Metabolic alkalosis decreases bone calcium efflux by suppressing osteoclasts and stimulating osteoblasts

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Bushinsky, David A. Metabolic alkalosis decreases bone calcium efflux by suppressing osteoclasts and stimulating osteoblasts. *Am. J. Physiol.* 271 (*Renal Fluid Electrolyte Physiol.* 40): F216–F222, 1996.—In vivo and in vitro evidence indicates that metabolic acidosis, which may occur prior to complete excretion of end products of metabolism, increases urinary calcium excretion. The additional urinary calcium is almost certainly derived from bone mineral. Neutralization of this daily acid load, through the provision of base, decreases calcium excretion, suggesting that alkali may influence bone calcium accretion. To determine whether metabolic alkalosis alters net calcium efflux (J^+_{Ca}) from bone and bone cell function, we cultured neonatal mouse calvariae for 48 h in either control medium (pH $\approx$ 7.4, $[HCO_3^-] \approx 24$), medium simulating mild alkalosis (pH $\approx$ 7.5, $[HCO_3^-] \approx 31$), or severe alkalosis (pH $\approx$ 7.6, $[HCO_3^-] \approx 39$) and measured J^+_{Ca} and the release of osteoclastic β-glucuronidase and osteoblastic collagen synthesis. Compared with control, metabolic alkalosis caused a progressive decrease in J^+_{Ca}, which was correlated inversely with initial medium pH (pH_i). Alkalosis caused a decrease in osteoclastic β-glucuronidase release, which was correlated inversely with pH_i and directly with J^+_{Ca}. Alkalosis also caused an increase in osteoblastic collagen synthesis, which was correlated directly with pH_i and inversely with J^+_{Ca}. There was a strong inverse correlation between the effects alkalosis on osteoclastic β-glucuronidase release and osteoblastic collagen synthesis. Thus metabolic alkalosis decreases J^+_{Ca} from bone, at least in part, by decreasing osteoclastic resorption and increasing osteoblastic formation. These results suggest that the provision of base to neutralize endogenous acid production may improve bone mineral accretion.

calcium flux; osteodystrophy; osteoporosis

CLINICAL STUDIES INDICATE that the addition of animal protein to the diet results in an increased acid load to the body leading to augmented net acid excretion (4, 32). These additional acids derive from the metabolism of protein to metabolic acids, which are initially buffered by bicarbonate and must result in decreased serum bicarbonate concentration and systemic pH, so-called metabolic acidosis, prior to the acid being excreted by renal mechanisms (6). The severity of the resulting metabolic acidosis appears to be mitigated by skeletal buffering of these additional hydrogen ions (6). In the process of skeletal buffering, calcium is released from the bone mineral (6, 9). Indeed, under carefully controlled conditions, clinical studies indicate that additional acid loads either from dietary protein or NH_4Cl cause an increase in urine calcium excretion without a change in intestinal calcium absorption, resulting in a decrease in net calcium retention or balance (4, 25–27, 32). Ongoing chronic metabolic acidosis, due to ingestion of high-animal-protein diets, has been suggested as cause of osteoporosis (3). Alkali administration to subjects eating animal protein diets has been shown to significantly decrease urine calcium excretion and enhance net calcium retention (28, 33). As bone is the repository for over 99% of body calcium, any increase in calcium retention almost certainly leads to an increase in bone mineral stores (38).

In vitro evidence from this (5, 7–10, 12–18, 24, 34) and other laboratories (2, 21, 30) clearly indicates that metabolic acidosis increases net calcium efflux from bone. The calcium efflux is secondary to initial physicochemical effects of a lowered pH causing dissolution of the bone mineral (8, 11, 14, 15, 17) and to a subsequent increase in osteoclastic bone resorption and a decrease in osteoblastic bone formation (7, 13, 24, 34). However, an independent effect of metabolic alkalosis on bone calcium flux and cellular activity has never been determined. Previous studies from our laboratory suggest that metabolic alkalosis may have a marked effect on bone calcium flux and cellular activity (14). At a constant pH, whether neutral or acidic, variations in bicarbonate concentration regulate net calcium flux; there is greater calcium efflux at decreased bicarbonate concentrations (14). There is net calcium efflux from bones cultured under conditions of metabolic (low bicarbonate) compared with isohydric respiratory (normal bicarbonate) acidosis (5, 16). During metabolic, but not respiratory, acidosis there is increased osteoclastic activity and decreased osteoblastic activity (7, 34).

To determine whether metabolic alkalosis alters net calcium flux, osteoclastic, and/or osteoblastic activity, we cultured neonatal mouse calvariae in alkalotic medium and determined the effect on calcium flux, osteoclastic β-glucuronidase release into the medium, and osteoblastic collagen synthesis. We found that not only did metabolic alkalosis decrease net calcium efflux from cultured calvariae, but it decreased osteoclastic activity and increased osteoblastic activity as well.

METHODS

Organ culture of bone. Neonatal (4–6 day old) CD-1 mice (Charles River, Wilmington, MA) were killed, their calvariae were removed by dissection, the adherent cartilaginous material was trimmed, and the periosteum was left intact (5, 7–11, 13–14, 16–18, 24, 34). Exactly 2.8 ml of Dulbecco's modified Eagle's medium (M. A. Bioproducts, Walkersville, MD) containing heat-inactivated (1 h, 56°C) horse serum (15%), sodium heparin (10 U/ml), and potassium penicillin (100 U/ml) was preincubated at a fixed chosen P_{CO_2} at 37°C for 3 h in 35-mm petri dishes. We have found that 3 h is sufficient for P_{CO_2} equilibration between the incubator and the medium (9). After preincubation, 1.0 ml was removed to determine initial medium pH (pH_i), P_{CO_2}, and total calcium concentration, and two calvariae were placed in each dish on a stainless-steel

wire grid. Total bone content in each culture dish was controlled by using pups that were the same age and size, by using a standardized dissection procedure, and by placing two bones in each dish. Experimental and control cultures were performed in parallel and in random order. For determinations of collagen synthesis and β-glucuronidase release, bones were cultured at 37°C for 48 h; after the first 24 h, calvariae were transferred to preincubated fresh medium. After each 24-h period, a sample of culture medium was removed and analyzed for pH, P_{CO_2}, and calcium. After the second 24-h incubation, an additional aliquot was used for assay of β-glucuronidase activity. The incubator used (model 3154; Forma Scientific, Marietta, OH) maintains a constant temperature (±0.02°C) and a constant P_{CO_2} (±0.1%) at an ambient O_2 concentration of 21%.

Osteoclastic β-glucuronidase activity. To measure cellular β-glucuronidase activity, calvariae were studied in the following three groups: control (Ctl), mild metabolic alkalosis (M-Alk), and severe metabolic alkalosis (S-Alk). In the Ctl group, calvariae were cultured in basal medium [pH ≈ 7.40; P_{CO_2} ≈ 40 mmHg; and medium bicarbonate concentration ($[HCO_3^-]$) ≈ 24 mM]. In the M-Alk group medium pH was increased to ≈7.50 by increasing the $[HCO_3^-]$ to ≈31 mM at a constant P_{CO_2} of 40 mmHg. In the S-Alk group medium pH was increased to ≈7.60 by increasing the $[HCO_3^-]$ to ≈39 mM at a constant P_{CO_2} of 40 mmHg. β-Glucuronidase activity of culture medium collected in the second 24 h of the 48-h incubation was determined colorimetrically using phenolphthalein glucuronidate (Sigma Chemical, St. Louis, MO) as a substrate and incubating in 75 mM phosphate buffer at 37°C for 72 h (7, 13, 24).

Osteoblastic collagen synthesis. To measure collagen synthesis, calvariae were again studied in the same three groups (i.e., Ctl, M-Alk, and S-Alk). The culture medium of all three groups was modified to include 100 μg/ml ascorbic acid and an additional 0.6 mM phosphate. Ascorbic acid and additional phosphate are necessary for optimal collagen synthesis (7, 23, 24). In previous studies, we have added an additional 1 mM phosphate; however, alkalosis decreases the solubility of calcium phosphate complexes (11), and we were concerned that the alkalosis would not allow an additional 1 mM phosphate to remain in solution. In preliminary studies we found that, after adding an additional 1 mM phosphate to the S-Alk medium (without calvariae), there was a fall in medium calcium at 48 h indicating precipitation (data not shown). There was no fall with an additional 0.8 or 0.6 mM phosphate, and we chose the latter to ensure that there would be no precipitation. The medium becomes more acidic with time in culture, as the calvariae generate metabolic acids, so any calcium phosphate complexes would become more soluble over the duration of the experiment (7).

Calvariae were incubated with [^{3}H]proline (5 μCi/dish) (52 Ci/mmol, Dupont-NEN, Boston, MA) during the last 3 h of the second 24-h incubation. At the end of the 3-h incubation, bones were removed from culture, rinsed three times in ice-cold 5% trichloroacetic acid (TCA), once in cold acetone, and once in cold ether. The calvariae were dried thoroughly (60°C overnight) and then processed for determination of [^{3}H]proline incorporation into collagenase-digestible protein using purified bacterial collagenase (Worthington Biochemical) (23). The dried calvariae were homogenized in 0.5 M acetic acid, incubated for 24 h at 4°C, and then neutralized. An aliquot of the homogenate was combined with an equal volume of 10% TCA-0.5% tannic acid to precipitate protein and determine the amount of unincorporated (free) [^{3}H]proline in the supernatant. Duplicate aliquots of homogenate were then incubated with 0.2 mg/ml purified collagenase in 240 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid buffer, pH 7.2, containing 1 mM $CaCl_2$ and 10 nM tosyl-L-lysyl chloromethyl ketone for 60 min at 37°C. Samples were then placed on ice, and protein was precipitated with 10% TCA-0.5% tannic acid. [^{3}H]proline in the supernatant was designated as collagenase-digestible protein, and the remaining label in the precipitate was designated noncollagen protein. Results obtained after liquid scintillation counting were corrected for the relative abundance of proline in collagen versus noncollagen protein and were initially expressed as disintegrations per minute (dpm) per microgram dry weight of tissue (7, 13, 23, 24). Final results are presented as percent incorporation in collagen relative to total [^{3}H]proline incorporation.

Measurements of pH, P_{CO_2}, and calcium. pH and P_{CO_2} were measured with a blood-gas analyzer (model ABL 30; Radiometer, Copenhagen, Denmark). Medium $[HCO_3^-]$ was calculated from pH and P_{CO_2} using the Henderson-Hasselbalch equation as previously described (9). Total calcium concentration was measured by automatic fluorometric titration (Calcette; Precision Systems, Sudbury, MA), which we have shown to give results similar to those obtained by atomic absorption spectroscopy (8).

Net calcium flux (J_{Ca}) was calculated as $V_m([Ca]_f - [Ca]_i)$, where V_m is medium volume (1.8 ml) and $[Ca]_f$ and $[Ca]_i$ are the final and initial medium calcium concentrations (9), respectively. A positive flux value (J^+_{Ca}) indicates movement of calcium from the bone into the medium, and a negative value (J^-_{Ca}) indicates movement from the medium into the bone. Fluxes are expressed as nanomoles per bone per unit time.

Statistical analysis. Tests of significance, calculated by analysis of variance, and linear regression analysis were performed using conventional computer programs (BMDP; UCLA, Los Angeles, CA) on a digital computer. All values are presented as means ± SE.

RESULTS

Net calcium flux and osteoclastic β-glucuronidase activity. To determine whether metabolic alkalosis has an effect on net calcium flux (J_{Ca}) and, if so, does it also alter osteoclastic function, we utilized a system in which we could simultaneously measure J_{Ca} and osteoclastic β-glucuronidase activity (7, 13, 24).

Compared with Ctl, in both 0- to 24- and 24- to 48-h incubations pH_i was increased with M-Alk and increased further with S-Alk because of a progressive increase in initial medium bicarbonate concentration ($[HCO_3^-]_i$) (Table 1). The initial medium partial pressure of carbon dioxide ($P_{CO_{2i}}$) was not different in any group.

Over the first 24 h, there was a decrease in net calcium efflux (J^+_{Ca}) with M-Alk and with S-Alk compared with Ctl; however, there was no difference between M-Alk and S-Alk (Table 1). Over the second 24 h, there was a decrease in J^+_{Ca} with M-Alk ($P < 0.001$ vs. Ctl) and a further decrease with S-Alk ($P < 0.001$ vs. Ctl and $P < 0.05$ vs. M-Alk) (Fig. 1*A*). Over this time period there was a significant inverse correlation between J^+_{Ca} and pH_i. When J^+_{Ca} from the two time periods were added, there was a decrease in cumulative J^+_{Ca} with M-Alk and a further decrease with S-Alk compared with Ctl (Table 1).

Compared with Ctl, β-glucuronidase release into the medium did not change with M-Alk but was decreased with S-Alk ($P < 0.005$), and β-glucuronidase release

Table 1. *Medium pH, Pco_2, $[HCO_3^-]$, and Ca values for β-glucuronidase experiments*

Group	pH_i	Pco_{2i}, mmHg	$[HCO_3^-]_i$, meq/l	Ca_i, mg/dl	pH_f	Pco_{2f}, mmHg	$[HCO_3^-]_f$, meq/l	J_{Ca}, nmol·bone^{-1} ·24 h^{-1}	Cumulative J_{Ca}, nmol·bone^{-1} ·48 h^{-1}
					0–24 h				
Ctl	7.393 ± 0.004	39.8 ± 0.4	24.0 ± 0.3	7.43 ± 0.03	7.298 ± 0.008	41.1 ± 0.4	19.7 ± 0.4	302 ± 35	
M-Alk	7.507 ± 0.004*	39.4 ± 0.3	31.2 ± 0.5*	7.47 ± 0.03	7.390 ± 0.008*	42.5 ± 0.5	25.4 ± 0.4*	138 ± 50*	
S-Alk	7.600 ± 0.006*†	39.2 ± 0.2	38.9 ± 0.6*†	7.43 ± 0.04	7.490 ± 0.007*†	41.6 ± 0.6	31.7 ± 0.5*†	52 ± 37*	
					24–48 h				
Ctl	7.403 ± 0.006	39.9 ± 0.6	24.6 ± 0.2	7.43 ± 0.01	7.239 ± 0.015	41.9 ± 0.4	17.4 ± 0.6		723 + 47
M-Alk	7.507 ± 0.003*	40.4 ± 0.4	32.0 ± 0.3*	7.39 ± 0.02	7.340 ± 0.013*	42.3 ± 0.5	22.5 ± 0.8*		350 ± 59*
S-Alk	7.592 ± 0.003*†	41.2 ± 0.4	40.1 ± 0.3*†	7.42 ± 0.01	7.446 ± 0.009*†	42.0 ± 0.4	28.7 ± 0.6*†		150 ± 64*†

Values are means ± SE. pH, medium pH; i, initial; f, final; Pco_2, partial pressure of carbon dioxide; $[HCO_3^-]$, medium bicarbonate concentration; Ca, medium calcium concentration; J_{Ca}, net calcium flux; Ctl, calvariae cultured in basal medium; M-Alk, calvariae cultured in medium in which the pH was increased to ≈7.5 by the addition of $NaHCO_3$; S-Alk, calvariae cultured in medium in which the pH was increased to ≈7.6 by the addition of $NaHCO_3$. *$P < 0.05$, different from Ctl same time period. †$P < 0.05$, different from M-Alk same time period.

into the medium was correlated inversely with pH_i (Fig. 1*B*). There was a significant direct correlation between osteoclastic β-glucuronidase release and J_{Ca}^+ (Fig. 2). Calvariae cultured in Ctl demonstrated substantial β-glucuronidase release and substantial J_{Ca}^+. There was less β-glucuronidase release and less J_{Ca}^+ from calvariae incubated in M-Alk and even less release of β-glucuronidase and less J_{Ca}^+ from calvariae incubated in S-Alk.

Net calcium flux and osteoblastic collagen synthesis. To determine whether metabolic alkalosis affects osteoblastic activity, we utilized a system in which we could measure osteoblastic collagen synthesis and J_{Ca} simultaneously (7, 13, 23, 24).

Compared with Ctl, in both 0- to 24- and 24- to 48-h incubations, pH_i was increased with M-Alk and increased further with S-Alk because of a progressive increase in $[HCO_3^-]_i$ (Table 2). The Pco_{2i} was not different in any group.

Over the first 24 h, there was a decrease in J_{Ca}^+ with M-Alk and a further decrease with S-Alk compared with Ctl (Table 2). Over the second 24 h, there was a decrease in J_{Ca}^+ with M-Alk ($P < 0.001$ vs. Ctl) and a further decrease with S-Alk ($P < 0.001$ vs. Ctl and $P < 0.005$ vs. M-Alk) (Fig. 3*A*). Over this time period, there was a significant inverse correlation between J_{Ca}^+ and pH_i. When J_{Ca}^+ values from the two time periods were added, there was a decrease in cumulative J_{Ca}^+ with M-Alk and a further decrease with S-Alk compared with Ctl (Table 2).

Compared with Ctl, percent collagen synthesis increased with M-Alk and with S-Alk (each $P < 0.05$ vs. Ctl), and there was a significant direct correlation between percent collagen synthesis and pH_i (Fig. 3*B*). There was a significant inverse correlation between percent collagen synthesis and J_{Ca}^+ (Fig. 4). Calvariae

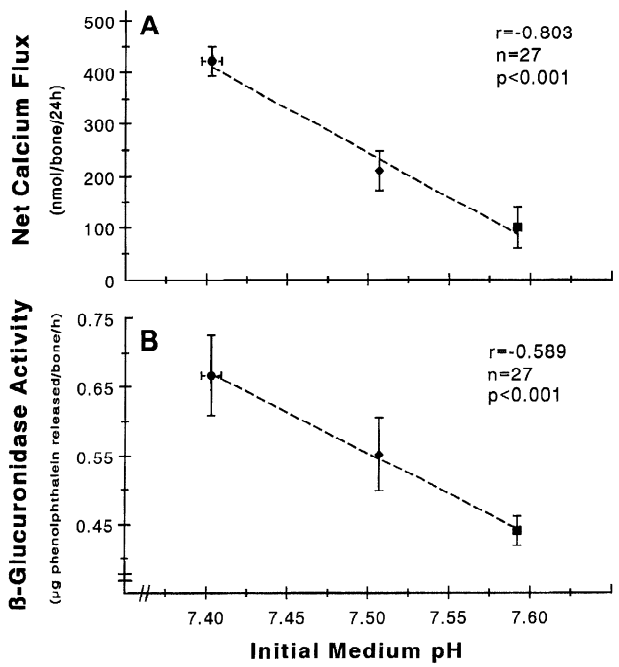

Fig. 1. Effect of metabolic alkalosis on net calcium flux (*A*) and β-glucuronidase activity (*B*). Calvariae were cultured in control medium (Ctl, ●) or in medium in which the pH was increased to ≈7.5 (M-Alk, ◆) or to ≈7.6 (S-Alk, ■). Calvariae were cultured for 48 h; after the first 24 h, calvariae were transferred to similar preincubated fresh medium. Here and in Figs. 3 and 5, values are means ± SE; absence of error bars indicates that the SE was less than the width of the symbol. Initial medium pH was measured at the start of the 2nd incubation; net calcium flux was measured over the 2nd incubation, and β-glucuronidase activity was measured at the end of the 2nd incubation.

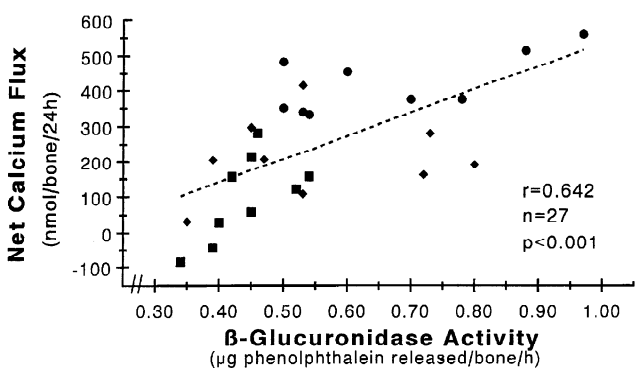

Fig. 2. Correlation between net calcium flux and β-glucuronidase activity. Calvariae were cultured in control medium (Ctl, ●) or in medium in which the pH was increased to ≈7.5 (M-Alk, ◆) or to ≈7.6 (S-Alk, ■). Calvariae were cultured for 48 h; after the first 24 h, calvariae were transferred to similar preincubated fresh medium. Net calcium flux was measured over the 2nd incubation, and β-glucuronidase activity was measured at the end of the 2nd incubation.

Table 2. *Medium pH, P_{CO_2}, $[HCO_3^-]$, and Ca values for collagen synthesis experiments*

Group	pH_i	$P_{CO_{2i}}$, mmHg	$[HCO_3^-]_i$, meq/l	Ca_i, mg/dl	pH_f	$P_{CO_{2f}}$, mmHg	$[HCO_3^-]_f$, meq/l	J_{Ca}, nmol·bone^{-1}·24 h^{-1}	Cumulative J_{Ca}, nmol·bone^{-1}·48 h^{-1}
					0–24 h				
Ctl	7.386 ± 0.002	39.7 ± 0.2	23.5 ± 0.1	7.50 ± 0.05	7.244 ± 0.008	40.3 ± 0.4	16.9 ± 0.2	336 ± 22	
M-Alk	7.498 ± 0.004*	39.5 ± 0.3	30.6 ± 0.4*	7.46 ± 0.04	7.372 ± 0.015*	39.7 ± 0.6	22.7 ± 0.6*	177 ± 27*	
S-Alk	7.598 ± 0.006*†	39.7 ± 0.6	39.2 ± 0.2*†	7.42 ± 0.05	7.466 ± 0.010*†	40.5 ± 0.8	29.0 ± 0.4*†	87 ± 32*†	
					24–48 h				
Ctl	7.384 ± 0.002	40.8 ± 0.4	24.0 ± 0.2	7.59 ± 0.02	7.204 ± 0.012	39.1 ± 0.3	14.9 ± 0.5		819 ± 30
M-Alk	7.496 ± 0.005*	40.1 ± 0.2	31.0 ± 0.4*	7.60 ± 0.02	7.290 ± 0.018*	40.1 ± 0.6	18.9 ± 0.8*		404 ± 79*
S-Alk	7.595 ± 0.002*†	40.1 ± 0.4	39.4 ± 0.3*†	7.53 ± 0.02	7.416 ± 0.031*†	39.6 ± 0.5	25.4 ± 0.2*†		203 ± 73*†

Values are means ± SE. See legend to Table 1 for descriptions of abbreviations and *P* values.

incubated in Ctl exhibited little collagen synthesis and substantial J_{Ca}^{+}. There was increased collagen synthesis and less J_{Ca}^{+} in calvariae incubated in M-Alk, whereas calvariae incubated in S-Alk had the highest level of collagen synthesis and the least J_{Ca}^{+}.

Correlation between β-glucuronidase activity and collagen synthesis. When mean data from the separate experiments were combined, there was a strong inverse correlation between the effects of M-Alk and S-Alk on osteoclastic β-glucuronidase release and osteoblastic collagen synthesis (Fig. 5). The greatest osteoclastic β-glucuronidase release into the medium and the lowest collagen synthesis was observed in calvariae incubated in Ctl. There was less release of osteoclastic β-glucuronidase and more osteoblastic collagen synthesis in calvariae incubated in M-Alk. Calvariae incubated in S-Alk had the least release of osteoclastic β-glucuronidase into the medium and the most collagen synthesis.

DISCUSSION

In this study, a model of metabolic alkalosis decreased net calcium efflux from bone, inhibited osteoclastic β-glucuronidase release, and stimulated osteoblastic collagen synthesis. These findings indicate that alkalosis can inhibit bone resorption and stimulate bone formation through alterations in bone cell function.

The alkalosis-induced decrease in calcium efflux from bone could be due to a decrease in osteoclastic function and/or an increase in osteoblastic bone formation. To determine whether alkalosis affects osteoclastic function, we utilized a culture system in which we could measure release of osteoclastic β-glucuronidase into the culture medium in conjunction with measurements of calcium flux (7, 13, 24). Although β-glucuronidase is found in lysosomes of many cell types, its release from bone into the medium is well correlated with osteoclast-mediated bone resorption (19, 20). The fall in medium

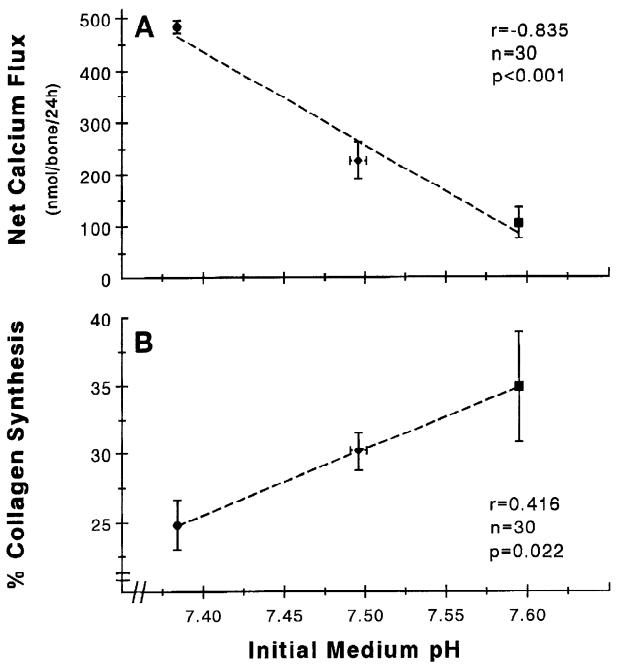

Fig. 3. Effect of metabolic alkalosis on net calcium flux (*A*) and percent collagen synthesis (*B*). Calvariae were cultured in control medium (Ctl, ●) or in medium in which the pH was increased to ≈7.5 (M-Alk, ◆) or to ≈7.6 (S-Alk, ■). Calvariae were cultured for 48 h; after the first 24 h, calvariae were transferred to similar preincubated fresh medium. Initial medium pH is measured at the start of the 2nd incubation, net calcium flux was measured over the 2nd 24-h incubation, and incorporation of [^{3}H]proline into collagenase-digestible protein in calvariae was measured during the last 3 h of the 2nd incubation.

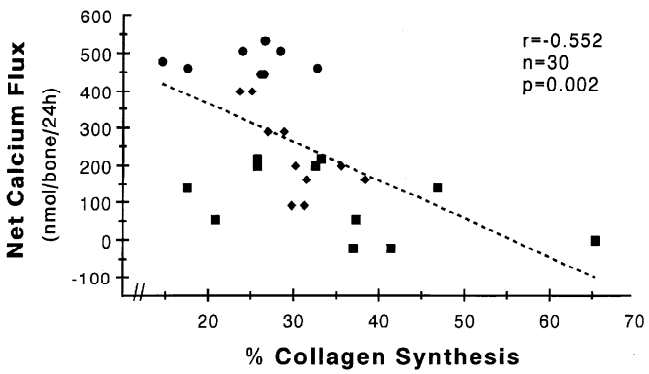

Fig. 4. Correlation between net calcium flux and percent collagen synthesis. Calvariae were cultured in control medium (Ctl, ●) or in medium in which the pH was increased to ≈7.5 (M-Alk, ◆) or to ≈7.6 (S-Alk, ■). Calvariae were cultured for 48 h; after the first 24 h, calvariae were transferred to similar preincubated fresh medium. Net calcium flux was measured over the 2nd 24-h incubation, and incorporation of [^{3}H]proline into collagenase-digestible protein in calvariae was measured during the last 3 h of the incubation.

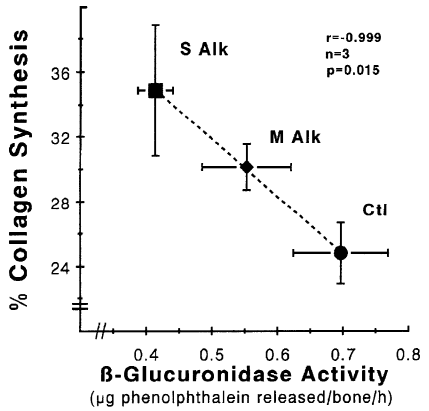

Fig. 5. Correlation between percent collagen synthesis and β-glucuronidase activity. Calvariae were cultured in control medium (Ctl, ●) or in medium in which the pH was increased to ≈7.5 (M-Alk, ◆) or to ≈7.6 (S-Alk, ■). Calvariae were cultured for 48 h; after the first 24 h, calvariae were transferred to similar preincubated fresh medium. Percent collagen synthesis and medium β-glucuronidase were measured in separate experiments, and the resultant means of each similar group are compared here. Incorporation of [^{3}H]proline into collagenase-digestible protein in calvariae was measured during the last 3 h of the 2nd 24-h incubation. At the end of the 2nd 24-h incubation, aliquots of medium were removed for assay of β-glucuronidase activity.

β-glucuronidase activity with alkalosis and the direct correlation of net calcium flux and medium β-glucuronidase activity support the role of the osteoclast in mediating calcium release from bone during metabolic alkalosis.

To determine whether alkalosis affects osteoblastic function, we utilized a modified culture system in which the effects of alkalosis on osteoblastic collagen synthesis could be determined simultaneously with measurements of calcium flux (7, 13, 23, 24). We found that metabolic alkalosis caused an increase in calvarial collagen synthesis in parallel with the decrease in net calcium flux from bone. Although fibroblast-like cells can produce very limited amounts of collagen (29), osteoblasts are the principal source of bone collagen in this system (31). The increase in calvarial collagen synthesis with metabolic alkalosis and the inverse correlation between net calcium flux and collagen synthesis support the role of the osteoblast in mediating calcium flux during metabolic alkalosis. An increase in collagen available for mineralization can only serve to decrease calcium efflux from bone.

Alkalosis appears to stimulate osteoblastic function in addition to its effect on osteoclastic activity. The inverse correlation between the effects of alkalosis on osteoclastic β-glucuronidase release and osteoblastic collagen synthesis suggest that alkalosis may alter the function of both cell types to a similar degree or that it modifies the function of one cell type which then alters the function of the other. The mechanism of the alteration of bone cell function is not clear from this study. Although acidosis has been shown to directly increase osteoclastic podosome formation (36), the independent effects of alkalosis on each cell type have never been studied. However, the increased concentration of medium bicarbonate in metabolic alkalosis may directly affect osteoclastic function. Osteoclasts secrete protons into the microenvironment between this resorbing cell and the bone mineral. For every hydrogen ion secreted, a bicarbonate is generated which must exit the osteoclasts via exchange with extracellular chloride to prevent intracellular alkalinity (22, 37). During metabolic alkalosis, the increased extracellular bicarbonate might suppress osteoclastic hydrogen ion secretion by inhibiting osteoclastic chloride for bicarbonate exchange, thereby inhibiting bone resorption.

In both the β-glucuronidase and collagen synthesis experiments there was a fall in medium pH during each 24-h incubation due to endogenous acid production by the bone cells. However, in all cases the most alkalotic groups (S-Alk) remained above the physiologically neutral pH of 7.40 throughout the entire experiment. To change the medium pH more frequently than every 24 h, in an effort to maintain a more consistent elevated pH, would lead to unacceptable fluctuations in medium P_{CO_2}. Further studies, perhaps utilizing a pH stat to maintain a constant medium pH, will be necessary to determine whether more consistent alkalosis will further influence bone cell function.

It is not entirely surprising that metabolic alkalosis (an elevated pH caused by an increased bicarbonate concentration) decreases net calcium efflux from bone, since the opposite condition, metabolic acidosis (a decreased pH caused by a reduced bicarbonate concentration), increases net calcium efflux from bone. At a constant pH, the concentration of bicarbonate appears to directly influence bone calcium stores; increasing medium bicarbonate decreased calcium efflux from cultured bone (14). At a constant neutral pH of ≈7.4, calcium efflux from bone is inversely correlated with $[HCO_3^-]$: the higher the medium bicarbonate, the less calcium efflux from bone. When osteoclastic activity is inhibited with calcitonin, the remaining calcium efflux remains inversely correlated with $[HCO_3^-]$, indicating that alterations in bicarbonate appear to have a non-osteoclast-mediated effect on the bone mineral. Whether, in addition to its effect on cell-mediated net calcium flux, metabolic alkalosis also affects physicochemical mineral dissolution and accretion remains to be determined.

That metabolic alkalosis can decrease bone resorption and perhaps even increase bone formation has been suggested by several clinical studies (4, 27, 32). Increasing dietary protein not only increases net acid excretion but results in increased urinary calcium excretion without a change in intestinal calcium absorption, indicating decreased net calcium retention. Since bone is the repository of over 99% of body calcium, decreased net calcium retention almost certainly implies loss of bone mineral. However, provision of oral alkali can reverse the excess calcium excretion (28, 33). When Lutz (28) fed subjects a high-protein diet, urine calcium excretion increased and the patients developed negative calcium balance. Provision of sodium bicarbonate decreased urinary calcium excretion and significantly improved calcium retention. In a more recent, larger study, Sebastian and co-workers (33) fed postmenopausal women potassium bicarbonate to neutralize endogenous acid production. Serum bicarbonate

significantly increased from 23.7 ± 1.3 to 25.6 ± 1.3 meq/l and net acid excretion fell, resulting in a decrease in urine calcium excretion from 238 ± 86 to 172 ± 81 mg/day and an increase in calcium retention. The improvement in calcium retention almost certainly indicates an increase in bone mineral stores.

Americans typically eat a diet that contains ample acid precursors, a so-called "acid-ash" diet, which, after metabolism, produces ~100 meq of acid each day (3). The initial response to this acid load is to buffer the additional hydrogen ions via the bicarbonate/carbonic acid buffer system. In this system protons combine with bicarbonate to form carbonic acid, which then dissociates into carbon dioxide and water (6). Increased minute ventilation restores the partial pressure of carbon dioxide toward the normal range. While some of this bicarbonate is in the systemic circulation, much of the bicarbonate and carbonate resides within bone and is readily accessible to the circulation (10, 11). Indeed, cultured calvariae are in equilibrium with the calcium and carbonate within the mineral (11), and in acidic medium bone carbonate becomes progressively depleted (10). With time, renal acid excretion increases and the additional acid is excreted through augmented ammonia production and ammonium excretion (6). However, with aging, the ability of the kidney to excrete an acid load diminishes (1). Acid ingestion has long been known to increase urine calcium excretion and has been implicated in the pathogenesis of osteoporosis (3, 25, 26). Neutralization of that acid with exogenous bicarbonate may serve to lessen physicochemical bone mineral dissolution and cell-mediated resorption, as suggested by the study of Sebastian and co-workers (33).

Thus, using cultured neonatal mouse calvariae, we have found that metabolic alkalosis decreases net calcium efflux from bone, decreases osteoclastic β-glucuronidase activity, and increases osteoblastic collagen synthesis. Neonatal mouse calvariae appear to be an excellent model of mammalian bone. They respond to calcium-regulating hormones, they have functioning osteoblasts and osteoclasts, and they synthesize DNA and protein; however, they are woven bone, compared with the mostly cortical bone in humans, and in culture they are not perfused by blood (35). In addition it is important to recognize that the initial increase in medium pH in the alkalotic groups was above that which could reasonably be expected to be achieved on a chronic basis in vivo. However, the continuum of acidosis promoting bone calcium dissolution (8, 11, 14, 15, 17) and resorption (7, 13, 24, 34) and the current findings that alkalosis decreases net calcium efflux from bone suggest that an increase in pH, produced by increasing the [HCO_3^-], will be advantageous in maintaining existing bone mineral. If the findings of calvariae in culture can be applied to human bone, then neutralization of an acidic environment may help to protect bone mineral stores.

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