Low Sodium Diet Decreases Stone Formation in Genetic Hypercalciuric Stone-Forming Rats

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Abstract

Background: Urine (u) calcium (Ca) excretion is directly dependent on dietary sodium (Na) intake leading to the recommendation for Na restriction in hypercalciuric kidney stone formers. However, there is no direct evidence that limiting Na intake will reduce recurrent stone formation. **Materials and Methods:** We used genetic hypercalciuric stone-forming (GHS) rats, which universally form Ca phosphate (P) kidney stones, fed either a low Na (LNa, 0.05%) or normal Na (NNa, 0.4%) Na diet (D) for 18 weeks. Urine was collected at 6-week intervals. Radiographic analysis for stone formation and bone analyses were done at the conclusion of the study. **Results:** Mean uCa was lower with LNaD than NNaD as was

uP and LNaD decreased mean uNa and uChloride. There were no differences in urine supersaturation (SS) with respect to calcium phosphate (CaP) or Ca oxalate (CaOx). However, stone formation was markedly decreased with LNaD by radiographic analysis. The LNaD group had significantly lower femoral anterior-posterior diameter and volumetric bone mineral density (vBMD), but no change in vertebral trabecular vBMD. There were no differences in the bone formation rate or osteoclastic bone resorption between groups. The LNaD group had significantly lower femoral stiffness; however, the ultimate load and energy to fail was not different. **Conclusion:** Thus, a low Na diet reduced uCa and stone formation in GHS rats, even though SS with respect to CaP and CaOx was unchanged and effects on bone were modest. These data, if confirmed in humans, support dietary Na restriction to prevent recurrent Ca nephrolithiasis.

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Introduction

Hypercalciuria (IH) is the most common metabolic abnormality observed in patients with calcium (Ca)-based kidney stones, the most prevalent stones formed by patients in the United States [1, 2]. Increased levels of urine (u) Ca increase the probability of growth of Ca oxalate (CaOx) and/or Ca hydrogen phosphate (brushite) crystals into clinically significant stones [1]. Patients with idiopathic IH, defined as excessive uCa without a demonstrable metabolic cause, generally have normal serum (s) Ca, normal or elevated serum 1,25 (OH)₂D₃ (s1,25D), normal or elevated serum parathyroid hormone (sPTH), normal or low serum phosphate, and low bone mass [1, 3, 4]. IH exhibits a polygenic mode of inheritance [3, 5].

The annual incidence of kidney stones in industrialized nations exceeds 1 per 1,000 persons, with a lifetime risk of ~7% in women and ~11% in men [6]. After an initial episode of nephrolithiasis 60–80% of patients form at least one recurrent stone. Several strategies to decrease stone recurrence have been devised, including potassium citrate [7–9], thiazide diuretics [10], and reduction of dietary sodium (Na) [11–16].

The rationale for preventing stone recurrence by limiting dietary Na relies on both Ca transport physiology and clinical studies. Renal Ca transport is Na dependent [17–19]. Clinically, there is an increased risk for kidney stones in women in the highest quintile of Na intake compared to the lowest quintile [20]. Comparison of a low-Ca diet with a low-Na, low protein, normal-Ca diet over a 5-year period demonstrated that the latter diet was more effective in reducing stone recurrence although the individual contribution of the reduction in dietary Na could not be determined [21]. In Ca stone-forming patients, the highest Na intake correlated with the lowest bone mineral density (BMD) [22]. Urinary Na has been shown to correlate with urinary Ca, suggesting that Na intake influences IH and stone formation [23].

To examine the effect of a low Na diet on urine supersaturation (SS), stone formation and bone quality, we utilized a model of human idiopathic IH, the genetic hypercalciuric stone-forming (GHS) rat [24]. GHS rats were generated by selectively inbreeding Sprague-Dawley rats for increased uCa excretion [24]. When fed a standard, ample Ca diet, each GHS rat now consistently excretes over tenfold more uCa than Sprague-Dawley rat controls [24]. The degree of IH has continued to increase with successive generations, although at a much slower rate than with earlier generations. Like patients with IH, GHS rats have normal serum Ca [25], increased intestinal Ca ab-

sorption [26] and enhanced bone resorption [27], decreased renal tubule Ca reabsorption [28], and normal s1,25D levels [26, 29, 30] in addition to decreased BMD [31, 32]. IH is also a polygenic trait in GHS rats [33]. When fed a standard Ca diet, all GHS rats develop kidney stones [34, 35] composed of calcium phosphate (CaP) [34, 36–38]. The addition of hydroxyproline to the diet of GHS rats results in CaOx stone formation [39–41]. In this study, GHS rats were fed either a normal Na diet (NNaD, 0.4%) or a low Na diet (LNaD, 0.05%). At the end of 18 weeks, urine and blood chemistry, urine SS, stone formation, and bone quality were evaluated.

Materials and Methods

Study Protocol

Three-month-old GHS rats from the 98th generation were divided into 2 groups (each $n = 12$). Both were fed a normal Ca diet (1.2% Ca) with either normal Na (0.40%, NNaD) or low Na (0.05%, LNaD). Na content of the LNaD diet was adjusted with NaCl to produce NNaD. At weeks 6, 12, and 18, each rat was weighed and 24-h urine collected over 4 days. For 2 collections, urine was acidified with HCl; for another 2 collections, urine was collected in thymol. Collections in thymol were used for pH, uric acid (UA), and chloride (Cl) and collections in HCl were used for all other measurements. Each rat received an intraperitoneal injection of 1% calcein green at 10 and 2 days prior to sacrifice for dynamic histomorphometry. At 18 weeks, rats were euthanized and blood was collected via cardiac puncture. Kidneys, ureters, and bladders were removed for X-ray imaging in a faxitron, while bones were obtained for histological studies and mechanical testing. Femurs, humeri, and vertebral columns were harvested and frozen at -20°C , while the right tibiae were placed in 70% ethanol and the left tibiae fixed in 10% neutral buffered formalin for 48 h and then decalcified in 10% EDTA. One rat from each group died during the study and therefore was not included (final $n = 11$). The University of Rochester Committee for Animal Resources approved all procedures.

Urine and Serum Chemistries

Urine Ca, magnesium, phosphate, ammonium, and creatinine were measured spectrophotometrically using a Beckman CX5 Pro autoanalyzer (Beckman Coulter, Brea, CA, USA). Urine potassium (K), Cl, and Na were measured by ion-specific electrodes on the Beckman CX5 and urine pH using a glass electrode. Urine citrate, oxalate, and sulfate were measured by ion chromatography using a Dionex ICS 2000 system (Dionex Corp., Sunnyvale, CA, USA). All urine solutes were measured at 6, 12, and 18 weeks and a mean value for each time period as well as overall mean value was calculated. Serum Ca and phosphate were determined colorimetrically (BioVision, Milpitas, CA, USA). sPTH, FGF23, osteocalcin, and propeptide of type 1 collagen (P1NP) were determined by enzyme immunoassay: intact PTH and FGF23 (Immutopics, San Clemente, CA, USA), OC (Alfa Aesar, Tewksbury, MA, USA) and P1NP (Fisher Scientific). All of these methods have been used previously [31, 36, 42–44].

Urine SS

With the measured solute excretion, the urinary SS with respect to CaOx, CaP, and UA solid phases were calculated using the computer program EQUIL2 [45] as we have done previously [37, 39, 44, 46, 47]. Ratios of 1 denote a urine at equilibrium, those >1 denote SS and those <1 denote undersaturation. There is excellent correspondence between calculated and experimentally measured saturation in urine and blood.

Kidney Stone Formation

The kidneys, ureters, and bladder were removed from each rat en bloc, frozen and imaged in a Faxitron radiography device (Tucson, AZ, USA) to determine extent of kidney stone formation. Three observers blinded to treatment scored all radiographs on a qualitative scale ranging from 0 (no stones) to 4 (extensive stones).

BMD: Dual Energy X-ray Absorptiometry

Dual energy X-ray absorptiometry with a Lunar PIXImus Bone Densitometer (Lunar GE, Mississauga, Canada) was used to determine tissue density and mineral content of the right femora and L6 vertebrae. The areal BMD, bone mineral content, and bone area were measured.

BMD: Micro-Computed Tomography

Micro-computed tomography (microCT) with an 1174 compact microCT (Skyscan, Kontich, Belgium) was used to measure volumetric BMD (vBMD) and microarchitecture of the mid-diaphysis of right femurs (cortical bone) and L6 vertebrae (trabecular bone). For vertebrae, density measured was the area between the growth plates, and for femurs, density measured at one millimeter above and below the mid shaft of the bone. Measured parameters include vBMD (Femoral vBMD), anterior-posterior (AP) diameter, trabecular vBMD, bone volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp).

Tissue Level Remodeling

Tissue-level remodeling was assessed via histomorphometry on both mineralized (undecalcified) and unmineralized (decalcified) bone thin sections. Stained sections are viewed at a high magnification and results quantified using computer software.

Undecalcified Histomorphometry

Undecalcified histomorphometry differentiates between mineralized and demineralized tissue in bone fixed in 70% ethanol. Right tibiae sections were used for static and dynamic histomorphometric analysis. Cross-sections of the left distal tibiae were used for back-scattered electron (BSE) microscopy.

Static Histomorphometry

Sections (5 μ m) of undecalcified right tibiae were stained with Goldner's Trichrome and quantified using the Bioquant Osteo 11.2.6 MIR software (Bioquant Image Analysis, Nashville, TN, USA). Trabecular bone was analyzed in the proximal tibia metaphysis. Parameters measured include percent BV (BV/TV), percent bone surface (BS/BV), Tb.N, Tb.Sp, Tb.Th, osteoid volume, percent osteoid volume per BV, osteoid surface, percent osteoid surface per BS, and osteoid width.

Dynamic Histomorphometry

Sections of calcein labeled undecalcified right tibiae were used for dynamic histomorphometry and quantified using Bioquant Osteo 11.2.6 MIR software (Bioquant Image Analysis). Parameters measured include percent mineralized surface per BS, mineral apposition rate, bone formation rate normalized over BS (BFR/BS), and BFR normalized over BV (BFR/BV).

Decalcified Histology

Sections (5 μ m) of decalcified left tibiae were stained for tartrate-resistant acid phosphatase to measure osteoclasts to assess bone resorption and counterstained with acid hematoxylin. Results were quantified using Bioquant Osteo 11.2.6 software. Trabecular bone of proximal tibial metaphysis was analyzed for osteoclast surface (Oc.S), percent Oc.S, number of osteoclasts (N.Oc), and N.Oc per mm Oc.S.

Biomechanical Properties

Biomechanics of femurs were assessed using an Instron 4465 mechanical testing instrument (Instron, Burlington, Canada) and Labview 5.0 data acquisition software (National Instruments, Austin, TX, USA) to define a load-displacement curve. Ultimate load, stiffness, ultimate displacement, and energy to break were calculated from the load-displacement curve. Data were normalized for specimen geometry to create a stress-strain curve to derive ultimate stress, failure strain, toughness, and Young's modulus.

Three-point bending. Three-point bending was performed on the right femurs to test the strength of the cortical bone.

Vertebral compression. Vertebral compression was measured on excised L6 vertebrae. Vertebral compression does not result in complete fracture; the failure point was determined by an 8–10% reduction in load.

Femoral neck fracture. The proximal end of the femurs was subjected to femoral neck fracture using an Instron 4465 to test mechanical properties of cortical and trabecular bone combined.

Degree of Mineralization

BSE microscopy on both undecalcified right tibiae and left distal tibiae cross-section samples was done with a scanning electron microscope (Philips, XL300ESEM, Best, the Netherlands). Images were taken using a solid state BSE detector (FEI Company, Hillsboro, OR, USA).

Statistical Analysis

Urine analytes, serum values, and stone formation were compared by ANOVA with a conventional computer program (Statistica, StatSoft, Tulsa, OK, USA) and expressed as mean $\pm$ SE. Bone parameters were analyzed using the SPSS Statistics 20 program (SPSS, Chicago, IL, USA) to perform independent-sample *t* tests and are expressed as mean $\pm$ SD. Significance for all determinations was considered at $p < 0.05$.

Results

Urine Chemistry

Overall, mean uCa was lower with LNaD than NNaD (Table 1) and uCa was reduced in the first two 6-week

Fig. 1. Urine calcium (Ca), phosphorus (P) and oxalate (Ox) excretion for GHS rats fed a normal (N) or low (L) sodium (Na) diet. Urine was collected for four 24-h periods at 6, 12, and 18 weeks

from GHS rats to determine solute levels as described in the Concise Methods. Values are mean $\pm$ SE for 11 rats in each group. * $p < 0.05$ for L compared to N at the same time period.

Table 1. Overall mean urine parameters

Solute	NNaD, mean $\pm$ SD	LNaD, mean $\pm$ SD
Ca, mg/day	17.3 $\pm$ 0.5	15.2 $\pm$ 0.55*
P, mg/day	16.5 $\pm$ 0.6	13.8 $\pm$ 0.4*
CaOx, mg/day	0.38 $\pm$ 0.03	0.43 $\pm$ 0.02
Na, mmol/day	2.48 $\pm$ 0.04	0.39 $\pm$ 0.02*
Cl, mmol/day	2.65 $\pm$ 0.13	1.20 $\pm$ 0.06*
Potassium, mmol/day	1.39 $\pm$ 0.02	1.27 $\pm$ 0.03*
Citrate, mg/day	60.24 $\pm$ 1.6	57.24 $\pm$ 1.6
Ammonium, mmol/day	1.07 $\pm$ 0.07	0.96 $\pm$ 0.03
Magnesium, mg/day	15.0 $\pm$ 0.3	14.1 $\pm$ 0.3
Creatinine, mg/day	11.65 $\pm$ 0.19	11.31 $\pm$ 0.26
pH	6.24 $\pm$ 0.07	6.17 $\pm$ 0.08
Volume, mL	29.6 $\pm$ 1.1	27.8 $\pm$ 1.5
UA, mg/day	1.35 $\pm$ 0.07	1.44 $\pm$ 0.10
Sulfate, meq/day	1.28 $\pm$ 0.06	1.22 $\pm$ 0.05
SS UA	0.09 $\pm$ 0.01	0.18 $\pm$ 0.03*
SS CaP	4.8 $\pm$ 0.4	4.6 $\pm$ 0.5
SS CaOx	3.2 $\pm$ 0.2	4.0 $\pm$ 0.4
Weight, g	298.0 $\pm$ 2.5	290.3 $\pm$ 2.7*

* $p < 0.05$ compared to the NNaD.

Na, sodium; NNaD, normal Na diet; LNaD, low Na diet; Ca, calcium; P, phosphate; CaOx, oxalate; Cl, chloride; UA, uric acid; SS, supersaturation.

time periods but not in the third (Fig. 1). Overall mean uP was lower with LNaD than NNaD and uP was reduced in the first 6 weeks, but not in the later time periods. Overall mean u oxalate (Ox) was not different between the 2 groups, though it was increased during the second time period with LNaD. Overall mean uNa and uCl were significantly lower in GHS fed LNaD compared to NNaD (Table 1) and both were lower during each time period (Fig. 2). Overall mean uK was lower with LNaD than NNaD and uK was reduced in the first two 6-week time periods but not in the third. Creatinine, pH, NH_4^+ , citrate, and volume did not differ with diet Na (Table 1). There were no significant differences in urine supersaturation (SS) with respect to the solid phases of CaP or CaOx (Fig. 3). Overall mean urine SS with respect to the solid phase of UA was not different between the groups and far less than one at all times; however, it was increased during the second time period with LNaD.

Stone Formation

X-rays of excised kidneys and ureters were examined radiographically (representative X-rays, Fig. 4a) and an overall stone formation score determined as we have done previously [44]. Radiographic analysis of kidneys demonstrated a significant decrease in stone formation in

Fig. 2. Urine sodium (Na), chloride (Cl) and potassium (K) excretion for GHS rats fed a normal (N) or low (L) sodium (Na) diet. Urine was collected for four 24-h periods at 6, 12, and 18 weeks

from GHS rats to determine solute levels as described in the Concise Methods. Values are mean $\pm$ SE for 11 rats in each group. * $p < 0.05$ for L compared to N at the same time period.

both the right and left kidneys with LNaD (scores: range of 0 [absence of calcification] to 4 [significant calcification] Fig. 4b).

Serum Chemistry

Serum Ca was slightly, but significantly, increased with LNaD (Fig. 5a). There were no differences in serum P (Fig. 5a), PTH, or FGF23 (Fig. 5b). To assess osteoblast activity and, indirectly, bone formation, serum levels of osteocalcin and the amino terminal P1NP were measured. P1NP levels were elevated with LNaD as compared to NNaD; however, there was no difference in the level of OC (Fig. 5c).

Bone Mineral Density

MicroCT revealed that the LNaD had significantly lower femoral cortical vBMD, but no change in vertebral trabecular vBMD (Table 2). However, dual energy X-ray absorptiometry did not demonstrate a difference in either the femurs or vertebrae for areal BMD, bone mineral content, or bone area between the NNaD and LNaD (data not shown).

Bone Microarchitecture

While there was no difference in cortical thickness, the femurs from rats fed the LNaD had significantly lower AP diameter when compared to NNaD (Table 2). MicroCT showed no significant differences between the 2 groups in the moment of inertia, porosity, or thickness of the cortical bone (data not shown). In the trabecular bone, microCT showed no significant differences in trabecular structural parameters, including percent BV, Tb.N, Tb.Th, and Tb.Sp (data not shown).

Mechanical Properties

There was significantly lower femoral stiffness in rats fed the LNaD; however, there was no difference in ultimate load, energy to fail, or Young's modulus (Table 2). Vertebral compression showed a trend toward lower yield displacement ($p = 0.083$) and lower stiffness (0.075) with LNaD compared to NNaD (Table 2). There was also a trend toward lower yield strain ($p = 0.079$) and lower fail stress ($p = 0.093$) with LNaD. Femoral-neck fracture showed no significant differences between LNaD and NNaD (data not shown).

Fig. 3. Urine supersaturation (SS) for calcium phosphate (CaP), calcium oxalate (CaOx), and uric acid (UA) for GHS rats fed a normal (N) or low (L) sodium (Na) diet. Urine was collected for four 24-h periods at 6, 12, and 18 weeks to determine solute levels that

were used to calculate relative SS as described in the Concise Methods. Values for relative SS are mean $\pm$ SE ($n = 11$) and are unitless. * $p < 0.05$ for L compared to N at the same time period.

Fig. 4. Kidney stone formation at the conclusion of the 18 week study. At the end of the 18-week study, the extent of kidney stone formation was determined by blinded 3 observers as described in the Concise Methods. **a** Representative radiographs of kidneys on a normal Na diet (NNaD) or a low Na diet (LNaD). Calcification

scores are provided as a reference. **b** Quantitation of stone formation and calcification in all GHS rats fed a NNaD or a LNaD. Values are mean $\pm$ SEM ($n = 11$). * $p < 0.05$ for LNaD compared to NNaD.

Fig. 5. Serum calcium (Ca), phosphorus (P), fibroblast growth factor 23 (FGF23), parathyroid hormone (PTH), osteocalcin (OC), and amino terminal propeptide of type 1 collagen (P1NP) at the conclusion of the 18-week study. Serum levels were determined as described in the Concise Methods. **a** Serum Ca and P on normal

Na diet (NNaD) compared to low Na diet (LNaD); **(b)** Serum FGF23 and PTH on NNaD compared to LNaD; **(c)** Serum OC and P1NP on NNaD compared to LNaD. Values are mean ± SEM ($n = 11$). * $p < 0.05$ for LNaD compared to NNaD.

Undecalcified Histomorphometry

Static histomorphometry. Rats fed LNaD had significantly lower Tb.Sp compared to NNaD (Table 2). There was also a trend toward lower Tb.Th ($p = 0.079$) with LNaD, as well as a trend toward a higher percent BS (BS/BV, $p = 0.069$) compared to NNaD.

Dynamic histomorphometry. There were no significant differences in percent mineralized BS or BFR between NNaD and LNaD (Table 2).

Decalcified Histology

Based on tartrate-resistant acid phosphatase staining, there were no significant differences in bone resorption between NNaD and LNaD. However, there was a trend toward a lower N.Oc per Oc.S ($p = 0.077$) with LNaD compared to NNaD (Table 2).

Bone Mineralization

BSE microscopy showed no significant differences in the mineralization of the bone between the 2 groups; however, there was a trend toward a higher peak grey level ($p = 0.074$) with LNaD compared to NNaD (Table 2).

Discussion

GHS rats have alterations in Ca homeostasis including increased intestinal absorption, reduced renal tubular reabsorption, and increased bone resorption, similar to those of many patients with idiopathic IH [24]. Hypercalciuria in the GHS rats leads directly to stone formation as it does in humans [1, 37, 39]. GHS rats also have reduced BMD, as do humans with nephrolithiasis, as well as lower trabecular volume and thickness compared to control Sprague-Dawley rats [32, 39]. Previous studies on the effect of dietary Na intake, performed on humans with IH, as well as normal rats [48, 49], demonstrate that increased dietary Na leads to increased hypercalciuria, as well as an increase in osteoporotic risk factors such as increased bone resorption and decreased BMD [48, 49]. In this study, we utilized GHS rats to determine the effect of a low Na diet, generally prescribed to help prevent recurrent stone formation [11–16, 50, 51], on urine SS, stone formation, and bone quality.

Feeding the hypercalciuric GHS rats a low Na diet effectively reduced uCa excretion. However, in spite of an eight-

Table 2. Bone parameters

	NNaD, mean ± SD	LNaD, mean ± SD
BMD, g/cm ³		
Femoral cortical vBMD	1.266±0.067	1.206±0.058*
Vertebral trabecular vBMD	0.704±0.077	0.712±0.112
Bone microarchitecture, mm		
Cortical thickness	0.69±0.04	0.69±0.02
Anterior-posterior diameter	3.60±0.12	3.49±0.11*
Cortical mechanical properties		
Stiffness, N/mm	417.91±27.82	377.58±45.27*
Ultimate load, N	151.86±10.52	146.55±10.79
Energy to fail, MPa	22.45±4.80	21.02±5.04
Young's modulus, GPa	1.16±0.05	1.12±0.15
Vertebral compression		
Stiffness, N/mm	719.07±201.13	583.67±80.20
Failure stress, MPa	40.58±6.30	36.23±5.23
Energy to fail, MPa	2.76±0.95	2.69±1.06
Young's modulus	740.22±225.56	646.15±199.98
Static histomorphometry		
BS (BS/BV), %	32.31±2.54	36.59±6.92
Tb.Sp, µm	0.29±0.05	0.23±0.05*
Tb.N	2.47±0.28	2.67±0.40
Dynamic histomorphometry		
MS/BS, %	23.31±6.06	19.91±6.18
Mineral apposition rate, µm/day	1.96±0.31	1.89±0.23
BFR/BS, µm/day/mm	0.02±0.01	0.01±0.01
BFR/BV, µm/day/mm ²	0.61±0.30	0.43±0.18
Decalcified histology		
Osteoclasts/OC.S, 1/mm	19.10±4.35	12.58±10.64
Bone mineralization		
Peak grey level	169.30±8.78	177.25±8.78

* $p < 0.05$ compared to the NNaD.

BMD, bone mineral density; NNaD, normal Na diet; LNaD, low Na diet; BS, bone surface; vBMD, volumetric bone mineral density; Tb.N, trabecular number; Tb.Sp, trabecular separation; OC.S, osteoclast surface; BFR, bone formation rate; BS, bone surface; bone volume.

fold reduction in dietary Na intake and a fall in uNa of 2.09 mmol, uCa fell by only 0.05 mmol. A similar decrease in uCa excretion was found in a previous study examining the effect of potassium citrate in GHS rats [44], while thiazides caused an even greater decrease in uCa [41] as well as improved BMD (20) compared to our findings in the current study [31]. The low Na diet also induced a reduction of uP, uCl, and uK but no differences in urine oxalate, citrate, ammonium, magnesium, pH, or volume. The low Na diet-induced reductions in urine Ca and P excretion were insufficient to alter SS with respect to CaP or CaOx, possibly due to the lower ionic strength of the urine leading to greater activity coefficients and therefore chemical activity of the critical ions. However, the GHS rats fed the low Na diet had a marked and unequivocal reduction in kidney stone for-

mation. Why stone formation fell with no change in SS is not clear from this study. The urine SS with respect to the CaP solid phase, the type of stones formed by the GHS rats on this diet [36], exceeded 4.5 in both groups indicating that there was a marked driving force for CaP stone formation. It may be that alterations in dietary Na intake influence inhibitors and/or promoters of stone formation or crystal-urothelial interactions. Further studies will be necessary to address this possibility. It has been suggested that, at least in humans, SS may not always be predictive of subsequent stone formation [52]; however, SS appears to be the best measure, available clinically, to predict stone recurrence [1]. It is possible that using a different program to estimate urine SS such as JESS [53] or LithoRisk [54] might bring better agreement between urine SS and stone formation.

However, we have shown that EQUIL2 is an excellent predictor of CaP stone formation in GHS rats [37], and a recent publication demonstrated that a fall in SS calculated only by EQUIL2, and not JESS or LithoRisk, was significantly associated with a lower risk of stone recurrence in humans [55].

The GHS rats fed a low Na diet had a small, but significant, increase in serum Ca, perhaps due to increasing passive proximal tubule Ca reabsorption, which follows Na and water reabsorption in this segment [18, 19]. Additionally in the thick ascending limb of Henle's loop, increased Na reabsorption through the Na/K/2Cl transporter leads to increased K back diffusion into the lumen leading to an increased luminal positive charge, which drives Ca reabsorption through paracellular pathways [18]. In spite of the increase in serum Ca, there were no changes in serum PTH or FGF23. Two markers of bone formation were studied, serum OC and P1NP. While there was no change in OC with low Na diet, P1NP increased, perhaps indicating increased bone formation in this group.

There were small but significant changes in bone quality in rats fed a low Na diet, including a significant reduction in femoral cortical vBMD and a reduction in AP diameter in the low, compared to the normal Na, diet. GHS rats fed a low Na diet had a reduction in cortical stiffness, which indicates that their bone is more ductile and resistant to fracture. However, there was no difference in ultimate load, energy to fail or Young's modulus. As opposed to these changes in cortical mechanical properties, there were no significant differences in these parameters in the L6 vertebrae. Static and dynamic histomorphometry of tibiae of the GHS rats fed a low Na diet demonstrated decreased Tb.Sp as well as a trend toward increased percent BS. There was no difference between the 2 groups for osteoid or BFR; however, there was a trend toward a lower percentage of osteoclasts per Oc.S in the rats fed a low Na diet, which would result in decreased bone resorption.

Bone is a large reservoir for body Na [56]. Our results indicate that reducing dietary Na results in more ductile bone with lower cortical vBMD and decreased Tb.Sp. However, the changes in the bone parameters were modest and suggest that while the low Na diet reduces urine Ca excretion, which may improve bone quality and quantity, the reduction in dietary Na may have negative effects on bone density and quality that offset positive effects of this diet in reducing urine Ca excretion. However, the low Na diet did not appear to be detrimental to bone. As there were several trends toward improved trabecular bone with a low Na diet, it is possible that analysis of larger sample size over a longer time will help define more accurate comparisons.

In summary, using the GHS rats we found that a decrease in dietary Na resulted in a significant decrease in urine Ca excretion with no change in urine SS with respect to the Ca containing solid phases of kidney stones. Even so, there was a marked reduction in kidney stone formation in the GHS rats fed the low Na diet. This low Na diet did not appear to adversely alter the bone density and may improve it. If these results obtained in a model of hypercalciuric nephrolithiasis can be replicated in humans, it suggests that a low Na diet may be beneficial in preventing recurrent stone formation even if urine SS is not altered.

Ethics Statement

All animal experiments conform to internationally accepted standards and have been approved by the appropriate institutional review body at the University of Rochester.

Disclosure Statement

The authors have no competing financial interests and nothing to disclose.

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Authors Contribution

Conception and study design and interpretation of data: N.S.K., M.G., J.R.A., K.K.F., and D.A.B.; Drafting or revising the article: N.S.K., M.G., A.V.E., J.R.A., K.K.F., and D.A.B.; Providing intellectual content: N.S.K., M.G., A.V.E., J.R.A., K.K.F., M.H.K., F.R., I.G., and D.A.B.; Final approval of manuscript: N.S.K., M.G., A.V.E., J.R.A., K.K.F., M.H.K., F.R., I.G., and D.A.B.

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