

Calcium-Regulated Renal Calcium Handling in Healthy Men: Relationship to Sodium Handling*

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ABSTRACT

Several studies have shown an increase in urinary calcium excretion in response to a calcium load. However, because of the inverse changes in PTH levels with a calcium load, the effect of changes in serum calcium *per se* on its own excretion is unclear in humans. In this study we used a PTH clamp protocol to further characterize calcium-regulated renal calcium and magnesium handling and the relationship of the former to sodium excretion.

Eight normal male subjects were evaluated using a calcium clearance protocol with graded calcium infusions under a PTH clamp while in balance during a high and then during a low sodium diet. The curves describing calcium and magnesium excretion as a function of serum ionized calcium on the high sodium diet were best fitted by

sigmoidal functions, with midpoints (the levels of calcium resulting in half-maximal increases in urinary cation excretion) of 1.51 and 1.49 mmol/L, respectively. The curve describing urinary sodium as a function of serum calcium was also sigmoidal on the high sodium diet, with a midpoint of 1.55 mmol/L.

Our data taken in conjunction with those of previous studies evaluating sodium and calcium excretion in diseases characterized by inactivating or activating mutations in the calcium receptor, are consistent with the hypothesis that PTH-independent, calcium-dependent changes in renal calcium, magnesium, and sodium handling may be mediated at least in part by this receptor, which is known to be located in the loop of Henle. (*J Clin Endocrinol Metab* **83**: 2366–2372, 1998)

THE KIDNEY plays a key role in the maintenance of mineral ion homeostasis, particularly that of calcium (Ca) (1, 2). Urinary calcium (UCa) excretion depends on the filtered load of Ca and on several other factors, including PTH levels, sodium (Na) excretion, serum concentrations of Ca and magnesium (Mg), Ca intake and absorption, and acid-base status (3). PTH enhances the tubular reabsorption of Ca, an important mechanism for the control of this cation's plasma concentration. In rodents, PTH acts on both the cortical thick ascending limb (CTAL), where about 25% of filtered Ca is reabsorbed, and the distal convoluted tubule, where another 8% is reabsorbed (4, 5). Volume expansion with saline causes a decline in both Na and Ca reabsorption by the kidney proximally and distally, thereby resulting in an increase in the excretion of both cations (6–8).

In vivo and *in vitro* studies have suggested that in addition to acting indirectly by inhibiting PTH secretion, elevated concentrations of Ca and Mg also directly inhibit Ca and Mg reabsorption in the CTAL (9–12). Indeed, several Ca clearance studies have shown a steep increase in UCa excretion in response to an oral or iv load of Ca (13–16). The role of changes in Ca *per se* on its own excretion was, however, unclear because of the concomitant decrements in PTH levels in response to the Ca

loads in the above studies. Nevertheless, the fact that this increase in UCa excretion is preserved in patients with surgical hypoparathyroidism but is lost in hypoparathyroid patients with familial hypocalciuric hypercalcemia (FHH), who carry heterozygous inactivating mutations in the extracellular Ca-sensing receptor (CaR) (14), points to a central role of this receptor in UCa handling. Indeed, the CaR not only plays a key role in regulating PTH secretion (17–20), but it is also present in regions of the rat kidney where PTH regulates renal tubular Ca reabsorption and may itself modulate renal tubular handling of Ca (5, 21, 22). In the present study, we have, therefore, further characterized the direct effects of Ca on its own excretion 1) under a PTH clamp and 2) with rigorously controlled salt balance, two factors with significant impact on Ca excretion that have not been controlled for in previous studies evaluating the effects of changes in serum Ca on renal calcium handling in humans. The effect of changes in serum ionized calcium (SCa_i) levels on Mg and Na excretion were also evaluated for the following reasons: 1) there is coupling of Ca and Mg excretion in micropuncture and in clearance studies (9, 11); 2) the CaR senses not only changes in serum Ca but also those in serum Mg levels (21); 3) there are often parallel changes in Na and Ca excretion in clearance studies (11, 23–27); and 4) the coupling in Ca and Mg excretion seems to operate in part via Ca-induced inhibition of an apical potassium (K) channel that is needed for recycling of K into the lumen of the CTAL for continued activity of the apical NaK₂Cl cotransporter (28).

Materials and Methods

Subjects

Eight healthy men were studied. Before enrollment, each volunteer underwent a physical examination and a laboratory evaluation that included a multichannel chemistry analysis, a serum TSH determina-

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tion, and a complete blood count with differential. The study was reviewed and approved by the committee for the protection of human subjects of the Brigham and Women's Hospital. Informed written consent was obtained from each subject before participation.

Experimental design

Because of the significant impact of Na excretion on renal Ca handling, our Ca clearance studies were performed sequentially on the same subjects while in balance [as assessed by 24-h urinary sodium (UNa) excretion] during a high and then during a low salt diet. Balance was usually achieved between days 4 and 5 of each diet. The diet was provided by the metabolic kitchen of the General Clinical Research Center and contained 1.0–1.3 g protein/kg BW, 900–1100 mg Ca, 1000–1400 mg phosphorus, either 200 or 10 mEq Na, 400 ± 80 IU vitamin D, and 200–400 mg Mg/day. Subjects consumed their meals at 0800, 1300, and 1800 h and had a snack at 2100 h, except during the clearance protocol when they fasted until 1300 h. On the day of admission, an iv catheter was inserted for the administration of Parathar after blood was drawn for baseline chemistries and intact PTH (iPTH) levels. The Parathar infusion, human PTH-(1–34) (Rorer Pharmaceuticals, King of Prussia, PA), at a dose of 0.2 U/kg·h ($0.035 \mu\text{g}/\text{kg}\cdot\text{h}$) was started at 0800 h on day 1. An additional catheter was placed at 0700 h on day 2 for the Ca clearance protocol, which was performed after an overnight fast at the General Clinical Research Center with the subjects in a recumbent position except while voiding. The protocol was a modification of that described by Attie *et al.* (14) and is summarized in Fig. 1. We previously demonstrated that within 12 h after initiation of Parathar administration, as described above, a steady state was reached where iPTH levels were suppressed and N-terminal PTH levels were in the upper part of the normal range [normal range, up to 25 pg/mL in PTH-(1–34) units] (El-Hajj Fuleihan, G., unpublished observations). Blood for determination of baseline chemistries and SCa_i and iPTH levels was drawn via the iv catheter. Subjects drank 200 mL distilled water at 0700 h and again every 30 min throughout the clearance study to maintain urinary flow. At 0900 h, a control clearance protocol lasting 180 min was started with urine collections every 30 min and blood sampling in the middle of the corresponding urine clearance period (*e.g.* urine clearance 0900–0930 h; blood sampling at 0915 h). The clearance protocol was discontinued at noon, and the subjects were allowed to eat and ambulate while the PTH infusion continued. On day 3, the clearance protocol was repeated during administration of a Ca infusion. Calcium chloride (CaCl_2) was infused at two rates: 75 μEq ($37.5 \mu\text{mol}$) elemental Ca/kg·h and then 100 μEq ($50 \mu\text{mol}$) elemental Ca/kg·h for three consecutive 30-min clearance periods for each dose. For all clearance periods, venous blood was obtained from an antecubital vein (in the arm opposite that used for the infusion) for measurement of Ca_i , total Ca, iPTH, Mg, and Na. UCa, UMg, UNa, and creatinine (UCr) were measured for all clearance periods.

Laboratory tests

Serum chemistry values were determined by the clinical chemistry laboratory; total serum Ca was measured with a colorimetric method

using an Olympus AU-5061 analyzer (Olympus Corp., Lake Success, NY). The intra- and interassay coefficients of variation (CV) for Ca in this method were 1.09% and 1.36%, respectively.

Blood for SCa_i determination was collected anaerobically and measured with an AVL 987-S electrolyte analyzer (AVL Scientific Corp., Roswell, GA), which has an intraassay CV of 0.39% and an interassay precision of 1.7–2.5% for Ca_i levels between 1.12–1.28 mmol/L (normal range, 1.15–1.33 mmol/L).

Serum iPTH was measured by the Allegro immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA). The detection limit of the assay is 1 pg/mL (normal range, 10–65 pg/mL), and the intra- and interassay CVs are 1.7% and 6.5% at iPTH levels of 37.7 and 41.1 pg/mL, respectively. 25-Hydroxyvitamin D₃ was measured by a competitive protein binding assay, with a normal range of 10–55 ng/mL.

SCa_i was measured on the day of the infusion, whereas all other serum samples were stored at -70°C . All samples from each patient were subsequently run in duplicate in a single assay.

Analyses

Cation excretion was expressed as a function of the glomerular filtrate (GF), *e.g.* for Ca it is expressed as $\text{UCa} \times \text{volume}/\text{GF}$, where GF is $\text{UCr} \times \text{volume}/\text{SCr}$; therefore, the Ca excretion/GF = $\text{UCa} \times \text{volume} \times \text{SCr}/\text{UCr} \times \text{volume} = \text{UCa} \times \text{SCr}/\text{UCr}$. The values are expressed as millimoles per L GF. This was calculated for each cation (Ca, Mg, and Na) for each subject during each clearance period. An average value for the eight subjects was then computed and plotted as a function of the average SCa_i , as ultrafilterable Ca was not calculated. We have, however, shown previously that in the case of the parathyroid gland, ionized rather than ultrafilterable Ca is sensed by the parathyroid cell (29). The data for each cation were fitted to a sigmoidal curve using the software GraphPad PRISM version 1.0 (GraphPad Software, San Diego, CA). The four parameters describing any sigmoidal curve (in this instance, urinary cation excretion) were determined by the program software for the eight subjects on both the high and low salt diets. They are as follows: 1) maximal cation excretion, 2) minimal cation excretion, 3) midpoint (SCa_i concentration resulting in a half-maximal change in urinary excretion), and 4) slope of the curve at the midpoint. Results are expressed as the mean \pm SEM unless specified otherwise.

Results

Baseline characteristics

Our subjects were young (30.6 ± 3.3 yr) and had normal serum chemistries and levels of calciotropic hormones. There were no differences in the 24-h calculated Cr clearances on the high and low salt diets, and the values were 110 ± 11 and 98 ± 14 mL/min, respectively. The 24-h values for UNa excretion measured 4–5 days after the start of the high and low salt diets were 184 ± 23 and 7.3 ± 1.9 mEq, respectively, thus confirming that the subjects were indeed in balance.

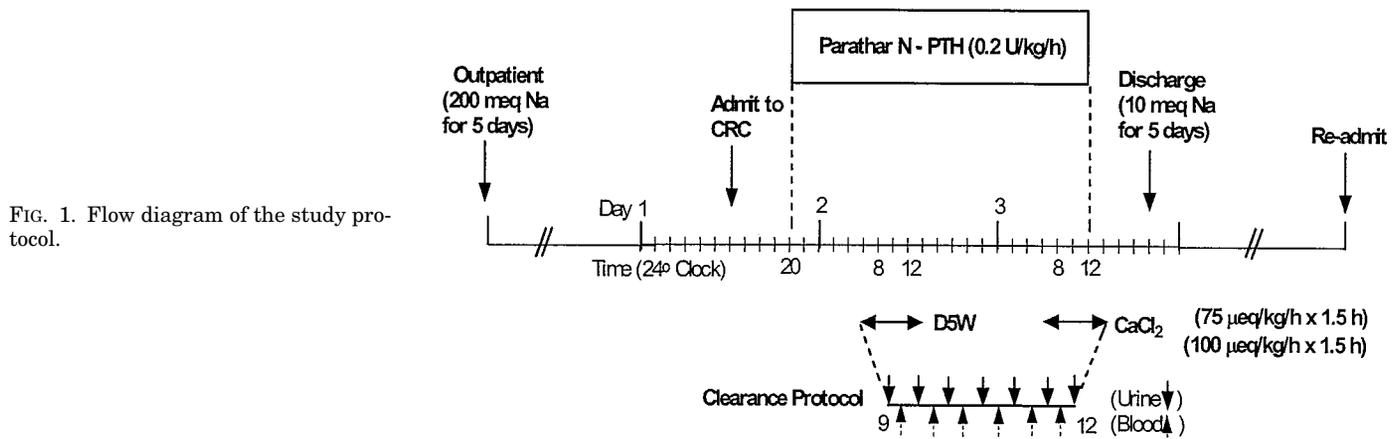


FIG. 1. Flow diagram of the study protocol.

Biochemical profile during the clearance protocols

As shown in Fig. 2, a gradual increase in SCa_i levels was inversely related to a gradual decline in $iPTH$ levels. $iPTH$ reached 1.53 ± 0.20 pmol/L by 0800 h on day 2 of the high

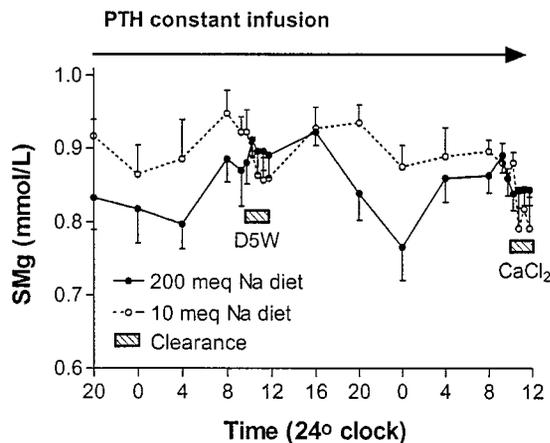
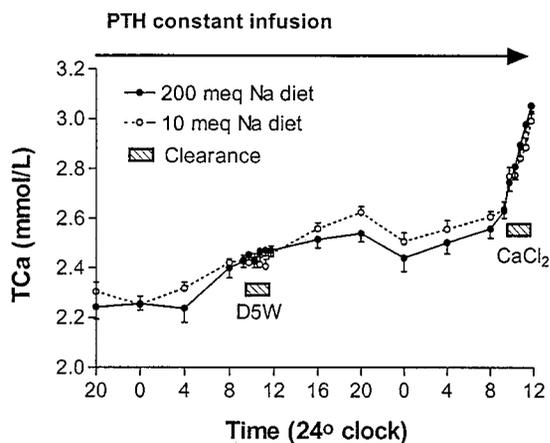
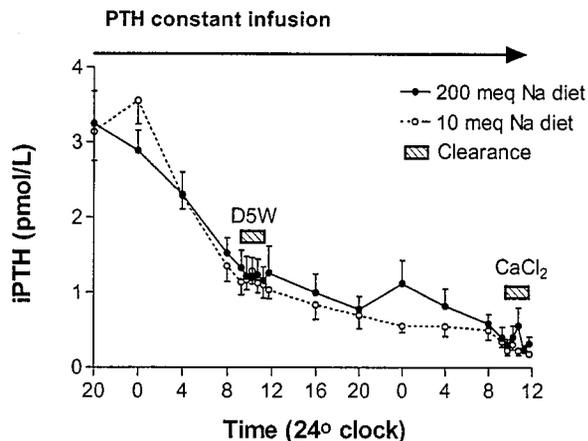


FIG. 2. Serum levels of $iPTH$, Ca, and Mg during the clearance protocol. The studies were performed on eight normal male subjects as described in *Materials and Methods*. Points indicate the mean (\pm SEM) as shown in the legend.

salt diet, at which time the first control clearance protocol took place. On day 3, $iPTH$ levels decreased further due to the additional increments in SCa_i , resulting from the Ca infusion. Serum Mg levels showed a diurnal rhythm during the high salt diet, with a nadir occurring between 0000–0400 h (Fig. 2).

Ca clearance

During the high salt diet, Ca excretion (millimoles per L GF) was tightly fitted to a sigmoidal curve, with a midpoint (SCa_i concentration resulting in a half-maximal change in Ca excretion) of 1.51 ± 0.009 mmol/L ($r^2 = 0.99$; Fig. 3). The characteristics of the curve are fully detailed in Table 1. Because the curve was not clearly sigmoidal during the low salt diet, a midpoint was not calculated (Fig. 3).

To evaluate whether the gradual increase in SCa_i levels may partially account for the observed flattening of the Ca excretion curve at high SCa_i , the results were also expressed as the fractional excretion of Ca: $Ca \text{ excretion}/GF \times SCa_i = (UCa \times SCr/UCr)/SCa_i = UCa \times SCr/SCa_i \times UCr$, which led to almost identical results (data not shown).

Mg excretion

During the high salt diet, Mg excretion (millimoles per L GF) was tightly fitted to a sigmoidal curve, with a midpoint (SCa_i concentration resulting in a half-maximal increase in Mg excretion) of 1.49 ± 0.01 mmol/L ($r^2 = 0.96$; Fig. 4). The characteristics of the curve are fully detailed in Table 1. As with the Ca data, the curve during the low salt diet was not clearly sigmoidal, and a midpoint was not calculated (Fig. 4). Maximal Mg excretion was slightly, but not significantly, higher during the low salt diet compared to that during the high salt diet ($P = 0.07$).

Na excretion

During the high salt diet, Na excretion (millimoles per L GF) was also tightly fitted to a sigmoidal curve, with a midpoint (SCa_i concentration resulting in a half-maximal increase in Na excretion) of 1.55 ± 0.02 mmol/L ($r^2 = 0.93$; Fig. 5a). As with the Ca data, the curve during the low salt diet

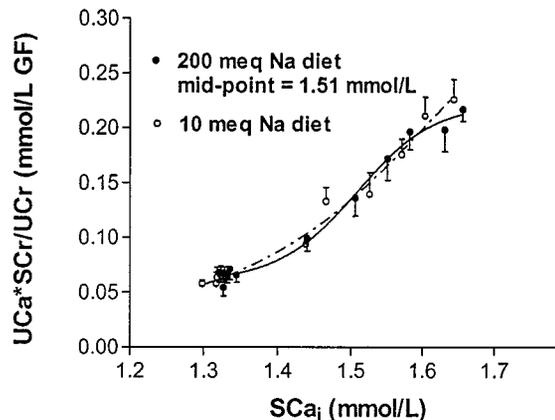


FIG. 3. Ca clearance (millimoles per L GF) during the high and low salt diets. The midpoint represents the SCa_i resulting in a half-maximal change in the excretion of UCa.

TABLE 1. Values of the four parameters describing Ca, Mg, and Na excretion as a function of SCa_i on the high salt diet

	Midpoint ^a	Max ^b	Min ^c	Slope ^d
UCa (mmol/L GF)	1.51	0.22	0.06	7.97
UMg (mmol/L GF)	1.49	0.08	0.05	13.05
UNa (mmol/L GF)	1.55	3.75	2.04	11.59

GF, Glomerular filtrate.

^a Serum ionized calcium concentration at which urinary calcium excretion is half-maximally inhibited.

^b Maximal cation excretion.

^c Minimal cation excretion.

^d Mmol urinary cation/mmol SCa_i .

was not clearly sigmoidal, and a midpoint was not calculated (Fig. 5b). Because of the close relationship between UCa and UNa excretion, the same data were also evaluated expressing the former as a function of the latter (Fig. 6). During both the high and low salt diets, UCa as a function of UNa was fitted to a steep sigmoidal curve, with $r^2 = 0.94$ and $r^2 = 0.93$, respectively (Fig. 6).

Discussion

To our knowledge, this is the first study to characterize Ca-dependent renal Ca handling during a PTH clamp in subjects studied while in salt balance, first during a high and then during a low salt diet. Under clamped PTH conditions, UCa, UMg, and UNa excretion were exquisitely sensitive to changes in SCa_i , increasing by 400%, 67%, and 150%, respectively, in response to a 25% increment in SCa_i . Previous investigations that did not control for fluctuations in PTH, a known modulator of UCa excretion, have demonstrated sharp increments in UCa excretion in response to increasing serum Ca concentrations (13–16). In our studies, the highest Ca excretion rate was 0.20 mmol/L GF (achieved when the total Ca level was 3.1 mmol/L), an excretion rate well within the range reported by Peacock *et al.* in their hyperparathyroid subjects (13) and documented by Mioni *et al.* in normal subjects for comparable levels of total Ca (16). The similarity of our results to those of Mioni *et al.* and Peacock *et al.* despite differences in PTH levels underscores the major contribution of SCa_i *per se* in modulating its own excretion. The slightly higher excretion rates reported in some previous studies can be explained by the concomitant hypoparathyroidism, whether surgical or iatrogenic due to the Ca infusion, and by the different Ca salts used, as Ca gluconate is less well reabsorbed than $CaCl_2$ (30). Our curve shows blunting of the increase in Ca excretion at the highest serum Ca concentrations achieved, which suggests a saturation phenomenon. Indeed, this was a computer-generated fit with an r^2 of 0.99. Furthermore, the clear saturation of Mg excretion at high Ca concentrations further supports the concept of a saturable phenomenon. Peacock *et al.* (13) projected the curves derived from their data points to increase linearly as serum Ca levels increased. A close examination of their original data, however, suggests a leveling off of UCa excretion in the hypo- and hyperparathyroid subjects studied at high serum Ca concentrations (13). Similar conclusions can be reached by examining the data reported Attie *et al.* for two of their three hypoparathyroid subjects (14).

UCa excretion varies as a function of salt balance, with Ca

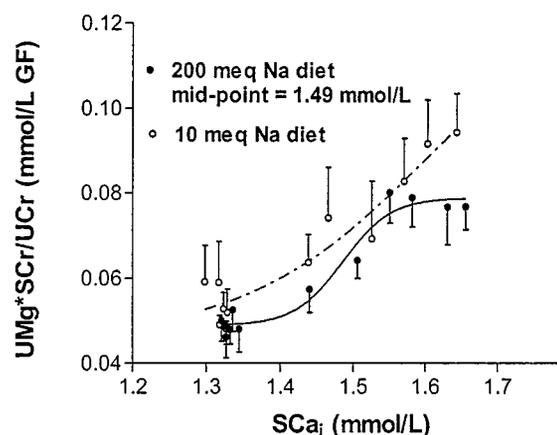


FIG. 4. Mg excretion (millimoles per L GF) during the high and low salt diets. The midpoint represents the SCa_i resulting in a half-maximal change in the excretion of UMg.

excretion increasing at higher levels of Na excretion (23–25). Although the patients reported by Attie *et al.* were studied during a high salt diet, the salt intake in many other studies was not specified (13, 15, 16). In our study, we carefully evaluated the impact of salt on Ca excretion by studying the same subjects while in balance during a high and then during a low salt diet. As shown in Fig. 6, Ca excretion increased concomitantly with increments in Na excretion in response to the Ca infusion. The sharp increments in Na excretion in response to the rising levels of SCa_i , doubling during the high and quadrupling during the low salt diets, have been previously observed in an animal model and *in vitro* (26, 27). The increment in Na excretion in response to the Ca load in our studies is comparable to that reported by Attie *et al.* in one hypoparathyroid patient (14). This Ca-induced natriuresis may contribute to the extracellular volume depletion and renal concentrating defect in patients with hypercalcemia (31).

Our observations may also elucidate the mechanism(s) by which oral Ca supplementation decreases blood pressure in patients with salt-sensitive hypertension (32). Indeed, several studies suggest that the effect of Na intake on blood pressure is determined by the adequacy of other minerals, such as Ca (33–35), Mg (36), and K (37). The pressor effect of NaCl seems to be expressed in subjects with the lowest intake of these minerals (32, 34, 38). The natriuretic effect of Ca may be mediated either through increases in serum and/or renal tubular Ca concentration. In Fig. 7, we propose a model that outlines the modulation of Ca, Mg, and Na excretion by serum Ca that takes into account available data, including our own.

Several lines of evidence suggest that the inhibition of both renal tubular Ca and Mg reabsorption by increases in serum Ca is mediated by the CaR located on the basolateral surface of the epithelial cells of the CTAL (5, 22). Quamme demonstrated that raising peritubular, but not luminal, Ca or Mg inhibits the reabsorption of both divalent cations in the CTAL (9). In our studies, the parallelism of Ca and Mg excretion in response to gradual elevations in SCa_i suggests that the locus of the Ca effect is the CTAL, as the excretion of these cations is dissociated in the distal convoluted tubule. In addition, Ca

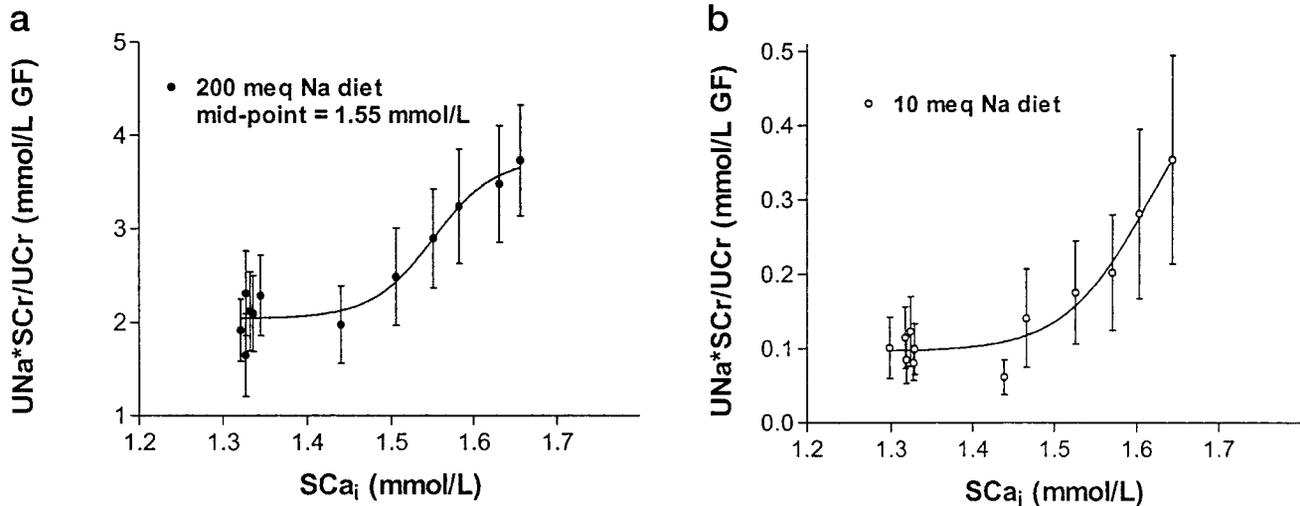


FIG. 5. Na excretion (millimoles per L GF) during the high (a) and low (b) salt diets. The midpoint represents the SCa_i resulting in a half-maximal excretion of UNa.

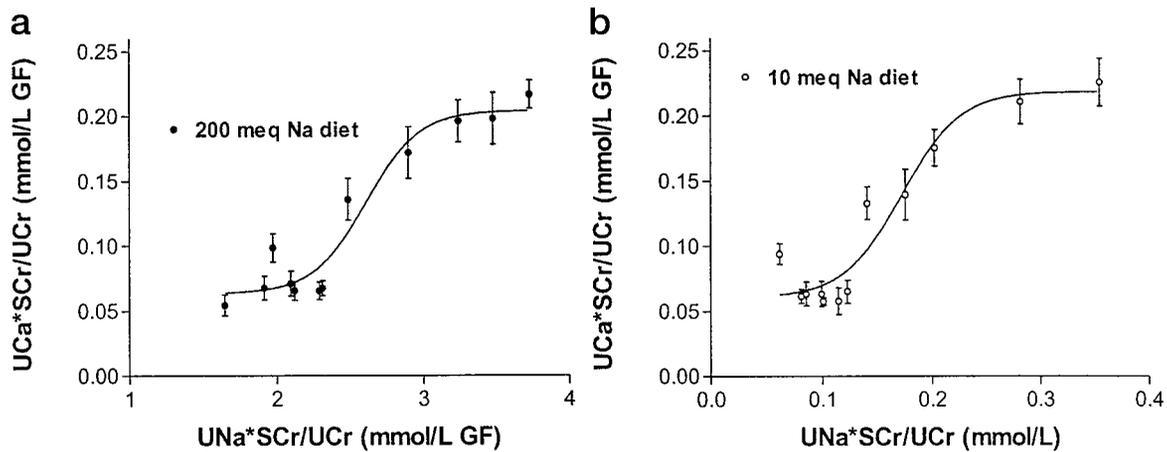


FIG. 6. Ca excretion (millimoles per L GF) as a function of Na excretion (millimoles per L GF) during the high (a) and low (b) salt diets.

is known to inhibit an apical K channel that recycles the K necessary to maintain both NaCl reabsorption via the NaK2Cl cotransporter and the resultant lumen-positive potential driving paracellular Na, Ca, and Mg reabsorption in this nephron segment (28). A tight coupling of the CaR to the NaK2Cl and/or NaCl cotransporters, therefore, might explain the observed characteristics of UNa excretion expressed as a function of SCa_i during the high salt diet in our studies (Fig. 7).

An essential role of the CaR is further suggested by human diseases in which activating or inactivating mutations in this receptor result in dramatic changes in urinary cation excretion. The excessively avid tubular reabsorption of Ca in FHH is only reversed with the use of loop diuretics that act on the CTAL where the receptor is known to be located, at least in rodents (22). Parathyroidectomized patients with familial hypocalciuric hypercalcemia carry heterozygous inactivating mutations in the CaR (39) and demonstrate a marked blunting of UCa and UNa excretion (14, 40) in response to a Ca infusion that is corrected by ethacrynic acid (14). These findings support the idea that CTAL is a major site of ex-

cessive reabsorption of Ca due to impaired CaR-mediated inhibition of the NaK2Cl cotransporter. The latter also presumably explains the failure of Ca infusion to increase UNa in these patients (14). In contrast, despite their higher PTH levels, untreated subjects with autosomal dominant hypocalcemia (who carry activating mutations of the CaR) exhibit rates of Ca excretion that are 2-fold higher than those in patients with classical hypoparathyroidism (41–43). The development and use of specific CaR agonists and antagonists in conjunction with a PTH clamp (as in this study) will further clarify the role of renal CaRs in regulating various aspects of renal function.

Conclusion

In summary, several important factors contribute to UCa excretion, including PTH levels and salt and acid-base status. In this study, we rigorously controlled PTH levels with the use of a PTH infusion, strictly controlled salt balance, and were able to characterize a direct serum Ca-dependent effect on UCa handling. The steep increases in UCa, UMg, and UNa

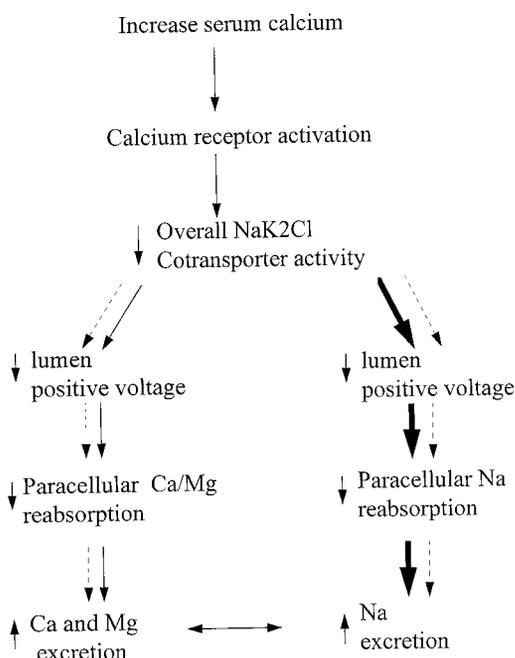


FIG. 7. Model outlining the interactions between serum calcium and Na, Ca, and Mg excretion during the high (—) and low (---) salt diets. The thicker arrow for Na excretion during the high salt diet emphasizes the greater importance of salt balance on Na excretion than on Ca and Mg excretion.

excretion with increases in SCa_i during a high sodium diet are best represented by sigmoidal curves and are consistent with CaR-mediated modulation of Ca, Mg, and Na excretion. With appropriate modifications, this protocol will provide a useful paradigm for elucidating the mechanisms underlying normal and abnormal renal handling of Ca, Mg, and Na as well as the effects of several drugs on the renal handling of these ions.

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**Festschrift In Honor of Maria I. New
September 23-24, 1998**

To honor Maria New for her lifetime achievements in pediatric endocrinology, a one day symposium will be held at the Villa Medicea "La Ferdinanda" in Artimino near Florence, Italy on 9/23-9/24/98. This conference will be held in conjunction with the annual meeting of the European Society for Pediatric Endocrinology in Florence.

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