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Decreased Bone Formation and Increased Mineral Dissolution During Acute Fasting in Young Women*

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ABSTRACT

Severe chronic undernutrition is associated with decreased bone turnover and significant bone loss. However, little is known about the short-term effects of nutritional deprivation on bone turnover. To investigate the effects of short-term fasting on bone metabolism and the contribution of acidosis to these changes, 14 healthy women ages 18–26 (mean, 21 ± 2 (SD years) were randomized to potassium bicarbonate ($KHCO_3$, 2 meq/kg/day in divided doses) to prevent acidosis or control (potassium chloride, 25 meq/day) during a complete 4-day fast. Bone turnover was assessed using specific markers of formation [osteocalcin (OC) and Type I procollagen carboxyl-terminal propeptide (PICP)] and resorption [pyridinoline (PYRX) and deoxypyridinoline (DPYRX)].

Serum bicarbonate levels fell significantly from 27.0 ± 3.2 to 17.3 ± 2.6 mmol/L ($P < 0.01$) in the control group and were decreased compared to patients receiving $KHCO_3$ [17.3 ± 2.6 *vs.* 23.4 ± 2.4 mmol/L, ($P < 0.001$)]. Serum total and ionized calcium increased significantly in the control group [9.1 ± 0.1 to 9.4 ± 0.2 mg/dL ($P < 0.01$) and 1.20 ± 0.03 to 1.23 ± 0.03 mmol/L ($P < 0.05$), respectively], but not in patients receiving $KHCO_3$. In addition, serum parathyroid hormone (PTH) levels decreased from 32 ± 17 to 16 ± 10 pg/mL ($P < 0.05$) and urinary calcium excretion increased [86 ± 51 to 182 ± 103 mg/day ($P = 0.01$)] in the control group, but not in patients receiving $KHCO_3$. Serum osteocalcin (OC) and procollagen carboxyl-terminal propeptide (PICP) levels decreased significantly after 4 days of fasting from 9.1 ± 3.4 to 5.5 ± 4.2 ng/mL ($P < 0.01$) and 121 ± 21 to 46 ± 13 ng/mL ($P = 0.0001$) respectively in the patients receiving bicarbonate, and from 10.1 ± 3.3 to 4.0 ± 2.9 ng/mL ($P < 0.01$) and from 133 ± 22 to 47 ± 19 ng/mL ($P < 0.001$) respectively in the control group. The decrease in osteocalcin and PICP during fasting was comparable in both treatment groups. By contrast, urinary excretion of PYRX and DPYRX did not change significantly in either group with 4 days of fasting.

These data are the first to demonstrate that markers of bone formation decline significantly with short-term fasting, independent of changes in acid-base status. By contrast, these data demonstrate a direct effect of acidosis in stimulating calcium release from bone during short-term fasting and suggest that acidosis may increase mineral dissolution independent of osteoclast activation and PTH in this experimental model of acute starvation. (*J Clin Endocrinol Metab* **80:** 3628–3633, 1995)

SEVERE CHRONIC UNDERNUTRITION is associated with decreased bone formation, decreased bone resorption, and osteoporosis (1–3). However, little is known of the effects of short-term starvation on bone turnover. Previous studies have demonstrated that metabolic acidosis and hypercalciuria occur simultaneously in short-term fasting and suggest that acidosis may have important effects on calcium metabolism in severe undernutrition (4). *In vitro* studies demonstrate that metabolic acidosis stimulates osteoclast activity and simultaneously inhibits osteoblast function, resulting in a net loss of calcium from bone (5). Acidosis also directly enhances mineral dissolution independent of osteoclast activation *in vitro* (5, 6). Taken together, these data suggest that metabolic acidosis may affect bone turnover *in vivo* and contribute to bone demineralization in severe undernutrition.

To determine the effects of short-term starvation on bone metabolism and the role of starvation-induced metabolic acidosis in mediating these effects, young healthy women were randomized to potassium bicarbonate in sufficient doses to neutralize metabolic acidosis or to control during a 4-day complete fast. Specific markers of bone formation, osteocalcin and procollagen carboxyl-terminal propeptide (PICP) and resorption [pyridinoline (PYRX) and deoxypyridinoline (DPYRX)] were investigated. Serum and urine calcium and phosphorus levels as well as parathyroid hormone levels were assessed to determine calcium metabolism. In addition, levels of insulin-like growth factor-I (IGF-I), a nutritionally regulated hormone with potent effects on bone formation, were measured in response to fasting and acid-base manipulation (7–12). Our data demonstrate a profound decline in markers of bone formation and a simultaneous increase in calcium release from bone independent of PTH and osteoclast activation in an experimental model of short-term starvation.

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Subjects and Methods

Experimental subjects

We studied 14 women with a mean age of 21 ± 2 y, range 18–26 y. All patients gave written consent as approved by the Subcommittee on Human Studies of the Massachusetts General Hospital. Subjects weighed between 91–119% of ideal body weight (mean 105, ± 8%), as defined by the Metropolitan Life Insurance Tables of Height and Weight. Each participant was in good health without any acute or chronic med-

ical conditions, was on no medication at the time of the study, and had not received glucocorticoids or any other medication known to affect bone metabolism. All subjects had normal menstrual function assessed by clinical history and normal thyroid function determined by clinical exam and a thyroid stimulating hormone level. Patients with a history of oral contraceptive use within 3 months of the study or history of recent trauma or bone fracture were excluded.

Experimental design and endpoints

Subjects were admitted to the General Clinical Research Center of the Massachusetts General Hospital. They underwent a total fast for 4 days except for water *ad libidum*. Allopurinol 200 mg/day was administered daily to prevent hyperuricemia. In addition, a standard multivitamin containing 400 USP units of ergocalciferol but no calcium was administered daily throughout the protocol to provide essential vitamins and minerals. Patients were encouraged to ambulate throughout the protocol. Subjects were randomized to receive either oral potassium chloride (25 meq daily) or potassium bicarbonate [($KHCO_3$), 2 meq/kg daily up to a maximum dose of 150 meq/day] in divided oral doses 4 times a day. Serum electrolytes, including potassium and bicarbonate levels were monitored daily. Serum calcium, ionized calcium, venous pH, phosphorus, ketones, IGF-I and insulin-like growth factor binding protein-3 (IGFBP-3), osteocalcin, PICP, and PTH levels were measured at baseline and at the termination of the study after 4 days of fasting. Twenty-four h urine measurements for calcium, phosphorus, PYRX, and DPYRX were made from 0800 h to 0800 h on Days 1–2 and 4–5. Urine creatinine excretion was measured to confirm that 24-h collections were complete. Weight was measured daily and percent ideal body weight (%IBW) was calculated before and after fasting. Potassium bicarbonate was chosen over sodium bicarbonate to avoid an effect of sodium on calciuresis, volume status, and GFR.

Laboratory methods

Serum total and ionized calcium, phosphorus, electrolytes, and PTH, as well as urine calcium, phosphorus, and creatinine were measured using published methods (13). Serum IGF-I was measured after an acid-alcohol extraction using an RIA kit with an intraassay coefficient of variation 2.4–3.0% (Corning Nichols Institute, San Juan Capistrano, CA). PICP was measured using a RIA kit with an intraassay coefficient of variation of 2.1–3.2% (Incstar, Stillwater, MN). Osteocalcin was measured using an RIA kit with an intraassay coefficient of variation of 5.7–8.1% (Corning Nichols Institute). Urinary excretion of PYRX and DPYRX were measured according to the methods of Uebelhart *et al.* and modified by Corning Nichols Institute using high pressure liquid chromatography with an intraassay coefficient of variation of 9.8–10.7% for PYRX and 11.6–13.3% for DPYRX (14). Insulin-like growth factor binding protein-3 (IGFBP-3) was measured using an RIA kit with an intraassay coefficient of variation of 5.3–6.7% (Diagnostic Systems Laboratories, Webster, Texas). All samples for hormone determinations from a single individual were measured in duplicate and run in the same assay. Normal values for each assay are shown in Table I.

Statistical methods

The effects of potassium bicarbonate administration on experimental endpoints were determined using the Student *t*-test. Clinical endpoints were compared between treatment groups using an unpaired two-tail

TABLE 1. Clinical endpoints at baseline and after a 4-day complete fast

Variable	Baseline	Day 5	*P vs.* Baseline [a]	*P vs.* Control [b] (Day 5)	Normal Range [c]
Weight (kg)					
Bicarbonate	57.6 ± 4.3	54.5 ± 4.3	0.0001	0.166	
Control	62.6 ± 7.6	59.3 ± 7.6	0.0002		
% Ideal body weight					
Bicarbonate	106 ± 9	100 ± 10	0.0001	0.869	
Control	106 ± 8	100 ± 8	0.0001		
Venous pH (pH units)					N/A
Bicarbonate	7.36 ± 0.03	7.38 ± 0.01	0.329	0.001	
Control	7.37 ± 0.03	7.33 ± 0.03	0.033		
BUN (mg/dL)					8–25
Bicarbonate	13 ± 3	14 ± 3	0.435	0.682	
Control	12 ± 4	13 ± 4	0.462		
Creatinine (mg/dL)					0.6–1.5
Bicarbonate	0.8 ± 0.1	1.2 ± 0.2	0.0004	0.277	
Control	0.8 ± 0.0	1.1 ± 0.2	0.0046		
Ionized calcium (mmol/L)					1.14–1.30
Bicarbonate	1.21 ± 0.04	1.15 ± 0.05	0.010	0.003	
Control	1.20 ± 0.03	1.23 ± 0.03	0.020		
Serum phosphorus (mg/dL)					2.6–4.5
Bicarbonate	3.5 ± 0.3	3.5 ± 0.3	0.923	0.633	
Control	3.5 ± 0.3	3.6 ± 0.4	0.780		
Urinary phosphorus (mg/day)					N/A
Bicarbonate	416 ± 78	736 ± 256	0.013	0.389	
Control	558 ± 162	836 ± 148	0.006		
IGF-I (μg/L)					112–450
Bicarbonate	361 ± 95	200 ± 55	0.0001	0.347	
Control	325 ± 52	229 ± 58	0.0001		
IGFBP-3 (μg/L)					2000–4000
Bicarbonate	3580 ± 654	3398 ± 667	0.303	0.175	
Control	3990 ± 988	3991 ± 858	0.991		
PYRX (nmol/mmol Cr)					22–89
Bicarbonate	83 ± 74	77 ± 81	0.902	0.300	
Control	106 ± 89	43 ± 22	0.141		
DPYRX (nmol/mmol Cr)					4–21
Bicarbonate	21 ± 19	23 ± 30	0.892	0.406	
Control	29 ± 25	13 ± 8	0.188		

BUN, blood urea nitrogen; Cr, creatinine; PYRX, pyridinoline; DPYRX, deoxypyridinoline; IGFBP-3, insulin-like growth factor binding protein-3; N/A, normal range not applicable during fasting.

[a] *P vs.* baseline by paired *t*-test.

[b] *P vs.* control on Day 5 by unpaired *t*-test.

[c] Normal range is for adult females.

t-test. Change within each group was compared using a paired two-tail *t*-test. Unless noted otherwise, results are expressed as mean ± SD.

Results

Acid-base status and renal function

Mean serum bicarbonate levels decreased from 27.0 ± 3.2 to 17.3 ± 2.6 mmol/L in the control group [($P < 0.01$), normal range 22–26 mmol/L] and from 26.2 ± 2.3 to 23.4 ± 2.4 in the $KHCO_3$ group ($P < 0.05$), but differed significantly between treatment groups at the end of the study ($P < 0.001$, Fig. 1).

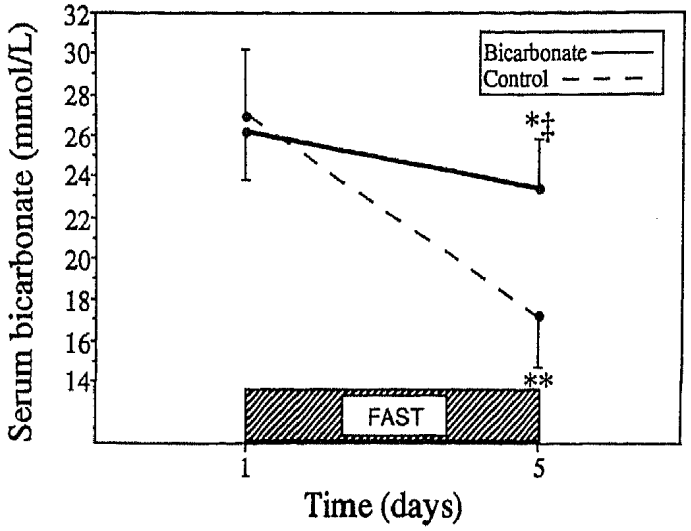

FIG. 1 Serum bicarbonate levels in response to a complete 4 day fast. Error bars represent mean ± 1 SD. *$P < 0.05$ *vs.* baseline by paired *t*-test. ** $P < 0.01$ *vs.* baseline by paired *t*-test. ‡ $P < 0.01$ *vs.* control by unpaired *t*-test. Normal range for serum bicarbonate is 22–26 mmol/L.

Venous pH levels decreased from 7.37 ± 0.03 to 7.33 ± 0.03 pH units ($P < 0.05$) in the control group, but did not change significantly in patients receiving $KHCO_3$, and again differed significantly between the groups at the end of the study [7.38 ± 0.01 *vs.* 7.33 ± 0.03 pH units, ($P < 0.01$, Table 1)]. Blood urea nitrogen levels did not change with fasting in either treatment group (Table 1). Serum creatinine levels increased in both groups, but remained well within the normal range (Table 1).

Fasting induced changes in IGF-I and IGFBP-3

Mean weight loss after 4 days of fasting was 3.3 ± 0.8 kg. Percent IBW decreased from 106 ± 9% to 100 ± 10% ($P = 0.0001$) in patients receiving bicarbonate and from 106 ± 8% to 100 ± 8% ($P = 0.0001$) in the control group (Table 1). Serum IGF-I levels decreased significantly in the control and bicarbonate treatment groups [325 ± 52 to 229 ± 58 μg/L ($P = 0.0001$) and 361 ± 95 to 200 ± 55 μg/L ($P = 0.0001$) respectively, Table 1]. Serum IGFBP-3 levels did not change in either treatment group (Table 1).

Calcium and phosphorus metabolism

Serum total and ionized calcium levels increased significantly in the control group but not in patients receiving $KHCO_3$ (Table 1, Fig. 2). Urinary calcium levels increased and PTH levels decreased in the control group, but did not change significantly in patients receiving bicarbonate. Serum phosphorus levels did not change with fasting in either treatment group, but urinary phosphorus excretion increased significantly in both groups (Table 1). Comparison of urinary calcium excretion indexed for creatinine confirmed a signif-

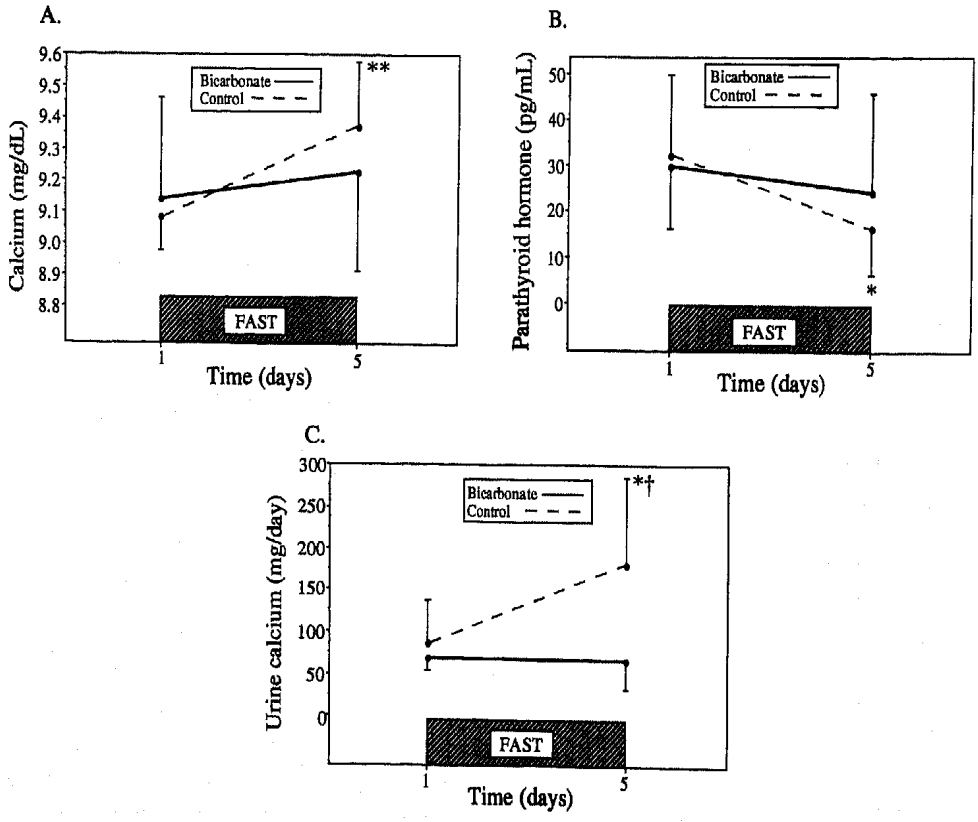

FIG. 2 Serum total calcium, urine calcium, and PTH in response to a complete 4-day fast. Error bars represent mean ± 1 SD. (A) calcium. (B) parathyroid hormone. (C) urine calcium. * $P < 0.05$ *vs.* baseline by paired *t*-test. ** $P < 0.01$ *vs.* baseline by paired *t*-test. † $P < 0.05$ *vs.* control by unpaired *t*-test. ‡ $P < 0.01$ *vs.* control by unpaired *t*-test. Normal range for serum calcium is 8.5–10.5 mg/dL. Normal range for parathyroid hormone is 10–60 pg/mL. Normal range for urinary calcium in fasting conditions is 5–40 mg/day.

icant increase in calcium excretion in the control group compared with patients receiving bicarbonate.

Bone turnover

Serum OC and PICP, markers of bone formation, decreased significantly after 4 days of fasting in both treatment groups and did not differ between treatment groups at the end of the study (Fig. 3). Urinary excretion of two markers of bone resorption, PYRX and DPYRX did not change significantly in either group but decreased to a greater extent in the control group (Table 1).

Discussion

We investigated the effects of short-term starvation and metabolic acidosis on bone turnover and calcium metabolism in young women. Our data demonstrate that markers of bone-formation decline significantly with short-term fasting, independent of changes in acid-base status. In addition, these data indicate that fasting-induced metabolic acidosis directly effects the removal of calcium from the bone independent of PTH and osteoclast activation. These data are the first to demonstrate the effects of severe acidosis on bone metabolism in short-term starvation and suggest a mechanism whereby repeated cycles of fasting might affect bone metabolism in chronic undernutrition.

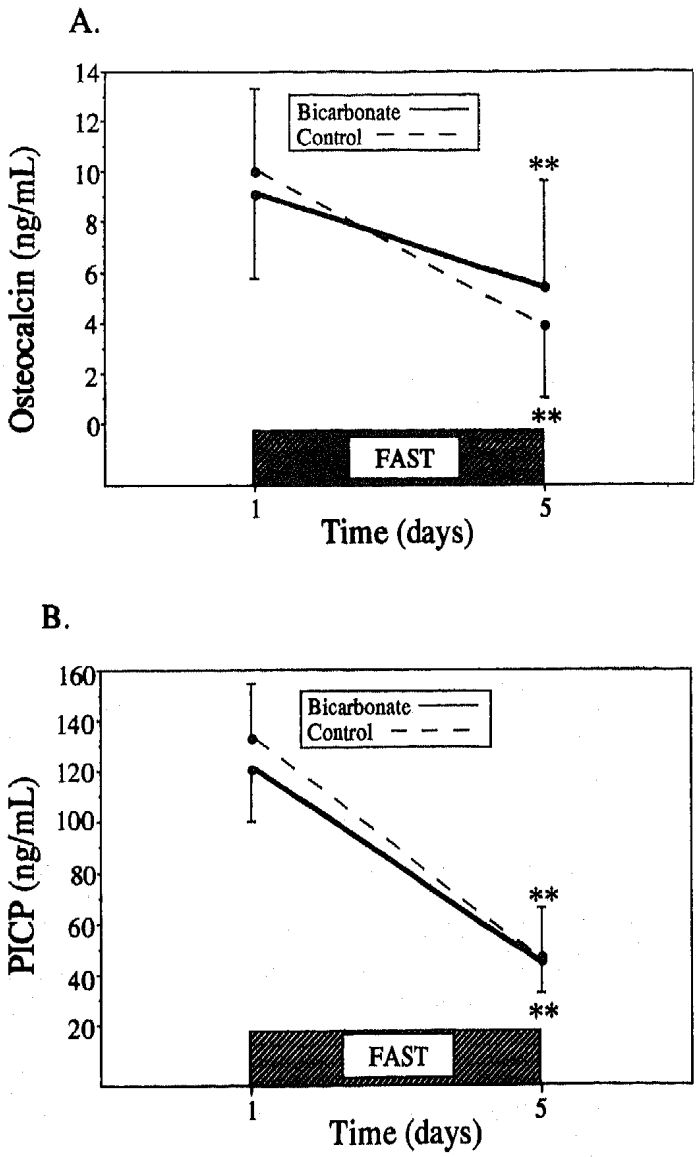

FIG. 3 Biochemical markers of bone formation in response to a complete 4-day fast. Error bars represent mean ± 1 SD. (A) osteocalcin. (B) PICP. ** $P < 0.01$ *vs.* baseline by paired *t*-test. Normal range for osteocalcin in adult females is 0.4–8.2 ng/mL. Normal range for PICP in adult females is 50–170 ng/mL.

A critical observation in this study is the significant fall in biochemical markers of bone formation, OC and PICP, with only 4 days of fasting. Although we have assessed bone formation indirectly during fasting with serum markers of osteoblast function and collagen formation, the significant and consistent decline in these markers suggests a potent effect of starvation to acutely decrease rates of bone formation. This novel finding may help explain the observation of low bone turnover osteoporosis associated with conditions of severe chronic undernutrition, such as anorexia nervosa. The fall in markers of bone formation was equivalent in both treatment groups, indicating that in this experimental model, factors other than metabolic acidosis are responsible for decreased bone formation. These observations are in agreement with recent animal data, in which bone collagen messenger RNA expression declined significantly after 4 days of fasting, and provide further evidence for the important effects of acute undernutrition on bone metabolism (15–17). One factor with potential importance in this regard is IGF-I, a nutritionally dependent bone trophic hormone with potent effects on osteoblast function and collagen synthesis *in vitro* and *in vivo* (7, 12, 18). Serum levels of IGF-I fell by 37% in both treatment groups with four days of fasting. The decline in serum levels of IGF-I is the result of an acquired resistance to the action of growth hormone on IGF-I production in the liver (8–10) and is likely to contribute to decreased osteoblast function in short-term fasting. Decreased osteoblast function could also be caused by a decline in the fraction of free IGF-I because of a change in the ratio of IGF-I to IGFBP-3 levels, whereby IGF-I levels fall and IGFBP-3 remains constant (19). Although the mechanism of decreased bone turnover in undernutrition remains unknown, declining IGF-I levels may contribute to this effect.

A second novel observation in this study is the potent effect of metabolic acidosis in releasing calcium from bone during short-term fasting, independent of PTH and osteoclast activation. Metabolic acidosis was severe in the control group, and serum bicarbonate and venous pH levels were significantly lower in this group compared with patients receiving bicarbonate (Fig. 1 and Table 1). Serum total and ionized calcium levels increased in association with metabolic acidosis in patients not receiving bicarbonate. Our data suggest that the increased serum calcium observed during acidosis was mobilized from bone, as oral calcium intake was eliminated and urinary calcium excretion was increased in this experimental model. Serum levels of PTH fell significantly in the control group in response to increased serum calcium, contributing to increased urinary calcium excretion in this group. In contrast, patients who received bicarbonate to maintain normal acid base status demonstrated no significant changes in serum calcium, PTH, or urine calcium during short-term fasting. Furthermore, urinary excretion of

PYRX and DPYRX, sensitive markers of bone resorption and collagen degradation, did not change significantly with acidosis. Taken together, these data suggest that acidosis increases calcium efflux from bone independent of PTH and osteoclast activation during fasting. Our study was not designed to assess directly the effects of acidosis on bone mineral matrix. However, the absence of any evidence for osteoclast activation strongly supports the hypothesis that acidosis results in increased mineral dissolution in acute starvation. These data are in accord with *in vitro* data demonstrating that metabolic acidosis increases mineral dissolution and alters the physiochemical properties of calcium at the bone-mineral surface (5, 6). An alternative mechanism to explain these findings may relate to the abrupt decline in bone formation and a relative shift of mobilizable calcium out of the bone.

Our data contrast with previous *in vitro* data, which demonstrate that metabolic acidosis increases osteoclast activation as determined by osteoclastic-B glucoronidase activity in calvarial cell culture (5). However, *in vitro* and *in vivo* models of acidosis differ significantly. For example, serum PTH and IGF-I levels declined significantly in the acidotic patients, potentially affecting osteoclast activity in this *in vivo* model. In addition, an effect of acidosis on osteoclast function may be delayed beyond the time frame of this study. However, PYRX and DPYRX do not increase but instead decrease with acidosis, arguing against a delayed effect of acidosis to increase collagen degradation. These data demonstrate that severe-fasting-induced metabolic acidosis results in increased serum and urine calcium levels independent of osteoclast activation and collagen resorption *in vivo*. Of note, parameters of bone formation and resorption were at the upper end of normal or slightly increased in our normal volunteers. Normal ranges for these markers are determined in women age 18 and above, but higher levels are noted during adolescence (12–17 yr). We postulate that the slightly elevated baseline levels were related to the relatively young age of our patients, 6 of whom were either 18 or 19.

To control for variability in urinary calcium excretion, urinary calcium was indexed for urine creatinine. A significant increase in urinary calcium excretion in the control group was confirmed by this method of analysis. Furthermore, the low levels of calcium that we observed are consistent with the expected range in fasting (20). Serum creatinine levels rose with fasting but remained within the normal range in all patients, and BUN was unchanged. The slight increase in serum creatinine likely reflects measurement of elevated serum ketone bodies in the creatinine assay. Serum acetone levels rose significantly with fasting in all patients and cause an overestimate of creatinine by 0.2–0.4 mg/dL in the standard creatinine assay (20).

One final and unexpected observation from this study was the significant increase in urinary phosphate excretion in both treatment groups with fasting (Table I). Data from hypoparathyroidectomized rats demonstrate an increase in renal phosphate excretion over three days of fasting which was prevented with adequate sodium bicarbonate to neutralize metabolic acidosis (21). In contrast, our data demonstrated no effect of potassium bicarbonate in preventing the significant increase in phosphate excretion observed with 4 days of starvation and suggest that other factors are responsible for the phosphaturia in this experimental model. Serum phosphorus levels did not change in either group, suggesting a primary renal mechanism as opposed to increased excretion caused by an increased filtered load. PTH levels decreased in the acid group and would have been expected to cause a decrease in urinary phosphate excretion. One hypothesis is that the significant decline in serum IGF-I levels is responsible for the increased urinary phosphate excretion. IGF-I is known to have a stimulatory effect on renal phosphate transport (22). In conditions of GH excess such as acromegaly, serum levels of IGF-I are increased and renal phosphate excretion is decreased. Importantly, renal phosphate excretion increases with decreased IGF-I levels after cure of acromegaly (23). Taken together, these data suggest, but do not prove, that decreased serum levels of IGF-I contribute to increased phosphate excretion in short-term fasting.

This protocol is the first to examine the effects of metabolic acidosis on bone turnover in short-term fasting and demonstrates the potent effects of caloric deprivation on markers of osteoblast function and collagen formation. Administration of neutralizing base does not prevent the acute decline in bone formation with fasting, suggesting that this effect is not dependent on acid-base disturbances and changes in the calcium-PTH axis but is a result of other factors. One such important factor may be the decline in circulating and/or local levels of IGF-I, which occurs with fasting. In addition, the data demonstrate a novel effect of acidosis in releasing calcium from the bone independent of PTH and markers of collagen degradation in short-term starvation. The data also suggest a direct effect of acidosis in enhancing mineral dissolution unrelated to osteoclast activation. These data suggest a potential mechanism whereby repetitive cycles of fasting may cause bone demineralization and decreased bone formation in chronic undernutrition.

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