Kidney International, Vol. 28 (1985), pp. 951–958

Hydrochlorothiazide inhibits bone resorption in men despite experimentally elevated serum 1,25-dihydroxyvitamin D concentrations

JACOB LEMANN, JR., RICHARD W. GRAY, WILLIAM J. MAIERHOFER, and HERMAN S. CHEUNG

Departments of Medicine and Biochemistry and the Clinical Research Center, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Hydrochlorothiazide inhibits bone resorption in men despite experimentally elevated serum 1,25-dihydroxyvitamin D concentrations. We evaluated the effects of hydrochlorothiazide administration in relation to Ca balance, the PTH and vitamin D endocrine systems, acid-base balance, and bone. We studied six healthy men fed constant diets providing only 5.1 ± 0.7 SD mmoles Ca/day. Three of the men were also given calcitriol, 0.5 μg 6-hrly throughout their studies. All subjects were observed during 18 control days and then during 18 days of hydrochlorothiazide (HTZ) administration, 25 mg 12-hrly. Observations during control days 11 through 16 were compared to those during days 7 through 18 of HTZ administration, inclusively. Directional changes during HTZ did not differ among subjects not given or given calcitriol. For all six subjects, control net intestinal Ca absorption, serum 1,25-$(OH)_2$-D concentrations, serum iPTH concentrations, and daily urine cAMP excretion averaged 0.5 ± 2.2 mmoles/day, 162 ± 51 pM, 4.3 ± 2.2 μl Eq/ml and 4.2 ± 0.9 μmoles/day, respectively; none changed during HTZ. As expected, HTZ administration was accompanied by a fall in urinary Ca excretion, averaging − 1.4 ± 0.8 mmoles/day; $P < 0.01$. HTZ administration was also accompanied by less negative Ca balances, averaging + 1.6 ± 1.0 mmoles/day; $P < 0.025$, and by a fall in daily urinary hydroxyproline excretion averaging − 0.13 ± 0.09 mmoles/day; $P < 0.025$. We interpret these data to indicate that HTZ administration is accompanied by an inhibition of bone resorption. HTZ administration also raised serum HCO_3 concentrations by + 2.7 ± 0.5 mEq/liter; $P < 0.001$ and blood pH by + 0.05 ± 0.02 units; $P < 0.005$. Since HTZ administration did not change either serum iPTH or 1,25-$(OH)_2$-D concentrations nor urinary cAMP excretion, inhibition of bone resorption may be mediated by either relative alkalosis, a reduced skeletal sensitivity to PTH or 1,25-$(OH)_2$-D, or, possibly, by a direct effect of HTZ on bone.

L'hydrochlorothiazide inhibe la résorption osseuse chez l'homme en dépit de concentrations expérimentalement élevées de la 1,25-dihydroxy-vitamine D sérique. Nous avons étudié les effets de l'administration de l'hydrochlorothiazide sur la balance calcique, la PTH, la vitamine D, la balance acido-basique et l'os. Nous avons étudié 6 sujets sains recevant un régime constant amenant seulement 5,1 ± 0,7 SD mmoles de calcium par jour. Trois de ces hommes recevaient également du calcitriol, 0,5 μg toutes les 6 heures tout au long de l'étude. Tous les sujets ont été étudiés pendant une période contrôle de 18 jours et ensuite pendant 18 jours d'administration de l'hydrochlorothiazide (HTZ), 25 mg toutes les 12 heures. On a comparé les résultats obtenus entre les jours 11 et 16 inclus de la période contrôle et ceux obtenus 7 et 18 inclus de l'administration de HTZ. Les modifications directionnelles pendant l'HTZ n'ont pas été différentes selon que les sujets ont reçu ou non du calcitriol. Pour les 6 sujets, l'absorption intestinale nette contrôle du calcium, les concentrations de 1,25-(OH)2-D sérique, les concentrations de iPTH sérique et l'excrétion quotidienne urinaire de cAMP étaient de 0,5 ± 2,2 mmoles/jour, 162 ± 51 pmoles, 4,3 ± 2,2 μlEq/ml et 4,2 ± 0,9 μmoles/jour, respectivement; aucune n'a été modifiée par l'HTZ. Comme prévu, l'administration d'HTZ s'est accompagnée d'une chute de l'excrétion urinaire du calcium, de 1,4 ± 0,8 mmoles/jour ; $p < 0,01$. L'administration d'HTZ a aussi été accompagnée par des balances du calcium moins négatives, de + 1,6 ± 1,0 mmoles/jour ; $p < 0,025$, et par une chute de l'excrétion urinaire quotidienne d'hydroxyproline de − 0,13 ± 0,09 mmoles/jour; $p < 0,025$. Nous interprétons ces résultats comme indiquant que l'administration d'HTZ est accompagnée par une inhibition de la résorption osseuse. L'administration d'HTZ a aussi augmenté les concentrations sériques de HCO3 de + 2,7 ± 0,5 mEq/l ; $p < 0,001$ et le pH sanguin de + 0,05 ± 0,02 unités, $p < 0,005$. Dans la mesure ou l'administration d'HTZ n'a pas modifié l'iPTH sérique ou la 1,25-(OH)2-D, ni l'excrétion urinaire de cAMP, l'inhibition de la résorption osseuse pourrait être provoquée soit par une alcalose relative, soit par une diminution de la sensibilité du squelette à la PTH ou à la 1,25-(OH)2-D ou peut-être par un effet direct de l'HTZ sur l'os.

Chronic administration of the thiazide diuretics has recently been observed to be accompanied by increased bone density in humans [1]. These drugs are well known for their ability to reduce urinary calcium excretion [2] by augmenting distal renal tubular calcium reabsorption [3]. However, net intestinal calcium absorption in humans is apparently not altered by the acute administration of thiazides [4, 5]. The combination of reduced urinary calcium excretion and unaltered net intestinal calcium absorption would necessarily result in the retention of calcium in the body, presumably in bone. The present studies were undertaken to re-evaluate the effects of thiazide diuretics on the components of calcium balance and bone resorption in relation to serum 1,25-$(OH)_2$-D concentrations as well as serum parathyroid hormone levels and acid-base homeostasis in healthy men.

Methods

We studied six healthy men, aged 22 to 29 years, in the Medical College of Wisconsin Clinical Research Center with their consent using a protocol approved by the Medical College of Wisconsin Human Research Review Committee. Each subject ate a constant whole-food diet throughout his study that provided for the group an average of only 5.1 ± 0.7 SD mmoles Ca/day. Two menus of comparable composition were alternated

Received for publication February 22, 1985,
and in revised form June 3, 1985

daily and duplicate diets were analyzed. The diets also contained 58.0 ± 4.2 mmoles PO_4/day, 18.8 ± 1.0 mmoles Mg/day, 178 ± 5 mmoles Na/day, 85 ± 4 mmoles K/day, 156 ± 9 mmoles Cl/day, and an estimated 3250 ± 330 kcal/day (47 ± 4 kcal/kg/day). The diets did not contain gelatin. Three of the six subjects were also given calcitriol, 0.5 μg, 6-hrly (at 0600, 1200, 1800 and 2400 hours) beginning 7 days before they started the constant diet while they ate a low-calcium diet at home and continuing throughout their studies. We chose to use the low-calcium diet because we wished to observe the subjects when net bone resorption must have been occurring. Healthy men cannot achieve calcium balance on such low calcium intakes [6] and Ca balance becomes even more negative during 1,25-$(OH)_2$-D administration because of the effect of this hormone to stimulate net bone resorption [6]. In addition, a low Ca intake would provide a setting in which a small effect of the thiazides to alter fecal Ca excretion and net intestinal Ca absorption might become detectible. Each subject was adapted to his constant diet for 4 days and then observed during two 6-day control periods and two additional days to permit passage of the carmine used to demarcate the end of the second control stool period. The subjects were then given hydrochlorothiazide (HTZ), starting at a dose of 12.5 mg, 12-hrly (at 0600 and 1800 hours) for 3 days and then 25 mg, 12-hrly while observations were continued during three 6-day experimental periods. Three subjects complained of weakness while receiving HTZ and, because of hypokalemia (serum K 3.3–3.5 mEq/liter), these three subjects were given supplementary KCl 28, 31, and 35 mEq/day. Although carmine was used to mark the 6-day stool periods, feces were actually pooled every 2 to 4 days to permit more frequent estimates of fecal mineral excretion and thus net intestinal mineral absorption. Twenty-four-hr urines were collected and, in addition, 6-day urine pools were prepared for some analyses. Fasting morning blood specimens were collected on five control and six experimental days and additional bloods were collected during the day at times corresponding to 2 and 4 hrs after the last meal or the last dose of calcitriol on four occasions during control and four occasions during HTZ administration.

The methods employed for the analyses of diets, urine, feces, and serum have been described [6, 7]. Parathyroid hormone was measured using antibody detecting both intact PTH and carboxy-terminal fragments kindly provided by Dr. Eduardo Slatopolsky, Washington University School of Medicine, St. Louis, Missouri, USA. Serum 1,25-$(OH)_2$-D concentrations were measured using the calf thymus receptor for competitive binding assay after extraction of the serum samples with acetonitrile and purification using sequential C_{18} and silica "Sep-paks" [8]. Twenty-three serum specimens with 1,25-$(OH)_2$-D concentrations ranging from 35 to 190 pM using HPLC purification and the chick duodenal receptor [7] exhibited similar values when also analyzed by the calf-thymus method; $r = 0.75$.

Results are presented as individual data, individual means ± SD, or the average of these individual means for the group ± SD. The means of the individual changes from control for the group during HTZ were evaluated by the paired t test. Fecal Ca excretion during control and during HTZ were also compared by two-way analysis of variance [9]. Because of the delay in adaptation to a low-calcium diet [6] and to the steady-state

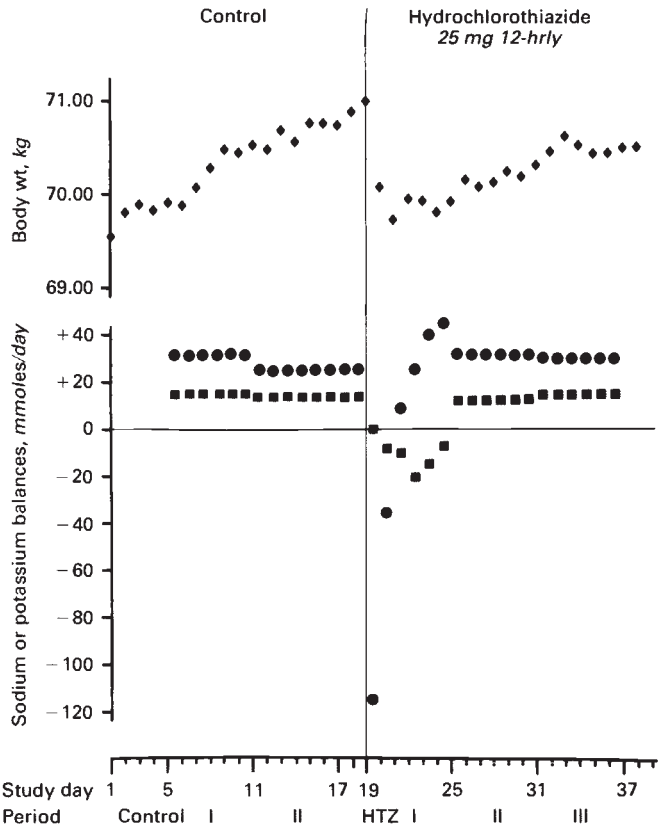

Fig. 1. *Mean daily body weights (♦) and mean daily sodium (●) and potassium balances (■) for the six subjects during control and during HTZ administration.* Urinary Na and K excretion rates were measured in 6-day urine pools during control and during days 7 through 18 of HTZ and daily during days 1 through 6 of HTZ.

effects of HTZ, average balances during the second control period were compared to those during the second and third HTZ periods. Since the directional changes from control during HTZ were similar regardless of the administration of calcitriol, data for all six subjects were pooled to simplify their presentation.

Results

The results are summarized in Figures 1 through 4 and Tables 1 through 4.

Control conditions

As shown in Figure 1, average body wts for the group increased slightly during control, but were nearly stable during the second control period (Table 2). Sodium and potassium balances were positive but stable (Fig. 1 and Table 2). The other measured constituents of the blood, feces, and urine were also stable during the second control period (Figs. 2 and 3, Tables 1 through 4). We could not detect a diurnal variation in serum 1,25-$(OH)_2$-D concentrations [10, 11]. For the three subjects who ate the low-calcium diet alone, control serum 1,25-$(OH)_2$-D concentrations, without regard to time of day, averaged 121 ± 26, 109 ± 19, and 124 ± 20 pM. During the second control period, these subjects exhibited rates of net intestinal calcium absorption that averaged − 1.5 ± 0.3 mmoles/day (Fig. 2), a

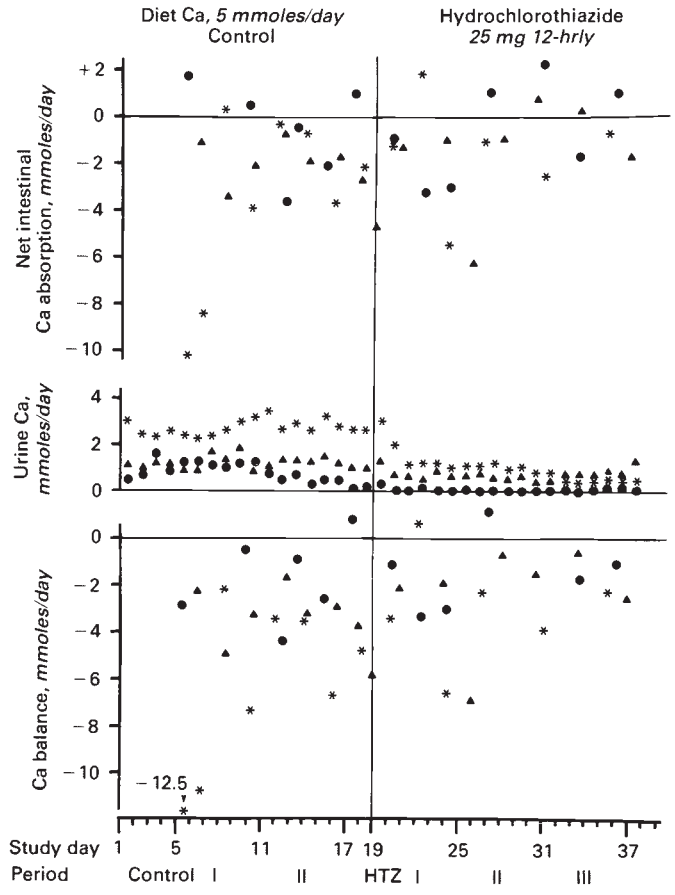

Fig. 2. *Net intestinal Ca absorption, urinary Ca excretion, and Ca balances before and during hydrochlorothiazide administration for the three subjects who ate the low-Ca diet alone.* Each *symbol* identifies a subject.

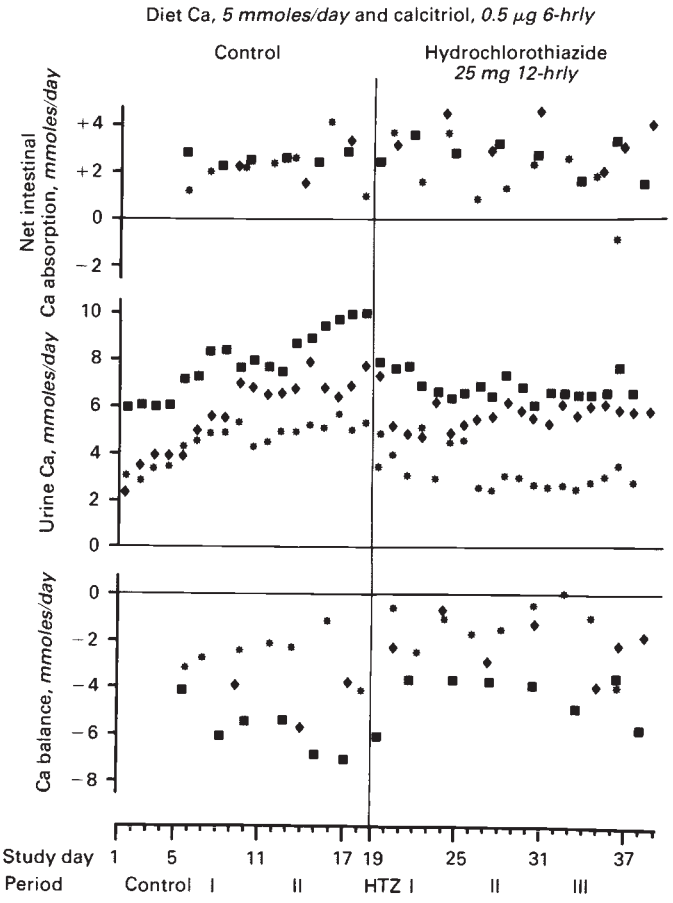

Fig. 3. *Net intestinal Ca absorption, urinary Ca excretion, and Ca balances before and during hydrochlorothiazide administration for the three subjects who ate the low-Ca diet and also were given calcitriol 0.5 μg 6-hrly continuously.* Each *symbol* identifies a subject.

value significantly less than zero but within the range of − 2 to + 1 mmoles/day expected for subjects adapted to a low-calcium diet [6]. Calcium balances for these three subjects averaged − 3.0 ± 1.5 mmoles/day. For the three subjects who also received calcitriol, control serum 1,25-$(OH)_2$-D levels averaged 181 ± 47, 226 ± 27, and 211 ± 26 pM. During the second control period, these three subjects exhibited, as expected, positive rates of net intestinal calcium absorption that averaged 2.6 ± 0.6 mmoles/day (Fig. 3), a significantly positive value ($P <$ 0.025). Nevertheless, because of a greater increase in urinary calcium excretion, their calcium balances averaged − 4.4 ± 2.5 mmoles/day, a value that was slightly more negative than that observed for the subjects who ate the low-calcium diet alone. [6].

Effects of hydrochlorothiazide

As shown in Figure 1, the administration of HTZ was accompanied, as expected, by transiently more negative Na and K balances and a loss in body wt. The maximum loss in body wt occurred during the first two days of HTZ administration and averaged − 1.27 kg for all six subjects. During these two days, Na balance became more negative by an average total of 203 mEq when expressed as the cumulative change from control. Potassium losses continued after the sodium diuresis. Cumulative potassium balances during the first six days of HTZ averaged for the group − 145 mEq, corrected for average daily control balances. During the second and third 6-day periods of HTZ administration, body wt rose slightly as in the second control period and Na and K balances were similar to control. As shown in Figures 2 and 3, HTZ administration promptly reduced urinary calcium excretion in all subjects regardless of calcitriol administration, an effect that was sustained during the second and third HTZ periods, at the end of which body wt had been restored to levels indistinguishable from control (Table 2). HTZ had no detectable effect on net intestinal calcium absorption (Figs. 2 and 3, Table 2). Thus, as a consequence of the persistent decrease in urinary calcium excretion, average calcium balances became significantly less negative during the second and third HTZ periods by + 1.6 ± 1.0 mmoles/day; $P <$ 0.025 (Table 2). Hydrochlorothiazide had no effect on fecal or urinary excretion rates or the balances of the other minerals during the second and third HTZ periods (Table 2). However, average daily fecal wet weight fell significantly (Table 2).

As shown in Table 3, the administration of HTZ was accompanied by a significant fall in daily urinary hydroxyproline excretion. Daily urinary creatinine excretion did not change, indicating that urine collections were appropriate and complete. However, because of the slight but significant rise in serum

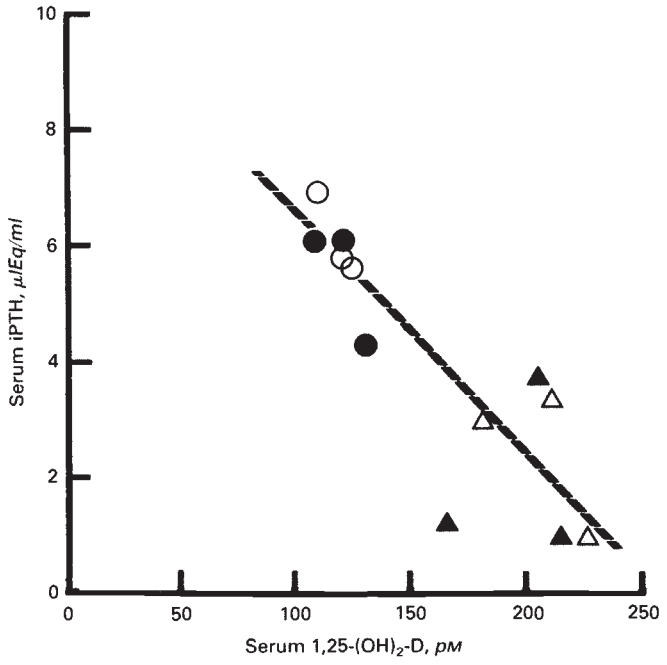

Fig. 4. *Individual mean serum iPTH concentrations as a function of individual mean serum 1,25-$(OH)_2$-D concentrations during control* (open symbols) *and during hydrochlorothiazide administration* (solid symbols) *for the subjects who ate the low-Ca diet alone* (circles) *and the subjects also given calcitriol* (triangles). y = 10.9–0.0423x, $r = -0.85$; $P < 0.001$.

creatinine (Table 1), estimated endogenous creatinine clearances fell slightly but significantly (Table 3). Neither serum phosphate concentrations (Table 1), nor the components of phosphate balance (Table 2) changed.

Hydrochlorothiazide had no effect on serum 1,25-$(OH)_2$-D concentrations (Table 1). Hydrochlorothiazide also had no effect on serum PTH concentrations (Table 1) nor on daily urinary cAMP excretion (Table 3). However, as shown in Figure 4, serum PTH concentrations were inversely correlated to serum 1,25-$(OH)_2$-D concentrations [7, 12, 13] regardless of the administration of HTZ.

Table 4 presents the effects of HTZ on serum bicarbonate concentrations, pCO_2, and blood pH as well as the components of acid balance during the second control and third HTZ periods. Hydrochlorothiazide administration, as expected, was accompanied by the development of a significant rise in serum bicarbonate without a change in pCO_2 and thus a rise in blood pH. The components of fixed acid production were unaffected by HTZ administration. The urine was slightly, but not significantly, more alkaline during HTZ administration and this change was accompanied by a slight, but significant, rise in urinary bicarbonate excretion. Offsetting this rise in urinary bicarbonate excretion was a slight, but significant, rise in urinary ammonium excretion. As a consequence of these opposite directional changes, net acid excretion did not change. Thus, since neither acid production nor acid excretion changed, acid balance, which was not different from zero during the control period, was unchanged during HTZ administration.

Discussion

Since the administration of HTZ was accompanied by less negative calcium balances together with a decrease in daily urinary hydroxyproline excretion, it would appear that HTZ inhibits bone resorption. The validity of the indirect estimates of net bone resorption in humans upon which this interpretation is based require consideration.

Calcium balances became significantly less negative during HTZ due solely to the fall in urinary calcium excretion, an effect that was sustained (Figs. 2 and 3) despite the slight rise in serum total calcium concentrations (Table 1) and the apparent restoration of extracellular fluid volume during days 7 through 19 of HTZ administration as judged by the return of body wt to control levels at the end of the studies and unchanged Na balances (Fig. 1 and Table 2). The possibility remains that we could have failed to detect a quantitatively equivalent decline in net intestinal calcium absorption because of the variability of this measurement (Figs. 2 and 3). When net intestinal Ca absorption for all six subjects during control and during HTZ were compared using two-way analysis of variance, we found that net intestinal absorption of Ca would have had to have decreased by an average of − 1.2 mmoles/day to detect a statistically significant ($P < 0.05$) compensatory fall in intestinal Ca absorption. Such a small change could have escaped detection. That HTZ has no effect to inhibit basal or 1,25-$(OH)_2$-D-stimulated net intestinal calcium transport in these men is consistent with previous studies in both humans [4, 5] and in rats [14].

It also might be argued that the mean retention of 1.6 mmoles Ca/day relative to control for the 12 days of the second and third HTZ periods (Table 2), cumulatively totaling 19.2 mmoles, might have been retained at sites other than bone. This possibility also seems unlikely. The rise in total serum calcium of 0.05 mmoles/liter, if distributed in the extracellular fluid (neglecting protein binding of calcium in plasma) could account for less than 5% (less than 1 mmoles) of the retained calcium. If calcium were retained within cells it would have to be continuously sequestered in a normally calcium-rich compartment such as mitochondria, but there is no evidence for such a potentially pathological process. Since long-term thiazide administration has not been reported to be accompanied by ectopic calcification, pathological sites of calcium deposition are also unlikely.

Daily excretion of total urinary hydroxyproline reflects both collagen synthesis and degradation in bone as well as in other tissues such as the skin and also has been adopted as a rough index of both bone turnover and bone resorption [15]. We have previously observed that daily urinary hydroxyproline excretion rates are inversely correlated to calcium balances in healthy subjects studied at varying levels of dietary calcium intake and, in addition, when net bone resorption was stimulated by the administration of calcitriol to subjects fed a low-calcium diet [6, 7]. These considerations thus also support the interpretation that the decline in daily urinary hydroxyproline excretion during HTZ administration accompanying the less negative calcium balances reflected inhibition of bone resorption.

Although the present studies do not provide precise insights

Table 1. Mean blood composition in six men during control and during hydrochlorothiazide administration

	Control	HTZ	Δ	*P*
Serum total Ca, *mmoles/liter*	2.36 ± 0.07	2.42 ± 0.08	0.06 ± 0.02	< 0.005
PO_4, *mmoles/liter*	1.48 ± 0.07	1.48 ± 0.11	0 ± 0.05	NS
Mg, *mmoles/liter*	0.89 ± 0.07	0.90 ± 0.10	0.01 ± 0.06	NS
iPTH, *μlEq/ml*	4.4 ± 1.8	3.6 ± 1.9	−0.8 ± 0.9	NS
1,25-$(OH)_2$-D, *pmoles/liter*	162 ± 51	157 ± 45	−5 ± 8	NS
25-OH-D, *nmoles/liter*	60 ± 17	60 ± 17	0 ± 6	NS
Creatinine, *μmoles/liter*	99 ± 8	105 ± 9	6 ± 4	< 0.01
Na, *mmoles/liter*	142 ± 1	142 ± 1	0 ± 1	NS
K, *mmoles/liter*	4.0 ± 0.2	3.5 ± 0.2	−0.5 ± 0.2	< 0.001
Cl, *mmoles/liter*	106 ± 1	100 ± 1	−6 ± 1	< 0.001
Total protein, *g/dl*	6.5 ± 0.4	6.4 ± 0.4	−0.1 ± 0.4	NS

Table 2. Body weight, fecal weight, and mineral balances

	Control (Period II)	HTZ (Periods II and III)	Δ	*P*
Initial body wt, *kg*	70.53 ± 13.41	69.93 ± 13.20		
Final body wt, *kg*	70.75 ± 13.15	70.49 ± 13.29		
Δ wt, *kg/day*	+0.04 ± 0.07	+0.05 ± 0.03	+0.01 ± 0.08	NS
Fecal wt, *g/day*	126 ± 25	99 ± 29	−27 ± 24	< 0.05
Diet Ca, *mmoles/day*	5.1 ± 0.7	5.1 ± 0.7	0	NS
Fecal Ca, *mmoles/day*	4.6 ± 2.4	4.4 ± 2.6	−0.2 ± 1.1	NS
Net intestinal Ca absorption, *mmoles/day*	+0.5 ± 2.2	+0.7 ± 2.3	+0.2 ± 1.1	NS
Urine Ca, *mmoles/day*	4.3 ± 3.3	2.9 ± 2.7	−1.4 ± 0.8	< 0.01
Ca balance, *mmoles/day*	−3.8 ± 2.0	−2.2 ± 1.3	−1.6 ± 1.0	< 0.025
Diet PO_4, *mmoles/day*	58.0 ± 4.2	58 ± 4.2	0	NS
Fecal PO_4, *mmoles/day*	16.7 ± 2.5	15.0 ± 4.7	−1.7 ± 4.5	NS
Net intestinal PO_4 absorption, *mmoles/day*	41.3 ± 3.9	43.0 ± 7.1	+1.7 ± 4.5	NS
Urine PO_4, *mmoles/day*	32.7 ± 5.4	35.0 ± 3.8	2.3 ± 2.6	NS
PO_4 balance, *mmoles/day*	+8.6 ± 4.1	+8.0 ± 7.6	−0.6 ± 4.9	NS
Diet Mg, *mmoles/day*	18.8 ± 1.0	18.8 ± 1.0	0	NS
Fecal Mg, *mmoles/day*	13.2 ± 2.4	11.6 ± 2.9	−1.6 ± 3.0	NS
Net intestinal Mg absorption, *mmoles/day*	5.6 ± 2.7	7.2 ± 2.5	+1.6 ± 3.0	NS
Urine Mg, *mmoles/day*	4.4 ± 0.8	5.0 ± 0.6	+0.6 ± 0.6	NS
Mg balance, *mmoles/day*	1.2 ± 2.4	+2.2 ± 2.4	+1.0 ± 3.2	NS
Diet Na, *mmoles/day*	178 ± 5	178 ± 5	0	NS
Fecal Na, *mmoles/day*	3 ± 1	1 ± 1	−2 ± 2	NS
Net intestinal Na absorption, *mmoles/day*	175 ± 6	177 ± 5	+2 ± 2	NS
Urine Na, *mmoles/day*	149 ± 12	145 ± 12	−4 ± 9	NS
Na balance, *mmoles/day*	+26 ± 7	+32 ± 9	+6 ± 10	NS
Diet K, *mmoles/day*	85 ± 4	100 ± 18	+15 ± 17	NS
Fecal K, *mmoles/day*	15 ± 2	13 ± 3	−2 ± 4	NS
Net intestinal K absorption, *mmoles/day*	70 ± 4	87 ± 19	+17 ± 19	NS
Urine K, *mmoles/day*	56 ± 5	73 ± 11	+17 ± 17	NS
K balance, *mmoles/day*	+14 ± 5	+14 ± 11	0 ± 9	NS
Diet Cl, *mmoles/day*	156 ± 9	172 ± 22	+16 ± 17	NS
Fecal Cl, *mmoles/day*	1 ± 1	1 ± 1	0 ± 1	NS
Net intestinal Cl absorption, *mmoles/day*	155 ± 8	171 ± 22	+16 ± 17	NS
Urine Cl, *mmoles/day*	132 ± 10	138 ± 14	+6 ± 15	NS
Cl balance, *mmoles/day*	+23 ± 16	+33 ± 21	+10 ± 10	NS

into the mechanism or mechanisms by which HTZ administration apparently inhibits bone resorption, several possibilities can be considered.

First, if the rise in serum total calcium concentration can be taken as an index of ionized calcium (and not a rise in the concentration of protein-bound calcium due either to persistent diuretic-induced volume depletion or alkalosis), such a rise might theoretically directly inhibit bone resorption. However, elevation of medium Ca concentrations did not inhibit either basal or PTH-stimulated bone resorption in cultured fetal bone [16].

Second, if a rise in ionized calcium occurred, such an increase could have indirectly inhibited bone resorption by a suppression of PTH secretion. Some previous studies have suggested that PTH is necessary for the anti-calciuric effects of the thiazide diuretics [17, 18], but not for the anticalciuric effects of chlorthalidone [19]. PTH was clearly present among our subjects, but lower among those given calcitriol (Fig. 4). These variations in basal serum PTH levels did not alter the response to HTZ. Moreover, we could not detect changes in either serum PTH concentrations, daily urinary cAMP excretion rates, serum phosphate concentrations, or daily urinary phosphate excretion during HTZ administration. Thus it appears unlikely that the apparent decline in bone resorption

Table 3. Daily urine volume, creatinine excretion, creatinine clearance, hydroxyproline, cAMP, and oxalate excretion during control and during hydrochlorothiazide administration

	Control	HTZ	Δ	*P*
Volume, *ml/day*	2480 ± 570	2660 ± 600	+180 ± 300	NS
Creatinine, *mmoles/day*	16.7 ± 1.5	16.9 ± 1.6	+0.2 ± 0.6	NS
Creatinine clearance, *liters/day*	166 ± 19	161 ± 19	−5 ± 4	< 0.05
Hydroxyproline, *mmoles/day*	0.39 ± 0.04	0.26 ± 0.08	−0.13 ± 0.09	< 0.025
cAMP, *μmoles/day*	4.2 ± 0.9	4.5 ± 1.2	+0.3 ± 0.8	NS
Oxalate, *mmoles/day*	0.46 ± 0.04	0.46 ± 0.04	0.00 ± 0.12	NS

Table 4. Blood pH, serum HCO_3, and the components of acid balance during control and during hydrochlorothiazide administration

	Control	HTZ	Δ	*P*
Blood pH	7.36 ± 0.03[a]	7.41 ± 0.03	+0.05 ± 0.02	< 0.005
H^+, *μEq/liter*	43.1 ± 2.8	39.1 ± 2.2	−4.0 ± 1.9	< 0.005
pCO_2, *mm Hg*	47 ± 5	47 ± 5	0 ± 2	NS
HCO_3, *mEq/liter*	26.0 ± 1.9	28.7 ± 1.6	+2.7 ± 0.5	< 0.001
Diet unmeasured anion,[b] *mEq/day*	50 ± 16	50 ± 16	0	NS
Fecal unmeasured anion,[b] *mEq/day*	23 ± 4	19 ± 5	−4 ± 5	NS
Urine SO_4, *mEq/day*	37 ± 4	41 ± 4	+4 ± 5	NS
Urine organic anion, *μEq/day*	48 ± 10	49 ± 10	+1 ± 3	NS
Net fixed acid production, *mEq/day*	58 ± 24	59 ± 23	+1 ± 10	NS
Urine pH	5.94 ± 0.41	6.11 ± 0.36	+0.17 ± 0.23	NS
Urine NH_4^+, *mEq/day*	43 ± 4	49 ± 5	+6 ± 3	< 0.01
Urine titratable acid, *mEq/day*	23 ± 7	22 ± 6	−1 ± 4	NS
Urine HCO_3^-, *mEq/day*	3 ± 3	6 ± 3	+3 ± 2	< 0.025
Renal net acid excretion,[c] *mEq/day*	63 ± 7	65 ± 6	+2 ± 5	NS
Acid balance, *mEq/day*	−5 ± 24	−6 ± 20	+1 ± 10	NS

[a] Standard deviation.
[b] Unmeasured anion in diet and in feces calculated as (Σ Na + K + Ca + Mg, *mEq/day*) − (Σ Cl *mEq/day* + 1.8 P *mmoles/day*).
[c] Renal net acid excretion = NH_4 + titratable acid − HCO_3.

during HTZ could have been mediated by suppression of PTH secretion.

Third, elevated serum 1,25-$(OH)_2$-D levels stimulate bone resorption when dietary calcium intake is low [16]. Since serum 1,25-$(OH)_2$-D levels did not fall during HTZ neither interference with renal synthesis of the hormone in the three subjects not given calcitriol nor accelerated catabolism of the 1,25-$(OH)_2$-D in any of the subjects could have occurred. Thus, a fall in serum 1,25-$(OH)_2$-D levels could not have accounted for the apparent inhibition of bone resorption accompanying HTZ administration.

Fourth, relative alkalosis may have played a role. We have reviewed 39 previous balance studies that we have carried out among healthy volunteers eating a variety of diets providing normal quantities of calcium while the subjects ate the diet alone or were also given ammonium chloride [20–23]. In those studies (Fig. 5), we have observed that serum bicarbonate concentrations were inversely correlated to daily net external acid balance: serum bicarbonate, mEq/liter = 24.5 − 0.087 acid balance, mEq/day; $r = -0.56$. Calcium balances were also inversely correlated to acid balances: calcium balance, mmoles/day = − 0.23 − 0.18 acid balance, mEq/day; $r = -0.79$. Thus, calcium balances were directly correlated to serum bicarbonate concentrations: calcium balance, mmoles/day = − 23.4 + 0.93 serum bicarbonate, mEq/liter; $r = 0.64$. If the changes in serum bicarbonate concentrations participate in mediating the changes in calcium balance, then the slope of the latter equation would predict that a rise in serum bicarbonate concentration of 2.7 mEq/liter (the mean change during HTZ in the present studies, Table 4) should be accompanied by more positive calcium balances averaging + 2.5 mmoles/day. Such a change is similar in magnitude to the mean change in calcium balance of + 1.6 mmoles/day that accompanied HTZ administration. This effect is consistent with recent observations showing the effect of oral $NaHCO_3$ administration to improve calcium balance when acid production is augmented by increasing dietary protein intake [24].

Fifth, consideration must be given to the possibility that HTZ either blunted the sensitivity of bone to the resorbing effects of PTH or 1,25-$(OH)_2$-D or in some manner directly reduced bone resorption. No studies of the effects of HTZ in cultured bone are apparently available. The thiazide diuretics do have carbonic anhydrase inhibitory activity and it has recently been observed that acetazolamide inhibits 1,25-$(OH)_2$-D-stimulated bone resorption in organ culture [25] and that PTH stimulation of bone resorption in organ culture is accompanied by increased carbonic anhydrase activity [26]. Thus, such a mechanism may mediate the effect of HTZ to inhibit bone resorption.

Finally, the present studies provide further support for the view that chronic HTZ administration either augments or preserves bone mass in human beings. The less negative calcium balance of 1.6 mmoles/day if cumulated over one year would be approximately 580 mmoles of calcium, or approximately 2.3% of the 25,000 mmoles of calcium contained in the skeleton of an adult of average body size. It seems likely that there is eventual escape from the effect of HTZ to promote Ca retention. Although chronic HTZ therapy is accompanied by a

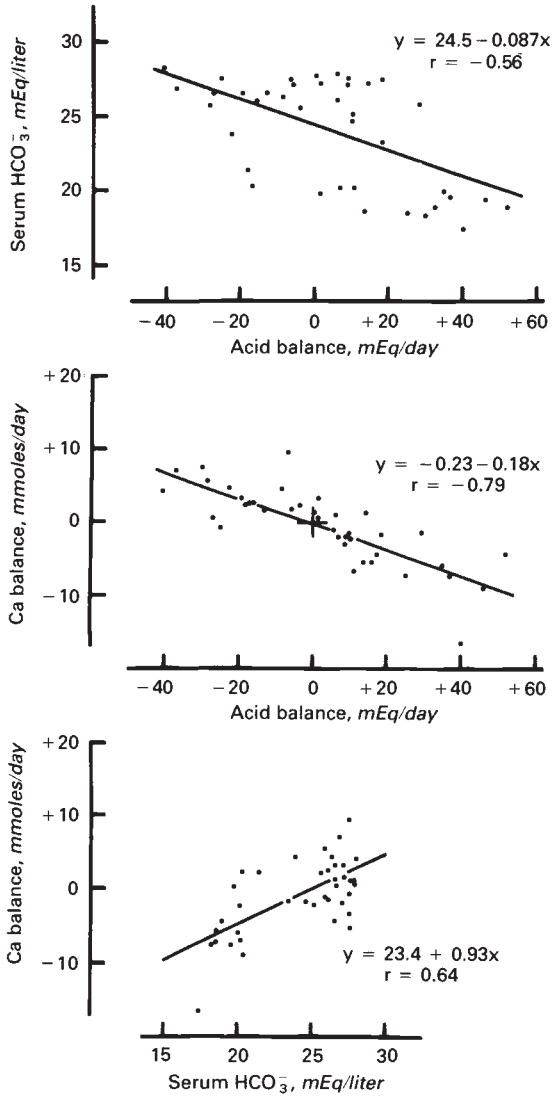

Fig. 5. *Serum HCO_3 in relation to daily acid balance,* (upper panel) *Ca balance in relation to acid balance,* (middle panel), *and Ca balance in relation to serum HCO_3* (lower panel). Previous studies in subjects eating normal diets or also given NH_4Cl [20–23].

sustained reduction in urinary Ca excretion [4], intestinal Ca absorption is often [27, 28], but not always, [4, 27] reduced.

Acknowledgments

The authors gratefully acknowledge the expert planning and production of the diets provided by Miss Joan Pleuss and her staff, the nursing care provided by Miss Bernadette Klapinski and her staff, and the skilled analyses provided by the Medical College of Wisconsin Clinical Research Center, as well as the advice and assistance of Raymond G. Hoffmann, Ph.D., of the Medical College of Wisconsin Biostatistics Center.

This study was supported by United States Public Health Service Grants RR-00058, RO1 AM-15089, RO1 AM-22014, and KO4 AM-01065 (Dr. Cheung).

Reprint requests to Dr. J. Lemann, Jr., Professor of Medicine, Chief Nephrology Section, Medical College of Wisconsin, Department of Medicine, Nephrology Section, Froedtert Memorial Lutheran Hospital, 9200 West Wisconsin Avenue, Milwaukee, Wisconsin 53226 USA

References

1. Wasnich RD, Benfante RJ, Yano K, Heilbrun L, Vogel JM: Thiazide effect on the mineral content of bone. *N Engl J Med* 309:344–347, 1983
2. Lichtwitz A, Parlier R, De Seze S, Hioco D, Miravet L: L'effet hypocalciurique des sulfamide diuretiques. *Sem Hos Paris* 37:2350–2362, 1961
3. Costanzo LS, Windhager E: Calcium and sodium transport by distal convoluted tubule of the rat. *Am J Physiol* 235:F492–F506, 1978
4. Yendt ER, Gagni RJA, Cohanim M: The effects of thiazides in idiopathic hypercalciuria. *Am J Med Sci* 251:449–460, 1966
5. Harrison AR, Rose GA: The effect of bendrofluazide on urinary and faecal calcium and phosphorus. *Clin Sci* 34:343–350, 1968
6. Maierhofer WJ, Gray RW, Cheung HS, Lemann J Jr: Bone resorption stimulated by elevated serum 1,25-$(OH)_2$-vitamin D concentrations in healthy men. *Kidney Int* 24:555–560, 1983
7. Maierhofer WJ, Lemann J Jr, Gray RW, Cheung HS: Dietary calcium and serum 1,25-$(OH)_2$-vitamin D concentrations as determinants of calcium balance in healthy men. *Kidney Int* 26:752–759, 1984
8. Reinhardt TA, Horst RL, Orf JW, Hollis BW: A microassay for 1,25-dihydroxyvitamin D not requiring high performance liquid chromatography: application to clinical studies. *J Clin Endocrinol Metab* 58:91–98, 1984
9. Snedocor GS, Cochran WG: *Statistical Methods* (7th ed), Ames, Iowa, Iowa State Press, 1980
10. Adams ND, Gray RW, Lemann J Jr: The effects of oral $CaCO_3$ loading and dietary calcium deprivation on plasma 1,25-dihydroxyvitamin D concentrations in healthy adults. *J Clin Endocrinol Metab* 48:1008–1016, 1979
11. Manolagas SC, Deftos LJ: No diurnal variations in calcitonin and 1,25-$(OH)_2$-D. *N Engl J Med* 312:122–123, 1985
12. Portale AA, Booth BE, Halloran BP, Morris RC Jr: Effect of dietary phosphorus on circulating concentrations of 1,25-dihydroxyvitamin D and immunoreactive parathyroid hormone in children with moderate renal insufficiency. *J Clin Invest* 73:1580–1589, 1984
13. Slatopolsky E, Weerts C, Thielan J, Horst R, Harter H, Martin KJ: Marked suppression of secondary hyperparathyroidism by intravenous administration of 1,25-dihydroxycholecalciferol in uremic patients. *J Clin Invest* 74:2136–2143, 1984
14. Favus MJ, Coe FL, Kathpulin SC, Porat A, Sen PK, Sherwood LM: Effects of chlorothiazide on 1,25-dihydroxyvitamin D_3, parathyroid hormone and intestinal calcium absorption in the rat. *Am J Physiol* 242:G575–G581, 1983
15. Dull TA, Henneman PH: Urinary hydroxyproline as an index of collagen turnover in bone. *N Engl J Med* 268:132–134, 1963
16. Raisz LC: Bone resorption in tissue culture: factor influencing the response to parathyroid hormone. *J Clin Invest* 44:103–116, 1965
17. Brickman AS, Massry SG, Coburn JW: Changes in serum and urinary calcium during treatment with hydrochlorothiazide: studies on mechanims. *J Clin Invest* 51:945–954, 1972
18. Parfitt AM: The interactions of thiazide diuretics with parathyroid hormone and vitamin D: *J Clin Invest* 51:1879–1888, 1972
19. Porter RH, Cox BG, Heaney D, Hostetter TH, Stinebaugh BJ, Suki WN: Treatment of hypoparathyroid patients with chlorthalidone. *N Engl J Med* 298:577–581, 1978
20. Lennon EJ, Lemann J Jr, Litzow JR: The effects of diet and stool composition on the net external acid balance of normal subjects. *J Clin Invest* 45:1601–1607, 1966
21. Lemann J Jr, Litzow JR, Lennon EJ: The effects of chronic acid loads in normal man: further evidence for the participation of bone mineral in the defense against chronic metabolic acidosis. *J Clin Invest* 45:1608–1614, 1966
22. Weber HP, Gray RW, Dominguez JH, Lemann J Jr: The lack of effect of chronic metabolic acidosis on 25-OH-vitamin D metabolism and serum parathyroid hormone in humans. *J Clin Endocrinol Metab* 43:1047–1055, 1976
23. Adams ND, Gray RW, Lemann J Jr: The calciuria of increased fixed acid production: evidence against a role for parathyroid

hormone and 1,25-$(OH)_2$-vitamin D. *Calcif Tissue Int* 27:233–239, 1979
24. LUTZ J: Calcium balance and acid-base status of women as affected by increased protein intake and by sodium bicarbonate ingestion. *Am J Clin Nutr* 39:281–288, 1984
25. HALL GE, KENNEY AD: Role of carbonic anhydrase and 1,25-dihydroxycholecalciferol-induced bone resorption *(abstract). Calcif Tissue Int* 36:461, 1984
26. HALL GE, KENNEY AD: Parathyroid hormone increases carbonic anhydrase activity in cultured mouse calvaria *(abstract). Calcif Tissue Int* 35:682, 1983
27. EHRIG U, HARRISON JE, WILSON DR: Effect of long-term thiazide therapy on intestinal calcium absorption in patients with recurrent renal calculi. *Metabolism* 23:139–149, 1974
28. SAKHAEE K, NICAR MJ, GLASS K, ZERWEKH JE, PAK CYC: Reduction in intestinal calcium absorption by hydrochlorothiazide in postmenopausal osteoporosis. *J Clin Endocrinol Metab* 59:1037–1043, 1984