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Effect of cinacalcet on urine calcium excretion and supersaturation in genetic hypercalciuric stone-forming rats

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Idiopathic hypercalciuria is the most common metabolic abnormality in patients with nephrolithiasis. Through successive inbreeding, we have developed a strain of rats whose urine calcium (UCa) excretion is ~8–10-fold greater than that of control rats and who spontaneously form kidney stones. We have termed these rats genetic hypercalciuric stone-forming (GHS) rats. The physiology of the hypercalciuria in the GHS rats closely parallels that of man. We have recently shown that the GHS rat kidneys have an increased number of receptors for calcium (CaR) compared to Sprague–Dawley rats, the strain of rats originally bred to develop the GHS rats. Calcimimetics, such as cinacalcet (Cin), increase the sensitivity of the CaR to Ca. The effects of Cin on UCa are complex and difficult to predict. We tested the hypothesis that Cin would alter urinary (U) Ca and supersaturation with respect to calcium hydrogen phosphate ($CaHPO_4$) and calcium oxalate (CaOx). GHS or control rats were fed a normal Ca diet (0.6% Ca) for 28 days with Cin (30 mg/kg/24 h) added to the diet of half of each group for the last 14 days. The protocol was then repeated while the rats were fed a low Ca (0.02% Ca) diet. We found that Cin led to a marked reduction in circulating parathyroid hormone and a modest reduction in serum Ca. Cin did not alter UCa when the GHS rats were fed the normal Ca diet but lowered UCa when they were fed the low Ca diet. However, Cin did not alter U supersaturation with respect to either CaOx or $CaHPO_4$ on either diet. If these findings in GHS rats can be confirmed in man, it suggests that Cin would not be an effective agent in the treatment of human idiopathic hypercalciuria and resultant stone formation.

Kidney International (2006) **69,** 1586–1592. doi:10.1038/sj.ki.5000324; published online 22 March 2006

KEYWORDS: nephrolithiasis; hypercalciuria; calcium receptor

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Received 19 July 2005; revised 21 December 2005; accepted 12 January 2006; published online 22 March 2006

Hypercalciuria is the most common metabolic abnormality found in humans with nephrolithiasis.[1–5] Hypercalciuria raises urine saturation with respect to the solid phases of calcium hydrogen phosphate ($CaHPO_4$, brushite) and calcium oxalate (CaOx), enhancing the probability of nucleation and growth of crystals into clinically significant kidney stones.[1,2,4]

We have established a model of hypercalciuria and nephrolithiasis by successively inbreeding 67 generations of the most hypercalciuric progeny of the most hypercalciuric Sprague–Dawley rats found on an initial screen. Each rat now excretes 8–10 times as much urinary calcium (UCa) as similarly fed controls.[6–23] The hypercalciuria is due to increased intestinal Ca absorption[6,7] coupled to a defect in renal tubular Ca reabsorption[7,14] and enhanced bone mineral resorption,[11] suggesting a systemic dysregulation of Ca homeostasis.[8] Human stone formers and these rats share many metabolic features in common; many human stone formers also have increased intestinal Ca absorption, increased bone resorption, and decreased renal tubule Ca reabsorption.[1,3] Virtually all of these hypercalciuric rats form kidney stones while there was no evidence of stone formation in controls.[10] We have termed the rats genetic hypercalciuric stone-forming (GHS) rats.[10,12,13,15,17–20] The stones formed by the GHS rats fed standard rat chow contain only Ca and phosphorus (P).[10,13,18,19] The dietary addition of hydroxyproline, a common amino acid and an oxalate precursor,[24] results in the formation of CaOx kidney stones.[17,20]

The Ca receptor (CaR) is a seven membrane-spanning protein that is part of the G-coupled protein family of plasma membrane receptors.[25] The CaR is expressed in a wide variety of tissues including parathyroids, kidney, and gastrointestinal tract.[26–28] There is marked homology between the parathyroid and kidney CaR in a variety of animals including man and rat.[25,28] In the thick ascending limb of the loop of Henle, the secretion of potassium into the lumen, through the potassium channel ROMK, increases the lumen positive voltage and drives Ca reabsorption through the paracellular space.[26] At this tubular site, elevation of the blood Ca level is detected by the CaR, located on the plasma (anti-luminal) membrane, decreasing potassium traffic through this channel

resulting in decreased luminal positivity, decreased Ca reabsorption, increased UCa, and a reduction in the concentration of serum Ca.

The GHS rats have been found to have elevated levels of vitamin D receptors (VDRs) in the intestinal mucosa, bone, and renal cortex.[8,11,16,23] Analogously, human stone formers have also been shown to have an elevated number of VDRs in their circulating monocytes.[29] CaR contains vitamin D response elements in its promoter region.[30] We found that there was increased CaR mRNA and protein in the GHS rat kidney and that $1,25(OH)_2D_3$ increased CaR levels through both elevated CaR gene expression and prolonged tissue half-life.[21]

The calcimimetics, such as cinacalcet (Cin), are small organic molecules that act as allosteric activators of the CaR, increasing the sensitivity of the CaR to serum Ca and substantially lowering parathyroid hormone (PTH) levels.[31–33] In patients with secondary hyperparathyroidism treated with Cin, there is a marked reduction of PTH ($\sim$50%) and a modest reduction of serum Ca ($\sim$10%).[31,33] The effect of Cin on human UCa and supersaturations with respect to common solid phases responsible for kidney stones has not been reported.

The effects of Cin on renal tubular Ca reabsorption and resulting UCa are complex. A reduction in PTH should increase UCa; in addition, increasing the sensitivity of the renal CaR to Ca should lead to an increase in UCa. However, the lowered filtered load of Ca from Cin-induced hypocalcemia should lower UCa. In this study, we utilized the GHS rats to test the hypothesis that Cin would alter urine Ca excretion and supersaturation with respect to $CaHPO_4$ and CaOx.

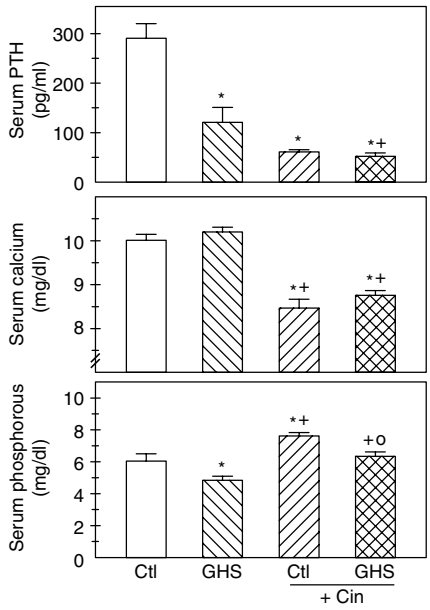

Figure 1 | Serum PTH, Ca, and P at the conclusion of the experiment. Fourteen 67th generation female GHS and 14 Ctl rats were placed in metabolic cages. From days 1–14, each rat in each group was fed NCD. From days 15 to 28, half of each group (seven GHS and seven Ctl rats) was continued on NCD and the other half (seven GHS and seven Ctl rats) were fed NCD supplemented with Cin. From days 29 to 42, all GHS and Ctl rats were fed LCD. No rat received Cin. From days 43 to 56, half of each group was continued on LCD without modification and the other half (the same rats that had previously received Cin) was fed LCD supplemented with Cin. Blood was then drawn at the conclusion of day 56. Abbreviations: Ctl, Sprague–Dawley rats; GHS, genetic hypercalciuric stone-forming rats; NCD, 0.6% Ca and 0.65% P; LCD, 0.02% Ca and 0.65% P; Cin, cinacalcet; *, $P<0.05$ vs Ctl; +$P<0.05$ vs GHS; o, $P<0.05$ vs Ctl + Cin.

RESULTS

Serum PTH, Ca, and P

At the conclusion of the experiment, when all rats were being fed low Ca diet (LCD), the serum PTH was significantly lower in the GHS rats compared to the control (Ctl) rats (Figure 1, top). The addition of Cin led to a significant fall in PTH both in the Ctl and in the GHS rats compared to respective non-Cin-fed rats. There was no difference in serum PTH between the two Cin-fed groups. There was no difference in serum Ca between the GHS and the Ctl rats not fed Cin; however, the addition of Cin led to a significant reduction in Ca both in the Ctl and in the GHS rats (Figure 1, middle). There was no difference in serum Ca between the two Cin-fed groups. Compared to the Ctl rats, serum P was significantly lower in the GHS rats; the addition of Cin led to an increase in serum P both in the Ctl and in the GHS rats (Figure 1, bottom). Serum P was lower in the GHS rats fed Cin compared to the Ctl rats fed Cin.

Urine Ca excretion

While being fed the normal Ca diet (NCD, 0.6% Ca), the GHS rats excreted significantly more UCa compared to the Ctl rats (Figure 2, top). With the addition of Cin to half of each group, UCa remained significantly elevated in the GHS rats compared to the Ctl rats whether or not they received Cin. Cin did not alter UCa in either group. On the LCD (0.02% Ca) without Cin, the GHS rats continued to excrete significantly more UCa than the Ctl rats (Figure 2, bottom). With the addition of Cin to the same rats that had received Cin previously, UCa remained significantly elevated in the GHS rats compared to the Ctl rats whether or not they received Cin. Cin significantly lowered UCa in the GHS, but not in the Ctl rats.

Urine P excretion

While being fed NCD the GHS rats excreted slightly, but significantly, more P compared to the Ctl rats (Figure 3, top). With the addition of Cin to half of each group, urine phosphorus (UP) remained significantly greater in the GHS rats compared to the Ctl rats whether or not they received Cin. Cin did not alter UP in the Ctl or in the GHS rats. On the LCD without Cin, the GHS rats continued to excrete significantly more UP than the Ctl rats and there was a significant increase in the P excretion for both Ctl and GHS rats compared to NCD (Figure 3, bottom). With the addition of Cin, UP was not different between the GHS and the Ctl rats. Cin did not alter UP in the Ctl or in the GHS rats.

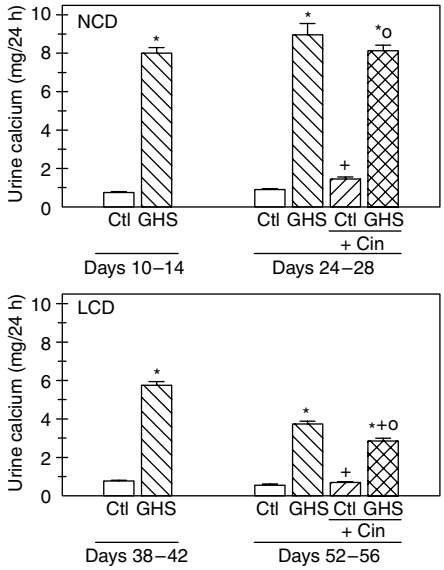

Figure 2 | Urine Ca excretion at the four different urine collection periods. Fourteen 67th generation female GHS and 14 Ctl rats were placed in metabolic cages. From days 1 to 14, each rat in each group was fed NCD. During the last 5 days of this period (days 10–14), five successive 24-h urine collections were obtained. From days 15 to 28, half of each group was continued on NCD and the other half were fed NCD supplemented with Cin. During the last 5 days of this period (days 24–28), five successive 24-h urine collections were obtained. From days 29 to 42, all GHS and Ctl rats were fed LCD. No rat received Cin. During the last 5 days of this period (days 38-42), five successive 24-h urine collections were obtained. From days 43 to 56, half of each group was continued on LCD without modification and the other half (that had previously received Cin) was fed LCD supplemented with Cin. During the last 5 days of this period (days 52–56), five successive 24-h urine collections were obtained. Abbreviations: Ctl, Sprague–Dawley rats; GHS, genetic hypercalciuric stone-forming rats; NCD, 0.6% Ca and 0.65% P; LCD, 0.02% Ca and 0.65% P; Cin, cinacalcet; *$P<0.05$ vs Ctl, same time period; +$P<0.05$ vs GHS, same time period; o, $P<0.05$ vs Ctl + Cin, same time period.

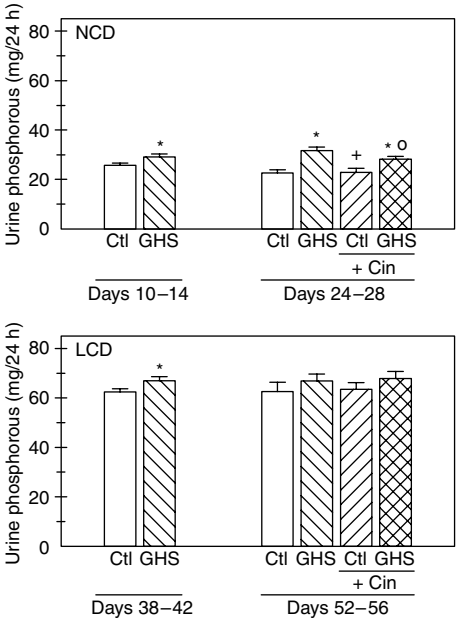

Figure 3 | Urine P excretion at the four different collection periods. Protocol and abbreviations as in Figure 2.

Urine oxalate excretion

While being fed the NCD, the GHS rats excreted significantly less oxalate (Ox) compared to the Ctl rats (Figure 4, top). With the addition of Cin to half of each group, urine oxalate (UOx) remained significantly lower in the GHS rats compared to the Ctl rats whether or not they received Cin. Cin led to a significant increase in UOx in the Ctl, but not the GHS, rats. On the LCD without Cin, the GHS rats continued to excrete significantly less UOx than the Ctl rats (Figure 4, bottom). With the addition of Cin, UOx remained significantly lower in the GHS rats compared to the Ctl rats. Cin significantly increased UOx in the Ctl and in the GHS rats.

Urine $CaHPO_4$ supersaturation

While being fed NCD, the GHS rats had a significantly higher urinary supersaturation (USS) for $CaHPO_4$ (brushite) compared to the Ctl rats (Figure 5, top). With the addition of Cin, USS for $CaHPO_4$ remained significantly elevated in the GHS rats compared to the Ctl rats whether or not they received Cin and Cin did not alter USS for $CaHPO_4$ in either group. On the LCD without Cin, the GHS rats continued to have a significantly higher USS for $CaHPO_4$ than the Ctl rats (Figure 5, bottom). With the addition of Cin, USS for $CaHPO_4$ remained significantly elevated in the GHS rats compared to the Ctl rats whether or not they received Cin. Cin did not alter USS for $CaHPO_4$ in the GHS or in the Ctl rats.

Urine CaOx supersaturation

While being fed NCD, the GHS rats had a significantly higher USS for CaOx compared to the Ctl rats (Figure 6, top). With the addition of Cin, USS for CaOx remained significantly elevated in the GHS rats compared to the Ctl rats whether or

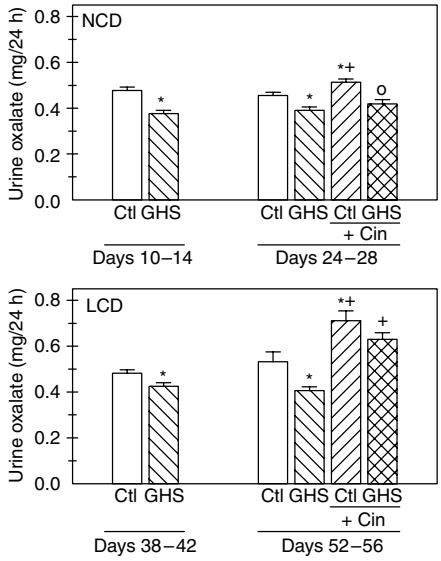

Figure 4 | Urine Ox excretion at the four different collection periods. Protocol and abbreviations as in Figure 2.

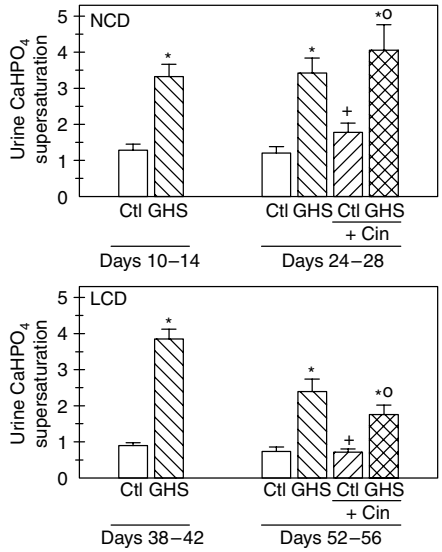

Figure 5 | Urine supersaturation with respect to the $CaHPO_4$ (brushite) solid phase at the four different collection periods. Protocol and abbreviations as in Figure 2.

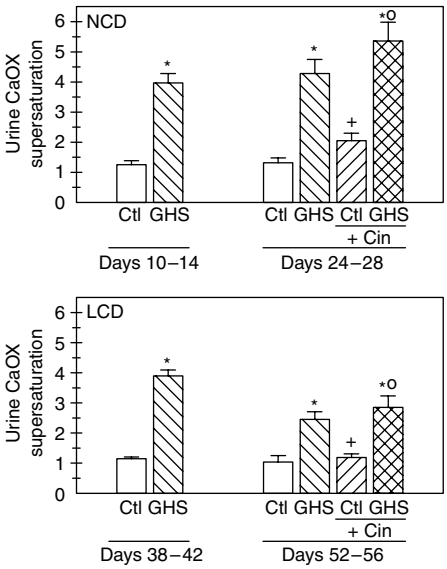

Figure 6 | Urine supersaturation with respect to the CaOx solid phase at the four different collection periods. Protocol and abbreviations as in Figure 2.

not they received Cin and Cin did not alter USS for CaOx in either group. On the LCD without Cin, the GHS rats continued to have a significantly higher USS for CaOx than the Ctl rats (Figure 6, bottom). With the addition of Cin, USS for CaOx remained significantly elevated in the GHS rats compared to the Ctl rats whether or not they received Cin. Cin did not alter USS for CaOx in the GHS or in the Ctl rats.

Urine volume, creatinine clearance, and filtered load of Ca

The GHS rats were polyuric (32 ± 1.7 ml/24 h) compared to the Ctl rats (21.9 ± 2.4, $P < 0.05$) on NCD and on LCD (19.4 ± 0.9 ml/24 h GHS vs 14.6 ± 0.9 Ctl, $P < 0.05$) and Cin did not alter urine volume. There was no difference in the creatinine clearance between the GHS (0.95 ± 0.05 ml/min) and Ctl (1.09 ± 0.05) rats on LCD and Cin did not alter the creatinine clearance (0.96 ± 0.03 ml/min, GHS + Cin vs 1.07 ± 0.08 Ctl + Cin). There was no significant difference in the filtered load of Ca between the GHS (72.9 ± 3.0 μg Ca/min) and Ctl (78.7 ± 3) rats on LCD; however, Cin significantly lowered the filtered load of Ca in both the GHS (60.8 ± 2.3) and the Ctl (57.2 ± 1.4) rats compared to similar rats not fed Cin (both $P < 0.05$).

DISCUSSION

The GHS rats were bred for hypercalciuria, the most common metabolic abnormality in patients with nephrolithiasis. Studies of the pathophysiology of the hypercalciuria in the GHS rats reveal that, similar to many patients with idiopathic hypercalciuria, they have increased intestinal Ca absorption,[6,7] reduced renal Ca reabsorption,[7,14] and excessive bone demineralization.[11] The dysregulation of Ca transport at these sites suggests a systemic abnormality in Ca homeostasis.[8] Indeed, we have shown that there is an increase in the number of VDR in intestine, bone, and kidney[8,11,16,23] and CaR in kidney[21] of the GHS compared to non-hypercalciuric Sprague–Dawley rats, the parental strain of the GHS rats.

Where there is a reduction in renal tubular Ca reabsorption, the overriding mechanism for promoting hypercalciuria in the GHS rat, we would expect to observe a fall in serum Ca leading to an increase in PTH.[34] Indeed, kidney stone patients with so-called 'renal hypercalciuria' have elevated levels of PTH.[1,2] On LCD, the serum level of PTH in the GHS rat was lower than that in Ctl rats, suggesting that even on this reduced level of dietary Ca, the hypercalciuria in the GHS rat was driven more by enhanced intestinal Ca absorption and/or bone demineralization than by a reduction in renal tubular Ca reabsorption. Serum Ca was not different between the GHS and Ctl rats and serum P was slightly, but significantly, lower in the GHS compared to the Ctl rats. The reason for this decrease in serum P is not apparent but is consistent with a decrease in serum P found in descriptions of some hypercalciuric patients.[1,2]

The relationship of serum Ca to PTH secretion seems to be shifted to the right in the GHS rats compared to Ctl, since lower levels of serum PTH are required to maintain similar levels of serum Ca. This may be due to a higher density of CaR in the parathyroid glands of the GHS rat, leading to an amplification of the serum Ca signal. The higher density of CaR in the kidneys of GHS rat could also play a role in the lower tubular Ca reabsorption found in the GHS rat. The lower serum PTH levels in the GHS rat compared to Ctl might also be explained by the potential for higher parathyroid gland VDR levels in the GHS rats.[8,11,16,23] Since calcitriol suppresses PTH secretion, elevated VDR in the parathyroid glands would accentuate calcitriol action. The factors that are most important in determining the relationship between serum Ca and PTH in the GHS rat are not clear at this time.

Recently, small molecules that increase the sensitivity of the CaR to Ca, the calcimimetics, have been developed for the treatment of human primary and secondary hyperparathyroidism.[31–33] The calcimimetic approved for use in man, Cin, has been shown to markedly reduce serum PTH levels and modestly reduce levels of serum Ca.[31,33] Because of the effects of both the filtered load of Ca and the level of PTH to alter renal tubular Ca reabsorption, the effects of a calcimimetic on UCa are difficult to predict. However, if Cin were to reduce UCa and supersaturation with respect to a crystal solid phase such as CaOx and/or $CaHPO_4$, it might be useful in the pharmacological therapy of hypercalciuric stone formers. We found that although Cin reduced serum PTH and Ca in the GHS and control rats, it did not alter urine supersaturation with respect to CaOx or $CaHPO_4$ in either strain.

Cin increases the sensitivity of the CaR to Ca, thereby shifting the sigmoidal-shaped relationship between Ca and PTH to the left, resulting in a reduction of PTH secretion and serum levels.[31–33] Cin led to a marked reduction in PTH and serum Ca in both the GHS and Ctl rats. This is consistent with findings in humans treated with this calcimimetic.[31–33] As PTH is phosphaturic, the reduced PTH should lead to an increase in serum P as shown in this study.[34]

Urine Ca in the GHS rats far exceeded that in the Ctl rats on both NCD and LCD. On LCD, UCa in the GHS rats exceeded that of available dietary Ca, indicating that bone must have contributed to the excessive UCa in the GHS rats. This is consistent with our previous observations that the GHS rats are in negative Ca balance on LCD[7] and alendronate, a bisphosphonate which inhibits bone resorption, markedly reduced UCa in GHS rats fed LCD.[15] That UCa fell with Cin in the GHS rats fed LCD suggests that PTH is necessary to resorb the bone mineral that contributes to the hypercalciuria of the GHS rats fed LCD. UCa was not appreciably different in the Ctl rats fed NCD compared to LCD, indicating that in Ctl rats even on LCD there is adequate dietary Ca to maintain urinary Ca excretion. That the Ctl rats fed Cin did not lower their UCa on NCD or LCD, in spite of a decreased filtered load of Ca, suggests that Cin also may have affected the renal CaR, resulting in a decreased renal tubular Ca reabsorption.

The effect of Cin on UCa is difficult to predict. PTH induces the renal tubule to increase Ca reabsorption from the glomerular filtrate indicating that a reduction in PTH induced by Cin should increase UCa.[34] A fall in serum Ca induced by Cin should reduce the filtered load of Ca and lead to a decrease in UCa.[34] However, if Cin influences the renal CaR, increasing its sensitivity to Ca may lead to an increase in UCa. Cin did not alter UCa excretion in either the GHS or Ctl rats fed NCD and lowered UCa in the GHS rats fed LCD but not in the Ctl rats fed LCD. Further studies, perhaps using parathyroidectomized GHS and Ctl rats perfused with a constant amount of Ca as we have previously done with GHS rats not given Cin,[14] will be necessary to understand the multiple interactions between PTH, Cin, and the filtered load of Ca.

Urine P was higher in the GHS rats than in the Ctl rats on both the NCD and LCD and, as noted above, serum P was lower in the GHS rats despite a reduced level of the phosphaturic hormone PTH. Whether other phosphaturic compounds are increased in the GHS rat is not known at this time. UP excretion increased substantially in both groups of rats when they were changed from NCD to LCD. Dietary Ca binds intestinal P, reducing P absorption,[35] so that a reduction in dietary Ca would be expected to result in an increase in UP. LCD will also increase calcitriol production, which will increase intestinal P absorption. It is unclear why UOx in the GHS rats was lower than that of Ctl rats when fed either NCD or LCD and why the addition of Cin led to an increase in UOx in the Ctl rats fed NCD and in the Ctl and GHS rats fed LCD.

In this study, we found that Cin did not alter supersaturation with respect to either solid phase in either the GHS or Ctl rats when fed the NCD or LCD. If these findings in the GHS rats can be confirmed in man, it suggests that Cin would not be an effective agent in the treatment of idiopathic hypercalciuria in humans.

MATERIALS AND METHODS

Establishment of hypercalciuric rats

Adult Sprague–Dawley rats (Charles River Laboratories, Kingston, NY, USA) were initially screened for hypercalciuria by placing the rats in individual metabolic cages, feeding them a constant amount of a standard Ca diet, and measuring urine Ca excretion. The most hypercalciuric male and female rats were used to breed the next generation. A similar protocol was used for screening and inbreeding of subsequent generations.[6,8,10,16,17,20–23]

Study protocol

Fourteen 67th generation female GHS rats and 14 female Sprague–Dawley Ctl rats, initially weighing on average 238 g, were placed in metabolic cages. From days 1 to 14, each rat in each group was fed 13 g/day of a NCD (0.6% Ca and 0.65% P, Harlan Teklad, Madison, WI, USA). We have previously shown that rats of this size completely consume this amount of diet on a daily basis.[15,17–20] During the last 5 days of this period (day 10–14), five successive 24-h urine collections were obtained. Three (first, second, fourth) were collected in concentrated HCl (0.5 ml) for all measurements except for pH, uric acid, and chloride and two collections (third and fifth) were collected in the presence of thymol for measurement of pH, uric acid, and chloride. All samples were refrigerated at 4°C until measurement and all measurements were completed within 2 weeks.

From days 15 to 28, half of each group (seven GHS and seven Ctl rats), chosen at random, was continued on NCD without modification and the other half (seven GHS and seven Ctl rats) was fed NCD supplemented with Cin (30 mg/kg/day) (Amgen Inc., Thousand Oaks, CA, USA). This dose has been shown to significantly inhibit PTH in normal rats.[36] In humans, the terminal half-life of Cin is 30–40 h and steady-state drug levels are reached in 7 days.[36,37] During the last 5 days of this period (day 24–28), five successive 24-h urine collections were obtained as during days 10–14.

From days 29 to 42, all GHS and Ctl rats were fed 13 g/day of a LCD (0.02% Ca and 0.65% P, Harlan Teklad, Madison, WI, USA). No rat received Cin. LCD was utilized to remove the contribution of

appreciable intestinal Ca absorption to UCa excretion. During the last 5 days of this period (day 38–42), five successive 24-h urine collections were obtained as during days 10–14.

From days 43 to 56, half of each group (seven GHS and seven Ctl rats) was continued on LCD without modification and the other half (the same seven GHS and seven Ctl rats that had previously received Cin) was fed LCD supplemented with Cin (30 mg/kg/day). During the last 5 days of this period (day 52–56), five successive 24-h urine collections were obtained as during days 10–14.

Each rat had access to deionized distilled water *ad libitum*. Any rat that ate less than 12 g of food per day or drank less than 15 ml of water on any day would have been excluded from the study; however, all rats met these prospective criteria throughout the study.

At the conclusion of the experiment, each rat received an intraperitoneal injection of sodium pentobarbital (Nembutal) to induce complete anesthesia. Blood was collected through the carotid artery, in uncoated centrifuge tubes for determination of serum Ca, P, and creatinine and in ethylenediaminetetraacetic acid-coated tubes for determination of PTH. After centrifugation and separation of serum, the samples were stored at 4°C (Ca/P) or −70°C (PTH) until analysis. The kidneys were not examined for stones.

Urine chemical determinations

UCa, creatinine, inorganic P, uric acid, magnesium, ammonia, sodium, potassium, chloride, oxalic acid, citric acid, pH, and sulfate were measured using methods previously described.[15,17–20,22] The filtered load of Ca was calculated by multiplying the serum Ca times the fraction of Ca that is ultrafiltrable times the creatinine clearance. We have previously determined that the ultrafiltrable Ca in the GHS rat is 0.725 times the total Ca.[14]

Urine supersaturation

The CaOx and the $CaHPO_4$ ion activity products were calculated using the computer program EQUIL developed by B Finlayson and associates.[38–40] The computer program calculates free ion concentrations using the concentrations of measured ligands and known stability constants. Ion activity coefficients are calculated from ionic strength using the Davies modification of the Debye–Huckel solution to the Poisson–Boltzman equation. The program simultaneously solves for all known binding interactions among the measured substances. Oxalate, P, and Ca ion activities were used to calculate the free-ion activity products. The relative supersaturation for CaOx is calculated as the ratio of the free-ion activity product of Ca and Ox in the individual urine to the solubility of CaOx. The relative supersaturation for $CaHPO_4$ is calculated as the ratio of the free-ion activity product of Ca and P in the individual urine to the solubility of $CaHPO_4$. Ratios of 1 connote a sample at equilibrium, above 1 supersaturation, and below 1 undersaturation. We have used this computer program previously and found excellent correspondence between calculated and experimentally measured saturation in urine and blood.[7,9,10,12,13,15,17–20,22]

Blood determinations

Serum Ca was measured by atomic absorption using a Perkin–Elmer AA 100 (Perkin–Elmer Inc., Wellesley, MA, USA). Serum P and creatinine were measured on a Beckman Synchron CX5CE autoanalyzer (Beckman Instruments, Brea, CA, USA) by the phosphomolybdate method and the alkaline picrate method, respectively. Serum PTH was determined using a rat bioactive intact PTH enzyme-linked immunosorbent assay (Immutopics, Inc., San Clemente, CA, USA).

Statistical analyses

All values are expressed as mean ± s.e.m. Tests of significance were calculated by *t* tests and analysis of variance, as appropriate, using conventional computer programs (BMDP, University of California, Los Angeles, CA, USA). $P < 0.05$ was considered significant.

ACKNOWLEDGMENTS

This work was supported in part by grants DK 56788, DK 57716, and AR 46289 from the National Institutes of Health.

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