

Neutralization of Western diet inhibits bone resorption independently of K intake and reduces cortisol secretion in humans

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Maurer, Marc, Walter Riesen, Juergen Muser, Henry N. Huter, and Reto Krapf. Neutralization of Western diet inhibits bone resorption independently of K intake and reduces cortisol secretion in humans. *Am J Physiol Renal Physiol* 284: F32–F40, 2003. First published September 24, 2002; 10.1152/ajprenal.00212.2002.—A Western-type diet is associated with osteoporosis and calcium nephrolithiasis. On the basis of observations that calcium retention and inhibition of bone resorption result from alkali administration, it is assumed that the acid load inherent in this diet is responsible for increased bone resorption and calcium loss from bone. However, it is not known whether the dietary acid load acts directly or indirectly (i.e., via endocrine changes) on bone metabolism. It is also unclear whether alkali administration affects bone resorption/calcium balance directly or whether alkali-induced calcium retention is dependent on the cation (i.e., potassium) supplied with administered base. The effects of neutralization of dietary acid load (equimolar amounts of NaHCO₃ and KHCO₃ substituted for NaCl and KCl) in nine healthy subjects (6 men, 3 women) under metabolic balance conditions on calcium balance, bone markers, and endocrine systems relevant to bone [glucocorticoid secretion, IGF-1, parathyroid hormone (PTH)/1,25(OH)₂ vitamin D and thyroid hormones] were studied. Neutralization for 7 days induced a significant cumulative calcium retention (10.7 ± 0.4 mmol) and significantly reduced the urinary excretion of deoxyypyridinoline, pyridinoline, and *n*-telopeptide. Mean daily plasma cortisol decreased from 264 ± 45 to 232 ± 43 nmol/l ($P = 0.032$), and urinary excretion of tetrahydrocortisol (THF) decreased from $2,410 \pm 210$ to $2,098 \pm 190$ μg/24 h ($P = 0.027$). No significant effect was found on free IGF-1, PTH/1,25(OH)₂ vitamin D, or thyroid hormones. An acidogenic Western diet results in mild metabolic acidosis in association with a state of cortisol excess, altered divalent ion metabolism, and increased bone resorptive indices. Acidosis-induced increases in cortisol secretion and plasma concentration may play a role in mild acidosis-induced alterations in bone metabolism and possibly in osteoporosis associated with an acidogenic Western diet.

glucocorticoid; acid-base; potassium; acidosis; osteoporosis

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CHRONIC METABOLIC ACIDOSIS (CMA) is a frequent acid-base disturbance generated by extrarenal loss of base (e.g., diarrhea), increased acid production (e.g., organic acidosis such as ketoacidosis), or impaired renal acid excretion (i.e., renal failure and inherited or acquired forms of renal tubular acidosis).

CMA has a well-established potential for a catabolic effect on bone. In addition to renal phosphate wasting (28, 35, 36), experimentally induced CMA also results in hypercalciuria and negative calcium balance, attributable to calcium efflux from bone (10, 34). CMA is associated with a poorly characterized metabolic bone disease (23), growth retardation (40), and calcium nephrolithiasis (7). In animal models, CMA results in a decrease in bone calcium and gravimetrically determined bone mass (2), decreased wet tissue femur density (41), accelerated rates of cortical and trabecular bone resorption (2, 20, 29, 41), and diminished rates of bone formation (24), resulting in reduced trabecular bone volume (29, 41).

In vitro studies have demonstrated that metabolic acidosis (imitated by the use of media with low ambient pH and bicarbonate concentrations) is a potent stimulator of bone resorption and inhibitor of bone formation (11, 32), suggesting that CMA acts directly at the tissue level to affect bone metabolism. However, CMA also might affect bone metabolism indirectly, i.e., via numerous well-characterized alterations in endocrine function that include parathyroid, thyroid, adrenal and growth hormone (GH)/IGF-1 dysfunction.

CMA decreases free serum IGF-1 levels during CMA in rats and humans (8, 15) due to GH insensitivity (8), results in a mild form of hypothyroidism (9), and increases the serum 1,25(OH)₂ vitamin D [1,25(OH)₂D] concentration (due to renal phosphate wasting) in humans, resulting in a decreased serum parathyroid hormone (PTH) concentration (28).

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Table 1. Steady-state plasma acid-base and electrolyte composition during control, experimental, and recovery periods

Study Period	pH	P _a CO ₂ , mmHg	HCO ₃ ⁻ , mmol/l	Unmeasured Anions, mmol/l	Na ⁺ , mmol/l	K ⁺ , mmol/l	Cl ⁻ , mmol/l	Ca ²⁺ , mmol/l	PO ₄ ⁻ , mmol/l	Mg ²⁺ , mmol/l	Weight, kg	Creatinine Clearance, ml/s
Control	7.404 ± 0.003	40.5 ± 0.6	24.8 ± 0.3	12.4 ± 0.4	139.5 ± 0.2	4.19 ± 0.04	106.5 ± 0.1	1.21 ± 0.02	1.38 ± 0.04	0.79 ± 0.02	71.1 ± 1.8	2.12 ± 0.10
Experimental	7.411 ± 0.003	41.2 ± 0.8	25.6 ± 0.3*	12.7 ± 0.7	139.9 ± 0.3	4.18 ± 0.03	105.8 ± 0.7	1.19 ± 0.02	1.37 ± 0.04	0.82 ± 0.01	71.6 ± 1.8	2.18 ± 0.11
Recovery	7.402 ± 0.004	40.6 ± 0.7	24.8 ± 0.4	12.6 ± 0.4	139.7 ± 0.3	4.25 ± 0.04	106.6 ± 0.2	1.20 ± 0.03	1.42 ± 0.03	0.80 ± 0.02	70.9 ± 1.7	2.22 ± 0.12

Values are means ± SE, the last 2 days of every study period. P_aCO₂, arterialized PCO₂. To convert values for P_aCO₂ to kPa, multiply by 0.1333. Unmeasured anions were calculated as (Na⁺ + K⁺) - (Cl⁻ + HCO₃⁻) mmol/l. *P < 0.05.

In addition, a hyperglucocorticoid response to CMA has been demonstrated in humans (39) and rats (48). The hyperglucocorticoid response has generated substantial interest because it might explain the negative nitrogen balance of CMA reported in normal rats (39) and humans (1). Support for this possibility is that the catabolic muscle proteolytic effect of CMA demonstrable in vitro in muscle from normal rats was not found in muscle from adrenalectomized rats with CMA (39).

The modern Western-type diet in humans, which is rich in animal protein, has been implicated as a cause of lifelong mild CMA with secondary bone catabolism caused by the induction by this diet of an obligatory daily acid load (endogenous acid production), due largely to endogenous oxidation of cationic and sulfur-containing amino acids (46, 53). Although still within the broad range of normal values, plasma bicarbonate concentration decreases progressively when endogenous acid production is increased by menu changes among normal foodstuffs in normal subjects (33). In support of the hypothesis that ongoing metabolism of the Western diet can result in net bone catabolism, it was demonstrated that prolonged neutralization of endogenous acid production in postmenopausal women resulted in calcium and phosphate retention, reduced markers of bone resorption, and an increase in serum osteocalcin concentration, a marker of bone formation (46). Several uncontrolled observational studies have provided evidence that loss of bone mineral density (BMD) in elderly humans is less while they are ingesting a presumed alkali-rich diet with higher levels of estimated fruit and vegetable intake (42, 47, 51). These observational studies are intriguing but not compelling, however, both because acid excretion was not measured and because the Framingham database examined over the identical time interval that suggested both static and dynamic BMD protection with high fruit/vegetable intake (51) also provided seemingly contrary evidence that high animal protein (but not

nonanimal protein) intake is protective for BMD loss, even after correction for multiple covariates (25).

In addition, there is considerable debate on the issue of whether an increase in potassium intake (typical of a vegetable-/fruit-rich diet) rather than the alkali per se is responsible for the salutary effects on bone. Indeed, studies that administered NaHCO₃ and sodium citrate have found little effect on urinary calcium excretion (34, 38, 45), whereas those in which KHCO₃ or potassium citrate were administered have found large, significant reductions (34, 45, 46).

The present study was designed to assess the possibility that even the mildest CMA of the magnitude reported with the Western diet might be sufficient to cause significant abnormalities in at least one of the reported bone-active endocrinopathies of CMA described above. Hyperglucocorticoidism of very small magnitude was viewed as a particularly likely candidate as an effector for diet-induced bone catabolism because a recent retrospective cohort study found that even very low glucocorticoid doses within the physiological range (i.e., <2.5 mg oral prednisolone daily) significantly increased both vertebral and nonvertebral fracture risk relative to age- and gender-matched controls (52).

METHODS

The protocol was designed to measure the renal and systemic electrolyte, acid-base, and endocrine response to neutralization of endogenous acid production by oral ingestion of HCO₃⁻ in nine normal subjects. Nine nonsmoking subjects [6 men, 3 women, 22.1 ± 1.4 (SD) yr; body wt 70.6 ± 6.8 kg], who were taking no concurrent medication, ate a constant whole-food metabolic diet during all three consecutive study periods (control, neutralization of endogenous acid production, recovery). The diet was administered under metabolic ward conditions and provided the following total content per kilogram of body weight per day, inclusive of the electrolyte supplements described below: 1.42 mmol Na (32.7 mg), 1.18 mmol K, 0.412 mmol Ca, 0.58 mmol PO₄, 16.1 mmol N, 0.24 mmol methionine, 36 kcal, and 47 ml H₂O.

Table 2. Urinary acid-base and electrolyte composition during control, experimental, and recovery periods

Study Period	pH	NH ₄ ⁺ , mmol/24 h	Titrateable Acidity, mmol/24 h	HCO ₃ ⁻ , mmol/24 h	Net Acid, mmol/24 h	Na ⁺ , mmol/24 h	K ⁺ , mmol/24 h	Cl ⁻ , mmol/24 h	Ca ²⁺ , mmol/24 h	PO ₄ ⁻ , mmol/24 h	Mg ²⁺ , mmol/24 h	FE _{Ca} , %	PO ₄ clearance, ml/min
Control	5.83 ± 0.09	61 ± 4	24 ± 2	4.7 ± 0.7	83 ± 5	153 ± 3	119 ± 4	228 ± 3	3.6 ± 0.3	34.6 ± 1.0	5.1 ± 0.4	1.84 ± 0.09	19.2 ± 1.6
Experimental	7.07 ± 0.05*	28 ± 2*	5 ± 1*	3.0 ± 0.3	12 ± 3*	156 ± 5	113 ± 4	133 ± 4†	3.0 ± 0.3†	34.2 ± 1.1	5.2 ± 0.3	1.65 ± 0.08†	18.9 ± 1.8
Recovery	5.83 ± 0.07	64 ± 3	24 ± 1	4.1 ± 0.6	86 ± 3	165 ± 9	126 ± 4	235 ± 2	4.3 ± 0.4	34.0 ± 1	5.1 ± 0.3	1.92 ± 0.09	19.8 ± 1.9

Values are the means ± SE, the last 2 days of every study period. FE_{Ca}, fractional excretion of Na. *P < 0.001. †P < 0.05.

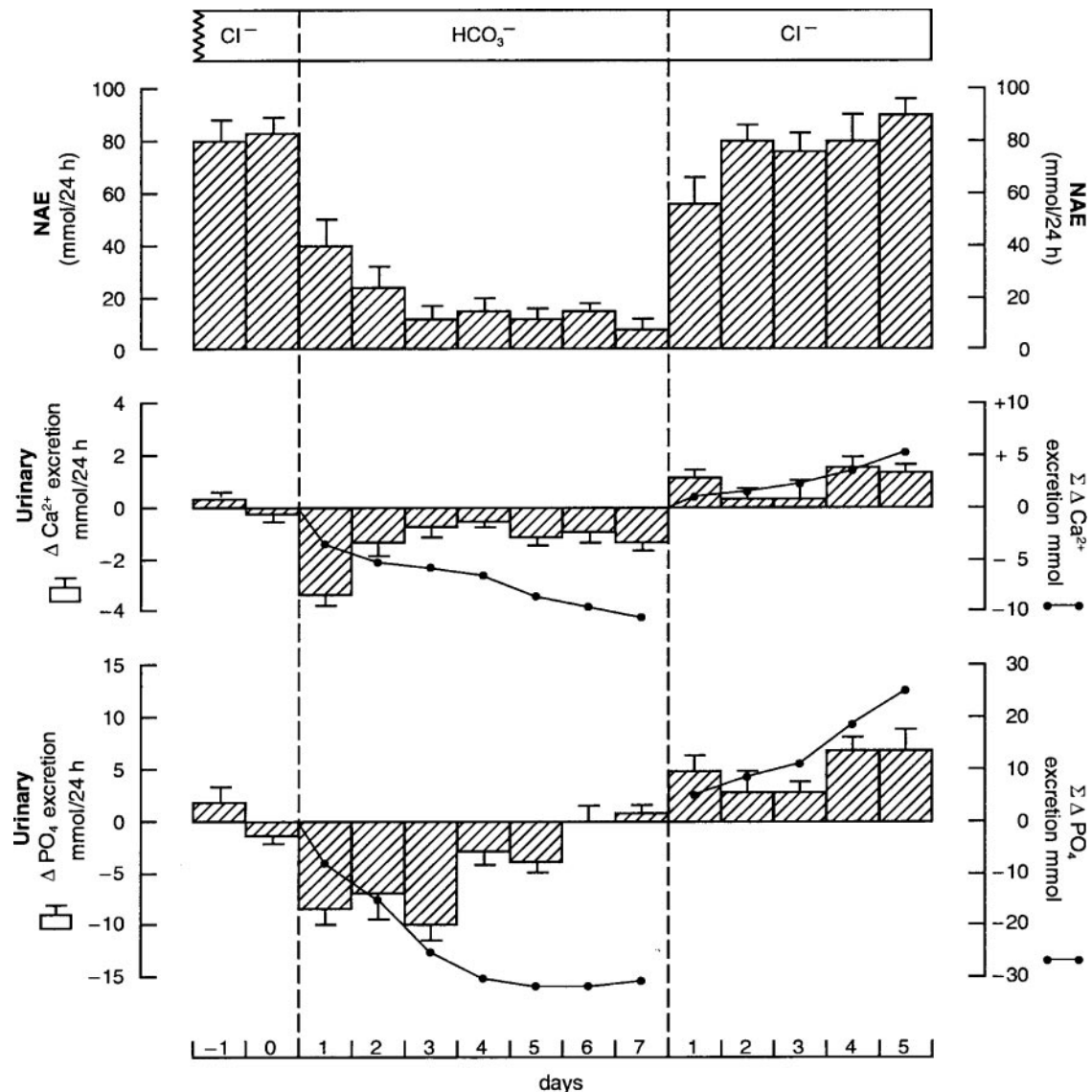


Fig. 1. Effect of HCO_3^- administration on net acid excretion (NAE; mmol/24 h), urinary calcium (Ca^{2+}), and urinary phosphate (PO_4) excretion. Δ , Excretion for calcium and phosphate, daily changes in urinary excretion compared with the previous steady-state periods; $\Sigma\Delta$, cumulative change (sum of the daily difference for a given period) in urinary excretion rates.

All subjects ingested 1.10 mmol chloride salt supplement/kg body wt $^{-1}\cdot\text{day}^{-1}$ during the control (9 days) and the recovery periods (5 days). The daily chloride supplement provided was equimolar (0.55 mmol/kg NaCl, 0.55 mmol/kg KCl). To experimentally neutralize endogenous acid production, a 7-day neutralization period followed the control period wherein equimolar NaHCO_3 was substituted for the NaCl supplement and equimolar KHCO_3 was substituted for KCl. All salts were administered in gelatin capsules in six divided doses daily.

Fasting arterialized venous blood samples (22) were obtained in a heparin-coated syringe from a heated hand or forearm vein. Blood samples were accepted only when PO_2 was >70 Torr (9.3 kPa) and were obtained at 8 AM unless otherwise specified.

All volunteers were paid for their participation and gave informed consent. The study protocol was approved by the ethics committee of the Kantonsspital, St. Gallen, Switzerland.

Analytic procedures. All measurements were performed in duplicate. Acid-base parameters in blood and urine were determined as described elsewhere (27). Analysis of hormones and their metabolites was performed as described previously (8, 9, 28, 48). Biochemical bone markers were determined using ELISA assays for deoxypyridinoline and pyridinoline (21) and *n*-telopeptide of type I collagen (24).

All steady-state values represent the mean of the last 2 days of the corresponding study period. Results are reported as means \pm SE. Statistical analysis was performed by ANOVA for repeated measurements. Slope and intercept testing for plasma cortisol on time was performed using the general linear model procedure for two-way ANOVA with treatment and subject effects (SAS Institute, Cary, NC).

RESULTS

All volunteers tolerated the protocol well. There were no significant differences in body weights (Table

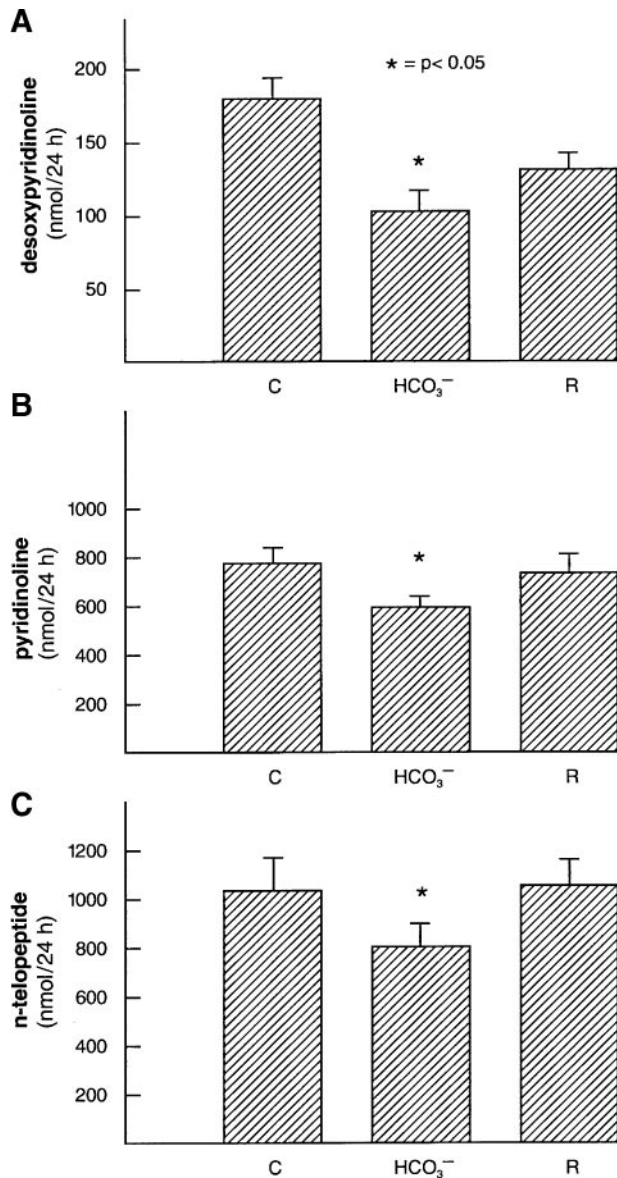


Fig. 2. Effect of HCO_3^- administration on 24-h urinary excretion rates of deoxypyridinoline (A), pyridinoline (B), and *n*-telopeptide (C) of collagen type I. C, control; R, recovery.

1) and blood pressure (not shown) during any of the three study periods.

Administration of HCO_3^- resulted in a small but significant increase in the plasma HCO_3^- concentration (Table 1) from 24.8 ± 0.3 to 25.6 ± 0.3 mmol/l ($P < 0.005$), whereas renal acid excretion (net acid excre-

tion) decreased significantly from 83 ± 5 to 12 ± 3 mmol/24 h (Table 2, Fig. 1, $P < 0.001$).

As shown in Fig. 1 and Table 2, urinary calcium excretion decreased immediately, reversibly, and significantly during HCO_3^- administration and remained decreased throughout the 7-day HCO_3^- period, resulting in significant cumulative calcium retention of 10.7 ± 0.4 mmol/7 days ($P < 0.025$). Urinary phosphate excretion also decreased significantly and reversibly, resulting in a cumulative phosphate retention of 29.9 ± 1.2 mmol/7 days ($P < 0.025$). However, in contrast to calcium excretion, phosphate excretion values were decreased only transiently and returned to values not different from control and recovery values by days 6 and 7 of HCO_3^- administration (Fig. 1, Table 2). Consistent with the unchanged cation intake during the protocol, the cumulative changes in sodium and potassium excretion averaged only $+23 \pm 17$ and -18 ± 7 mmol, values that did not differ significantly from zero.

As shown in Table 2, the fractional renal excretion of calcium, computed from the filtered load of ionized calcium, also decreased significantly from 1.84 ± 0.09 (control) to $1.65 \pm 0.08\%$ during HCO_3^- administration and rose again to $1.92 \pm 0.09\%$ in the recovery period ($P < 0.05$). In contrast, urinary phosphate clearance was not different among the steady-state days of the three periods. Thus increased renal tubular reabsorption of calcium accounted, at least in part, for the observed HCO_3^- -induced urinary calcium retention.

Figure 2 illustrates that markers of bone resorption (i.e., the urinary excretion of deoxypyridinoline, pyridinoline, and *n*-telopeptide of type I collagen) decreased significantly during HCO_3^- administration. The observed reduction in the excretion rates of bone resorption markers and sustained calcium retention in young adult subjects thus greatly extend the previous observation in postmenopausal women that neutralization of endogenous acid production leads to inhibition of bone resorption and positive calcium balance (46).

As neutralization of endogenous acid production might inhibit bone resorption by direct local acidification (\pm paracrine/autocrine effectors) and/or indirectly via alterations in endocrine systems known to be modulated by exogenous acid loads, i.e., the GH/IGF-1 axis, $1,25(\text{OH})_2\text{D}$ and PTH, thyroid hormones, and glucocorticoid activity (see the beginning of this study), we assessed these endocrine systems during steady states, i.e., the last 2 days of each study period.

As shown in Table 3, there were no significant differences in the serum concentrations of free IgF-1,

Table 3. Plasma hormone composition during control, experimental, and recovery periods

Study Period	Free IGF-1, nmol/l	I-PTH, ng/l	$1,25(\text{OH})_2\text{D}$, pmol/l	Free T3, nmol/l	Free T4, nmol/l	TSH, mU/l
Control	48.6 ± 6.0	27.2 ± 4.1	85.6 ± 6.3	1.63 ± 0.06	97.4 ± 3.8	2.07 ± 0.30
Experimental	48.1 ± 4.9	27.4 ± 3.6	83.7 ± 7.4	1.56 ± 0.06	91.6 ± 3.2	1.79 ± 0.25
Recovery	52.3 ± 6.7	26.7 ± 3.5	86.2 ± 5.3	1.60 ± 0.09	91.3 ± 3.5	2.01 ± 0.29

Values are means \pm SE. PTH, parathyroid hormone; $1,25(\text{OH})_2\text{D}$, $1,25(\text{OH})_2$ vitamin D.

Table 4. *Effect of HCO₃⁻ administration on diurnal plasma ACTH values and diurnal plasma cortisol concentrations*

	Control	HCO ₃ ⁻
<i>Diurnal plasma ACTH values</i>		
Time		
0700	32.0 ± 2.8	31.3 ± 3.8
1130	30.1 ± 2.9	26.8 ± 2.6
0400	26.0 ± 2.4	25.6 ± 2.9
0900	14.4 ± 3.4	15.6 ± 2.9
<i>Diurnal plasma cortisol concentrations</i>		
Time		
0700	483 ± 39	416 ± 23*
1130	295 ± 56	293 ± 56
0400	200 ± 17	190 ± 16
0900	73 ± 6	69 ± 6

Values are means ± SE. **P* < 0.05.

1,25(OH)₂D, and intact PTH among the three periods. Similarly, serum TSH, free T3, and free T4 concentrations were also not affected significantly by HCO₃⁻ administration. Dynamic testing of any of these endocrine systems was not attempted during this study.

Table 4 demonstrates the diurnal changes in plasma ACTH and plasma cortisol concentrations. No demonstrable effect of alkali was noted on plasma ACTH levels throughout the day. However, plasma cortisol concentration at 7 AM was reduced significantly during the HCO₃⁻ period compared with control (416 ± 23 vs. 483 ± 39 nmol/l, *P* = 0.046), and the difference remained significant over all four measured diurnal values in the 24-h cycle (232 ± 43 vs. 264 ± 45 nmol/l, *P* = 0.032). Plots of the nine pairs of linear regression lines for plasma cortisol on time demonstrated a pattern of parallel slopes for cortisol decay throughout the day, and this pattern is also apparent in the homogeneity of individual slope values (see Table 5). When tested by two-way ANOVA, the mean slope for cortisol decay did not differ significantly during the HCO₃⁻ and

Cl⁻ periods; however, the mean *y*-intercept cortisol value during the control/Cl⁻ period significantly exceeded the corresponding mean value in the HCO₃⁻ period, providing for a shift in the elevation of plasma cortisol decay throughout the day (Table 5).

To further analyze the effect of neutralization of endogenous acid production on glucocorticoid activity/production, cortisol and cortisol metabolites were determined in 24-h urine collections. As shown in Table 6, HCO₃⁻ administration decreased urinary free cortisol excretion significantly. Because tetrahydrocortisol (THF), cortisol's metabolite, is neither secreted nor reabsorbed in the renal tubule, altered tubular handling that could occur in the case of cortisol cannot account for decreased THF excretion (19). Figure 3 thus concentrates on the excretion values for THF. As illustrated, HCO₃⁻ administration decreased 24-h THF excretion from 2,410 ± 210 mg/24 h for control to 2,098 ± 190 mg/24 h during HCO₃⁻ administration (*P* < 0.05) and recovered to 2,290 ± 220 mg/24 h (*P* < 0.05 compared with HCO₃⁻ period, not significant vs. control period). There was no significant change in the THF+allo-THF/tetrahydrocortisone (THE) ratio during all study periods (Table 6).

DISCUSSION

These results furnish the first evidence that a very mild Western diet-induced CMA (a degree of acidosis that would not be recognized by applying diagnostic acid-base criteria found in textbooks) results in a state of increased cortisol secretion and plasma concentration and provides several novel findings in humans regarding the possible causality of the Western diet in the etiology of osteoporosis. The present results demonstrate that ingestion of neutralizing alkali per se, as exchanged for chloride in the absence of other experimental maneuvers (e.g., concomitant potassium supplement), can result in urinary calcium retention and suppression of biochemical markers of bone resorption. Finally, the present study demonstrates that neutral-

Table 5. *Linear regression analysis of diurnal plasma cortisol concentration: intercept and slope values during Cl⁻ and HCO₃⁻ bicarbonate periods*

Subject No.	Control/Cl ⁻ Period			HCO ₃ ⁻ Period		
	<i>y</i> -Intercept, nmol/l	Slope, nmol·l ⁻¹ ·24 h ⁻¹	<i>r</i> ²	<i>y</i> -Intercept, nmol/l	Slope, nmol·l ⁻¹ ·24 h ⁻¹	<i>r</i> ²
1	446	-27.5	0.98	410	-24.3	0.97
2	461	-22.0	0.95	465	-24.4	0.95
3	460	-26.0	0.99	371	-22.3	0.81
4	394	-21.2	0.78	374	-19.9	0.92
5	509	-28.2	0.93	486	-28.2	0.93
6	717	-48.2	0.89	521	-31.3	0.99
7	394	-23.1	0.74	275	-16.2	0.80
8	508	-27.4	0.97	499	-29.8	0.98
9	407	-25.2	0.90	411	-22.4	0.73
Mean	477	-27.6		423	-24.3	
SD	100	8.1		78	4.8	

Intercept, slope, and *r*² values reflect characteristics of the linear regression for each subject's paired plasma cortisol value on a single day at the end of each dietary period using values drawn at 0700, 1130, 1600 and 2100. Values in bold represent *P* < 0.05 for *y*-intercept difference and *P* = 0.0129 for slope difference. The use of an additional general linear ANOVA model that fixed all slopes for both treatments to an identical value revealed additional evidence for a *y*-intercept difference (*P* = 0.0675).

Table 6. Effect of neutralization of endogenous acid production on urinary excretion of glucocorticoid metabolites

Study Period	Cortisol, nmol/24 h	THE, $\mu\text{g}/24\text{ h}$	THF, $\mu\text{g}/24\text{ h}$	Allo-THF, $\mu\text{g}/24\text{ h}$	THP+allo-THF/THE
Control	461 \pm 115	3,346 \pm 406	2,410 \pm 210	1,606 \pm 151	1.20 \pm 0.12
Experimental	383 \pm 94*	2,738 \pm 386*	2,098 \pm 190*	1,380 \pm 100*	1.27 \pm 0.14
Recovery	426 \pm 104	3,113 \pm 425	2,290 \pm 220	1,540 \pm 130	1.23 \pm 0.15

Values are means \pm SE. THE, tetrahydrocortisone; THF, tetrahydrocortisol. * $P < 0.05$.

izing alkali administered to very youthful male and female adults during the bone-anabolizing interval before achievement of peak bone mass can reproduce the bone metabolic effects of alkali plus potassium reported in postmenopausal osteoporotic women (46).

This study establishes that the arithmetically trivial degree of Western diet-induced CMA is part of an endocrine-metabolic continuum that includes the well-established hyperglucocorticoidism of moderate-to-severe CMA (39, 44, 48, 54). When even very modest CMA, of the magnitude produced by a Western diet, can result in increased cortisol secretion and plasma concentration, the intriguing possibility arises that idiopathic osteoporosis and/or postmenopausal osteoporosis might be modulated, at least in part, by hypercortisolism. That mild hyperglucocorticoidism of long duration can lead to osteoporosis is supported by a recent retrospective cohort observation that even very low glucocorticoid doses (i.e., <2.5 mg oral prednisolone daily) significantly increased both vertebral and nonvertebral fracture risk relative to age- and gender-matched controls (52). Because the increased cortisol secretion and associated increase in plasma concentration demonstrated in the present study are

probably of smaller magnitude than the net glucocorticoid effect achieved even by quite modest prednisone dosing, its contribution to long-term putative bone loss incurred by the Western diet would presumably require many years of adrenal hypersecretion. Importantly, the pathophysiology of glucocorticoid-induced osteoporosis in humans shares with postmenopausal osteoporosis the two fundamental features found in experimentally induced CMA, namely, decreased trabecular bone formation/osteoblast recruitment rate and a component of early accelerated resorption (14, 49).

The finding that the urinary ratio [THF+allo-THF]/THE is unchanged in the prolonged transition from/to hypercortisolism of diet-induced CMA suggests that 11β -hydroxysteroid dehydrogenase type 1 isoform (11β -HSD1) activity in liver and adipocytes and renal 11β -HSD2 activity are grossly normal. However, the skeletal activity of either or both HSD isozymes is not known to be reflected in the excretion rates of urinary metabolites. 11β -HSD1 is strongly expressed in normal human bone in both osteoblasts and osteoclasts, whereas 11β -HSD2 is weakly expressed and only in osteoblasts (16, 18). The glucocorticoid receptor may only be expressed in osteoblasts (5). The finding that administration of carbenoxolone, a potent inhibitor of both 11β -HSD isozymes, to normal subjects resulted in a significant decrease in pyridinoline and deoxypyridinoline excretion (18) suggests that variation in the activity of these isozymes in osteoblasts or osteoclasts or both can result in important alterations in glucocorticoid receptor-mediated action on bone metabolism. The recent *in vitro* findings that 11β -HSD1 (cortisol-generating) activity in human osteoblasts is increased by increasing ambient cortisol concentrations and that its osteoblastic activity is increased as a function of a subject's age provide evidence that even very small increases in plasma cortisol concentrations in humans may be subject to autocrine amplification loops deleterious to skeletal function (17). Thus the effects of CMA on these isozymes in bone remains an important unanswered question.

The present results provide the first evidence in any species that the alkali (as exchanged for chloride) vs. acid content of a diet per se, rather than the specific effect of a coadministered alkali-associated cation (sodium or potassium), modulates bone resorption and the associated alterations in calcium and phosphate homeostasis. Whether alkali per se has a clear role has remained a question because studies in which NaHCO_3 and sodium citrate were administered have found little

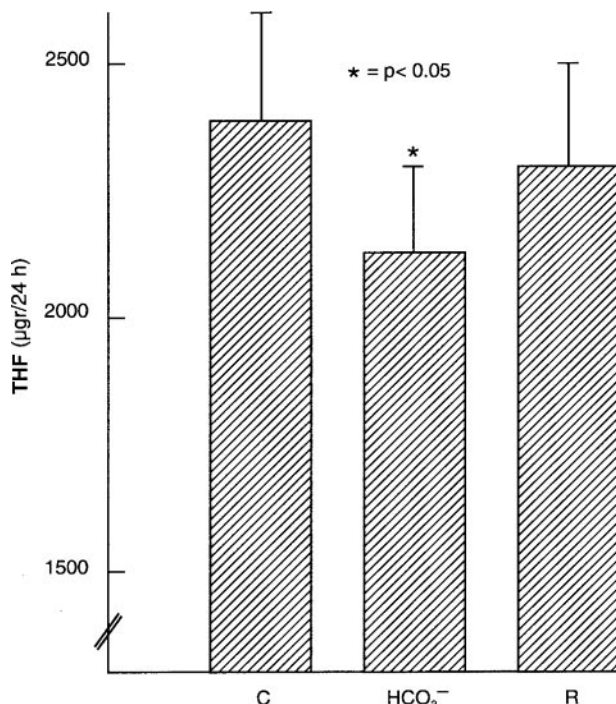


Fig. 3. Effect of HCO_3^- administration on 24-h urinary excretion rates of tetrahydrocortisol (THF).

effect on calcium excretion (34, 38, 45), whereas those administering KHCO_3 or potassium citrate have found significant reductions of large magnitude (38, 45, 46). The present study, by holding cation intake constant and exchanging equimolar HCO_3^- for chloride, has eliminated the argument that modest alkali treatment per se might be insufficient to cause urinary calcium retention in the absence of a concomitant increase in potassium intake. A specific role for the reduction in chloride intake in producing renal calcium and phosphate retention and decreased markers of bone resorption was not apparent in the present study, but it was not rigorously excluded.

Part of the confusion over the relative hypocalciuric roles of alkali and coadministered cation has arisen because of the interpretation of a study in adult male subjects ingesting a normal diet in which sequential 4-day periods of KCl and then KHCO_3 administration were undertaken (37). Both the authors of that study and others (12) have interpreted those data as indicating that KCl as well as KHCO_3 administration to potassium-replete subjects resulted in decreased urinary calcium excretion, yet the reported data for the 4 days of KCl administration (unlike the KHCO_3 results) showed no significant difference in calcium excretion relative to paired control values in the same subjects despite a similar magnitude of potassium retention with both potassium salts (37). Furthermore, in contrast to significant hypercalciuria produced by prolonged NaCl loading, no effect of prolonged KCl loading on calcium excretion was reported in healthy young women (3). On the basis of the in vivo literature to date, small alkali-independent effects of primary alterations in potassium balance on calcium retention would be difficult to detect in initially normokalemic animals or humans and have not been reported. However, by the use of a very low extracellular fluid potassium concentration of 1.0 mM, cultured murine calvariae exhibited an effect of low medium potassium concentration to increase calcium efflux, to increase a bone resorption marker, and to decrease bone collagen synthesis (12) in the absence of detectable acid-base change. The applicability of the in vitro data in calvariae to human potassium depletion is uncertain because the significant hypercalciuria reported in diet-induced potassium depletion in normal subjects was accompanied by renal NaCl retention and weight gain, suggesting a role for extracellular fluid volume expansion in the etiology of hypercalciuria (26). Thus whether the bone catabolism findings for a potassium-depleting environment in vitro predict an in vivo bone anabolic effect of potassium loading in potassium-replete humans awaits future studies.

The present studies do not exclude cortisol-independent mechanisms for mild CMA-induced reversible effects on bone metabolism. Local mechanisms in bone have been elucidated that might explain the effect of CMA in causing bone loss. In mature mouse osteoclasts in culture, acidified medium results in upregulation of both carbonic anhydrase II and calcitonin receptor, the former being associated with increased resorptive ac-

tivity and the latter with suppressed osteoclastic activity (12). In cultured murine calvariae, acidification of the medium results in calcium efflux accompanied by enhanced PGE_2 production (30), and calcium efflux is inhibited by both nonselective cyclooxygenase (COX) inhibitors and COX-2-selective agents (31). Cultured osteoclasts also exhibit important morphological and functional changes to acidified media that include formation of the resorbing clear zone podosomes (4) as well as augmentation of the final step in resorption, activity of the V-type plasma membrane H^+ -ATPase (43). Thus CMA-induced bone catabolism might conceivably be mediated within bone by a variety of plausible mechanisms. We also cannot exclude the possibility that alkali-induced increases in distal HCO_3^- delivery might have resulted in suppressed bone resorption as mediated by tubular calcium retention. We believe that this is an unlikely mechanism in the present study because we found no alkali-induced suppression of PTH levels nor ionized hypercalcemia, which are recognized as the mediators of the small suppressive effects of calcium loading on bone resorption markers reported in young adults (6).

We did not find evidence for an effect of neutralization of acidogenic diet on other endocrine axes [i.e., GH/IGF-1, PTH/1,25(OH) $_2$ D, and thyroid hormones], which are important to bone integrity and are affected by acidosis (Table 3). However, subtle regulatory alterations cannot be excluded, i.e., altered sensitivity of feedback loops and end-organ hormone (i.e., GH) sensitivities.

In summary, we have provided novel evidence that ingestion of an ordinary acidogenic Western diet to normal young adult subjects results in a mild CMA in association with a state of increased cortisol secretion and plasma concentration, altered divalent ion metabolism, and increased bone-resorptive indices. Because mild hyperglucocorticoidism is reported to result in an osteoporotic state that shares numerous qualitative and quantitative histomorphometric features with postmenopausal osteoporosis and with experimental CMA in animals, it is proposed that CMA-induced cortisol excess may play a role in mild CMA-induced alterations in bone metabolism in humans and possibly in osteoporosis associated with the Western acidogenic diet.

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