# Ethnic differences in titratable acid excretion and bone mineralization

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**ABSTRACT**

VAITKEVICIUS, H., R. WITT, M. MAASDAM, K. WALTERS, M. GOULD, S. MACKENZIE, S. FARROW, and W. LOCKETTE. Ethnic differences in titratable acid excretion and bone mineralization. *Med. Sci. Sports Exerc.*, Vol. 34, No. 2, pp. 295–302, 2002. **Purpose:** To test our hypothesis that differences in urinary calcium excretion among blacks and whites may be secondary to ethnic variations in acid ($H^+$) metabolism and to prove that increases in titratable acid excretion would be found among individuals predisposed to the development of stress fractures. **Methods:** We administered 8 g $NH_4Cl$ acutely to 11 black and 18 white healthy volunteers and measured urinary sodium, calcium, and acid excretions. We measured the $Na^+/H^+$ antiporter activity using acid-loaded platelets as surrogate markers for this exchanger expressed in renal epithelial cells. We also compared differences in titratable acid excretion among a cohort of subjects with, and without, a history of stress fracture. **Results:** $NH_4Cl$-induced increases in titratable urinary acid correlated with changes in the renal excretion of calcium and sodium, and stimulated acid excretion correlated with basal acid loss. Despite comparable changes in plasma pH, whites, when compared to blacks, had much greater basal acid excretion and $NH_4Cl$-induced acid excretion. Whites also had much greater baseline calcium excretion rates when compared to blacks. Following acid loading, whites continued to exhibit greater calcium excretion rates than blacks. Acid loading significantly decreased sodium excretion in whites but not in blacks. Blacks also had significantly attenuated $Na^+/H^+$ exchange activity. In a cohort of resting, athletic students, we found enhanced basal $H^+$ and phosphate excretion among subjects who experienced stress fractures during their rigorous physical training when compared to those individuals who did not. **Conclusion:** Blacks may have a greater endogenous buffering capacity than whites, or the reported ethnic differences in sodium and calcium excretion rates between blacks and whites may be secondary to racial variations in renal $H^+$ excretion. We conclude that both ethnic differences in bone mineralization and bone integrity in athletes are mediated by heritable differences in titratable acid excretion. **Key Words:** OSTEOPOROSIS, CALCIUM, EXERCISE, SODIUM-HYDROGEN ANTIPORTER, AFRICAN AMERICAN, HUMAN

It is known that American blacks consume and absorb less dietary calcium than whites (10,25). Despite these differences in calcium intake, blacks maintain increased rates of bone mineralization, greater bone density, and a decreased prevalence of osteoporosis and fractures relative to whites (2,3,11,17,24,26,30). The selective advantage for this racial difference in calcium output is unknown. The disparate calcium excretion between blacks and whites has been attributed to ethnic differences in dietary habits, or variation in mineral absorption, or fundamental disparities in the renal transport of calcium. We recognized that the extracellular pH is a primary driving force in the determination of net calcium balance in humans. Furthermore, prolonged metabolic acidosis, as is found in chronic renal insufficiency, is associated with bone demineralization and osteodystrophy. Although other investigators have proposed plausible explanations for racial differences in calcium metabolism, such as contrasting parathyroid hormone or vitamin D activities (4,16,27), no one has suggested that differences in calcium metabolism among blacks and whites may be secondary to ethnic variations in acid ($H^+$) metabolism. We tested our hypothesis that differences in urinary calcium excretion and bone mineralization among blacks and whites may be secondary to ethnic variations in acid ($H^+$) excretion. Given the known relationship between distal sodium delivery in the kidney and $H^+$ loss, these disparate findings in acid excretion between whites and blacks could be explained on the basis of racial differences in renal $Na^+/H^+$ antiporter activity (19,36,38). We next determined whether or not titratable acid excretion correlated with sodium excretion rates in our subjects. Using platelets as a surrogate marker for renal distal tubules, we were able to measure net $Na^+/H^+$ antiporter activity in our black and white volunteers.

It has been anecdotally reported that the development of stress fractures in some athletes has an ethnic bias. We postulated that ethnic contrasts in the prevalence of stress fractures results from racial differences in acid-dependent bone mineralization in athletes. Stress fractures occur not only from the result of trauma to bone, but also from an inadequate reparation process. We reasoned that deposition of calcium within the mineral matrix of injured bones is

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made difficult in the presence of an acid milieu such as that which would develop during exercise. We postulated that if this were true, individuals with a history of stress fractures should have higher metabolic acid production and titratable acid excretion. We recruited a cohort of similarly trained athletes with, and without, a history of stress fractures. Specifically, we identified 33 subjects who had successfully completed the first phase of the grueling Naval Special Warfare training known as BUD/S (Basic Underwater Demolition/SEAL). These trainees run a minimum of 6 miles each day, and there is a high attrition rate because of stress fractures. We tested our hypothesis that those BUD/S students with a history of stress fractures would have significantly greater titratable acid output when compared with their similarly trained counterparts without known stress fractures.

In summary, we suggest that a strong relationship exists between the prevalence of stress fractures, and perhaps bone mineralization, and the renal excretion of titratable acid. Such findings would be significant, as it is possible that the alkali is more important than the calcium in mineral supplements taken to increase bone mineralization in athletes or an aging population prone to the development of osteoporosis.

## METHODS

To first determine whether or not ethnic differences in titratable acid excretion exist, we recruited 18 white and 11 black subjects; of this group, 22 were men and 7 were women. The volunteers were taking no prescriptions or over-the-counter medications and their race categorization was determined by self-reporting of all four grandparents. These studies were approved by our institutional committees for the protection of humans in research, and volunteers gave informed written consent. Subjects were fed an identical light breakfast supplemented with 8 g $NH_4Cl$ p.o. with 1.5 L of water over 1 h. In humans, $NH_4Cl$ induces acidification by being converted to urea in the liver in a reaction that consumes bicarbonate. We measured pH on baseline samples of venous plasma using a commercial blood gas analyzer. Acid excretion was measured on double void urine samples by immediate titration with NaOH. Sodium and calcium were measured using an autoanalyzer. After the $NH_4Cl$ load, blood and urine from the subjects were sampled every 30 min, and the cumulative excretion rates for $H^+$, calcium, and sodium over 2–3 h were determined. $Na^+/H^+$ exchange activity was measured with cell sizing of platelets loaded with propionic acid (37). Briefly, platelets isolated from healthy subjects were suspended in a physiologic salt solution in which 140 mM NaCl was replaced with 140 mM sodium propionate. At pH 6.7, propionic acid freely diffuses into platelets where it dissociates, and it causes a fall in intracellular pH. The $Na^+/H^+$ antiporter becomes activated, $H^+$ is extruded from the cell in exchange for osmotically active extracellular $Na^+$, and the platelets swell. Differences in propionic acid-induced changes in platelet size were measured in the presence or absence of

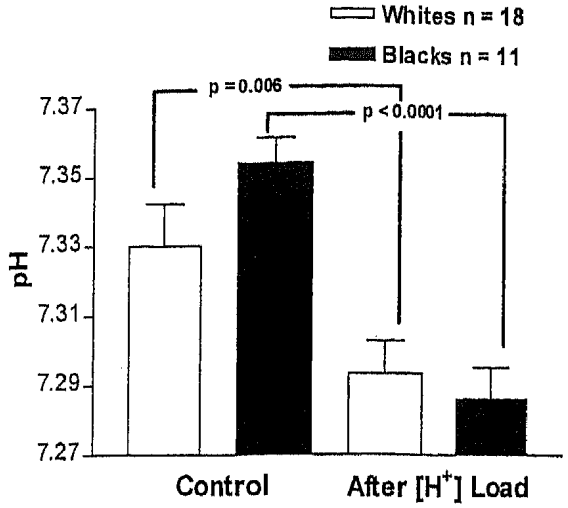


**FIGURE 1—Effect of $NH_4Cl$ loading on plasma pH. $NH_4Cl$ is converted to urea in the liver with a net consumption of bicarbonate; this metabolism of $NH_4Cl$ induces a gradual, systemic, metabolic acidosis. Blacks and whites had similar basal venous plasma pH. Oral loading with 8 g $NH_4Cl$ resulted in identical, significant falls in venous blood pH (values expressed in pH units ± SEM) from 7.35 ± 0.01 to 7.29 ± 0.01 in blacks, and from 7.33 ± 0.01 to 7.29 ± 0.01 in the white subjects.**

$10^{-6}$ M dimethyl amiloride (DMA), an agent that blocks the $Na^+/H^+$ exchanger. The net $H^+$ efflux shifts the propionic acid equilibrium toward the dissociated form and favors the entry of more undissociated acid. Accordingly, the cells in the propionate media are effectively pH clamped, and the maximum increase in platelet size for a given time interval reflects $Na^+/H^+$ antiporter rate (37).

To determine whether differences in $H^+$ excretion were associated with bone mineralization and a predilection for stress fractures, we recruited an additional 33 subjects who by completion of the rigorous first phase of U.S. Navy BUD/S training did, or did not, develop stress fractures. Medical charts and radiographic studies were reviewed, and all volunteers were examined by a medical officer. Subjects were classified according to the presence or absence of history of stress fractures during the first phase of BUD/S training. Acid excretion rates were measured by investigators blinded to the medical history of the subjects. Rested subjects were fed an identical light meal, and the urinary excretion rates for titratable $H^+$ and bone minerals were measured. Statistical analyses were performed with the GraphPad Prism computer program (GraphPad Software, Inc., San Diego, CA). Mean values were compared within subjects with a paired *t*-test, and group differences were evaluated using an unpaired *t*-test. When unequal variances were found between groups, a nonparametric test was used. Ethnic differences in $Na^+/H^+$ exchange were evaluated with a repeated measures analysis of variance.

## RESULTS

There were no ethnic differences in baseline, or post-ammonium chloride plasma pH (Fig. 1), which dropped significantly over 2 h from 7.34 ± 0.01 to 7.28 ± 0.01 ($P < 0.0001$). Despite comparable plasma pH values, white

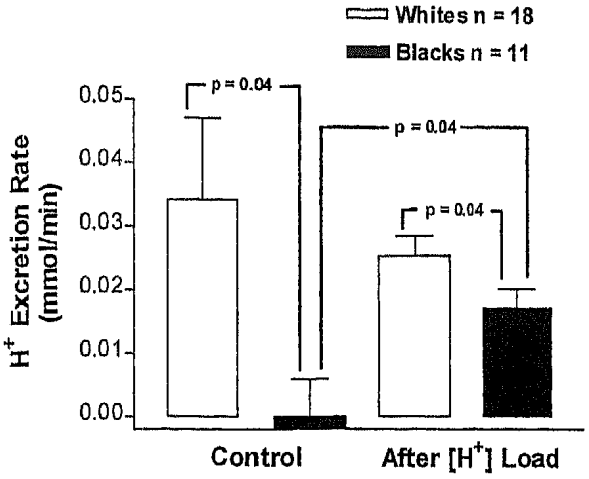


FIGURE 2—Effect of $NH_4Cl$ loading on titratable $H^+$ excretion rates in blacks and whites. Blacks had significantly lower basal $H^+$ rates when compared with whites (values in mmol·min$^{-1}$ ± SEM), 0.00 ± 0.008 vs 0.034 ± 0.013, $P < 0.05$, and following an identical acid load, 0.017 ± 0.003 vs 0.025 ± 0.003, $P < 0.05$.

subjects had significantly greater baseline urinary $H^+$ excretion compared to blacks (Fig. 2). This ethnic group tended to increase their titratable acid excretion following ammonium chloride; the white subjects did not. These contrasting results are most likely because of the already high baseline acid excretion rates from the white subjects. It appears that the titratable acid excretion is near maximal values before acid loading in whites or, alternatively, $NH_4Cl$ is less effective at inducing acidosis in whites when compared to blacks. However, if that were true, we would expect significant ethnic differences in plasma pH following the oral administration of ammonium chloride. However, this did not occur despite comparable diets (Fig. 1). Baseline calcium and sodium excretions tended to be higher in the whites compared to blacks, and acidification tended to increase calcium excretion in blacks, but not whites (Figs. 3 and 4). The rates of calcium and sodium excretion also correlated positively with titratable $H^+$ excretion (Figs. 5 and 6).

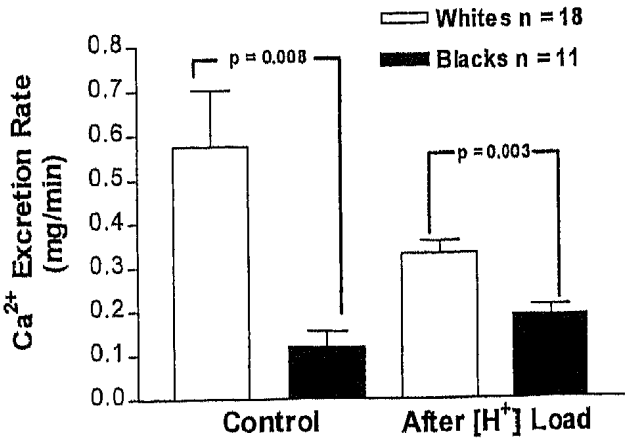


FIGURE 3—Effect of $NH_4Cl$ loading on calcium excretion rates in blacks and whites. Blacks had significantly lower basal calcium excretion rates when compared with whites (values in mg·min$^{-1}$ ± SEM), 0.12 ± 0.04 vs 0.58 ± 0.13, $P < 0.008$, and following an identical acid load, 0.19 ± 0.02 vs 0.33 ± 0.03, $P < 0.003$.

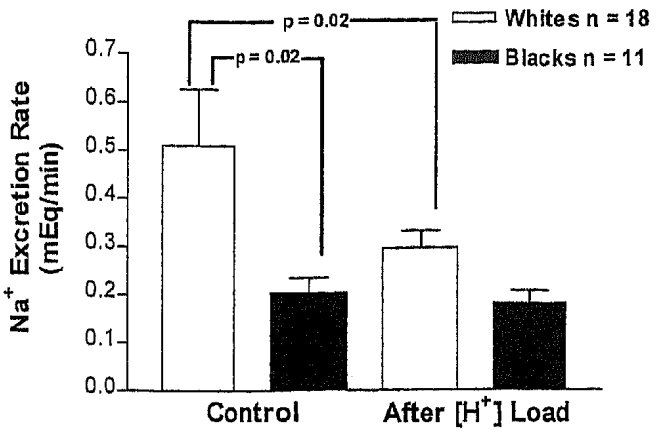


FIGURE 4—Effect of $NH_4Cl$ loading on sodium excretion rates in blacks and whites. Blacks had significantly lower basal sodium excretion rates when compared with whites (values expressed in mEq·min$^{-1}$ ± SEM), 0.20 ± 0.03 vs 0.51 ± 0.12, $P < 0.02$, and following an identical acid load, 0.18 ± 0.03 vs 0.30 ± 0.04, $P < 0.05$.

Titratable acid excretion by the distal tubule is mediated by the apical $Na^+/H^+$ antiporters. We postulated that the greater sodium excretion rates in whites would be coupled to the enhanced acid excretion by the $Na^+/H^+$ exchanger in the distal renal tubule (Fig. 7). Indeed, using platelets that appear to have the same $Na^+/H^+$ isoform (NHE3) as that found on the apical surface of the distal tubule (9,28,40), we report that comparable intracellular acidification is coupled to greater amiloride-sensitive $Na^+/H^+$ exchange in whites when compared to blacks (Fig. 8).

We next measured $H^+$, calcium, and phosphate excretion in subjects with, and without, a history of stress fractures. It was of interest that stimulated excretion rates of titratable acid could be predicted on the basis of an individual's baseline, unstimulated acid excretion. As shown in Figure 9, $NH_4Cl$-induced acid excretion inversely correlated with baseline acid excretion. It appears that humans are very close to renal acid balance. Those individuals that had high renal excretion rates of acid have the least enhancement of titratable acid excretion by $NH_4Cl$. Conversely, those with

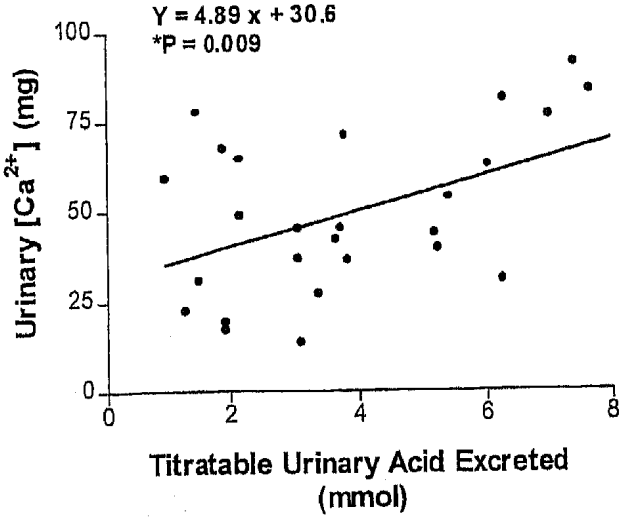


FIGURE 5—Titratable urinary acid excretion correlates with urinary calcium excretion. Following $NH_4Cl$, titratable acid excretion was variable between the black and white subject. However, the greater the urinary acid excretion, the greater an individual's calcium excretion.

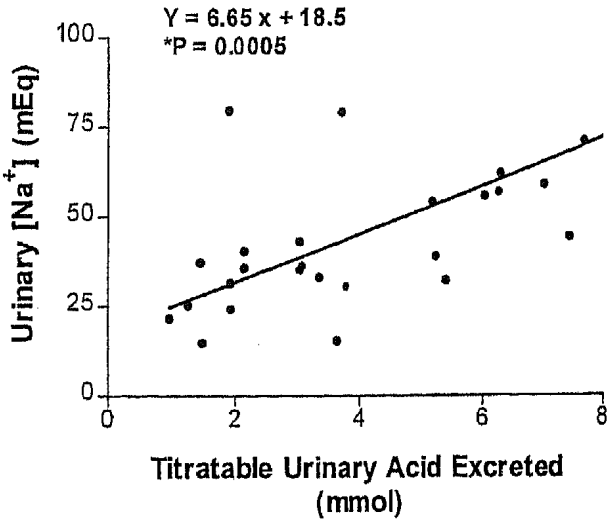


FIGURE 6—Titratable urinary acid excretion also correlates with urinary sodium excretion. Following an acid load, an increase in urinary $H^+$ excretion was highly correlated with an increasing urinary sodium excretion. As yet, it is unknown whether high urinary $H^+$ excretion drives high renal sodium output, or conversely, whether or not high urinary sodium excretion drives high renal $H^+$ output.

the least basal acid excretion had the greatest augmentation in acid output by the ammonium salt. With this in mind, we postulated that even basal acid excretion rates may be a marker for those at risk for stress fractures. We found that volunteers with a history of stress fractures averaged a 54% greater excretion of titratable acid when compared to similarly trained BUD/S students (Fig. 10). Most of the titratable acid was buffered by phosphate, and as a result, urinary excretion of this anion is tightly coupled to acid excretion (Fig. 11). Those subjects with a history of stress fractures also tended to have higher calcium and phosphate excretion rates when compared to their counterparts without such injuries (Fig. 12).

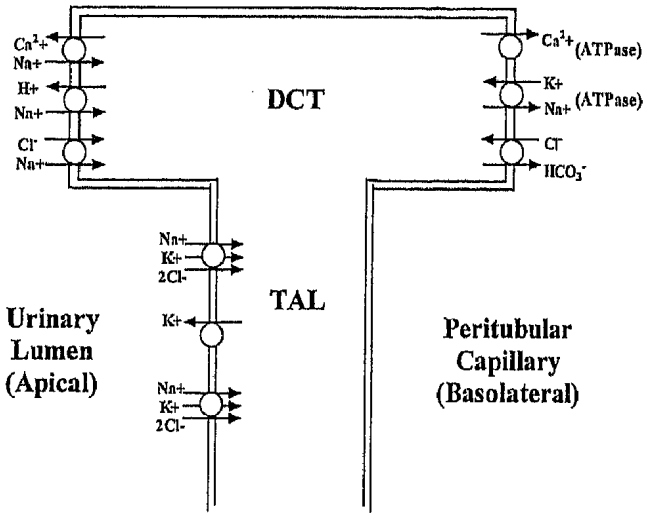


FIGURE 7—Proposed representation of the effect of variable $H^+$ or sodium delivery on the distal tubule. Blacks have decreased urinary $H^+$ excretion when compared with whites. One of the primary cellular mechanisms for $H^+$ excretion in the kidney is the $Na^+/H^+$ antiporter. Blacks may have greater endogenous buffering capacity; if this were true, then blacks would have less active $Na^+/H^+$ antiporter activity in the distal tubule and greater sodium excretion rates. Similarly, intrinsic, heritable decreases in $Na^+/H^+$ exchange activity would be expected to result in higher sodium excretion rates in blacks. However, a primary decrease in delivery of sodium to the distal tubule of blacks would be expected to result in diminished $H^+$ and calcium excretion. A decrease in sodium delivery to the distal tubule for whatever reasons would decrease $H^+$ and calcium excretion through the $Na^+/H^+$ antiporter and $Na^+/Ca^{2+}$ exchanger, respectively. If these assumptions are correct, it is more likely that net sodium delivery to the distal tubule drives $H^+$ excretion, and therefore, ethnic differences in $H^+$ and calcium metabolism are secondary to ethnic variation in sodium excretion.

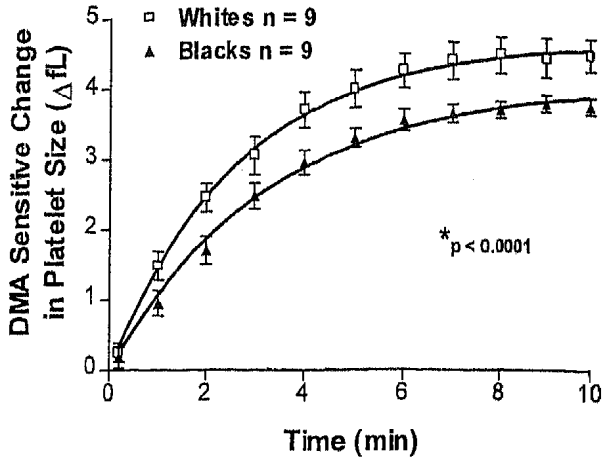


FIGURE 8—Given the demonstrated relationship between distal sodium delivery in the kidney and $H^+$ excretion, ethnic contrasts in acid excretion could be explained by racial differences in $Na^+/H^+$ activity. We next measured sodium-dependent acid transport in platelets isolated from 18 volunteers. Platelets were suspended in a physiologic salt solution in which 140 mM NaCl was replaced with equimolar sodium propionate. At pH 6.7, propionic acid freely diffuses into platelets, which causes a drop in intracellular pH. The $Na^+/H^+$ antiporter becomes activated, and $H^+$ is extruded from the cell in exchange for the extracellular $Na^+$. The influx of sodium down its concentration gradient causes an osmotic gain of water into the platelets, and the thrombocytes swell. Differences in propionic acid-induced changes in platelet size were measured in the presence and absence of amiloride, an agent that specifically blocks this antiporter. As expected, propionic acid induced a markedly greater rate in platelet swelling in whites; this finding is suggestive of greater acid-stimulated $Na^+/H^+$ antiporter activity in platelets and bone reabsorbing osteoclasts in this ethnic group.

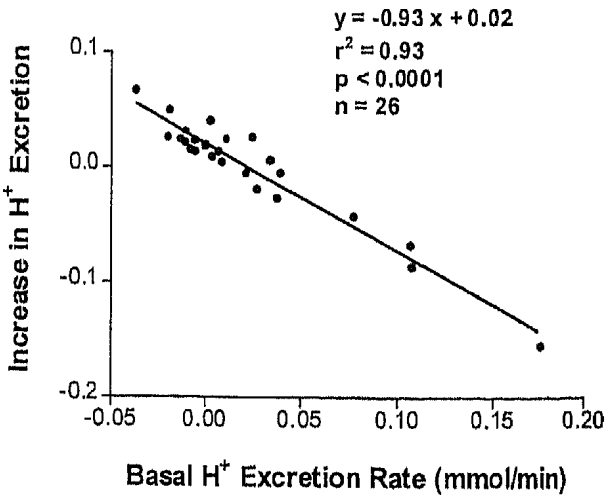


FIGURE 9—We found that $NH_4Cl$-induced increases in titratable acid excretion were highly correlated with baseline acid excretion ($N = 26$, $P < 0.0001$). Accordingly, we next determined whether a history of stress fractures was associated with basal, unstimulated titratable acid excretion.

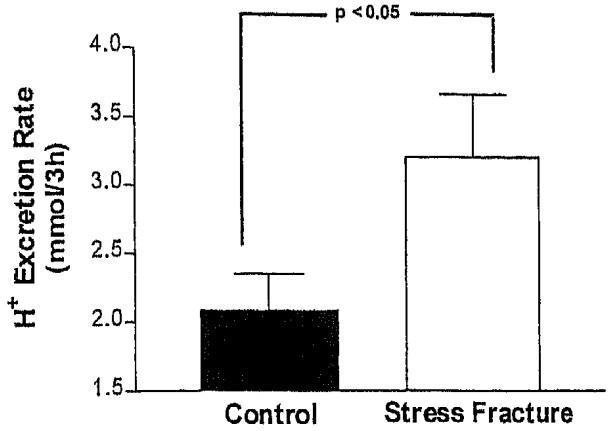


FIGURE 10—Despite similar intakes in dietary calcium, older blacks have increased bone mineralization (calcium + phosphate) when compared to age-matched whites. It has anecdotally been reported that blacks also have a smaller prevalence of stress fractures when compared to similarly trained white athletes. We hypothesized that ethnic differences in the prevalence of osteoporosis in the aged, and stress fractures in young athletes, could be attributable to heritable differences in acid excretion. Indeed, basal, titratable acid excretion was significantly greater in students undergoing the physically rigorous Basic Underwater Demolition/SEAL training at the Naval Special Warfare Center in San Diego, California, who had a history of stress fractures when compared to their counterparts who did not have such a history (values expressed in $H^+$ mM·(3 h)$^{-1}$ ± SEM, 3.20 ± 0.46 vs 2.08 ± 0.27, $P < 0.05$, $N = 33$).

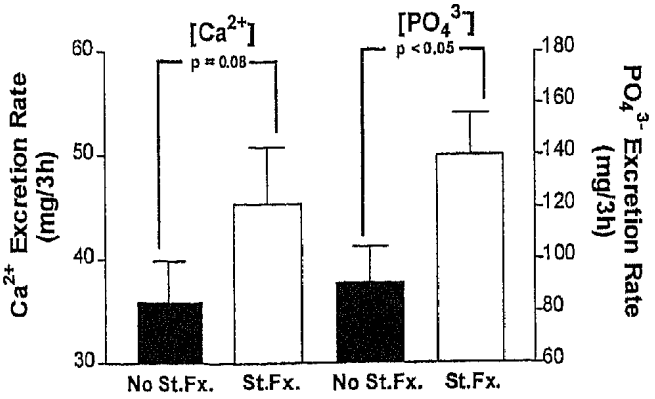


FIGURE 12—When compared to trainees with no history of stress fractures, BUD/S students with stress fractures had greater excretion rates of calcium (values expressed in mg·(3 h)$^{-1}$ ± SEM, 45.4 ± 5.4 vs 35.9 ± 4.0, $P = 0.08$) and phosphate (values expressed in mg·(3 h)$^{-1}$ ± SEM, 140 ± 16 vs 91 ± 14, $P < 0.05$).

## DISCUSSION

Oral $NH_4Cl$ loading induces a systemic metabolic acidosis in humans. $NH_4Cl$-stimulated $H^+$ excretion inversely correlated with basal, unstimulated acid excretion. We did not measure changes in the respiratory compensation for the acid load resulting from the feeding of ammonium chloride to our subjects. Yet, despite whatever ventilatory compensation may occur, we found significant ethnic differences in titratable acid excretion between blacks and whites. The evolutionary advantages for these contrasting racial responses to acid are unknown.

An adequate dietary calcium intake is necessary for healthy bone mineralization, and enhancing dietary calcium intake has been reported to play a protective role against the development of osteoporosis. At the same time, well-mineralized bones supply a source of endogenous calcium when dietary access is limited. One would expect to find a negative cross-cultural association between dietary calcium and osteoporosis and fractures. However, those types of epidemiological studies have found just the opposite; the rate of osteoporosis and hip fractures increases with an increase in dietary calcium intake (1). These studies are in contrast to reports that calcium supplementation indeed lessens the prevalence of osteoporosis and subsequent fractures within the same population. It can be suggested from this consistently reported contradiction that calcium intake is likely a surrogate marker for another factor that is causally related to fracture prevalence. We are now exploring titratable acid excretion for its influence on calcium excretion.

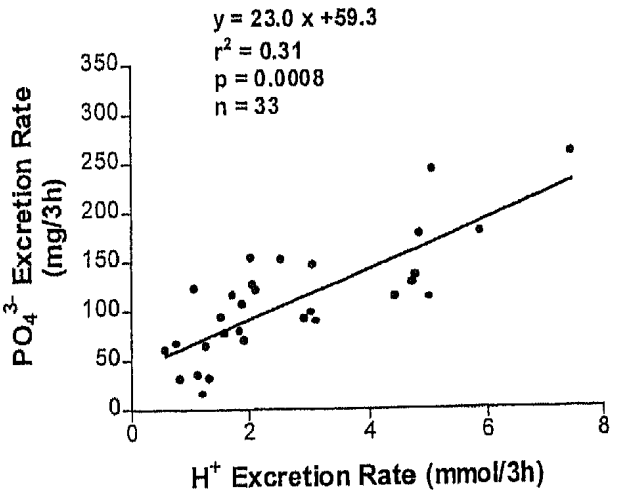


FIGURE 11—The loss of inorganic phosphate from bone is the primary mechanism by which the kidney excretes titratable acid. As expected, the excretion rate of $H^+$ was highly correlated with phosphate excretion in BUD/S students.

We postulate that this confounding variable is, in fact, a difference in $H^+$ balance across different populations. Over the past century, changes in the Western diet have led to an increase in acid consumption that occurs predominantly in the form of high intake of animal protein. Unlike vegetable protein, meats have much, much higher quantities of sulfated amino acids, and the metabolism of these proteins results in a measurable oral acid load for Westerners. Unfortunately, diets rich in calcium are also more likely to be rich in acid (1). If a population were to increase its acid consumption without evolving a comparable mechanism to excrete this acid load, endogenous buffer stores such as bone will be called on to maintain extracellular pH. We hypothesize that ethnic differences in acid excretion result from contrasting racial abilities to buffer dietary acid by bone and from heritable differences in the ability to excrete a dietary acid load.

In humans, renal $H^+$ secretion is mediated in the proximal tubule, the ascending loop of Henle, distal tubule, and the intercalated cells of the collecting duct system. Within the proximal tubule, $H^+$ and $OH^-$ ions are generated from water, and the $H^+$ is exchanged for sodium through a luminal $Na^+/H^+$ antiporter; the hydroxyl ion combines with $CO_2$ to form a bicarbonate ion in a reaction catalyzed by

carbonic anhydrase. Bicarbonate exits the cell at the basolateral surface by an exchange with chloride, or through a sodium-dependent bicarbonate transporter. Apical and basolateral membrane $Na^+/H^+$ exchangers play a pivotal role in regulation of renal acid excretion. In the distal tubule and collecting duct, active $H^+$-ATPase, or $H^+/K^+$-ATPase, as well as a luminal $Na^+/H^+$ exchanger are also found, and these transporters mediate the final, net excretion of protons into the urine (38). It has been demonstrated that alterations in distal sodium delivery affect the rate of excretion of an acute, exogenous acid load (36). In response to a high-protein diet, the activity of the $Na^+/H^+$ antiporter also rises dramatically, and an increase in sodium reabsorption by the luminal $Na^+/H^+$ antiporter would be accompanied by enhanced acid extrusion into the lumen (19). Indeed, we found that in whites, acid loading was accompanied by enhanced sodium reabsorption (i.e., a fall in sodium excretion), whereas our black subjects had a low baseline sodium excretion that remained unchanged in the face of a significant acidemia (Fig. 4).

Given the known relationship between distal sodium delivery in the kidney and $H^+$ excretion, these disparate findings in titratable acid loss between whites and blacks could be explained on the basis of racial differences in $Na^+/H^+$ activity. Using platelets, we were able to measure net $Na^+/H^+$ antiporter activity in blacks and whites. Others have demonstrated that intracellular acidification is associated with an activation of the $Na^+/H^+$, and this exchange activity is dependent on the availability of extracellular sodium. Accordingly, we measured sodium-dependent $Na^+/H^+$ antiporter activity in acid-loaded platelets isolated from volunteers in each ethnic group. As expected, propionic acid induced a markedly greater rate in platelet swelling in whites; this finding is suggestive of greater acid-stimulated $Na^+/H^+$ antiporter activity in the platelets, a surrogate marker for polarized, distal tubule $Na^+/H^+$ exchange, of this ethnic group.

The bone reabsorbing osteoclast, in many ways (31,32), resembles the renal epithelial cells of the distal tubule. We next reasoned that an increased availability of $H^+$ to the osteoclast for secretion into the ruffled border to effect bone reabsorption may contribute to enhanced bone demineralization in whites. Conversely, an increased availability of $H^+$ in the extracellular milieu may also prevent the physiochemical deposition of calcium and phosphate into bone. These ethnic contrasts in the handling of hydrogen ion may contribute to the increased bone demineralization found in older whites, and the increased prevalence of microfractures (i.e., stress fractures) found in younger whites when compared to age-matched, similarly fit black individuals. We speculated that an increased predisposition to stress fractures would be found in subjects with excess $H^+$ available for secretion by the osteoclast or the kidney.

Despite having diminished $Na^+/H^+$ exchange activity, the black volunteers did not have a greater acidemia than the whites (Fig. 1). From these data, it can be suggested that blacks have a greater capacity to buffer an exogenous acid load. It has been shown that over the past century, changes in American dietary habits resulted in an increase in dietary acid consumption, and matching net acid excretion, by approximately 20–40 $mEq \cdot d^{-1}$ (1). Such a finding implies that Americans are very close to acid balance, and any increase in acid intake must be offset by a corresponding increase in acid excretion, or reserves of endogenous buffers must be called on to offset an additional acid load. We propose that the increasing dietary $H^+$ load calls on the buffering capacity of bone. If this were true, then any increase in acid presentation to humans should be accompanied by an augmented excretion of calcium and phosphate into the urine as these minerals are released from the bone matrix to buffer the acid; such is likely the case. In addition to the cellular complement and collagenous matrix support, bone is composed of three salts: apatite ($Ca_{10}(PO_4)_6(OH)_2$), brushite ($CaHPO_4$), and calcium carbonate ($CaCO_3$). Exposure of bone to a low pH results in a direct dissolution of these salts from the collagenous matrix in an effort to buffer the increased concentrations of protons in the extracellular milieu. It has long been recognized that the skeleton acts as a buffer reservoir during acute and chronic metabolic acidosis. Lehmann et al. first noted that with sustained acid loading, serum bicarbonate ultimately stabilized at some reduced level despite continuing acid retention, and they concluded that an additional buffer system is titrated (23). It was suggested that such additional quantities of buffer could arise from the slow dissolution of bone mineral during chronic metabolic acidosis. In those well done balance studies, a chronic metabolic acidosis was induced with ammonium chloride feeding. Most of the ensuing acid load was excreted by the kidneys, but their subjects maintained a positive acid balance despite subsequent return of the serum bicarbonate to stable control levels. The simultaneous persistence of a negative calcium balance supported the argument that bone mineral such as $CaCO_3$ is an important buffer reservoir in the defense against chronic metabolic acidosis. These findings are also compatible with the well known finding of renal osteodystrophy in patients with chronic, pathologic metabolic acidosis. In this severe, chronic form of acidemia, bone calcium is released to not only maintain the plasma calcium concentration, but also to buffer against the endogenous production of acid that cannot be excreted by the kidneys.

Acute acidosis is also associated with $H^+$ buffering by bone. More recently, Grinspoon et al. demonstrated increased mineral dissolution during the mild ketoacidosis that occurs during acute fasting in young women (18). After only 4 d of fasting, the mean serum bicarbonate level fell 37% in these volunteers, and this metabolic acidosis was associated with a significant increase in the plasma ionized calcium concentration despite a fall in parathyroid hormone levels. It was suggested that the increased serum calcium observed during the acidosis was mobilized from bone, as oral calcium intake was eliminated and urinary calcium excretion was increased. All of these changes were prevented when subjects were given an oral supplement of potassium bicarbonate. Furthermore, in these subjects, the urinary excretion of pyridinoline and deoxypyridinoline,

markers of osteoclast-induced bone resorption, did not change. Using alternative methodologies such as radioactive tracer exchange experiments, Bettice and Gamble demonstrated that on induction of metabolic acidosis, the sodium content of the mineral matrix was exchanged for $H^+$ (5). This cation exchange of sodium for hydrogen in the extracellular compartment of the bone matrix helped to buffer the extracellular pH. Bushinsky subsequently showed that acid could induce calcium efflux from bone by dissolving calcium carbonate and generating additional buffer for the extracellular pH, and this process was not necessarily dependent on osteoclasts (6,7). In aggregate, these findings demonstrated that the development of metabolic acidosis can directly increase the physiochemical dissolution of bone. These findings are consistent with our hypothesis that any increase in endogenous acid production or exogenous acid consumption may be deleterious to bone composition.

These findings are of no small consequence. It is suggested that alkali supplementation, rather than calcium administration *per se*, is responsible for the enhanced bone mineralization seen in patients given oral calcium compounds. In fact, it is possible that the protective effect of estrogen on bone health may also be mediated by its effect on pH rather than the effect of this hormone on gene expression. It has been recently shown that women taking supplemental estrogen develop a significant respiratory alkalosis (29), and bicarbonate supplementation without calcium may increase skeletal integrity (14,33).

In our study, blacks had less titratable acid excretion and less capacity to excrete acid through the $Na^+/H^+$ antiporter. It is possible that blacks either consume less acid in their diet or, alternatively, produce less endogenous acid. The acid content of the diet is dependent on the type of protein consumed. Blacks tend to have less protein intake than whites, and ethnic contrasts have been reported for the sources and types of protein intake (21,35).

Most endogenous metabolic acid production comes from the generation of $H^+$ from the hydration of $CO_2$ with water by carbonic anhydrase. It is interesting to note that mice with genetic disruption of carbonic anhydrase develop osteopetrosis (8,22,34); a deficiency of carbonic anhydrase in these animals is associated with increased bone mineralization. Finally, there is strong evidence in support of lower basal metabolic rate (BMR) in blacks when compared to whites (12,20), and differences in bone mineralization may reflect underlying differences in metabolic acid production. Consistent with this hypothesis is the observation that obese individuals who expend less metabolic energy have a diminished prevalence of osteoporosis (39).

Finally, age-related declines in acid excretion could also contribute to the progression of osteoporosis. It has been observed that with increasing age, there is a significant increase in the steady-state blood pH (13,15). In summary, all of our findings are consistent with our hypothesis that acid-base metabolism may be as important as dietary calcium in governing bone density and health.

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