Information

Current Concepts of Calcium Absorption

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Although the biological activity and the therapeutic usefulness of vitamin D has been recognized for almost fifty years, it is only within the last decade that the specific metabolic effects of its role in calcium absorption have been elucidated. With advanced understanding of the mechanism of action of vitamin D, and the isolation of the active metabolite of this vitamin, it has been possible to test our hypothesis of the actual mechanics of calcium absorption in the gut. The isolation of a calcium-binding protein from the intestinal mucosal cell, and the demonstration of a calcium-dependent ATPase system active in the immediate transport of calcium have all subsequently followed these initial studies.

Historical

Mellanby, in 1919, was one of the earliest investigators to demonstrate the relationship between rickets and vitamin D insufficiency. Having produced rickets in dogs by maintaining weaned six-weeks-old puppies on a cereal diet in a room devoid of sunlight, he was able to reverse the symptoms of rickets by administration of cod liver oil. In 1922, McCollum named this active substance found in cod liver oil vitamin D, and showed that it was different from the recently isolated fat soluble vitamin, vitamin A. In 1924, Steenbock expanded the understanding of vitamin D metabolism with the discovery that ultra-violet radiation to both food and animals produced anti-rachitic activity. Subsequent to these reports, vitamin D was isolated and was shown to be a sterol. It was chemically synthesized and since then its therapeutic usefulness has become well known.

Vitamin D

The initial steps in understanding calcium absorption were elucidated with the advent of radioactive vitamin D. In 1955, Kodicek, using the first radioactive-labeled vitamin D preparation (a C14-labeled ergocalciferol), studied vitamin D absorption. He found the administered radioactivity was primarily localized to the blood, liver and kidneys. In 1963, H-vitamin D was prepared by Norman and DeLuca. A similar compound was prepared by Thompson and associates and used in humans to measure absorption of vitamin D. These investigators showed that the vitamin was fat-soluble, was taken up by the intestinal lymphatic chain and was primarily absorbed in the ileum. DeLuca corroborated these findings, showing 45 to 100 percent of the radioactivity of the plasma localized to the chylomicron lipoprotein fraction after absorption from the intestine.

In 1967, Avioli and his group further demonstrated the intestinal absorption of the vitamin via the lymphatics. In their studies with thoracic duct cannulation in normal patients, radioactivity appeared in the lymph quite early after oral ingestion of radioactive vitamin D3. They also reported on patients with bowel fistulas and common bile duct obstruction, and showed that vitamin D was not absorbed in the absence of bile salts. Similar presumptive evidence for the obligatory role of bile salts in vitamin D absorption had been shown by DeLuca et al and by Thompson and co-workers.

Active Metabolite

25-Hydroxycholecalciferol

From the preceding studies, we see that the initial step in understanding calcium absorption was elucidated with the advent of radioactive

CALIFORNIA MEDICINE 91
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vitamin D. Vitamin D was shown to be absorbed from the gastrointestinal tract via the lymphatics and from there transported to the liver in the chylomicron fraction of the plasma. DeLuca et al, in 1966, showed that vitamin D was hydroxylated in the liver to an active metabolite, 25-hydroxycholecalciferol (25-HCC). In his studies he showed that after administration of radioactive vitamin D₃, the radioactivity was localized in the chylomicron lipoprotein fraction of the plasma. He separated this radioactivity into five bands, four of which were lipoproteins and the fifth an alpha-2 globulin. Purification of this alpha-2 globulin band recovered essentially all the active metabolic form of the vitamin D. This fraction proved to be one and a half to two times biologically more active in restoring normal calcium balance in vitamin D-deprived animals and was equally effective in mobilizing calcium from the bone of animals that had been kept on low calcium diet. Chemical isolation showed this band to be 25-hydroxycholecalciferol (25-HCC). From previous studies it had been known that there was an 8- to 10-hour delay to stimulate calcium transport in rachitic animals when vitamin D was given alone. With 25-HCC, the onset of action was only three hours.

To reiterate, following absorption from the gut, vitamin D is transported to the liver, where it is hydroxylated to 25-HCC, the active metabolic form. From the liver, 25-HCC is transported bound to an alpha-2 globulin. After 8 to 10 hours, much of the radioactivity of the labeled vitamin D is found within the mucosal cell nuclei of the small intestine. It is presumed that the bound 25-HCC is attached to the nuclear membrane of the mucosal cell. As was previously said, the "lag" which exists following the administration of vitamin D to rachitic animals, for the enhanced calcium absorption, is only partially accounted for by the formation of 25-HCC. This implies that there are additional steps which occur between the interaction of vitamin D and the subsequent enhanced calcium absorption.

Calcium-Binding Protein

Wasserman and Taylor, in 1966, isolated a protein which was produced in the intestinal mucosa of animals which had been stimulated by vitamin D after having been reared on a vitamin D-deprived diet. This protein had specific calcium-binding properties and was present in low levels in the rachitic animals. When vitamin D was given to these animals, the protein was increased several fold. This protein, labeled calcium-binding protein (caBP), was isolated from the kidneys, liver and muscle in addition to the mucosa of the duodenum, jejunum, ileum and colon. Of interest was the finding that vitamin D stimulation of the mucosa of rachitic animals produced elevated levels of caBP only in those areas of the gastrointestinal tract where calcium was absorbed, the duodenum, jejunum and ileum. The conclusions of Wasserman and Taylor were that the calcium-binding protein was produced in the intestinal mucosa following stimulation by vitamin D, and this protein facilitated the movement of calcium across the intestinal mucosa. They isolated the protein and found that it consisted of not one but actually several closely associated proteins capable of binding calcium. To prove the association between the calcium-binding protein and the absorption of calcium, they made the following observations:

- The calcium-binding protein which was present in very low levels increased in the rachitic intestinal mucosa about the same time as the demonstrated increase in calcium absorption occurred following vitamin D administration.
- In vitamin D deprived chicks, the lowered level of caBP and the decrease of calcium absorption were of the same order of magnitude.
- Mucosal caBP concentration varied as the calcium absorption efficiency of the gastrointestinal tract varied—that is greater in the duodenum than in the jejunum and greater in the jejunum than the ileum.
- Younger chicks, those with a higher growth rate and more need for calcium, had more mucosal caBP than older or more mature chickens which had completed growth.
- Laying hens or pregnant rats had more caBP in their intestinal mucosa than non-laying chicks or non-pregnant rats. (This effect was not reduplicated by estrogen replacement.)
- Low calcium diet which stimulated an increase in calcium absorption, normally also produced an increase in caBP in chickens.
• In chickens, where vitamin D₃ has a greater physiological effect than vitamin D₂, there was a higher mucosal content of caBP following administration of vitamin D₃ than vitamin D₂.

Calcium-Dependent ATPase System

The time between vitamin D replacement in rachitic animals and the enhanced calcium absorption still is not completely accounted for by the hydroxylation of vitamin D to form 25-HCC or by the formation of caBP. The final step was proposed by Martin and DeLuca in 1969 when they demonstrated that immediate calcium uptake across the brush border of the intestinal mucosal cell was oxygen-dependent, required active transport, and included a calcium-dependent ATPase system.¹ This ATPase has been shown to be induced by vitamin D and its active metabolite 25-HCC, and is located at the site of calcium absorption—that is, the brush border of the intestinal mucosal cell. It has been shown to be present in low amounts or absent in vitamin D-deprived animals and it increases synchronously with the appearance of vitamin D-induced calcium transport in these animals. Magnesium, which has recently been shown to be important in calcium transport, is required for the ATPase system. DeLuca and his co-workers concluded that both the caBP and the calcium-dependent ATPase systems were the proteins induced by vitamin D replacement and were responsible for calcium transport. They expressed belief that the caBP is playing its major role in transcellular calcium movement and absorption regulation in calcium deficiency states, while the calcium-dependent ATPase system plays its role in the immediate absorption of calcium across the intestinal brush border.

Summation of the Physiology of Absorption

What is known thus far of the physiology of calcium absorption may be summarized briefly by stating that calcium absorption occurs with the oral ingestion of the fat soluble vitamin D which is absorbed across the intestinal mucosa into the lymphatics. It is carried in the chylomicron portion of the plasma to the liver, where it is hydroxylated to the active form (25-HCC) and then transported via an alpha-2 globulin to the nuclear membrane of the intestinal mucosal cell. At this site it may act on messenger RNA-DNA synthesis of two proteins—one of them the calcium-binding protein (caBP) which is thought to be responsible for the transcellular calcium transport and the facultative absorption regulation of calcium, and the other the calcium-dependent ATPase protein which may be responsible for the immediate absorption of calcium across the intestinal brush border. The requirements for calcium absorption may then be listed as: (1) an intact absorptive surface, (2) the presence of vitamin D, (3) the ability to convert vitamin D to its active metabolite 25-HCC, (4) the presence of caBP, and finally (5) the calcium-dependent ATPase system.

Clinical Applications

Clinical malabsorption of calcium may occur because of an interruption of the intestinal absorptive surface, or the inability of the liver to form the active metabolite of vitamin D, or through failure of the absorption of the fat-soluble vitamin D itself. The malabsorbptive states, including adult celiac disease, diffuse inflammatory processes such as regional enteritis, and the infiltrative processes such as lymphoma or Whipple’s disease, are clinical examples of interruption of the intestinal absorptive surface. Primary malabsorption may also be the result of bacterial overgrowth, as in the “blind loop” syndrome, or following surgical removal as in the postgastrrectomy states. Steatorrhea with primary failure of absorption of the fat-soluble vitamin D occurs from a variety of causes, such as those described above or from failure of pancreatic or biliary function.

In a study attempting to discern the relative importance of vitamin D absorption and calcium absorption, Thompson et al (1966) studied 12 patients with adult celiac disease or pancreatic or biliary insufficiency. In one group there were five patients with adult celiac disease, representing malabsorption, and a second group of seven patients with pancreatic or biliary insufficiency representing malnutrition. In each group, vitamin D absorption was studied and all patients were found to malabsorb vitamin D. It was only in the first group, the patients with adult celiac disease, that decreased calcium absorption was demonstrated. These investigators concluded that the primary role of vitamin D deficiency on calcium malabsorption was to affect intestinal mu-
coal protein synthesis—that is calcium-binding protein synthesis. This effect was more pronounced in patients with intestinal mucosal abnormalities than in those with pancreatic or biliary malabsorption. A similar study was done by Sjöberg and Nilsson (1970). They studied 11 patients with regional enteritis and 12 patients with pancreatic insufficiency and found that calcium absorption was significantly less in patients with reduced intestinal absorptive surface (regional enteritis) than in the malabsorption produced by pancreatic insufficiency.

The studies demonstrating significant calcium malabsorption with adult celiac disease or regional enteritis as compared with pancreatic insufficiency should not imply that vitamin D deficiency is not a significant cause of calcium malabsorption. It is well known that hypocalcemia may be induced by primary vitamin D deficiency. In clinical practice today in most parts of the world, vitamin D deficiency is generally secondary to malabsorption associated with tropical or non-tropical sprue. Malabsorption of calcium may also be a feature of "vitamin D-resistant" rickets, osteomalacia, parathyroid insufficiency, hyperadrenal corticism, or hyperthyroidism. Pharmacological malabsorption of calcium may be produced chemically, by ingestion of calcium chelating agents such as sodium phytate, sodium phosphate or EDTA (ethylenediaminetetra acetic acid). There are probably other dietary or chemical factors thus far not described which may influence the absorption of dietary calcium.

Recently, Avioli et al (1969) in studying patients with chronic renal disease found that most of the patients normally absorbed vitamin D, but there was a significant decrease in the biologically active form of vitamin D-25-hydroxycholecalciferol. They thought this decrease in 25-HCC to be responsible for the defective intestinal absorption of calcium. Interestingly, this malabsorption of calcium is not reversed by hemodialysis, but is corrected by renal allograft or homotransplant. When they studied the concentration of calcium-binding protein activity in the duodenal mucosa in uremic rats, they found that the activity of the protein was significantly decreased. Treating the animals with the active metabolite (25-hydroxycholecalciferol) brought about an increase in intestinal transport of calcium and an increase in the calcium-binding protein activity in the intestinal mucosa. The effect of renal insufficiency on gastrointestinal transport of calcium was also studied in vitro by Baerg et al (1970), and in experimental animals by Kessner and Epstein in 1965. Calcium malabsorption in humans with chronic renal failure was demonstrated by Messner et al. These investigators showed that despite hemodialysis and parenteral vitamin D, calcium malabsorption persisted and could only be corrected by renal homotransplant or extremely high doses of vitamin D.

Kehayoglou et al (1968) studied the intestinal absorption of calcium in cirrhotic rats. They found that in rats with chronic cirrhosis or in animals that had chronic ligation of the common bile duct, there was a significant decrease in calcium absorption. During the same period, Avioli and his group, in studying four patients with cirrhosis, found a slow disappearance of vitamin D from the plasma and a decreased rate of intestinal absorption of calcium. Metabolic studies utilizing 3H-D3 showed not only slow disappearance of this vitamin from plasma but also a decrease in the quantity of vitamin D metabolites recovered from the urine. The speculation was that the biological transformation of vitamin D3 to its active metabolite (25-HCC) in the liver was impaired and the lack of this substance contributed to the calcium malabsorption. It remains only for a study to show that giving 25-hydroxycholecalciferol to cirrhotic patients results in a correction of calcium malabsorption.

The clinical disturbances of calcium absorption that are frequently seen may be summarized as follows: Malabsorption of calcium is seen in (1) vitamin D deficiency states as well as in conditions of (2) malabsorption of the vitamin. Calcium is also malabsorbed where there is (3) significant loss of intestinal mucosa as in adult celiac disease or regional enteritis. The metabolic states of (4) parathyroid insufficiency, (5) hyperadrenal corticism and (6) hyperthyroidism may be associated with calcium malabsorption. Calcium malabsorption may result from (7) the ingestion of pharmacological agents which bind calcium and remove it from dietary absorption. Malabsorption has been demonstrated in (8) in patients with chronic renal disease and (9) in patients with liver cirrhosis.

Understanding the basic physiology and biochemical requirements for the intestinal absorption of calcium will enable the clinician to more
fully utilize his clinical laboratory for demonstration of calcium malabsorption and his clinical applications will permit treatment of the disorder where possible.

REFERENCES