Metabolic and Biochemical Considerations of Bone*

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ABSTRACT

Recognition of the dynamic aspects of bone metabolism can lead to a unified concept involving endocrine and nutritional influences. Although most hormones can influence bone metabolism directly or indirectly, the principal ones involved in skeletal metabolism are parathyroid hormone, calcitonin and 1,25-dihydroxy-vitamin D. The actions of parathyroid hormone and 1,25-dihydroxy-vitamin D result in elevations of circulating extracellular fluid calcium concentration through actions directly on bone, intestine, and kidney. Calcitonin leads to decreases in calcium concentration, primarily by action on bone and kidney. The absorption and retention of calcium by the organism is further influenced by the dietary content of calcium, phosphorus, protein, and fluoride. Chronic dietary deficiencies of calcium and excesses of phosphorus may lead to chronic nutritional secondary hyperparathyroidism with resulting skeletal demineralization. In both experimental animals and in man, the earliest manifestation of this condition may be demineralization of the jaw with resultant paradentosis. Experimental studies in animals and in man have shown that this form of demineralization may be completely reversed by increasing dietary calcium and decreasing dietary phosphorus.

Introduction

Bone, in its function as the rigid structural support for the vertebrate, has fascinated philosophers, artists and scientists since earliest times. In its rock-like solidity, it offered a permanent memorial long after the flesh had decayed. Its seeming immutability provided a basis for magical and religious speculation for centuries before modern scientific research turned its tools to exploring the function of bone in the physiology of the organism.

In 1564, Lemnius came to the astounding conclusion that bone was a living, changing substance, reflecting the environment and nutrition of the animal, as shown by the deposition of red coloring in the bones of sheep after the animals were fed for several days on madder root. Some 200 years later, the vital staining of bone with the red dye of madder, later identified as...
alizarin, was rediscovered and, subsequently, eighteenth century investigators showed that new bone was continuously being deposited, while older bone was continuously resorbed. In the past 50 years, with the increasing use of radioactive isotopes and with newer concepts of enzymology and endocrinology, it has become apparent that bone, like every other tissue, is dynamic, is continuously being remodeled throughout life and is under the control of the internal and external environment.

Bone or skeletal metabolism is usually equated to mineral metabolism. Bone mineral, calcium hydroxyapatite, is deposited on or removed from a collagenous matrix with the formation of the collagen and the movement of minerals, both under the active control of the bone cell. The bone cell is histologically derived from fibroblasts or mesenchymal cells which differentiate into (1) osteocytes, presumably a resting stage, (2) osteoblasts, presumably a formation phase and (3) osteoclasts, presumably resorptive cells. The interconversion of the bone cells, as well as their activities, are under the control of the various hormones. Reactions of some of the endocrine secretions on bone metabolism have been clearly defined and confirmed, whereas those of others are only presumed. Of the pituitary hormones, growth hormone may have a direct action on bone metabolism by controlling longitudinal growth of the bone during adolescence; linear growth is accompanied by remodeling of the bone, removal of mineral from previously formed endosteal and periosteal surfaces by osteoclastic activity and redeposition at epiphyseal areas by osteoblastic activity.

Effect of Hormones on Bone Metabolism

Thyroid Hormones

The thyroid hormones, by stimulating total metabolism and increasing total turnover, increase both bone formation and bone resorption rates. Generally, bone resorption rate is increased to a greater extent than bone formation rate under the influence of thyroid, with the net result of diminution of calcium in the skeleton and movement out of bony areas.26,33

Adrenocortical Hormones

The adrenocortical hormones, primarily hydrocortisone in man, act by inhibition of enzymatic processes. In skeletal metabolism, therefore, inhibition of both bone formation and bone resorption are seen. Bone formation is inhibited to a greater extent than is bone resorption, with the result of loss of calcium from the organism because of increased activity or secretion of the adrenocortical hormones.15,32 Direct renal effects of hydrocortisone may lead to losses of calcium, primarily from extraskelatal circulating sources which in turn lead to further skeletal depletion.

Gonadal Hormones

There is considerable controversy over the effects of gonadal hormones on skeletal metabolism. No effect of androgens have been demonstrated on either bone formation or bone resorption; no effects have been shown on overall calcium metabolism in well controlled long term metabolic balance studies.1,26 In surgically castrated women, estrogens have been demonstrated to prevent further bone loss if administered within a short period of time after oophorectomy has been performed.3 When administered in conjunction with kinetic studies and carefully controlled balance studies, estrogens have been shown to produce an initial increase in bone formation and decrease in bone resorption which, however, is not sustained beyond the first month of therapy.49 Even with large doses of estrogen, no persistent effects can be seen in calcium balance or in bone kinetics.36,42 When estro-
gens are administered to patients with hypercalciuria, a significant decrease in urinary calcium can be shown which is related to the sodium retention effects of the steroid.21,42

**Hormones Involved in Calcium Metabolism**

Three hormones are involved specifically in calcium metabolism and bone biochemistry, in an interrelated and interdependent fashion. These are parathyroid hormone, calcitonin, and 1,25-dihydroxycholecalciferol (1,25-DH-Vitamin D).

**Parathyroid Hormone**

Parathyroid hormone, a polypeptide with a molecular weight of 9,000 to 10,000, is secreted by the parathyroid glands. The first 34 amino acid sequence of the polypeptide has been synthesized and shown to have all the biologic activity of the natural hormone. Many fragments of the intact 84 amino acid polypeptide have been found to be present in the normal circulation with varying degrees of biologic activity. The initial effect of parathyroid hormone on bone metabolism is to increase bone resorption, probably by increasing the number of active osteoclasts, and to decrease bone formation, probably by decreasing active osteoblasts. With time, however, bone formation increases secondary to the sustained increase in osteoclastic activity.47 Parathyroid hormone is secreted by the parathyroid glands in response to decrease in serum or extracellular calcium concentrations. Conversely, its secretion is inhibited by elevations in the calcium concentration. At the cellular level, parathyroid hormone has at least two primary effects. It stimulates adenylate cyclase, leading to an increase in cyclic-AMP concentration, which in turn stimulates the efflux of calcium from mitochondria and activates various protein kinases. It also enhances the rate of calcium entry into the cell. The combined effects lead to an increase in calcium ion concentration within the cell, which in turn either stimulates or inhibits various enzyme activities within the bone cell which may be responsible for bone resorption.47

**Calcitonin**

Calcitonin is also a polypeptide hormone. The 32 amino acid chain is secreted by the parafollicular cells of the thyroid which are of ultimobranchial gland origin. Calcitonin secretion is stimulated by rises in serum calcium and is inhibited by decreases in the extracellular fluid concentration of this ion. The initial effect of calcitonin is to enhance bone formation and to decrease bone resorption.46 With time, formation also decreases, thus resulting in a decrease in skeletal remodeling. Calcitonin functions, just as parathyroid hormone does, through the mediation of bone cell cyclic-AMP concentration. The ultimate result of calcitonin action, however, is opposite to that of parathyroid hormone. Parathyroid hormone activity leads to an increase in extracellular fluid concentration while calcitonin activity leads to a fall.

Both parathyroid hormone and calcitonin influence urinary electrolyte excretion as well. Parathyroid hormone acts on both the proximal and distal tubules of the kidney. It inhibits reabsorption of calcium ion, sodium ion and monohydrogen phosphate in the proximal tubule; it increases calcium ion, hydrogen ion and sodium ion reabsorption and decreases potassium ion reabsorption in the distal tubule.2 The combined effect is a slight increase in sodium excretion, a decrease in calcium and hydrogen excretion, an increase in potassium excretion and a very striking increase in monohydrogen phosphate excretion. Urinary cyclic AMP excretion also increases.9 Calcitonin, on the other hand, produces an increased excretion of monohydrogen phosphate, sodium ion and chloride ion, but
very little change in hydrogen or potassium excretion and an increase in calcium excretion.\textsuperscript{5}

Considerable controversy still exists concerning a possible effect of either calcitonin or parathyroid hormone on intestinal absorption of calcium. Parathyroid hormone may increase the rate of entry of calcium into intestinal mucosal cells from the intestinal lumen, thus influencing absorption from the gut. No similar action has been shown for calcitonin.

\textbf{1,25-DH-Vitamin D}

The third specific calcium influencing hormone, 1,25-DH-Vitamin D, is synthesized by the kidney through a series of complex reactions and transport. Vitamin D is obtained by the organism either from the diet or by conversion in the skin of 7-dehydrocholesterol to the vitamin. Vitamin D must be activated metabolically before it can function physiologically. In very high concentrations, in excess of those commonly attributed to vitamin activity, vitamin D can act directly on intestinal mucosal cells to induce the formation of a calcium binding protein.\textsuperscript{10} The concentration necessary for this action is so high that it is unlikely that it is of physiological significance. Primarily through the efforts of the laboratory of DeLuca, it was shown that vitamin D must be hydroxylated at the 25-carbon before it can carry out its biologic functions.\textsuperscript{12} This hydroxylation takes place predominantly in the liver. In the chicken, some hydroxylation also occurs in the intestine.\textsuperscript{52} The control of the hydroxylase reaction in the liver may be through the concentration of vitamin D available to the enzyme.\textsuperscript{13} Increasing doses of vitamin D lead to only slight increases in circulating 25-hydroxyvitamin D. The fully biologically active hormone, 1,25-DH-Vitamin D, is synthesized in the kidney by a 1-hydroxylase enzyme from the 25-hydroxyvitamin D previously formed in the liver.\textsuperscript{16}

The hydroxylation is under the control of dietary levels of calcium. Low calcium diets markedly stimulate the production of 1,25-DH-Vitamin D, whereas high calcium diets inhibit it.\textsuperscript{6} The 1,25-DH-Vitamin D is, at present, considered to be the active form of the vitamin, or the specific calcium hormone. 1,25-DH-Vitamin D functions at the intestinal level specifically to increase calcium absorption.\textsuperscript{17} At present, the mechanism of this action remains unknown. It may be by stimulation of the formation of a calcium binding protein,\textsuperscript{56} by the stimulation of synthesis of a calcium dependent ATP-ase,\textsuperscript{44} or by some entirely different mechanism. In the bone, 1,25-DH-Vitamin D increases bone resorption, owing either to activation of new osteoclasts or to an increase in the functional activity of osteoclasts already present.\textsuperscript{4} In addition, 1,25-DH-Vitamin D may have a direct action on the bone cell, initiating bone mineral deposition, since in the absence of vitamin D or its metabolites, normal osteoid mineralization does not occur.\textsuperscript{57}

It is of additional clinical interest that insofar as intestinal absorption of calcium is concerned, parathyroid hormone is not necessary nor does it enhance the effect of 1,25-DH-Vitamin D.\textsuperscript{17} However, in the absence of parathyroid hormone, 1,25-DH-Vitamin D has little or no ability to mobilize calcium from the bone.

For practical purposes, in the otherwise healthy individual or animal, vitamin D can be used as a normal precursor for the hormone. In the presence of liver disease or in patients taking antiepileptic drugs which influence the 25-hydroxylation reaction by the liver, vitamin D is insufficient and the 25-hydroxyvitamin D must be administered. In chronic renal disease, hypoparathyroidism and certain congenital conditions where the 1-hydroxylase activity of the kidney is missing, the only active substance is 1,25-DH-Vitamin D.
Absorption and Excretion of Calcium

The previous discussion of the control of calcium movement to and from bone presupposes the presence of adequate amounts of mineral within the skeletal tissue. More than 99 percent of the body calcium is in the skeleton in the normal mammalian organism. The small amount of calcium in extracellular fluid, in organelles intracellularly and in membranes account for less than 1 percent of the total. This amount of calcium is the critical amount, being involved in the physiologic control of the endocrine systems and transport mechanisms. The previous discussion has dealt with the mechanisms of movement of calcium into and out of the supportive structures. There is only one mechanism whereby calcium enters the organism and that is by absorption from the gut of the calcium which has been ingested through the diet. As has been seen by us, the absorption of the dietary calcium may be controlled to some extent by the endocrine system. There are, in addition, several mechanisms whereby calcium may be continuously lost from the body. These, too, are controlled to a certain extent by the endocrines. Renal excretion is relatively fixed for most individuals and ranges from 100 to 200 mg per day under normal circumstances, relatively independent of dietary intake.\textsuperscript{11} Endogenous fecal losses, that is, calcium that is secreted in the bile and the pancreatic juices and not reabsorbed, are about 140 to 175 mg per day.\textsuperscript{50} Dermal losses of calcium, including those in desquamated skin and those through active sweating, average about 20 mg per day under various conditions of activity, when measured over long periods of time.\textsuperscript{18}

If the losses of calcium are smaller than the amount of calcium being absorbed from the gut from the dietary intake, then there is excess calcium which can be deposited into the skeleton. If, however, the losses exceed the gains, then calcium must be mobilized from the skeleton in order to maintain the homeostatic concentration necessary for life in the extracellular fluid.\textsuperscript{41}

Nutritional Factors

In addition to the endocrine factors controlling absorption from the diet, various nutritional factors are also involved. The role of vitamin D, after its conversion to an active hormone, has already been discussed. Equally or even more important is the role of dietary calcium. The efficiency of absorption of calcium from the diet is inversely proportional to the amount being ingested over a long period of time. At very low dietary intakes, less than 150 mg per day, the efficiency of absorption in otherwise normal healthy adults ranges from 20 to 80 percent with a mean at about 60 percent.\textsuperscript{47} As dietary intake increases, this efficiency falls rapidly. At intakes of the order of magnitude of the National Research Council Recommended Daily Allowances, 800 mg per day, the efficiency ranges from 5 to 50 percent with a mean at about 27 percent. Calculating from the known values of averages losses of calcium by the kidney, intestine and skin, it can be seen that the average individual may barely be in balance at these intakes. A significant proportion of the population, whose intakes may very well be considerably below the recommended daily allowances or whose efficiencies of absorption may be considerably below the average, will be in slight negative balance of calcium for most of their adult lives. Small daily negative balances of the order of 20 to 30 mg per day over the course of 20 to 30 years of adult life can lead to losses of mineral from the skeleton sufficient to produce radiographically diagnosable osteopenia or osteoporosis.\textsuperscript{44}

Dietary Factors

Food disappearance data from the U.S. Department of Agriculture for 1955\textsuperscript{18} dem-
onstrate that the primary source of calcium in the American diet is milk; the remainder of the diet provides very little calcium. Since 1955, there have been significant changes in the American dietary pattern.\textsuperscript{54} Milk consumption has decreased, resulting in still lower intakes of calcium. Dietary surveys of individuals indicate that most adults, particularly adult women homemakers in this country, consume about 400 mg per day of calcium.\textsuperscript{8} Thus, it may be concluded that chronic long-term dietary deficiencies of calcium do exist and may result in clinically significant disease. The relationship of chronic dietary deficiency of calcium to clinical disease, primarily osteoporosis, has been derived principally from retrospective data. Long-term dietary intakes of calcium in patients with osteoporosis generally are lower than those in comparable populations without clinically diagnosed disease.\textsuperscript{84} Furthermore, when patients with longstanding osteoporosis are placed on diets of increasing calcium intake, the metabolic balance of calcium becomes progressively more positive as the intake increases. Although the efficiency of absorption decreases with increased intake, the absolute amount of calcium absorbed increases.\textsuperscript{43} Kinetic studies with radioactive isotopes indicate that bone formation rates are within the normal range in these patients but bone resorption rates are greatly increased. As the dietary intake of calcium is increased, the bone resorption rates fall.\textsuperscript{22,51} However, after several years at high intakes of calcium, new equilibrium points are achieved and no further calcium is retained.

Other dietary factors can influence calcium absorption. In experimental animals, feeding of diets with low calcium:phosphorus ratios results in nutritional secondary hyperparathyroidism, both at low calcium intakes and at adequate intakes.\textsuperscript{27,28,29} The diets with excessively high phosphate content lead to hyperplasia of the parathyroids and bone changes identical to those seen in human clinical osteoporosis. The food disappearance data quoted previously indicate a high phosphorus intake in the American diet. Phosphorus is provided by milk, poultry, fish, and meat primarily. In 1955, the calcium:phosphorus ratio in the American diet was about 1:2.8. The decrease in milk consumption in the years since then has been accompanied by an increase in meat consumption with a resulting increase in phosphorus intake. Furthermore, the decreased milk consumption has been compensated for by an increase in the consumption of soft drinks. Industry data show that the primary soft drinks consumed in the United States are of the cola variety.\textsuperscript{45} Recent analyses have shown that the cola beverages are exceedingly high in phosphorus, in the range of 20 mg per 100 ml, with virtually no calcium.\textsuperscript{28} Thus the phosphate content of the American diet has increased markedly, leading to a calcium:phosphorus ratio at present approaching 1:4. This is of the same order of magnitude used in the experimental animal studies to produce secondary hyperparathyroidism, where diets with ratios of 1:5 to 1:20 have produced the dramatic changes.

In a prospective animal study, adult beagles were placed on diets with calcium:phosphorus ratios of 1:10.\textsuperscript{22} After one year of this diet, animals were sacrificed. Chemical analyses of various bones demonstrated a hierarchy of disappearance of calcium. The jaw bone was the most labile and also has the highest content of trabecular bone. The vertebrae were next, followed by the ribs and finally the long bones. Demineralization of the jaw bones resulted in the development of paradentosis in all the animals. This was accompanied by changes typical of osteoporosis in other skeletal tissue. The parathyroids of these animals showed hyperplastic changes indicative of hypersecretory phases.
When animals who had been depleted in this fashion were placed on diets higher in calcium, with calcium:phosphorus ratios of 1.2:1, repair of all the changes were noted. After eight weeks of repletion, trabecular bone within the jaw reappeared. By 16 weeks, the jaw bones were completely normal. Similar changes were present in the humeri of the same animals. After depletion, large resorption cavities were present. After repletion feeding for 12 weeks, most of these cavities had disappeared with new bone formation quite evident. By 28 weeks, the humerus was indistinguishable from normal dog bone.

In the human, coincidental appearance of severe periodontal disease with disruption of trabecular bone structure in the jaw, associated vertebral fractures and osteoporosis elsewhere in the body, has been demonstrated. This observation suggests that some forms of periodontal disease with bone resorption may be the long-sought preosteoporotic condition, where the patient may still be capable of regenerating bone before collapse of the vertebrae has occurred and before the disease has become irreversible.

In a prospective study, 90 human patients with mild degrees of periodontal disease were selected as cases of preosteoporosis. Bone density was measured at the start of the study and at monthly intervals thereafter by photondensitometry. The patients were then divided into groups that received either a calcium supplement of one g per day or a similarly appearing placebo for 12 months. The addition of calcium resulted in no significant differences in bone density of the radius (a bone with a high cortical to trabecular bone ratio) over this interval. Similarly, there was very little change in the density of the ulna during the 12 months, in the group on calcium supplementation. In the group receiving placebo, there was a border-line-significant decrease in the bone density of the ulna, suggesting that the portion of the ulna being measured was more sensitive to changes and had a higher trabecular content than the radius. In the jaw, much more change was seen. The group receiving placebo showed no significant changes during the 12 months, but the group on calcium supplements showed a statistically significant increase in bone density, approximately 12.5 percent, as a result of supplementation with dietary calcium.

Another dietary factor that has been implicated in bone metabolism is protein. It has been shown that individuals consuming diets high in protein excrete more calcium in the urine consistently than those receiving normal or low protein intakes. This factor becomes of increasing importance with the increase in protein consumption seen in recent decades.

**Fluoride Content in Diet**

Still another factor of importance is the fluoride content of the diet. Fluoride is incorporated into the bone mineral as calcium fluoroapatite. At intakes of 1 to 2 mg per day, the resulting bone is more resistant to both chemical and biological resorption. At intakes between 2 and 10 mg per day, the bone becomes increasingly resistant to resorption, but is still capable of responding when necessary to maintain extracellular calcium concentrations. At intakes considerably above this in the range of 15 to 40 mg per day, the bone, because of its resistance to normal resorptive stimuli, cannot be appropriately remodeled and becomes abnormal. Since bone turnover in the human is relatively slow, the effectiveness of dietary fluoride is apparent only with long term low dose supplementation. Fluoride has been used as a therapeutic agent in the treatment of osteoporosis at higher doses, but it may be predicted that such effects would be of relatively low significance and apparent only when dietary calcium is adequate as well.
Summary

To summarize, metabolic considerations of bone and mineral metabolism must take into account various dietary and endocrine factors. The dietary intake of calcium must be adequate,— at least 800 mg per day and probably higher in many individuals. As dietary phosphate intake increases, the requirement for dietary calcium increases as well and, if not met, can result in stimulation of bone resorption. When dietary protein is increased much above the normal level, the requirement for calcium again increases. Dietary fluoride should be maintained, for optimal metabolic activity, within the normal range of 1 to 2 mg per day.

When dietary calcium is absorbed, extracellular fluid concentration of calcium rises evanescently. This rise then stimulates secretion of calcitonin and inhibits secretion of parathyroid hormone. Calcitonin stimulates osteoblastic activity and inhibits osteoclastic activity. The absorption of calcium from the diet is under the control of 1,25-DH-Vitamin D which functions as a hormone.

Owing to the continuing excretion of calcium by the kidney, intestine, and skin, the concentration of calcium in the extracellular fluid falls when dietary calcium is low. This inhibits the secretion of calcitonin and stimulates the secretion of parathyroid hormone. Parathyroid hormone functions primarily at the level of the bone, in conjunction with 1,25-DH-Vitamin D, to increase bone resorption and to decrease bone formation, thus liberating additional calcium to maintain the homeostatic concentration. When dietary calcium ingestion is chronically low, or when pathologic losses of calcium occur chronically, there is long term hyperactivity of parathyroid hormone with hypersecretion by the parathyroid gland leading to chronic demineralization of the skeleton or osteoporosis.

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