Review

Nutritional biochemistry of calcium and phosphorus

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The biological importance of calcium (Ca) and inorganic phosphorus (Pi), two minerals required daily from foods in reasonably large quantities, ranks along side the macronutrients. Both bulk or macro-elements play critical roles in cellular regulatory mechanisms as well as in extracellular tissues, such as the skeleton and teeth. The concentration of Ca ions in extracellular fluids, it should be emphasized, is approximately 10^3 greater than within cells, and the ratio for free Pi ions is similar. This huge gradient of Ca requires cells to pump, i.e., actively transport, Ca ions that leak inside, to the extracellular fluid to preserve the low intracellular concentration. This concentration difference also serves advantageously first for the entry of these ions into the bone fluid compartment, a special extracellular pool, and then for the precipitation of calcium phosphate and closely related hydroxyapatite salts on the organic matrix of skeletal tissues.

Major topics addressed in this review on calcium and phosphorus are: food sources and dietary intakes of the minerals; bioavailability and intestinal absorption; blood homeostasis; bone tissue dynamics; and perspectives on human needs. In this report emphasis, which is placed on adult humans, and animal studies when necessary, is focused on the changes of Ca and Pi across the life cycle, especially as changes in the intakes of these macrominerals contribute to osteoporosis.[1]

Food sources and dietary intakes

As a general rule, only a few foods are rich in Ca, but nearly all foods contain phosphorus (P) as phosphates, both organic and inorganic. Thus, the problem of consumers is in obtaining sufficient amounts of Ca to meet physiological and biochemical requirements, estimated to range between 400 to 500 mg per day for adults by Kanis and Passmore[2] and between 500 and 600 by Nordin and Marshall.[3] This intake requirement must equal the Ca losses in urine, endogenous fecal excretions, and dermal losses. Since the efficiency of intestinal Ca absorption in adults is considerably less than 100%,[4] a much greater amount of calcium must be consumed to meet the daily requirement. A daily allowance, such as the recommended dietary allowances (RDA), should then be set at a value that exceeds the requirement. In the United States the adult Ca RDAs are established to be 1200 mg for young adults (18–24) and 800 beyond this age, except for pregnant and lactating women whose allowances are increased by an additional 400 mg per day.[5] Phosphorus allowances are identical to the Ca RDAs,[5] although no rationale for this equivalency exists.

Since dairy products provide roughly 50 to 75% of Ca in the diets of Americans,[6] more Ca (%) for men from dairy products than women,[7] the other food groups seemingly have much less significance in meeting daily allowances. Nevertheless, as dairy consumption declines among females as they pass from adolescence to adulthood, other food sources, such as cereals, vegetables, and mixed foods, do take on increased significance in meeting Ca requirements, as is clearly the case for older women.[8] Low-fat milks and calcium-enriched products, including orange juice, have not been available long enough to have improved appreciably the Ca consumption patterns of adult women, but recent data suggest a partial reversal of the trend of relatively low intakes of Ca by American women.[7]

The low Ca consumption by most American women is contrasted by high phosphorus (P) ingestion, which is contributed by all foods; dairy and other animal products provide less than 50% of the typical P intake of Americans.[6] Mean P intakes of adult women range between 1200 and 1600 mg per day, on the high side for large consumers of animal products. For those individuals who achieve the Ca RDAs, the dietary Ca:P ratio approximates 0.70 to 0.75, but for those who consume at about 50 to 60% of the calcium RDAs, the dietary ratio of the two mineral elements dips to 0.50 or lower, reflecting an inadequate amount of dietary Ca. When the ratio approaches 0.25, Ca intakes clearly force hormonally governed homeostatic adjustments that draw Ca from the skeleton (see below), and they are, thus, considered unhealthful. An estimated

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10–20% of adult women in the United States fall in this latter category as they consume less than 400 mg of Ca per day.[9]

Bioavailability and intestinal absorption

Ca bioavailability from dairy products, other foods, and diverse types of meals is consistent, except for spinach, and presumably rhubarb, because of their high oxalate content.[10] The adverse effects of other food constituents on calcium bioavailability, such as phosphates, phytates, fats, dietary fiber, and others, are considered minimal in healthy individuals, despite reports presenting data on statistically significant declines in calcium absorption.[11] Conversely, other dietary factors, such as lactose, have only a minor enhancing effect on Ca absorption via a passive mechanism in the lower small intestine,[12] but this extra amount may be important, especially in older adults.[13] Vitamin D, through conversion to its hormonal form, 1,25-dihydroxy vitamin D, can exert a significant influence on Ca bioavailability by stimulating absorption in the duodenum via an active transport mechanism, but the vitamin D–mediated adaptational mechanism only operates when dietary Ca intake per se is low.[14] The hormonal form of vitamin D probably also enhances calcium absorption in the other segments of the small intestine, but the increases are considerably less than in the duodenum.

The bioavailability of P, as inorganic phosphates ($HPO_4^=$ and $H_2PO_4^-$), is high, but it always exceeds that of Ca by two- to threefold. The only exceptions to this rule are that several food phosphate additives, including polyphosphates and pyrophosphates used by the food industry, solubilize very poorly in the gastrointestinal tract and, therefore, are poorly bioavailable.[15]

Most of the Ca absorbed from foods is passively moved across the gastrointestinal barrier paracellularly, i.e., around cells rather than through them, especially in the jejunum and ileum. The active Ca absorption mediated by 1,25-dihydroxy vitamin D, however, is transcellular and saturable.[16] The bulk flow of calcium via the paracellular route appears to be directly related to the Ca concentration in the gut lumen up to a threshold level, beyond which calcium absorption is limited by the capacity for water (bulk) flow.[16] The typical net absorption efficiency of Ca by adults from a meal, as measured by either intrinsic or extrinsic labels, is 25 to 35%,[4] regardless of the food source of Ca except for Ca from high-oxalate foods. Thus, dairy Ca, cereal Ca, and vegetable Ca are absorbed with the same efficiency.[17] Only Ca as the calcium citrate malate complex (CCM) has a slightly higher efficiency of absorption,[18] and as the Ca citrate it also may be slightly higher,[19] but other supplemental Ca salts, such as Ca carbonate, have approximately the same absorption efficiency as milk Ca.[20-22] The concurrent consumption of a meal and a calcium supplement may enhance Ca absorption because of increased gastric acid production, especially in elderly individuals.[17,23] The role of citrate and other Ca chelators in foods and beverages in relaxing the tight junctions of the small intestinal mucosa, and thereby enhancing the paracellular absorption of Ca and other divalent cations, requires further study.

In elderly subjects with achlorhydria, Ca absorption from Ca carbonate and most other Ca supplements is greatly depressed, as shown by Recker,[23] but Ca from Ca citrate supplements in fasted achlorhydric subjects is absorbed more efficiently by older subjects.

Pi, on the other hand, is absorbed highly efficiently by the small intestine, i.e., at a rate of 60–70%.[24] It is also absorbed primarily by the passive route, but transcellular Pi absorption can be enhanced by 1,25-dihydroxy vitamin D, presumably when intakes are low, an unlikely possibility considering typical food consumption patterns.

Illustrations of human Ca and Pi absorption patterns as functions of intake amounts are given in *Figure 1*. Note the linear absorption of Pi compared to

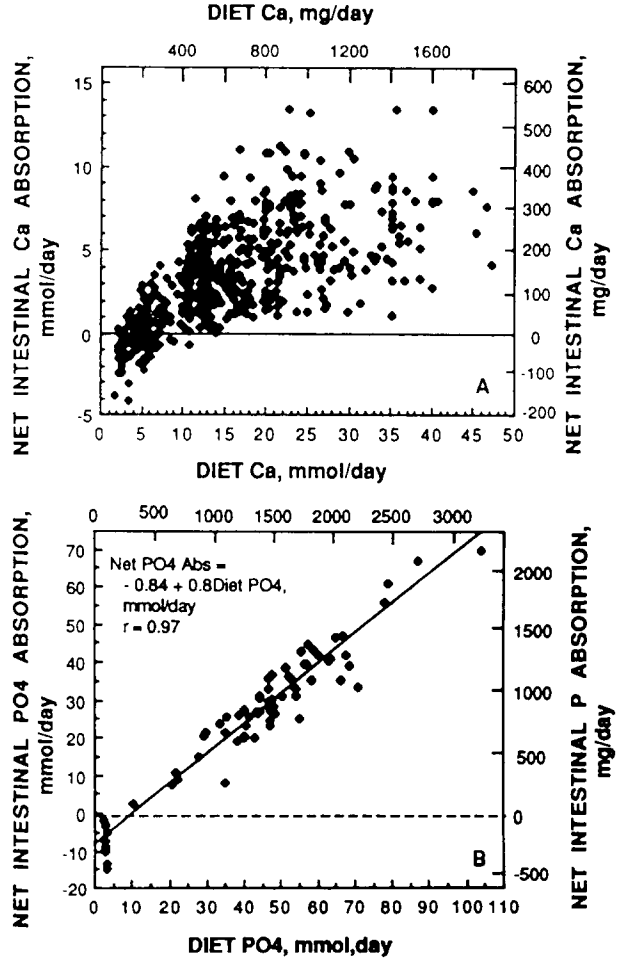

Figure 1 (A) Net intestinal calcium (Ca) absorption as measured by the metabolic balance method in relation to dietary Ca intake among healthy adults; (B) net intestinal phosphate (PO_4) absorption in relation to dietary PO_4 intake among healthy adults. (Reprinted with permission from *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism* [M.J. Favus, ed.], pp. 33, 35, American Society for Bone and Mineral Research, Kelseyville, CA, 1990)

the threshold-limited absorption of calcium.[25] Similar results have been reported by other investigators.[24]

Blood calcium concentration and homeostasis

The blood Ca concentration is highly regulated at a set level, i.e., 100 mg/L (2.50 mmol/L) in all healthy individuals across the life cycle, although a modest decline in total Ca concentration occurs from childhood to late adulthood, i.e., from 105 to 90 mg/L (2.62 to 2.25 mmol/L). (Ionic Ca concentration falls in a similar manner, but less so than total, over a lifetime.) Part of this age-related decline results from a reduction in the total calcium-binding capacity of serum proteins, especially from a modest decline in hepatic albumin synthesis by the elderly,[26] and part most likely from a reduced efficiency of the hormonally regulated Ca homeostasis.

Ca homeostasis is controlled primarily by parathyroid hormone (PTH), although several other hormones can also affect the regulation of blood Ca. PTH, the major calciotropic hormone, acts directly on the bone and the kidneys to increase Ca in the blood and on the kidneys to reduce serum Pi, and indirectly on the small intestine through 1,25-dihydroxy vitamin D to enhance Ca absorption when dietary Ca intake is low (*Figure 2*). The direct actions of PTH on skeletal and renal tissues to maintain the blood Ca concentration at 100 mg/L (2.50 mmol/L), i.e., Ca conservatory actions, are thought to occur mainly during the fasting or intermeal periods, whereas the indirect action of PTH on the gut via preformed 1,25-dihydroxy vitamin D happens during meals, primarily when chronic conditions of inadequate Ca consumption exist (see above). Supplemental vitamin D has been shown recently to improve the bone mass of postmenopausal women consuming low-calcium diets.[27]

The Ca homeostatic events during the three- to four-hour period after food ingestion are not clearly understood, but Ca and Pi removal from blood and subsequent uptake by tissues during this postprandial period are closely linked. In studies during which radioactive labels of both Ca and Pi have been simultaneously administered by mouth, Pi is much more rapidly absorbed than calcium, and the ^{32}P label peaks earlier and disappears more rapidly from the blood than the ^{45}Ca tracer (Anderson and Talmage, unpublished data). It is generally accepted that during the first hour or so of the postprandial period the additional absorbed Pi ions complex in some way with Ca ions in the blood and that the two ions in this complex are removed together from the blood and taken up by both soft and mineralized tissues. This downhill transfer or influx of Ca and Pi ions is passive because of the large concentration gradient between blood and cells or the bone fluid compartment. The net result is to decrease transiently the serum Pi concentration resulting from the absorption of food phosphorus and *also* the total serum Ca concentration, particularly the ionic Ca fraction. The latter decline, which is difficult to demonstrate during the postprandial period, in turn,

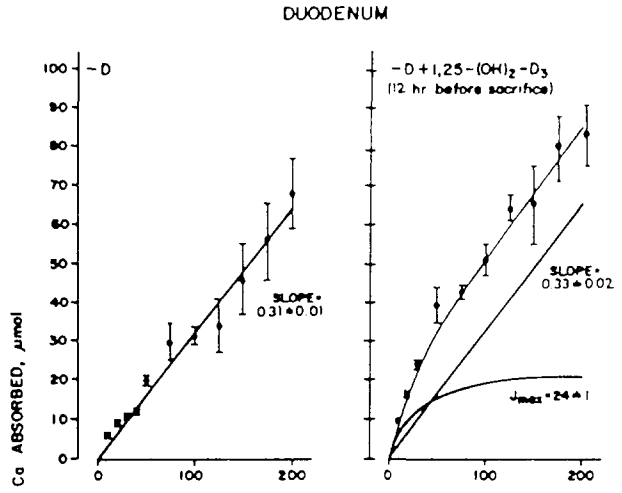

Figure 2 Calcium (Ca) absorption in the duodenum of vitamin D-deficient rats before (*left*) and after (*right*) treatment with 1,25-dihydroxy vitamin D_3. The linear portion of each plot represents the nonsaturable paracellular absorption, whereas the curved portion of the right plot represents the additional Ca absorbed in response to vitamin D by the transcellular saturable mechanism. In situ loops were used. (Reprinted with permission from Pansu, D., Bellaton, C., Roche, C., and Bronner, F. (1983). Duodenal and ileal calcium absorption in the rat and effects of vitamin D. *Am. J. Physiol.*, **244**, G696–G700)

stimulates PTH release, which acts to conserve Ca, especially at the distal nephron sites of action.[28] PTH does *not* appear to increase bone release of Ca ions (and Pi ions) at this time. This block of Ca transfer (efflux) from bone fluid to blood presumably occurs because of the elevated meal-related calcitonin concentration, as the effect of calcitonin is considered to dominate the skeletal action of PTH during the postprandial period.[29] Infusion studies of animal models support this concept.[30]

During the fasting or intermeal periods, however, an elevation of the PTH concentration clearly stimulates the metabolic activity of the bone cells, especially osteoblasts and lining cells.[31] PTH has two major effects on bone tissue, one that is homeostatic and the other that directs bone remodeling. The homeostatic function involves the action of bone lining cells, also known as resting osteoblasts, which presumably "pump" Ca ions, and indirectly inorganic Pi ions, from the bone fluid compartment to the extracellular fluid. The rate of efflux of Ca from bone is high and, over a 24-hour period, it is estimated to amount to 6000–7000 mg of Ca.[32] (In healthy individuals, the rate of Ca influx equals the efflux over a 24-hour period.) Although the precise mechanism governing this uphill transfer of ions is not established, PTH controls it,[33] and it disappears in parathyroidectomized animal models. The remodeling function of PTH is carried out by osteoclasts, through PTH-stimulated osteoblastic production of local bone factors,[34] but the level of osteoclastic activity in adult bone is generally low. Thus, the ratio of the fluxes transferring Ca ions from bone to blood by these two cellularly mediated processes

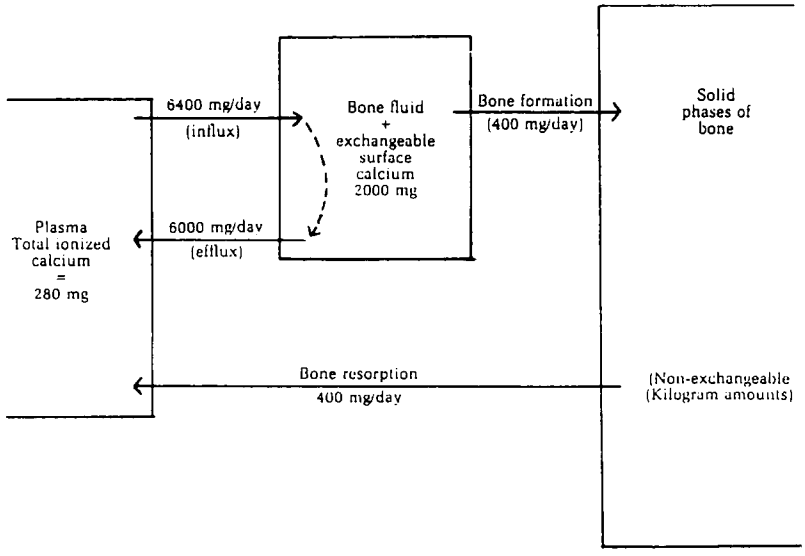

Figure 3 Approximate rates of calcium (Ca) fluxes between serum and bone (24 hour totals in a 70 kg adult). By far the major Ca movements are the two-way fluxes between serum and bone fluid; the flux from osteoclastic bone resorption is more than 15 times lower than the homeostatic flux from bone to blood. (Reprinted with permission from Talmage, R., Grubb, S., and VanderWiel, C. (1983). Physiologic processes in bone. In *The Muscoloskeletal System: Basic Processes and Disorders*, 2d ed. [F.C. Wilson, ed.], pp. 109–125, J. B. Lippincott, Philadelphia)

governed by PTH is roughly 15 to 20, greatly favoring the homeostatic role of this hormone (*Figure 3*).

Because of the relatively high solubility of Ca and Pi ions in blood and the concentration gradients from blood to the bone fluid compartment, the concentrations of these ions in blood are supersaturated compared to their concentrations at the bone mineral surfaces. Thus, at the usual concentration gradients precipitation will occur even in the absence of the activity of living bone lining cells.

Bone tissue dynamics

Bone tissue exists in two major types during adulthood, i.e., cortical (compact) and cancellous (trabecular), but all bone tissue surfaces throughout the body are covered by lining cells or other cells. Osteoclasts are also found on bone surfaces, but rarely in adulthood. Both osteoblasts and osteoclasts are derived from osteoprogenitor or precursor cells in the bone marrow. As stated above, the bulk of the Ca moved from bone to blood occurs via the presumed pumping or metabolic action of lining cells, which by far are the most common cells found on bone surfaces throughout the adult skeleton.[33]

Osteoblasts decrease in number as a function of adult age, and they only exist at sites of bone formation during normal remodeling. The number of osteoclasts on bone surfaces of adults, although very low, probably increases relative to osteoblasts during late life, i.e., after age 50 (or the menopause) in women and somewhat later in men. Osteoclasts act first during the course of a bone remodeling cycle by producing resorption cavities on either cortical or cancellous bone surfaces. Then osteoblasts are recruited to the resorption cavities to form new bone matrix and thereby to fill in the cavities. (The matrix subsequently mineralizes, and the net result is new healthy bone tissue.) In younger adults, the amount of old bone resorbed is equivalent to the quantity of new bone formed, but beyond the menopause and in physically inactive men (beyond age 60 or so), skeletal resorption exceeds formation.[35] Thus, the remodeling cycle may yield new stronger bone in the elderly, but it is at the expense of a net loss in bone mass. *Figure 4* schematically illustrates the bone remodeling cycle that is initiated by one or more unidentified activating factors.[34]

Bone mass declines by an estimated 1–2% per year for the first decade following the menopause and by 1% or less thereafter in both older women and men until late-life senility, when the rate of loss probably surpasses 1% per year. In late life, not only does the quantity of bone decline, but so does the structural quality diminish. The lower quality of cancellous bone, i.e., loss of microarchitectural vertical support columns and horizontal connecting struts, is considered highly subject to fragility break down and, thus, fractures at different skeletal loci.[36-38] Common sites of fractures late in life, i.e., vertebrae, hips, and wrists, contain fairly large proportions of cancellous tissue.[39]

The role of an adequate intake of Ca in countering the gradual loss of bone mass of older adults is now generally accepted to be minimal, though not inconsequential. Ca intakes need to meet or modestly exceed the RDA (800 mg per day) in later life to optimize the uptake of Ca ions by bone surfaces and to try to achieve Ca balance. The best outcome that can be expected in postmenopausal women and older men is for Ca balance to improve from the more negative to less negative rather than to zero balance.[40] In a pro-

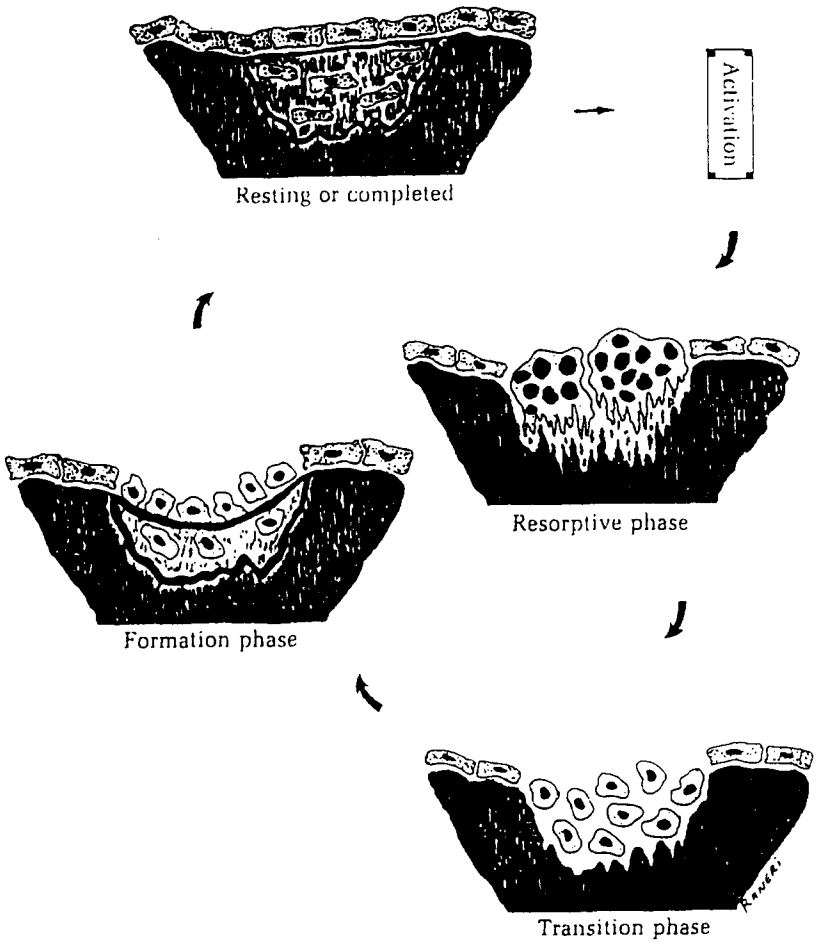

Figure 4 Diagrammatic representation of the remodeling of trabecular bone. Following activation, the process begins with resorption by the osteoclasts that move along the surface of bone trabeculae. Then osteoblasts move into the cavity and replace the osteoclasts. Failure to complete the remodeling of the resorbed cavity leads to a net loss of bone mass, a phenomenon commonly observed in elderly subjects. (Reprinted with permission from Talmage, R., Grubb, S., and VanderWiel, C. (1983). Physiologic processes in bone. In *The Musculoskeletal System: Basic Processes and Disorders*, 2d ed. [F.C. Wilson, ed.], pp. 109–125, J. B. Lippincott, Philadelphia)

spective five-year study,[41] elderly women (N = 189), despite an adequate Ca intake of over 900 mg per day, still lost bone mass at approximately 1% per year. The reasons for the high loss rate of elderly women, and probably also of men, have not been satifactorily established, but the rate of loss relates to several factors, including a decline in the efficiency of intestinal absorption with age,[42] a marked fall in circulating estrogens (women) or androgens (men), declining renal function,[43] and, perhaps most importantly, a diminution in lean body mass indirectly linked to restricted physical activity.[44] The elderly are also less able to adapt to a low calcium intake than young adults, presumably through an increase in the hormonal form of vitamin D, as demonstrated by Ireland and Fordtran.[45]

Bone tissue maintenance in older individuals over a period of one to two years has only been demonstrated by treatment with estrogens or by prescription of an exercise program; but Ca intake alone, including supplementation of Ca to meet or even exceed the RDA, has not been shown to retain bone mass over a similar time frame.[46]

Perspectives on human needs

The most important dietary factors affecting bone mass throughout the life cycle, especially during the periods of bone building and skeletal maturation, continue to be Ca and Pi. Assuming adequate intakes of all other nutrients, peak bone mass is optimized by an adequate intake of Ca, a concept supported by the cross-sectional data of Matkovic et al.[47] Although dietary P is essential, too much P can alter the calcium homeostatic mechanisms and contribute to low bone mass over an extended period. When the dietary Ca : Pi ratio approaches 1 : 4, secondary hyperparathyroidism, a disorder well known in growing animals[48] and in advanced renal patients,[28] will occur.

It has been difficult to establish nutritional secondary hyperparathyroidism in humans because of the long period of observation required, but it has been simulated in an eight-day study,[49] using typical foods consumed by adult females. In a second, longer investigation by Calvo et al.,[50] the serum ionic Ca concentration was reduced slightly, but significantly, in the

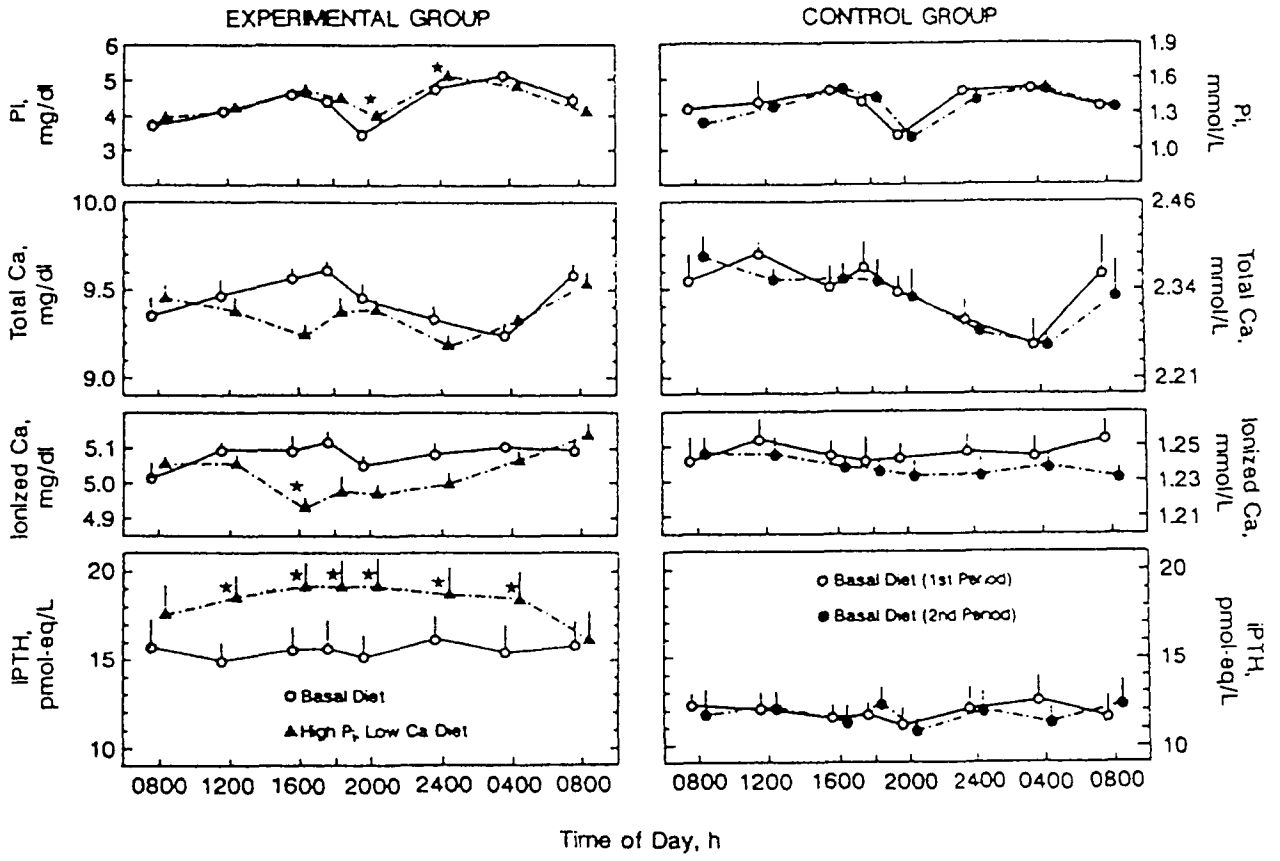

Figure 5 Mean (±SE) serum phosphorus (Pi), total calcium (Ca), ionized Ca, and immunoreactive parathyroid hormone (iPTH) concentrations during ingestion of the basal diet (O—O) and the high phosphorus, low-calcium diet (△---△) after the experimental protocol (*left, N* = 10) or during the first 4 weeks (0—0) and second four weeks 0--0) of basal diet ingestion after the control protocol (*right, N* = 5). *, *P* = 0.006, within-group comparison, versus test or 8-week basal diet. (Reprinted with permission from Calvo, M., Kumar, R., and Heath, H., III [1990]. Persistently elevated parathyroid hormone secretion and action in young women after four weeks of ingesting high phosphorus, low calcium diets. *J. Clin. Endocrinol Metab.* **70,** 1334–1340)

high P group. As a consequence, the blood concentration of PTH hormone was also elevated in the high P group (*Figure 5*). Other markers of Ca and bone metabolism were appropriately modified, including elevations of urinary cAMP and hydroxyproline. Because a sufficient change in bone mass could not have been detected over the 28-day period of observation, it was not practical to measure this important response variable. Nevertheless, if the low Ca–high P diet had been continued by these subjects, it is most likely that a net loss of bone mass would have been detected by 6 to 12 months. Thus, a typical high P intake by women from animal products (but not including dairy sources) and other food groups is presumed to be an important risk factor contributing to low bone mass late in life.

Finally, mention needs to be made of the potential adverse effect of a diet high in animal protein, but low in dairy products. Amino acids derived from intestinal protein digestion are rapidly absorbed and they, along with glucose, stimulate insulin release by β cells. (Liquid protein diets and intravenously administered amino acids also act in the same manner.[51] By themselves amino acids also trigger the release of glucagon during the postprandial period (*Figure 6*). Different

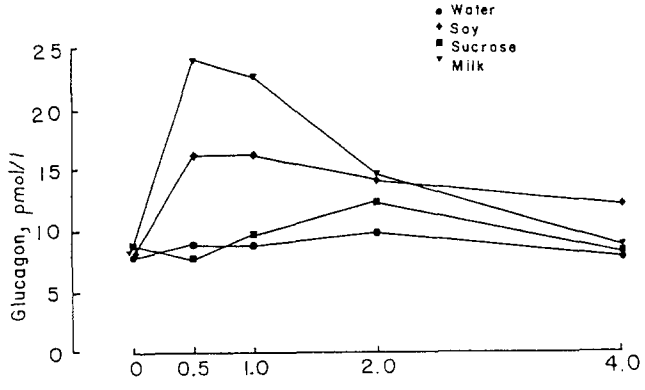

Figure 6 Plasma glucagon concentrations as a function of time for the four different meal types. (The water meal consisted of approximately 100 kcal less than the others, which were three 150 calorie meals.) The number of subjects (*N* = 12) was the same for each point. (Reprinted wtih permission from Anderson, J., Thomsen, K., and Christiansen, C. [1987]. High protein meals, insular hormones and urinary calcium excretion in human subjects. In *Osteoporosis 1987* [C. Christiansen, ed.], p. 243, Osteopress ApS, Copenhagen)

Table 1 Areas under the curves of glucose, insulin, and glucagon

Variable	Meal Type			
	Water	Sugar	Milk protein	Soy protein
Glucose	18.6 ± 2.6	18.6 ± 2.1	17.2 ± 1.8	17.3 ± 1.7
Insulin	77.4* ± 19.9	105.9* ± 31.8	86.3 ± 20.1	82.1 ± 26.0
Glucagon	35.7*† ± 11.4	40.2 ± 12.7	63.2* ± 29.9	55.8† ± 25.0

Note: Data are given as mean ± SD. Cross-Row Comparisons: *†, $P < 0.05$ or better, Duncan's multiple range test.
Reprinted with permission from Anderson, J., Thomsen, K., and Christiansen, C. (1987). High protein meals, insular hormones and urinary calcium excretion in human subjects. In *Osteoporosis 1987* (C. Christiansen, ed.), p. 243, Osteopress ApS, Copenhagen.

meal types have differential effects on serum glucagon (*Table 1*). It is speculated that the elevated glucagon,[52] or another hormone or factor,[53] then acts on distal renal tubular cells to block partially the reabsorption of Ca. The net result is an elevation of urinary calcium in the immediate postprandial period and in the total 24-hour collection.[52] A number of studies, but not all,[54] have produced the protein-induced hypercalciuria,[55] but it remains to be established over a long period of time how significant this loss of Ca is in the face of the antagonistic effect of a high P intake, which produces a reduction of urinary calcium.[56] Well-designed prospective studies are needed to separate the opposing effects of P and protein on long-term Ca metabolism and bone turnover.

Summary

The increased longevity of Western populations, especially of women, has forced a reexamination of the nutritional biochemistry of Ca and P, especially how these nutrients relate to bone mass over the increasingly extended period of late adulthood. The current epidemic of hip fractures in northern Europe and probably in North America,[57,58] with an impending rise in the early twenty-first century, impels us to: (1) optimize nutritional intakes of both Ca and P throughout life; (2) to reduce, perhaps, animal protein in the diet to promote the attainment of adult peak bone mass prior to the menopause; and (3) to maintain as much of that peak bone mass as possible in the face of the postmenopausal phase of ovarian cessation, and the inexorable bone loss related to the loss of estrogen production, and the inactivity and loss of skeletal muscle function of both superannuated men and women.[44]

Acknowledgments

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