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ORIGINAL CONTRIBUTION

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Acid-base imbalance and the skeleton

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Summary Humans generally consume a diet that generates metabolic acids leading to a reduction in the concentration of systemic bicarbonate and a fall in pH. In vitro experiments indicate that this metabolic acidosis causes a release of calcium from bone that initially is simply due to physicochemical dissolution of the mineral. On a more chronic basis metabolic acidosis alters bone cell function; there is an increase in osteoclastic bone resorption and a decrease in osteoblastic bone formation. Concomitant with the dissolution and resorption of the bone mineral there is buffering of the addition protons by bone leading to restoration of the systemic pH. Interestingly respiratory acidosis, caused by an increase in the partial pressure of carbon dioxide induces far less bone dissolution and resorption and the additional hydrogen ions are not buffered by bone. As we age we are less able to excrete these metabolic acids due to the normal decline in renal function. We hypothesize that a slight, but significant, metabolic acidosis leads to greater loss of bone mineral and increase potential to fracture.

Key words Calcium – Bone – Acidosis – Osteoclast – Osteoporosis

Dietary acid intake

On a daily basis the human diet contains food that upon metabolism generates or consumes hydrogen ions [1, 2]. Acid may be generated by the following reactions:

methionine or cystine $\rightarrow$ glucose + urea + SO_4^{2-} + 2 H^+

arginine$^+$ $\rightarrow$ glucose + urea + H^+

R-H_2PO_4 + H_2O $\rightarrow$ ROH + 0.8 HPO_4^{2-} / 0.2 H_2PO_4 + 1.8 H^+

Acid may be consumed, or base generated, by the following reaction:

glutamate$^-$ + H^+ $\rightarrow$ glucose + urea

lactate$^-$ + H^+ $\rightarrow$ glucose + CO_2

citrate$^-$ + 4.5 O_2 $\rightarrow$ 5CO_2 + 3H_2O + HCO_3^-

The net result is that on a normal Western diet adult humans generate approximately 1 meq of acid per day [3–5]. The more acid precursors our diet contains, the greater degree of systemic acidity [3]. As we age there is a decrease in overall renal function, including a decrease in the ability to excrete acid [6]. The reduction in renal acid excretory capacity with increasing age, in conjunction with the continued intake of acid precursors results in a slight, but significant, acidemia in the elderly [6, 7].

Effects of acid on calcium homeostasis

Metabolic acidosis (Fig. 1), an increase hydrogen ion concentration caused by a fall in the concentration of the principal extracellular buffer bicarbonate, results in a marked increase in urine calcium excretion with no change in intestinal calcium absorption [8–10]. The vast

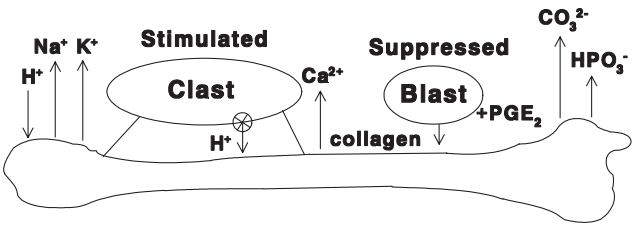

Fig. 1 Metabolic acidosis. Schematic diagram of the mechanisms by which protons lead to the release of bone calcium and are buffered by the bone mineral during chronic metabolic acidosis [1, 2]. Clast = osteoclast, Blast = osteoblast

majority of body calcium is contained within the mineral phase of bone suggesting an osseous source for the increased urine calcium excretion [11, 12].

The acid-induced increase in urine calcium excretion could be due to a decrease in renal calcium reabsorption inducing secondary hyperparathyroidism and bone mineral resorption or to a direct effect of acid on bone. Sutton and colleagues utilized micropuncture to study the effect of acidosis, and its correction, on calcium transport in thyroparathyroidectomized dogs [13]. They found that metabolic acidosis, pH = 7.22, increased the ratio of nonreabsorbed calcium to nonreabsorbed sodium in the distal tubule and in the final urine, but not in the proximal tubule. Correction of acidosis with sodium bicarbonate rectified the defect in calcium reabsorption. Thus, acidosis clearly decreases renal calcium reabsorption. We undertook a series of studies to determine whether an increase in acidity affects bone dissolution and/or resorption [1, 2, 5, 15–21, 25–32, 38–45, 48, 51, 58–61].

Acid effects on bone

Acute calcium release

When mineral acid is infused into nephrectomized animals there is a rapid increase in serum calcium implying dissolution of bone mineral [14]. Using cultured neonatal mouse calvariae we determined that a physiologic decrease in medium pH of 3 h duration, a model of acute metabolic acidosis, causes bone mineral dissolution [15, 16].

Studies in calvariae indicate that the mechanism by which hydrogen ions cause the release of bone calcium during this short time period appears due to alterations in the physicochemical factors that govern the deposition and dissolution of the bone mineral and not to cell-mediated alterations in bone resorptive activity [16]. To confirm this hypothesis, we cultured synthetic carbonated apatite disks in physiologically acidic medium [17]. The synthetic carbonated apatite disks are an accurate, cell-free model of bone mineral. There is calcium release from cultured disks in response to a physiologic decrement in pH, supporting the hypothesis that excess hydrogen ions directly induce physicochemical calcium release from bone [17].

The type of bone mineral in equilibrium with the medium, and thus altered by the physicochemical forces resulting in calcium release could be carbonate or phosphate in association with calcium. To determine whether either of these forms were involved, we cultured calvariae in medium in which the driving forces for crystallization with respect to the solid phase of the bone mineral were altered by changing medium pH [18]. With respect to calcium and carbonate but not calcium and phosphate, there was bone formation in a supersaturated medium, no change in the bone mineral when cultured in a saturated medium and bone dissolution into an undersaturated medium. Thus bone carbonate, apparently in the form of carbonated apatite, appears to be solubilized during a reduction in medium pH leading to a release of calcium.

Further support for the role of carbonate in proton-mediated bone mineral dissolution comes from studies in which we demonstrated that at a constant pH, whether physiologically neutral or acid, calcium efflux from bone is dependent on the medium bicarbonate concentration; the lower the bicarbonate concentration, the greater the calcium efflux [19].

Hydrogen ion buffering

Acidosis not only increases calcium efflux from bone, but some of the additional protons are apparently buffered by its mineral phases restoring the pH toward the normal range. The evidence that bone buffers acute acid loads derives principally from the acid-induced loss of bone sodium [20–23] and the depletion of bone carbonate [18, 24, 25]. Bone sodium loss suggests proton for sodium exchange and carbonate loss suggests consumption of this buffer by protons. The *in vitro* evidence is derived from proton flux studies into bone [15, 18, 20, 26, 27] and microprobe evidence for a depletion of bone sodium during acidosis [20, 21].

Proton for sodium exchange

Bone is a reservoir for sodium and potassium and its surface has fixed negative sites which normally complex with sodium, potassium and hydrogen ions; the sodium appears to exchange freely with the surrounding fluid [11, 12]. A decrease in pH causes the additional hydrogen ions to displace sodium and potassium from the mineral surface resulting in an efflux of these ions and a reduction of the systemic acidity (buffering) [22, 23].

The indirect evidence that bone is a hydrogen ion buffer, based on the loss of bone sodium, is supported by

our *in vitro* studies that demonstrate that when neonatal mouse calvariae are cultured in acidic medium there is a net influx of hydrogen ions into the bone. This influx decreases the medium hydrogen ion concentration (increases the medium pH) indicating that the additional hydrogen ions are being buffered by bone [15, 18, 20, 26, 27]. Examination of calvariae with a high resolution scanning ion microprobe demonstrates that the surface of the bone is rich in sodium and potassium relative to calcium [20, 21, 28–32]. After incubation in an acidic medium there is loss of surface sodium and potassium relative to calcium in conjunction with proton buffering, suggesting sodium and potassium for proton exchange on the bone surface [20, 21, 33].

Fall in bone carbonate

Bone contains approximately 80 % of the total body carbon dioxide [34]. Approximately two-thirds of this is in the form of carbonate complexed with calcium, sodium and other cations, and is located in the lattice of the bone crystals where it is relatively inaccessible to the systemic circulation. The other third consists of bicarbonate which is located in the hydration shell of hydroxyapatite and is readily available to the systemic circulation.

Acute metabolic acidosis decreases total carbon dioxide in bone. Bettice [24] found that bone total carbon dioxide fell with acidosis and that the fall was directly proportional to the decline in the extracellular fluid pH and bicarbonate concentration. The loss of bone carbon dioxide, presumably from the readily available bicarbonate pool, suggests that bone is actively buffering the increased hydrogen ion concentration. A reduction of medium pH has also been shown to induce the release of calcium and carbonate from the bone [18]. When we culture neonatal mouse calvariae in acidic medium modeling metabolic acidosis, there is a progressive loss of bone carbonate [25].

Chronic acidosis

Calcium release

Lemann and coworkers fed normal subjects protein, NH_4Cl and $NaHCO_3$ and measured urine calcium excretion [8]. Protein and NH_4Cl, which are metabolized into metabolic acids, led to a marked increase, while the base $NaHCO_3$ led to a decrease, in renal calcium excretion. There was no measurable change in intestinal calcium absorption with any treatment. Balance studies performed on patients given NH_4Cl demonstrated that much of the acid was retained, resulting in a fall in serum bicarbonate concentration [9]. Twelve days after the conclusion of the NH_4Cl administration there was an equivalence of the hydrogen ions retained to calcium excreted suggesting that bone buffered the additional hydrogen ions, raising the plasma bicarbonate and pH, and was the source of the additional urinary calcium.

The mechanism by which chronic metabolic acidosis induces the release of bone calcium appears to be direct physicochemical dissolution of the bone mineral, as in acute metabolic acidosis, as well as enhanced cell-mediated bone resorption. Studies in rats have shown stimulated cell-mediated bone calcium resorption during prolonged acidosis [14, 35].

Arnett and Dempster studied the effects of alterations in pH on cell-mediated bone resorption [36]. Using isolated rat osteoclasts cultured on slices of polished bovine femur they found increased areas of resorption in the acidic, compared to physiologically neutral, pH medium. There is evidence for cell-mediated bone resorption when calvariae are cultured in acidic medium as well [37, 38]. Goldhaber and Rabadjija described enhanced calcium release from calvariae cultured for one week in acidic medium that was suppressed by the osteoclastic inhibitor calcitonin [37]. We demonstrated cell-mediated bone mineral resorption after 99 h of culture in acidic medium [38]. In addition acidosis has been shown to increase osteoclastic and inhibit osteoblastic activity [39, 40]. Release of the osteoclastic enzyme β-glucuronidase was stimulated while osteoblastic collagen synthesis and alkaline phosphatase activity were inhibited by metabolic acidosis [39]. Since many patients with renal failure are acidemic and have elevated levels of parathyroid hormone we determined if acidosis and PTH have additive effects on calcium release from bone [41]. Acidic medium and PTH independently stimulated calcium release from bone; however, the combination caused a greater calcium efflux than either alone. Osteoclastic activity, as determined by β-glucuronidase release, was increased with both acidosis and PTH individually but was most pronounced with the combination. Increasing medium [HCO_3^-] and pH, metabolic alkalosis, results in a decrease in osteoclastic resorption and an increase in osteoblastic bone formation [42].

We have examined the effects of metabolic acidosis on the RNA levels of several genes known to be expressed in osteoblasts. After acute stimulation with serum, metabolic acidosis selectively inhibits expression of *Egr-1* and type 1 collagen RNA when compared to stimulation at neutral medium pH [43]. In contrast, expression of *c-fos*, *c-jun*, *junB*, and *junD* RNA were not affected by a similar decrement in medium pH. In chronic bone cell cultures, maintained up to 6 weeks, metabolic acidosis inhibited expression of matrix Gla protein and osteopontin RNA relative to expression in neutral medium [44]. Expression of osteonectin, transforming growth factor β, and glyceraldehyde–3-phosphate dehydrogenase were not affected by acidosis.

Further evidence that metabolic acidosis inhibits os-

teoblastic function was obtained utilizing cultured bone cells, principally osteoblasts. Isolated osteoblasts cultured for three weeks form collagen and actual nodules of apatitic bone. Acidic medium leads not only to fewer nodules, but decreased calcium influx into these nodules [45].

Metabolic alkalosis, results in a decrease in osteoclastic resorption and an increase in osteoblastic bone formation [42].

Hydrogen ion buffering

Bone appears to buffer hydrogen ions during acid administration. When patients with normal renal function were fed an acid load they did not quantitatively excrete the administered acid, yet their serum bicarbonate concentration stabilized [9]. As bone is a predominant source of buffer in the body, this observation suggested that bone is the likely hydrogen ion buffer [9]. As with acute acidosis the imposition of a chronic acid load appears to decrease bone carbonate suggesting that bone carbonate may be a physiologic hydrogen ion acceptor [46, 47].

We studied the effect of chronic acidosis on the bone hydrogen ion buffers phosphate and bicarbonate. We found that one week of mild metabolic acidosis led to a fall in mineral phosphates and bicarbonate, indicating buffering of the excess protons and returning the systemic pH toward normal [48].

Relationship between calcium release and hydrogen ion buffering

During acute metabolic acidosis a reduction in pH causes both bone calcium release and proton buffering by bone. If all buffering were the result of mineral dissolution, there should be a 1:1 ratio of protons buffered to calcium released in the case of calcium carbonate, 5:3 for apatite and 1:1 for brushite [49, 50]. However, with cultured calvariae the ratio was found to be 16–21 to 1 indicating that proton buffering could not simply be due to mineral dissolution [15]. That calcium release is only one component of proton buffering by bone is demonstrated by the microprobe studies which show substantial sodium and potassium exchange for protons [20, 21, 28, 32, 51] and loss of bone phosphate and bicarbonate with acidosis [48].

Mechanism of acid-induced, cell-mediated bone resorption

In non-osseus cells metabolic acidosis increases the levels of prostaglandins [52–55]. Any increase in prostaglandin levels by bone cells is important as prostaglandins are potent local stimulators of bone resorption and appear to mediate resorption induced by a variety of cytokines and growth factors [54, 56]. Goldhaber et al. [37] first demonstrated that the prostaglandin inhibitor indomethacin inhibits acid-induced, cell-mediated calcium efflux from bone and Rabadjija et al. [57] subsequently demonstrated that protons stimulate release of prostaglandin E_2 from neonatal mouse calvariae. We demonstrated that incubation of bone cells in medium simulating metabolic acidosis led to an increase in the level of medium PGE_2 [58] and that incubation of calvariae in similarly acidic medium led to a parallel increase in PGE_2 levels and net calcium efflux.

Metabolic vs. respiratory acidosis

Most *in vivo* and *in vitro* studies have utilized HCl or NH_4Cl to lower serum bicarbonate concentration as a model of metabolic acidosis. This non-anion gap acidosis mimics clinical disorders such as renal tubular acidosis, in which the kidney is unable to maintain systemic pH in the normal range or diarrhea, in which there is a loss of the proton buffer bicarbonate through the gastrointestinal tract. *In vitro* the type of acidosis, metabolic vs. respiratory, appears to be critical in determining the magnitude of both the bone calcium release and hydrogen ion buffering. This first became evident when we found a clear distinction between the effects of metabolic and respiratory acidosis on cultured bone [4, 15–21, 25–28, 38, 39, 59–61]. During acute incubations there is a far greater net calcium efflux during metabolic, compared to respiratory, acidosis [26]. With respiratory acidosis there is not only less calcium efflux but there appears to be deposition of medium calcium on the bone surface [60]. This is consistent with the observation that over this short time period acidosis affects the physicochemical driving forces for formation and dissolution of the bone mineral [16, 18, 19, 21]. During metabolic acidosis the low $[HCO_3^-]$ favors the dissolution, while during respiratory acidosis the increased Pco_2 and $[HCO_3^-]$ favors the deposition of carbonated apatite. Indeed there is no net hydrogen ion influx into bone during respiratory acidosis [26].

During more chronic incubations we found net cell-mediated calcium efflux from bone during models of metabolic, but not respiratory, acidosis [38]. Many investigators have shown that metabolic acidosis stimulates osteoclastic resorption [35–39, 51, 60, 62]. We found that respiratory acidosis does not appear to alter osteoclastic β-glucuronidase release, osteoblastic collagen synthesis or alkaline phosphatase activity as does metabolic acidosis [40]. Utilizing a high resolution scanning ion microprobe, respiratory acidosis does not

appreciably alter the surface ion concentration of bone [28].

Given the marked differences in the osseous response to metabolic and respiratory acidosis, we hypothesized that incubation of neonatal mouse calvariae in medium simulating respiratory acidosis would not lead to the increase in medium PGE_2 levels which is observed during metabolic acidosis [58]. We recently found that metabolic, but not respiratory, acidosis increased bone culture medium PGE_2 levels and net bone calcium release. There was a strong, direct correlation between bone culture medium PGE_2 levels and net calcium release.

Clinical observations

Sebastian and coworkers have shown that bone is affected by normal endogenous acid production [63]. These investigators fed 18 postmenopausal women a constant diet and then nearly completely neutralized endogenous acid production with potassium bicarbonate. They found that the administration of alkali resulted in a decrease in urine calcium and phosphorus excretion and that the overall calcium balance became less negative or more positive (Table 1). In addition there was reduced urinary excretion of hydroxyproline, a marker of bone breakdown, and an increased excretion of serum osteocalcin, a marker of osteoblastic bone formation. This study supports a role for daily endogenous acid production and low level acid retention in the development of osteoporosis in patients with normal renal function [63, 64].

There is substantial evidence that acidosis, either directly or through hormonal or ionic factors, severely inhibits bone growth [65–67].

Hypothesis

Thus, bone appears to decrease the magnitude of the fall in serum [HCO_3^-] and blood pH during metabolic acidosis. The acidosis may be mild and secondary to the consumption of food rich in acid precursors. Initially there is physicochemical sodium for hydrogen and potassium for hydrogen exchange on the mineral surface in conjunction with dissolution of carbonate and release of bone calcium (Fig. 1). This is followed by stimulation of cell mediated osteoclastic resorption and inhibition of osteoblastic collagen deposition. The increased resorption releases calcium and the buffers carbonate and phosphate. The fall in bone formation prevents calcium uptake and blocks the hydrogen ion release that accompanies bone mineral formation.

Table 1 Calcium balance in 18 postmenopausal women. Data from [63]

Calcium mg/day/60kg	Before $KHCO_3^-$	During $KHCO_3^-$	After $KHCO_3^-$
Intake	652±188	652±188	652±188
Stool	608±143	616±134	592±138
Urine	236±86	172±81*	224±70**
Balance	−180±124	−124±76	−148±96

* different from before $KHCO_3^-$, ** different from during $KHCO_3^-$

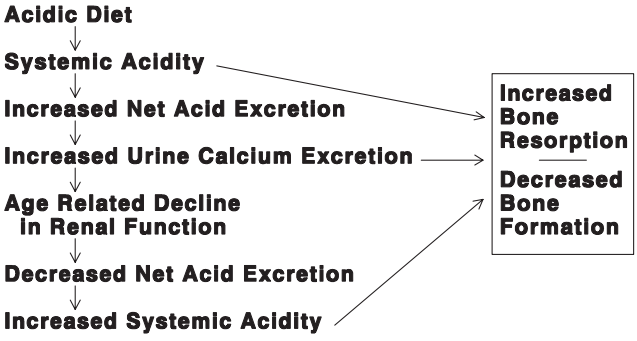

Fig. 2 Hypothesis for the mechanisms leading to dietary acid-induced increased bone resorption and decreased bone formation [1].

With adequate renal function mild metabolic acidosis leads to an increase in urine calcium excretion, evidence for bone mineral dissolution and resorption, with buffering of the additional hydrogen ions [1] (Fig. 2). However, as we age renal function slowly deteriorates decreasing the ability of the kidney to excrete the daily acid load. Exchange of hydrogen ions for bone sodium and potassium and release of carbonate all help to restore the decrease in pH. Bone carbonate is replaced by phosphate adding base to the extracellular fluid. Hydrogen ions are exchanged for bone sodium and potassium. Osteoclastic bone resorption is further stimulated and osteoblastic bone formation is further suppressed. Bone mineralization continues to decrease setting the stage for a fracture.

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