Evidence that postprandial reduction of renal calcium reabsorption mediates hypercalciuria of patients with calcium nephrolithiasis

Elaine M. Worcester,1 Daniel L. Gillen,2 Andrew P. Evan,3 Joan H. Parks,1 Katrina Wright,1 Linda Trumbore,4 Yasushi Nakagawa,1 and Fredric L. Coe1

1Department of Medicine and 4General Clinical Research Center, University of Chicago, Chicago, Illinois; 2Department of Statistics, University of California, Irvine, California; and 3Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, Indiana

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Worcester EM, Gillen DL, Evan AP, Parks JH, Wright K, Trumbore L, Nakagawa Y, Coe FL. Evidence that postprandial reduction of renal calcium reabsorption mediates hypercalciuria of patients with calcium nephrolithiasis. Am J Physiol Renal Physiol 292: F66–F75, 2007; doi:10.1152/ajprenal.00115.2006.—Idiopathic hypercalciuria (IH) is common among calcium stone formers (IHSF). The increased urinary calcium arises from increased intestinal absorption of calcium, but it is unclear whether increased filtered load or decreased renal tubular reabsorption of calcium is the main mechanism for the increased renal excretion. To explore this question, 10 IHSF and 7 normal subjects (N) were studied for 1 day. Urine and blood samples were collected at 30- to 60-min intervals while subjects were fasting and after they ate three meals providing known amounts of calcium, phosphorus, sodium, protein, and calories. Fasting and fed, ultrafiltrable calcium levels, and filtered load of calcium did not differ between N and IH. Urine calcium rose with meals, and fractional reabsorption fell in all subjects, but the change was significantly higher in IHSF. The changes in calcium excretion were independent of sodium excretion. Serum parathyroid hormone levels did not differ between N and IHSF, and they could not account for the greater fall in calcium reabsorption in IHSF. Serum magnesium and phosphorus levels in IHSF were below N throughout the day, and tubule phosphate reabsorption was lower in IHSF than N after meals. The primary mechanism by which kidneys ferry absorbed calcium into the urine after meals is via reduced tubule calcium reabsorption, and IHSF differ from N in the magnitude of the response. Parathyroid hormone is not likely to be a sufficient explanation for this difference. Idiopathic hypercalciuria; urine calcium excretion; parathyroid hormone.

GENETIC, SO-CALLED IDIOPATHIC hypercalciuria (IH) is common among calcium stone formers (3), and its treatment with thiazide diuretic agents is a proven method of reducing stone recurrence (8). The surplus urine calcium in IH arises mainly from abnormally complete intestinal absorption of dietary calcium (8), although in the absence of an adequate dietary calcium supply, bone mineral is readily lost in the urine (9). How the excess of absorbed calcium finds its way into the urine is not fully known. Fasting calcium reabsorption in IH patients is slightly below that in normal subjects (11, 23), suggesting a component of reduced tubule reabsorption. Blood calcium levels rise slightly in IH and normal subjects after oral calcium loads (27), suggesting that a component of increased filtered load may be present.

Whether increased filtered load or reduced renal calcium reabsorption is the main mechanism that links increased intestinal calcium absorption to hypercalciuria is of general interest, as the knowledge would help complete the pathogenetic linkages for IH. In addition, the mechanism might bear on the forces that drive formation of the interstitial renal medullary and papillary apatite deposits often called Randall’s plaque (13). Plaque appears to offer an anchoring site on which calcium oxide (CaOx) stones can grow to a clinically relevant size and is therefore of considerable potential importance in the pathogenesis of the most common kind of kidney stone (16). Given that kidneys of IH patients must ferry abnormally large amounts of calcium from blood to urine with each meal, how they do this could offer hints as to why these patients form more plaque (13) than normal subjects do.

For these reasons, we have constructed experiments to determine whether the calcium loads are removed from normal foods into the urine mainly by increased filtered load or reduced tubule calcium reabsorption, and how calcium stone formers (SF) with IH (IHSF) might differ from sex- and age-comparable normal subjects (N).

METHODS

Patients and Normal Subjects

Ten IHSF patients were compared with seven N (Table 1). No patient had any systemic or renal disease as a cause or consequence of stones apart from IH, which was diagnosed by 24-h urine calcium excretion rates >140 mg calcium/g urine creatinine, normal blood calcium on three determinations, and exclusion of sarcoidosis, vitamin D excess, malignant neoplasm, renal tubular acidosis, or calcium supplement use at the time of testing (8). No N had a personal history of stone disease, known illnesses, or required any chronic medication apart from birth control and routine daily vitamins.

One IHSF included in the analysis (Table 1, IHSF 9) had Dunning type 1 lipodystrophy, which is not known to be a cause of stones or hypercalciuria (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM). Her serum calcium levels were slightly above our normal limits (10.6, 10.6, and 10.3 mg/dl); her serum parathyroid hormone (PTH) was low (21 pg/ml) and 1,25D high (71 ng/ml). Her daughter, with the same syndrome, was also hypercalciuric (165 mg calcium/g urine creatinine) and normocalcemic (9.36 mg/dl).

Experimental Design

Our first aim was to compare the contributions of changes in filtered load and tubule reabsorption to an increase in urine calcium
after meals in IHSF vs. N to determine which mechanism was predominant in producing the higher urine calcium in IHSF. Our second aim was to determine whether changes in calcium reabsorption could be ascribed to a fall in serum PTH levels with meals. Our third aim was to document postprandial changes in sodium, phosphate, and magnesium excretion and reabsorption as a way of extending our knowledge of renal responses to meals.

Our approach was to calculate calcium filtered load, urine calcium excretion, and calcium reabsorption during a three-meal day during which IHSF and N ate diets that matched in calcium and other critical nutrients. Changes in urine calcium and calcium reabsorption were compared between fasting and fed periods, and between each of the four periods of the day (fasting, breakfast to lunch, lunch to supper, supper to the end of the protocol) with appropriate adjustments detailed below (Statistical approaches). Additional measurements were made of magnesium, phosphate, and sodium filtration, excretion, and reabsorption and sequential serum PTH levels.

Protocol

Subjects were admitted to the General Clinical Research Center at the University of Chicago between 6 and 7 AM. Two fasting urine samples were collected (Fig. 1). Thereafter, three meals were provided accompanied by 1-h urine samples and then blood samples as illustrated. Blood was drawn at the end of each urine collection (14 serum samples) and at the midpoints of urine samples 3, 4, 7, 8, 12, and 13 (total of 20 serum samples). From one normal subject (normal subject 1, Table 1) we obtained only the second fasting urine.

In some subjects (Table 1), glomerular filtration was estimated using iothalamate clearance (C_{\text{GFR}}) as well as creatinine clearance (C_{\text{Cr}}) to control for possible confounding effects of creatinine secretion. In those patients, a loading dose of nonisotopic iothalamate (Conray 60, Mallinckrodt, St. Louis MO) was given to achieve a blood concentration of 10 \mu g/ml, assuming a volume of distribution of 0.25 l/kg body wt (2.5 mg/kg body wt) between 6 and 7 AM, followed by continuous infusion of iothalamate in 0.45% normal saline at 0.25 mg/kg/h starting 2 hours before the end of the protocol, Cit could only be obtained on one of the fasting urine samples vs. two urine samples for C_{\text{Cr}}.

### Diets

The study diet consisted of three isocaloric meals, estimated by calculation to contain calcium, phosphorus, and sodium evenly distributed among the meals (Table 2). Diet planning and analysis were performed using Nutritionist IV, version 4.1 (N-Squared Computing, San Bruno, CA). The goal for the 1,800-kcal base diet was to provide 1,000–1,200 mg calcium/day. Phosphorus was to be supplied in a 1:1 ratio with calcium. The base diet as analyzed in Nutritionist IV provided 1,000 mg calcium and 1,070 mg phosphorus/day. The goal for sodium content was <2,000 mg/day in the base diet. At the 1,800-kcal level, 1,900 mg of sodium were provided, according to the computer analysis. Subjects were stratified to one of three caloric levels (1,800, 2,100, or 2,400 kcal/day) based on an estimate of individual energy needs using the Schofield equation (26).

Meals were composed of common American foods, purchased locally from a major grocery supplier. Storage space and shelf-life limitations prevented the purchase of all the food for the study in advance, but consistency in purchase specifications and production methods was strictly enforced throughout. All meals were prepared under Registered Dietitian supervision in the University of Chicago General Clinical Research Center Metabolic Kitchen. The nutritionist (L. Trumbore) interviewed each subject before the study day to ascertain food allergies that might limit food choices and to instruct the subjects on a prestudy diet that approximated the study diet. Subjects were instructed to avoid supplements, particularly of vitamin D and calcium, during the week before the study day.

A laboratory analysis was performed of the three meals of the base diet (Table 2). For this purpose, the base diet was produced and each meal was homogenized, weighed, and frozen. Aliquots of each homogenized meal were analyzed for calcium, phosphorus, magnesium, sodium, and potassium, as well as total dry solids as a percentage of

### Table 1. Patients and normal subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>Sex</th>
<th>Weight, kg</th>
<th>CACR, mg/g</th>
<th>Stone Type</th>
<th>C_{\text{Cr}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>38</td>
<td>F</td>
<td>68</td>
<td>—</td>
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<tr>
<td>Normal</td>
<td>36</td>
<td>M</td>
<td>74</td>
<td>27–94</td>
<td>None</td>
<td>No</td>
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<tr>
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<td>52</td>
<td>F</td>
<td>61</td>
<td>110–155</td>
<td>None</td>
<td>Yes</td>
</tr>
<tr>
<td>Normal</td>
<td>23</td>
<td>F</td>
<td>70</td>
<td>74</td>
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<td>Yes</td>
</tr>
<tr>
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<td>M</td>
<td>74</td>
<td>57–98</td>
<td>None</td>
<td>Yes</td>
</tr>
<tr>
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<td>29</td>
<td>F</td>
<td>61.4</td>
<td>103</td>
<td>None</td>
<td>Yes</td>
</tr>
<tr>
<td>Normal</td>
<td>37</td>
<td>M</td>
<td>89.6</td>
<td>—</td>
<td>None</td>
<td>Yes</td>
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<tr>
<td>IHSF 1</td>
<td>41</td>
<td>F</td>
<td>46</td>
<td>116–155</td>
<td>BR (5)</td>
<td>No</td>
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<tr>
<td>IHSF 2</td>
<td>43</td>
<td>M</td>
<td>80</td>
<td>184–263</td>
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<tr>
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<td>M</td>
<td>89</td>
<td>137–170</td>
<td>CaOx (2)</td>
<td>Yes</td>
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<tr>
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<td>45</td>
<td>F</td>
<td>86</td>
<td>466–546</td>
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<td>Yes</td>
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<tr>
<td>IHSF 5</td>
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<td>84</td>
<td>227–285</td>
<td>CaOx</td>
<td>Yes</td>
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<tr>
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<td>47</td>
<td>F</td>
<td>56</td>
<td>131–152</td>
<td>BR</td>
<td>Yes</td>
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<tr>
<td>IHSF 7</td>
<td>47</td>
<td>F</td>
<td>75</td>
<td>162–281</td>
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<td>Yes</td>
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<tr>
<td>IHSF 8</td>
<td>45</td>
<td>M</td>
<td>79.5</td>
<td>207–232</td>
<td>BR</td>
<td>Yes</td>
</tr>
<tr>
<td>IHSF 9</td>
<td>42</td>
<td>F</td>
<td>80</td>
<td>350–484</td>
<td>Apatite</td>
<td>Yes</td>
</tr>
<tr>
<td>IHSF 10</td>
<td>56</td>
<td>M</td>
<td>94</td>
<td>159–232</td>
<td>BR (4)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

F, female; M, male; IHSF, stone formers with idiopathic hypercalciuria; CACR, urine calcium-to-creatine ratio; CA, stones reported as calcium oxalate; BR, stones reported as brushite (calcium monohydrogen phosphate); apatite, stone analysis shows calcium phosphate as hydroxyapatite. C_{\text{Cr}}, idiopathic stone analysis

### Table 2. Composition of base diet

<table>
<thead>
<tr>
<th></th>
<th>Breakfast</th>
<th>Lunch</th>
<th>Supper</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Total weight, g</td>
<td>519</td>
<td>432</td>
<td>481</td>
<td>1,432</td>
</tr>
<tr>
<td>Fraction dry solids</td>
<td>0.267</td>
<td>0.276</td>
<td>0.257</td>
<td></td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>407</td>
<td>455</td>
<td>297</td>
<td>1,160</td>
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<tr>
<td>Phosphorus, mg</td>
<td>377</td>
<td>334</td>
<td>529</td>
<td>1,240</td>
</tr>
<tr>
<td>Magnesium, mg</td>
<td>86</td>
<td>48</td>
<td>84</td>
<td>218</td>
</tr>
<tr>
<td>Sodium, mg</td>
<td>499</td>
<td>683</td>
<td>959</td>
<td>2,141</td>
</tr>
<tr>
<td>Potassium, mg</td>
<td>1,086</td>
<td>596</td>
<td>744</td>
<td>2,427</td>
</tr>
</tbody>
</table>

A full-day base diet (see Methods) was prepared for analysis; each meal was homogenized, weighed, and frozen. Aliquots were measured using inductively coupled plasma atomic emission spectrometry (Covance Laboratories, Madison WI).

#### Fasting Urine

<table>
<thead>
<tr>
<th>Urine #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

Fig. 1. Protocol of the experiment. Over a 14-h period (bottom row), we collected 14 (1-h) urine samples (top row), organized around fasting and 3 meals (middle row). Numbers of urine samples differed, as shown, between the meal periods. Serum samples were collected at the end of each urine collection and at the midpoints of urine samples 3, 4, 7, 8, 12, and 13.
total weight (Covance Laboratories, Madison, WI). Our overall plan-
ning goals for calcium, magnesium, phosphorus, and sodium were
reasonably well accomplished for the day, with some variation be-
tween meals.

Laboratory Measurements

Blood and urine calcium, phosphate, magnesium, creatinine, and
sodium, and urine volume were measured in our laboratory as de-
scribed elsewhere (1, 22). Serum intact PTH was measured by
electrochemiluminescence immunoassay (Roche, Elecsys, Indianapo-
lis, IN). Serum samples were ultrafiltered using a 10-kDa MW cutoff
membrane (Amicon Ultra-4, Millipore, Bedford, MA); calcium, phos-
phate, magnesium, and creatinine were measured in the ultrafiltrate
using the same methods as for serum. Ultrafiltrated (UF) and urine
iothalamate concentration were measured by reverse-phase HPLC
using a LiChrospher 100 RP18 5-μm, 250 × 4.6-mm column, eluted
with isocratic 50 mM NaH2PO4 containing 0.5 mM tetrabutyl ammo-
nium phosphate and methanol (87:13), at a flow rate of 0.8 ml/min
with detection at 239 nm.

Calculations

Urine excretions were calculated in milligrams per hour (calcium,
magnesium, phosphate, and creatinine) or milliequivalents per hour
(sodium); CCr was calculated in milliliters per hour using the ratio of
total creatinine excretion during the urine collection to the average
value of serum UF creatinine for the serum samples at the beginning
and end of the urine samples, or in the special cases of urine samples
3, 4, 7, 8, 12, and 13, the three samples available (beginning, midpoint
of collection, and end). For the fasting urine samples, we used only the
serum 1 and serum 2 creatinine values, respectively. Filtered loads
(mg/h) were calculated for calcium, magnesium, and phosphate as the
product of their UF concentration and CCr; for this purpose, we used
the same serum-averaging protocol as for CCr. For filtered load of
sodium (meq/h), we used serum sodium concentration. Fractional
re absorptions for all materials except sodium were calculated as 1 −
[U(i) × UF(cr)/UF(i) × U(cr)], where cr is creatinine concentration,
and i is the material being reabsorbed. For sodium, we used serum
values as noted. When iothalamate measurements were made, we
calculated filtered loads and re absorptions identically except that UF
and urine iothalamate concentrations replaces those of creatinine.

Statistical Approaches

General analytic approach. As the data consist of repeated mea-
sures on individuals over time, statistical methods for correlated data
were used to analyze the mean difference in each laboratory measure-
ment of interest between IHSF and N. In particular, generalized
estimating equations (10) utilizing compound symmetric covariance
structures were used to compare laboratory values between IHSF and
N. To determine whether the mean difference in each laboratory value
comparing IHSF and N changed with respect to mealtime, multipli-
cative interactions between the covariates were modeled. For simplic-
ity, mealtimes were modeled as fasting vs. nonfasting. In addition, to
compare the effect of IHSF by specific mealtimes, we also categorized
mealtimes into four groups: fasting, postbreakfast, postlunch, and post-
supper. Interaction terms were tested using multivariate Wald tests. In
individuals of interest between IHSF and N. In an additional
analysis of calcium reabsorption, adjustment was added for
serum PTH. Serum PTH was adjusted for age and sex. In an additional
analysis of serum PTH, adjustment was added for serum total calcium
concentration. No adjustment for multiple comparisons has been made
so that inferential P values should be interpreted accordingly.

Because creatinine is secreted and may be an imprecise measure of
glomerular filtration rate (GFR), secondary analyses adjusting for CCr
in place of Ccr, were conducted to investigate potential residual
confounding of GFR in the relationship between IHSF status and each
case of interest.

RESULTS

Fall in Tubule Calcium Reabsorption Accounts for
Postprandial Hypercalciuria

Fasting and fed UFCa levels did not differ between IHSF and
N (Fig. 2, top left; Table 3), nor did the mean differences
between fasting and fed values even though the mean change
in UFCa from fasting to fed was significant in IHSF and not in
N (Table 3). Filtered load of calcium did not increase with meals,
neither between IHSF and N (Fig. 2, bottom left; Table 3). Urine calcium adjusted for age, sex, sodium excre-
tion, height, weight, UFCA, and CCr was higher in IHSF vs. N
in the fed but not the fasting state (Fig. 2, top right; Table 3); the
difference from the fasting to fed state was significant in IHSF
and not in IHSF vs. N (Table 3). Correspond-
ingly, although tubule calcium reabsorption (Fig. 2, bottom
right; Table 3) fell with meals in IHSF and N, the fall was
greater in IHSF vs. N. Calcium reabsorption differed between
IHSF and N in the fasting and fed states, but differed more in
the fed than in the fasting state (Table 3). Altogether, the in-
crease in urine calcium after eating is greater in IHSF than
N, and the difference cannot be accounted for by differences in
UFCA, CCr, height, weight, age, sex, or sodium excretion, but by
a fall in calcium reabsorption.

Individual meal periods differed significantly from each
other. IHSF and N did not differ in adjusted urine calcium
excretion in the fasting period (Table 3) or in the periods after
breakfast (Fig. 2, top right) but did differ in the periods after
lunch and supper. Fractional reabsorption paralleled the behav-
or of adjusted urine calcium except that IHSF and N differed
even in the fasting period (Fig. 2, bottom right). Values of
mean differences for adjusted urine calcium excretion (mg/h)
comparing IHSF to N were 4.46 (−2.84, 11.76), 10.21 (2.42,
18.00), and 11.5 (4.52, 18.48) after breakfast, lunch, and
supper, respectively; 95% confidence intervals (CI) are in
parentheses (fasting values in Table 3). Neither fasting nor
postbreakfast values differed significantly (CI values include
0). Corresponding values of mean differences in fractional
reabsorption comparing IHSF to N were −0.015 (−0.029,
−0.001), −0.029 (−0.46, −0.012), and −0.030 (−0.046,
−0.014) after breakfast, lunch, and supper, respectively. In
other words, adjusted fractional reabsorption differed between
IHSF and N fasting (Table 3) and in the periods following each
of the meals; moreover, the difference between IHSF and N
increased with breakfast and lunch (Fig. 2, bottom right).

If the time sequence is collapsed into fasting and fed periods,
one can visually detail individual data points (Fig. 3). Individ-
ual values for fasting and fed UFCA (Fig. 3, top left) and filtered
load of calcium (Fig. 3, bottom left) overlap completely be-
 tween N and IHSF; values for the individual with Dunnigan
type 1 lipodystrophy, represented by circled points in Fig. 3,
are within the values from other IHSF. In contrast, the overlap between N and IHSF is minimal with respect to urine calcium (Fig. 3, top right) and fractional calcium reabsorption (Fig. 3, bottom right). Once again, the values from the Dunnigan patient fall within the points from other IHSF.

Natriuresis Did Not Accompany Hypercalciuria

Urine calcium increased with meals, and the greater increase in IHSF vs. N occurred despite adjustment for urine sodium excretion, as already noted. Similarly, fractional calcium reabsorption fell significantly in IHSF vs. N and did not change in N. There were no significant changes in fractional calcium reabsorption in IHSF with meals.

Table 3. Primary analysis

<table>
<thead>
<tr>
<th></th>
<th>Fasting</th>
<th>Fed</th>
<th>N</th>
<th>IHSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>UF calcium</td>
<td>-0.05 (-0.33, 0.23)</td>
<td>0.06 (-0.23, 0.35)</td>
<td>0.06 (-0.06, 0.18)</td>
<td>0.17 (0.04, 0.3)‡</td>
</tr>
<tr>
<td>FL calcium</td>
<td>0.7 (-71.73)</td>
<td>28 (-36.92)</td>
<td>-19 (-48, 9)</td>
<td>3.02 (0.3, 5.74)‡</td>
</tr>
<tr>
<td>Urine calcium</td>
<td>0.7 (-5.84, 7.24)</td>
<td>9.12 (1.96, 16.28)*,†</td>
<td>-0.026 (-0.041, -0.011)*,†</td>
<td>11.44 (6.66, 16.22)‡§</td>
</tr>
<tr>
<td>FR calcium</td>
<td>-0.012 (-0.023, -0.001)*</td>
<td>-0.026 (-0.041, -0.011)*,†</td>
<td>-0.010 (-0.015, -0.005)*,†</td>
<td>-0.024 (-0.033, -0.015)‡§</td>
</tr>
<tr>
<td>UF magnesium</td>
<td>-0.18 (-0.28, -0.08)*</td>
<td>-0.16 (-0.25, -0.07)*</td>
<td>-0.01 (-0.04, 0.02)</td>
<td>0.02 (-0.02, 0.06)</td>
</tr>
<tr>
<td>FL magnesium</td>
<td>-11.8 (-45.22)</td>
<td>-4.5 (-31.22)</td>
<td>-8.7 (-17, -7)‡</td>
<td>-1.4 (-16.5, 13.7)</td>
</tr>
<tr>
<td>Urine magnesium</td>
<td>0.06 (-2.06, 2.18)</td>
<td>1.10 (-0.47, 2.67)</td>
<td>2.19 (0.77, 3.61)‡</td>
<td>3.23 (2.43, 4.03)‡</td>
</tr>
<tr>
<td>FR magnesium</td>
<td>0.005 (-0.007, 0.017)</td>
<td>-0.005 (-0.019, 0.009)</td>
<td>-0.019 (-0.031, -0.007)‡</td>
<td>-0.030 (-0.039, -0.021)‡‡</td>
</tr>
<tr>
<td>UF phosphate</td>
<td>-0.59 (-1.08, -0.1)*</td>
<td>-0.47 (-0.78, -0.16)*</td>
<td>0.29 (-0.02, 0.6)</td>
<td>0.42 (0.22, 0.62)‡‡</td>
</tr>
<tr>
<td>FL phosphate</td>
<td>-39.8 (-83.7, 4.1)</td>
<td>-19.1 (-62.7, 24.5)</td>
<td>9.0 (-19.5, 37.5)</td>
<td>29.7 (4.0, 55.4)‡‡</td>
</tr>
<tr>
<td>Urine phosphate</td>
<td>10.82 (-3.71, 25.35)</td>
<td>10.35 (-1.28, 21.98)</td>
<td>3.39 (-4.45, 11.23)</td>
<td>2.92 (-3.09, 8.93)</td>
</tr>
<tr>
<td>FR phosphate</td>
<td>-0.031 (-0.078, 0.016)</td>
<td>-0.025 (-0.057, -0.007)*</td>
<td>-0.015 (-0.043, 0.013)</td>
<td>-0.009 (-0.032, 0.014)</td>
</tr>
</tbody>
</table>

Values are adjusted means with 95% confidence interval in parentheses. UF, ultrafiltrate; FL, filtered load; FR, fractional reabsorption; IHSF, stone formers with idiopathic hypercalciuria; N, non-stone formers. Columns 2 and 3 depict changes between IHSF and N within fasting and fed states. Columns 4 and 5 depict changes between fed and fasting states within N and IHSF. *Difference between IHSF and N within fasting or fed states, P < 0.05. †Changes between IHSF and N groups differ between fasting and fed states, P < 0.05. ‡Difference between fed and fasting within N and IHSF groups, P < 0.05. §Changes between fed and fasting states differ between N and IHSF groups, P < 0.05.
sorption fell, despite adjustment for urine sodium excretion. Because independence from sodium is of great importance, we illustrate these findings here. A plot of calcium excretion vs. urine sodium excretion (Fig. 4, left) and of calcium fractional reabsorption vs. sodium reabsorption (Fig. 4, right) illustrates that postprandial calcium excretion and reabsorption in IHSF are above and below those in N, respectively, despite overlapping values of sodium excretion and reabsorption.

Magnesuria was Present But Not Different Between IHSF and N

Serum UF\textsubscript{Mg} of IHSF were below N (Fig. 5, top left; Table 3) both fasting and fed and did not change with meals. Filtered loads were not different and increased with meals in N but not IHSF (Fig. 5, bottom left; Table 3). Urine magnesium excretion adjusted for sex, age, height, weight, sodium excretion, UF\textsubscript{Mg}, and C\textsubscript{Cr} rose with meals but did not differ significantly between IHSF and N (Fig. 5, top right; Table 3). Magnesium reabsorption fell with meals in both IHSF and N; IHSF and N did not differ in adjusted magnesium reabsorption rates (Fig. 5, bottom right; Table 3).

Phosphate Responses Differed from Those of Calcium and Magnesium

Throughout the day, UF\textsubscript{Phos} of IHSF was below N (Fig. 6, top left; Table 3). Serum UF\textsubscript{Phos} levels and phosphate filtered

Fig. 3. Renal calcium handling in individual subjects. Values of serum ultrafiltrable calcium (top left), urine calcium excretion (top right), filtered load of calcium (bottom left), and tubule calcium reabsorption (bottom right) are shown for N (○) and IHSF (●) during fasting and fed periods. Results from 1 subject with Dunnigan type 1 lipodystrophy are represented by encircled symbols.

Fig. 4. Lack of relationship between hypercalciuria and natriuresis. Unadjusted urine calcium excretion (left, y-axis), is higher in IHSF (●) vs. N (○) during the 3 postprandial periods despite overlapping values for urine sodium excretion rate (x-axis). Calcium excretions overlapped in the fasting period. Unadjusted fractional calcium reabsorption (right, y-axis) was lower in IHSF vs. N during the 3 postprandial periods despite overlap of sodium fractional reabsorptions (x-axis). Fractional reabsorptions overlapped in the fasting period. Values are means with 1.96 SE.
load rose with meals in IHSF but not N (Fig. 6, top and bottom left; Table 3). Urine phosphate excretion did not differ between IHSF and N, nor did it change with meals. Tubule phosphate reabsorption did not change with meals but was lower in IHSF than N during meals (Fig. 6, bottom right; Table 3). This difference in adjusted tubule reabsorption between IHSF and N was significant after all three meals (results not shown).

Serum PTH does not account for the lower calcium reabsorption of IHSF vs. N. Serum PTH levels did not differ between IHSF and N in the fasting or fed states, without (Table 4, PTH) (1) or with (Table 4, PTH) (2) adjustment for serum calcium concentration (Fig. 7, left). Unadjusted serum PTH fell with food in IHSF and N (Table 4, PTH) (1), but when adjusted for serum calcium PTH did not fall significantly with feeding in N (Table 4, PTH) (2). Change in urine calcium excretion adjusted for height, weight, age, sex, sodium excretion, Ccr, UFca, and PTH differed between IHSF and N in the fed vs. fasting state because adjusted urine calcium rose with feeding in IHSF but not N (Table 4). Calcium fractional reabsorption adjusted for age, sex, sodium excretion, and PTH fell with meals in IHSF and N and was lower in IHSF vs. N both fasting and fed (Table 4 and Fig. 7, right). In other words, adjustment for serum PTH does not account for the observed difference in fractional calcium reabsorption or urine calcium excretion between IHSF and N. This is visually evident in Fig. 7, right; unadjusted calcium reabsorption values of N rise above those of IHSF at clearly overlapping unadjusted serum PTH levels.

**Comparison of Iothalamate to Ccr as an Adjustment Variable**

In the n = 13 subjects for whom we have both measurements, mean Ccr did not exceed clearance of CIt (mean Ccr was 7.97 l/h vs. mean CIt of 7.57 l/h; P = 0.340). Differences between Ccr and CIt were not sufficiently differential between IHSF and N to influence our conclusions concerning adjusted UFca, UFmg, UFphos, urinary excretion rates, or fractional reabsorptions, with the exception of a significant change in the mean difference in UFca between IHSF and N by mealtime. With adjustment for CIt, it was estimated that the mean difference in UFca between IHSF and N while fasting was −0.10 vs. 0.09 after feeding; the P value of interaction between IHSF status (IHSF vs. N) and the feeding state (fasting vs. fed) was 0.004. In contrast, with adjustment for Ccr it was estimated that the mean difference in UFca between IHSF and N in the fasting state was −0.05 vs. 0.06 after feeding (Table 3, P = not significant). This minor change does not influence any of our conclusions concerning mechanisms of hypercalciuria and indicates that iothalamate offered no advantages vs. creatinine as a filtration marker in these experiments.
Discussion

Eating normal foods reduces calcium reabsorption, more in IHSF than N

The outcomes of our first aim seem clear; in IHSF the large amount of extra calcium that is known to enter the blood from absorption of dietary calcium after eating (4-7) is ferried into the urine mainly by reduction of renal tubule calcium reabsorption. Differences in filtered load of calcium between IHSF and N and between fasting and fed conditions do not account for the observed changes in urine calcium excretion. Others, using single semisynthetic meal preparations with a large added calcium load (27), found prompt urine calcium increases with modest changes in blood calcium, compatible with our findings. Oral calcium loads without foods and large amounts of high-calcium foods (15, 17, 27, 28) produce only modest increases in blood calcium, but in none of these studies was tubule reabsorption measured. In two studies of fasting IHSF and normal controls, tubule reabsorption of IH patients was slightly lower, as we found here (11, 23). During 9 days of a very-low-calcium diet, many IHSF patients lost more calcium in the urine than they ingested despite the absence of a significant change in serum calcium from the control diet, indicating renal losses of bone mineral (9).

Our findings do not comment on the long history of “renal” and “absorptive” IH physiologies (21). Fasting urine calcium excretions, serum PTH values, cAMP levels fasting and after oral calcium loads, and rates of urine calcium excretion after fasting IHSF and normal controls, tubule reabsorption of IH patients was slightly lower, as we found here (11, 23). During 9 days of a very-low-calcium diet, many IHSF patients lost more calcium in the urine than they ingested despite the absence of a significant change in serum calcium from the control diet, indicating renal losses of bone mineral (9).

Table 4. Secondary analysis of PTH and calcium

<table>
<thead>
<tr>
<th></th>
<th>Fasting</th>
<th>Fed</th>
<th>N (Fed-Fasting)</th>
<th>IHSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum PTH (1)</td>
<td>7.01</td>
<td>-0.60</td>
<td>-3.71 ( -5.18, -2.24)‡</td>
<td>-11.32 ( -19.70, -2.94)‡</td>
</tr>
<tr>
<td>Serum PTH (2)</td>
<td>6.88</td>
<td>1.60</td>
<td>-1.89 ( -5.32, 1.54)‡</td>
<td>-7.17 ( -11.65, -2.69)‡</td>
</tr>
<tr>
<td>Urine calcium</td>
<td>1.50</td>
<td>8.30</td>
<td>1.10 (2.60, 4.80)</td>
<td>7.9 (3.30, 12.50)§§</td>
</tr>
<tr>
<td>FR calcium</td>
<td>-0.018</td>
<td>-0.027</td>
<td>-0.007 ( -0.013, -0.001)‡</td>
<td>-0.016 ( -0.024, -0.008)‡</td>
</tr>
</tbody>
</table>

Values are adjusted means with 95% confidence interval in parentheses. Serum PTH (1), serum PTH adjusted for age and sex; serum PTH (2), serum PTH adjusted for age, sex, and total serum calcium. Columns are defined as in Table 3. *Difference between IHSF and N within fasting or fed states, P < 0.05. †Changes between IHSF and N groups differ between fasting and fed states, P < 0.05. ‡Difference between fed and fasting within N and IHSF groups, P < 0.05. §Changes between fed and fasting states differ between N and IHSF groups, P < 0.05.
such calcium loads and during low dietary calcium intakes have been used to classify patients into these two categories. Any or all of these physiologies could be based on a model in which renal tubule calcium reabsorption varied with meals, as this particular measurement has not been made as part of published classification schemes. In fact, our particular patients were not selected based on such a classification. In a sense, all IH may well be, in part, "renal," if what we have found here, in unselected calcium stone formers with IH, is found to be true in other populations.

**PTH is not the Main Mediator of Postprandial Hypercalcuria or IH**

Our second specific aim concerned PTH, an established regulator of renal tubule calcium reabsorption (14). Before adjustment for total serum calcium, serum PTH levels fell with meals in both IHSF and N. With adjustment for total serum calcium, decreases in serum PTH after feeding were only observed in IHSF. Nonetheless, adjustment for serum PTH did not affect the significance of the difference in calcium reabsorption between IHSF and N. A simple plot of unadjusted calcium reabsorption against unadjusted serum PTH illustrates the large differences in reabsorption despite overlapping values for serum PTH in IHSF vs. N (Fig. 7, right). Nor did a change in PTH account for the fall in tubule calcium reabsorption with meals in either IHSF or N. Therefore, even though the simultaneous fall in PTH and calcium reabsorption with meals points to some PTH regulation of calcium reabsorption, PTH changes are not a sufficient explanation for the greater reduction in IH and therefore for their greater postprandial hypercalcuria, nor for the postprandial fall in tubule calcium reabsorption in IHSF or N.

**Changes in Sodium and Magnesium Reabsorption Hint at Effects of Cortical Thick Ascending Limb**

Our third aim was to infer from changes in sodium, magnesium, and phosphate reabsorption what nephron segments might possibly be responsible for the postprandial fall in calcium reabsorption and for the differential responses of IHSF vs. N.

**Sodium reabsorption does not parallel that of calcium.** We found a fall in renal calcium reabsorption despite no changes in renal sodium reabsorption, and as a corollary urine calcium increased without a change in urine sodium excretion. This is compatible with the functioning of the cortical thick ascending limb (CTAL) and distal convoluted tubule, both of which can dissociate changes in calcium and sodium reabsorption (2, 14).

**Magnesium reabsorption, CTAL, and the cell surface calcium receptor.** CTAL specifically responds to activation of the cell surface calcium receptor (CaSR) with a reduction of calcium reabsorption that is not accompanied by changes in sodium reabsorption (20). It is also known to reabsorb both calcium and magnesium (20) and could therefore produce the modest magensuria we observed in subjects after meals. This segment also responds to PTH with increased calcium reabsorption (20), so the correlated reductions in calcium reabsorption and PTH after meals would also be reasonable. In a single study of PTH clamping of normal men (12), urine calcium excretion per unit of GFR varied directly with serum calcium, indicating indirect evidence for CaSR regulation of calcium reabsorption independently of PTH; urine calcium values for given ranges of serum calcium were the same at 10- and 200-meq sodium intakes. This work supports our idea that PTH is not the main cause of the postprandial reduction of calcium reabsorption and also hints at CTAL as a likely regulation site. Since serum calcium rose with meals in at least our IHSF, a signal exists for CaSR regulation.

**Phosphate Reabsorption is Low in IHSF vs. N after Meals and Does Not Parallel Changes in Calcium Reabsorption**

Why tubule phosphate reabsorption is low in IHSF vs. N after meals is not clear. An attractive possibility is a higher level of one or more phosphotinins, such as FGF 23 (19, 24, 25). We have no measurements to offer here. PTH is clearly not an explanation, given that serum PTH values did not significantly differ between IHSF and N.

**Relationship of Our Findings to Apatite Plaque Formation**

If the main objectives of our three aims was to add detail to the pathophysiological map of IH, our more general objective was to gather clues as to why high urine calcium excretion rates are linked to high plaque abundance (18). That we now know it is mainly tubule calcium reabsorption changes that mediate the renal removal of absorbed calcium gives a first clue as to how to proceed.

Hadh postprandial increases in serum calcium been more marked in IHSF than N, increased lumen calcium concentration in thin loops, where plaque first forms, might be part of the...
mechanism. Similarly, increased descending vas recta calcium concentration could play a role given their close proximity to plaque areas. We did not find this. Had filtered load of calcium increased more in IHSF than N, one might have speculated that increased delivery of calcium to the medullary thick ascending limbs led to increased interstitial calcium concentrations that could increase descending vas recta calcium concentrations. We did not find differential changes in filtered load between IHSF and N. Needed are experiments in humans to attempt a more complete identification of the tubule segments involved, a matter for another time.

Subject with Dunnigan Lipodystrophy

Strictly, we should not have included this subject in that she had slightly increased serum calcium levels, but we chose her inclusion for multiple reasons. This genetic disorder has no known associations with hypercalciuria, yet she and her daughter with Dunnigan lipodystrophy are hypercalciuric. Her low PTH level and high serum 1,25D level are usual in IH (8). There was no clinical evidence for sarcoidosis. The higher serum calcium is not explained. In fact, her UFCa values are not the highest in our group, nor are her filtered loads (her points are identified in Fig. 3). All of her results are enclosed by those of other IH subjects. We hope that including her may prompt others to study this genetic disorder further in relation to hypercalciuria.

Study Limitations

Our IHSF were mostly chosen from our outpatient clinic using the criteria described in METHODS. Apart from the requirement that all exhibit hypercalciuria, they would not differ remarkably from any other sample of calcium stone formers. Three patients (IHSF subjects 4, 6, and 8, Table 1) met our criteria but had also participated in a separate research project involving papillary intraoperative biopsy. The biopsy procedure had been performed months to years before the present study. However, all three formed calcium phosphate stones whereas CaOx stones are by far more common. As a result, 6 of our 10 IHSF formed calcium phosphate stones (Table 1).

Because this is an observational study with respect to IHSF, there may be unmeasured confounders when IHSF are compared with N. This being said, we have adjusted for the most common potential confounders such as age and sex, and in relation to filtered load and excretion rates we also adjusted for height and weight. Since one cannot randomly allocate subjects to being IHSF vs. N, this is an unavoidable limitation.

Other limitations of the protocol include the lack of a diet equilibration period, some variability in the timing of sample collections, and possible shifts in food nutrient contents. Each subject was interviewed by one of us (L. Trumbore) with respect to diet and was instructed to follow a diet similar to our test diet for 4 days at home before the study to minimize the discrepancies between the at-home and test diets. Because of our addition of iothalamate measurements which require a loading dose and equilibration period, our protocol went through three iterations with respect to clock times for starting and ending the experiment. Thus the meal clock times varied by as much as 1 h between protocol iterations, but never varied with respect to the interval of time between meals, the number of samples obtained, or the intervals between sample collections and meals.

Summary

Limitations notwithstanding, we are reasonably confident that the primary mechanism by which kidneys ferry absorbed calcium into the urine after meals is via reduced tubule calcium reabsorption, and that IHSF differ from N mainly with respect to the magnitude of their tubule response. The linkages between eating and reduction of calcium reabsorption are not known but our work strongly suggests that PTH is not likely to be a sufficient explanation. The behavior of magnesium reabsorption parallels that of calcium reabsorption, but IHSF and N were not different. Because calcium and magnesium responses were similar, and because sodium reabsorption did not change along with calcium reabsorption, tubule adjustments may well involve sites at which regulation of reabsorption of both ions can occur without change in sodium reabsorption, such as CTAL. Finally, as others have found, IHSF exhibit lower serum phosphorous and lower tubule reabsorption than N. The present work was not intended to explore this matter in further detail; however, it is of interest that eating did not alter phosphorous reabsorption.

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