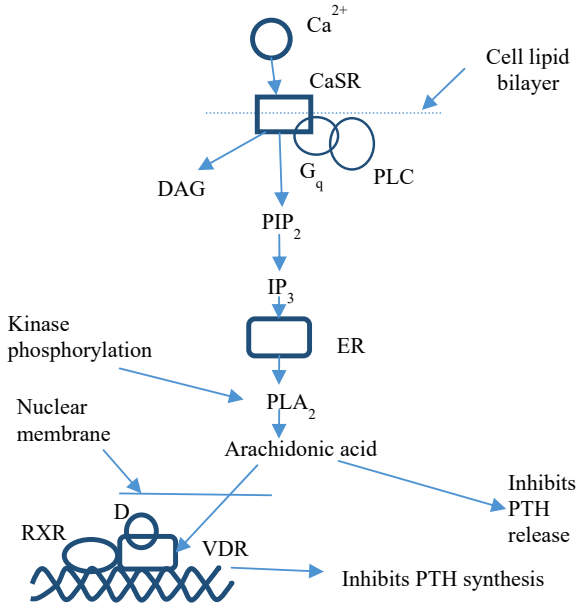

Fig. 1 Signaling pathway of CaSR in the parathyroid gland

The CaSR is expressed on parathyroid cell surface, enabling the parathyroid gland to respond to changes in extracellular Ca. The CaSR is linked to several intracellular signalling pathways, and its activation leads to an increase in intracellular calcium [38].

The CaSR on the cell membrane of the chief cells of the parathyroid glands detects extracellular calcium and alters PTH release. There are several pathways within the cell in which the CaSR couples to such as PLC and protein kinase B [39].

CaSR regulates parathyroid function by affecting the processes of PTH release from secretory vesicles, formation of PTH and the growth pattern of the parathyroid gland

An increase in plasma calcium level causes a drop in PTH release via CaSR activation, resulting in negating the increase in serum calcium level, as illustrated in Fig. 1. This response is mediated by arachidonic acid metabolites generated via Gαq and Phospholipase A2 (PLA_2) activation [40]. CaSR also inhibits PTH synthesis. The main regulator of PTH gene transcription is 1,25$(OH)_2$-vitamin D, represented by D in Fig. 1. Binding of 1,25$(OH)_2$-vitamin D to the VDR causes a decrease in pre-pro-PTH mRNA levels creating a negative-feedback loop. Serum calcium upon binding to CaSR can synergise the inhibitory actions of 1,25$(OH)_2$-vitamin D on PTH gene transcription by upregulating VDR expression [41].

This occurs via increasing arachidonic acid metabolites and activating the MAPK pathway, which results in increased VDR mRNA levels.

VI. Indirect Effect of Magnesium on Calcium via Vitamin D

There is an important functional link between magnesium and calciotropic hormones. PTH stimulates magnesium reabsorption in the kidney, absorption and release of magnesium in the gut and from bone, respectively. Magnesium is essential for the maintenance of normal function of parathyroid glands, bioconversion of vitamin D to its active form and maintaining target tissues' normal response to PTH and active metabolites of vitamin D. Magnesium deficiency is usually associated with hypoparathyroidism, low production of active vitamin D metabolites, in particular $1,25(OH)_2$-vitamin D, and resistance to PTH and vitamin D. Excess magnesium inhibits PTH secretion. Bone metabolism is impaired under positive as well as under negative magnesium balance. Attention to the early diagnosis of magnesium imbalance is important as magnesium plays in the regulation of calcium homeostasis [42].

VII. Ratio of Calcium and Magnesium

A person's calcium and magnesium ratio and mineral status can be influenced by imbalanced diets and supplementations, organ damage, infections, drug use as well as internal factors such as hormonal balance, so there is optimal calcium to magnesium ratio. The calcium and magnesium ratio can also be affected by other minerals such as zinc, potassium and copper. Different ratios of these other minerals can also influence calcium to magnesium ratio.

If zinc intake is high, and potassium intake is low, then taking extra magnesium will usually lower calcium requirements, since magnesium supports zinc, but lowers potassium, which is a calcium synergist. Likewise, if potassium intake is high, and zinc intake is low, then taking magnesium will lead to higher calcium absorption as well. Vitamin D increases calcium, phosphorus, and hence-magnesium absorption. If magnesium intake is higher than that of calcium, then the risk of calcium deficiency is more likely to be elevated [44].

An intake of higher potassium and higher copper levels synergistically lead to a rise in calcium level within cells. Low intake levels of manganese, phosphorus, zinc, nickel and vitamin C will lead to an increase in intracellular calcium ratio-wise, and will raise the risk for soft tissue calcification. When increasing the intake of manganese, phosphorus, zinc, vitamin C, and bringing stomach acid to normal levels, calcium uptake is optimized and soft tissue calcification risks are significantly reduced. However, increasing these same co-factors to above-normal levels will increase the risk for calcium loss [44].

Vitamin D is converted into its active form with the aid of magnesium to boost calcium absorption. Calcitonin is also stimulated by magnesium, aiding in preservation of the skeletal structure, with the withdrawal of calcium from the blood and soft tissues into the bones, thereby reducing osteoporosis risk and other health ailments. Calcification and related health ailments such as osteoporosis are brought about by high calcium - low magnesium ratio, in increasing scientific proof [43].

VIII. Calcium: Phosphorus Ratio in Bone

Most discussions on bone health mainly focus on dietary calcium. However, the importance of phosphorus in bone metabolism should not be ignored because the main constituent in bone is hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ which comprises of both minerals, with a calcium-to-phosphorus ratio of 1.67 (mol:mol), corresponding to 2.15 (wt:wt) [15].

The dietary amount of calcium and phosphorous and the relative ratio of calcium to phosphorous affect calcium metabolism and hence bone health [22]. A ratio of <1 was unfavourable for bone compared with a ratio of 2. However, when the Ca:P ratio of 2 was maintained, a diet with 5 g phosphorus/kg diet had a more favorable effect on weight gain, plasma PTH, and bone mineral content than a formulation with 2.5 g phosphorus/kg. These results indicate that an increase of dietary calcium as an osteoporosis prevention strategy should include sufficient intake of both calcium and phosphorus. This point should be given more emphasis in light of a growing demand in the market for calcium supplements [45].

IX. Discussion

Calcium on its own will be poorly absorbed and may actually cause harm. Many who take health supplements high in calcium but low in other nutrients may not realize that this does not prevent or reduce osteoporosis. Our gastrointestinal tract can absorb only a fraction of the oral calcium consumed. There are other nutrients or compounds that may increase calcium absorption directly and indirectly. For example, studies have shown that fructooligosaccharides such as inulin, when fermented in the colon, produce acids that lower pH in the large intestine, causing a rise in calcium solubility so that calcium bioavailability is increased [12]. Some of these acids also help increase calcium absorption via a cation exchange mechanism [13]. Inulin and other prebiotics when combined with probiotic strains such as Lactobacillus improve mineral absorption from the gut. Health supplement manufacturers should look to the prospect of incorporating prebiotics and probiotics into their products to boost calcium absorption.

Bone does not comprise of calcium alone. Bone is made up of hydroxyapatite $(Ca_{10}(PO_4)_6(OH)_2)$ which is a compound of calcium and another mineral phosphorus. Maintaining a proper ratio of calcium to phosphorus in the diet is also important for bone health. Adverse effects on bone health occur as a result of improper calcium to phosphorous ratios. [45]. So people should not consume solely high calcium nutritional supplements but also include phosphorous in their diet and in moderation.

A. Factors Affecting Calcium Homeostasis

Vitamin D is required for the absorption of calcium and other minerals in the intestine. Vitamin D upon absorption is eventually converted into its bioactive form calcitriol or $1,25(OH)_2$-vitamin D in the kidney. Calcitriol then binds to its receptor, VDR which heterodimerizes with the RXR [19] which then upregulates certain genes. Hence Vitamin D is required to increase transcription and eventually translation of calcium transport proteins in the intestine and kidney to boost calcium absorption and reduce calcium excretion respectively, maintaining calcium balance in the body. A study in 1988 supports the importance of Vitamin D in calcium absorption in the food [20].

The body has its own regulatory system in the form of hormones such as PTH and calcitonin to keep serum calcium levels in check. Unless there is hormonal imbalance or genetic defect, there will not be an imbalance of calcium in the body due to ingestion of high levels of calcium from the diet.

Based on literature review, magnesium is needed for calcium absorption. Although there is some concern that magnesium will compete with calcium for the same absorption sites in the intestine especially when ingested in high amounts, magnesium can increase calcium absorption. Magnesium lowers parathyroid activity [46] and upregulates Vitamin D activity [42].

Most nutritionists or dietitian experts believe that a calcium to magnesium ratio of 2:1 is optimal for bone health. However, they do not take into account various factors, namely other nutrients and minerals that may affect the bioavailability of calcium and magnesium.

B. Age and Chronically Ill

Nutritional supplements targeted at the elderly and chronically sick individuals especially those with kidney ailments should incorporate higher calcium and vitamin D content. Vitamin D, endogenous or dietary-sourced, is converted into its bioactive form calcitriol or $1,25(OH)_2$-vitamin D by an enzyme called 1α-hydroxylase which is found in the kidney. In people with kidney conditions such as kidney failure, expression of this enzyme is impaired and hence calcium absorption is decreased in these individuals. Furthermore, as people age, expression of this enzyme decreases and hence calcium absorption decreases. Elderly people are less mobile and thus are less exposed to sunlight. UV radiation in sunlight as highlighted [2] is required for the production of vitamin D in the skin. Furthermore, calcium intake is lower in the elderly population.

C. Genetics across Different Races

Most research on calcium homeostasis and factors affecting it focused mainly on Caucasian populations. There is a possibility that calcium homeostasis varied to different levels in other populations or race as compared to the Caucasians. Moreover, some research are carried out on animal models rather than on human models. Animal systems may be similar to human systems anatomically but they may function differently from those of human origin. More research is required to look into the hormonal changes associated with calcium balance in other races and in human-derived cell models.

D. Nutrition Health Supplement for Increased Nutrient Bioavailability

Early nutrient health supplements came in single-nutrient formulations. But with increasing understanding of the interactions between the nutrients in affecting their bioavailability, more nutritionists and health supplement manufacturers are coming up with multi-vitamin and multi-mineral formulations based on different nutrient ratios.

X. Conclusions

Understanding calcium pathways, whether taken as dietary meals or health supplements, must be understood in order to better synergize nutritional formulas. It is shown that nutrients, such as calcium in this case, do not work in isolation. Synergizing the various components well can increase calcium's bioavailability even if lower amounts are ingested. This is especially so when benefiting bone density whilst minimizing negative side effects. Other factors such as hormonal system, genetics, age and diseases is to be considered to promote more holistic synergy. With the above considerations, it is deemed that calcium guides, such as the dietary reference intake (DRI), should be revisited in light of combinative interactions of ingredients and factors over those of a one-size-fits all guideline.

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