EFFECTS OF DIETARY FACTORS ON SKELETAL INTEGRITY IN ADULTS: CALCIUM, PHOSPHORUS, VITAMIN D, AND PROTEIN

September 1981

Prepared for

BUREAU OF FOODS
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
WASHINGTON, D.C. 20204

under

Contract Number FDA 229-79-2275
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by

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FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in specific areas of biology and medicine.

This technical report was prepared for the Bureau of Foods, Food and Drug Administration (FDA), by Herman I. Chinn, Ph.D., Senior Staff Scientist, LSRO, FASEB, in accordance with the provisions of Contract No. FDA 223-79-2275.

The LSRO acknowledges the contributions of the investigators and consultants who assisted with this study. The report reflects the opinions expressed by participants in an ad hoc study group that met at the Federation on January 12 and 13, 1981. A judicious attempt has been made to incorporate the various viewpoints and opinions. The report was reviewed by these consultants; however, the listing of their names in Section IX does not imply that they endorse the study. The author and LSRO accept responsibility for the contents of the report.

The report was reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent Society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures, the report was approved and transmitted to FDA by the Executive Director, FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of each individual member of the FASEB constituent Societies.

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office
ABSTRACT

Bone undergoes continuous remodeling as new bone is deposited and existing bone is resorbed throughout life. During middle age and in later years, the resorptive processes generally predominate, causing a net loss and weakening of the bones. If sufficiently pronounced, such loss of mineral content leads to osteoporosis, a relatively common disability of the elderly, especially postmenopausal women. While many factors including racial, genetic, hormonal, nutritional, and physical aspects may contribute to the etiology of osteoporosis, this report focuses on the role of certain of the nutritional factors believed important in maintaining normal bone homeostasis, namely, calcium, phosphorus, protein, and vitamin D. The interrelations of these dietary constituents among themselves and with the various calcitropic hormones are discussed. The calcium requirements of adults remain controversial and a vast literature on the subject reveals widely differing opinions. An attempt is made to evaluate critically relevant studies and to identify reasons associated with these discrepancies. Calcium intakes and bone status have been studied by a number of epidemiologic, metabolic balance, absorptiometric, radiologic, tomographic, and other techniques. The advantages, reliability, and utility of these methods are described briefly. There is evidence to support the prophylactic use of calcium supplementation among postmenopausal women, but its therapeutic utility remains controversial. Specific topics requiring additional research are identified.
SYNOPSIS

Osteoporosis is an age-related disorder characterized by excessive bone loss. The elderly, especially postmenopausal women, are the principal victims. The condition results from disturbance of the delicate homeostatic mechanisms which maintain normal bone status. Various genetic, nutritional, hormonal, and physical factors interact in a complex manner to ensure skeletal integrity. Marked disturbances in any of these parameters may contribute to the etiology of osteoporosis. This report considers certain nutritional or dietary factors in bone homeostasis, namely, calcium, phosphorus, vitamin D, and protein.

Different calcium intakes have been recommended by various national and international organizations. In the United States, the recommendation for the ostensibly normal middle-aged and elderly adult populations is 800 mg/d. National surveys indicate that a substantial percentage of North American adults do not consume sufficient calcium-rich foods, such as dairy products, to meet this recommended intake.

Although a number of hormones influence calcium and phosphorus metabolism, homeostatic control of these elements is maintained primarily through the delicate interaction of three calcitropic hormones: parathyroid hormone (PTH); 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃]; and calcitonin. Plasma levels are usually kept within normal limits by the action of one or more of these hormones in controlling intestinal absorption, urinary excretion, or bone deposition or resorption.

Data from several surveys of the United States population indicate that the calcium intake of males varies from about 550-1300 mg/d with the greatest intake during late adolescence (mean about 1100 mg/d) and the least by men 50 yr and older (mean about 700 mg/d). The range for women is 400-1050 mg/d, with lowest intakes after 35 yr of age (mean about 540 mg/d).

Calcium is absorbed both by simple diffusion and by an active process involving 1,25-(OH)₂D₃. Calcium absorption is more efficient when its intake is low, thus favoring adaptation to reduced dietary levels. Both the efficiency of absorption and the ability to increase absorption in response to low calcium intake decrease as the individual ages. Some passive calcium absorption continues even after saturation of the vitamin D-mediated absorptive process. It is estimated that such passive diffusion approximates 15% of the ingested intake.

Some ingested calcium is rendered unavailable in the intestinal tract by phytates, oxalates, and fiber found in certain vegetables and grains. However, the reduction in calcium absorption by these agents is generally slight and appears to be of little nutritional significance in North American diets. Lactose enhances absorption of calcium in the rat but this effect has not
been confirmed in man. Some investigators have speculated that lactase deficiency, and the resulting reduction in intake of dairy products, may be a predisposing factor to osteoporosis. Supporting evidence for this hypothesis is inconclusive.

Despite an extensive literature on the subject, the calcium requirements for man remain a matter of dispute. There are wide variations in the intakes of different populations which presumably maintain normal skeletal status. Cross-sectional comparisons among groups are complicated by ethnic, cultural, socio-economic, dietary, and other factors. Further complications are posed by the varied techniques employed to assess bone status and by the demonstrated ability of most, but not all, individuals to adapt to different calcium intakes.

Most epidemiological surveys have failed to demonstrate a clear relationship between calcium intake and bone mass. However, one recent survey conducted on population groups of the same ethnic origin of the same country, living under similar rural conditions, but with markedly different calcium intakes, showed significantly greater bone mass and a reduced number of proximal femur fractures among the high calcium group.

A number of analytical techniques have been developed to assess bone status, but none is entirely satisfactory for all circumstances. There are several advantages and limitations associated with these techniques and no single method is utilized universally.

Individuals form and lose bone at different rates. The bone mass at maturity, the age of onset of net loss, and the rate of loss are all determinants of whether osteoporosis will develop. Age-related bone loss tends to follow an exponential course, so that the rate of loss is proportional to the initial mass of bone at onset of net loss.

Among white, perimenopausal women, a correlation has been noted between calcium intake and calcium balance. Women on higher intakes exhibit less negative balances, although zero balance has not been achieved. Investigators have calculated that an average daily intake of 0.99 g calcium for estrogen-treated and 1.50 g for untreated women would be required to achieve zero balance. Calcium supplementation significantly reduces bone loss among elderly women, as demonstrated by radiographic and photon absorptiometric measurements.

The recommended daily intake in the United States of phosphorus for the middle-aged and elderly population is 800 mg/d. American males have an estimated daily intake of phosphorus of 850-1700 mg/d and females about 700-1200 mg/d. Approximately 60-70% of phosphate from normal diets is absorbed. Phytate phosphorus is less readily available because of the relative deficiency of the enzyme phytase in the intestine.
Phosphorus is a ubiquitous constituent of food, so that its dietary deficiency in man is unlikely. Such a condition, however, may be induced inadvertently in patients receiving aluminum hydroxide for prolonged periods.

High intakes of phosphorus in several animal species have caused secondary hyperparathyroidism with significant bone loss. An elevated serum phosphate level is thought to induce a transient hypocalcemia which stimulates PTH secretion and causes eventual bone resorption. Urinary calcium is decreased, caused apparently by a shift of endogenous calcium excretion from urine to feces.

The secondary hyperparathyroidism and bone loss observed in certain animals receiving diets rich in phosphorus have not been demonstrated in man. Large doses of phosphorus stimulate PTH secretion temporarily, but it is uncertain whether its level is persistently increased by diets rich in phosphorus. Also, the net and long-term effects on bone of moderate increases of PTH is not known.

High phosphorus intakes induce decreased urinary excretion of calcium. Whether the calcium retained by the kidney is excreted in the feces, deposited in bone, soft tissue, or both has not been established. An increased rate of bone remodeling has been demonstrated, but there is no evidence that this causes bone loss.

In rodents, low calcium and high phosphorus intakes cause bone loss. The actual ratio producing this effect varies with the absolute intakes of the elements. On the basis of animal studies and of the relative contents in bone, a Ca:P ratio for human diets of 1:1 to 2:1 has been suggested as desirable. Survey data indicate a current ratio of about 1:1.6 in the United States dietary. There is no convincing evidence that phosphate intakes by man, which might reasonably be expected, are harmful provided the calcium intakes are adequate. Nevertheless, effects of long-term, high dietary phosphorus are unknown and prudence suggests that large intakes be avoided until more definitive data are available.

High protein diets in both man and animals cause increased calcium excretion in the urine, resulting primarily from reduced re-absorption in the renal tubules. The hypercalciuric effect occurs within 24 h after increasing protein intake and may continue for long periods. Some investigators found no tendency for urinary calcium to decrease during 48 d on a high protein diet; others reported an exponential decrease in calcium excretion beginning after 6-12 d of high protein feeding. The effect is dependent, at least partially, upon the sulfur-containing amino acids of the protein. The hypercalciuric effect, largely abolished when natural protein sources such as meat rather than purified proteins are fed, is generally attributed to the counteracting effect of their high phosphorus content.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreword</td>
<td>iii</td>
</tr>
<tr>
<td>Abstract</td>
<td>v</td>
</tr>
<tr>
<td>Synopsis</td>
<td>vii</td>
</tr>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>II. Normal Bone Structure and Homeostasis</td>
<td>3</td>
</tr>
<tr>
<td>A. Bone composition</td>
<td>3</td>
</tr>
<tr>
<td>B. Hormonal control of bone minerals</td>
<td>5</td>
</tr>
<tr>
<td>C. Methodology</td>
<td>9</td>
</tr>
<tr>
<td>D. Summary</td>
<td>11</td>
</tr>
<tr>
<td>III. Calcium and Skeletal Integrity</td>
<td>13</td>
</tr>
<tr>
<td>A. Dietary intake</td>
<td>13</td>
</tr>
<tr>
<td>B. Absorption</td>
<td>16</td>
</tr>
<tr>
<td>C. Calcium balance</td>
<td>19</td>
</tr>
<tr>
<td>D. Excess intakes</td>
<td>21</td>
</tr>
<tr>
<td>E. Epidemiologic surveys</td>
<td>23</td>
</tr>
<tr>
<td>F. Longitudinal studies</td>
<td>26</td>
</tr>
<tr>
<td>G. Calcium supplementation</td>
<td>27</td>
</tr>
<tr>
<td>H. Summary</td>
<td>28</td>
</tr>
<tr>
<td>IV. Phosphorus and Skeletal Integrity</td>
<td>31</td>
</tr>
<tr>
<td>A. Intake levels</td>
<td>31</td>
</tr>
<tr>
<td>B. Absorption and excretion</td>
<td>34</td>
</tr>
<tr>
<td>C. Phosphorus deficiency</td>
<td>34</td>
</tr>
<tr>
<td>D. High phosphorus intakes</td>
<td>35</td>
</tr>
<tr>
<td>E. Calcium:phosphorus ratio</td>
<td>38</td>
</tr>
<tr>
<td>F. Summary</td>
<td>39</td>
</tr>
<tr>
<td>V. Protein and Bone Metabolism</td>
<td>41</td>
</tr>
<tr>
<td>A. Hypercalciuric effect of high protein diets</td>
<td>41</td>
</tr>
<tr>
<td>B. Duration of hypercalciuric effect</td>
<td>43</td>
</tr>
<tr>
<td>C. Opposing effects of protein and phosphorus</td>
<td>44</td>
</tr>
<tr>
<td>D. Mechanism of hypercalciuria induction</td>
<td>44</td>
</tr>
<tr>
<td>E. Effect on skeletal integrity</td>
<td>47</td>
</tr>
<tr>
<td>F. Summary</td>
<td>48</td>
</tr>
<tr>
<td>VI. Conclusions</td>
<td>49</td>
</tr>
<tr>
<td>VII. Suggestions for Future Research</td>
<td>53</td>
</tr>
<tr>
<td>VIII. Literature Cited</td>
<td>57</td>
</tr>
<tr>
<td>IX. Study Participants</td>
<td>73</td>
</tr>
</tbody>
</table>
I. INTRODUCTION

Bone loss is a major health problem of the geriatric population, especially among white, postmenopausal women. Of the six million spontaneous fractures reported each year in the United States, approximately five million occur within this vulnerable group (Albanese, 1977). The incidence of such fractures increases with age; after the age of 60, the number of femoral neck fractures in women doubles every 5 yr (Alffram, 1964). The medical and economic costs exacted by this bone deterioration are enormous. Such costs are apt to become even more oppressive in the future since the number of elderly is increasing more rapidly than other segments of the American population. These considerations, entirely apart from humanitarian concerns, provide strong stimuli to search for causes of bone loss and for possible ameliorative measures.

Osteoporosis is the predominant form of excessive bone loss in this country. The term has been the source of considerable confusion because it has been defined in different ways by various investigators. In this report, osteoporosis refers to a condition in which bone has a reduced mass but an essentially normal mineral-to-matrix ratio, although there may be minor bone compositional changes (Arnold et al., 1966). Skeletal strength is impaired with an increased risk of fracture. In a related bone condition, osteomalacia, the mineral content is reduced, but the amount of organic matrix remains essentially normal. Osteoporosis has a multifactorial etiology involving racial, genetic, hormonal, physical, and nutritional factors (Avioli, 1980). Calcium and phosphorus constitute the principal minerals of the bone and consequently are the obvious candidates to be considered for nutritional interventions. Calcium, especially, has been the focus of numerous attempts to relate dietary intake in patients with osteoporosis.

The Food and Drug Administration (FDA) is the federal agency responsible for ensuring the safety and adequacy of the American food supply and for providing nutritional information to the public. The FDA has become increasingly concerned with the possibility that dietary inadequacy or imbalance might be contributing to bone fragility among elderly Americans. This concern has been heightened by the realization that nutrient intakes below recommended levels are more prevalent in this group than among younger adults. Several factors contribute to this situation, including the lower levels of physical activity of the elderly, their susceptibility to chronic or recurring illness, and irregular eating habits associated with living alone. Under these circumstances, both caloric and calcium intakes decline, although the need for calcium does not decrease proportionately to that for calories.
Intake of essential nutrients by large numbers of the American population at levels considered inadequate by recommended standards has prompted the FDA to reexamine current food fortification practices. It is currently reviewing available information as a basis for considering revisions of fortification policies. As part of this continuing exercise, the Associate Director for Nutrition and Food Sciences of the Bureau of Foods requested that the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (FASEB) review and evaluate the role of dietary calcium and phosphorus in bone homeostasis and skeletal integrity. This report focuses on calcium and phosphorus, and the effects of vitamin D and protein on their metabolism. A later meeting may be convened to review the influence of other dietary components in bone homeostasis and skeletal integrity in adults.
II. NORMAL BONE STRUCTURE AND HOMEOSTASIS

A. BONE COMPOSITION

Bone consists largely of a collagenous matrix in which calcium and phosphorus have been deposited as crystals of hydroxyapatite $[\text{Ca}_10(\text{PO}_4)_6(\text{OH})_2]$. Some amorphous calcium phosphate is present, as well as magnesium, zinc, sodium, carbonate, and fluoride (Raisz, 1977). Bone is not a passive tissue but undergoes continuous remodeling throughout life as new bone is deposited and existing bone is resorbed. According to Parfitt (1979) this continuous turnover of bone is brought about by specialized groups of cells termed bone remodeling units. New bone formation occurs largely at sites of former bone resorption. Locally excessive bone resorption or inadequate deposition by these bone remodeling units results in bone loss. Each cycle of bone remodeling activity begins with bone resorption by osteoclasts followed by new bone formation by osteoblasts. The difference between the amount of bone resorbed and the amount of bone formed by each cycle of bone remodeling activity has been termed the remodeling imbalance (Parfitt, 1979). A markedly negative remodeling imbalance occurs on the endosteal surface and a much smaller positive imbalance on the periosteal surface. The net bone loss at the endosteal surface is approximately ten times the gain at the periosteal surface. For a representative white, 40-70 yr woman, Parfitt (1980) calculated that the external metacarpal diameter increased by 0.3 mm, while the internal diameter increased by 3.0 mm, resulting in an overall decrease of 2.7 mm in cortical thickness. Little is known of the mechanism which couples bone resorption with bone formation.

During childhood and into early adulthood, bone accretion continues until skeletal maturity is reached. In young adults, a relatively steady state is achieved, with bone formation roughly equaling bone resorption. During middle age, bone resorption begins to exceed new bone formation, and slow bone loss then generally continues for the rest of life. As much as 30-50% of the mature bone mass may be lost. This loss begins earlier in women than in men, and the rate usually accelerates after menopause (Heaney et al., 1978a). The approximate gain or loss of calcium at various ages is shown in Table 1.

Scientific literature and clinical experience provide ample documentation that bone is lost during aging from all parts of the skeleton in both men and women of all races. Newton-John and Morgan (1968) analyzed 30 reports relating bone content to age and sex. All reports indicated a generalized bone loss, beginning at about age 35-45 yr in women and 45-65 yr in men. In men, the decrease in bone content was essentially linear with age. In women, the loss was greater immediately following menopause, with a somewhat slower decline thereafter. Although loss from all skeletal sites occurs during aging, the rate of loss varies with the site and nature of the bone.
Table 1. Approximate Calcium Balance with Age*

<table>
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<tr>
<th>Age (yr)</th>
<th>Calcium Balance (mg/d)</th>
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<tr>
<td>&lt; 1</td>
<td>+ 80</td>
</tr>
<tr>
<td>1-10</td>
<td>+150</td>
</tr>
<tr>
<td>10-20</td>
<td>+250</td>
</tr>
<tr>
<td>20-45†</td>
<td>0</td>
</tr>
<tr>
<td>45+†</td>
<td>- 40</td>
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* Modified from Avioli, 1980.
† Bone loss occurs earlier in women than in men.

Since the review by Newton-John and Morgan (1968) on bone loss during aging, considerable additional data have been obtained with more sophisticated methodology than was then available. Mazess (1981a) has recently analyzed the literature on age-related changes in trabecular and compact bone. From his review of absorptiometric data on several thousand subjects, he concludes that compact bone is lost at a rate of about 3% per decade in both sexes beginning at about age 40. In women after menopause, this loss increases to about 9% per decade until about age 75 when the rate of loss again reverts to about 3% per decade. Results with trabecular bone are more variable. According to Mazess (1981a), most results indicate a loss of 6-8% per decade for both sexes beginning in young adulthood. He states the general belief that trabecular bone is lost more rapidly after menopause may be erroneous. In any event, it is clear that trabecular and compact bone should be considered separately.

Newton-John and Morgan (1968) concluded that, not only did all persons lose bone with aging, but also the amount lost was approximately the same. They considered osteoporosis to be a consequence of senescence manifesting itself more frequently in individuals whose bone mass at skeletal maturity was less than normal. According to this concept, the dietary intake of calcium would be of greater importance during growth and early adulthood than after the individual had reached skeletal maturity. This does not imply that adequate calcium intakes are not important in adult life. Individuals with large bone mass acquired in early life, however, would be less likely to suffer fractures or other signs of bone depletion in their later years than persons with lesser bone content. The findings of various investigators that blacks have denser bones (Trotter et al., 1960) and fewer fractures (Gyepes et al., 1962; Nordin, 1966; Solomon, 1968) than age- and sex-matched whites are in accord with this hypothesis.
Johnston et al. (1979) however, have demonstrated that age-related bone loss is more likely to follow an exponential course rather than the linear model proposed by Newton-John and Morgan (1968). This concept implies that the rate of loss of bone is proportional to the initial mass of bone present at onset of loss, as contrasted to the linear model where there is no relationship to initial mass. Thus, individuals lose bone at different rates, with the risk of fracture depending not only upon the bone mass at maturity, but also upon the age of onset of net loss and the rate of loss. Consequently, it is impossible to determine at adult skeletal maturity who will be at greatest risk of low bone mass in old age.

Calcium levels in blood and tissues are normally maintained despite widely varying dietary intakes. Bone contains approximately 99% of the body's calcium and serves as a reservoir to meet varying demands (Avioli, 1980). Consequently, even a modest but persistent excretion of calcium in excess of intake (negative calcium balance) resulting from inadequate calcium intake, reduced intestinal absorption, or increased urinary excretion may eventually cause serious loss of bone tissue.

B. HORMONAL CONTROL OF BONE MINERALS

Three hormones are primarily involved in maintaining the homeostatic control of calcium and phosphorus: parathyroid hormone (PTH), 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃], and calcitonin, a hormone produced mainly by the thyroid gland, but also by the thymus and parathyroid glands. The interrelationships among these hormones in maintaining calcium homeostasis have been represented diagrammatically by DeLuca (1979) (Figure 1).

The parathyroid gland is sensitive to the level of serum calcium; hypocalcemia stimulates and hypercalcemia depresses the secretion of PTH. To maintain normal levels of calcium, PTH acts directly or indirectly on the three target organs largely involved in such control: intestine, bone, and kidney. It stimulates the production of 1,25-(OH)₂D₃ in the kidney, which in turn promotes increased calcium absorption from the intestine (DeLuca, 1979). It increases tubular reabsorption of calcium in the kidney, while decreasing the reabsorption of phosphate, both measures tending to maintain the calcium levels of serum. Finally, it stimulates resorption of bony tissue to restore calcium in blood and intercellular fluids to physiological levels (Avioli, 1980; DeLuca, 1979). Parfitt and Klererekoper (1980a) postulate that PTH stimulates bone turnover, increasing the rate both of periosteal gain and endosteal loss. Since the latter process is usually the greater, the net result is bone loss.

Vitamin D is first hydroxylated in the liver to 25-hydroxyvitamin D₃ (25OHD₃) which is then further hydroxylated in the kidney to 1,25-(OH)₂D₃ (DeLuca, 1979). This latter metabolite acts upon
Figure 1. The Calcium Homeostatic System (DeLuca, 1979).

In response to hypocalcemia, the parathyroid glands (PTG) secrete PTH, which in turn binds to the kidney and bone. In the kidney, it stimulates production of 1,25-(OH)$_2$D$_3$, which then stimulates the mobilization of calcium from bone and renal reabsorption of calcium. The resultant rise in extracellular fluid (ECF) (plasma) calcium shuts off secretion of PTH. If calcium rises above the normal value of 10 mg/dl, calcitonin (CT) is secreted from the "C" cells of the thyroid. This hormone blocks the mobilization of calcium from bone, thereby suppressing plasma calcium concentration.
the intestine, bone, and kidney and is primarily responsible for the effects originally attributed to "vitamin D". The primary function of 1,25-(OH)_2D_3 is the mobilization of calcium from various sites to maintain physiological concentrations in the circulatory system and intercellular fluids. Towards this end, it enhances the absorption of calcium (and of phosphorus) from the intestine, liberates calcium by bone resorption, and stimulates the reabsorption of calcium by the renal tubules (Haussler and McCain, 1977a,b). It has an apparently close, but as yet ill-defined, relationship with PTH and calcitonin. Low calcium intakes stimulate the production of 1,25-(OH)_2D_3, a response which requires the presence of vitamin D as well as the parathyroid glands (Hughes et al., 1975). Serum phosphate, independently of the presence of PTH, also affects the circulating levels of 1,25-(OH)_2D_3; low phosphate levels increase 1,25-(OH)_2D_3 concentrations (Haussler et al., 1977). Paradoxically, high serum phosphate may also lead to high 1,25-(OH)_2D_3 levels (Siu and Draper, 1981). This effect is explained by the depression in serum calcium which accompanies hyperphosphatemia. Any inhibitory effect of excess phosphate on the 1α-hydroxylase enzyme is overcome by the stimulating effect of depressed serum calcium.

The relationship of 1,25-(OH)_2D_3 to calcitonin is still unclear. Contrary to expectations, injection of calcitonin into rats increased rather than decreased the production of 1,25-(OH)_2D_3 (Spanos and MacIntyre, 1980). Because this effect depends upon the presence of parathyroid glands, it may merely reflect the action of PTH. Spanos and MacIntyre (1980) pointed out that under conditions of physiological stress, such as growth, lactation, and pregnancy, serum calcitonin and 1,25-(OH)_2D_3 are both elevated. They speculated that under these conditions, calcitonin "...by opposing the resorptive action of 1,25-(OH)_2D_3 on bone, preserves the integrity of the skeleton, thus directing the action of 1,25-(OH)_2D_3 to the gut to meet the need for calcium."

Because both low serum calcium and low serum phosphate cause an increased production of 1,25-(OH)_2D_3, which then stimulates the absorption of both ions, it is puzzling how an excess of one is avoided while the normal level of the other is being restored, that is, why does hypocalcemia not induce a hyperphosphatemia, and vice versa? The answer appears to reside in the opposite effects PTH has upon the renal reabsorption of these ions: inhibiting the reabsorption of phosphate while stimulating that of calcium (Haussler and McCain, 1977a,b). Hypocalcemia, by stimulating PTH production, favors renal reabsorption of calcium and urinary excretion of phosphorus. Hypophosphatemia has opposite effects. In both cases, the PTH action tends to restore normal blood levels of calcium and phosphorus.

The role of vitamin D in rickets and osteomalacia is well known. In addition, disturbances in the vitamin D system may be important in the development of various other bone disorders including those found in renal osteodystrophy, vitamin D-resistant
rickets, hypoparathyroidism, and perhaps in osteoporosis (DeLuca, 1979). The plasma 1,25-(OH)₂D₃ levels of postmenopausal, osteoporotic women are about 30% lower than age- and sex-matched controls, which corresponds closely with the level of reduced calcium absorption by these women. Administration of small amounts (0.5 µg/d) of 1,25-(OH)₂D₃ to postmenopausal women caused an increased intestinal absorption of calcium and improved calcium balance (DeLuca, 1979).

Calcitonin is an antihypercalcemic hormone elaborated mainly by the parafollicular cells of the thyroid gland but also by multiple extrathyroidal sources, including the thymus and parathyroid glands. Its secretion increases during hypercalcemic states and decreases markedly when plasma ionized calcium is depressed (Austin and Heath, 1981). Its best known effects are the lowering of plasma calcium and inorganic phosphorus concentrations by acting on bone and kidney. It also has diverse, but poorly understood, effects on various other organs including the gastrointestinal tract, liver, and pancreas, but little is known as yet of their significance. It is a potent inhibitor of osteoclastic bone resorption, manifesting its inhibiting effects on resorbing surfaces as early as 15 min after administration. In the kidney, calcitonin decreases renal tubular reabsorption of calcium and phosphate, which also tends to lower serum levels and combat hypercalcemic states. The prompt and striking inhibition of bone resorption by calcitonin has prompted its trial in certain bone disorders, including osteoporosis (Holtrop et al., 1974). Clinical trials with calcitonin alone have been inconclusive, but additional studies are in progress in which calcitonin is combined with calcium supplements (Chestnut et al., 1980). Thyroidectomized patients, however, are able to maintain normal ionized calcium levels despite the lack of detectable calcitonin in the blood (Grubb et al., 1979).

Other hormones, especially estrogens and glucocorticoids, may also modify calcium or phosphorus metabolism. A relationship between menopause and bone loss has long been recognized, suggesting that estrogens are important in normal bone maintenance. Estrogen therapy is widely used to inhibit bone turnover in postmenopausal women. It has been reported to prevent the accelerated bone loss normally occurring after menopause (Lindsay et al., 1980) and to reduce substantially the incidence of vertebral, femoral neck, and wrist fractures (Hutchinson et al., 1979). Young and Nordin (1967) reported that plasma calcium and phosphorus tend to increase after menopause and can be reduced significantly with estrogen treatment (Riggs et al., 1969). Nordin et al. (1970) have suggested that estrogen may act by decreasing the sensitivity of bone to the resorptive action of PTH.

Estrogen may also be involved in the production of 1,25-(OH)₂D₃. In birds, estrogen administration markedly stimulated the conversion of 25OHĐ₃ to 1,25-(OH)₂D₃ by renal 25OHĐ₁α-hydroxylase (DeLuca, 1980). The levels of plasma 1,25-(OH)₂D₃ are
markedly elevated and are probably responsible for the stimulation of intestinal calcium absorption to provide the calcium necessary for eggshell formation.

Other hormones including glucocorticoids, growth hormone, androgens, thyroxine, insulin, and glucagon may also be involved in bone homeostasis (Raisz, 1977). Their effects and mechanisms of action are still poorly understood and are not considered in this report.

C. METHODOLOGY

A number of techniques have been utilized in studying bone status. These can be broadly divided into balance studies, epidemiologic surveys, bone mass determinations, bone turnover or remodeling rates, and morphometric techniques. In general, simple measurements adaptable to mass surveys suffer from lack of precision or accuracy, while highly precise measurements are limited to smaller experimental cohorts.

Epidemiologic studies of osteoporosis have generally relied on simple indices of bone strength, such as the incidence of fractures, or x-ray measurements of bone mass. Bone fracture depends not only on bone strength but also the severity of trauma, which is uncontrollable and unquantifiable. Nevertheless, this event has been widely used in surveys (Chalmers and Ho, 1970; Matkovic et al., 1979; Moldawer et al., 1965) as a criterion of osteoporosis because of its unequivocal nature and its ready identification from even unsophisticated medical records.

Similarly, radiographs provide only relatively crude measures of bone mass and strength. Variations in film exposure and development, the quantity and nature of the overlying soft tissue, and the subjective impressions of observers all affect the final interpretation. Lachman (1955) estimated that a bone loss of 30% was necessary before it could be detected by routine skeletal x-rays. According to Mazess (1979), radiogrammetry of the hands does not reflect actual bone mass or intracortical porosity and consistently underestimates bone loss by 20-30%. Although it is less accurate and precise than mineral measurements, it has the advantage of high anatomic specificity and of allowing changes at the periosteal and endosteal surfaces to be distinguished (Parfitt, 1980). It is adaptable to mass surveys and, when the analyses are performed by the same investigators under standardized conditions, useful comparisons between different population groups are possible (Garn, 1970).

A number of techniques have been developed to provide more sensitive and precise measures of bone status. These include photon absorptiometry, radionuclide uptake, neutron activation, Compton scattering, computed tomography, and histomorphometry.
Single-photon absorptiometry and computed tomography provide measures of peripheral bone mass with a precision error less than 3% (Mazess, 1979). The former technique is widely available and provides an accurate index of changes in cortical bone. However, both techniques have certain disadvantages. Photon absorptiometry does not discriminate between the trabecular bone of the vertebral bodies and the bone of the vertebral processes. Also, it could detect calcium in paraspinal ligaments, aorta, and other soft tissues, although the procedures used by various groups generally minimize soft-tissue contributions. The cost, accessibility, and radiation dose, limit the wide use of computed tomography. Also, single energy, computed tomography of trabecular bone is subject to significant errors because of variability in the lipid content of bone marrow. Precise trabecular bone measurements, including dual-photon absorptiometry, computed tomography, and Compton scattering, are still largely confined to research laboratories although commercial equipment is now available for dual-photon absorptiometry of bone. Neutron activation provides a useful technique for determining total body calcium but does not differentiate between bone and extracellular calcium. Histomorphometry offers valuable etiologic information, but is an invasive technique not adaptable to surveys or to repeated measurements on the same individuals. Furthermore, the data offer little information on the bone mass or strength in certain critical skeletal areas.

All measures of the skeletal status suffer from the inherent difficulty that bone loss is not uniform throughout the skeleton. Measurements of one bone cannot be extrapolated with confidence to another bone, or indeed, to other sites of the same bone. Loss in the axial skeleton may differ markedly from that in the appendicular skeleton. Loss of trabecular bone with aging has been reported to occur both more slowly (Madsen, 1977) and more rapidly (Cann et al., 1980) than cortical bone.

Riggs et al. (1981) have recently studied the patterns of bone loss in the axial and appendicular skeletons in normal and in osteoporotic men and women. In normal women, the bone mineral density of the lumbar spine (trabecular bone) began to diminish in young adulthood and continued in linear fashion during aging. In the midradius (mainly cortical bone), bone diminution did not occur until age 50, then accelerated somewhat until age 65, after which it decelerated. In normal men, bone loss, both vertebral and appendicular, was minimal with aging. The cumulative diminution of bone mineral density in the vertebrae, between young adulthood and extreme old age, was 47% for women and 14% for men. The investigators concluded that a disproportionate loss of trabecular bone from the axial skeleton is a distinguishing characteristic of spinal osteoporosis and represents an extreme form of the normal aging process. By age 65, half the normal women studied had vertebral bone mineral density below the 90th percentile of women with vertebral fractures (0.965 g/cm³). By age 85, virtually all women had vertebral bone mineral densities below this level and could be considered to have "asymptomatic
osteoarthritis". Riggs and coworkers (1981) suggested that cortical and trabecular bone appear to function as separate compartments which differ both in their onset and rate of bone loss, and possibly are subject to different homeostatic controls.

Another example of differences in bone from various skeletal sites is the response of osteomalacic patients to vitamin D. In such patients, mineralized bone in the ilium increased 50%, whereas the mineral content of radii increased only 2% during the same period (Mathews et al., 1980).

At present there is no simple measurement available to assess the status of spinal trabecular bone. Changes in vertebral shape often occur from microscopic fractures and are about ten times as frequent as overt fractures of the spine. These changes in vertebral shape might serve as useful early indications of osteoporosis (Horsman, 1976).

Alveolar bone appears to be especially susceptible to resorption so that loss at this site may be an early manifestation of more generalized osteopenia. Alveolar bone has a much higher rate of turnover than other bony sites. In the rat, the alveolar bone turnover is 100 times that of the tail bone (Vignery and Baron, 1981). Henrikson (1968) demonstrated loss of alveolar bone radiographically in adult dogs on low calcium diets before losses from other bones could be detected. Krook et al. (1975) have described a metabolic bone disease, termed nutritional secondary hyperparathyroidism, which occurs spontaneously in many animal species and is characterized by enhanced progressive bone resorption. The excessive bone loss occurs first in the jaw bones, notably of the alveolar bone. Krook et al. (1972) necropsied four osteoporotic humans and found greater bone loss from their jaws than from ribs, vertebrae, or long bones.

D. SUMMARY

Bone consists largely of a collagenous matrix in which calcium and phosphorus have been deposited as hydroxyapatite. The skeleton acts as a massive calcium reservoir to meet varying body demands. There is continual resorption and deposition of bone throughout life, a process termed bone remodeling, and which is brought about by specialized groups of cells termed bone remodeling units. During childhood and into early adulthood, accretion processes predominate. During early adult life, the opposing processes are in rough equilibrium. Bone loss begins at about age 40 for women and about age 50 for men; it is especially marked in postmenopausal women. Calcium and phosphorus metabolism are regulated primarily by sensitive interactions of three calcitropic hormonal systems: 1,25-(OH)2D3, PTH, and calcitonin. These act upon the target organs, intestine, kidney, and bone, to modify the absorption and excretion of calcium and phosphorus and to maintain normal levels of these elements in the extracellular fluid. Other
hormones, especially estrogens, glucocorticoids, and growth hormone, also have roles in bone metabolism but are not discussed in detail in this report.

The techniques employed to measure bone status are briefly reviewed and some of their advantages and shortcomings are discussed. Although all bone undergoes loss during aging, the responses of trabecular and cortical bones differ and must be evaluated separately. Data obtained with one form or at a given site cannot be extrapolated with confidence to another form or site.
III. CALCIUM AND SKELETAL INTEGRITY

A. DIETARY INTAKE

Numerous surveys have been conducted in many countries to determine the calcium intakes of different population groups (Garn et al., 1967; Irwin and Kienholz, 1973). Current estimates of calcium intake by the American population are based largely on two periodic surveys, the Nationwide Food Consumption Survey (NFCS) (U.S. Department of Agriculture, 1980) and the Health and Nutrition Examination Survey (HANES) (Abraham et al., 1979; National Center for Health Statistics, 1981). The most recent NFCS study was conducted in 1977-1978 and included a 24-h recall and a 2-d diary from individuals in households within the contiguous 48 states. The intakes of selected nutrients were calculated from standard food composition tables. Because error is inevitable in converting some 4500 separate food items and servings of various sizes into quantitative data, the calculated range and mean values represent only rough approximations of actual calcium intakes (Pao, 1980). A preliminary analysis of the intake of 9620 individuals is summarized in Table 2. These values represent only spring quarter data for 1977 and may reflect seasonal dietary variations. Calcium intakes among males were greatest during early and mid-teen years and lowest among men over 75 yr of age. Calcium intakes of females gradually but steadily decreased from the immediate pre- and early-teen years to the perimenopausal years.

The National Center for Health Statistics has conducted two studies: HANES I in 1971-1974 and HANES II in 1978-1980. Data were obtained on approximately 20,000 noninstitutionalized subjects by 24-h dietary recall, coupled with physical, x-ray, blood, and urine examinations. The dietary data reflect a single day's intake as elicited by trained personnel during a 20-min interview. The subjects were grouped according to age, sex, and economic status. Although data for HANES II are not yet available, values from HANES I (Table 3) agree roughly with those obtained by NFCS. Direct comparison is impossible because of differences in the analytic groupings. Again, the calcium intake of women was markedly below that of men, with the lowest intakes by any group occurring among adult black women. As with the NFCS values, these data reflect approximate calcium intakes because of inherent methodological shortcomings.

In addition to these two nationwide surveys, the FDA also conducts a Total Diet Study which includes the analysis of selected minerals (Harland et al., 1980). These so-called "market basket" samples are collected throughout the United States for three age groups: the 6-mo-old infant, the 2-yr-old toddler, and the 15- to 20-yr-old male. Food purchases reflecting the representative 4-wk diet of a 15- to 20-yr-old male and a 2-wk diet for infants and toddlers are made every year in four geographic regions of the United States. Composite food samples
<table>
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<td>&lt; 1*</td>
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<tr>
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* Total; no differentiation by sex.
Table 3. Average Calcium Intakes (mg/d) (National Center for Health Statistics)*†

<table>
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<th>Female</th>
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</table>

* Unpublished data from HANES I, 1971-1974, presented by Dr. Jean Pennington at ad hoc conference, January 12-13, 1981, with permission from National Center for Health Statistics.

† The first paired value in all cases is for individuals below the poverty level and the second for those above this level. The poverty level varies depending on site of residence, sex of family head, and family size. It approximates $2000/yr for individuals and $3700/yr for families.
are analyzed in the Kansas City Field Office Laboratory for some essential minerals. Calcium and phosphorus were analyzed in 1974, 1975, 1978, and 1979 for adult diets and in 1975, 1976, 1978, and 1979 for infant and toddler diets, all based on food consumption data from U.S. Department of Agriculture's NFCS of 1965. The estimated calcium contents in infant, toddler, and male teenage diets in 1979 were 840, 816, and 1122 mg/d, respectively. The calculation for the teenage male group assumed a 2800 kcal/d intake.

Page and Friend (1978) utilized national food supply statistics to study the changing pattern of the United States diet during this century. They estimated the current dietary content of calcium to be about 930 mg/d, compared with 820 mg/d at the beginning of the century and 980–1000 mg/d when consumption of dairy products was at a peak (1947–1959). Data on age and sex distribution were not shown. It must be pointed out, that these values refer to the calcium available for consumption in the food supply and do not indicate the actual amount consumed.

B. ABSORPTION

The intestinal absorption of calcium is a complex process influenced by the amount of calcium ingested; its chemical and physical nature; the age, sex, and vitamin D status of the individual; the presence of other dietary constituents; and the intake of certain drugs.

Calcium is absorbed both by active transport and by diffusion (Wasserman, 1981). The vitamin D metabolite 1,25-(OH)_{2}D_{3} plays an important role in the former process, presumably through its induction of a calcium-binding protein in the intestine. Fractional calcium absorption, within limits, varies inversely with the calcium intake. Calcium is absorbed more efficiently when the dietary intake is low than when it is high. Spencer et al. (1969) determined the absorption of {sup 47}Ca in subjects receiving diets low (209 mg/d) and high (2108 mg/d) in calcium. Each subject served as his own control. The average {sup 47}Ca absorption on the low calcium diet was 63.6% but was only 30.5% with the high calcium intake. Even in a calcium-replete individual, some calcium will be absorbed by diffusion processes from a high calcium diet (Harrison, 1959).

Both the efficiency of calcium absorption and the ability to increase absorption in response to low intake of calcium decrease as an individual ages (Gallagher et al., 1979). A recent report (Slovik et al., 1981) suggests that this decreased absorptive ability in the elderly may be due to an impaired production of 1,25-(OH)_{2}D_{3} in response to PTH stimulation. Slovik et al. (1981) reported significantly lower levels of serum 1,25-(OH)_{2}D_{3} among elderly subjects after PTH infusions than in normal young adults.
Harrison (1959) reviewed data from reports in the literature and concluded that absorption decreased from about 70% of intake among premature infants to about 20% among adults. Bullamore et al. (1970) gave $^{45}$Ca or $^{47}$Ca by mouth to individuals ranging in age from 20–95 yr. They found that absorption began to fall at about 55–60 yr in women and at 65–70 yr in men. Avioli et al. (1965) reported absorption to be significantly less among postmenopausal (55–85 yr) than among premenopausal (16–32 yr) women. Spencer et al. (1964a,b) reported that patients with osteoporosis have a decreased ability to absorb calcium during high calcium intakes compared with young persons or patients of comparable age without osteoporosis.

Various dietary constituents may alter the bioavailability of calcium. The relationships of calcium with phosphorus and protein are considered in more detail later (see Sections IV and V). In addition, calcium absorption may be influenced by lactose, phytates, fat, oxalates, magnesium, fluoride, and fiber. Although fecal calcium increases in patients with steatorrhea, dietary fat within the normal range has little effect on calcium absorption (Aub et al., 1937; Riggs et al., 1967).

Phytates and oxalates form insoluble salts with calcium; excessive dietary intakes can reduce significantly the absorption of calcium. McCance and Widdowson (1942) found that subjects absorbed 38% of ingested calcium when consuming a diet containing white bread, but only 7% on a similar diet with the same amount of brown bread (containing 99 mg phytic acid phosphorus per 100 g bread). However, Bronner et al. (1956) found no significant difference in calcium absorption among boys fed oatmeal containing 80 mg phytate phosphorus or farina containing no phytate. Wasserman (1960) expressed the general belief that phytate posed no significant problem in this country.

The presence of oxalates in spinach, kale, rhubarb, cocoa, and other foodstuffs has also evoked concern that calcium absorption might be seriously reduced, especially among individuals on restricted diets. Experimental results have been equivocal. Bonner et al. (1938) failed to demonstrate any effect on calcium absorption when spinach containing about 700 mg oxalic acid was added to the daily diet of preadolescent children. Johnston et al. (1952) compared fecal calcium excretion in six young women consuming a basal diet before and after adding spinach containing 600 mg oxalic acid. After spinach ingestion, the fecal calcium increased from 673 mg/d to 838 mg/d, but the calcium balance was not significantly affected. Most investigators agree that the oxalate content in normal North American diets poses no serious problem.

In contrast to the action of phytates and oxalates, lactose has been reported to enhance calcium absorption in the rat (Armbrecht and Wasserman, 1976; Lengemann, 1959; Wasserman, 1964),
but this effect has not been proved in human subjects (Greenwald et al., 1963). Dietary lactose, but not glucose, significantly improved the absorption and retention of calcium during rat growth as reflected by increased femur mass after 42 wk (Schaafsma and Visser, 1980). The exact mechanism of such stimulation remains uncertain, but lactose appears to influence the passive or diffusional process rather than the active, energy-dependent process. Armbrecht and Wasserman (1976) incubated everted gut sacs from the rat ileum with lactose or saline controls for 45 min and then measured the tissue uptake of added $^{45}$Ca. The calcium uptake by the lactose-incubated tissue was markedly greater than by the control gut and was dependent upon the length of the incubation period. The presence of lactose was not necessary during the actual uptake period, suggesting that the increased absorption resulted from an interaction of lactose with the intestinal mucosa, rather than by the formation of a calcium-lactose complex.

The effects of lactose on calcium absorption in man are equivocal. A slight, but not significant, improvement in calcium balance was reported by Mills et al. (1940) when a daily dose of 36 g lactose was given to boys from 5–7 yr of age. Greenwald et al. (1963) reported an increased absorption of about 10% when $^{45}$Ca was fed to an elderly male subject receiving 50 g lactose daily in three divided doses for several weeks. The calcium balance of three other subjects was not improved when 20–50 g/d lactose were given for 18–34 d.

The role of lactose in calcium absorption has aroused clinical as well as theoretical interest because lactase deficiency is more prevalent among osteoporotic patients than among age- and sex-matched cohorts. This relationship was first reported by Birge et al. in 1967, and more recently by Newcomer et al. (1978), suggesting that lactase deficiency might be a predisposing factor to osteoporosis, because individuals with this condition would presumably curtail their consumption of dairy products, the major source of calcium. Nevertheless, the relationship between lactase deficiency and osteoporosis remains tenuous. As pointed out in a recent editorial (Anonymous, 1979), American blacks are more prone to lactase deficiency than whites, yet exhibit a considerably lower frequency of osteoporosis.

The usual 50 g test load of lactose may overestimate the significance of lactose intolerance as a nutritional problem. Of Alaskan Eskimos tested with this load, 70–80% were judged to be lactase deficient, but only about 10% showed any signs of deficiency with the amount of lactose in a glass of milk (about 10 g) (Bell et al., 1973). One of the participants at the FASEB study commented that the calcium intakes of blacks may be higher than generally assumed because of their liberal use of dairy products in cooking and baking.
C. CALCIUM BALANCE

The calcium requirements of human adults and the relation of the calcium intake to osteoporosis have been subjects of sharp controversy for more than a generation. The calcium requirements for adults have been estimated by different investigators to range from 300 mg/d to four or more times that amount.

The failure of investigators to agree on calcium requirements can be attributed to various reasons. For example, the analytical techniques for measuring bone loss or status vary markedly in their precision (Mazess, 1979). Grossly diverse dietary patterns and cultural habits of different ethnic groups may modify requirements (Draper and Scythes, 1981). There are wide individual variations of calcium needs. Activity and other nondietary factors may influence calcium requirements (Avioli, 1980). Short-term studies often give misleading results. Man has a striking ability to adapt to different calcium intakes. All of these factors have contributed to the continuing controversy and the wide range of reported requirements.

The effect of calcium restriction in animals can be demonstrated by direct bone examination or analysis. More than a half-century ago, Bauer et al. (1929) reported that the thickness of bone trabeculae of the cat varied with calcium intake. Similarly, rats with high intakes of calcium showed greater bone calcium content (Campbell et al., 1935; Sherman and Booher, 1931) and bone density (Ellinger et al., 1952; Schraer and Schraer, 1956) than those on lower intakes of calcium.

For studies with human subjects, investigators have utilized a variety of indirect techniques in attempts to relate calcium intake to bone integrity. A substantial literature is available on the utility and shortcomings of these various methods (Avioli, 1980; Mazess, 1975, 1980). For estimates of human requirements, calcium balance has been the method most frequently employed. The difference between calcium intake and excretion represents retention or loss with a zero balance reflecting maintenance requirements. Wedon (1959, 1964) has discussed in detail the inherent errors of this method and the precautions that must be employed to minimize them.

A wide range of calcium intakes has been reported in which subjects have apparently maintained normal skeletal integrity. The variation of apparent individual requirements is well illustrated by the early short-term studies of Steggerda and Mitchell (1951) who maintained adult males on controlled diets for 8-20 d. Calcium balances among these men ranged from -2.70 to +1.99 mg/kg body weight/d. Positive balances were achieved in some subjects with daily intakes of calcium as low as 2-3 mg/kg (<200 mg total) whereas other subjects remained in negative balance with intakes of 9-10 mg/kg (600-700 mg).
The balance data usually cited as reflecting low requirements of calcium are those of Hegsted et al. (1952) who studied the calcium intake and excretion of 10 male inmates, 30-56 yr of age, of a Peruvian prison. Calcium balances were determined when varying amounts of calcium were given for 10-d dietary periods. The mean calcium requirement was estimated to be very low, either 126 or 216 mg/d, depending on the mathematical treatment of the data. This study has been subject to criticism for ignoring calcium intakes between meals and for employing dietary periods too short to allow adaptation to the calcium intake. As Whedon (1959, 1964) has pointed out, calcium balance studies require careful dietary control, as well as measurement for a relatively prolonged period of calcium intake and excretion.

Malm (1958) utilized considerably longer periods of observation by studying the calcium balance of 26 male Norwegian prison inmates, 20-69 yr of age (average 45 yr), for over 2 yr. Twenty-three of the subjects were still able to achieve calcium balance when their intake was reduced from an original level of about 930 mg to 430 mg/d, indicating an ability to adapt to a reduced intake by more efficient absorption of ingested calcium. Also calcium absorption is enhanced by previous calcium restrictions (Nordin et al., 1979). In Malm's study, however, three of the subjects on low intakes of calcium remained in negative calcium balance, suggesting that most, but not all, individuals can adapt to reduced intakes.

Subjects in these early studies were mostly adult males. These results cannot be extrapolated uncritically to other population groups, especially to perimenopausal women whose calcium requirements may be markedly different. This has been demonstrated by Heaney et al. (1977) who studied 130 normal women, aged 35-50 yr, on their usual, self-selected, dietary calcium intakes. The women exhibited an average negative balance of 31 mg daily on a mean intake of 661 mg calcium. Since dermal loss was not measured, the investigators estimated that the actual negative balance was probably 40-50 mg daily. A positive correlation was noted between calcium intake and balance; women on higher intakes exhibited less negative balances. The investigators calculated that an average daily intake of 1.24 g calcium would be required to maintain a zero balance. They also pointed out that although this calculated requirement was considerably higher than most published values, it represented only an average value for the perimenopausal women they studied. It should be noted (Table 2) that this is more than twice the average intake of calcium for U.S. women in this age group (515 mg/d).

The women in the perimenopausal group of Heaney and co-workers (1977) were of mixed estrogen status. In a subsequent study with a larger group of subjects (Heaney et al., 1978b), women were separated into pre- and postmenopausal categories. In 207 studies of calcium balance with premenopausal women, mean age 42.3 yr, an average negative balance of 19.9 mg/d occurred
with an intake of 646 mg/d calcium. Forty-one untreated postmeno-
pausal women, mean age 46.5 yr, receiving an average of 659 mg/d
calcium had a mean negative calcium balance of 42.7 mg/d. No
difference could be detected between premenopausal women and
estrogen-treated postmenopausal women. The calculated calcium
intakes which theoretically would produce zero balance were
990 mg/d for estrogen-replete women and 1.504 g/d for untreated
postmenopausal women. This demonstration that calcium require-
ments are related to estrogen status suggests the need for in-
creased calcium intakes by postmenopausal women. Most of the
ad hoc review panel agreed that generous calcium intakes by this
group are desirable.

Nordin et al. (1979) have collected from the literature
details of 212 calcium balances of normal subjects (age range and
sex not specified) receiving various calcium intakes. The average
level at which calcium intake and excretion were equal was 578 mg.
They also calculated that a daily intake of 900 mg calcium was
necessary to ensure that 95% of normal adults would be in calcium
balance. This value approximates an earlier independent estimate
by Whedon (1964), based on various published reports, that 1.09 g/d
was the average requirement for adults. For postmenopausal women,
Whedon (1964) indicated that even larger, but unspecified, intakes
of calcium would be required to maintain calcium balance.

Heaney et al. (1977) compared the recommended calcium in-
takes (in mg/kg) of various animals with their adult body weight.
When plotted on full-logarithmic coordinates, the data closely fit
an inverse power function which could be represented by a straight
line. Data on animals within a broad weight range adhered closely
to this curve except for swine and man. The recommended calcium
intake for swine was about three times and that for man about one-
fifth the values predicted by the curve (Figure 2).

D. EXCESS INTAKES

The continuing controversy over calcium requirements has
primarily concerned the intake levels adequate for normal indi-
viduals of different age groups, especially postmenopausal, white
women. There has been relatively little concern of possible excess-
ive intakes. Since calcium absorption becomes progressively less
efficient as the intake increases, an endogenous control mechanism
appears to be in place to prevent excessive body levels (Avioli,
1980). However, some passive calcium absorption continues even
after saturation of the vitamin D-mediated absorptive process
(Stanbury, 1980). Heaney et al. (1975) gave high intakes of
calcium to normal individuals which suppressed the formation of
1,25-(OH)₂D₃. Absorption by passive diffusion approximated 15% of
that ingested. Similarly, uremic patients, whose ability to
produce 1,25-(OH)₂D₃ is virtually or totally destroyed, still ab-
sorbed up to 1 g daily by passive diffusion when doses of 8-10 g
calcium were administered (Clarkson et al., 1970).
Figure 2. Recommended Calcium Intakes Expressed as a Function of Body Weight (Heaney et al., 1977).
Some individuals can absorb calcium much more efficiently at given intake levels than their age- and sex-matched counterparts. At least in some cases, this superior absorptive capability may be due to relatively high levels of circulating 1,25-(OH)$_2$D$_3$. In 30 of 50 patients with primary hyperparathyroidism, Broadus et al. (1980) found hyperabsorption of calcium, striking hypercalciuria, marked elevations in 1,25-(OH)$_2$D$_3$, and a high incidence of renal stones. Increased levels of 1,25-(OH)$_2$D$_3$, as a consequence of prolonged exposure to the sun, have also been postulated as the probable explanation for the high prevalence of renal stones detected by Better et al. (1980) among lifeguards in Israel. These investigators reported that the hormonal level was twice that of a control group, and the incidence of kidney stones among lifeguards was ten times that of the general population.

Because the body calcium is largely concentrated in bone, changes in calcium balance are generally assumed to indicate accretion or resorption of bone. As one member of the study group pointed out, however, the fractional calcium content in extraosseous tissue increases markedly with age, a factor which could influence interpretation of calcium balance and of neutron activation studies. Thus, despite limited data, the possibility of calcium changes in nonosseous tissues cannot be ignored.

E. EPIDEMIOLOGIC SURVEYS

Epidemiologic studies have attempted to answer the critical question of whether diminished skeletal integrity is a normal consequence of aging dependent on genetic or ethnic factors, or if it is a reflection of the amount and nature of calcium intake. If bone loss is a universal phenomenon of aging, relatively independent of calcium intake, the rationale for calcium supplementation would be seriously weakened.

The scientific literature and clinical experience document the loss of bone during aging in both men and women of all races. Furthermore, bone is lost from all parts of the skeleton with increasing age. Epidemiologic studies have attempted to identify factors in addition to age, especially calcium intakes, which relate to bone loss.

Chalmers and Ho (1970) compared, in several geographic areas, the incidence of hip fractures among women 65-75 yr of age with differing dietary and cultural habits. The incidence of such fractures per 100,000 women was: 290-491 in Sweden; 159 in Great Britain; 103 in Hong Kong; 69 in Singapore; and 12-16 among the Bantus in South Africa. Thus, the incidence of fractures among the Chinese of Hong Kong and Singapore, and especially among the Bantus of South Africa was much lower than that of the British or Swedes, despite the higher intakes of calcium of the latter groups. Similarly, Moldawer et al. (1965) found the incidence of hip fractures among American white males to be 5.6 times that of their
black counterparts, and among white women to be 3 times that of black women. Mayor et al. (1980) reported that both black men and women had greater bone density than age-matched whites.

Garn (1970) and Garn et al. (1967) strongly support the concept that bone loss is a general aging phenomenon, beginning at about age 40 and progressing more rapidly in women than in men. They contend that within very broad limits, calcium intakes bear little relation to bone density. These investigators employed radiogrammetric measurements of the second metacarpal bone as an index of cortical bone status. They surveyed over 13,000 subjects from seven countries who subsisted on markedly different diets and calcium intakes. The rate of bone loss with age was roughly the same among the various groups, namely, about 0.8% per yr among women and 0.3% per yr among men. There was no significant difference which could be attributed to the calcium intakes. Individuals with an average calcium intake of 300-500 mg/d showed no difference in bone density from persons receiving 900-1500 mg/d.

Similarly, among more than 2000 ambulatory women, aged 45 yr or older, Smith (1967) and Smith and Frame (1965) could detect no correlation between calcium intake and loss of either appendicular or axial bone. Women receiving an average of less than 300 mg/d calcium showed no greater diminution in cortical thickness or diameter of the second metacarpal bone or femur than age- and weight-matched women receiving 1500 mg/d calcium. A similar lack of correlation was evident between calcium intake and the presence or absence of vertebral wedging or compression.

On the other hand, Thorangkul et al. (1959) reported phalangeal bone density to be 12% greater in individuals ingesting more than 1250 mg/d of calcium than in persons whose intakes were 350-750 mg/d. Albanese et al. (1981) compared the dietary calcium intake of 52 postmenopausal women aged 53-60 yr with their phalanx density measured radiodensitometrically. The bone densities of 17 of 23 women with intakes less than 800 mg Ca/d were below the mean values for this age group, whereas only 3 of 29 women receiving more than 800 mg Ca/d were below this age mean value. Jowsey (1973) reported preliminary results on 12 osteoporotic patients receiving 2 g calcium supplements daily and 50,000 IU vitamin D twice weekly. Bone biopsies after 3 mo showed a decrease of both bone formation and resorption. The PTH of six of eight patients tested had decreased during this treatment period.

Equivocal results were reported by Hurxthal and Vose (1969) who attempted to relate lifetime calcium intakes of subjects with their vertebral density. The investigators made rough estimates of the lifetime calcium intake of 398 subjects between 15-90 yr of age on the basis of dietary recall. They found a significant relationship between lifetime calcium intake and vertebral mineralization for women between 60-69 yr of age but for no other age group. Among men, no significant correlation could be made for any decade of life. However, when all age groups were combined, a low but significant correlation was claimed for both men and women.
Among 53 osteoporotic patients, the vertebral mineralization was 60% lower, and their mean estimated lifetime calcium intakes 21% lower than age- and sex-matched controls. However, vertebral densities of subjects on low intakes of calcium (<350 mg/d) were actually somewhat (but not significantly) greater in both men and women than subjects on relatively high calcium intakes (>800 mg/d). Because it is difficult to use dietary recalls to obtain information for more than the preceding 24 h, lifetime estimates by this method can provide, at best, only extremely rough indications of dietary intake.

The failure to establish a clear relationship of calcium intake to bone status has elicited a variety of explanations. Because skeletal status is the result of bone accumulation and bone loss, which are separate processes influenced by different factors, individual differences in bone mineral content and strength are to be expected. These differences may be emphasized by varying environmental, cultural, dietary, and other conditions. The assumption that calcium requirements of different populations are similar has not been established. Nordin et al. (1979) pointed out that many of the populations with low intakes of calcium, especially in tropical and subtropical areas, have considerably smaller skeletons and correspondingly lower calcium requirements than those in temperate zones. Harrison et al. (1980) have demonstrated a relationship between body size and calcium content and have emphasized the importance of adequate "normalization" for body size in interpreting bone mass data. Nordin et al. (1979) also speculated that the availability of sunlight in rural areas near the equator would improve the vitamin D status of the inhabitants, increasing the efficiency of calcium absorption, and reducing calcium requirements.

The diets of different cultural groups differ not only in their calcium content, but also in other important respects. The typical North American diet is rich in phosphorus and protein with an acidic metabolic residue (Draper and Scythes, 1981). In most developing countries, rice or other cereal is generally the dietary staple, which is lower in protein and phosphorus and has a neutral or alkaline "ash". Thus, differences in dietary habits, ethnic characteristics, and cultural traditions preclude simple comparison of calcium requirements.

These cross-cultural and ethnic differences were largely avoided in two relatively recent surveys in which populations with markedly different calcium intakes were identified within the same country. The first study was conducted in Switzerland by Donath et al. (1975). They measured the bone mineral content by single photon absorptiometry of 3000 healthy inhabitants of Geneva and of 800 members of a Swiss mountain village. The reported calcium intakes were 1100 mg/d in the city and 2150 mg/d in the village for the male inhabitants, and 870 and 1270 mg/d, respectively, for the females. There was no significant difference in the bone mineral content of either sex residing in these two settlements.
It is apparent, however, that although the intakes were markedly different between urban and rural inhabitants, the absolute amounts were relatively generous in both settlements. The diets and physical activity of the two groups, factors which might also influence bone calcium, were not reported.

The second survey was conducted in Yugoslavia by Matkovic et al. (1979). Two regions were chosen for study in which the daily calcium intake of one was about twice that of the other (800–1100 vs. 350–500 mg/d). Both population groups were of the same ethnic origin, lived in similar rural conditions, and had comparable physical exertion. The calcium:phosphorus ratios in both districts were similar (about 0.6). The total number of proximal femur fractures during a 6-yr period was 396 and 187 in the low and high calcium districts, respectively. Both men and women had higher fracture rates in the low calcium district, although the difference was more marked among the women. There was no significant difference between the two districts in fracture rates of the distal forearm. The cortical bone mass, as determined by hand radiographs and morphometric measurements, was greater in the high calcium district among both sexes and all age groups. Although the calcium intake was believed to be the essential difference between the two groups, those subjects in the high calcium district also consumed more protein, fat, calories, and phosphate than those in the low calcium district. The effect, if any, of these dietary differences on bone mass and strength is not known.

F. LONGITUDINAL STUDIES

Cross-sectional studies tend to obscure individual changes; for example, exceptionally high or low bone mineral in a few individuals might skew results to reflect spurious group averages. To preclude this possibility, Garn et al. (1967) studied serial radiographs of 34 men and 53 women over average periods of 15 and 23 yr respectively, and found no significant relationship between dietary calcium and bone loss. Christiansen et al. (1980, 1981) reported a practically linear decline in mineral content of the radius and ulna among 103 healthy women in their early menopause, despite a calcium supplement of 500 mg/d for 2 yr. Bone mineral content was measured by photon absorptiometry (125I) every 3 mo. The bone loss averaged 3.3% during the 2-yr period. These studies, however, reflect only compact bone; trabecular bone may be affected differently.

Heaney and colleagues (1978a, b) determined the calcium balance of 15 untreated women before menopause and 5 yr later, after menopause had occurred. The negative calcium balance in the postmenopausal study was 29 mg/d greater than in the premenopausal period. Among 11 transmenopausal women treated with estrogen, the increased postmenopausal loss of calcium was prevented. The investigators concluded that bone remodeling increases slightly during menopause, with resorption significantly greater than accretion, and that low dosage of estrogen preserves a premenopausal level of remodeling.
G. CALCIUM SUPPLEMENTATION

In the few reported studies in which nonosteoporotic, postmenopausal women received calcium supplementation, the rate of bone loss was reduced. As indicated earlier, Heaney et al. (1977) reported a positive correlation between calcium intake and balance in 130 normal women, 35-50 yr of age, consuming their normal, self-selected diets. Women on higher intakes exhibited less negative calcium balances than women selecting lower intakes. Recker et al. (1977) supplemented the diet of elderly women on normally low calcium intakes with 1 g/d calcium for a 2-yr period and concluded from radiographic and photon absorptiometric measurements that bone loss was significantly reduced.

Horsman et al. (1977) supplemented the normal diet of 24 postmenopausal women with 800 mg/d calcium for 2 yr. Bone loss from both the ulna and radius was reduced, although the retardation in the radius was not statistically significant. The mean rate of decrease in mean cortical area of the metacarpals was less than half that in the control group, with the difference approaching significance (p<0.10).

Albanese et al. (1975) reported an increase in bone density of a finger phalanx determined radiographically among aged female subjects receiving calcium supplements. Twelve subjects (average age 80 yr) were maintained on about 450 mg/d calcium, while 17 others (average age 82 yr) received a calcium supplement of 750 mg/d together with 375 IU of vitamin D. After 36 mo, the average density of the supplemented group, expressed in arbitrary units based on an aluminum wedge, increased from 90.1 to 96.1 units whereas the density of the controls decreased from 90.3 to 84.2 units. In another study, Albanese (1977) supplemented the normal diets of 31 female subjects (ages 39-73, average 55.4 yr) with 750 mg calcium and 375 units of vitamin D$_2$ per d for periods of 4-5 yr. The coefficient of bone density increased significantly whereas the control group showed significant declines. In a subsequent report, Albanese and coworkers (1981) state that "... longitudinal studies with 619 postmenopausal women (36-75 yr) for periods of 1-10 yr strongly support the use of calcium supplements as a safe and effective modality for the management of incipient or advanced osteoporosis."

Results, similar to those of Albanese and coworkers (1981), were reported by Smith et al. (1981), who found a significant increase in bone mineral content, as determined by photon absorptiometry, among aged white women (average, 80.7 yr) receiving 750 mg calcium and 400 IU of vitamin D daily for 36 mo. They reported a decline in bone mineral content of 3.29% among the control group and an increase of 2.29% among women receiving the calcium and vitamin D supplement. The possibility that some women might have had osteomalacic changes and had benefited from the vitamin D supplementation was not discussed.
In contrast to these studies suggesting that calcium supplements slow the rate of age-related bone loss in nonosteo-
porotic subjects, other evidence indicates that such supplements are ineffective in increasing the bone mass of already osteo-
porotic patients. Shapiro et al. (1975) found in 10 osteoporotic women that a total intake of 2400 mg/d calcium and 2200 mg/d phos-
phorus did not increase the radial mineral content as measured by photon absorptiometry. Nordin et al. (1980) reported a slightly, but not significantly, reduced rate of bone loss, compared with un-
treated women, among 20 osteoporotic women receiving an additional 1200 mg/d calcium for an average period of 28 mo. There were no significant effects on fracture incidence or spine scores.

Lee et al. (1981) recently claimed a significant mean increase (10%) of bone density in elderly osteoporotic women (mean age 70.5 yr) receiving supplements to their self-selected diets averaging 710 mg calcium and 405 IU vitamin D daily for 6 mo. The total calcium intake during this period averaged 1152 mg/d. Bone density of the phalanx was measured from a hand x-ray taken with a standard aluminum step wedge. The investigators claimed that 11 of the 20 subjects demonstrated increased bone densities after 6 mo, three had no changes, and six had reduced bone densities. This study has been criticized because of serious methodological shortcomings (Mazess, 1981b).

SUMMARY

The calcium intakes of American males vary from about 550-1300 mg/d, with the intake greatest during late adolescence and least by men 50 yr and older. The range for American females is 400-1050 mg/d with lowest intakes after 35 yr of age.

Calcium is absorbed both by an active process involving 1,25-(OH)2D3 and by simple diffusion. Calcium absorption is more efficient with low than with high intakes, so that most persons can adapt to some extent to reduced dietary levels. Absorption of calcium is reduced in the elderly as is their ability to in-
crease absorption in response to low calcium intake. Various dietary constituents, especially phytates and oxalates, may inter-
fere with the absorption of calcium, but these effects appear to be of little nutritional significance in American diets.

Calcium requirements remain controversial. Many epidemi-
ological studies conducted in various parts of the world revealed little correlation between bone loss or fractures and calcium intakes. Comparison between groups in different countries is seriously complicated by ethnic, cultural, and socioeconomic differences as well as by dietary factors. In a recent survey in which these differences were largely avoided, a low calcium-
consuming cohort had significantly more fractures and lesser bone mass than subjects from the same country, ethnic origin, and rural background receiving approximately twice as much dietary calcium.
The calcium balance of perimenopausal women reflects their calcium intake, with women on higher intakes exhibiting less negative balances. Zero balance was not achieved by these women; an intake of 1.24 g/d calcium was the calculated requirement to reach this level. Untreated (but not estrogen-treated) postmenopausal women had more negative calcium balances than premenopausal women.

Calcium supplementation to non-osteoporotic women improves their calcium balance and slows the rate of bone loss, but evidence that supplementary calcium can restore bone already lost in osteoporotic patients is conflicting.
IV. PHOSPHORUS AND SKELETAL INTEGRITY

A. INTAKE LEVELS

Phosphorus, as well as calcium, is essential for bone mineralization and other vital physiologic processes. A dietary deficiency of phosphorus seems highly unlikely, for practically all foods contain available phosphorus. This is reflected by the relative constancy of phosphorus availability in food (1500–1600 mg/capita/d) during the past 70 yr, despite marked shifts in the American dietary during this period (Page and Friend, 1978).

The average intake of phosphorus and the resultant ratio of calcium to phosphorus have also been estimated in the NFCS and HANES I surveys described earlier. Tables 4 and 5, which summarize their findings, are not completely comparable because of differences in age groupings. However, it is evident that the phosphorus intake of both males and females is greatest during adolescence, that intake by males is consistently greater than by females, and that whites of both sexes and all age groups consume more phosphorus than their black counterparts. This difference is evident in both white and black groups regardless of income level. There is a decline in the Ca:P ratio with age in all groups.

Analysis of the so-called "market basket" content representing typical diets in 1979 for 6-mo infants, 2-yr toddlers, and teenage males revealed phosphorus intakes of 813, 1076, and 1644 mg/d, respectively. The corresponding Ca:P ratios were 1.03, 0.76, and 0.68. Values for the teenager have been extrapolated to an assumed diet of 2800 kcal/d.

Much of the apparent variability of calcium and phosphorus intakes among the different surveys can be attributed to differences in total caloric intakes. Greger (1980a) has compared the phosphorus and caloric intakes of "average" American diets as determined from four surveys (NFCS, Market Basket, HANES I, National Food Situation). From these data, Greger (1980a,b) has calculated an overall average phosphorus intake of 471–621 mg/1000 kcal. For the adult male consuming 2700 kcal/d, the phosphorus intake would be 1270–1680 mg; for the adult female consuming 2000 kcal/d, the phosphorus intake would be 940–1240 mg. The data from NFCS and HANES I indicate that adults receive 25–40% of their daily phosphorus intakes from meat, fish, and eggs; about 20–30% from milk products; and 12–20% from grain. Infants receive about two-thirds of their intake from milk (Greger, 1980a,b).

The amount of phosphorus contributed by food additives is unknown, but has been estimated by Greger (1980a,b) to be about 25% of the total intake of the adult. On the basis of a 1977 survey of the food industry, the Committee on GRAS List Survey—Phase III
Table 4. Average Phosphorus Intakes (mg/d) and Ca:P Ratio (U.S. Department of Agriculture, 1980)*

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Male</th>
<th></th>
<th></th>
<th>Female</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subjects</td>
<td>P</td>
<td>Ca:P</td>
<td>Subjects</td>
<td>P</td>
<td>Ca:P</td>
</tr>
<tr>
<td>&lt; 1†</td>
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<td>654</td>
<td>1.24</td>
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<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1-2†</td>
<td>264</td>
<td>840</td>
<td>0.83</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>3-5†</td>
<td>437</td>
<td>930</td>
<td>0.75</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>6-8†</td>
<td>469</td>
<td>1134</td>
<td>0.75</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>9-11</td>
<td>216</td>
<td>1267</td>
<td>0.72</td>
<td>241</td>
<td>1161</td>
<td>0.72</td>
</tr>
<tr>
<td>12-14</td>
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<td>1467</td>
<td>0.71</td>
<td>309</td>
<td>1193</td>
<td>0.70</td>
</tr>
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<td>15-18</td>
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<td>1691</td>
<td>0.68</td>
<td>402</td>
<td>1112</td>
<td>0.68</td>
</tr>
<tr>
<td>19-22</td>
<td>287</td>
<td>1601</td>
<td>0.60</td>
<td>337</td>
<td>1008</td>
<td>0.60</td>
</tr>
<tr>
<td>23-34</td>
<td>770</td>
<td>1461</td>
<td>0.56</td>
<td>949</td>
<td>993</td>
<td>0.59</td>
</tr>
<tr>
<td>35-50</td>
<td>784</td>
<td>1397</td>
<td>0.54</td>
<td>942</td>
<td>922</td>
<td>0.54</td>
</tr>
<tr>
<td>51-64</td>
<td>634</td>
<td>1289</td>
<td>0.54</td>
<td>792</td>
<td>948</td>
<td>0.56</td>
</tr>
<tr>
<td>65-74</td>
<td>295</td>
<td>1246</td>
<td>0.57</td>
<td>377</td>
<td>930</td>
<td>0.61</td>
</tr>
<tr>
<td>75+</td>
<td>127</td>
<td>1137</td>
<td>0.60</td>
<td>197</td>
<td>880</td>
<td>0.65</td>
</tr>
</tbody>
</table>

* These values represent only spring quarter data for 1977 and may reflect seasonal dietary variations.

† Total subjects; no differentiation by sex.
Table 5. Average Phosphorus Intakes (mg/d) and Ca:P Ratio (National Center for Health Statistics)**†

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>White Male</th>
<th></th>
<th>Black Male</th>
<th></th>
<th></th>
<th>White Female</th>
<th></th>
<th>Black Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>Ca:P</td>
<td>P</td>
<td>Ca:P</td>
<td></td>
<td>P</td>
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<td>P</td>
</tr>
<tr>
<td>1-5</td>
<td>1101</td>
<td>0.80</td>
<td>893</td>
<td>0.80</td>
<td></td>
<td>1015</td>
<td>0.90</td>
<td>850</td>
</tr>
<tr>
<td></td>
<td>1081</td>
<td>0.84</td>
<td>957</td>
<td>0.83</td>
<td></td>
<td>999</td>
<td>0.91</td>
<td>923</td>
</tr>
<tr>
<td>6-11</td>
<td>1368</td>
<td>0.78</td>
<td>1098</td>
<td>0.76</td>
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<td>1203</td>
<td>0.85</td>
<td>1006</td>
</tr>
<tr>
<td></td>
<td>1415</td>
<td>0.86</td>
<td>1202</td>
<td>0.80</td>
<td></td>
<td>1221</td>
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<td>0.70</td>
<td></td>
<td>1004</td>
<td>0.79</td>
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<tr>
<td></td>
<td>1678</td>
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<td>1236</td>
<td>0.72</td>
<td></td>
<td>1121</td>
<td>0.84</td>
<td>978</td>
</tr>
<tr>
<td>18-44</td>
<td>1451</td>
<td>0.67</td>
<td>1233</td>
<td>0.57</td>
<td></td>
<td>922</td>
<td>0.68</td>
<td>777</td>
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<tr>
<td></td>
<td>1574</td>
<td>0.68</td>
<td>1345</td>
<td>0.57</td>
<td></td>
<td>940</td>
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<td>751</td>
</tr>
<tr>
<td>45-65</td>
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<td>0.70</td>
<td>1030</td>
<td>0.59</td>
<td></td>
<td>901</td>
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<td>677</td>
</tr>
<tr>
<td></td>
<td>1288</td>
<td>0.66</td>
<td>1062</td>
<td>0.56</td>
<td></td>
<td>879</td>
<td>0.68</td>
<td>739</td>
</tr>
<tr>
<td>65+</td>
<td>986</td>
<td>0.63</td>
<td>849</td>
<td>0.66</td>
<td></td>
<td>759</td>
<td>0.72</td>
<td>685</td>
</tr>
<tr>
<td></td>
<td>1069</td>
<td>0.70</td>
<td>907</td>
<td>0.60</td>
<td></td>
<td>820</td>
<td>0.72</td>
<td>682</td>
</tr>
</tbody>
</table>

* Adapted from unpublished data presented by Dr. Jean Pennington at ad hoc conference, January 12-13, 1981, with permission from National Center for Health Statistics.

† The first paired value in all cases is for individuals below the poverty level and the second for those above this level (see Table 3 for definition of poverty level).
(1979) of the National Academy of Sciences identified a number of different phosphorus-containing food ingredients routinely consumed by adults. For example, approximately 50 mg phosphorus are present in a 12-oz serving of the various "cola" or "pepper" carbonated beverages (Stewart and Higgs, 1980).

B. ABSORPTION AND EXCRETION

Phosphorus is efficiently absorbed from the small intestine, usually in the form of free orthophosphate (Avioli, 1980). Intestinal absorption of phosphorus probably takes place via three different mechanisms: calcium-coupled, vitamin D-dependent; non-calcium-coupled, vitamin D-dependent; and noncalcium-coupled, vitamin D-independent (Parfitt and Kleerekoper, 1980b). Approximately 60-70% is absorbed from normal diets and as much as 90% when phosphorus intake is low. Zemel and Linkswiler (1981) reported that 80.5% of administered polyphosphate (sodium hexametaphosphate) is hydrolyzed by man to orthophosphate, and absorbed. Because the intestine is relatively deficient in the enzyme phytase, phytate phosphorus of certain seeds and cereals is less readily available to man. Nevertheless, of that consumed, about 50% is absorbed by human subjects (Parfitt et al., 1964).

Low blood phosphorus causes marked elevations of plasma 1,25-(OH)2D3 in both man and animals, which directly stimulate the phosphate transport mechanism in the small intestine (DeLuca, 1979). Phosphate is normally cleared efficiently by the kidney glomeruli with 85-95% reabsorbed by the renal tubules. PTH plays a role in phosphate regulation, although the exact mechanism is not well understood. In the kidney, PTH blocks tubular reabsorption and stimulates phosphate diuresis by a vitamin D-independent mechanism (Forte et al., 1976).

C. PHOSPHORUS DEFICIENCY

Phosphorus depletion causes striking impairment of bone growth and increased bone resorption, both in experimental animals and in tissue cultures (Baylink et al., 1971; Raisz and Niemann, 1969). As would be expected, phosphorus requirements are greatest during periods of rapid growth. Baylink et al. (1971) maintained weanling rats on a low phosphorus diet (0.2%) for 11 d before sacrificing. A number of bone changes were observed, the most striking of which were related to endosteal bone resorption. The length of the resorbing surface was increased, part of which was due to the conversion of forming to resorbing surfaces. It has been known for more than a half century (Steenbock and Black, 1925) that experimental rickets could not be produced consistently in rats unless their diet was deficient in phosphorus. Thus, experimental rickets in the rat is predominantly a hypophosphatemic, rather than a hypocalcemic, disease. In human osteomalacia resulting from vitamin D
deficiency, there may be hypocalcemia or hypophosphatemia, or both; however, the most severe bone changes are seen mainly in those patients who are hypocalcemic. The greatly increased demands for phosphorus during actively growing periods have been demonstrated in human infants as well as in experimental animals. Hypophosphatemic rickets has been reported in a premature infant fed exclusively on breast milk (Rowe et al., 1979) which has a relatively low content of phosphorus.

Although nutritionally induced phosphorus deficiencies are possible, they are highly unlikely among individuals with access to normal food supplies. However, serious phosphorus deficiencies have been induced unwittingly in some patients treated for other ailments, especially in those receiving aluminum hydroxide as an antacid for prolonged periods (Spencer and Lender, 1979). The antacid precipitates phosphate in the gastrointestinal tract, increases its excretion in the feces, and reduces its level in the blood. Bloom and Flinchum (1960) reported a case of osteomalacia with pseudofractures in a 50-yr-old woman who had ingested generous amounts of aluminum hydroxide during a 6-yr period. Lotz et al. (1964) reported a similar case of excessive ingestion of a nonabsorbable antacid in which osteomalacia and other abnormalities were noted, characteristic of phosphorus depletion. Subsequently, these investigators (1968) deliberately induced a phosphorus depletion syndrome in normal volunteers by prolonged treatment with aluminum-magnesium hydroxides. Increased skeletal resorption was evident together with bone pain, weakness, malaise, and other pathological signs and symptoms. Even relatively short exposures cause significant losses of calcium. Spencer and Lender (1979) administered 90 ml/d of an aluminum hydroxide preparation to subjects for approximately 3 wk. The urinary excretion of calcium approximately doubled during this period and remained elevated for 18 d after discontinuance of the antacid. Urinary excretion of phosphorus was markedly reduced. Osteomalacia from phosphate depletion has also been induced by hemodialysis (Pierides et al., 1976) and after renal transplantation (Moorhead et al., 1974).

D. HIGH PHOSPHORUS INTAKES

The ready availability of phosphorus in commonly consumed foodstuffs and its widespread use in food processing have focused attention on the possible deleterious effects of its excessive intake. Animal studies have shown that high intakes of phosphorus can initiate a series of homeostatic adjustments which may result in bone loss (Krook, 1968). Excessive dietary phosphorus causes an increase of plasma phosphorus and a decline in serum calcium. The resulting hypocalcemia stimulates secretion of PTH which in turn increases the rate of bone resorption. This sequence of events has been induced experimentally in a number of animal species. Krook et al. (1975) state that bone loss occurs commonly among house pets and zoo animals, especially those fed on table
scraps of bread and meat, which are poor in calcium but rich in phosphorus. Monkeys fed fruit or bread and meat scraps develop a similar condition, known as simian bone disease.

Diets rich in phosphates have led to progressive bone resorption in mice (Krishnarao and Draper, 1972), rats (Draper et al., 1972), dogs (Laflamme and Jowsey, 1972), rabbits (Jowsey and Balasubramaniam, 1972), horses (Krook, 1968), and pigs (DeLuca et al., 1976). Moderate phosphate excess increased the rate of bone resorption in the rat, but with no detectable bone loss; the increased resorption was apparently compensated by increased bone deposition (Draper and Bell, 1979). A larger phosphate excess led to significant bone loss.

Urinary excretion of calcium is usually reduced with high phosphate intakes although they had no effect in reducing calcium loss in the urine of immobilized patients (Hulley et al., 1971). It was originally believed that the reduced urinary excretion reflected a decreased intestinal absorption of calcium, resulting from the formation of insoluble, unabsorbed calcium phosphate (Regsted, 1968). Spencer et al. (1975), however, demonstrated that 47Ca absorption in human subjects was not influenced by high phosphate intakes. The mechanism and significance of the reduced urinary excretion of calcium after high phosphate intakes remain uncertain. Animal studies suggest that reduced calcium excretion is mediated by an increased PTH secretion which is stimulated by increased phosphate. The elevated PTH level presumably allows more efficient reabsorption of calcium from the renal tubule with increased retention in the body. The reduced calcium excretion in dogs receiving high phosphorus intakes has also been attributed to action by PTH (Laflamme and Jowsey, 1972). After long-term phosphate supplementation, increased PTH levels were detected, together with increased resorption and loss of bone. Concomitantly, the calcium content of kidney, tendon, heart, and thoracic aorta increased significantly in these phosphorus-replete dogs. Calcification could be demonstrated histologically in the kidney and in the lens of the eye. Soft-tissue calcification is a well-known long-term consequence of hyperphosphatemia, but the dietary intake of phosphate by human adults is rarely great enough to raise plasma phosphate levels appreciably (Parfitt and Kleerekoper, 1980a).

Although high phosphate intakes have been shown to increase bone loss in experimental animals, the validity of extrapolating these findings to man has been questioned. Much of the reported data have been collected on aging rats, and rat bone differs from human bone in several important respects. Rat bone does not undergo epiphyseal closure until late adult life; it is not actively remodeled; and its loss occurs mainly by cortical thinning. DeLuca and coworkers (1976) selected the pig as a more appropriate animal model, for the pig undergoes epiphyseal plate closure and bone remodeling more comparable to man. Adult pigs
were maintained for 6 mo on diets containing 0.65% calcium and one, two, or three times this amount of phosphorus. Calcium:phosphorus ratios of 1:2 or 1:3 resulted in diminished growth, slight hypocalcemia, significant hyperphosphatemia, elevated immunoreactive PTH, and increased bone turnover rate. At a Ca:P ratio of 1:3 (but not at 1:2) the animals showed increased total kidney calcium and phosphorus, increased lumbar vertebral marrow, decreased rib ash, and decreased femoral cortical bone formation. Anderson et al. (1977) fed cinnamon ringtail monkeys high phosphorus diets (Ca:P ratio 1:4) for 3-88 mo with only minor microscopic bone changes not detectable with conventional radiography or absorptiometry. The results suggest that primates may tolerate a greater phosphate load than lower species without incurring bone loss.

Large oral doses of phosphorus increase serum PTH level in man, but the effect appears to be transitory. Two quite different interpretations have been proposed for the rise in serum PTH after phosphorus administration. One postulates the production of a modest hypocalcemia induced by the phosphorus intake, which in turn stimulates the parathyroid glands. The other suggests that the primary effect of the elevated serum phosphorus is a depression of the bony response to PTH, thereby stimulating increased PTH secretion. The former mechanism would result in negative skeletal balance, whereas the latter would tend to have the opposite effect.

Reiss et al. (1970) administered 1 g doses of phosphorus orally (as buffered sodium phosphate) to five adult subjects. They observed that PTH concentrations increased 60-125% within 1 h, but returned to base levels within 2 h. The effect of long-term repeated intakes of smaller doses is not known. Bell et al. (1977a) fed volunteer subjects a diet containing 0.7 g/d calcium and 2.1 g/d phosphorus for 4 wk. Serum and urinary phosphorus increased and serum and urinary calcium decreased. No direct estimate of PTH was possible because of the insensitivity of the assay procedure. However, reduced serum calcium and increased urinary hydroxyproline and cyclic AMP suggested enhanced parathyroid activity. Other investigators, however, question whether high dietary intakes of phosphorus, within broad limits, induce significant increases of PTH. Van den Berg et al. (1980) reported only a slight increase in serum immunoreactive PTH in patients with idiopathic hypercalciuria after oral administration of 2 g/d of phosphate phosphorus for 2 wk. The values, though somewhat elevated, remained within the normal range. The treatment did not change serum calcium or phosphorus levels.

Goldsmith et al. (1976) gave seven postmenopausal or osteoporotic women supplements of 1.0 g/d phosphate phosphorus for 3-20 mo. No significant changes in serum PTH could be detected. In common with other investigators, they noted that the phosphate supplementation had improved the calcium balance. The increased
calcium retention was accompanied by a decrease in the surface of the iliac crest undergoing new bone formation and an increase in resorbing surfaces. No evidence of calcinosis was apparent. The investigators could detect no band keratopathy, roentgenographically demonstrable vascular calcification, or nephrocalcinosis. They suggested that the difference between clinical observations and animal findings could be explained by the proportionately larger phosphate doses (on a per kilogram basis) administered experimentally. These were sufficient to induce marked hyperphosphatemia in animals but not in human subjects. Nevertheless, they concluded that it was prudent not to increase phosphorus intake in osteoporotic patients. Smith et al. (1973) administered supplemental phosphate phosphorus (2-3 g/d) to patients as a renal lithiasis treatment for as long as 10 yr with no evidence of calcinosis.

There is little epidemiologic evidence relating bone integrity to phosphorus intakes. Identification of populations on diets normally high in phosphorus is difficult and is further complicated by their coincident high intake of protein, which also modifies calcium metabolism. The meat diets of Eskimos provide high phosphorus intakes; surveys taken 15-20 yr ago (Heller, 1964; Mann et al., 1962) indicated a phosphorus intake of 1000-2000 mg/d. Mazess and Mather (1974) reported that Eskimos of both sexes over 40 yr of age had 10-15% less bone than whites of the same age. The loss of mineral from the forearm bones, measured by direct photon absorptiometry, occurred earlier and more rapidly in Eskimos than in white counterparts. The Arctic Eskimos of both Alaska and Canada underwent a rate of bone loss 15-20% greater than the white population of the United States. In a more recent survey (Health and Welfare Canada, 1975), it was reported that serum phosphate levels of adult Eskimos were considerably higher and calcium levels were lower than age- and sex-matched Canadian controls on more conventional diets.

E. CALCIUM:PHOSPHORUS RATIO

There is insufficient evidence to establish an optimal calcium:phosphorus ratio, or indeed, to determine whether this ratio is of dietary significance in man. A Ca:P ratio of 1:1 to 2:1 has been recommended for man (National Research Council, 1980; Select Committee on GRAS Substances, 1975), but additional studies seem necessary to validate this recommendation. As already described, bone loss can be induced in experimental animals by high phosphorus diets even when calcium intake is normal. Establishing an "optimal" Ca:P ratio, however, is difficult for the ratio varies with the absolute intakes of the minerals. For example, with a low calcium diet (0.3%), there is no discernible difference of bone mass in mice receiving a Ca:P ratio of 1:2 or of 1:1. With 1.2% calcium in the diet, bone mass is depressed with a Ca:P ratio of 1:1, and with very high calcium diets (2.4%), decreased bone mass can be
produced even at a ratio of 2:1 (Bell et al., 1980). Draper and Scythes (1981) attribute the marked shift in apparent bone responsiveness of the mice on different Ca:P ratios to the relative absorptive efficiencies of calcium and phosphorus at different levels of intake. The efficiency of calcium absorption decreases markedly with increasing levels of intake, while that of phosphorus is relatively unaffected by the intake level. Thus, much more phosphorus than calcium is absorbed when the same amounts of each are added to the diet. According to Draper and Scythes (1981), this accounts for the paradoxical situation in which the plasma calcium concentration is often more responsive to dietary phosphorus than to the intake of calcium itself.

Loss of bone mass by man on low Ca:P ratios has not been demonstrated. However, as already described, indications of enhanced parathyroid activity were observed among young, adult human subjects maintained for 4 wk on a Ca:P ratio of 1:3 (Bell et al., 1977a). Measurements of bone mass were not performed.

Zemel and Linkswiler (1981) maintained eight young male subjects for consecutive 15-d periods on diets with differing Ca:P ratios. High phosphorus intakes (1835 mg/d) stimulated PTH secretion as indicated by increased urinary excretion of cyclic AMP, both with low (399 mg/d) and high (1199 mg/d) intakes of calcium. Bone resorption measured by urinary hydroxyproline was not affected by high phosphorus intakes. With high calcium, high phosphorus diets, both urinary cyclic AMP and hydroxyproline decreased, suggesting a decrease in PTH-mediated bone resorption.

One of the panel participants decried the attempt to recommend a specific Ca:P ratio for human subjects. He pointed out that the ratio of Ca:P in bone (about 2:1) may have some dietary justification during growth since a large percentage of both minerals go into bone. When adulthood is reached, however, the ratio shifts sharply in favor of phosphorus because of the greater requirement for this element in soft tissue metabolism. This shift is reflected in the self-selection of phosphorus by animals, which choose a ratio approaching that of bone during rapid growth (1.8:1), but a ratio favoring phosphorus as adults (1:1.2).

**F. SUMMARY**

Phosphorus is widely distributed in commonly consumed foodstuffs and dietary deficiency of this element is unlikely. However, inadvertent and unwitting phosphorus deficiencies have occasionally resulted from long-term administration of aluminum hydroxide as an antacid.

The average phosphorus intake among the United States population varies from approximately 650–1700 mg/d for males and from 650–1200 mg/d for females, depending largely upon total
caloric intake. Teenagers of both sexes have the greatest intake and the elderly (over 65 yr) have the least. The intake by males of all age groups is greater than of females, and whites of both sexes and all age groups consume more than their black counterparts.

High phosphorus intakes induce secondary hyperparathyroidism in various animal species with progressive resorption and loss of bone. Such bone loss from high intakes of phosphorus has not been demonstrated in man. Large doses of phosphorus increase serum PTH levels in man but opinions differ on the magnitude, duration, and significance of the parathyroid stimulation which might result from relatively high intakes of phosphorus in the American diet.

Urinary calcium excretion is reduced with high phosphorus intakes. Calcium absorption is normal under these conditions; the increased retention presumably results from more efficient reabsorption of calcium from the renal tubules. Calcium deposition was detected in soft tissues of dogs after long-term phosphate supplementation, but this has not been reported in man as a result of large dietary phosphorus intakes.

High phosphorus intakes in some animal species result in bone loss even with normally adequate intakes of calcium. The Ca:P ratio at which bone loss occurs varies with the absolute intakes of the individual elements; a ratio of 1:2 prevents bone loss when calcium intakes are low, whereas bone loss occurs even at a ratio of 2:1 when intakes are high. Various expert groups have recommended a Ca:P ratio of 1:1 to 2:1 for man, but there is insufficient evidence at present to support the establishment of a firm ratio.

There is little epidemiologic evidence relating bone mass in man to phosphorus intakes. Eskimos, whose diet is rich in phosphorus, have been reported to have higher serum phosphate levels and lower bone mass than age-matched whites. Eskimo diets, however, are rich in protein, which also modifies calcium metabolism.
V. PROTEIN AND BONE METABOLISM

A. HYPERCALCIURIC EFFECT OF HIGH PROTEIN DIETS

Although high protein diets have been shown repeatedly to induce hypercalciuria in both man and animals, questions remain concerning the magnitude, duration, mechanism, and significance of this effect. Differing experimental and clinical responses are not surprising when it is realized that calcium excretion varies not only with levels of protein and calcium intake, but also with the amount of dietary phosphorus, and to some extent, with the nature of the protein consumed. In fact, urinary calcium may vary spontaneously even during a constant intake of calcium.

As early as 1920, Sherman observed that in human subjects, the addition of meat to low calcium diets caused increased urinary excretion of calcium. Originally, this increase was attributed to greater calcium absorption from the intestine (Kunerth and Pittman, 1939; McCance et al., 1942). Later workers were divided between those claiming an increased calcium absorption on high protein diets (Bell et al., 1975) and those denying any significant effect (Allen and Hall, 1978; Allen et al., 1979a; Spencer et al., 1978). These differences reflect the variability of dietary composition and subject selection in the various studies, as well as the marginal nature of the protein effect on calcium absorption. Schuette et al. (1980) found no change in the mean absorption of calcium among eleven adult males and females on diets low or high in protein. With high protein diets, the apparent absorption of calcium decreased in five and increased in six subjects. Further, Schuette et al. (1980) found no difference in the levels of circulating 1,25-(OH)₂D₃ among subjects on either high or low protein diets. However, it seems unlikely that modest changes in calcium absorption would be reflected in detectable increases of 1,25-(OH)₂D₃.

High protein intakes increase the urinary excretion of calcium at all levels of calcium ingestion. Linkswiler et al. (1974) studied calcium balances in young adult males receiving three levels of protein (47, 95, and 142 g/d) at each of three levels of calcium intake (500, 800, and 1400 mg/d). Each experimental period was of 15-d duration. Each subject served as his own control. Phosphorus intakes were the same as those of calcium or slightly greater in each case. The low protein diet was a mixed diet of ordinary foods. Casein, lactalbumin, wheat gluten, and gelatin were added in the medium and high protein diets in amounts necessary to maintain the same relative proportion of the eight essential amino acids as well as cystine and tyrosine. The results are summarized in Table 6. At the lowest protein intake, the subjects remained in calcium balance at all levels of calcium intake. At the intermediate protein level, all subjects receiving 500 mg/d calcium were in negative calcium balance, but were in positive balance at 800 mg/d calcium. When the highest level
of protein was ingested (142 g/d) all subjects were in negative balance on a daily calcium intake of 800 mg; 12 of 15 subjects had negative calcium balances even with intakes of 1400 mg/d calcium. An increase of dietary protein caused a rapid and dramatic increase of urinary calcium excretion. An increase in protein intake from 47 to 142 g/d by a subject receiving 800 mg calcium raised the excretion of calcium in the urine from 184 to 394 mg within 24 h.

Table 6. Effect of Protein and Calcium Intakes on Calcium Balance*

<table>
<thead>
<tr>
<th>Protein Intake g/d</th>
<th>Calcium Intake mg/d</th>
<th>Calcium Balance mg/d</th>
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<tbody>
<tr>
<td>47</td>
<td>500</td>
<td>+ 31</td>
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<tr>
<td></td>
<td>800</td>
<td>+ 18</td>
</tr>
<tr>
<td></td>
<td>1400</td>
<td>+ 20</td>
</tr>
<tr>
<td>95</td>
<td>500</td>
<td>- 58</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>+ (&quot;slightly positive&quot;)</td>
</tr>
<tr>
<td></td>
<td>1400</td>
<td>- 27</td>
</tr>
<tr>
<td>142</td>
<td>500</td>
<td>- 120</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>- 85</td>
</tr>
<tr>
<td></td>
<td>1400</td>
<td>- 65 (12 of 15 subjects had negative balance)</td>
</tr>
</tbody>
</table>

* Adapted from Linkswiler et al., 1974.

Even more striking effects on urinary calcium excretion were noted by Margen et al. (1974) in subjects on extremely high protein diets. Subjects receiving 2.3 g calcium and 12-13 g nitrogen (about 80 g protein) daily excreted an average of 81 mg/d calcium. When the protein intake was raised to 90 g nitrogen (about 560 g protein) subjects excreted almost five times (380 mg) as much calcium as they had on the low protein diet. Such extremely high protein intakes, however, would rarely be achieved in normal dietaries. Other subjects receiving 1.6 g/d calcium excreted 46 mg calcium in the urine on an essentially protein free diet (<1 g/d nitrogen) but 318 mg calcium when they received 62 g/d nitrogen (about 390 g protein).
The calcium intakes cited above (500-2300 mg/d) represent moderate to generous dietary levels. Similar effects of protein on urinary calcium excretion, however, have been noted even with extremely low intakes of calcium. Chu et al. (1975) varied the protein intake of young male volunteers maintained for periods of 15 d on diets containing only 100 mg/d calcium. The urinary excretion of calcium increased from 51 to 164 mg/d when the subjects were transferred from a protein free (0.9 g/d nitrogen) to a protein rich (24 g/d nitrogen) diet.

B. DURATION OF HYPERCALCIURIUS EFFECT

The duration of the hypercalciuric effect of high protein intake is of practical as well as of academic interest. If subjects adapted to such diets and their urinary calciums returned rapidly to normal, effects on the calcium reserves of the body would be minimal. If negative balances persisted for lengthy periods, however, the drain on bone calcium could be significant. Results from short-term studies are inconclusive because fecal calcium is slow to reflect dietary intakes, and this delay tends to obscure results. Ingestion of a changed diet for 5-10 d is necessary before fecal excretion becomes representative of the diet (Allen et al., 1979a). Allen and Hall (1978) found that the calciuriic effect of high protein diets in 56-d-old male rats diminished as the study continued. Within 29 d, on high protein diets, urinary calcium excretion had returned to control levels. This finding was not confirmed by Whiting and Draper (1981) who reported that rats receiving high protein diets maintained hypercalciuria for at least 123 d. Similar observations were reported in man by Johnson et al. (1970) who found that urinary calcium of six adult male subjects consuming high protein diets remained elevated during a 45-d period, with no tendency to decline. Allen et al. (1979a) also found consistently elevated urinary calcium during a 48-d period among subjects fed high protein diets. Hegsted and Linkswiler (1981) have also confirmed the persistent calciuriic effect of high protein diets. Young adult women received diets containing either 46 or 123 g/d protein for 60 d and were then fed the alternate diet for 15 d. Calcium, phosphorus, and magnesium intakes were kept constant at 500, 900, and 350 mg/d respectively throughout the study. The urinary excretion of calcium at each protein level remained constant during the 60-d period. Negative calcium balances of 121 mg/d resulted with the high protein diet and 14 mg/d with the low protein diet.

In contrast to these findings, Spencer et al. (1978) reported that high protein (meat) intakes caused no significant increase in urinary calcium in subjects receiving low or normal calcium intakes and only moderate increases among subjects with high calcium intakes. These increases occurred in the initial phase of the high protein intake; the urinary calcium decreased progressively while the subjects remained on the high protein

43
intake reaching baseline levels within 50–60 d. In a later study, 2 g protein as meat per kg body weight were given to patients for 90–132 d (Spencer et al., 1981). The calcium absorption and urinary and fecal calcium excretions did not differ from controls. However, since meat is rich in phosphorus, ingestion of phosphorus as well as protein was increased by this high meat diet. Phosphorus is known to decrease calcium excretion and would thus tend to counteract the hypercalciuric action of the protein. It seems likely these compensatory actions on urinary calcium by protein and phosphorus account, at least in part, for the experimental differences reported.

C. **OPPOSING EFFECTS OF PROTEIN AND PHOSPHORUS**

Because phosphorus and protein have opposing effects on calcium excretion, it is difficult to interpret studies unless the intakes of both nutrients are carefully controlled. Such a study has recently been attempted by Hegsted et al. (1981), who examined the effects on urinary calcium of different levels of phosphorus at constant protein intakes, and vice versa. Eight men, aged 19–25 yr, received combinations of low or high phosphorus (1010 and 2525 mg/d) and protein (50 and 150 g/d) diets in four consecutive 12-d periods. The dietary calcium level was maintained at 500 mg/d. An increase in phosphorus intake from 1010 to 2525 mg/d reduced urinary calcium by 40% with both levels of protein intake. Conversely, calcium excretion increased with the high protein intakes at both phosphorus levels but, as expected, calciuresis was more marked with the low phosphorus diet. The data suggested that the percentage increase in urinary calcium induced by increased protein intake was essentially constant for any given level of calcium and phosphorus. The investigators also analyzed data from published reports with varying protein intakes in which calcium and phosphorus intakes were maintained at constant levels. Regression analysis of these data indicated a high degree of correlation between the percentage increases of dietary protein and urinary calcium. Doubling the protein intake increased urinary calcium by 50%; tripling the protein doubled the urinary calcium.

D. **MECHANISM OF HYPERCALCIURIA INDUCTION**

The mechanism by which high protein diets induce hypercalciuria has stimulated considerable speculation among investigators. Because intestine, bone, and kidney are intimately involved in the maintenance of calcium balance, changes at any of these sites might induce the increased urinary calcium, thus raising a number of theoretical possibilities. As Allen and Hall (1978) have pointed out, the increased urinary calcium from high protein diets could be induced by increased absorption of dietary calcium, by decreased endogenous fecal excretion of calcium, by
increased bone resorption, by decreased renal tubular reabsorp-
tion, by decreased bone accretion of calcium, or by various com-
binations. To these possibilities could be added possible actions
by PTH. Regardless of the mechanism, all additional calcium ex-
creted in sustained hypercalciuria must originate either from
increased net intestinal absorption or from increased net bone
resorption (Parfitt and Kleerekoper, 1980b).

As discussed earlier, opinions are divided whether high
protein ingestion causes increased calcium absorption. Another
possible effect of high protein intakes is to shunt endogenous
calcium excretion from feces to urine. This shunt has been demon-
strated in rats. Only 2-6% of endogenous calcium was excreted in
the urine of rats receiving 10% protein, but 20-30% was excreted
by this route with diets containing 40% protein (Bell et al., 1975).
This shift in the route of endogenous calcium excretion has been
confirmed by Whiting and Draper (1981). Increased urinary $^{45}$Ca
in rats fed high protein diets was accompanied by a comparable
decrease in endogenous fecal $^{45}$Ca. Thus, in the rat, the total
calcium excretion (fecal plus urinary) did not increase on high
protein diets, in contrast to man, who experienced negative cal-
cium balances on such diets. This difference is another indica-
tion that rats may not be the most suitable animal model to study
calcium interactions in man.

There is no evidence that diets high in protein increase
parathyroid secretion. No difference in fasting serum concentra-
tions of calcium or of PTH was detected in six young adult men
fed 47 or 142 g protein for 10-d periods (Kim and Linkswiler,
1979). All subjects received the 47 g protein diet during the
first 10-d period and the 142 g protein diet during the second
10-d period. Similarly, no difference in the concentration of
PTH was noted in adult males maintained on two protein levels
(12 or 36 g nitrogen) for 47 and 48 d, respectively (Allen et al.,
1979a). Finally, Schuette et al. (1980) estimated parathyroid
function in men and women aged 44-86 yr receiving diets low
(47 g/d) and high in protein (112 g/d). No difference could be
detected between the two groups in circulating levels of immuno-
reactive PTH or in urinary excretion of cyclic AMP, an indirect
measure of parathyroid activity.

The panel members cautioned that care should be exercised
in attempting to equate the actions of phosphorus ingestion from
inorganic and organic sources. Insufficient information is avail-
able to conclude that the effects on the parathyroid or other
physiological parameters are the same from inorganic phosphate
as from phosphoproteins or other organic phosphates, such as found
in meats.

Recent data suggest that altered kidney function is the
likely cause of protein-induced hypercalciuria. Increased protein
intake in man has been shown to increase glomerular filtration and
to reduce fractional renal tubular reabsorption. Both of these processes contribute to the increased urinary calcium. Hegsted and Linkswiler (1981) reported that increasing the protein intakes of young adult women from 46 to 123 g/d increased glomerular filtration rate by 12% and reduced tubular reabsorption of calcium by approximately 0.9% (from 98.5 to 97.6%). Similar results were found in male subjects (Kim and Linkswiler, 1979). The diminution of tubular reabsorption is small but, coupled with the large volume of glomerular filtrate, it appears to account for the observed urinary calcium excretion with surprising accuracy. This is demonstrated by the calculated and the observed urinary calcium content on different protein intakes (Hegsted and Linkswiler, 1981). On the low protein diet, daily glomerular filtrate contained 7.435 g of calcium, of which 98.5% was reabsorbed by the renal tubules, leaving a calculated value of 112 mg calcium to be excreted in the urine; the calcium excretion determined experimentally was 113 mg. On the high protein diet, the filtrate contained 8.333 g calcium, of which 97.6% was reabsorbed, for a calculated excretion of 200 mg; the amount found analytically was 212 mg. Allen et al. (1979b) studied the effects of protein meals on the renal reabsorption of calcium. Both male and female subjects (aged 26-48 yr) were fed meals containing 18 g and 54 g protein at 3-9 d intervals. The protein level did not affect the glomerular filtration rate, but tubular reabsorption of calcium was significantly reduced after the high protein meal, resulting in higher urinary calcium excretion between 2-4 h after consumption of the high protein meal.

Increased endogenous acid production is known to inhibit calcium reabsorption of the renal tubules (Lemann et al., 1967). Most endogenous acid from protein is produced by the catabolism of sulfur-containing amino acids (Chan, 1974). Whiting and Draper (1981) fed various diets high in protein to adult rats, and observed that the hypercalcuria induced was proportional to the sulfur content of the diets. A high lactalbumin diet, rich in sulfur amino acids, induced a urinary calcium excretion after 2 d almost five times as great as that from an isonitrogenous diet of casein, which has a low sulfur content. Urinary calcium decreased somewhat with longer dietary periods, but even after 8 wk, rats on the lactalbumin diet excreted twice as much calcium as did those on the casein diet. When the casein diet was supplemented with sulfur amino acids to approximate the methionine and cysteine content of lactalbumin, a calcuiuria was induced comparable to that produced by lactalbumin. The addition of inorganic sulfate to casein also markedly increased urinary calcium. There was a linear relationship between urinary calcium and sulfate excretion in rats fed different dietary regimens; the authors propose that the production and excretion of sulfate is a major factor in the hypercalcuiuria of high protein feeding. Schuette et al. (1980) found a positive correlation in older men and women on various diets between the increased urinary calcium on high protein intakes and urinary sulfate and total acid excretion.
The hypercalciuric effect of sulfur-containing amino acids has been confirmed in human subjects maintained for extended periods on sulfur-rich diets, but this was not observed on short-term exposures (Block et al., 1980). No effect was noted postprandially on the urinary excretion of calcium or on its reabsorption by the kidney when diets were supplemented with sulfur amino acids. Urinary calcium increased within 30 min after a high protein (45 g) meal and remained significantly higher for 3 h than after a control meal (15 g protein). Supplementing the control meal with sufficient sulfur-containing amino acids to equal their content in the high protein meal had no significant effect on urinary calcium. The sulfur amino acid content of the diet had no significant effect on urinary pH, titratable acidity, ammonium ion, or net acid excretion. The calciuric effect of sulfur-containing amino acids was clearly evident, however, when subjects were maintained for 51 d on various diets (Zemel et al., 1981). Thus, factors in addition to their sulfur content appear to be responsible for the hypercalciuric effects of high protein diets. Other studies lend support to this suggestion.

Zemel et al. (1981) reported a relationship between sulfur amino acid intake by man and urinary calcium. However, the increase in calcium excretion by subjects receiving 150 g/d protein was approximately twice that in subjects fed a low protein diet (50 g/d) supplemented with sulfur amino acids to equal their content in the high protein diet. Preliminary studies by Allen and coworkers (1980) suggest that the acute effect of high protein diets may be mediated in part by stimulation of insulin secretion. They detected a significant increase of serum insulin after a high protein meal, which correlated well with the percentage increase of urinary calcium. Changes in growth hormone and glucocorticoid secretion after high protein diets have also been suggested as possible contributory factors to the observed calciuria (Zemel et al., 1981).

E. EFFECT ON SKELETAL INTEGRITY

The long-term effect of high protein diets on bone structure is still unknown. Wachman and Bernstein (1968) suggested that long-term ingestion of high protein diets, with the consequent metabolic production of an "acid ash", imposes a continuous demand on the buffering capacity of bone, and might thus be a factor in human osteoporosis. Acting on this suggestion, Ellis et al. (1972) compared by x-ray densitometry, the bone density of fingers of vegetarians, whose diet generally yields an "alkaline ash", with that of omnivores whose production of metabolic acid is considerably greater. They reported a greater bone density among the vegetarian group. However, a later survey by the same investigators using improved techniques and a larger group of subjects failed to confirm the original observations (Ellis et al., 1974). Sanchez et al. (1980) found no statistical difference in bone mineral ratio
between 50-59 yr old vegetarian and omnivore females. In older individuals (60-87 yr), however, long-term vegetarians had significantly greater values than their omnivore peers, matched according to age, weight, and height. They concluded that the reduced loss could, in part, be explained by the low acid ash content of the vegetarian diet.

Bell et al. (1977b) could detect no difference in bone resorption in rats fed diets yielding either "neutral" or "acid ash". Similarly, a 50% increase of fruits and vegetables failed to influence the negative calcium balance induced by high protein diets of young men (Anand and Linkswiler, 1974).

F. SUMMARY

Diets rich in purified protein induce a calciuria in both animals and human subjects. When meat is the protein source, its high phosphorus content seems to counteract to a large extent the calciuric action of protein. High protein diets have caused persistent hypercalciuria for as long as 123 d in rats and 60 d in man.

The long-term calciuric effectiveness of a protein depends at least in part on its content of sulfur-containing amino acids. Renal tubular reabsorption of calcium is reduced, presumably because of the increased acid production from the metabolism of these amino acids. This renal effect, although small, seems sufficient to account for the increased calcium excretion in the urine.

Other factors may also be involved in the protein induced hypercalciuria, especially in the acute postprandial stage. Some evidence suggests that the rise in urinary calcium after meals high in protein may be related to an increased secretion of insulin.

There is no evidence that PTH secretion is increased by high protein diets. The long-term effect of high protein diets on bone structure is not known.
VI. CONCLUSIONS

- Osteoporosis is a major public health problem of the elderly, especially among postmenopausal white women. The bone density of elderly individuals depends upon the peak mineral content achieved at skeletal maturity and the rate of subsequent bone loss. Both mass and loss of bone vary widely among individuals. Bone remodeling continues throughout life. The rates of bone accretion and resorption are dependent on various genetic, hormonal, dietary, and other factors whose mechanisms of action and interrelationships are not completely understood.

- The calcium intakes of American males vary from about 550-1300 mg/d with the highest level occurring during teenage years (mean about 1100 mg/d) and the lowest by individuals 50 yr of age and older (mean about 700 mg/d). The corresponding range for females is 400-1050 mg/d with lowest intakes after 35 yr of age (mean about 540 mg/d). Large-scale survey statistics usually based on short-term dietary recall and diary are insufficiently sensitive to identify subgroups with particularly low or high calcium intakes.

- The efficiency of calcium absorption from the intestine varies inversely with the level of intake. As calcium intakes decrease, the proportion absorbed increases. This adaptive process, present in variable degrees among individuals, is an important protective mechanism against reduced calcium intakes. After the age of about 50, both basal calcium absorption and the ability to increase absorptive efficiency in response to low calcium intake decline progressively.

- Various dietary factors such as fat, oxalates, phytate, and lactose have been shown experimentally to influence calcium absorption. These effects appear to be of little nutritional significance in typical mixed diets consumed in the United States.

- Many epidemiological surveys conducted in various parts of the world have failed to demonstrate, unequivocally, a significant relationship between calcium intake and bone loss or fracture. The ad hoc review panel emphasized that comparison of groups in different geographic areas may be misleading because of differences in body size, cultural tradition, ethnic characteristics, physical activity, and sunlight exposure, as well as markedly different dietary habits. Most of the panel believed the results of a recent epidemiological study, in which these differences were largely avoided, demonstrated a
clear relationship between calcium intake and bone strength. Two groups within the same country were identified on markedly different calcium intakes. Both groups were of the same ethnic origin and had similar rural backgrounds and work habits. The cohorts on low calcium intake experienced significantly more proximal femoral fractures and lesser bone mass than those receiving approximately twice as much dietary calcium. However, other members of the review panel believed more convincing evidence is necessary before a relationship between calcium intake and bone mass can be considered established.

Perimenopausal women of mixed estrogen status receiving high calcium intakes exhibited less negative (but not zero) calcium balances than those on lower intakes. A calcium intake of 1.24 g/d was the calculated zero balance requirement for these women. Premenopausal and estrogen-treated postmenopausal women had a calculated zero calcium balance of 0.99 g/d. The corresponding value for untreated postmenopausal women was 1.50 g/d. No difference in calcium balance was apparent between premenopausal and estrogen-treated postmenopausal women.

Calcium supplementation improves the calcium balance of perimenopausal women. Most of the ad hoc panel agreed that generous calcium intakes by these women are desirable. Some members of the review panel, however, believed that it may not restore bone already lost by osteoporotic patients.

In age-related bone loss, all bones of the skeleton lose minerals, but the relative loss between cancellous and cortical bones differs among individuals. Changes in bone mass with age or treatment require comparison at the same bone site. Data from cortical bone should not be extrapolated to cancellous bone nor vice versa. Even more hazardous is the extrapolation of data from animal studies to man, for no completely satisfactory animal model has been demonstrated.

The bone mass at skeletal maturity is an important, but not the only, determinant of bone mass and strength in old age. The age of onset of net loss and the rate of bone loss also determine the ultimate bone mass. Thus, one cannot predict in early adulthood who will be at greatest risk of osteoporosis.

The variety of techniques for bone measurement makes data comparison difficult. Agreement on acceptable methodologies for pertinent bone parameters should be encouraged.
The phosphorus intake in the United States is considered adequate; it varies from approximately 650-1700 mg/d for males and 650-1200 mg/d for females of different ages. For both sexes, teenagers have the greatest intake and adults over 65 yr have the least. For most adults, dietary phosphorus deficiency is highly unlikely but its deficiency and eventual bone loss have been inadvertently induced in some individuals by habitual use of aluminum hydroxide antacids.

High phosphorus diets induce secondary hyperparathyroidism in certain animal species with significant bone loss. Bone loss in man from such diets has not been demonstrated. The ad hoc review panel believed that up to 2 g/d phosphorus could be safely consumed. However, the long-term effects of high intakes are still unknown and require further study.

The mechanism by which phosphate administration increases serum PTH has not been established. Some investigators believe the increase results from a phosphate-induced hypocalcemia; others attribute the rise to a reduction by phosphorus of the bony responsiveness to PTH, thus stimulating further PTH secretion.

The significance of the hypocaliuria induced by high phosphorus diets is unsettled. The known effect of phosphorus in suppressing bone resorption and stimulating bone formation suggests that the retained calcium is deposited in bone. In animals on high phosphorus diets, however, calcium deposition has been demonstrated in soft tissues. Although this has not been observed in man, the possibility cannot be excluded.

The importance of the dietary Ca:P ratio in man is debatable. The average North American diet provides a Ca:P ratio less than that generally recommended (1:1 to 2:1), with no evidence of harm. Most of the ad hoc review panel believed that the Ca:P ratio is of secondary importance to the adequacy of calcium intake and that attempts to establish a recommended Ca:P ratio are premature and unproductive.

The hypercalciuric effect of high protein intake appears to result from a reduced calcium reabsorption by the renal tubules. The effect depends at least in part on the sulfur-containing amino acids of the protein.

The hypercalciuric effect of high protein diets is partially counteracted by high phosphorus intake. Urinary calcium changes only slightly when natural protein sources, such as meat, which is rich in phosphorus, are consumed.
VII. SUGGESTIONS FOR FUTURE RESEARCH

- The most valuable, but admittedly the most difficult, studies that are now needed are long-term prospective experiments in which dietary intakes, bone measurements, and other relevant analyses are conducted on the same individuals for long periods, perhaps as much as 20-25 yr. Different dietary, hormonal, or therapeutic interventions should be correlated with appropriate bone measurements, calcium balances, and blood studies. Members of the review panel recognized the formidable nature of multifaceted, longitudinal studies, but believed that if such efforts were not initiated, the same questions would continue to plague succeeding generations of investigators.

- A less intimidating protocol which would provide useful information involves periodic monitoring among selected subjects of their current bone status, and calcium and phosphorus intakes. Long-term studies would relate these intakes with the ultimate development or avoidance of osteoporosis.

- Retrospective studies, although more equivocal than prospective studies, are still valuable and should be utilized whenever possible. Attempts should be made to identify individuals who have had relatively constant intakes (either high or low) of calcium or phosphorus for many years, and to correlate these intakes with relevant precise bone measurements.

- Studies on absorption of calcium should be extended, especially among the elderly. At present, the recommended dietary allowance for calcium is the same for all persons 19 yr of age and older, except for pregnant and lactating women. If the absorption of calcium changes significantly with age, it may be necessary to establish more critical recommendations for different adult age groups.

- The calcium requirements during pregnancy should be re-examined, as well as the susceptibility to osteoporosis of women who have had multiple pregnancies and who have breast fed their infants.

- Based on relative body weights, the recommended dietary allowance of calcium for man is considerably below the requirements recognized for other animals. It would be useful to learn the effect of high calcium intakes (2-3 g/d) in man. Since possibly harmful effects of such amounts of calcium have not been ruled out, it would be desirable to screen potential subjects to exclude those who might be susceptible to renal stones, and to monitor all subjects carefully.
If higher dietary allowances of calcium for specific population groups are deemed desirable, additional research on the sources of calcium supplementation will be necessary, especially for individuals for whom milk and cheese are not practical dietary sources.

Individuals vary widely in the rate at which their bone mineral is lost. Attempts should be made to identify subjects at high risk for more intensive study.

Early detection of osteoporosis would be of value. Some studies have suggested that bone loss may occur in the alveolar bone before changes can be detected in the spine. Practical, standardized techniques should be developed to explore this possibility. Other possible indices of early bone loss should also be sought. The development of spinal densitometry of sufficient sensitivity to detect prefracture osteoporosis would be especially valuable.

Current methodology for determination of bone mass is oriented more toward cortical than trabecular bone. Sensitive techniques for trabecular bone studies are needed.

The metabolism and remodeling rates of trabecular and cortical bone may differ. The effects of age, menopause, calcium intakes, and other factors should be evaluated independently for each type of bone.

Fractures ordinarily related to osteoporosis may have different origins and etiologies; more intensive examination of etiologies and differences of these fractures would be instructive.

Further studies are necessary to identify the factors which determine the bone mass at skeletal maturity. Since the greatest increase in bone mass occurs during growth and through young adulthood, intensive studies on bone deposition during these periods are indicated.

High intakes of purified proteins, especially of nonmeat origin, are known to increase urinary calcium excretion, which may induce negative calcium balances. The protein intake of adults in the United States is 75-105 g/d for men and 54-67 g/d for women over age 18 yr. The calcium and phosphorus intakes necessary to maintain calcium balance at these levels should be determined for different types of dietary protein.

The effect of various levels and sources of protein intake on bone mass in adolescents of both sexes and in osteoporotic women is not known and should be investigated.
• Preliminary studies suggest that blood levels of 1,25-(OH)₂D₃ are decreased in osteoporotic patients. These studies should be repeated and extended, and the levels of other vitamin D metabolites determined.

• Vitamin D deficiency causes malabsorption of calcium, secondary hyperparathyroidism, increased bone turnover, and accelerated loss of cortical bone long before osteomalacia occurs. The cortical bone status of patients with hip fractures as indicated by several indices, are lower than age-, sex-, and race-matched controls. These findings, together with surveys indicating reduced plasma levels of an active vitamin D metabolite, emphasize the need for further research on the possible role of subclinical vitamin D deficiency in the pathogenesis of hip fractures.

• Estrogen is widely used among elderly women to counteract menopausal symptoms. It increases calcium balance, decreases bone loss, and reduces the incidence of certain fractures. It is important that these benefits be evaluated critically against possible harm of continued treatment.

• The variation of PTH secretion with age is not known. The possibility that bone loss in the elderly is related to prolonged increases of PTH secretion should be explored.

• Further studies are necessary to elucidate the mechanism of the rise of serum PTH after phosphate administration. In this connection, the possible role of phosphate in modifying the responsiveness of bone to PTH should be explored.

• It was recognized that the current review considered only a limited number of the dietary factors involved in bone homeostasis and osteoporosis. Various minerals, vitamins, and other dietary constituents appear to play roles in bone formation and/or resorption; these should be similarly addressed in subsequent reviews. For example, both calcium and magnesium are divalent ions and both markedly influence PTH stimulation, yet they are antagonistic in many physiological processes. These interactions and their ultimate effect on bone homeostasis require clarification. Both vitamin D and fluoride markedly affect bone metabolism and deserve more extensive consideration than was possible in this review. The role of osteocalcin, the vitamin K-dependent protein found in bone, should be explored, as should the effect of zinc and perhaps other dietary constituents, on bone remodeling.
VIII. LITERATURE CITED


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