Calcium Kinetics in the Hyperprostaglandin E Syndrome

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ABSTRACT. Metabolic investigations, including the use of stable isotopes of calcium, were used to study calcium kinetics in three children with the hyperprostaglandin E syndrome. The studies were performed both during indomethacin treatment and in the absence of therapy. Off therapy, each child had hypercalciuria (mean urinary calcium excretion 0.478 mM/kg/d), hyperprostaglandinuria, and elevated serum calcitriol concentration. All had diminished bone density and were euparathyroid. Indomethacin treatment was associated with a marked reduction in serum calcitriol concentration, as well as decreased prostaglandin E excretion. Mean urinary calcium excretion fell to 0.135 mM/kg/d. The stable isotope studies defined two components to the hypercalciuria of this disease: an indomethacin-sensitive dietary contribution and a relatively indomethacin-resistant bone resorptive element. Bone densitometry confirmed the presence of the resorptive element by demonstrating skeletal demineralization. (*Pediatr Res* 33: 92–96, 1993)

Abbreviations

iPTH, intact parathyroid hormone
PGE_2, prostaglandin E_2
Va, calcium absorption
Vou, component of 24-h urinary calcium excretion derived from recent diet
Vi, calcium intake
Vu, 24-h urinary calcium excretion
Vbu, endogenous, or tissue-derived, component of total 24-h urinary calcium excretion

Elevation in urinary calcium excretion in childhood can be modest, as is often seen with idiopathic hypercalciuria, or extreme, as is seen in a variety of systemic disorders. Among the latter are Bartter's syndrome and its variants, which share several laboratory features including hypokalemia, hyperprostaglandinuria, and subnormal fractional distal chloride reabsorption under conditions of hypotonic diuresis (1, 2). In 1985, Seyberth *et al.* (3) described a distinct congenital hyperprostaglandinuric tubular syndrome with massive hypercalciuria that resembled

Bartter's syndrome, but differed in that the children demonstrated normal fractional distal chloride reabsorption and each had had a history of premature labor and polyhydramnios. Previously, a 6-y-old child diagnosed with severe idiopathic hypercalciuria had been described with features of the hyperprostaglandin E syndrome (4). Unlike the children reported by Seyberth *et al.*, this child lacked evidence of an abnormality in potassium regulation. Although elevated plasma renin activity and aldosterone concentrations have been reported consistently in these patients, these findings are generally considered a consequence of renal prostaglandin synthesis, because they respond to therapy aimed at reducing this (3–11).

In children with these hyperprostaglandinuric syndromes, therapy with the cyclooxygenase inhibitor, indomethacin, favorably affects most of the clinical and the biochemical abnormalities (3–8, 12–19). In some patients with massive hypercalciuria and nephrocalcinosis, however, the reduction in the urinary calcium excretion by this therapy is incomplete (3, 5, 6, 12, 13). The facts that marked hypercalciuria persists for years in these children despite maximal dosages of indomethacin, but not in others on therapy with similar degrees of nephrocalcinosis (20), suggests that the hypercalciuria does not reflect dissolution of the calcinosis, but rather continued calcium excretion by established or alternative mechanisms. Such severe hypercalciuria places these children at risk of developing progressive nephrocalcinosis and renal failure (21, 22). To treat this disorder more effectively, it is necessary to understand the mechanisms mediating the excessive calcium loss.

A previous analysis of calcium balance, using ^{47}Ca, concluded that three out of the four children with this disorder were in positive calcium balance (22). This is in agreement with more recent studies that suggested excessive enteric calcium absorption contributed to the hypercalciuria (4, 6). Most reports, however, have implicated a primary renal reabsorptive defect (4, 22, 23). To investigate this problem further, we used a recently developed technique employing stable isotopes to measure calcium absorption and to determine the component of urinary calcium excretion derived directly from the diet. This was performed in three boys with massive hypercalciuria, hyperprostaglandinuria, and nephrocalcinosis, both on and off therapy with indomethacin.

MATERIALS AND METHODS

Patients. The patients are followed at Children's Hospital Medical Center, Cincinnati, OH, and State University of New York Health Sciences Center, Syracuse, NY. Each has polyuria, a urine concentrating defect, hypercalciuria, hyperprostaglandinuria, and nephrocalcinosis. The hypercalciuria partially but incompletely resolves with maximally tolerated dosages of in-

domethacin. Patients 1 and 2 were included in a previous study from this laboratory, and their clinical features are detailed in that report (6). Patient 3 was recently investigated at age $4^9/_{12}$ y for polyuria, polydipsia, and nephrocalcinosis. During hypotonic saline diuresis, his fractional distal tubular chloride reabsorption, calculated at the moment of maximal free water clearance (24, 25), was 81%. He had a normal physical examination, plasma renin activity, and serum concentrations of electrolytes and aldosterone.

Three healthy children, ages 5.4 to 7.9 years, underwent the stable isotope protocol at USDA/ARS Children's Nutritional Research Center, Baylor College of Medicine, Houston, Texas. These children, who represent the age-matched control population for the stable isotope studies, had normal values for serum calcium, phosphate, albumin, creatinine, and alkaline phosphatase activity.

Informed consent was obtained from the parents of every child, and the investigational protocol was reviewed and approved by the respective institutional review boards.

Protocol. Sterile solutions of the chloride salts of ^{42}Ca, ^{44}Ca, and ^{46}Ca were prepared by the National Institutes of Health pharmacy and tested for pyrogenicity and sterility before use. Calcium isotopes were obtained from Oak Ridge National Laboratory, Oak Ridge, TN.

Each patient was studied both off and on indomethacin. The drug was administered every 8 h at the largest dose tolerated without adverse gastrointestinal symptoms (range: 2.4-5.0 mg/ kg/d). Studies were performed in patient 3 prior to beginning indomethacin therapy; in the other patients, the initial studies were performed 72-96 h after the last dose of indomethacin. Our experience and that of others (3, 13, 26) suggest that this is an adequate washout period, since symptoms recurred in all patients, and it is consistent with the drug's $t_{1/2}$ of 4-5 h. The children were given only water and indomethacin (when indicated) after the evening meal. 12.5 mM/kg of ^{44}Ca was allowed to equilibrate in 8 ounces of milk overnight. After the first morning urine was collected, blood was drawn by venipuncture for measurement of iPTH and vitamin D metabolites, and 2.5 mM/kg ^{42}Ca, diluted to 10 mL in half isotonic saline, was infused slowly. This was followed by breakfast, during which the isotope-equilibrated milk was drunk. The milk cartons were rinsed twice with water, and the the water was also drunk. Urine was collected for 24 h, and dietary assessment of 24-h calcium intake was performed from nursing and parental records by a staff dietitian. In the control subjects, an identical protocol was performed except that ^{46}Ca was used as the oral tracer.

Analytical methods. Aliquots from the 24-h urine collections were analyzed at the National Institutes of Health or at Baylor College of Medicine, where the enrichment of ^{42}Ca and ^{44}Ca (or ^{46}Ca in control children) relative to ^{48}Ca (^{43}Ca in controls) was determined by thermal ionization mass spectrometry. This was used to calculate the fractional absorption of calcium and the fraction of each dose of isotope excreted in the urine (27). These values allow the determination of Va and the component of the urinary Vou directly derived from the diet:

$$Va = Vi \times \text{fractional absorption of calcium}$$

$$Vou = Vi \times \text{fraction of oral isotope excreted in urine}$$

Urine calcium concentration was measured by flame atomic absorption spectrophotometry and was used to determine Vu. Vbu is the difference between Vou and Vu. For paired data, t test was used to analyze the response of these parameters to therapy.

Serum and urine electrolytes, calcium, phosphate, creatinine, and complete blood counts were measured in the clinical laboratory. Urine concentrations of cAMP and PGE_2 were measured as previously described (6).

Vitamin D metabolites were extracted from serum by solid-phase extraction cartridges, and the calcitriol concentration was measured by a competitive radioligand assay described previously (28). Coefficients of variance are 5.2% intraassay and 6.4% interassay.

Serum PTH concentration was determined by a two-site immunoradiometric assay (29). This assay measures the intact PTH molecule and has a sensitivity of 1 pg/mL (0.1053 pmol/L). Intra- and interassay coefficients of variance are 4 and 8%, respectively (Allegro Intact PTH Immunoassay, Nichols Institute, San Juan Capistrano, CA).

Bone mineral content and bone width were determined by x-ray densitometry in patient 2 and by single-beam photon absorptiometry in patients 1 and 3. Measurements were taken from the left radius at the "midshaft" site, which is actually one-third the length between the ulnar styloid and the olecranon. These measurements were performed using a Hologic QDR-1000/W whole-body x-ray bone densitometer (Hologic Inc., Waltham, MA) in patient 2, and with a Lunar Bone Mineral Analyzer (Lunar Radiation Inc., Madison, WI) in patients 1 and 3. Bone mineral content:bone width ratios were compared with age, gender, and race-specific normal values (30) for the photon absorptiometry measurements and with an age-matched population of 13 normal children for the x-ray densitometry determinations (Bonny Specker, Ph.D., personal communication).

RESULTS

Calcium metabolism. Every patient was normocalcemic and hypercalciuric (Vu >0.1 mM/kg/d) both off and on cyclooxygenase inhibitor therapy (Table 1). Serum phosphate concentration was normal in patient 1 and mildly low in patient 3, whether or not indomethacin was taken. PTH concentrations were normal in each child not receiving indomethacin. Both phosphate and PTH concentrations were normal off therapy and slightly elevated during therapy in patient 2, probably because of renal insufficiency. Serum calcium concentrations decreased in each patient with therapy; ionized calcium concentrations were not obtained, but this was probably low in patient 2 during therapy, since his severe concentrating defect and alkalosis was refractory to indomethacin. Tm_{PO4}/GFR was normal in patient 1 and reduced in patient 3; in neither child was the ratio appreciably affected by indomethacin. During therapy, this value rose to normal in patient 2. Urine cAMP was measured in patient 3 to assess renal PTH activity. The urine cAMP/creatinine ratios, off and on indomethacin respectively, were 10.3 and 9.3 nM/mg. The range of normal ratios, (2 SD below and above the mean) are 5.0-15.0 nM/mg.

Serum concentrations of calcitriol were elevated in every child off indomethacin (Table 1). These values returned to normal during therapy in patients 1 and 3; in patient 2, the calcitriol concentration decreased nearly 50% but remained slightly elevated. Similarly, each child off therapy was hyperprostaglandinuric, with PGE_2 excretion exceeding the upper limit of 0.154 mM/h/1.73 m^2 (2 SD above mean of normals) (6). Urine PGE_2 excretion, as measured by PGE_2/creatinine ratios, decreased with therapy in each patient, returning to normal in patients 1 and 2. Indomethacin was associated with a rise in the osmolality of the first morning urines and with a decrease in creatinine clearance in each child.

Except for patient 3, who had a calcium-rich diet, Vi was comparable with the age-matched controls (Table 2). Va was increased to approximately twice the control value; whereas Vu, Vou, and Vbu were much more elevated in the patients than in the controls. Indomethacin was associated with an over 50% reduction in Vou in all ($p = 0.01$ by paired t test), similar to the reduction in serum concentration of calcitriol. There was a less dramatic decline in Va and Vu with therapy. In contrast, the effect of therapy on Vbu was variable: in two patients, it decreased approximately 33%; in the other, it increased. The response of Va, Vu, and Vbu to therapy failed to achieve statistical significance.

Table 1. *Metabolic studies in three hypercalcemic children, off and on indomethacin therapy*

	Patient 1		Patient 2		Patient 3		
	Off	On	Off	On	Off	On	Normal
Urine PGE_2/Cr (nM/mM)	1.04	0.41	2.08	1.08	2.94	2.79	
Calcitriol (pM/L)	339	195	4.02*	212*	252	80	112–212
PTH (pM/L)	3.8	3.7	†	9.8	1.7	0.8	1.2–7.1
Calcium (mM/L)	2.68	2.50	2.40	2.30	2.45	2.38	2.15–2.68
Phosphate (mM/L)	1.47	1.38	1.6	1.73	1.28	1.31	1.32–1.70‡
Tm_{PO_4}/GFR‡ (mg/dL)	3.9	3.7	3.3	3.9	3.0	3.0	3.53–4.65‡
Cr Cl (mL/min/1.73 m²)	95	84	54	32	170	100	
Urine osmolality§ (mosmol/kg)	183	254	81	83	273	286	

* Calcitriol concentrations in sera from patient 2 were performed by Endocrine Sciences (Calabasas Hills, CA). Normal values reported from an adult population by this laboratory range 52–120 pM/L.

† Serum PTH concentration when patient 2 was off indomethacin was performed by SmithKline and Beacham Laboratories (Van Nuys, CA). Both carboxy-terminal and midmolecule measurements were normal.

‡ Brodehl *et al.* (45), fasting norms.

§ Samples were obtained from the first morning void.

Table 2. *Stable calcium isotope studies in three patients and age-matched healthy children off and on indomethacin therapy**

	Patient 1		Patient 2		Patient 3		
	Off	On	Off	On	Off	On	Controls†
Vi	1.228	1.985	0.925	0.925	2.483	1.565	1.253 (0.798–1.523)
Va	0.74	0.535	0.748	0.475	0.775	0.52	0.373 (0.27–0.455)
Vu	0.333	0.293	0.61	0.373	0.493	0.283	0.048 (0.038–0.058)
Vou	0.09	0.025	0.133	0.05	0.095	0.038	0.005 (0.003–0.007)
Vbu	0.293	0.268	0.478	0.323	0.398	0.245	0.043 (0.033–0.053)

* All values are in mM/kg/d.

† Mean (range).

Bone density and growth. The BMC/BW ratio was low in each child, ranging from 67.6–84.5% of the mean values of control children (Table 3). In contrast, only the eldest boy was less than the fifth percentile in height. Patients 1 and 2 were each below the fifth percentile when initially evaluated; the height of patient 3 was at the the 50th percentile. The linear growth velocities of patients 1 and 2 increased from below the 3rd percentile during the 6-mo interval preceding indomethacin therapy to above the 97th percentile during the initial 6 mo with this therapy. In contrast, there was no significant change in the growth velocity of patient 3, which remained at approximately the 50th percentile. Skeletal radiographs did not reveal rickets or hyperparathyroidism; either initially or after therapy with indomethacin had been undertaken.

DISCUSSION

Royer *et al.* (31) were the first to report hypercalciuria in some patients with chronic idiopathic hyperkaliuric hypokalemia, a congenital electrolyte disorder initially described by Rosenbaum and Hughes (32). Several reports soon followed, confirming the findings of hypercalciuria and nephrocalcinosis in a subset of these patients (5, 22, 23). Previously, Arant *et al.* (21) had described two patients with a similar electrolyte disorder who developed interstitial nephritis and terminal renal failure; nephrocalcinosis was evident in one patient, although urinary Vou was never measured.

Table 3. *Bone density results in three hypercalciuric children*

Patient	Age (y)	Wt (kg)	Ht* (cm)	BMC/BW† (gm/cm²)
1	4.2	16.5	100.2, (25)	0.262, (78.7)
2	7.8	18.9	113.0, (<5)	0.376, (84.5)
3	4.8	18.5	109.3, (50)	0.225, (67.6)

* Numbers in parentheses are percentiles.

† Numbers in parentheses are percentages of mean BMC/BW in matched, normal children.

The therapy of hypercalciuria in this disease has not been satisfactory. Diuretics (4, 5, 12, 22, 23), modification of dietary sodium (22) or calcium (3–5, 22), oral phosphate (22, 23), prednisolone (3), and calcitonin have either been unsuccessful or associated with unacceptable complications.

Recognition of hyperprostaglandinuria in this syndrome led to attempts at treatment with cyclooxygenase inhibitors. Such treatment usually corrected polyuria and electrolyte disorders; its effect on calciuria, however, was less uniform (3–9, 12–14, 33). Over the long term, hypercalciuria is associated with progressive nephrocalcinosis and renal insufficiency. Thus, there is a critical need to establish an effective therapy. Before such therapy can be designed, however, a better understanding of the mechanisms of hypercalciuria is clearly needed.

We have used a new technique to quantitate the proportion of urinary calcium excretion derived from recent diet and that which is mobilized from tissue stores, presumably bone. Our results confirmed previous suggestions by us (6) and others (4, 22) that enhanced enteric calcium absorption contributed to hypercalciuria, and that this component was sensitive to indomethacin therapy. The enhanced enteric absorption correlated with elevated serum concentrations of calcitriol, which also decreased with cyclooxygenase inhibitor therapy. This is similar to a study in which either aspirin or indomethacin therapy decreased calcitriol concentrations and reduced the incremental calciuric response to an oral calcium load (4).

Although indomethacin therapy reduced both total (Vu) and recent diet-derived (Vou) urinary calcium excretion, calciuria continued to be well above normal in all. The balance of the urine calcium must have been mobilized from tissue stores, largely bone. The presence of skeletal hypomineralization in all three children supports this conclusion. The cause of the increased bone turnover is unclear, although several possibilities can be excluded by our data.

Increased serum concentrations of calcitriol may exert a calcium-mobilizing effect on bone. Although all patients had elevated serum calcitriol levels in the absence of treatment, these

values normalized on indomethacin. Despite a significant reduction in the absorptive component of calcium excretion, massive hypercalciuria persisted.

Hyperparathyroidism is another potential cause of increased bone turnover in these children. Leonhardt and coworkers (26) found mean levels of iPTH elevated in a group of nine children with this syndrome while receiving indomethacin. Their patients, however, had a substantial degree of renal insufficiency with therapy [creatinine clearances ranging from 37–95 (median 54) mL/min/1.73 m^2], making interpretation of their parathyroid status problematic. Our patients were euparathyroid, as evidenced by a highly specific two-site radioimmunoassay (29, 34–36). Similarly, patients with this disease and normal renal function reported by Ohlsson et al. (37) and Kohout and Bachmann (14) also had normal serum PTH concentrations.

The Tm_{PO4}/GFR ratios are not consistent with PTH-related peptide activity (38, 39). Although Tm_{PO4}/GFR was slightly diminished in patient 3, PTH-related peptide activity is not likely because he was subsequently shown to have normal urinary cAMP excretion. The mildly reduced Tm_{PO4}/GFR values may represent an additional feature of the calcium metabolic derangement that prevents hyperphosphatemia, a common feature of resorptive hypercalciuria (40).

The absence of hyperparathyroidism also excludes a "renal leak" of calcium as a primary disturbance, with tissue calcium mobilization a secondary phenomenon. Such a mechanism requires a secondary increase in PTH (41) and would be expected to result in calcipenic rickets (42) if enteric absorption were unable to compensate for the excessive calciuria. Rickets is rarely observed in these hypercalciuric children, however, unless additional tubular defects are involved or renal insufficiency supervenes (3, 5, 6, 21, 22).

Hypercalciuria has also been described as a consequence of a primary phosphate leak in two familial syndromes with differing modes of inheritance (43, 44). Each is characterized by normal or suppressed PTH concentrations and elevated concentrations of calcitriol. Hypophosphatemia is an invariable feature which distinguishes these patients from ours.

Seyberth et al. (3, 26) have speculated that increased bone resorption, probably mediated by PGE_2, contributed to hypercalciuria. Although we believe that this is likely, other mediators may also be involved. Because PGE_2 excretion was normalized in two patients during therapy, it is unlikely that excessive systemic prostanoid activity solely is responsible for increased bone turnover. Others have observed a lack of association between hypercalciuria and renal or systemic PGE_2 hyperactivity (13). Our data clearly show that the predominant effect of indomethacin in this disease is on enteric calcium absorption.

In summary, the significant reduction in recent diet-derived calciuria, but not the tissue-derived component, supports the hypothesis that additional mediator(s) of bone resorption are active in children with this syndrome. More importantly, these findings suggest that indomethacin therapy may worsen the bone mineralization status if the calcitriol-stimulated absorption is a protective feature.

This novel methodology is useful in following response to therapy, and it is likely to be beneficial in promoting a better understanding of the various mechanisms of hypercalciuria. Such understanding may permit development of strategies to treat children with this heterogeneous disease. Additionally, they may be widely applicable in other hypercalciuric states.

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