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6th INTERNATIONAL SYMPOSIUM ON MATERNAL & INFANT NUTRITION

論文集

SYMPOSIUM PROCEEDINGS

Heinz

亨氏營養科學研究所．廣州．中國

HEINZ INSTITUTE OF NUTRITIONAL SCIENCES, GUANGZHOU CHINA

Heinz Institute of Nutritional Sciences

6th International Symposium
On
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PROCEEDING

Date	: October 28, 29, 30, 1991
Location	: Administration Buiding, 3rd Floor Harbin Medical University Harbin, Heilongjiang, China.
Theme	: Vitamin D – Deficiency Rickets and Calcium Deficiency.
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CURRENT KNOWLEDGE OF CALCIUM METABOLISM AND FUNCTIONS

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(RESUME)

Dr. Tammy Bray received her undergraduate degree in Nutrition and Food Science at the Fu-Ren University, Taipei, Taiwan. She completed her postgraduate degrees from the Washington State University in Pullman, Washington in the United States. In 1978 she joined the Department of Nutritional Sciences at the University of Guelph in Canada and in 1989 she was promoted to Full Professor.

Dr. Bray is a member of numerous professional associations. Currently she is the President of the Canadian Society for Nutritional Sciences and is Associate Editor of the Canadian Journal of Physiology and Pharmacology.

(ABSTRACT)

Calcium is the most abundant element in the human and animal body. Calcium is involved in many physiological and biochemical processes through out the body. There are two major types of biological functions of calcium: (1) structural functions in the formation of bone, and (2) regulatory functions in the metabolic processes of cellular events. The crucial role of calcium in the regulation of cell functions is mediated by the oscillation of calcium gradients across the membrane and the second messenger's pathway. It takes two polypeptide hormones, parathyroid hormone and calcitionin, and a sterol hormone, ($1,25(OH)_2D_3$) and a specific intestinal vitamin D-dependent calcium binding protein to maintain homeostasis of plasma calcium. It takes numerous types of intracellular calcium binding proteins to regulate intracellular calcium levels and carry out various cellular events. Understanding the metabolism and cellular functions of calcium will provide the scientific basis for dietary intervention and manipulation of calcium for health promotion and disease prevention. Abnormality in calcium metabolism could have serious health consequences. Calcium nutrition is a very controversial topic, and is still an unsolved nutritional issue, especially its role in osteoporosis and the dietary requirement of calcium.

INTRODUCTION

Calcium is the fifth most abundant element in the human and animal body. Calcium, together with phosphorus, plays a central role in biological system. It is widely involved in many physiological and biochemical processes through out the body. There are two major types of biological functions of calcium: (1) structural functions involving

the formation of skeleton and soft tissues, and (2) regulatory functions involving the processess of cellular secretion, signal transduction, neuromuscular transmission of chemical and electrical stimuli, blood clotting and oxygen transport. The crucial role of calcium in the regulation of cell functions has undergone an explosive growth for the last 10 years due to the discovery of the widely distributed intracellular calcium binding proteins that mediate most of the effects of calcium. Abnormality in calcium metabolism could have serious health consequences. Calcium nutrition is a very controversial topic, which has attracted a great deal of concerns, and is still an unsolved nutritional issue, especially its role in osteoporosis and the dietary requirement of calcium. The purpose of this paper is to review our current understanding of the metabolism and functions of calcium at biochemical and cellular level.

PHYSIOLOGY AND BIOCHEMISTRY OF CALCIUM

Calcium makes up 1.5–2% of total body weight. More than 99% of body calcium is present in the skeleton[1]and the rest 1% is in the extracellular and intracellular fluid. In addition to the dietary calcium, 200–500 mg is added to the intestinal contents by intestinal secretions. This intestinal secretions brings the total calcium present in the lumen of the intestine up to 1,200–1,500 mg, 45% (about 700 mg) is absorbed or reabsorbed into the bloodstream, leaving the remaining to be excreted in the faeces[2]. Swear contributes to a small amount loss of calcium from the body. Some physiological conditions, such as pregnancy (300 mg / d) and lactation (20 mg / d), also causes a loss of calcium from the body[2]. The calcium retention varies with age and physiological conditions. during the first 10 years of life, calcium is retained about 100 mg / d, during puberty the daily net retention increased to 200 mg / d for females and 280 mg / d for males. This amount is decreased to only 30 mg / d during adult stage[4]. The glomerulus of the kidney filtes about 10,000 mg calcium / day, but the renal tubular reabsorption is so efficient that under normal circumstances only between 100–200 mg of calcium appears in the urine[2].

Plasma calcium (10 mg / dl) is the most important fraction in the extracellular calcium pool and is kept within a relatively narrow limits (7–12 mg / dl). They are present in three major forms: ionized (47%), protein (47%) and complexed (6%). The ionized form calcium is the only biologically active form. The protein–bound fraction is biologically inert. However, it provides a readily available reservoir as a first–line defence against hypocalcemia. The fraction of calcium complexed with organic or inorganic acids is diffusible. They has little quantitative importance in against hypocalcemia, but in states of hyperphosphatemia such as may exist in chronic renal failure, excessive complexing of calcium with phosphate may contribute to the decrease in plasma ionized calcium observed in this condition[1]. The calcium in th extracellular pool is in constant exchange with the calcium already present in the extracellular and intracellular fluids of the body and in certain compartments of the bone and glomerular filtered. The entire extracellular calcium pool turns over between 40–50 times per day. The intracelluar calcium fundamentally carries signals to a large number of biological activities in the various subcellular compartments[5]although quantitatively it is a very small pool, some 100 to 100,000 times lower than those in extracellular fluids[6]. The relationship between extracelluar calcium and the signalling calcium inside cells is not well understood, but it is evident that the extracellular pool provides a relatively large reservoir from which cal-

cium is drawn and made a flow into the cell[5].

EXTRACELLULAR FLUID CALCIUM: HOMEOSTASIS

The plasma calcium level is highly regulated within a narrow range (7 to 12 mg / dl). The regulation of plasma calcium is highly integrated and complex involving two polypeptide hormones: parathyroid hormone (PTH) and calcitonin (CT), and a sterol hormone: 1,25–dihydroxycholecalciferol $(1,25(OH)_2D_3)$. Biosynthesls and secretion of the polypeptide hormones are regulated by a negative feedback mechanism that involves the activity of ionic calcium in the extracellular fluid. The blosynthesis of $1,25(OH)_2D_3$ from the major circulating metabolite of vitamin D, 25–hydroxycholecalciferol $(25(OH)D_3)$, takes place in the kidney and is regulated by PTH and CT as well as by extracellular fluid concentration of calcium and phosphate. Under physiologic conditions there are small fluctuations in plasma calcium. Decreases in plasma calcium increase PTH secretion and decrease CT secretion. These changes in hormone secretion lead to increased bone resorption, decreased renal excretion of calcium, and increased intestinal calcium absorption via PTH stimulation of $1,25(OH)_2D_3$ production. As a consequence of these events, plasma calcium increases slightly above its physiologic concentrations, inhibiting PTH secretion and stimulating CT secretion. These changes in plasma hormone concentrations decreased bone resorption, increase renal excretion of calcium, and decrease intestinal absorption of calcium to decrease slightly below the physiologic concentration. This sequence of events occurs within milliseconds and is constantly repeated so that plasma calcium is maintained at physiologic concentrations with minimal oscillation[1].

The amount of dietary calcium required to maintain metabolic balance vary with the physiological needs, the ability of the intestine to absorb them and the ability of the kidneys to conserve them. Dietary calcium induces adaptive changes in the production and secretion of the calciotropic hormones that minimize the development of negative balance of calcium. Our understanding of the molecular details of vitamin D–induced calcium absorption by the intestine has been greatly clarified by the discovery of calcium–binding protein (CaBP) present in the intestine and induced by $1,25(OH)_2D_3$. However, the exact functional role of the intestinal CaPB has not been established. The CaBP may act as a cytosolic buffer to modulate changes in intracellular calcium, to it may facilitate intracellular calcium diffusion. Recently, Nemere et al.[7] have provided experimental evidences to suggest that a vitamin D–dependent CaBP (28 kDa) in the chick intestine may act like a classical receptor protein. More recently, Leathers et al,[8] have shown that this vitamin D–dependent CaBP has a capacity to bind five or six Ca^{++}ions with high affinity. After the binding, these CaBP, which are on the surface of the cell, are internalized as endocytic vesicles. These endocytolic vesicles then presumably fuse with lysosmes. Calcium is released by the acidic lysosomal interior and the free calcium appears to exit the cell via the basal–lateral membrane while the CaBP could return to the cell surface[7]. In mammalian intestine, the vitamin D–dependent CaBP is 9 kDa with a similar function of 28 Kda CaBP in the avian intestine.

Vitamin D control the synthesis of the CaBP at the level of DNA. Vitamin D binds to the specific receptor in the nucleus forming a receptor–1, $25(OH)_2D_3$ complex. This complex, then, activates the transcription qf the specific genes resulting in increased CaBP synthesis. These three events are closely correlated and takes place within two hours[9]. In addition to $1,25(OH)_2D_3$, evidence suggests that serum calcium level, together

with serum phosphate level, may independently influence synthesis of intestinal CaBP–28k in the chicks. The pronounced effects of serum calcium phosphate concentrations on intestinal CaBP–28k levels must be exerted by affecting translation or stability of the CaBP–28k–mRNA[10].

When plasma calcium concentration below 7 mg / dl reflect clinically significant hypocalcemia, and the values beyond 12 mg / dl reflect hypercalcemia. Hypocalcemia produces a mgriad of symptoms and when severe can result in tetany and possibly convulsions[11]. Hypercalcemia can produce functional changes in most organ systems, and these changes may lead to a confusing variety of symptome and objective finding[12].

INTRACELLULAR CALCIUM: REGULATOR

In human cells, the total cell calcium can vary from as little as 0.02 mmol / kg cell water in erythrocytes with no organelles, to more than 5 or 10 mmol / kg cell water in cells such as muscle or platelets with large stores of intracellular calcium. In the cell, more than 99.9% of calcium is bound, mainly within intracellular organelles. The cytoplasm of a resting cell contains about 10 UM calcium of which only about 0.1 UM is free calcium. The high extracellular calcium concentration and the low intracellular calcium concentration create an enormous electrochemical gradient of calcium across the cell membrane, greater than any of the other major ions in the cell. This enormous electrochemical gradient of calcium determine that calcium is so ideally suited to acting as a "switch" for cell activation. A small increase in the permeability of the cell membrane, or a small fractional release of calcium from an internal store, will lead to a large fractional rise in cytoplasmic calcium, to a level some several fold greater than that of the non–activated cell.

It is now well established that cell increase their cytosolic levels of calcium in response to many stimuli and that increase is the primary event that triggers the cellular response. No case is known in which the response to a stimulus leads to a decrease in the cytosolic concentration of calcium[17]. Cytosolic Calcium therefore acts as a "second messenger" that delivers to the effector system the signal of the stimulus. In eukaryotic cells, the role of calcium as a second messenger is as important as the more widely known messenger role of cAMP. This nucleotide and calcium may mediated, and responses to different stimull, but frequently the response to both are coordinated, and in some cases, cAMP and calcium affect in different ways the same effector system. The main experimental support for the role of calcium as a second messenger comes from the studies that; 1) demonstrate a correlation between the changes in the cytosolic calcium and the time course of a stimulus; 2) show that a cellular response can by reproduced in the absence of its physiological stimulus by artificially raising the cytosolic concentration of calcium, and 3) show a direct dependence on calcium of an enzyme or other intracellular system.

Depending on the cell and on the stimulus, cytosolic calcium concentration may increase, either as consequence of the inflow of extracellular calcium or because calcium is released from intracellular stores[13]. When cytosolic calcium concentration rises, specific sites in calcium–binding protein become occupied. The complex between calcium and the calcium–binding proteins may trigger the response either directly or through the activation of a protein kinase which catalyses the phosphorylation of the system in charge of the response. Protein kinase C also participates. Calpains are calcium–dependent proteases. Their role in calcium–mediated cell responses is not yet established.

Recently, evidence suggests that Ca^{++}ions, in addition to their role in the stimulation of energy–requiring cytoplasmic processes, may also stimulate intramitochondrial oxidative metabolism and thus actively promote the synthesis of the necessary ATP[14]. There are three exclusively intramitochondrial dehydrogenases in higher animals whose activity can be enhanced several–fold by increasing calcium concentrationin in a range of 0.1–10 UM. They are pyruvate dehydrogenase (PDH), NAD^{+}–isocitrate dehydrogenase (NAD–ICDH) and 2–oxoglutarate dehydrogenase (OGDH). All of these three dehydrogenases are generally important in the regulation of oxidative metabolism.

CONCLUSION

Calcium is the most abundant mineral in the body. More than 99% of body calicum functions as a structural component and is stored in bone while 1% of body calcium functions as regulator in metabolic processes and is located in soft tissue and the extracellular fluids. It takes two polypeptide hormones, PTH and CT, and a sterol hormone, $(1,25(OH)_2D_3)$ and a specific intestinal vitamin D–dependent CaBP to maintain homeostasis of plasme calcium. It takes numerous types of intracellular calcium binding proteins to regulate intracellular calcium levels and carry out various cellular events. Understanding the metabolism and cellular functions of calcium will provide the scientific basis for dietary intervention and manipulation of calcion for health promotion and disease prevention.

REFERENCES

1. C.D. Arnaud and S.D. Sanchez. 1990. Present Knowledge in Nutrition. p. 212.
2. A.W. Norman. 1990. Am. J. Clic. Nutr. 51. p. 290.
3. M.C. Linder. 1985. Nutritional Biochemistry and Metabolism–with clinical applications. Elsevier. p. 144.
4. S.M. Garn. 1970. The earlier and the later loss of cortical bone.
5. E. Carafoli. 1987. Ann. Rev. Biochem. 56:395.
6. W.G. Robertson. 1988. In: Calcium in Human Biology. Ed. B.E.C. Nordin. Springer–Verlag. p.1
7. I. Nemere, V. Leathers and A.W. Norman. 1986. J. Bio. Chem. 261: 16106.
8. V.L. Leathers, S. Linse, S. Forsen and A.W. Norman. 1990. J. Bio. Chem. 265: 9838.
9. G. Theofan, A.P. Ngnyen and A.W. Norman. 1986. J. Bio. Chem. 261:16943.
10. G. Theofan, M.W. King, A.K. Hall and A.W. Norman. 1987. Mol. Cell Endocrinol 54:135.
11. A.M. Parfitt. 1989. In. Endocrinology. vol.2, 2nd. Ed. L.J. DeGroot, G.M. Besser, G.F. Cahaill. W.B. Saunders, Philadelphis. p. 1049.
12. J.F. Habener and L.T. Potts, Jr. 1989. In: Endocrinology. vol.2, 2nd. Ed. L.J. DeGroot, G.M Besser, G.F. Cahaill. W.B. Saunders, Philadelphia. P. 954.
13. R.H. Kretsinger. 1981. neurosci. Res. Program Bull. 19:217
14. J.G. McCormack and R.M. Dentun. 1986. Trends in Biocheml. Scl. (11). p.258.

CURRENT KNOWLEDGE OF VITAMIN D METABOLISM, FUNCTION AND TOXICITY

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RESUME

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ABSTRACT

Vitamin D_3 is also known as calciferol. It is a lipid–soluble sterol produced in the skin by the action of sunlight (ultraviolet light) on 7–dehydrocholesterol. Vitamin D may also be ingested. Vitamin D_2 is the activated plant sterol ergocalciferol. It is produced by ultraviolet irradiation of ergosterol in plants. Vitamin D_2 and D_3 are equally antirachitic. The vitamin and its two physiologically important metabolites are carried on a specific binding protein (D binding protein). The fat–soluble vitamin is stored largely in liver and fat and is hydroxylated in the liver at the 25–position to 25–OH–D_3 (calcidiol). Calcidiol has weak physiologic activity but is important chiefly as a precursor of the active vitamin D hormone 1,25–$(OH)_2D_3$ (calcitriol). Vitamin D_2 and D_3 are rapidly cleared from the blood stream, so blood levels do not reflect nutritional status. The circulatin concentration of 25–OH–D_3 reflects an individual's vitamin D nutritional status, so that compound, rather than the native vitamin (D_3) is assayed when vitamin D nutrition needs to be assessed. The normal range is 10 to 60 ug / ml.

Vitamin D brings about mineralization of bone primarily by the elevation of plasma calcium and phosphorus to levels that are supersturating and that are required not only for the mineralization of skeleton but also for the prevention of hypocalcemic teany, the most serious disorder of vitamin D deficiency. Low blood calcium results in the convulsive state called hypoclacemic teany. The body's sensitive control of calcium metabolism guards against this life–threatening convulsive state. In the absence of vitamin D, the body cannot mobilize calcium thus the infant or child is at high risk of developing hypocalcemic tetany.

The most important role of vitamin D is the maintenance of blood–plasma levels of calcium. Vitamin D brings about the elevation of plasma calcium and phosphorus by three basic mechanisms:

1) It is the only substance known that stimulates the enterocytes of the small intestine to transport calcium from the lumen to the plasma compartment. It also brings about transport of phosphorus in the plasma compartment from the small intestine.

2) The body also uses the sketleton as a ready source of calcium since the presence of dietary calcium (and hence intestinal absorption) is not absolutely reliable. To mobilize calcium from the sketleton, two hormones working together and involved–the vitamin D hormone and the parathyroid hormone. The mechanism whereby they moblilize calcium from the skeleton has not been identified. Nevertheless, it is a very rapid process and probably does not involve osteoclastic–mediated bone resorption.

3) In the distal renal tubule, calcium is reabsorbed under the influence of paratheyroid hormone and vitamin D. Again, both hormones are involved in this process, unlike the intestinal absorption of calcium which only involves vitamin D.

Plasma calcium and phosphorus are elevated because of these three actions, and thus new bone is mineralized and hypocalcemic tetany is averted.

Vitamin D Toxicity

Although the metabolism of vitamin D to 1,25–$(OH)_2D_3$ is regulated, the fact that severe hypercalcemia can occur in those ingesting very high doses of vitamin D indicates that the control of vitamin D metabolism is not absolute. The deleterious clinical manifestations of vitamin D overdose include soft–tissue calcification in vital organs and diffuse demineralization of bones. The first clue to the possible cause of vitamin D toxicity was the finding that 25–OH–D_3 could stimulate the 1,25–$(OH)_2D_3$ hormone at the intestinal receptor when present in excessive amounts. Moreover, concentrations of 25–OH–D_3 one hundred to 500 times greater than 1,25–$(OH)_2D_3$ were capable of mobilizing bone calcium in vitro. This data along with the knowledge of limited regulaton of the liver lahydroxylase enzyme suggested that 25–$(OH)_3$ rather than 1,25–$(OH)_2D_3$ was responsible for the symptom of vitamin D overdose. The observation that anephric patients who are incapable of synthesizing 1,25–$(OH)_2D_3$ can becone vitamin D intoxicated as well as the observation that vitamin D intoxicated patients with intact kidneys have normal or low circulation levels of 1,25–$(OH)_2D_3$ and markedly elevated levels of 25–OH–D_3 (30 times above normal) proves that 25–OH–D_3 rather than 1,25–$(OH)_2D_3$ is responsible for the symptoms of vitamin D overdose.

Vitamin D_3 is also known as calciferol. It is a lipid–soluble sterol produced in the skin by the action of sunlight (ultraviolet light) on 7–dehydrocholesterol. Vitamin D may also be ingested. Vitamin D_2 is the activated plant sterol ergocalciferol. It is produced by ultraviolet irradiation of ergosterol in plants. Vitamins D_2 and D_3 are equally antirachitic. The vitamin and its two physiologically important metabolites are carried on a specific binding protein (D binding protein). The fat–soluble vitamin is stored largely in liver and fat and is hydroxylated in the liver at the 25–position to 25–OH–D_3 (calcidiol). Calcidiol has weak physiologic activity but is important chiefly as a precursor of the active vitamin D hormone 1,25–$(OH)_2D_3$ (calcitriol). Vitamins D_2 and D_3 are rapidly cleared from the blood stream, so blood levels do not reflect

nutritional status. The circulating concentration of 25–OH–D_3 reflects an individual's vitamin D nutritional status, so this compound, rather than the native vitamin (D_3) is assayed when vitamin D nutrition needs to be assessed. The normal range is 10 to 60 μg / ml.

Rickets is the vitamin D deficiency disease of the young. In the late 1800's and the beginning of this century, this disease appeared in epidemic proportions in Northern Europe, North America and Northern Asia. The discovery in 1919–1924 of vitamin D and its production in skin and foods by UV irradiation led to the elimination of rickets as a major medical problem in North America. The identification and chemical preparation of vitamin D in the next decade provided large quantities of vitamin D to the physician for the treatment of a variety of metabolic bone diseases.

Early in the 1960's, little was known about the role of vitamin D in mineralizing the skeleton and hence in preventing the disease rickets in children and osteomalacia in adults. With the application of modern tools of biochemistry came the discovery that vitamin D must first be modified by 25–hydroxylation in the liver followed by 1a–hydroxylation in the kidney to produce the vitamin D hormone 1a,25–dihydroxyvitamin D_3 [1,25–$(OH)_2D_3$]. This process is strongly feedback–regulated and is one of the major endocrine systems regulating plasma calcium and phosphorus concentrations. Furthermore, it is the major endocrine system regulating bone mass and state.

Bone is primarily made of organic matrix (collagen) and minerals. Rickets and osteomalacia, the vitamin D deficiency diseases, are characterized by a failure of the organic matrix to acquire the hydroxyapatite mineral. The absence of mineral leaves the collagen fibrils in a soft and pliable state that gives rise to the overt symptoms of rickets. Although it was originally believed that some form of vitamin D worked directly on the bone–forming cells (osteoblasts) to play a role in the synthesis of the organic matric of bone, it is now understood that the role of vitamin D is only in the mineralization process. Thus the presence in the plasma of adequate amounts of calcium and phosphorus brings about normal bone growth and mineralization.

Vitamin D brings about mineralization of bone primarily by the elevation of plasma calcium and phosphorus to levels that are supersaturating. Vitamin D is not only required for the mineralization of skeleton but also for the prevention of hypocalcemic tetany, the most serious disorder of vitamin D deficiency. Low blood calcium results in the convulsive state called hypocalcemic tetany. The body's sensitive control of calcium metabolism guards against this life–threatening convulsive state. In the absence of vitamin D, the body cannot mobilize calcium thus the infant or child is at high risk of developing hypocalcemic tetany.

The most important role of vitamin D is the maintenance of blood–plasma levels of calcium. Vitamin D brings about the elevation of plasma calcium and phosphorus by three basic mechanisms:

1) It is the only substance known that stimulates the enterocytes of the small intestine to transport calcium from the lumen to the plasma compartment. It also brings about transport of phosphorus to the plasma compartment from the small intestine.

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from the sdeleton has not been identified. Nevertheless, it is a very rapid process and probably does not involve osteoclastic–mediated bone resorption.
3) In the distal renal tubule, calcium is reabsorbed under the influence of parathyroid hormone and vitamin D. Again, both hormones are involved in this process, unlike the intestinal absorption of calcium which only involves vitamin D.
Plasma calcium and phosphorus are elevated because of these three actions, and thus new bone is mineralized and hypocalcemic tetany is averted.

METABOLISM OF VITAMIN D

Until the mid 1960's it was believed that the action of vitamin D was direct. However, it has now been proved that vitamin D must first be 25–hyroxylated in the liver and subsequently 1a–hydroxylated in the kidney before it can carry out the three classical functions as just described.

Some 35 metabolites of vitamin D have been isolated and identified. The significance of the majority of these metabolites remains to be established. Besides the 25–and 1a–hydroxylation pathway which is considered the activation pathway, 25–OH–D can be either 24–hydroxylated or it can be 26–hydroxylated and further oxidized to the 26,23 lactone. Ultimately, the vitamin D compounds are excreted through the bile and into the feces. A major route of metabolism of $1,25–(OH)_2D_3$ is to the 23–glucuronic bile acid called calcitronic acid.

The kidney is the exclusive site of synthesis of $1,25–(OH)_2D_3$ in non–pregnant mammals under normal circumstances. The placenta produces $1,25–(OH)_2D_3$; thus, pregnant animals that are anephric maintain an ability to synthesize $1,25–(OH)_2D_3$. Only in pathological states, such as with sarcoidosis and some lymphomas, does the ectopic production of $1,25–(OH)_2D_3$ occur, primarily in the macrophages and granulomatous tissue. Furthermore, in chromically anephric humans maintained on dialysis, it seems likely that some $1,25–(OH)_2D_3$ is synthesized in other tissues because there are measurable amounts of $1,25–(OH)_2D_3$ in the blood.

(i) Regulation of vitamin D metabolism.

As expected, the production of $1,25–(OH)_2D_3$ is tightly regulated by the need for calcium and phosphorus of the organism. Low blood calcium stimulates the parathyroid glands to secrete parathyroid hormone, which in turn increases production of $1,25–(OH)_2D_3$ in the proximal convoluted tubule cells of the kidney. The $1,25–(OH)_2D_3$ is then transported to the target organs where it initiates the transport of calcium and phosphorus in the small intestine, the mobilization of calcium from bone, and renal reabsorption of calcium in the distal tubules. The rise in blood calcium suppreses parathyroid secretion, which in turn suppresses production of the vitamin D hormone. Of particular importance is that the parathyroid hormone is required for the mobilization of calcium from bone, and is required for renal conservation of calcium, but is not required for intestinal absorption of calcium after stimulation of production of the vitamin D hormone.

Production of the vitamin D hormone ($1,25–(OH)_2D_3$) is also stimulated by hypophosphatemia and thus the need for phosphorus is translated by the vitamin D endocrine system to improve intestinal absorption of phosphorus and to mobilize phosphorus from bone. The 1a–hydroxylase is very strongly regulated by plasma inorganic phosphorus concentration such that hypophosphatemia also stimulates the 1a–hydroxylase. Phosphate deprivation also inbibits destruction of $1,25–(OH)_2D_3$, thus regulation by phosphorus may be more complex than merely stimulating the 1a–hydroxylase.

Perhaps the most important role of the vitamin D endocrine system, as previously mentioned

is in regulating intestinal absorption of calcium. When calcium is needed, intestinal calcium absorption is markedly elevated. The mechanism of this elevation is as follows: when there is a need for calcium, there is slight hypocalcemia. Parathyroid hormone secretion is stimulated, which in turn stimulates production of 1,25–$(OH)_2D_3$. The elevated 1,25–$(OH)_2D_3$ levels then lead to increased efficiency of intestinal calcium absorption. In this way, dietary calcium is avidly used to supply the needs of the organism. However, if there is no calcium in the diet, despite the stimulation of intestinal absorption mechanisms calcium homeostasis will not be accomplished. With continuing low levels of calcium in the blood, the parathyroid glands continue to secrete high levels of parathyroid hormone. This in combination with the elevated levels of blood 1,25–$(OH)_2D_3$ lead to the mobilization of bone calcium. Renal reabsorption of calcium is also increased raising blood calcium primarily at the expense of bone calcium. In this way, critical hypocalcemia (leading to hypocalcemic tetany) is averted. Thus calcium physiology is constructed such that the skeleton will be sacrificed to prevent the more serious hypocalcemic tetany. If this mechanism persists because of an inadequate intake of calcium or because the intestinal absorption mechanism is not operative, the skeleton will be sacrificed until it becomes low enough in mass to present serious structural problems. Fractures result and give rise to the disease osteoporosis. Therefore the ability of the intestine to adjust its absorption of calcium to meet the needs of the organism is an essential function in protecting skeletal mass.

Vitamin D Toxicity

Although the metabolism of vitamin D to 1,25–$(OH)_2D_3$ is regulated, the fact that severe hypercalcemia can occur in those ingesting pharmacologic (very high) doses of vitamin D indicates that the control of vitamin D metabolism is not absolute. The deleterious clinical manifestations of vitamin D overdose include soft-tissue calcification in vital organs and diffuse demineralization of bones. The first clue to the possible cause of vitamin D toxicity was the finding that 25–OH–D_3 could stimulate the 1,25–$(OH)_2D_3$ hormone at the intestinal receptor when present in excessive amounts. Moreover, concentrations of 25–OH–D_3 100 to 500 times greater than 1,25–$(OH)_2D_3$ were capable of mobilizing bone calcium in vitro. This data along with the knowledge of limited regulation of the liver la-hydroxylase enzyme suggested that 25–OH–D_3 rather than 1,25–$(OH)_2D_3$ was responsible for the symptoms of vitamin D overdose. The observation that anephric patients who are incapable of synthesizing 1,25–$(OH)_2D_3$ can become vitamin D intoxicated as well as the observation that vitamin D intoxicated patients with intact kidneys have normal or low circulating levels of 1,25–$(OH)_2D_3$ and markedly elevated levels of 25–OH–D_3 (30 times above normal) proves that 25–OH–D_3 rather than 1,25–$(OH)_2D_3$ is responsible for the symptoms of vitamin D overdose.

Summary

Vitamin D, one of the four nutritionally essential fat soluble vitamins, can be produced in skin by the photoactivation of 7–dehydrocholestrol or ingested in the diet. During the 70 years since Vitamin D was discovered to be an essential molecule in the prevention of rickets, much effort has been put into finding out its physiologic function and mechanism of action. We now know that vitamin D undergoes metabolic hydroxylations to produce endocrine factor (hormones) which control calcium homeostasis. It is now known that the vitamin D metabolite, 1,25–$(OH)_2D_3$, is the primary hormone involved in the stimulus of intestinal calcium and phosphate absorption, and calcium and phosphate transport in bone and kidney. This hormone is

closely regulated by levels of calcium and phosphours in the blood and parathyroid hormone.

New roles for vitamin D in reproduction, parathyroid function, skin proliferation and differentiation, in myeloid cell differentiation and as anti-cancer agents are all currently under investigation. There is no doubt that our understanding of the mechanisms of action of vitamin D will increase in the next decade as new functions of the vitamin D system are uncovered.

Three excellent review articles which summarize our current understanding of vitamin D metabolism are:

(1) DeLuca HF. The vitamin D story: a collaborative effort of basic science and clinical medicine. FASEB J2:224-236; 1988.

(2) DeLuka HF, Krisinger J, and Darwish H. The vitamin D system; 1990. Kidney International 38, Suppl 29. S-2-S-8, 1990.

(3) Gertner JM. Disorders of calcium and phosphate homeostasis. Pediatr Clin of North America 37;1441-65, 1990.

REFERENCES

1. DeLUCA HF, KRISINGER J, DARWISH, H: The vitamin D system; 1900. Kidney International 38, Suppl 29;S2-S8, 1990.
2. REICHEL H, KOEFFLER HP, NORMAN, AW: The role of the vitamin D endocrine system in health and disease. New Eng J Med 320;909-991, 1989.
3. FRASER D, KOOH SW, SCRIVER C: Rickets, osteomalacia and vitamin D. Med N Am 12;1247-1255, 1981.
4. FELDMAN KW,K MARCUSE EK: Nutritional rickets. AFP 42;1311-1318, 1990.
5. DeLUCA HF: The vitamin D story: a collaborative effort of basic science and clinical medicine. FASEB J 2;224-236, 1988.
6. GERTNER JM: Disorders of calcium and phosphorus homeostasis. Pediat Clin N Am 37;1441-1465, 1990.
7. SEELIG MS: VITAMIN D-Risk vs benefit. J Am Coll Nutr 2;109-110, 1983.

DISCUSSIONS(MORNING OF OCTOBER 28)

Question: Dr.David L. Yeung

Let me ask you a question Dr. Bray. You talk about the absorption of calcium and you talk about inhibitors and enhancers. You know that there are questions about the various types of calcium preparations. Is there any difference in the absorption and bioavailability of the various forms of calcium preparations? There is also a question about differences in the bioavailability of calcium between cow's milk and human milk. Do you have any answers to these questions?

Anwer: Dr.T.M.Bray

I leave the bioavialability to Lousise Lennard. I would like to say that there is relationship between Zinc and Calcium. It is very difficult to control deficiency of both zinc and calcium, because calcium is very difficult to absorb. And the vitamin D level would change according to the calcium status.

Answer: Ms.Lousie Lennard

Calcium in breast milk is more available than calcium in cow's milk.

Question: Dr.Feng Ze-Kung

According to Medical books, Vitamin D treatment with 5,000-10,000 IU / day, 10,000-30,000 IU / day, and 30,000-50,000 IU / day or megadose treatment with 2 doses of 300,000 units every other month. My first question to Dr.Zlotkin is whether the absorption of Vitamin D can last a long period of time especially by the megadose mthod, and whether this treatment can result in vitamin D toxicity? My second question is to Dr. Bray. Would the utilization of Vitamin D, especially by megadose Vitamin D therapy enhance the absorption of calcium?

Answer: Dr.S.Zlotkin

The use of injection of large dose of Vitamin D has been a traditional practice for may years. In fact the doses are even higher sometimes more than 300,000 IU / dose. They go up to as high as 500,000 IU per dose for the treatment of Vitamin D deficiency rickets. This form of treatment has not been associated with Vitamin D toxicity. In terms of the timing of the dose whether that large dose should be used on a monthly basis or 6 montlyy basis or yearly basis, I do not have any information that looked at Vitamin D stores in the liver based on that particular dose. So I cannot give you a specific answer to that question. It is my sense that with the high dose, especially if injected in the infant, that most of these will be indeed stored in the liver and that the liver stores will last for a considerable period of time probably in the range of 6 to 8 months.

Answer: Dr.T.M.Bray

If one only uses a high dose of Vitamin D without calcium it is of no use. From human studies, We know that if an extremely high dose of both Vitamin D and calcium is used there is a possibility of calcification of soft tissues. If only a large dose of calcium is given without Vitamin D, it is useless. Therefore, the interrelationship between calcium and Vitamin D is very important.

Answer: Dr.S.Zlotkin

Vitamin D toxicity will not occur if there is an inadequate amount of calcium in the diet. So similar to my comment about the treatment of Vitamin D deficiency rickets, the toxicity of Vitamin D is related to the absorption of calcium. You can take an extremely large dose of Vitamin D if there is a small amount of calcium in the diet. In this situation, Vitamin D toxicity will not be a significant problem.

Question: Dr.Zheng De-yuan

We have studied rickets for the last ten years, but we still have a lot of questions about its cause. We know that vitamin D deficiency is one of the causes of rickets. I have studied rickets in children under three, and a expert also has done a study on infants between 4-11 months using 3 assessment methods including clinical, X ray and blood chemistry. The prevalence rate is 34.4%. I still think that the prevalence rate of rickets is too high. From our clinical experience, we found that infantile rickets is high, and these infants were given vitamin D supplements. Therefore, I would like to ask, beside Vitamin D, What is the relationship between protein metabolism and the prevalence rate of rickets?

Answer: Dr.T.M.Bray

In basic metabolism, absorption always require protein synthesis. If you do not have enough protein in your diet the absorption and metabolism of calcium is not very effective. It will be the same as using Vitamin B_1 to treat beriberi. If only vitamin B_1 injection is given to a patient without