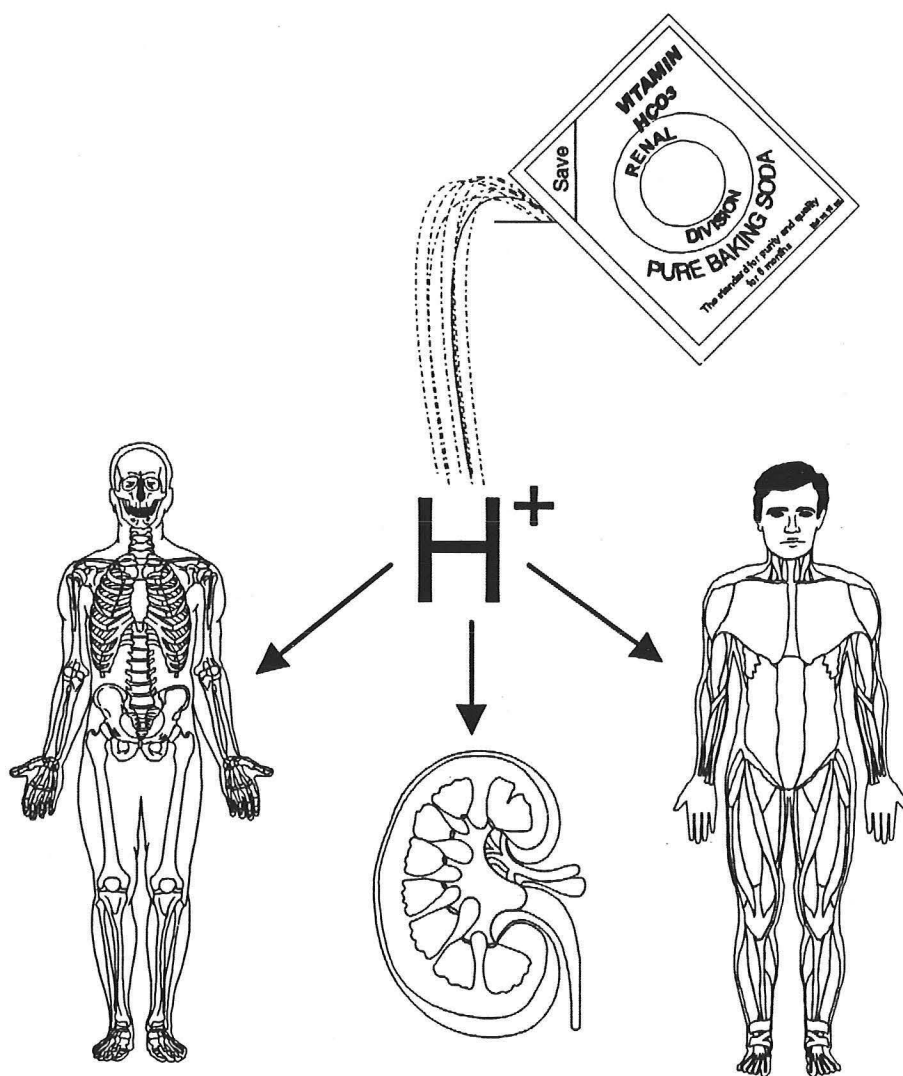


CHRONIC ACIDOSIS: AN INSIDIOUS CAUSE OF SIGNIFICANT MORBIDITY

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The University of Texas
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Internal Medicine Grand Rounds
April 11, 1996

While it is appreciated by most physicians that severe metabolic acidosis poses significant danger to the patient, the dangers of mild metabolic acidosis are less well appreciated. In this article we will first define metabolic acidosis. We emphasize that metabolic acidosis is a process rather than an abnormal plasma HCO_3^- concentration. We will then review the physiologic response to chronic metabolic acidosis, which in large part leads to the amelioration or correction of changes in blood pH and serum HCO_3^- concentration. However, inherent in the correction of acidosis are a number of trade-offs, whereby correction of acidosis is associated with consequences leading to significant patient morbidity.

Definition of Chronic Metabolic Acidosis

Chronic metabolic acidosis refers to a process whereby an excess nonvolatile acid load is chronically placed on the body due to: excess acid generation, or diminished acid removal by normal homeostatic mechanisms. Table 1 lists the most common causes of chronic metabolic acidosis. These conditions generally lead to significant decreases in the plasma HCO_3^- concentration and blood pH, and thus lead to overt metabolic acidosis.

Significant decreases in plasma HCO_3^- concentration are frequently associated with only small decreases in blood pH because of respiratory

CHRONIC METABOLIC ACIDOSIS: Etiologies	
1.	Gastrointestinal HCO_3^- loss
2.	Renal tubular acidosis
3.	Renal insufficiency

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| 1. | Gastrointestinal HCO_3^- loss |
| 2. | Renal tubular acidosis |
| 3. | Renal insufficiency |

compensation. While respiratory compensations typically do not return blood pH to 7.4, they frequently return blood pH to values close to or within the normal range. Perhaps less well appreciated is the fact that conditions associated with an excess nonvolatile acid load also may be associated with a normal serum HCO_3^- concentration. In this review, we will emphasize that the term

metabolic acidosis should be applied to the process of generating excess acid, and not be reserved for patients with abnormal serum HCO_3^- concentrations. It is well appreciated that a normal serum HCO_3^- concentration may be seen with a mixed metabolic acidosis/alkalosis, as in a patient with renal insufficiency who develops vomiting. In this case, the existence of a normal serum HCO_3^- concentration does not invalidate the diagnosis of metabolic acidosis. It should also be appreciated that in a patient with a mild metabolic acidotic process, that homeostatic mechanisms may bring the serum HCO_3^- concentration back to within the normal range. Nevertheless, an abnormal acidotic process is still present, which is likely of significant clinical importance.

An example of the potency of these homeostatic mechanisms can be found in the data of Kurtz et al (1), where diet-induced changes in net acid production from 0 to 150 mEq/day produced minimal changes in the plasma HCO_3^- concentration. In fact, only when a correction for the blood PCO_2 was made could a significant plasma HCO_3^- concentration change of 1 mEq/l be detected over this range of net acid production. Thus, significant changes in net acid production lead to undetectable changes in the serum HCO_3^- concentration.

This problem is compounded by the fact that the limits of normal for the serum HCO_3^- concentration are frequently very wide. At our hospital the limits for normal serum HCO_3^- are 22-31 mEq/liter. Such a wide range of normal is common in most clinical laboratories. There are a number of potential reasons for variability in the serum HCO_3^- concentration. First, a subject's serum HCO_3^- concentration varies during the day, affected in large part by the alkaline tide. Ingestion of food leads to secretion of acid into the stomach, generating HCO_3^- which is added to the bloodstream raising the serum HCO_3^- . Later, this HCO_3^- is secreted into the intestine to neutralize the acid, returning serum HCO_3^- to normal. There is also variability from patient to patient. This variability in large part can be attributed to variability in the blood PCO_2 (1,2). However, neither of the above two factors can

explain the wide range of normal used by most clinical laboratories. This probably needs to be reevaluated with current methods of measurement. Nevertheless, even if the limits of normal are narrowed, it is likely that many acidotic processes will continue to be associated with a "normal serum HCO_3^- concentration and blood pH". This is in large part due to the significant number of homeostatic mechanisms available to maintain HCO_3^- concentration normal.

Concept of Acid-Base Balance

To appreciate the mechanisms responsible for acid-base homeostasis, it is useful to consider

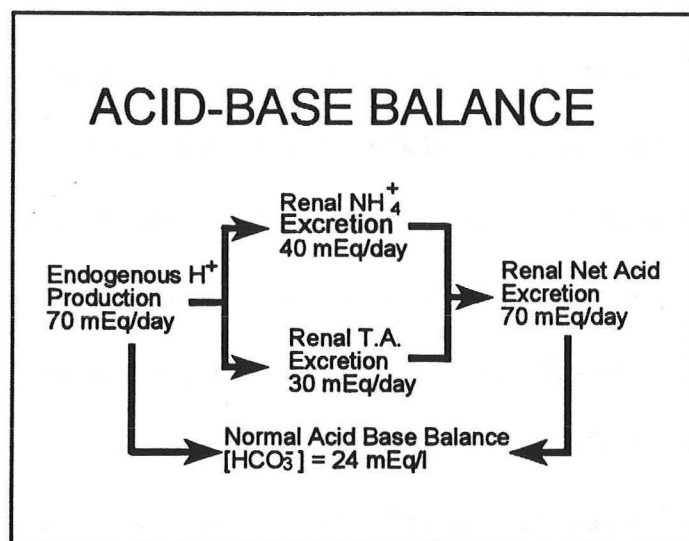


Figure 1

acid-base balance. Figure 1 shows a typical example of acid-base balance. An average 70 kg adult ingests a diet which generates approximately 70 mEq/day of acid. The kidney responds to this acid load by excreting approximately 70 mEq/day of acid. In the example shown in Figure 1, this is excreted in the form of 40 mEq of ammonium per day and 30 mEq of

titratable acid per day. In the steady state, renal net acid excretion is equal to endogenous acid production, and thus acid-base balance is neutral. This balance is associated with a normal serum HCO_3^- concentration of 24 mEq/l.

Figure 2 shows what happens when an individual increases endogenous acid production. This increase in endogenous acid production would typically be related to an increase in dietary protein intake. Initially, renal ammonium and titratable acid excretion rates continue as in Figure 1, leading

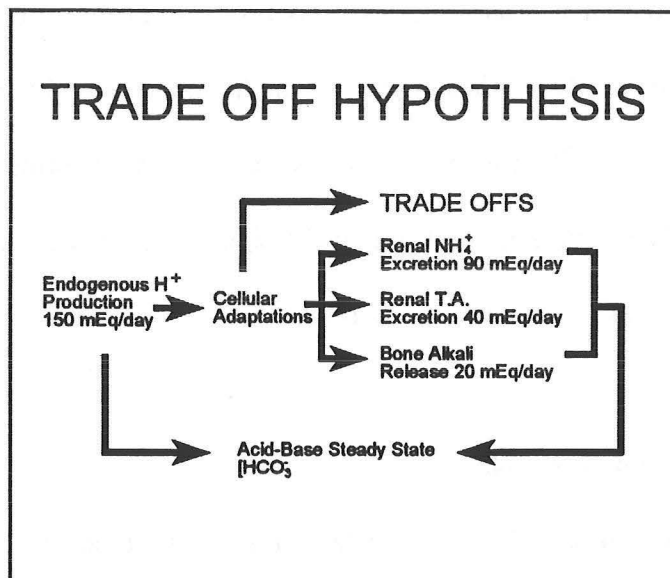


Figure 2

to a net positive acid balance and a decrease in the serum HCO₃⁻ concentration. However, this decrease in serum HCO₃⁻ concentration and blood pH then activate homeostatic mechanisms which serve to defend acid-base balance. The net result, shown in Figure 2, is that urinary ammonium excretion and titratable acid excretion increase to return acid-base balance toward normal.

However, as shown in this figure the increase in urinary ammonium and titratable acidity is not of a magnitude equal to the increase in endogenous acid production, leading to a positive acid balance in the body. Nevertheless, in this setting the serum HCO₃⁻ concentration does stabilize because of a continuous release of alkali from bone. At this point, serum HCO₃⁻ concentration and blood pH are stable, with net acid production equaling the sum of net acid excretion and alkali release from bone buffers. The serum HCO₃⁻ concentration and blood pH will be slightly lower than that present on a normal protein diet, but will generally be within the limits of normal. The small decrease in serum HCO₃⁻ concentration serves as a continued stimulus to maintain the adaptations in acid homeostatic mechanisms. However, because of the sensitivity of these mechanisms, the change in serum HCO₃⁻ required is extremely small and is not detectably outside the range of normal. From the standpoint of blood pH regulation, the system is highly efficient and accomplishes its goals.

Unfortunately, activation of these homeostatic mechanisms leads to certain negative side effects, which we refer to in Figure 2 as **trade-offs**. This is analogous to the trade-off hypothesis first proposed by Neil Bricker with regard to calcium and phosphate metabolism in renal insufficiency.

The normal physiologic response to prevent acidosis activates homeostatic mechanisms which return blood pH to normal, but have a number of negative consequences or trade offs. If these trade offs become significant, then even mild metabolic acidosis, could result in significant patient morbidity. Table 2 lists a number of conditions

EUBICARBONATEMIC METABOLIC ACIDOSIS	
1.	Mild GI HCO_3^- loss
2.	Incomplete distal RTA
3.	Mild renal insufficiency
4.	Excessive meat ingestion
5.	Excessive exercise
6.	Aging

Table 2

associated with a mild metabolic acidotic process, which we refer to as **eubicarbonatemic metabolic acidosis**.

One intriguing possibility is that aging can cause a clinically significant metabolic acidosis. Aging is associated with a progressive loss of nephrons which results in a progressive decrease in glomerular filtration rate (GFR) (3). When the renal response to an acid load is examined, elderly patients show subnormal rates of renal acid excretion (4). Indeed, Frassetto et al (5) recently examined arterial blood gases in patients of varying age on a constant diet, and demonstrated a significant decrease in the plasma HCO_3^- concentration and blood pH with age. The decrease in plasma HCO_3^- concentration correlated with the age related decrease in GFR. Of note, although HCO_3^- concentration and blood pH decreased with age, these values were not outside the range of normal. These studies thus demonstrate a subtle metabolic acidosis associated with aging and likely attributable to the age related decrease in renal function. This is of significant importance when one considers that two common complications of chronic metabolic acidosis are bone demineralization and muscle wasting, two significant problems in elderly subjects.

Thus, when considered as a pathologic process rather than as an abnormal serum HCO_3^- concentration, chronic metabolic acidosis is a common clinical problem. Indeed, it may be ubiquitous

in elderly patients. To understand the clinical consequences of this process, we address the normal homeostatic response to metabolic acidosis.

Homeostatic Response to Metabolic Acidosis

The homeostatic responses to metabolic acidosis can be divided conveniently into four groups:

1) increased renal tubular acid secretion; 2) increased $\text{NH}_3/\text{NH}_4^+$ synthesis; 3) increased tubular growth; and 3) increased bone alkali release. We discuss each of these individually.

Increased Renal Tubular Acid Secretion

Increased Renal Tubular H^+ Secretion. In response to chronic metabolic acidosis, the kidney increases its capacity for net acid secretion in a number of ways. First, the rate of tubular H^+ secretion is increased. This adaptation occurs along the entire nephron, and has been shown specifically to involve the proximal tubule, the thick ascending limb, and the cortical collecting duct. In the proximal tubule, rates of H^+ secretion into the luminal fluid are greater in chronic metabolic acidosis than in more severe acute metabolic acidosis (6,7). This finding suggests an adaptation in tubule function. Similarly, thick ascending limbs dissected from rats fed an acid diet reabsorb HCO_3^- at a faster rate than thick ascending limbs from rats on a control diet (8).

The cortical collecting duct contains two $\text{H}^+/\text{HCO}_3^-$ transporting cells, referred to as intercalated cells. The type A intercalated cell secretes H^+ into the luminal fluid, while the type B intercalated cell secretes HCO_3^- into the luminal fluid. Cortical collecting ducts dissected from rats or rabbits fed an acid diet, mediate net luminal H^+ secretion, while cortical collecting ducts from animals fed an alkaline diet secrete HCO_3^- (9,10). This adaptation is mediated by changes in the relative activities of type A and type B intercalated cells, with acid ingestion increasing the relative activity

of the type A intercalated cell, and alkali ingestion increasing the activity of the type B intercalated cell. This occurs in the absence of cell death and/or cell proliferation (11). Similarly, incubation of cortical collecting ducts in acid media for 1 hour in vitro leads to an increase in net luminal acidification, which is due to increases in H^+ secretion rate and decreases in HCO_3^- secretion rate (12). Thus, changes in the acid/base content of the diet and thus in net acid generation lead to adaptations in the function and relative activities of type A and type B intercalated cells.

In the renal proximal tubule, ingestion of an acid diet has been demonstrated to increase the activities of the apical membrane Na/H antiporter and the basolateral membrane $Na/HCO_3^-/CO_3$ cotransporter (13,14). These mechanisms mediate the majority of transcellular H^+ secretion, and likely account for the adaptation in H^+ secretory capacity described above. In the renal proximal tubule and the thick ascending limb, apical membrane Na/H antiporter activity is mediated by an isoform of the Na/H antiporter gene family, NHE-3 (15). In rats ingesting an acid diet, NHE-3 protein abundance is increased in the proximal tubule and the thick ascending limb (Figure 3) (16).

To examine specifically whether changes in extracellular fluid pH directly modify NHE-3, studies have been performed in cultured proximal tubule cells expressing NHE-3. For these studies, tubule cells have been grown to confluence, rendered quiescent by the removal of serum, and incubated in control (pH 7.4) or acid (pH 6.6-7.0) media for 24 hours. The cells are then harvested and studied. These studies have demonstrated that incubation in acid media increases NHE-3 activity, protein abundance, and mRNA abundance (Figure 4) (17). These studies thus demonstrate that the effect of acidosis on NHE-3 is mediated at least in part through a direct effect of pH. These results likely apply to the adaptations in Na/H antiporter activity in the proximal tubule and thick ascending limb.

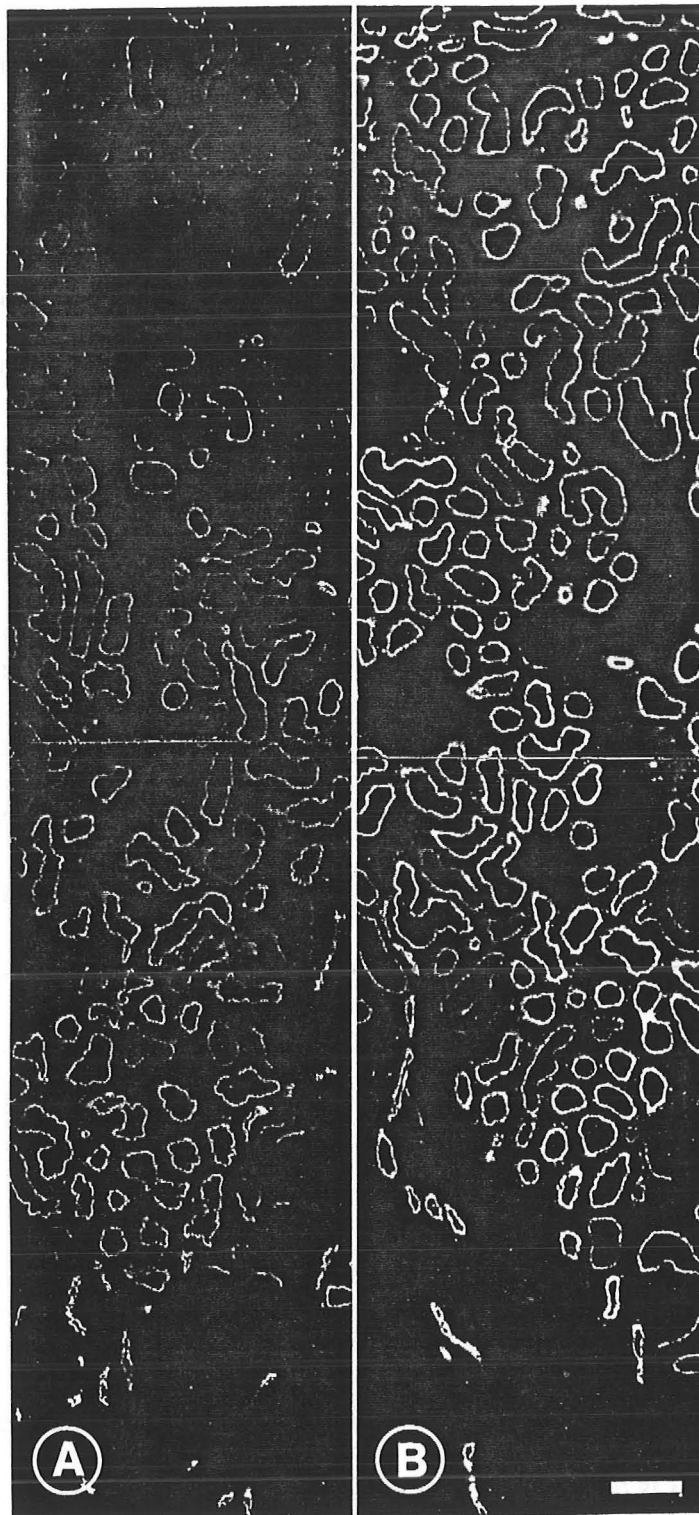


Figure 3: Distribution of NHE-3 immunoreactivity in the cortex and outer stripe of rat kidneys. A. Control rat; B. Acidotic rat. Immunostaining is restricted to the apical membrane of proximal tubules and thick ascending limbs. Labelling is increased in acidotic animals.

Increased Renal Tubular Citrate Absorption. A related acidosis-induced adaptation occurs in citrate transport and metabolism. Citrate is a molecule of intermediary metabolism that at pHs 7-7.4 possesses three negatively charged carboxyl groups. Its metabolism generates three HCO_3^- ions, and thus from the acid-base perspective it is equivalent to HCO_3^- . In response to chronic

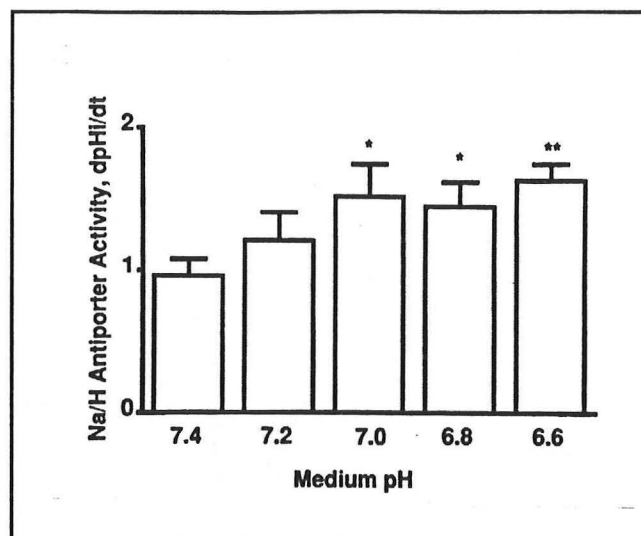


Figure 4a: Acid incubation increases Na/H antiporter activity. OKP cells were incubated in media at indicated pH for 24 h. Na/H antiporter activity (dpH/dt, where pH_i is cytosolic pH and t is time) is plotted as a function of media pH. *P<0.05; **P<0.01 vs. pH 7.4.

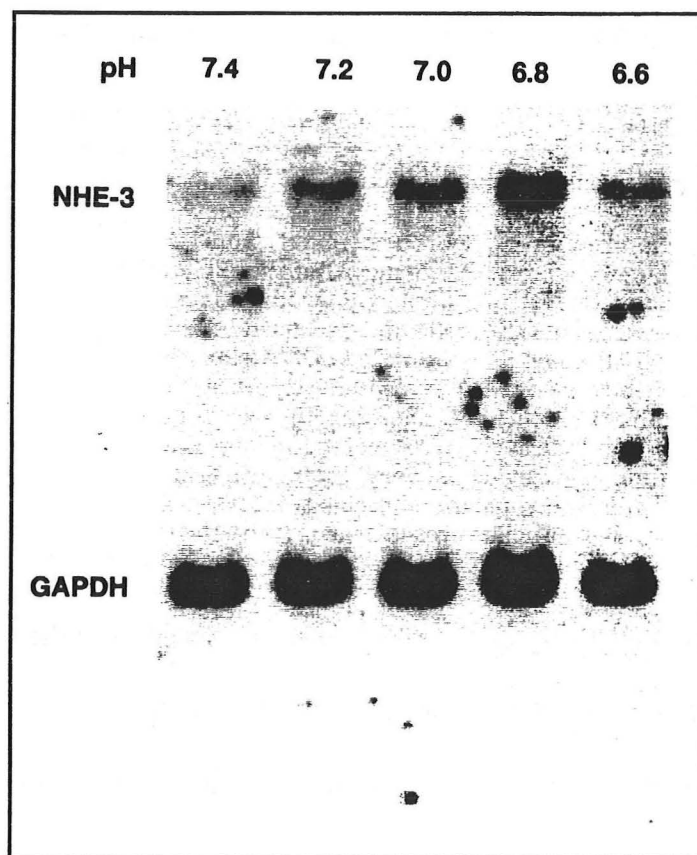


Figure 4b: Acid incubation increases NHE-3 mRNA abundance. OKP cells were incubated in media at indicated pH for 24 h. Fifteen μg of total RNA were probed with full-length OKP NHE-3 cDNA and full-length rat GAPDH cDNA. Typical blot.

metabolic acidosis, urinary citrate levels markedly decrease. This is of significant clinical importance because citrate is also responsible in the urine for complexation of Ca^{2+} , inhibition of renal stone formation, and prevention of nephrocalcinosis (18). Any condition which decreases urinary citrate levels increases the risk of nephrolithiasis and nephrocalcinosis.

Citrate is freely filtered at the glomerulus and its urinary excretion is determined by the amount of citrate absorption in the proximal tubule (19).

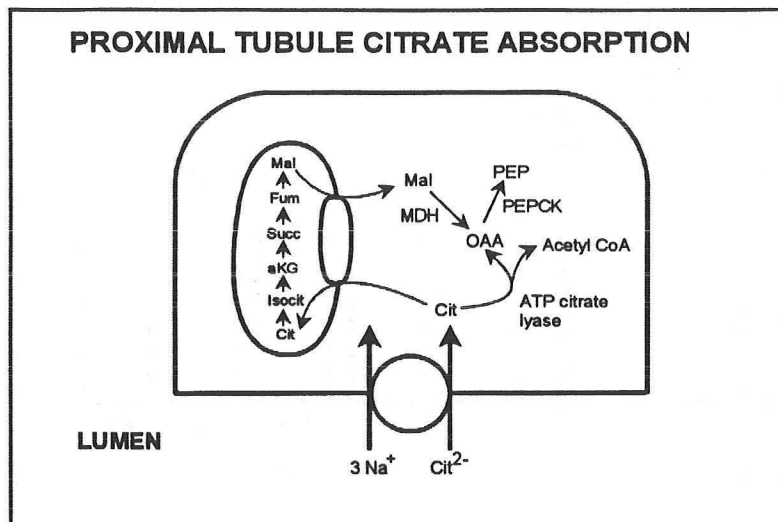


Figure 5

Figure 5 illustrates the possible mechanisms of citrate metabolism in the proximal tubule cell. Citrate is reabsorbed from the luminal fluid on a Na/citrate transporter which couples the transport of 3 Na⁺ ions with one citrate anion. This leads to accumulation of citrate within the proximal tubule cell.

Acidosis increases the rate of the Na/citrate cotransporter in two ways. First, the specific substrate for the cotransporter is the partially protonated Hcitrate²⁻, rather than citrate³⁻ (20). Decreases in luminal pH, as would be expected to occur in acidosis, increase the relative concentration of Hcitrate²⁻, and thus increase citrate transport into the cell. Secondly, it has been demonstrated that chronic metabolic acidosis leads to an adaptation in the activity of the Na/citrate transporter. Following chronic metabolic acidosis of 6 days duration, transporter activity assayed in vitro in the absence of an acidotic milieu, is increased (21).

Cytoplasmic citrate is then metabolized to HCO₃⁻ and either CO₂ and water or glucose. The specific site of metabolism has generally been considered to be the mitochondria (22). According to this scheme, citrate is transported into mitochondria on a citrate/malate exchanger (referred to as the tricarboxylic acid transporter) and is then metabolized within the tricarboxylic acid cycle, leaving the mitochondria as malate. Malate is then converted within the cytoplasm to oxaloacetate, then to phosphoenolpyruvate and finally can be converted to glucose.

Another possible pathway for citrate metabolism is cytoplasmic. In liver, citrate is produced

in mitochondria and exits mitochondria in exchange for malate on the tricarboxylic acid exchanger. Cytoplasmic citrate is then metabolized by ATP citrate lyase, forming acetyl CoA and oxaloacetate. The oxaloacetate is then metabolized to phosphoenolpyruvate and then to glucose. This represents the only source of cytoplasmic acetyl CoA, and is thus required for all fatty acid and sterol synthesis. The following results support an important role for cytoplasmic ATP citrate lyase in proximal tubule citrate metabolism (23). First, ATP citrate lyase protein is expressed in kidney cortex. Second, chronic metabolic acidosis of 7 days duration in rats increases renal cortical ATP citrate lyase activity and protein abundance. Of interest, hepatic ATP citrate lyase abundance is not increased in metabolic acidosis. Lastly, intraperitoneal administration of hydroxycitrate, a specific inhibitor of ATP citrate lyase, inhibits the hypocitraturia of metabolic acidosis. These data all support an important role for ATP citrate lyase in proximal tubular citrate metabolism, and an important role for up regulation of ATP citrate lyase in the hypocitraturia of metabolic acidosis. Nevertheless, an additional role for mitochondrial metabolism remains possible.

The importance of hypocitraturia in nephrolithiasis has been best demonstrated by Pak and his colleagues. These investigators have shown low levels of urinary citrate in a high proportion of patients with kidney stone disease (24). In patients with hypokalemic distal RTA, hypocitraturia has been demonstrated to clearly contribute to nephrocalcinosis and end stage kidney disease (25). Low levels of urinary citrate also have been found in patients with frequent nephrolithiasis due to absorptive hypercalciuria, renal hypercalciuria, enteric hyperoxaluria, uric acid lithiasis, and patients with frequent stones related to no metabolic abnormality (24). In the majority of these patients, hypocitraturia is associated with distal RTA or decreased net GI alkali absorption (26).

Increased $\text{NH}_3/\text{NH}_4^+$ Synthesis

One of the most important adaptations to chronic metabolic acidosis is an increase in renal NH_4^+ excretion. Quantitatively NH_4^+ provides the greatest component of the increase in renal net acid excretion. An increase in renal NH_4^+ excretion requires regulation of two separate processes. First, as discussed above, there must be an increase in net tubular H^+ secretion. This leads to a lower luminal pH in the medullary collecting duct and enhanced trapping of NH_4^+ by nonionic diffusion. A second, equally important, component of this response is enhanced synthesis of $\text{NH}_3/\text{NH}_4^+$ in the kidney. $\text{NH}_3/\text{NH}_4^+$ is synthesized in the renal proximal tubule by a sequence of events which includes glutamine transport into the cell on a Na/glutamine coupled transporter, glutamine transport into mitochondria, and then metabolism of glutamine (27). Decreases in pH acutely stimulate α ketoglutarate dehydrogenase, a key enzyme in glutamine metabolism (28). Chronic metabolic acidosis leads to increases in the activities of the Na/glutamine cotransporter, glutaminase, glutamate dehydrogenase, and phosphoenolpyruvate carboxykinase, all of which contribute to glutamine metabolism and ammonia synthesis (27,29,30). Incubation of cultured proximal tubule cells in acidic media leads to increases in the activities and mRNA abundances of glutaminase, glutamate dehydrogenase, and phosphoenolpyruvate carboxykinase (30,31). All of these adaptations serve to increase $\text{NH}_3/\text{NH}_4^+$ synthesis, increasing $\text{NH}_3/\text{NH}_4^+$ availability for trapping in the acidic lumen of the medullary collecting duct.

Increases in proximal tubule $\text{NH}_3/\text{NH}_4^+$ synthesis require increased availability of glutamine. In addition, other amino acids can be utilized for ammonia synthesis to a lesser extent (32). This may provide a teleologic reason for the enhanced breakdown of proteins observed in metabolic acidosis. Human subjects administered oral NH_4Cl develop a significantly negative nitrogen balance (33). The majority of this effect is due to enhanced protein degradation. Enhanced rates of muscle protein

degradation can be demonstrated in muscle isolated from rats with metabolic acidosis and in muscle cells exposed to an acid pH in vitro (34,35). Acidosis induced enhanced muscle breakdown is mediated by activation of an ATP-dependent pathway involving ubiquitin and proteasomes (34). Metabolic acidosis causes a 2.5-4 fold increase in mRNAs for ubiquitin, and for the C2 and C3 proteasome subunits in muscle (34). Kidney ubiquitin mRNA abundance did not change. A similar effect on muscle protein catabolism has been demonstrated in rats with renal failure, where it is prevented by administration of NaHCO_3 (36). This suggests an important role for acidosis in the causing the muscle wasting frequently observed in patients with renal failure. In addition to enhanced protein degradation, metabolic acidosis has been demonstrated to inhibit albumin synthesis in human subjects (33).

Evidence also suggests that the protein wasting seen with aging may be attributable to acidosis. As noted earlier, aging is associated with a decrease in glomerular filtration rate which may be associated with a subtle degree of metabolic acidosis. Sebastian and coworkers examined nitrogen balance in 14 postmenopausal women maintained on a constant diet in a clinical research center. Their studies demonstrated that provision of KHCO_3 to these women improved nitrogen balance (37).

Increased Renal Tubular Growth

In many cells, a drive toward increased work is associated with increased growth. This may provide a teleologic explanation for the initial observation of Lotspeich that chronic metabolic acidosis is associated with increased renal growth (38). This enhanced renal growth involves the tubules, and is mostly a hypertrophic process, with some hyperplasia. This is in marked contrast to most nonrenal tissues where acidosis is growth inhibitory. In that renal hypertrophy has been associated with progression of renal disease, it is possible that the acidosis seen in renal insufficiency

may contribute to enhancing the rate of progressive loss of renal function. Studies have suggested that acidosis can enhance the rate of progression of renal disease (39,40).

The ability of acidosis to promote renal growth may be most important in polycystic renal disease. The first suggestion that intracellular acidosis may contribute to cyst formation was made by Torres and coworkers who noted that patients with hyperaldosteronism and potassium deficiency (associated with intracellular acidosis) have renal cysts (41). They later demonstrated in the Han:SPRD rat, which spontaneously develops polycystic kidney disease, that administration of NH_4Cl enhances the rate of cyst formation, while administration of KHCO_3 slows the rate of cyst formation (42). Thus, polycystic kidney disease may represent one clinical disorder in which complete correction of metabolic acidosis is essential.

Increased Bone Alkali Release

The last component of the homeostatic response to acidosis is bone alkali release. This homeostatic mechanism has two interrelated components. First, metabolic acidosis directly regulates bone function by mobilizing Ca^{2+} and alkali. Second, metabolic acidosis directly inhibits renal Ca^{2+} absorption leading to Ca^{2+} removal from the body. These two effects are likely synergistic.

Chronic metabolic acidosis increases alkali mobilization from bone by two mechanisms. First, acidosis leads to the physicochemical dissolution of bone, causing release of HCO_3^- together with Na^+ and K^+ and a small amount of Ca^{2+} (43,44). Second, chronic metabolic acidosis leads to increases in the activity of osteoclasts and decreases in the activity of osteoblasts (45-48). This leads to net bone dissolution with mobilization of Ca^{2+} and alkali. The net result in chronic metabolic acidosis is protection of blood pH and HCO_3^- concentration at the expense of bone mineral content.

Mobilization of Ca^{2+} from bone would be expected to increase urinary Ca^{2+} excretion and thus

result in hypercalciuria. In addition, metabolic acidosis directly inhibits renal tubular Ca^{2+} reabsorption leading further to hypercalciuria and negative Ca^{2+} balance. Ca^{2+} is reabsorbed at three major sites along the nephron. In the proximal tubule Ca^{2+} absorption parallels NaCl and volume absorption and is largely passive. In the thick ascending limb Ca^{2+} absorption is also largely passive, driven by the lumen positive voltage. The distal nephron appears to provide a site whereby although only a small fraction of filtered Ca^{2+} is reabsorbed, it is highly regulated. Based on the comparison between proximal and distal RTA, it has been postulated that the key determinant of Ca^{2+} absorption in these settings is distal nephron luminal pH. This is based on the observation that patients with hypokalemic distal RTA who would be expected to have an acidic plasma pH and an acidic distal nephron luminal pH are hypercalciuric, while patients with proximal RTA who would be expected also to have an acidic plasma pH but an alkaline luminal pH have normal rates of Ca^{2+} excretion (25). In the presence of metabolic acidosis, increases in distal delivery of HCO_3^- have been demonstrated to increase renal Ca^{2+} absorption (49).

More direct analysis of this hypothesis has been made possible by the availability of cell cultures. Cultured rabbit connecting tubule/collecting duct cells exhibit transepithelial Ca^{2+} transport (50). In these cells, decreases in luminal pH inhibit Ca^{2+} transport, while decreases in serosal (interstitial) pH have no effect (50). Acidification of both luminal or serosal compartments leads to acidification of the cell. Thus, Ca^{2+} transport is not regulated by changes in cell or interstitial pH, but is regulated by luminal pH, confirming the clinical observations.

Thus, in summary, chronic metabolic acidosis leads to mobilization of alkali from bone which helps to defend blood pH. In the steady state, this likely explains a significant amount of the discrepancy between net acid production and renal net acid excretion. Unfortunately, this process is accompanied by mobilization of Ca^{2+} from bone and hypercalciuria. These processes occur secondary

to the ability of acidosis to physicochemically dissolve bone, to increase osteoclast activity, to inhibit osteoblast activity, and to directly impair renal distal nephron Ca^{2+} absorption.

The significance of this effect has been suggested in a number of studies. Sebastian and coworkers examined Ca^{2+} balance in 18 postmenopausal women. These women were found to be in negative Ca^{2+} balance. Administration of KHCO_3 decreased urinary Ca^{2+} excretion and improved net Ca^{2+} balance (51). Increases in protein intake increase urinary Ca^{2+} excretion, an effect which is reversed by administration of HCO_3^- (52). This effect of protein is likely mediated by the increased endogenous acid production associated with ingestion of protein. In a study of women from different areas of China, urinary Ca^{2+} was shown to vary as a function of dietary protein intake (53). In these subjects, urinary Ca^{2+} increased as urinary net acid excretion increased. As urinary net acid excretion correlates with endogenous acid production, these results again suggest that the effect of protein intake is related to endogenous acid production. It is also of significant interest that the incidence of hip fractures (related to osteoporosis) is high in industrialized countries where the population eats a

high protein diet, and is low in less industrialized countries where the population eats a lower protein diet.

Figure 6 shows a plot of the incidence of hip fractures as a function of dietary protein intake in a number of countries (54). These data suggest that excessive dietary protein intake as well as aging may be associated with a negative Ca^{2+} balance, osteoporosis, and an increased

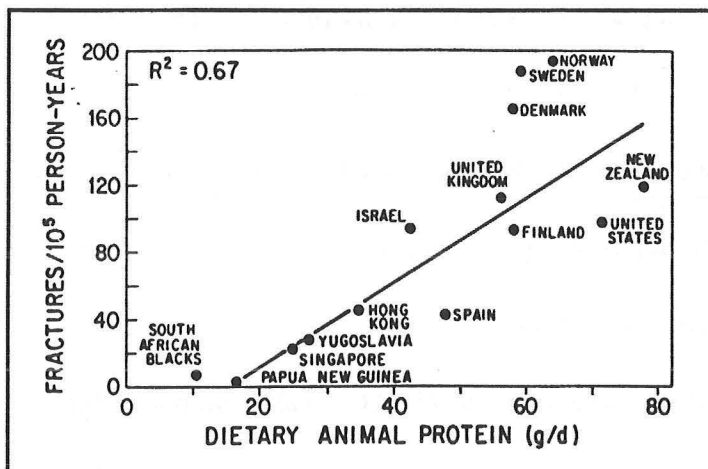


Figure 6: Incidence of hip fractures as a function of dietary animal protein intake in different countries. From Abelow et al, *Calcif. Tissue Int.* 50:16, 1992.

incidence of fractures. This has been referred to as the acidosis-osteoporosis hypothesis.

Acid Signaling

Thus, chronic metabolic acidosis has a large number of effects on cellular function. This involves regulation of renal epithelial cell transport, metabolism, and growth, regulation of muscle metabolism, and regulation of osteoclast and osteoblast function. It is unlikely that these effects are mediated by merely by deviations from the pH optima of various proteins. Changes in extracellular and cell pH are likely too small to elicit such effects. It is far more likely that a number of **pH sensitive proteins** exist that are designed to alter their function in response to small changes in cellular and/or extracellular pH. These proteins would thus function as **pH sensors**. One possible mechanism by which this could occur is if each pH-sensitive effector protein possessed its own pH sensor. In some situations such a scenario has been demonstrated to exist. The Na/H exchanger has an effector domain that transports Na and H, but also has domains which sense cell pH and allow Na/H antiporter rate to be exquisitely sensitive to cell pH (55).

A second mechanism for regulating cellular function, is to activate an **acid signaling system** whereby multiple proteins could be regulated. Given the large number of effects elicited by small changes in cell pH, this mechanism seems more likely. This has been best studied in the renal proximal tubule. In the proximal tubule cell, decreases in extracellular and intracellular pH have been demonstrated to increase activities of the apical membrane Na/H antiporter and basolateral membrane Na/HCO₃/CO₃ cotransporter (leading to enhanced transepithelial H⁺ secretion), to increase glutamine transport, glutaminase, glutamate dehydrogenase, and phosphoenolpyruvate carboxykinase activities (leading to enhanced ammoniagenesis), and to increase citrate transport and ATP citrate lyase activity (leading to enhanced citrate absorption). In addition, acidosis leads to growth of these cells. Chronic

potassium deficiency which is associated with extracellular alkalosis and intracellular acidosis (56), elicits a similar set of responses (27). Thus, it has been inferred that the key determinant of regulation is intracellular pH.

In order to study possible mechanisms by which changes in intracellular pH may modulate cell function, the effect of media acidification on cultured proximal tubule cells has been examined. When

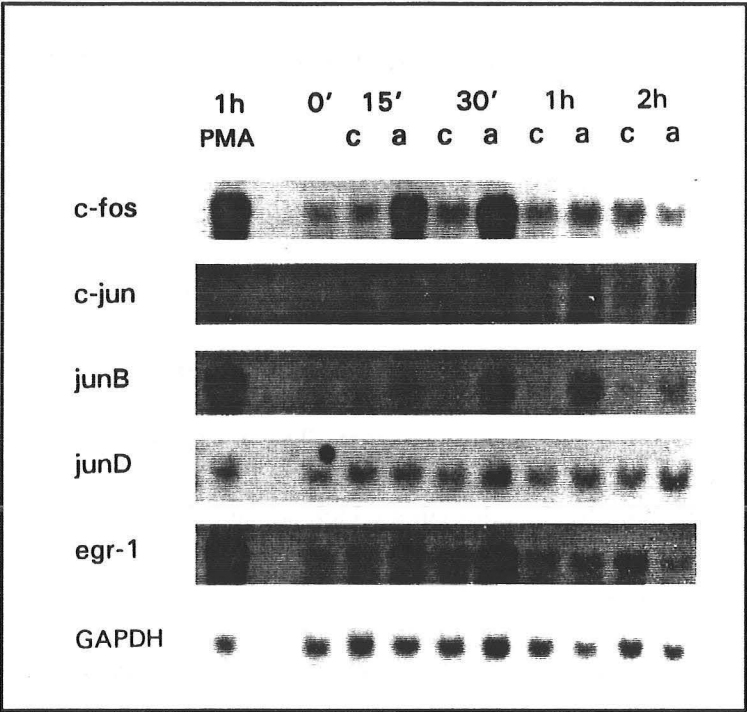


Figure 7: Effect of acid incubation or PMA on IE gene mRNA abundance in MCT cells. MCT cells were serum deprived for 48 h and then incubated in either control (c), or acid (a) media, or control media with 100 nM PMA (PMA). Cells were harvested at the times indicated and mRNA abundance measured on total RNA.

these cultured cells are exposed to acid medium, a number of immediate early genes are activated. Figure 7 shows northern blots for c-fos, c-jun, junB, junD, and egr1. Incubation of cultured proximal tubule cells in acid media leads to increases in the mRNA abundance of c-fos, c-jun, junB, and egr1 (57). These genes encode transcription factors, and are activated

in a number of physiologic circumstances where gene expression is regulated. This increase in mRNA

abundance is due to an increase in the rate of transcription of mRNA induced by acid incubation (57). In addition, it was shown that this effect is cell specific. While incubation of proximal tubule cells in acid media induced expression of these genes, incubation of fibroblasts in acid media did not induce expression (57). This result parallels results with the Na/H antiporter. Incubation of proximal tubule cells in acid media leads to increased expression of two isoforms of the Na/H antiporter, NHE-1 and

NHE-3; however incubation of fibroblasts in acid media leads to decreased NHE-1 expression (17,58).

The protein products of c-fos and c-jun form a heterodimer which functions as a transcription factor. This heterodimer, referred to as AP-1, binds a specific nucleotide sequence, TGA(C/G)TCA. To determine if acid-induced c-fos and c-jun expression is associated with functional AP-1 activity, proximal tubule cells were stably transfected with a reporter gene construct encoding chloramphenicol acetyl transferase driven by 6 AP-1 binding sites and a minimal interferon promoter. In these cells, any condition which increases AP-1 activity will increase the expression of the CAT gene and synthesis of CAT protein. Incubation of these cells in acid media was shown to increase CAT protein abundance within 3 hours (59).

Increases in c-fos and c-jun expression are frequently mediated through activation of members of the MAP kinase family. Incubation of cultured renal epithelial cells in acid media leads to a 4-fold increase in Erk-1/Erk-2 MAP kinase activity (60). Activation of MAP kinase and c-fos and c-jun expression typically occur either by activation of protein kinase C and/or by activation of tyrosine kinase pathways. Increased immediate early gene expression was not blocked by protein kinase C inhibition, but was blocked by tyrosine kinase inhibitors (57).

In studies examining the nature of the specific tyrosine kinase activated by acid, it was found that incubation of renal epithelial cells in acid media causes a 2-fold increase in c-src activity. c-src is a nonreceptor tyrosine kinase. Of interest, it is significantly expressed in proximal tubule cells and in osteoclasts. Csk (c-src kinase) is a tyrosine kinase that phosphorylates tyrosine 527 of c-src, leading to inhibition of c-src activity. To examine the role of c-src in mediating regulation of NHE-3 by acid, acid regulation was examined in renal cell lines overexpressing csk. In these cell lines, the abilities of acid to activate c-src, increase Na/H antiporter activity, and increase NHE-3 mRNA

abundance were all inhibited (61). These studies suggest that c-src plays a key role in acid activation of NHE-3.

Thus, these studies suggest the following scenario. Acidification of extracellular fluid leads to intracellular acidification which leads to activation of c-src. The mechanism by which c-src is activated has not yet been elucidated, but may involve activation of a tyrosine phosphatase which dephosphorylates tyrosine 527 of c-src. Activation of c-src then leads to activation of Erk-1/2 MAP kinase and increases in immediate early gene expression. These pathways play an important role in activation of NHE-3 by acid, and likely in other acid-induced effects.

Treatment

Thus, chronic metabolic acidosis causes a number of complications in patients. These include bone demineralization, nephrolithiasis, nephrocalcinosis, and muscle wasting. Acidosis-induced enhanced renal growth may be a problem in certain patients, particularly patients with polycystic kidney disease. In order to avoid these complications, it becomes important to correct metabolic acidosis.

The treatment of metabolic acidosis is relatively simple. One can utilize either HCO_3^- or citrate as oral alkali supplements. HCO_3^- can cause a feeling of bloating due to the reaction of gastric acid with HCO_3^- to form CO_2 . Therefore, citrate may be the preferred source of alkali. Citrate, however, can enhance aluminum absorption (62,63). Aluminum absorption is a major problem in patients with renal insufficiency. However, this is not a problem in patients with normal renal function (64).

A second choice is whether to give the alkali as a Na^+ or K^+ salt. The problem with the Na^+ salt is that Na^+ is more likely to expand extracellular fluid and intravascular volume. This will result in worsening hypertension in patients with renal insufficiency. In addition, volume expansion increases

urinary Ca^{2+} excretion which can further contribute to bone demineralization, nephrocalcinosis, and nephrolithiasis. Thus, in most patients KHCO_3 or K citrate is preferred. In patients with renal insufficiency, one generally administers NaHCO_3 to avoid the K^+ load and enhanced aluminum absorption.

A more difficult question is who should be treated. There is no question that metabolic acidosis should be aggressively corrected in patients with a serum HCO_3^- concentration <22 mEq/l. This would likely be seen in patients with significant GI HCO_3^- loss, renal tubular acidosis, or renal insufficiency. A more complicated question is whether to treat patients with “eubicarbonatemic metabolic acidosis,” where the maintenance of a normal serum HCO_3^- concentration is dependent on activation of homeostatic mechanisms with their consequent trade offs. This would occur in patients with:

1. Mild GI HCO_3^- loss
2. Incomplete distal RTA -- a condition where patients have mild distal RTA such that the serum HCO_3^- concentration is within the range of “normal.”
3. Mild renal insufficiency
4. Excess meat ingestion
5. Excess exercise
6. Elderly subjects

Patients with eubicarbonatemic metabolic acidosis may be difficult to detect. A history of diarrhea, excess meat ingestion, nephrolithiasis, or renal insufficiency would be suggestive of an excessive acid load. Increased urinary NH_4^+ excretion and decreased urinary citrate excretion would also be indicators of excessive acid. Increased urinary sulfate excretion would be an indicator of

excessive protein loads.

A key question is whether all elderly individuals should be treated with alkali. Osteoporosis and muscle wasting are very common in older individuals. Alkali treatment will likely improve Ca^{2+} and nitrogen balance in these subjects. However, it is premature to recommend this treatment for all elderly individuals. Many of these individuals are already on Ca^{2+} treatments, and the combination of Ca^{2+} with excessive alkali could lead to milk-alkali syndrome. Clinical trials need to be performed to examine whether small doses of KHCO_3 , sufficient to neutralize the endogenous acid production, are indicated.

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