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Research Article

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Chronic Metabolic Acidosis Increases the Serum Concentration of 1,25-Dihydroxyvitamin D in Humans by Stimulating Its Production Rate

Critical Role of Acidosis-induced Renal Hypophosphatemia

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Abstract

Chronic metabolic acidosis results in metabolic bone disease, calcium nephrolithiasis, and growth retardation. The pathogenesis of each of these sequelae is poorly understood in humans. We therefore investigated the effects of chronic extrarenal metabolic acidosis on the regulation of 1,25-(OH)₂D, parathyroid hormone, calcium, and phosphate metabolism in normal humans. Chronic extrarenal metabolic acidosis was induced by administering two different doses of NH₄Cl [2.1 (low dose) and 4.2 (high dose) mmol/kg body wt per d, respectively] to four male volunteers each during metabolic balance conditions. Plasma [HCO₃⁻] decreased by 4.5±0.4 mmol/liter in the low dose and by 9.1±0.3 mmol/liter (*P* < 0.001) in the high dose group. Metabolic acidosis induced renal hypophosphatemia, which strongly correlated with the severity of acidosis (Plasma [PO₄] on plasma [HCO₃⁻]; *r* = 0.721, *P* < 0.001). Both metabolic clearance and production rates of 1,25-(OH)₂D increased in both groups. In the high dose group, the percentage increase in production rate was much greater than the percentage increase in metabolic clearance rate, resulting in a significantly increased serum 1,25-(OH)₂D concentration. A strong inverse correlation was observed for serum 1,25-(OH)₂D concentration on both plasma [PO₄] (*r* = -0.711, *P* < 0.001) and plasma [HCO₃⁻] (*r* = -0.725, *P* < 0.001). Plasma ionized calcium concentration did not change in either group whereas intact serum parathyroid hormone concentration decreased significantly in the high dose group. In conclusion, metabolic acidosis results in graded increases in serum 1,25-(OH)₂D concentration by stimulating its production rate in humans. The increased production rate is explained by acidosis-induced hypophosphatemia/cellular phosphate depletion resulting at least in part from decreased renal tubular phosphate reabsorption. The decreased serum intact parathyroid hormone levels in more severe acidosis may be the consequence of hypophosphatemia and/or increased serum 1,25-(OH)₂D concentrations. (*J. Clin. Invest.* 1992. 90:2456-2463.) Key words: metabolic acidosis • 1,25-(OH)₂D • parathyroid hormone • metabolic clearance rate • production rate • hypophosphatemia • NH₄Cl feeding

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Introduction

Chronic metabolic acidosis occurs as the result of an increase in the systemic acid load (by either extrarenal loss of base or increase in acid production) or as the result of congenital or acquired defects in renal acid excretion (e.g., renal tubular or uremic acidosis). Metabolic acidosis profoundly affects calcium and phosphate metabolism resulting in calcium losses from bone (1) in association with hypercalciuria. Important clinical sequelae are a metabolic bone disease resembling osteomalacia (2-8) and calcium nephrolithiasis (9).

Reduced serum concentrations of the active metabolite of vitamin D, 1,25-(OH)₂D, have been implicated in mediating the acidosis-induced metabolic bone disease (8, 10, 11). The low 1,25-(OH)₂D serum concentrations have been pathogenetically attributed to diminished renal 1-alpha-hydroxylase activity (12-14), although not all studies have been consistent with an impaired conversion of 25-(OH)D to 1,25-(OH)₂D (15).

However, the results of measurements of serum 1,25-(OH)₂D concentration during experimentally induced metabolic acidosis have not been consistent: reported values have ranged from increased to unchanged to decreased mean concentrations (15-19). These inconsistencies may have been due in part to the experimental difficulties in controlling acidosis-induced alterations in the plasma concentrations of known modulators of 1,25-(OH)₂D production: phosphate (20-22), calcium (23), and parathyroid hormone (23, 24). Moreover, small doses of NH₄Cl were generally administered and therefore relatively mild degrees of acidemia and only minor reductions in plasma bicarbonate concentration were induced. An additional difficulty in demonstrating consistent and significant changes in serum 1,25-(OH)₂D concentration may be attributed to the reliance on unpaired observations rather than repeated measurements in the same subjects.

Recent preliminary data from our group (25) indicated that NH₄Cl-induced metabolic acidosis of moderate severity (plasma bicarbonate concentration ~ 12 mmol/liter) increased steady state 1,25-(OH)₂D serum concentration. We therefore have investigated the chronic regulation of 1,25-(OH)₂D metabolism in chronic extrarenal metabolic acidosis and administered NH₄Cl in two widely different doses for 8 d each in two groups of normal human subjects. The results indicate that serum 1,25-(OH)₂D concentration is increased greatly by moderate to severe metabolic acidosis. The increment in serum concentration is due to an increase in production rate (PR),¹ which at moderate to severe degrees of acidosis becomes of sufficient magnitude to override an increase in the

1. Abbreviations used in this paper: FE, fractional excretion; MCR, metabolic clearance rate; PR, production rate.

metabolic clearance rate (MCR). Hypophosphatemia, induced by renal phosphate losses, was found to be the stimulus for augmented 1,25-(OH)₂D production rate in human metabolic acidosis.

Methods

The protocol was designed to measure the effects of chronic NH₄Cl-induced metabolic acidosis on calcium, phosphate, and 1,25-(OH)₂D metabolism as well as serum parathyroid hormone levels in normal humans. These parameters were determined in eight normal male subjects during metabolic balance studies. None were smokers or were taking any drugs before and during the study. They ate a constant metabolic diet for ≥ 5 d before the study (prefeeding phase) and during the two study periods (control and acidosis). The diet provided the following for every kilogram of body weight per day: 1.91 mmol sodium, 1.45 mmol potassium, 0.395 mmol (15.8 mg) of calcium, 0.51 mmol (15.8 mg) phosphorus, 15.7 mmol (0.22 g) nitrogen, 35 kcal, and 43.1 ml of water.

Fasting arterialized venous blood samples (26) were obtained in a heparin-coated syringe from a heated hand or forearm vein at 7 a.m. Blood samples were accepted for acid–base analysis only if the partial pressure of oxygen was > 70 mmHg (9.3 kPa). Blood samples for 1,25-(OH)₂D and parathyroid hormone were obtained in nonheparinized syringes. Weight and oral temperatures were determined at the time of blood sampling; 24-h urine samples were collected in plastic bottles containing mineral oil and thymol-chloroform preservative. Exercise was limited to minimal ambulation throughout the study. During both study periods a subject was considered to be in a steady state when plasma values obtained on three consecutive days varied by no more than 1.5 mmol/liter for bicarbonate and by < 3 mmHg (0.4 kPa) for PaCO₂. To analyze the effect of metabolic acidosis on diurnal variation of parathyroid hormone, 1,25-(OH)₂D, calcium, and phosphate values, additional blood samples were drawn under nonfasting conditions at approximately 6 p.m. during steady state days.

All subjects volunteered for the study, were paid for their participation, and gave informed consent. The study protocol was approved by the ethics committee of the University of Berne School of Medicine.

Experimental design. To establish a normal baseline, the subjects were studied initially for 4 d while ingesting the metabolic diet without NH₄Cl (control period). After the control period two different degrees of metabolic acidosis were induced by NH₄Cl administration. Four male subjects (mean age [±SD] 30±6.4 y; body weight 60.7±2.4 kg) were fed 2.1 mmol NH₄Cl/kg in gelatin capsules in six divided doses. Four additional male subjects (mean age [±SD] 27.3±7.3 y; body weight 64.4±4.6 kg) were fed 4.2 mmol NH₄Cl/kg daily also in six divided doses. Observations were carried out for 8 d, during which period a new steady state of acid–base equilibrium was established in all subjects.

Analytical procedures. All measurements were performed in duplicate. Analysis of plasma and urine electrolyte and acid–base composition were performed as described previously (27). Plasma ionized calcium was measured with an ion-selective electrode at the prevailing blood pH under anaerobic conditions (intraassay coefficient of variation = 0.9%, interassay coefficient of variation = 1.1%, model 634, Ciba Corning, Medfield, MA). Serum intact parathyroid hormone (28) and 1,25-(OH)₂D (29) levels were measured with specific radioimmunoassay and radioreceptor kits, respectively (Nichols Institute, San Juan Capistrano, CA). MCR and PR of 1,25-(OH)₂D were estimated by the primed-infusion technique of Eastell et al. (30). 1,25-[³H-26,27](OH)₂D₃ (159 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, IL). A loading dose of 624±34 dpm/kg was infused over 2 min and the cannula flushed with 2 ml of 0.9% sterile saline. One tenth of this loading dose was then infused per hour over a period of 6 h (infusion rate [dpm/kg per h]:loading dose [dpm/kg] = 1:10). Radioactivity was determined in 1 ml of plasma. A constant

level of plasma radioactivity (linear regression slope of plasma concentration of ³H-1,25(OH)₂D₃ vs. time not significantly different from zero) was reached between 150 and 210 min. Plasma samples from blood drawn every 15 min between 300 and 360 min after administration of the loading dose were used for analysis. The MCR of endogenous 1,25-(OH)₂D is assumed to be equal to that of intravenously administered [³H]-1,25(OH)₂D₃. At steady state, MCR was calculated according to the relationship (31) MCR (ml/min) = (rate of infusion of [³H]-1,25(OH)₂D₃ [dpm/min])/(plasma concentration of [³H]-1,25(OH)₂D₃ [dpm/ml]). The value for plasma concentration of [³H]-1,25(OH)₂D₃ is the mean of five separate determinations of [³H]-1,25(OH)₂D₂ obtained at steady state. The intraassay coefficient of variation of [³H]-1,25(OH)₂D was 6.2%, the interassay coefficient of variation was 7.3% (tritium concentration 310 dpm/min). The PR of 1,25-(OH)₂D was calculated according to the relationship PR (μg/d) = MCR (ml/min) × serum concentration of endogenous 1,25-(OH)₂D (μg/ml) × 1440 min/d.

Results are reported as mean±SEM unless stated otherwise. Statistical significance was determined by simple and multiple linear regression, covariance analysis, and Student's *t* test for paired or unpaired data, as appropriate.

Results

Both groups of subjects tolerated the protocol well. The mean steady state plasma acid–base and electrolyte composition, albumin concentration, alkaline phosphatase activity, creatinine clearance rate, and body weight are shown in Table I. The steady state values for urinary acid–base and electrolyte excretion are given in Table II. The administration of NH₄Cl decreased the mean±SEM steady state plasma bicarbonate concentration by 4.5±0.3 mmol/liter in the group receiving 2.1 mmol (low dose group) and by 9.1±0.4 mmol/liter in the group receiving 4.2 mmol NH₄Cl/kg body wt per d (high dose group, *P* < 0.001). A similar dose-response to the two levels of oral acid load was observed in the rate of net acid excretion (NAE, Table II): NAE increased to 142.5±7.4 mmol/24 h in the low dose group, whereas subjects in the high dose group (ingesting twice the amount of NH₄Cl as the low dose group) excreted approximately twofold more acid (270.2±9.6 mmol/24 h, *P* < 0.001). All subjects in both groups lost weight significantly. NH₄Cl feeding decreased plasma potassium concentration and albumin concentration significantly only in the high dose group.

The effects of metabolic acidosis on calcium, phosphate, 1,25-(OH)₂D metabolism, and parathyroid hormone are illustrated in Table III and Figs. 1 and 2, which depict the mean daily values for the low and high dose groups. Steady state plasma-ionized calcium concentration did not change significantly in response to metabolic acidosis in either group, despite a large and significant increase in the total and fractional urinary excretions of calcium. However, as illustrated in Fig. 2, there was a transient small increase in calcium concentration during the adaptation to metabolic acidosis. This transient increase was observed only in the high dose group. Plasma phosphate concentration decreased in a dose-dependent fashion in response to metabolic acidosis: whereas hypophosphatemia was mild and not statistically significant in the low dose group, the decrement in plasma phosphate concentration of −0.26±0.05 mmol/liter in the high dose group was significant (*P* < 0.001). Acidosis-induced hypophosphatemia in the high dose group averaged 0.87±0.04 mmol/liter and was significantly more severe than that observed in the low dose group

Table I. Effect of Chronic NH₄Cl Administration on Fasting Plasma Acid-Base and Electrolyte Composition*

	H ⁺	P _a CO ₂	HCO ₃ ⁻	Unmeasured anions	Na ⁺	K ⁺	Cl ⁻	Albumin	Alkaline phosphate	Creatinine clearance	Weight
	nmol/liter	mmHg			mmol/liter			g/liter		ml/s	kg
Low dose group (2.1 mmol NH ₄ Cl/kg body wt per day, n = 4)											
Control	37.8±0.6	40.3±0.8	25.3±0.3	16.8±0.6	142.0±1.0	3.9±0.05	103.8±0.6	42.5±0.6	64±3	2.00±0.08	60.7±1.2
Metabolic acidosis, Days 6-8	41.8±0.4 [†]	36.6±0.5 [‡]	20.8±0.4 [‡]	14.7±0.9 [§]	139.4±0.6	3.9±0.06	107.6±0.5 [‡]	41.7±0.5	66±3	2.05±0.05	59.0±1.2 [‡]
High dose group (4.2 mmol NH ₄ Cl/kg body wt per day, n = 4)											
Control	37.9±0.9	40.1±0.5	25.5±0.4	17.6±0.6	142.3±0.9	4.0±0.04	103.7±0.7	43.8±0.5	78±3	2.11±0.07	64.4±2.3
Metabolic acidosis, Days 6-8	48.2±0.8 [‡]	33.0±0.7 [‡]	16.4±0.4 [‡]	15.3±0.7 [‡]	139.0±0.7	3.7±0.07	111.2±0.4 [‡]	42.3±0.6 [§]	84±3	2.24±0.08	62.5±2.3 [‡]

* Means±SEM. H⁺ denotes hydrogen ion, P_aCO₂ arterial carbon dioxide tension, HCO₃⁻ bicarbonate, Na⁺ sodium, K⁺ potassium, and Cl⁻ chloride. To convert values for P_aCO₂ to kP, multiply by 0.1333. Unmeasured anions were calculated as (Na⁺ + K⁺) - (Cl⁻ + HCO₃⁻)_{mmol/liter}.
[†] P < 0.001; [‡] P < 0.01; ^{||} P < 0.05 for the comparison to the control period within the same study group.

(1.00±0.02 mmol/liter, P < 0.005). The fact that urinary phosphate excretion was enhanced in the steady state despite a lower filtered load of phosphate (Table III, Figs. 1 and 2) indicates that metabolic acidosis-induced hypophosphatemia is at least in part of renal origin.

Low dose NH₄Cl resulted in a small, but nonsignificant increase in serum 1,25-(OH)₂D and a small, but nonsignificant decrease in intact parathyroid hormone concentration. In the high dose group, serum 1,25-(OH)₂D increased significantly by 15.8±2.9 pg/ml (P < 0.001) and parathyroid hormone decreased significantly by 4.9±1.8 pg/ml (P < 0.025, Table III, Figs. 1 and 2). Metabolic acidosis increased the

MCR of 1,25-(OH)₂D significantly and to a similar degree in both groups. The PR of 1,25-(OH)₂D increased by 0.61±0.25 μg/24 h (P < 0.025) in the low dose groups and by 1.14±0.4 μg/24 h in the high dose group (P < 0.005, Table III). Thus, the percentage stimulation of PR for high dose NH₄Cl was greater than the percentage increase in MCR, resulting in increased steady state serum 1,25-(OH)₂D concentrations in response to metabolic acidosis.

The relationship between steady state plasma phosphate and plasma bicarbonate concentrations are shown in Fig. 3 A, and the relationships between each of these parameters and serum 1,25-(OH)₂D concentration are depicted in Fig. 3 B and

Table II. Effect of Chronic NH₄Cl Administration on Urinary Acid-Base and Electrolyte Excretion*

	pH	NH ₄	Titrateable acidity	HCO ₃ ⁻	Net acid	Na ⁺	K ⁺	Cl ⁻
	mmol/24 h							
Low dose group (2.1 mmol NH ₄ Cl/kg body wt per day; n = 4)								
Control	6.30±0.07	19.5±2.2	15.3±1.5	4.5±1.4	30.3±2.8	141.3±8.7	86.8±3.2	144.9±7.3
Metabolic acidosis								
Day 1	4.99±0.11 [‡]	116.4±4.3 [‡]	22.2±1.5	1.1±0.5 [‡]	137.2±6.8 [‡]	156.1±4.2 [‡]	112.5±5.8	321.6±15.3 [‡]
Days 6-8	4.96±0.03 [‡]	119.4±5.2 [‡]	23.6±0.4	0.5±0.6 [‡]	142.5±7.3 [‡]	133.8±7.1	89.9±3.3	255.3±12.3 [‡]
High dose group (4.2 mmol NH ₄ Cl/kg body wt per day; n = 4)								
Control	6.22±0.13	20.7±1.2	16.2±1.4	5.1±1.2	31.8±3.1	145.5±8.4	91.4±3.2	150.5±8.1
Metabolic acidosis								
Day 1	4.76±0.03 [‡]	175.0±5.1 [‡]	31.3±1.6 [‡]	0.5±1.1 [‡]	205.9±8.9 [‡]	256.2±6.4 [‡]	142.3±4.7 [§]	428.1±11.9 [‡]
Days 6-8	5.02±0.03 [‡]	232.1±9.3 [‡]	38.5±1.6 [‡]	0.4±1.0 [‡]	270.2±9.6 [‡]	141.5±6.7	109.0±5.1	426.1±13.5 [‡]

* Mean±SEM. NH₄ denotes ammonia/ammonium.

[‡] P < 0.001; [§] P < 0.025; and ^{||} P < 0.05 for comparison with control steady state period (last 3 d of control period).

Table III. Mineral and Endocrine Data*

Group and study period	Ca _p ⁺⁺	PO _{4p}	Intact PTH	1,25(OH) ₂ vit D	Ca _u	PO _{4u}	FE _{Ca}	FE _{PO₄}	MCR 1,25(OH) ₂ vit D	PR 1,25(OH) ₂ vit D
	mmol/liter		pg/ml		mmol/24 h		%	%	ml/min	μg/24 h
Low dose group (NH ₄ Cl 2.1 mmol/kg body wt, n = 4)										
Control	1.33±0.01	1.06±0.04	21.4±6.3	32.3±3.1	6.08±0.62	26.0±1.9	1.74±0.24	16.8±1.2	32.5±2.6	1.35±0.1
Acidosis										
Day 1	1.33±0.01	1.13±0.05	25.3±5.1	27.8±3.4	8.07±1.51	32.8±3.9	2.00±0.50	20.0±1.6		
Days 6-8	1.30±0.01	1.00±0.02	23.3±4.5	34.3±3.1	9.82±1.08 [§]	30.2±1.5	2.76±0.48 [†]	20.1±1.2 [§]	41.7±2.2 [‡]	1.96±0.3
ΣΔ (day 8)					+21.0 ^{§§}	+31.9 ^{§§}				
High dose group (NH ₄ Cl 4.2 mmol/kg body wt; n = 4)										
Control	1.30±0.02	1.13±0.04	29.5±6.3	25.0±3.4	8.31±0.38	28.6±0.8	2.11±0.11	15.4±0.9	33.7±3.0	1.33±0.1
Acidosis										
Day 1	1.35±0.01	1.12±0.08	29.0±4.8	31.5±2.9	12.66±0.55	36.8±2.4	3.41±0.31	22.9±1.6 [†]		
Days 6-8	1.32±0.01	0.87±0.04 [‡]	20.9±3.2	40.3±3.5 [‡]	19.20±0.41	35.2±2.2	4.79±0.18 [‡]	23.5±1.3 [§]	42.3±3.2 [†]	2.47±0.4 [†]
ΣΔ (day 8)					+67.8 ^{§§††}	+91.3 ^{§§††}				

* Means±SEM. ΣΔ denotes the cumulative change in excretion, calculated as the accumulated sum of the daily differences from the previous steady state period. Ca²⁺ denotes ionized calcium, PO₄²⁻ phosphate, and suffixes P and U plasma and urine, respectively.

[‡] P < 0.001; [§] P < 0.005; ^{||} P < 0.025; [†] P < 0.05 for the comparison to the control period within the same study period; ^{**} P < 0.005;

^{††} P < 0.05 for the comparison between high and low dose group; ^{§§} significantly different from zero (P < 0.005).

C, respectively. Plasma phosphate concentration correlated positively and significantly with plasma bicarbonate concentration ($r = 0.72$, $P < 0.001$). A strong, negative correlation was observed between serum 1,25-(OH)₂D concentration and plasma phosphate concentration ($r = -0.711$, $P < 0.001$) as well as plasma bicarbonate concentration ($r = -0.725$, $P < 0.001$). Multiple regression analysis of the relationship of 1,25-(OH)₂D on plasma phosphate and bicarbonate concentrations revealed only slightly better correlation ($r = 0.756$, $P < 0.001$). Further partial correlation analysis of the relationship of serum 1,25-(OH)₂D concentration on a combination of parameters yielded the following partial correlation coefficients: (a) 1,25-(OH)₂D on plasma phosphate at constant plasma ionized calcium concentration ($r = -0.705$, $P < 0.001$), (b) 1,25-(OH)₂D on plasma phosphate at constant serum intact parathyroid hormone concentration ($r = -0.668$, $P < 0.001$), (c) 1,25-(OH)₂D on plasma ionized calcium at constant phosphate concentration ($r = -0.310$, $P > 0.05$).

Dietary potassium loading has been reported recently to produce renal hyperphosphatemia and a decrease in serum 1,25-(OH)₂D concentration in humans (32). Thus, the possibility might be considered that renal potassium wasting induced by chronic mineral acidosis could induce both hypophosphatemia and secondarily increased serum 1,25-(OH)₂D concentration. However, no significant correlation was obtained for the relation of steady state plasma phosphate on plasma potassium concentration ($r = 0.475$), although a significant inverse correlation does obtain for serum 1,25-(OH)₂D on plasma potassium concentration ($r = 0.542$, $P < 0.05$). This correlation is, however, substantially weaker than that for serum 1,25-(OH)₂D on plasma phosphate concentration (Fig. 3B).

To evaluate a possible influence of metabolic acidosis on the diurnal variation of plasma ionized calcium, plasma phosphate, serum 1,25-(OH)₂D, and serum intact parathyroid hormone concentrations, these values were determined at 1800 as well as at 0700 during steady state days during both the control and experimental periods. As illustrated in Fig. 4, metabolic

acidosis did not affect the evening values of any of these parameters, suggesting that diurnal variation was not changed significantly. However, a more detailed analysis of the diurnal pat-

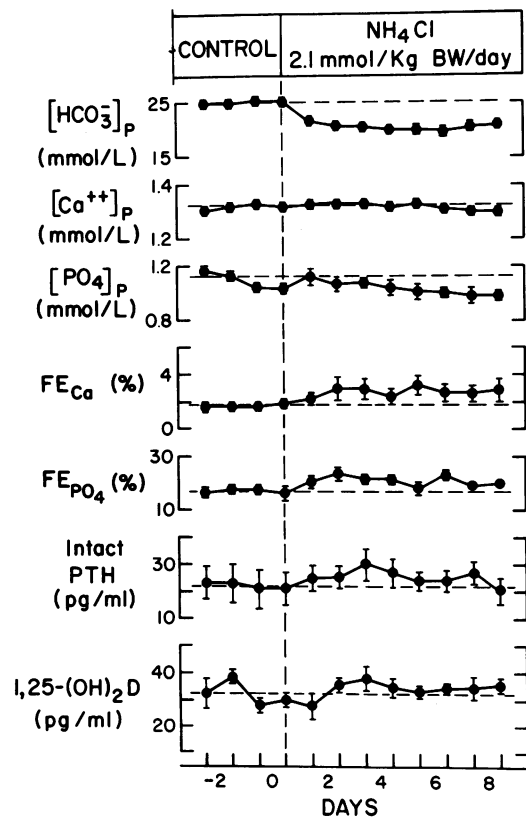


Figure 1. Effect of low dose NH₄Cl feeding (2.1 mmol/kg body wt per day) on the plasma bicarbonate (HCO₃⁻), ionized calcium (Ca⁺⁺), and phosphate (PO₄) concentrations, the fractional excretions of calcium (FE_{Ca}) and phosphate (FE_{PO₄}), and serum intact parathyroid hormone (PTH) and 1,25-(OH)₂D concentrations. Values are mean±SEM.

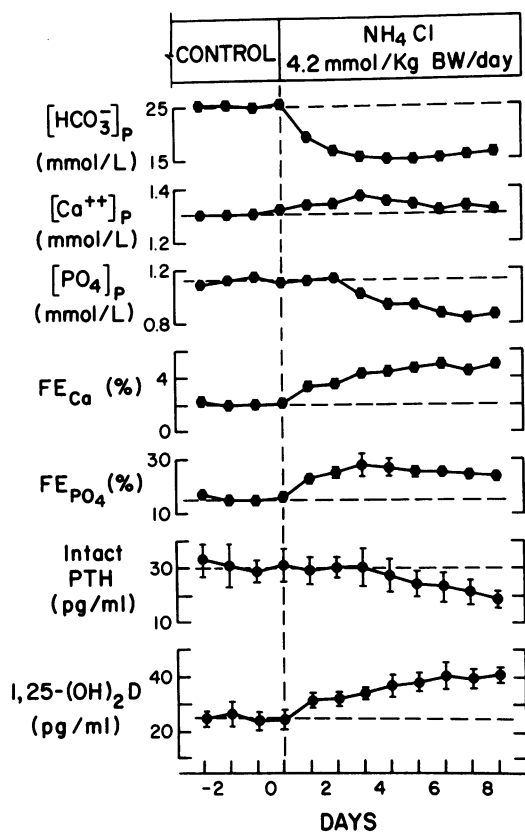


Figure 2. Effect of high dose NH_4Cl feeding (4.2 mmol/kg body wt per day) on the plasma bicarbonate (HCO_3^-), ionized calcium (Ca^{++}), and phosphate (PO_4) concentrations, the fractional excretions of calcium (FE_{Ca}) and phosphate (FE_{PO_4}), and serum intact parathyroid hormone (PTH) and $1,25\text{-(OH)}_2\text{D}$ concentrations. Values are mean \pm SEM.

tern of these parameters in response to metabolic acidosis was not performed nor was it a goal of these studies.

Discussion

Chronic metabolic acidosis is associated with clinically important osteomalacic and osteopenic bone disease, growth retardation, and calcium nephrolithiasis. To gain more insight into the mechanisms of these clinical syndromes, the present studies were designed to examine the regulation of $1,25\text{-(OH)}_2\text{D}$ metabolism, parathyroid hormone, and calcium and phosphate balance in metabolic acidosis. The novel findings are (a) metabolic acidosis significantly increases both the MCR and the PR of $1,25\text{-(OH)}_2\text{D}$; (b) at the lower plasma bicarbonate concentrations of more severe acidosis the increase in PR overrides the increase in MCR (Table III); thus, (c) resulting in a highly significant increase in serum $1,25\text{-(OH)}_2\text{D}$ concentration in metabolic acidosis of moderate severity (plasma $[\text{HCO}_3^-]$ ranging from 13 to 18 mmol/liter, high NH_4Cl dose group) and a smaller but nonsignificant increase in less severe degrees of acidosis (plasma $[\text{HCO}_3^-]$ ranging from 17 to 22 mmol/liter, low NH_4Cl dose group, Table III, Figs. 1 and 2); (d) metabolic acidosis induced hypophosphatemia of renal origin (Table III, Figs. 1 and 2) with a strong direct correlation of plasma phosphate with plasma bicarbonate concentration (Fig. 3 A) and an

equally strong inverse correlation of serum $1,25\text{-(OH)}_2\text{D}$ concentration with plasma phosphate concentration (Fig. 3 B); and (e) intact parathyroid hormone decreased significantly in response to metabolic acidosis in the high dose group (Table III, Fig. 2).

Our finding of an enhanced $1,25\text{-(OH)}_2\text{D}$ MCR represents the first report of this measurement in acidosis and is consistent with recent results of studies demonstrating enhanced degradation of labeled $1,25\text{-(OH)}_2\text{D}$ in vitamin D-deficient chicks with NH_4Cl -induced metabolic acidosis (33). The mechanism of the apparent acidosis-induced enhancement of MCR has not been investigated in this study. However, it is interesting to note that the MCR of another steroid hormone, aldosterone, is enhanced in response to dietary potassium depletion (34). The present observations indicate that the observed increase in the MCR can be overridden by an even greater increase in the PR of $1,25\text{-(OH)}_2\text{D}$. We are unaware of other metabolic disturbances that simultaneously increase both the PR and MCR of any hormone.

Dietary-induced decreases and increases in plasma phosphate concentration have been shown to increase and decrease serum $1,25\text{-(OH)}_2\text{D}$ concentration, respectively, in humans by altering its PR (20–22). Hypophosphatemia, hypocalcemia, and parathyroid hormone excess are the best-known stimulators of $1,25\text{-(OH)}_2\text{D}$ PR. When all steady state values of this study were analyzed (Fig. 3 B), a significant inverse correlation was demonstrable for the relation of serum $1,25\text{-(OH)}_2\text{D}$ as a function of plasma phosphate concentration. This observation coupled with the findings that plasma ionized calcium concentration did not change and that parathyroid hormone serum concentrations were unchanged (low dose group) or even decreased (high dose group) indicate a dominant role for hypophosphatemia and/or cellular phosphate depletion in acidosis-induced stimulation of $1,25\text{-(OH)}_2\text{D}$ production.²

The present studies also demonstrate a strikingly strong direct correlation of plasma phosphate with plasma bicarbonate concentration (Fig. 3 A), consistent with our previous observations in another group of humans (25). However, other studies in humans have not reported consistent acidosis-induced changes in plasma phosphate concentration, which was found to be either unchanged (35–37) or decreased (38, 39). Similar inconsistencies are reported in previous animal studies (rat, dog, and chick; references 12, 16–18, 33, 40–44). However, the results from the subjects in the low dose group (no significant decrease in plasma phosphate concentration) and the observation from other studies that the greatest reductions in plasma

2. Prevention of NH_4Cl -induced hypophosphatemia by either administering large oral doses of neutral phosphate or by pharmacologically increasing renal tubular phosphate reabsorption (e.g., by diphosphonates) might be considered to yield more direct evidence for the hypothesis that hypophosphatemia and/or cellular phosphate depletion is the sole determinant of elevated serum $1,25\text{-(OH)}_2\text{D}$ concentration during metabolic acidosis. However, even massive increases in oral phosphate intake (95.8 mmol/d) have been insufficient to increase fasting plasma phosphate concentration in humans with metabolic acidosis (41). Moreover, this approach would not be useful even if normophosphatemia was achievable inasmuch as phosphate loading of this magnitude would almost certainly result in severe hyperparathyroidism. Diphosphonates, on the other hand, have been shown to exert independent effects on intestinal calcium transport, vitamin D, and bone metabolism (61). Thus, currently available diphosphonates are of insufficient specificity to further address this question.

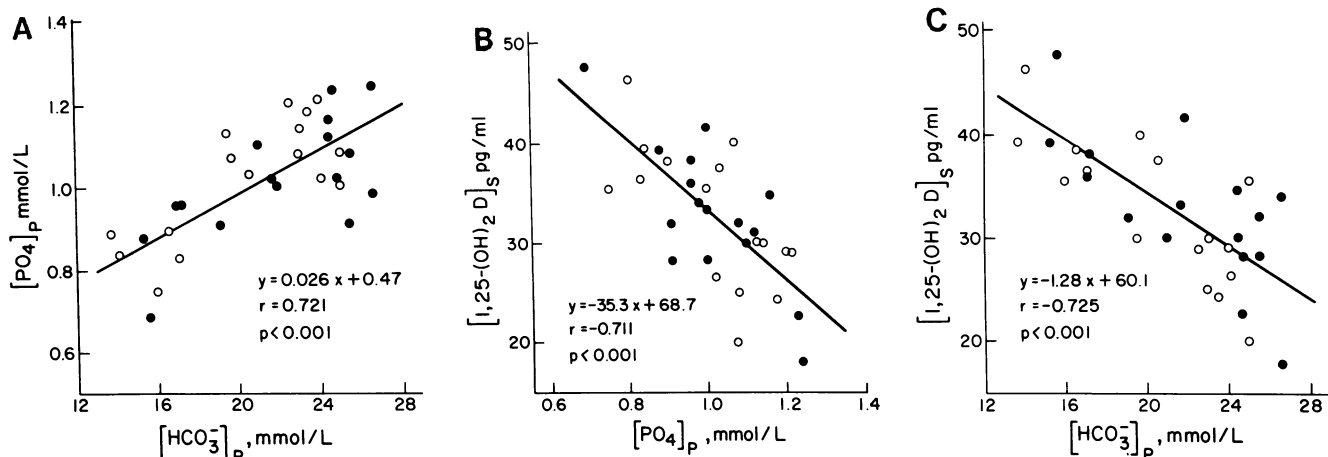


Figure 3. (A) Relation between steady state plasma phosphate (PO_4) and plasma bicarbonate (HCO_3^-) concentrations. Steady state data from all subjects in the high dose and low dose groups were pooled. Closed circles represent fasting, morning values (0700); open circles represent nonfasting, evening values (1800). (B) Relation between steady state serum 1,25-(OH) $_2$ D and plasma phosphate (PO_4) concentrations. Steady state data from all subjects in the high and low dose groups were pooled. Closed circles represent fasting, morning values (0700); open circles represent nonfasting, evening values (1800). (C) Relation between steady state serum 1,25-(OH) $_2$ D and plasma bicarbonate (HCO_3^-) concentrations. Steady state data from all subjects in the high and low dose groups were pooled. Closed circles represent fasting, morning values (0700); open circles represent nonfasting, evening values (1800).

phosphate concentration occurred at lowest plasma bicarbonate concentration indicate that the development of significant hypophosphatemia depends on the severity of metabolic acidosis. In this context, it is interesting to note that, of the previous reported measurements of serum 1,25-(OH) $_2$ D concentration during experimentally induced metabolic acidosis (15–19), the only study that found an acidosis-induced increase in serum 1,25-(OH) $_2$ D (16) also demonstrated the most severe degree of acidosis (mean plasma $[\text{HCO}_3^-] = 11$ mmol/liter) accompanied by significant NH_4Cl -induced hypophosphatemia. In addition, none of the previous reports has analyzed the correlation of steady state plasma phosphate and bicarbonate concentration.

Chronic NH_4Cl administration has been reported previously in humans to increase renal phosphate clearance (37), which accounts for most of the negative phosphate balance during prolonged acidosis (36). The present studies demonstrating increased fractional excretion of phosphate despite steady state hypophosphatemia are consistent with an acidosis-induced renal phosphate leak. The cellular mechanism(s) of decreased renal tubular phosphate reabsorption in response to acidosis is (are) currently unknown.

Chronic dietary potassium loading has been reported recently to result in chronic renal hyperphosphatemia and decreased 1,25-(OH) $_2$ D serum concentration (32). We therefore considered the possibility that the renal potassium wasting of mineral acidosis might be responsible for both hypophosphatemia and secondarily increased serum 1,25-(OH) $_2$ D concentration. However, no significant correlation was obtained for the relation of steady state values of plasma phosphate on potassium concentration ($r = 0.475$, $n = 16$). A statistically significant inverse correlation does obtain for serum 1,25-(OH) $_2$ D on plasma phosphate concentration ($r = 0.711$, $n = 16$; Fig. 3 B). These results suggest that other factors in addition to potassium homeostasis determine both plasma phosphate and serum 1,25-(OH) $_2$ D concentra-

tions in chronic mineral acidosis. The possibility of an ancillary direct phosphate-independent effect of potassium metabolism on 1,25-(OH) $_2$ D production in metabolic acidosis can not be excluded by the present results. However, there are no reported effects of potassium metabolism on 1,25-(OH) $_2$ D metabolism.

The present results in acidotic human subjects might appear to disagree with the repeated finding in rats that chronic mineral acidosis results in significant decreases in serum 1,25-(OH) $_2$ D concentration (17–19). However, in each of the protocols in rats, chronic metabolic acidosis was associated with increased plasma ionized calcium concentration (17–19). Chronic hypercalcemia is known in several species to be able to override the other potent stimuli that operate to increase the

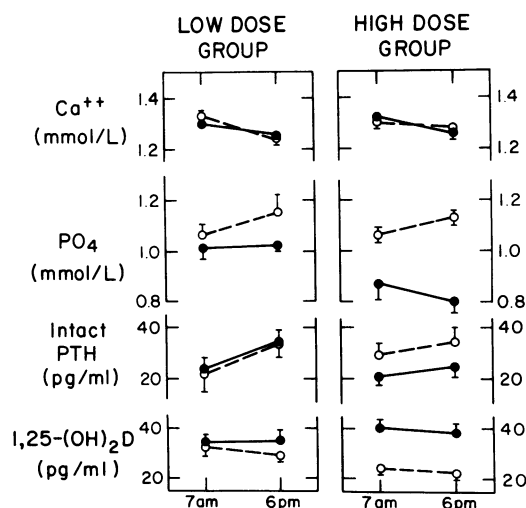


Figure 4. Effect of chronic metabolic acidosis on steady state diurnal variations in plasma ionized calcium (Ca^{++}) and phosphate (PO_4) concentrations and serum intact parathyroid hormone (PTH) and 1,25-(OH) $_2$ D concentrations. Open circles and broken line represent control; closed circles and solid lines represent experimental (NH_4Cl feeding) periods, respectively.

PR and serum concentration of 1,25-(OH)₂D (23, 45, 46). Only when hypophosphatemia/cellular phosphate depletion is severe has acidosis-induced hypercalcemia failed to suppress a hypophosphatemia-induced increase in serum 1,25-(OH)₂D concentration (40). In the present studies, hypercalcemia was not observed and, more importantly, a significant correlation of serum 1,25-(OH)₂D with plasma ionized calcium concentration ($r = 0.17$, linear slope = +29.1 pg/mL per mmol/liter) did not obtain.

The finding of a significant decrease (high dose group) in serum parathyroid hormone concentration in response to chronic metabolic acidosis is in agreement with a previous report in humans (35). However, other reports have found either increased (47, 48) or unchanged (15, 39) values. Although it is possible that the older parathyroid hormone assays were responsible for the failure to detect the small decreases in parathyroid hormone concentration detected herein, other explanations also need to be considered. Since the dose of NH₄Cl administered to the subjects of the present study (4.2 mmol/kg per 24 h, high dose group) was substantially greater than that employed previously, and since this large dose of NH₄Cl resulted in a greater decrement in steady state phosphate concentration, it is possible that hypophosphatemia-induced hypoparathyroidism contributed to the observed decrease in intact parathyroid hormone concentration in this group (49, 50). The sustained elevation of serum 1,25-(OH)₂D concentration per se may also have contributed to suppression of parathyroid hormone secretion and serum concentration (51).

The present experiments provide an opportunity to reinterpret previous observations on the role of parathyroid hormone in metabolic acidosis. On the basis of measurements of immunoreactive NH₂-terminal parathyroid hormone in chronic NH₄Cl-induced acidosis in humans (47, 48) and acute NH₄Cl-induced acidosis in rats (52), it was suggested that both acute and chronic acidosis elicits a state of hyperparathyroidism that, at least in rats (52), is critical to the full expression of the renal acid excretory response. Our findings herein using the modern two-site intact parathyroid hormone assay provide strong evidence that elevated levels of parathyroid hormone per se are not present over a wide range of values for depressed plasma bicarbonate concentration. Thus, increased parathyroid hormone secretion is not required for the renal adaptation to chronic metabolic acidosis in humans.

The elevated serum 1,25-(OH)₂D concentration in response to chronic metabolic acidosis raises the interesting possibility of a homeostatic role for 1,25-(OH)₂D metabolism in moderate to severe acidosis. That is, does the elevated serum 1,25-(OH)₂D contribute to the normal acid excretory response? This question will require further evaluation in view of the observations that vitamin D deficiency has been reported to cause metabolic acidosis in chicks (53) and chronic 1,25-(OH)₂D administration results in metabolic alkalosis (in part of renal origin) in thyroparathyroidectomized dogs (54, 55).

The results of the present studies help to integrate a number of interesting issues regarding the complex and interrelated pathophysiology of metabolic acidosis, hypophosphatemia, and metabolic bone disease. First, 1,25-(OH)₂D is a potent stimulus to bone resorption (56, 57). Second, mild phosphate depletion has been shown to enhance bone resorption and to decrease bone mineralization in rats (58). Moreover, the severity of hypophosphatemia-induced enhanced bone resorption was greatly attenuated by experimental vitamin D deficiency. Third, rats with chronic mineral acidosis and associated hypo-

phosphatemia exhibit increased bone resorption as well as decreased bone formation (59, 60). Fourth, hypophosphatemia is often present during chronic acidosis of either renal tubular or extrarenal etiology (59, 60) and the reversal of skeletal lesions by alkali treatment alone is associated with a markedly positive phosphate balance (3, 5). Taken together with the present findings that chronic metabolic acidosis induces acid load-dependent hypophosphatemia and increased serum 1,25-(OH)₂D concentration, the possibilities might therefore be considered that the renal hypophosphatemia/phosphate depletion of chronic metabolic acidosis plays a fundamental pathogenetic role in the development of metabolic bone disease and high levels of serum 1,25-(OH)₂D concentration may play a deleterious role in the development of metabolic bone disease in chronic acidosis.

In summary, chronic extrarenal metabolic acidosis increases both MCR and PR of 1,25-(OH)₂D in humans. At the lower plasma bicarbonate concentrations of more severe acidosis, the increase in production rate overrides the increase in MCR, resulting in a significant increase in serum 1,25-(OH)₂D concentration. The strong inverse correlation between serum 1,25-(OH)₂D and plasma phosphate concentration suggests that acidosis-induced hypophosphatemia and/or cellular phosphate depletion play a critical role in stimulating the PR of 1,25-(OH)₂D.

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