Diet, Bone Health and Aging

Khashayar Sakhaee, M.D.

Medical Grand Rounds

July 1, 1999

This is to acknowledge that Khashayar Sakhaee, M.D. has disclosed no financial interests or other relationships with commercial concerns related directly or indirectly to this program.

Name: Khashayar Sakhaee

Rank: Professor

Division Name: Mineral Metabolism

Clinical evaluation and research in areas of calcium, phosphorus and uric acid metabolism. Special emphasis on pathogenetic mechanisms of urinary acidity in gouty diathesis.

INTRODUCTION

Osteoporosis is a complex disorder which has a strong genetic component (1-6), and is also influenced by the dysregulation of calciotropic hormone metabolism and environmental risk factors (7). It has been shown in the adult that the skeletal peak bone mass is already attained at the age 16-18 years (8) but may continue during the third and fourth decades depending on the skeletal sites. Among other environmental factors, nutrition and especially calcium intake (7) are assumed to influence genetically determined peak bone mass. However, the role of other nutrients such as dietary salt and protein intake, which may influence the attainment of a positive calcium balance, have not been adequately explored (9).

The NIH consensus panel has recently acknowledged the importance of dietary salt and protein intake in development of an optimal calcium balance. But, the panel decided to simplify their recommendations on the basis of the assumptions that such dietary modifications may cause a great deal of confusion in the public domain. The objective of this review is to initially discuss the potential pathophysiological associations between dietary salt and protein intake and the development of skeletal bone loss.

PATHOGENESIS OF SALT-INDUCED SKELETAL BONE LOSS

Epidemiology

Calcium balance is known (10) to be influenced by (a) calcium intake, (b) intestinal calcium absorption, (c) urinary calcium excretion and (d) endogenous fecal loss of calcium. (Figure 1).

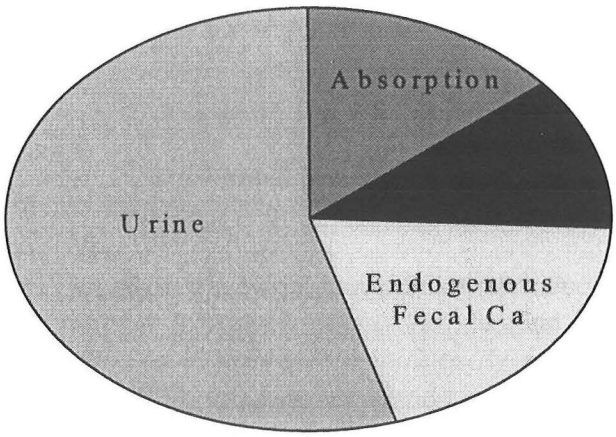


Fig 1-Changes in Calcium Balance

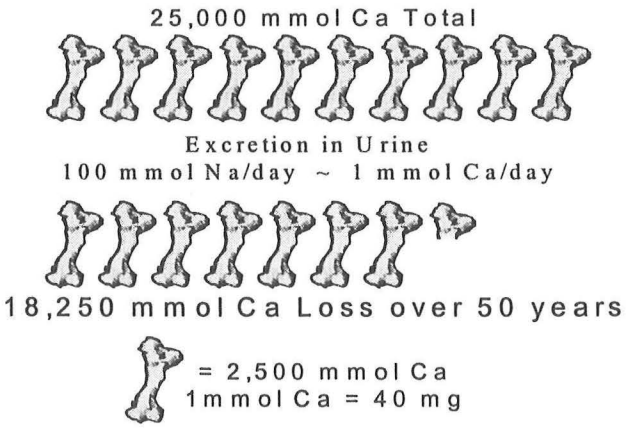
Intake and absorption of calcium account for 25% and urinary calcium excretion for 50% of change in calcium balance. The diet of current primitive cultures have lower sodium intakes of 20-40 mmol/day and potassium intakes of 150-290 mmol/day (11,12). In contrast, the American diet and diets of other industrialized societies contain a high amount of sodium at 80-250 mmol/day and low potassium of 30-70 mmol/day (13-17). The potassium:sodium ratio of <0.4 in western cultures is significantly lower than among current primitive cultures which were all found to have a high potassium:sodium ratio of more than 3

and closer to 10 (17). The impact of such a dietary composition on the low incidence of hypertension, coronary artery disease, heart failure and stroke has been shown in various epidemiological studies.

As compared with the recently published Third National Health and Nutrition Examination Survey (NHANES III 1988-1991), approximately one half of postmenopausal women have dietary intakes of sodium exceeding 120 mmol/day. Thus, dietary sodium intake may potentially play a significant role in the skeletal bone turnover and consequently the development of osteoporosis in this target population. This data was based on the twenty-four hour urinary sodium excretion, which is a more accurate measure of sodium intake than dietary recall (18).

Dietary sodium chloride ("salt") intake is one of the major determinants of the rate of urinary calcium excretion in humans (19-21). It has been long recognized that renal tubular reabsorption of calcium is dependent on that of sodium chloride, and when reabsorption of sodium chloride is inhibited so is that of calcium (22-24). Urinary sodium and calcium excretion have a strong positive correlation over a wide range of sodium intakes (25,26). It has been demonstrated in humans that there is 1 mmol of calcium excreted for every 100 mmol of sodium excreted (25). Thus, uncompensated urinary loss of calcium over a lifetime appears to result in a significant negative calcium balance (Table 1) and potentially skeletal bone loss.

Table 1-Estimated Skeletal Bone Loss



Pathogenetic Mechanisms of Salt-Induced Skeletal Bone Loss

Homeostatic adaptations to diets containing abundant sodium chloride are different in young men and women as compared to older human subjects and postmenopausal women (27). In young normal men and premenopausal women, a high sodium intake causes enhanced urinary calcium excretion, which leads to an increased parathyroid hormone secretion (PTH), an increase in serum 1,25-dihydroxyvitamin D (1,25-$(OH)_2D$) and a compensatory rise in intestinal calcium absorption (28). (Figure 2). Patients with hypoparathyroidism do not show an adaptive response, implying the importance of the role of PTH in stimulation of renal 1,25-$(OH)_2D$ synthesis.

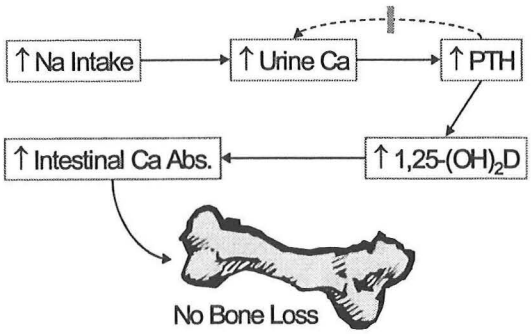


Fig 2-Salt Intake and Calcium Adaptation in Young Subjects

The homeostatic adaptation to an increased sodium chloride intake is also impaired in postmenopausal women (27). Enhanced 1,25-$(OH)_2D$ synthesis following dietary sodium loading is defective in postmenopausal women. This finding has been suggested to be the result of an impaired release of PTH or end organ response, rendering these women unable to compensate for urinary calcium losses induced by sodium. (Figure 3).

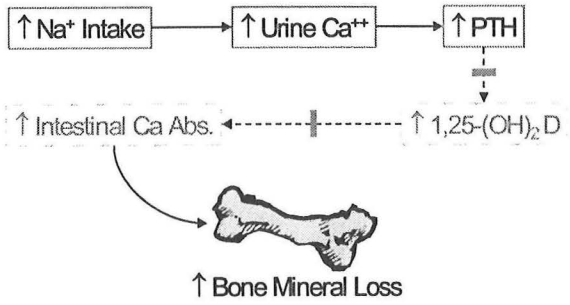


Fig 3-Salt Intake and Calcium Adaptation in Old Subjects

Several investigators have provided evidence for the lack of a rise in serum 1,25-$(OH)_2D$ concentration and an impaired intestinal calcium absorption in response to reduced dietary calcium intake following intravenous human PTH (1-34) administration in elderly patients with and without osteoporosis (29-31). The rise of serum 1,25-$(OH)_2D$ concentration following PTH infusion, and after salt loading, has been correlated inversely with age and directly with glomerular filtration rate (GFR) (31). These data would suggest an impaired ability of the aging kidney to synthesize 1,25-$(OH)_2D$. Moreover, the blunted adaptation to low calcium and high salt diets has been suggested to result from defective responsiveness of the aging intestine to 1,25-$(OH)_2D$. However, most studies in postmenopausal osteoporotic patients have found normal responsiveness of intestinal calcium absorption to small doses of synthetic oral calcitriol administration (29).

Recently several studies have provided evidence for a role of salt-induced calciura in changes of biochemical markers of skeletal bone turnover in humans and animals. Several studies have demonstrated a positive correlation between sodium and calcium excretion and urinary hydroxyproline (a bone resorption marker) in humans (32-34). Newer specific markers of skeletal bone turnover such as serum osteocalcin (an index of bone formation) have been

demonstrated to significantly increase following an increase of oral salt intake in healthy elderly human subjects (33).

Long-term studies are required to detect changes in bone mineral density following diets high in salt, however, only a few human studies have explored this mechanism. In a long-term longitudinal study in postmenopausal women, a negative correlation between urinary sodium excretion and changes in hip bone mineral density were found (35). At a sodium intake of 92 mmol/day or less, no change in hip bone mineral density occurred. Therefore, it was suggested that such a reduction in salt intake would achieve the same protective effect on bone as increasing dietary calcium by 22 mmol/day. Also demonstrated in a subset of postmenopausal osteoporotics with renal hypercalciuria and increased parathyroid activity (36), was that treatment with thiazide diuretics corrected the renal calcium leak, parathyroid stimulation, and increased bone turnover.

The effects of salt supplements on urinary calcium excretion and skeletal bone turnover have been studied in intact rats, and compared with thyroparathyroidectomized rates (TPTX) (37). Intact rats on high salt diets have increased urinary excretion of calcium, hydroxyproline, and cyclic AMP, suggesting an increased breakdown of collagen, the most abundant protein of the organic matrix of bone. However, in TPTX rats, salt augmented urinary calcium excretion but did not affect the urinary excretion of cyclic AMP or hydroxyproline. (Figure 4). This result suggests the pathogenetic importance of PTH in the development of skeletal bone loss following an excess dietary salt intake. Moreover, animals with high dietary intake of sodium have shown a defective skeletal bone calcium and phosphorus accretion, despite an adequate dietary calcium intake (38). Radioisotope labeling indicated that bone loss related to sodium chloride in these rats is due to a predominantly increased bone resorption (38).

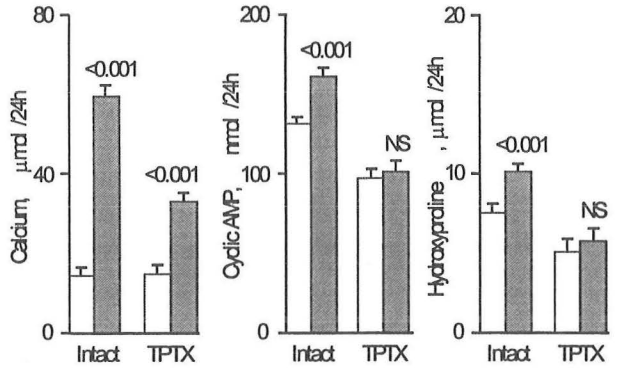


Fig 4-Effects of Salt Supplementation in Rats

Experimental study in rats has shown that increased salt intake causes an increased urinary excretion of calcium and hydroxyproline (39) and oophorectomy does not further aggrevate the loss, while maintained on a normal calcium diet. The loss of urinary calcium has been shown to be exaggerated while oophorectomized rats were maintained on a low calcium diet (40).

The results of the studies in human subjects as well as animal studies are consistent in that an increased habitual intake of sodium chloride (salt) may influence skeletal bone mineral loss. However, the role of other anions accompanied sodium has not been fully explored. Few studies

have shown sodium bicarbonate and sodium citrate (41) may not increase urinary calcium excretion when compared to sodium chloride. The fall in urinary net acid excretion, caused by neutralization of the dietary acid load following bicarbonate and citrate salt administration has been shown to blunt the rise of urinary calcium as compared with equimolar amount of sodium chloride, in salt-sensitive subjects (41,42). In addition, potassium chloride in contrast to sodium chloride has been shown to have no effect on urinary calcium excretion (42,43).

The independent effect of chloride on renal handling of calcium has not been fully investigated; however, sodium consumed with a metabolizing anion such as bicarbonate and citrate may be potentially effective against skeletal bone mineral loss. This effect is presumably exerted through the lowering of urinary calcium excretion.

PATHOGENESIS OF PROTEIN-INDUCED SKELETAL BONE LOSS

Epidemiology

The diet in industrialized societies is suboptimal in potassium, as well as deficient in naturally occurring anions, such as citrate, malate and gluconate. These anions accompanied with potassium in foods are known to subsequently be metabolized to bicarbonate in the body.

The recommended dietary allowance (RDA) for protein established by the World Health Organization and by the Food and Agriculture Organization is 0.8 gkg/day in adults, or approximately 50-70 g/day. In the United States, usual protein intake far exceeds this amount; the range is 90-100 g/day. In general, protein intake is lower in women than men. Nevertheless, there is an excess of dietary protein for most of the population. The metabolism of high protein diets delivers enough noncarbonic acid (44-46) (sulfuric acid) to the body to induce a subtle level of chronic metabolic acidosis. Thus, compared to the diet of nonindustrialized countries, the diets in industrialized nations tend to be rich in net acid load. The net acid load reflects the balance between acid-producing and base-producing components of the diet. Animal foods are the richest source of acid substrates: sulfur-containing amino acids of proteins which are known to release sulfuric acid when metabolism occurs. Vegetables and fruits are the richest source of base precursors; naturally occurring organic potassium salts (citrate, malate and gluconate) yield potassium bicarbonate (47).

Sodium, potassium and other electrolytes gain access into the extracellular fluid as a result of dietary ingestion. Acids and bases are end products of metabolism and rarely present in this form in orally ingested food products. Acids are normally presented into body fluid by the process of net acid production under normal circumstances, certain neutral precursor substrates are metabolized, releasing a variety of strong acids. The endogenous acids are derived either from the breakdown of the common constituents of the diet or the catabolism of body tissues.

The three metabolic processes that contribute to the endogenous acid load are oxidation of the sulfhydryl groups of cystine and methionine to from sulfuric acid; hydrolysis of phosphoesters to form phosphoric acid, and the incomplete breakdown of neutral carbohydrates, fats and proteins to form organic acids.

The presence of acids in the diet will augment the hydrogen load derived from endogenous acid production. In contrast, the ingestion of bicarbonate or other strong base, or if organic acid anions that may be converted to bicarbonate by cellular metabolism (citrate, gluconate, acetate), will tend to offset the endogenous hydrogen ion load.

Some endogenously generated organic acids are true end products of metabolism such as uric acid and creatinine and contribute only minimally, to the normal acid load. Other organic acids are metabolic intermediates (acetoacetic acid, beta-hydroxybutyric acid and lactic acid) and will contribute to the acid load when generated in excess, then can be metabolized to carbon dioxide and water.

In a cross-cultural comparison (48), the incidence of hip fractures was suggested to be related to protein consumption. The incidence of non-traumatic hip bone fractures related to osteoporosis is high in industrialized countries where the population consumes a high-protein diet, and is low in less industrialized countries where the population eats a lower-protein diet. (Figure 5).

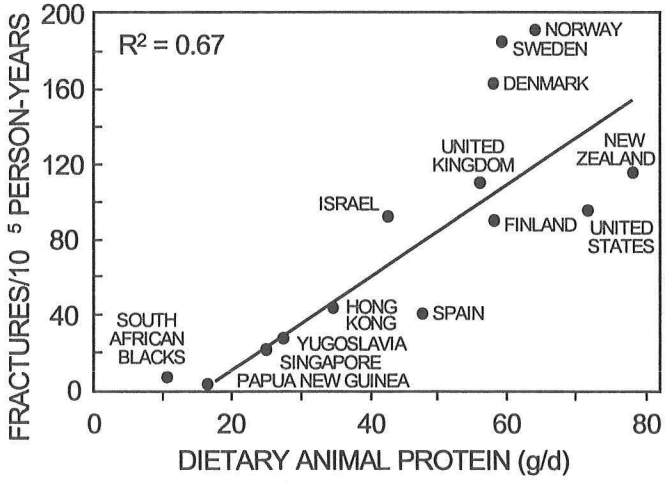


Fig 5-Hip Fracture Incidence Vs. Protein Intake

In a recent epidemiologic study (49), usual dietary intake was measured in a cohort of 85,900 women, aged 35-39 years, who were participating in the Nurse's Health Study. Protein consumption of more than 95 g/day was shown to be associated with an increased risk of forearm bone fracture compared with those who consumed less than 68 g/day. A similar increase was shown for animal protein, but no association was found for consumption of vegetable protein. Women who consumed five or more servings of red meat per week were shown to have significantly increased risk of forearm bone fracture compared with women who consumed red meat less than once per week.

In a study of women from different areas of China (50), urinary calcium was shown to vary as a function of dietary protein intake. In these subjects, urinary calcium increased as urinary net acid excretion increased.

Pathogenetic Mechanisms of Dietary Protein-Induced Skeletal Bone and Muscle Loss

In normal adult subjects consuming a net acid producing diet (American diets), systemic acid-base equilibrium is maintained within narrow limits (44,51-53). The regulation of acid-base equilibrium, is dependent on excretion of acid in urine (54). It has been shown that the kidney adjusts the rate of acid excretion keeping with the dietary changes in the net endogenous acid production. However, at all levels of renal function, the kidneys do not excrete the entire acid load (Figure 6) (44,45). Thus, healthy men and women develop a progressive low grade chronic metabolic acidosis (44) with the natural aging process. Renal function progressively declines with advancing age (55,56), and declines significantly between ages of 20 to 80 years in otherwise healthy subjects. Several investigators provided evidence for the role of aging in the development of a progressive increase in blood acidity and decrease in plasma bicarbonate concentration (57,58) (Figure 7).

Fig 6-Acid-Base Homeostasis

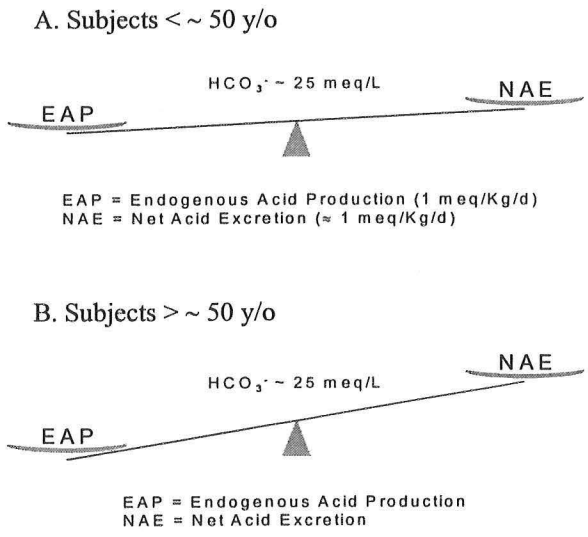
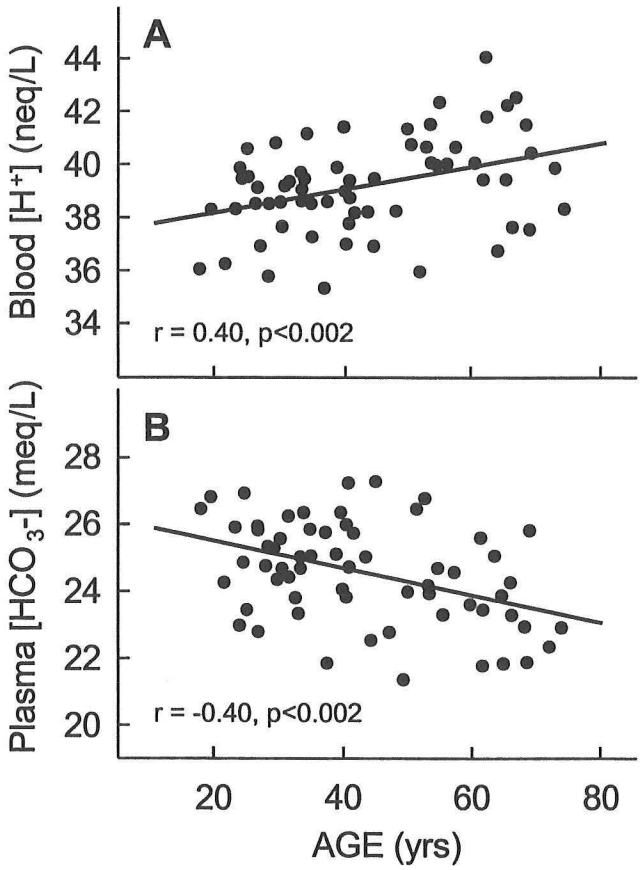


Fig 7-Changes in $[H^+]$ and $[HCO_3]$ With Age



The degree of metabolic acidosis in the systemic circulation is mitigated by the adaptations in the renal and extrarenal homeostatic mechanisms which serve to minimize the day-to-day alteration in blood acid-base composition (59). These adaptive mechanisms have been shown to

alteration in blood acid-base composition (59). These adaptive mechanisms have been shown to be deleterious to the body, as was recently suggested in the formulation of a "trade off" hypothesis (60). Under these circumstances, bone and skeletal muscle have been shown to participate as the effective buffer system to neutralize the dietary acid load (61-65). Bone contains a sufficient amount of alkali to neutralize the dietary acid load over a lifetime (66). It has been estimated that for each milliequivalent of acid buffered by bone, two milliequivalents of calcium is released into circulation. This amount of calcium loss will be sufficient to create a significant negative calcium balance and osteoporosis.

Diet-induced metabolic acidosis might also be a cause of age-related decline in skeletal muscle mass (67-71). Metabolic acidosis increases the rate of catabolism of skeletal muscle proteins (72,73), but not the rate of protein synthesis. A decrease in muscle mass in the elderly may increase the risk of falls and increase the occurrence of hip and wrist bone fractures. The risk for development of hip fractures in the elderly has been demonstrated to be directly dependent on the number of falls, and not only a function of the degree of osteopenia. The other long-term complications of diet-dependent chronic metabolic acidosis include decreased renal citrate production and excretion (59), renal hypercalciuria (74) and the progression of renal disease (75).

Role of Bone in Extrarenal Acid Buffering

There is substantial evidence supporting the role of bone and skeletal muscle in limiting the magnitude of the fall in serum bicarbonate concentration and blood pH during acute and chronic acidosis (66). In this review we focus on the homeostatic mechanism involved in extra renal acid buffering.

It has been demonstrated, that chronic metabolic acidosis increases skeletal bone alkali release by three different mechanisms: (a) physicochemical mechanism, (b) cell-medicated osteoclastic bone resorption and inhibition of osteoblastic bone formation and (c) by inhibition of renal tubular calcium reabsorption, PTH stimulation and perhaps alterations in vitamin D metabolism.

Physicochemical Mechanism

The skeleton contains 99% of the total body calcium, 35% of body sodium, 80% of the total carbon dioxide (including carbonate, bicarbonate and CO_2), 80% of the citrate and 60% of the body magnesium (Table 2) (76).

Table 2-Ionic Composition of Bone Mineral

Cations	Anions
Calcium (6.66)	Phosphate (4.02)
Sodium (0.32)	Carbonate (0.79)
Magnesium (0.18)	Citrate (0.05)
Potassium (0.02)	Chloride (0.02)

Values are indicated as mmol/g of dry fat-free bone

Approximately two-thirds of the carbon dioxide is in the form of carbonate complexed with sodium, calcium and other cations embedded in the lattice of bone crystals and is not readily available to the systemic circulation (77). The remaining one-third of carbon dioxide is in the form of bicarbonate (HCO_3^-) which is located on the surface of bone crystals (hydration shell) where it is readily available to the systemic circulation.

It has been shown that initially the physicochemical process of sodium for hydrogen exchange occurs on the mineral surface with the release of bone bicarbonate during acute metabolic acidosis (78). During this process, there is only a little calcium released from bone (79). Therefore, proton buffering in acute metabolic acidosis involves mainly reduction in bone sodium, potassium and CO_2 contents (Table 3).

	Metabolic Acidosis	
	Acute	Chronic
H^+ Influx	↑↑↑	↑↑↑
Ca^{++} Efflux	↑↑	↑↑↑
Efflux of Ions other than Ca^{++}	HCO_3^-, Na^+, K^+	CO_3^{-2},PO_4^{-3}

Table 3-Ionic Fluxes in Metabolic Acidosis

In the chronic metabolic acidosis, calcium carbonate efflux constitutes the main buffering mechanism. In this process carbonate and not phosphorus is the major anion accompanying calcium, therefore, its source could be hydroxyapatite. Thus, in chronic metabolic acidosis, in contrast to acute metabolic acidosis, there is significant reduction in the total mineral content of bone accompanied with the dissolution of the bone crystal (Table 3).

Balance studies in men demonstrated an equivalence between urinary calcium excretion and hydrogen retention in chronic metabolic acidosis induced by oral ammonium chloride (NH_4Cl) (61). The results suggested that during chronic metabolic acidosis increased bone dissolution occurs and is accompanied by the release of calcium into circulation. The loss of phosphorus was associated with acidosis but disappeared later, while calcium loss persisted beyond when NH_4C1 load was discontinued. This study supported evidence for the role of calcium carbonate as the main buffer constituent in chronic metabolic acidosis.

Cellular-Medicated Mechanism

Effect on Osteoclasts

It has been demonstrated that in chronic metabolic acidosis, the bone resorption occurs due to both the physicochemical effect of hydrogen ions on the bone mineral, as well as the enhanced cellular (osteoclastic) skeletal bone resorption (80). In vitro studies (81) using fetal mouse calvaria have demonstrated that acute exposure of bone to an acidic medium resulted in calcium efflux from both live and dead bone. In chronic acid exposure from 48 to 99 h, calcium efflux continued only in live bone, indicative of cell-medicated bone resorption. (Figure 8). In addition, metabolic acidosis stimulated the increased release of osteoclastic enzyme B-glucuronidase into medium (82) indicating an increase in osteoclastic activity, and also inhibition of osteoblastic collagen synthesis and alkaline phosphatase release. Calcitonin, an inhibitor of osteoclasts,

prevented the acidosis-stimulated calcium efflux from live bone (83). In an experimental model in which isolated osteoclasts were placed on slices of bovine cortical bone, the medium pH was reduced from 7.4 to 6.8 resulting in a significant increase in the number of resorption pits (84). The addition of PTH into the medium did not alter the osteoclastic response at either pH, but calcitonin inhibited osteoclastic bone resorption. Thus, acidic environment stimulates bone resorption both directly and also through PTH-independent cell-mediated mechanism.

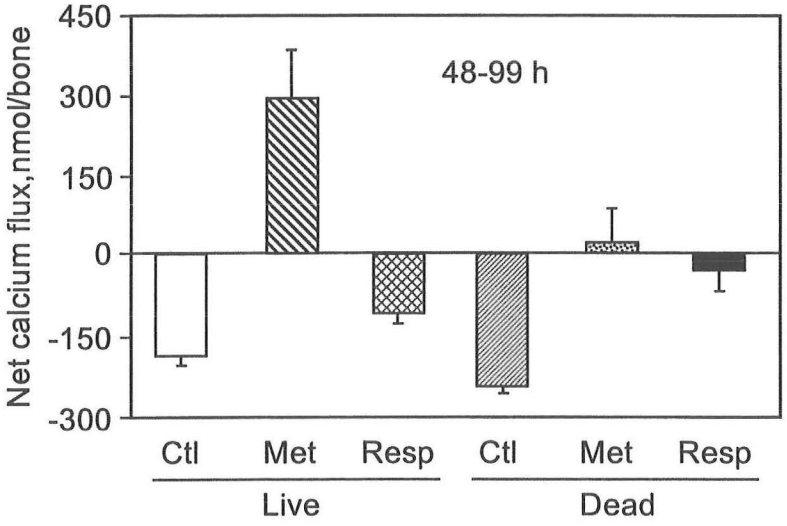


Fig 8-Net Calcium flux During Chronic Metabolic Acidosis

However, in vivo studies in nephrectomized thyroparathyroidectomized (TPTX) animals has confirmed the role of PTH in the extrarenal buffering of an acute acid load (85). The results of the study showed that TPTX nephrectomized animals were unable to tolerate an acid load, compared to intact nephrectomized animals. The TPTX nephrectomized animals developed acidosis with a significant fall in blood pH and bicarbonate concentration and a high mortality rate as compared to intact animals. However, TPTX nephrectomized animals pretreated with PTH, tolerated acid load like intact animals. The results of these studies suggested that PTH may play a role in extrarenal acid buffering.

Four possibilities have been proposed to explain the mechanism (s) of acid-induced osteoclastic bone resorption. First, an acidic environment at the bone cell-mineral interphase may facilitate removal of bone mineral and the exposure of bone matrix to acid hydrolases and collagenases elaborated by osteoclasts and macrophages (86). Second, an increased activity in the acid extruding transporters including Na^+/H^+ antiporter or H^+-ATPase, which in turn increases local H^+concentration at the cell-mineral interphase and provides an optimum medium for the release of hydrolases by the osteoclasts (66). Third, enhanced formation of podosomes, microfilament containing structures which are responsible for the adhesion of osteoclasts to the bone matrix (87). Lastly, coupling between osteoblasts and osteoclasts may play a role for acid-induced osteoclastic bone resorption (88).

Effect on Osteoblasts

It has been demonstrated that osteoblastic osteoid deposition is inhibited with metabolic acidosis. The decrease in bone formation blocks the hydrogen ion release that accompanies bone mineral formation. The protons released into the bone formation area during the process of hydroxyapatite formation must be either removed or neutralized in order for optimal bone formation to occur. Micropuncture analysis of the fluid microenvironment participating in the mineralization has shown a higher bicarbonate concentration compared to systemic circulation (89).

Acidosis and potassium deficiency in rats has been associated with low serum IGF-1 concentrations (90,91). Children and adults with chronic metabolic acidosis have low IGF-1 concentrations. IGF-1 is the best factor known to increase osteoblastic bone formation. It induces bone formation partially by increasing the pool of osteoblast precursors. However, it is not known whether the effect of potassium and acidosis on IGF-1 is exerted directly or mediated by changes in growth hormone secretion.

In children with the distal renal tubular acidosis and growth retardation, administration of alkali caused a significant increase in height and reversal of growth failure (92). The blunted release of GH in these children was previously documented following arginine infusion.

Hormonal Mechanisms

In humans, metabolic acidosis increases urinary calcium and phosphorus excretion without changing net intestinal calcium absorption and consequently causes a net loss of calcium from the body (61). As mentioned previously, during chronic acidosis the excess loss of calcium in the urine is from the bone (61). In a metabolic balance study following chronic oral NH_4Cl load, an average amount of acid was retained in the body at 192 meq. This was balanced with the amount of urinary calcium loss at 185 meq, implying that skeletal calcium mobilization has contributed to an excessive urinary calcium excretion. However, in some patients with chronic metabolic acidosis with a sufficient amount of salt intake, an increased urinary calcium excretion (93) is due to the inhibition of renal tubular calcium reabsorption, and consequently hypercalciuria is the cause of PTH stimulation. Thus, it is suggested that hypercalciuria caused by metabolic acidosis, and not metabolic acidosis alone is responsible for the stimulation of PTH secretion.

Under normal physiologic circumstances an increase in urinary calcium excretion and consequent fall in serum calcium leads to an increase in circulating PTH and 1,25-$(OH)_2D$. Intestinal calcium absorption is then expected to rise, resulting in restoration of serum calcium to normal. However, in some patients with metabolic acidosis, no change in the level of PTH or 1,25-$(OH)_2D$ occurs (94). But in other patients with metabolic acidosis the levels of PTH may increase (93) or decrease. The rise in ionized calcium concentration as the result of the fall in pH (94) is perhaps the responsible factor for the inhibition of both PTH secretion and 1,25- $(OH)_2D$ synthesis caused by acute metabolic acidosis.

The effect of acidosis on vitamin D metabolism is complicated, since it involves the contribution of multiple opposing regulatory factors. It has been well documented that under

normal physiological circumstances increased serum PTH and decreased serum calcium or phosphorus play a key role in the conversion of 25-hydroxyvitamin D to 1,25-$(OH)_2D$. The level of PTH generally is not altered in metabolic acidosis (94), despite hypercalciuric and hyperphosphaturic effects of metabolic acidosis (94,96). A low serum phosphorus caused by metabolic acidosis has been recently shown to increase 1,25-$(OH)_2D$ levels in normal human subjects (97). Hypophosphatemia in this study was shown to increase the production rate of 1,25-$(OH)_2D$ compared to its metabolic clearance rate. However, previous studies in human subjects found that circulating levels of 1,25-$(OH)_2D$, the major active vitamin D metabolite were not significantly decreased in metabolic acidosis (94,96). Moreover, in humans with metabolic acidosis, the rise of serum 1,25-$(OH)_2D$ in response to PTH infusion is not blunted. In the earlier study a low 1,25-$(OH)_2D$ serum concentration was attributed to diminished renal 1-hydroxylase activity (98) in acidotic rats. These experimental studies in the rats showed an impaired production of 1,25-$(OH)_2$ D in the isolated perfused kidney ensued with metabolic acidosis.

Thus, metabolic acidosis, generally alters the sensitivity and activity of feedback mechanism that normally adjusts calcium and phosphorus homeostasis by PTH and vitamin D. However, the degree of involvement of the key regulatory system appears to be influenced in the different species depending on the experimental conditions of the experiments.

Metabolic Balance Studies

The negative calcium balance resulting from an increased intake of dietary protein were detailed previously in several metabolic balance studies (63). In a recent study in 18 postmenopausal women, neutralization of the dietary net acid load with supplemental potassium bicarbonate, reduced urinary calcium and phosphorus losses. Administration of 60 to 120 mmol potassium bicarbonate per day resulted in a significant fall in urinary calcium excretion (236 ± 66 to 172 ± 81 mg/day, $P<0.001$), and a subsequent improvement of the net calcium balance from a negative to a less negative value (99). It also decreased bone resorption as assessed by the urinary markers of bone resorption and increased bone formation. Urinary hydroxyproline excretion decreased significantly (28.9 ± 12.3 to 26.8 ± 10.8 mg/day, $p<0.05$) and serum osteocalcin concentration increased (5.5 ± 2.8 to 6.1 ± 2.8 ng/ml, $p <0.01$) (Figure 9).

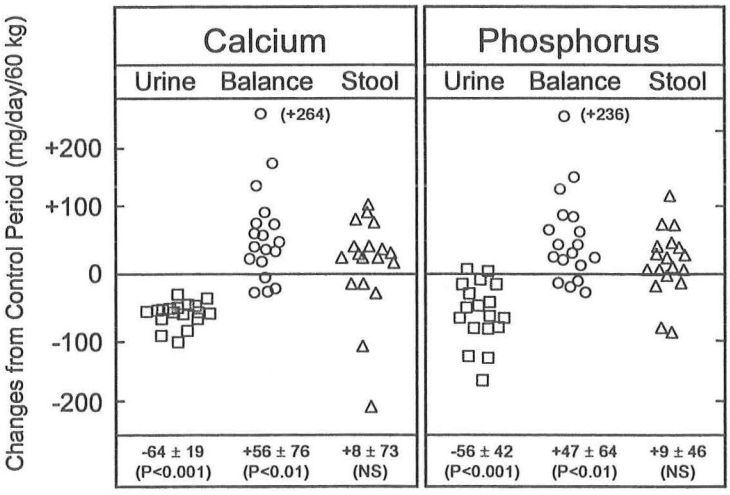


Fig 9-Effect of Potassium bicarbonate on Calcium and Phosphorous Balance

The result of this study is consistent with the previous studies using different forms of alkali salts in human subjects. In postmenopausal women, substitution of sodium bicarbonate for the dietary sodium chloride can improve calcium balance (100). In another study, healthy young normal male subjects who received potassium bicarbonate were shown to improve calcium and phosphorus balance (61). In contrast, institution of sodium bicarbonate was not effective in an improvement of calcium balance. The difference in calcium balance in this study was attributed to the calciuric effect of sodium when administered as sodium bicarbonate. In another study conducted in immobilized patients, a combined treatment with sodium bicarbonate and potassium bicarbonate were shown to induce a positive calcium balance.

The above findings, suggest that age-related accelerated bone turnover and potentially low bone mineral mass caused by the cumulative effect of the net acid-producing diet may be reversed by the provision of alkali.

Administration of potassium bicarbonate also improved phosphorus balance. The improvement of phosphorus balance was principally due to the reduction in urinary phosphorus excretion. Parallel improvement of calcium and phosphorus balances imply that the calcium and phosphorus retained is accumulated in the hydroxyapatite crystal of bone.

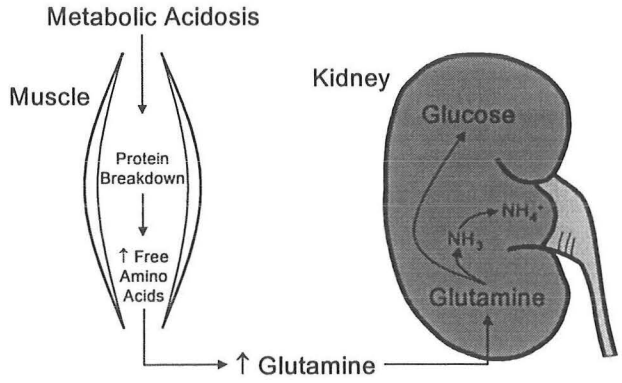
Role of Skeletal Muscle in Extrarenal Acid Buffering

Approximately one third of body weight is skeletal muscle. In chronic metabolic acidosis, protein breakdown in skeletal muscle is accelerated (101). An increased skeletal muscle protein catabolism and negative nitrogen balance has been typically encountered in patients with chronic renal failure.

It has recently been shown that the attendant low grade chronic metabolic acidosis of habitual ingestion of typical net acid producing diets might cause a sustained state of skeletal muscle protein catabolism and consequent nitrogen wasting (67-71). The gradual negative nitrogen balance in adult humans, may conceivably account for the normal progressive decrease in muscle mass in older population (102). It has been suggested that diet-induced skeletal muscle loss may be intensified with aging (102), from the normal age-related decline in the function of kidney.

Acidosis-induced skeletal muscle protein catabolism is thought to be an acid-base homeostatic adjustment mechanism. It has been suggested that nonbranched chain amino acids, especially glutamine in increased amounts are released into circulation during this process. The increased supply of glutamine is then available to the kidney for the generation and excretion of ammonium, thereby enhancing the degree of renal acid excretion. This adaptive mechanism occurs with an attendant increase in muscle nitrogen loss and perturbation of protein synthesis "Trade Off" (60). Moreover, chronic metabolic acidosis can cause an adaptive increase in renal glutamine extraction and ammonia production (103), and enhancement of hepatic production of glutamine, which also maintains renal glutamine extraction and utilization (104) (Figure 10).

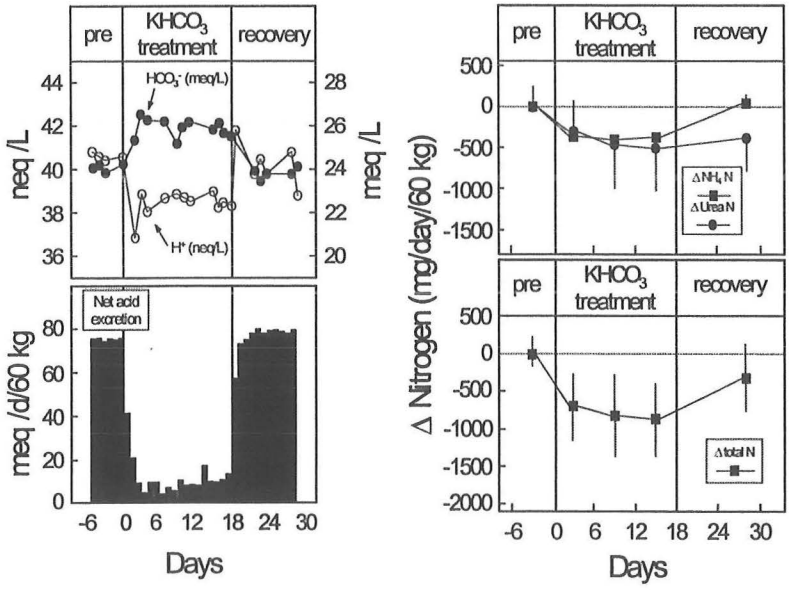
Fig 10-Role of Muscle in Acid-Base Homeostasis



The proteolytic effect of chronic metabolic acidosis is due to stimulation of an ATP and ubiquitin-dependent proteolytic mechanism, and an increased oxidation of the released branched chain amino acids (valine, leucine and isoleucine) preventing reuptake for protein synthesis (105). It has been shown that an increase in muscle proteolytic activity is glucocorticoid mediated (73). Acidosis without glucocorticoids or glucocorticoids without acidosis is known not to increase protein breakdown.

The disturbance of nitrogen balance has recently been shown to improve following administration of alkali (102), suggesting that nitrogen deficit arises directly from the acidosis. The effect of oral potassium bicarbonate was examined in 14 healthy postmenopausal women. The treatment of the attendant metabolic acidosis with oral potassium bicarbonate ($KHCO_3$; 60-120 mmol/day for 18 days) while they were maintained on a constant diet, significantly reduced blood hydrogen concentration and increased the plasma bicarbonate concentration suggestive of the neutralization of diet-dependent endogenous acid production. With these changes urinary ammonia nitrogen, urea nitrogen and total nitrogen significantly decreased (Figure 11). The total reduction of nitrogen excretion was found to be sufficient to prevent age-induced loss of muscle and correct the nitrogen deficit.

Fig 11- Urinary Acid and Nitrogen Excretion With Potassium Bicarbonate



References

1. Pocock NA, Eisman JA, Hopper JL, et al. Genetic determinants of bone mass in adults: A twin study. J Clin Invest. 1987; 80:706-710.

2. Harris SS, Eccleshall TR, Gross C, et al. The vitamin D receptor start codon polymorphism (Fok I) and bone mineral density in premenopausal American black and white women. J Bone Miner Res. 1997; 12:1043-1048.

3. Kobayashi S, Inoue S, Hosoi T, et al. Association of bone mineral density with polymorphism of the estrogen receptor gene. J Bone Miner Res. 1996; 11:306-311.

4. Grants FA, Reid DM, Blake G, et al. Reduced bone density and osteoporosis associated with a polymorphic SP1 binding site in the collagen type Iα1 gene. Nature Genet. 1996; 14:203-205.

5. Shiraki M, Shiraki Y, Aoki C, et al. Association of bone mineral density with apolioprotein E phenotype. J Bone Miner Res. 1997; 12:1438-1445.

6. Yamada Y, Miyauchi A, Goto J, et al. Association of a polymorphism of the transforming growth factor-β1 gene with genetic susceptibility to osteoporosis in Japanese women. J Bone Miner Res. 1998; 13:1569-1576.

7. Sowers MR, Galuska DA. Epidemiology of bone mass in premenopausal women. Epidemiol Rev. 1993; 15:374-398.

8. Matkovic V, Jelic T, Wardlaw GM, et al. Timing of peak bone mass in Caucasian females and its implications for the prevention of osteoporosis. J Clin Invest. 1994; 93:799-808.

9. Optimal calcium intake. NIH Consensus Development Conference. 1994; June 6-8.

10. Heany RP, Recker RR, Saville PD. Menopausal changes in calcium balance performance. J Lab Clin Med. 1978; 92:953-963.

11. Eaton SB, Konner M. Paleolithic nutrition. A consideration of its nature and current implications. N Engl J Med. 1985; 312:283-289.

12. Oliver WJ, Cohen EL, Neel JV. Blood pressure, sodium intake and sodium related hormones in Yanomamo Indians, a "no salt" culture. Circulation 1975; 52:146-151.

13. Frisancho AR, Leonard WR. Blood pressure in blacks and whites and its relationship to dietary sodium and potassium intake. J Chron Dis. 1984; 37:515-519.

14. Khaw KT, Barrett-Connor E. Dietary potassium and stroke-associated mortality. A 12-year prospective population study. N Engl J Med. 1987; 316:235-240.

15. Langford HG. Dietary potassium and hypertension: epidemiologic data. Ann Intern Med. 1983; 98:770-772.

16. McCarron DA, Morris CD, Henry HJ, Stanton JL. Blood pressure and nutrient intake in the United States. Science. 1984; 224:1392-1398.

17. Young DB, Lin H, McCabe RD. Potassium's cardiovascular protective mechanisms. Am J Physiol. 1995; 268:R825-837.

18. Schacter J, Harper PH, Radin ME, et al. Comparison of sodium and potassium intake with excretion. Hypertension. 1980; 2:695-699.

19. Kleeman CR, Bohannan J, Bernstein D, et al. Effect of variations in sodium intake on calcium excretion in normal humans. Proc Soc Exp Biol Med. 1964; 115:29-32.

20. Sakhaee K, Harvey JA, Padalino PK, et al. The potential role of salt abuse on the risk for kidney stone formation. J Urol. 1993; 150:310-312.

21. Sakhaee K, Nicar M, Hill K, Pak CYC. Contrasting effects of potassium citrate and sodium citrate therapies on urinary chemistries and crystallization of stone-forming salts. Kidney Int. 1983; 24:348-352.

22. Costanzo LS, Windhager EE. Renal regulation of calcium balance. In: The Kidney: Physiology and Pathophysiology: Seldin DW, Giebisch G Eds, 2nd ed. 1992; pp. 2375-2393. Raven Press, New York.

23. Freidman PA, Gesek FA. Calcium transport in renal epithelial cells. Am J Physiol. 1993; 264:F181-F198.

24. Sakhaee K. The effect of diuretics on calcium metabolism: physiologic and clinical effects. In: Diuretic Agents: Clinical Physiology and Pharmacology:Seldin DW, Giebisch G Eds, 1st ed. 1997; pp. 595-609. Academic Press, California.

25. Nordin BEC, Need AG, Morris HA, Horowitz M. The nature and significance of the relationship between urinary sodium and urinary calcium in women. J Nutr. 1993; 123:1615-1622.

26. Massey LK, Whiting SJ. Dietary salt, urinary calcium, and bone loss. J Bone Miner Res. 1996; 11:731-736.

27. Breslau NA, Sakhaee K, Pak CYC. Impaired adaptation to salt-induced urinary calcium losses in postmenopausal osteoporosis. Trans Assoc Am Phys. 1985; 98:107-115.

28. Breslau NA, McGuire JL, Zerwekh JE, Pak CYC. The role of dietary sodium on renal excretion and intestinal absorption of calcium and on vitamin D metabolism. J Clin Endocrinol Metab. 1982; 55:369-373.

29. Gallagher JC, Riggs BL, Eisman J, et al. Intestinal calcium absorption and serum vitamin D metabolites in normal subjects and osteoporotic patients. Effect of age and dietary calcium. J Clin Invest. 1979; 64:729-736.

30. Slovik DM, Adams JS, Neer RM, et al. Deficient production of 1,25-dihydroxyvitamin D in elderly osteoporotic patients. N Engl J Med. 1981; 305:372-374.

31. Tsai KS, Heath H III, Kumar R, et al. Impaired vitamin D metabolism with aging in women. Possible role in pathogenesis of senile osteoporosis. J Clin Invest. 1984; 73:1668-1672.

32. Chan ELP, Ho CS, McDonald D, et al. Interrelationships between urinary sodium, calcium, hydroxproline and serum PTH in healthy subjects. Acta Endocrinol. 1992; 127:242-245.

33. McParland BE, Goulding A, Campbell AJ. Dietary salt affects biochemical markers of resorption and formation of bone in elderly women. Br Med J. 1989; 299:834-835.

34. Need AG, Morris HA, Cleghorn DB, et al. Effect of salt restriction on urine hydroxyproline excretion in postmenopausal women. Arch Intern Med. 1991; 151:757-759.

35. Devin A, Criddle RA, Dick IM, et al. A longitudinal study of the effect of sodium and calcium intakes on regional bone density in postmenopausal women. Am J Clin Nutr. 1995; 62:740-745.

36. Sakhaee K, Nicar MJ, Glass K, et al. Postmenopausal osteoporosis as a manifestation of renal hypercalciuria with secondary hyperparathyroidism. J Clin Endocrinol Metab. 1985; 61:368-373.

37. Goulding A. Effect of dietary NaC1 supplements on parathyroid function, bone turnover and bone composition in rats taking restricted amount of calcium. Min Electrolyte Metab. 1980; 4:203-208.

38. Goulding A, Campbell DR. Effect of oral loads of sodium chloride on bone composition in growing rats consuming ample dietary calcium. Min Electrolyte Metab. 1984; 10:58-62.

39. Chan ELP, Swaminathan R. Effect of different amounts of sodium intake for four months on calcium metabolism in normal oophorectomized rats. J Bone Miner Res. 1993; 8:1185-1189.

40. Goulding A, Campbell DR. Dietary NaC1 loads promote calciuria and bone loss in adult oophorectomized rats consuming a low calcium diet. J Nutr. 1983; 113:1409-1414.

41. Kurtz TW, Al-Bander HA, Morris Jr RC. Salt sensitive essential hypertension in men. Is the sodium ion alone important? New Engl J Med. 1987; 317:1043-1048.

42. Sakhaee K, Alpern RJ, Jacobson HR, Pak CYC. Contrasting effects of various potassium salts on renal citrate excretion. J Clin Endocrinol Metab. 1991; 72:396-400.

43. Lemann Jr J, Gray RW, Pleuss JA. Potassium bicarbonate, but not sodium bicarbonate, reduces urinary calcium excretion and improves calcium balance in healthy men. Kidney Int. 1989; 35:688-695.

44. Kurtz I, Maher T, Hulter HN, et al. Effect of diet on plasma acid-base composition in normal humans. Kidney Int. 1983; 24:670-680.

45. Lennon EJ, Lemann Jr J, Litzow JR. The effect of diet and stool composition on the net external acid balance of normal subjects. J Clin Invest. 1966; 45:1601-1607.

46. Sherman HC, Sinclair JE. The balance of acid-forming and base-forming elements in foods. J Bio Chem. 1907; 3:307-309.

47. Breslau NA, Brinkley L, Hill KD, Pak CYC. Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. J Clin Endocrinol Metab. 1988; 66:140-146.

48. Abelow BJ, Holford TR, Insogna KL. Cross cultural association between dietary animal protein and hip fracture: A hypothesis. Calcif Tissue Int. 1992; 50:14-18.

49. Feskanich D, Willet WC, Stampfer MJ, et al. Protein consumption and bone fracture in women. Am J Epidemiol. 1996; 143:472-479.

50. Hu JF, Zhao XH, Parpia B, et al. Dietary intakes and urinary excretion of calcium and acids: A cross-sectional study of women in China. Am J Clin Nutr. 1993; 58:398-406.

51. Madias NE, Adrogue HJ, Horowitz GL, et al. A redefinition of normal acid-base equilibrium in man: Carbon dioxide as a key determinant of normal plasma bicarbonate concentration. Kidney Int. 1979; 16: 612-618.

52. Moller B. The hydrogen ion concentration of arterial blood. Acta Med Scand. 1959; 165:1-68.

53. Shock NW, Hastings AB. Studies of the acid-base balance of the blood. III. Variation in the acid-base balance of the blood in normal individuals. J Bio Chem. 1934; 104:585-600.

54. Schwartz WR, Hall PW, Hays RM, et al. On the mechanism of acidosis in chronic renal disease. J Clin Invest. 1959; 38:39-52.

55. Davies DF, Shock NW. Age changes in glomerular filtration rate, effective renal plasma flow, and tubular excretory capacity in adult males. J Clin Invest. 1950; 29:496-507.

56. Lindeman RD, Tobin J, Shock NW. Longitudinal studies on the rate of decline in renal function with age. JAGS. 1985; 33:278-285.

57. Frasetto LA, Morris Jr RC, Sebastian A. Effect of age on blood acid-base composition in adult humans: role of age-related renal functional decline. Am J Physiol. 1996; 271:F1114-F1122.

58. Frasetto LA, Sebastian A. Age and systemic acid-base equilibrium: analysis of published data. J Gerontol. 1996; 51A:B91-B99.

59. Alpern RJ, Sakhaee K. The clinical spectrum of chronic acidosis: Homeostatic mechanisms produce significant morbidity. Am J Kidney Dis. 1997; 29:291-302.

60. Alpern RJ. Trade-offs in the adaptation to acidosis. Kidney Int. 1995; 47:1205-1215.

61. Lemman Jr J, Litzow JR, Lennon EJ. The effects of chronic acid loads in normal man: further evidence for participation of bone mineral in the defense against chronic metabolic acidosis. J Clin Invest. 1966; 45:1608-1614.

62. Jaffe HL, Bodansky A, Chandler JP. Ammonium chloride decalcification, as modified by calcium intake. The relation between generalized osteoporosis and osteitis fibrosa. J Exp Med. 1932; 56:823-834.

63. Barzel US. The skeleton as an ion exchange system: implications for the role of acid-base imbalance in the genesis of osteoporosis. J Bone Miner Res. 1995; 10:1431-1436.

64. Mitch WE, Medind R, Grieber S, et al. Metabolic acidosis stimulates muscle protein degradation by activating the adenosine triphosphate-dependent pathway involving ubiquitin and proteasomes. J Clin Invest. 1994; 93:2127-2133.

65. Papadoyannakis NJ, Stefanidis CJ, McGowen M. The effect of correction of metabolic acidosis on nitrogen and potassium balance of patients with chronic renal failure. Am J Clin Nutr. 1984; 40:423-427.

66. Green J, Kleeman CR. The role of bone in regulation of systemic acid-base balance. Kidney Int. 1991; 39:9-26.

67. Frisancho AR. New standards of weight and body composition by frame size and height for assessment of nutritional status of adults and the elderly. Am J Clin Nutr. 1984; 40:808-819.

68. Aloia JF, McGowan DM, Vaswani AN, et al. Relationship of menopause to skeletal and muscle mass. Am J Clin Nutr. 1991; 53:1378-1383.

69. Pierson Jr RN, Lin DHY, Phillips RA. Total-body potassium in health: effects of age, sex, height, and fat. Am J Physiol. 1974; 226:206-212.

70. Rajagopalan B, Thomas GW, Beilin LJ, Ledingham JGG. Total body potassium falls with age. Clin Sci. 1980; 59:427s-429s.

71. Forsberg AM, Nilsson E, Wernerman J, et al. Muscle composition in relation to age and sex. Clin Sci. 1991; 81:249-256.

72. Hara Y, May RC, Kelly RA, et al. Acidosis, not azotemia, stimulates branched chain, amino acid catabolism in uremic rats. Kidney Int. 1987; 32:808-814.

73. Greiber S, Mitch WE. Mechanisms for protein catabolism in uremia: Metabolic acidosis and activation of proteolytic pathway. Miner Electrolyte Metab. 1992; 18:233-236.

74. Preminger GM, Sakhaee K, Pak CYC. Hypercalciuria and altered intestinal calcium absorption occurring independent of vitamin D in incomplete distal renal tubular acidosis. Metabolism. 1987; 36:176-179.

75. Nath KA, Salahudeen AK, Clark EC, et al. Role of cellular metabolites in progressive renal injury. Kidney Int. 1992; 38:Suppl:S109-S113.

76. Armstrong WD, Singer L. Composition and constitution of the mineral phase of bone. Clin Orthop. 1965; 38:179-190.

77. Pasquale SM, Messier AA, Shea ML, et al. Bone CO_2 titration curves in acute hypercapnia obtained with a modified titration technique. J Appl Physiol. 1980; 48:197-201.

78. Bushinsky DA, Krieger NS, Geisser DI, et al. Effects of pH on bone calcium and proton fluxes in vitro. Am J Physiol. 1983; 245:F204-F209.

79. Bushinsky DA, Levi-Setti R, Coe FL. Ion microprobe determination on bone surface elements: effects of reduced medium pH. Am J Physiol. 1986; 250:F1090-F1097.

80. Bushinsky DA. Acidosis and bone. Min Electrolyte Metab. 1994; 20:40-52.

81. Bushinsky DA, Goldring JM, Coe FL. Cellular contribution to pH mediated calcium flux in neonatal mouse calvariae. Am J Physiol. 1985; 298:F785-F789.

82. Krieger NS, Sessler NE, Bushinsky DA. Acidosis inhibits osteoblastic and stimulates osteoclastic activity in vitro. Am J Physiol. 1992; 262:F442-F448.

83. Kraut JA, Mishler DR, Kurokawa K. Effect of cochicin and calcitonin on calcemic response to metabolic acidosis. Kidney Int. 1984; 25:608-612.

84. Arnett TR, Dempster DW. Effect of pH on bone resorption by rat osteoclasts in vitro. Endocrinology. 1986; 119-124.

85. Farley DS, Adler S. An extrarenal role for parathyroid hormone in the disposal of acute acid loads in rats and dogs. J Clin Invest. 1979; 63:985-997.

86. Baron R, Neff L, Louvard D, et al. Cell mediated extracellular acidification and bone resorption: evidence for a low pH in resorbing lacunae and localization of 100-KD by so somal membrane protein at the osteoclast ruffie border. J Cell Biol. 1985; 101:2210-2222.

87. Teti A, Blair HC, Schlesinger P, et al. Extracellular protons acidify osteoclasts, reduce cytosolic calcium, and promote expression of cell-matrix attachment structures. J Clin Invest. 1989; 84:773-780.

88. McSheey PMJ, Chambers TJ. Osteoblastic cells mediate osteoclastic responsiveness to parathyroid hormone. Endocrinology. 1989; 118:824-828.

89. Cuervo LA, Pita JC, Howell DS. Ultramicroanalysis of pH, PCO_2 and carbonic anhydrase activity at calcifying sites in cartilage. Calcif Tissue Res. 1971; 7:220-231.

90. Challa A, Winn C, Kreig Jr RJ, et al. Effect of metabolic acidosis on the expression of insulin-like growth factor and growth factor receptor. Kidney Int. 1993; 44:1224-1227.

91. Flyvbjerg A, Droup I, Everts ME, et al. Evidence that potassium deficiency induces growth retardation through reduced circulating levels of growth hormone and insulin-like growth factor 1. Metabolism. 1991; 40:769-775.

92. McSherry E, Morris RC. Attainment and maintenance of normal stature with alkali therapy in infants and children with classic renal tubular acidosis. J Clin Invest. 1978; 61:509-527.

93. Coe FL, Firpo JS, Holland S, et al. Effect of acute and chronic metabolic acidosis on serum immunoreactive parathyroid hormone in man. Kidney Int. 1975; 8:262-273.

94. Weber HP, Gray RW, Dominguez JH, et al. The lack of effect of chronic metabolic acidosis on 25-OH vitamin D metabolism and serum parathyroid hormone in humans. J Clin Endocrinol Metab. 1976; 43:1047-1055.

95. Moore EW. Ionized calcium in normal serum, ultrafiltrates and whole blood determined by ion-exchange electrodes. J Clin Invest. 1970; 49:318-334.

96. Adams ND, Gray RW, Lemann Jr J. The calciuria of increased fixed acid production in humans: evidence against a role of parathyroid hormone and 1,25-$(OH)_2$-vitamin D. Calcif Tissue Int. 1979; 28:233-238.

97. Karpf R, Vetsch W, Hulter H. Chronic metabolic acidosis increases the serum concentration of 1,25-dihydroxyvitamin D in humans by stimulating its production rate. Critical role of acidosis induced renal hypophosphatemia. J Clin Invest. 1992; 90:2456-2463.

98. Lee SW, Russel J, Avioli LV. 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol: Conversion impaired by systemic acidosis. Science. 1976; 195:994-994.

99. Sebastian A, Harris St, Ottaway JH, et al. Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate. N Engl J Med. 1994; 330:1776-1781.

100. Lutz J. Calcium balance and acid-base status of women as affected by increased protein intake and by sodium bicarbonate ingestion. Am J Clin Nutr. 1984; 39:281-288.

101. Garibotto G, Russo R, Sofia A, et al. Muscle protein turnover in chronic renal failure patients with metabolic acidosis or normal acid-base balance. Miner Electrolyte Metab. 1996; 22:58-61.

102. Frassetto L, Morris Jr RC, Sebastian A. Potassium bicarbonate reduces urinary nitrogen excretion in postmenopausal women. J Clin Endocrinol Metab. 1997; 82:254-.

103. Pitts RF. Production and excretion of ammonia in relation to acid-base balance. In: Handbook of Physiology, Sec 8. Orloff J, Berliner RW, eds. Washington, DC. American Physiological Society. 1973; 455-496.

104. Cheema-Dhadli S, Jungas RL, Halprin ML. Regulation of urea synthesis by acid-base balance in vivo: role of NH_3 concentration. Am J Physiol. 1987; 252:F221-F225.

105. Baily JL. Metabolic acidosis and protein catabolism: mechanisms and clinical implications. Miner Electrolyte Metab. 1998; 24:13-19.