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Authors
Tasian, Gregory E
Ross, Michelle E
Song, Lihai
et al.

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Dietary Zinc and Incident Calcium Kidney Stones in Adolescence

Gregory E. Tasian, MD, MSc, MSCE,[1,2,3,4] Michelle E. Ross PhD,[4,5] Lihai Song MS,[3]
Robert W. Grundmeier MD,[6,7,8] James Massey RN, MPH[8]
Michelle R. Denburg MD, MSCE,[4,6,9] Lawrence Copelovitch MD,[6,9]
Steven Warner MPH,[1,3] Thomas Chi MD,[10]
David W. Killilea PhD,[11] Marshall L. Stoller MD,[10] Susan L. Furth MD, PhD[4,6,9]

[1] Department of Surgery, Division of Pediatric Urology, The Children's Hospital of Philadelphia, Philadelphia, PA
[2] Department of Surgery, Division of Urology; Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA
[3] Center for Pediatric Clinical Effectiveness, The Children's Hospital of Philadelphia, Philadelphia, PA
[4] Center for Clinical Epidemiology and Biostatistics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA
[5] Department of Biostatistics and Epidemiology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA
[6] Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA
[7] Department of Pediatrics, Division of General Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA
[8] Department of Biomedical and Health Informatics, The Children's Hospital of Philadelphia, Philadelphia, PA
[9]Department of Pediatrics, Division of Nephrology, The Children's Hospital of Philadelphia, Philadelphia, PA
[10] Department of Urology, University of California, San Francisco, San Francisco, CA
[11]Center for Nutrition and Metabolism, Children's Hospital Oakland Research Institute, Oakland, CA

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Corresponding Author:
Dr. Gregory Tasian
Children's Hospital of Philadelphia
Division of Urology, Wood Center, 3rd Floor
34th Street and Civic Center Boulevard
Philadelphia, PA 19104
Phone: 215.590.0317 Fax 215.590.3985 Email: tasiang@chop.edu

Abstract

Purpose To determine the association between dietary zinc intake and incident calcium kidney stones and examine the relationship between dietary zinc intake and urinary zinc excretion among adolescents.

Material and Methods This study was a nested case-control study conducted within a large pediatric healthcare system. Three 24-hour dietary recalls and spot urine chemistries were obtained for 30 participants aged 12-18 years with a first idiopathic calcium-based kidney stone and 30 healthy controls, matched on age, sex, race, and month of enrollment. Conditional logistic regression models were used to estimate the odds ratio (OR) between daily zinc intake and incident calcium kidney stones, adjusting for dietary phytate, protein, calcium, sodium, and oxalate. Multivariable linear regression was used to estimate the association between dietary and urine zinc, adjusting for urine creatinine and dietary phytate and calcium.

Results Cases had lower daily zinc intake (8.1mg) than controls (10mg; p=0.029). Daily zinc intake of boys and girls with calcium stones was 2mg and 1.2mg lower, respectively, than the daily intake of zinc recommended by the Institute of Medicine. There was a 13% lower odds of incident stones for every 1mg higher daily zinc intake (OR, 0.87; 95% CI, 0.75-0.99). There was an estimated 4.5μg/dL increase in urine zinc per 1mg increase in dietary zinc (p=0.009), with weak evidence of a smaller increase in urine zinc in cases than in controls (interaction p=0.08).

Conclusions Lower dietary zinc intake was independently associated with incident calcium nephrolithiasis in this population of adolescents.

Introduction

Calcium-based kidney stones reflect an underlying disorder of mineral homeostasis. The association between dietary intake of certain minerals, such as calcium, and kidney stone formation during adulthood is well established.[1] However, the role of trace minerals, such as zinc, is unclear.[2] This relationship is particularly uncertain for children, among whom little research has been done to identify modifiable risk factors for nephrolithiasis. Understanding the role of dietary minerals in pediatric nephrolithiasis is particularly important because of the rapid rise of kidney stone incidence and associated high recurrence rate during childhood.[3, 4] Identifying dietary determinants of kidney stone disease among adolescents may provide insight into why nephrolithiasis is now occurring at younger ages and lead to strategies to decrease the lifetime risk of nephrolithiasis.

Prior experiments have suggested that zinc, an essential trace mineral, affects calcium metabolism and kidney stone formation, and inhibits calcium phosphate (CaP) crystallization.[5-8] However, clinical studies of zinc and nephrolithiasis have yielded inconsistent results. In a study using the National Health and Nutrition Examination Survey (NHANES), dietary zinc intake was higher among older adults with nephrolithiasis, but lower among younger adults with nephrolithiasis.[9] Earlier studies examining urinary zinc levels in adults with and without nephrolithiasis also reported disparate results.[10, 11] These prior studies were cross-sectional, included only adults, and were likely confounded by unmeasured dietary intakes such as phytate, which binds zinc in the gut and decreases its bioavailability.[12]

Prospective cohort studies that have been utilized extensively to identify dietary risk factors for incident stones among adults, such as the Nurses' Health Study, do not

exist for children. We therefore performed a nested case-control study to determine the association between dietary zinc and incident calcium nephrolithiasis among adolescents. We chose this age group because adolescents have the highest incidence of kidney stones during childhood. Our secondary objective was to examine differences in dietary zinc intake and urinary zinc excretion between cases and controls.

Materials and Methods

We performed a case-control study nested within the Children's Hospital of Philadelphia (CHOP) healthcare system, which includes primary and specialty care clinics serving Eastern Pennsylvania and New Jersey. Participants were enrolled from July 2014 to March 2016. This study was approved by the CHOP Institutional Review Board.

Case definition

Consecutive patients aged 12-18 years who had spontaneous passage or surgical removal of a first calcium-based kidney stone were screened and enrolled within one month of diagnosis. A cut-point of 50% for oxalate and phosphate was used to classify stones as calcium oxalate (CaOx) or CaP, respectively. Study procedures (24-hour dietary recalls and spot urine chemistries) were completed before patients were counseled to modify diets to reduce stone recurrence risk.

Control definition

Controls were randomly sampled from patients aged 12-18 years attending well-child visits at two clinics in the CHOP Primary Care Network. Electronic medical record filters were used to screen clinic schedules and identify potentially eligible control participants. Eligible controls were matched 1:1 to cases by age (+/- 2 years), sex, and

race. Controls were enrolled within 2 months of cases to minimize seasonal variation in diet and urine chemistries. Controls were asked about a history of nephrolithiasis, but did not undergo imaging.

Exclusions

Patients with diabetes, malignancy, chromosomal anomalies, neurodevelopmental disorders, hypertension, immobility, inflammatory bowel disease, ureteropelvic junction obstruction, RTA, and hyperparathyroidism were excluded. Patients with personal or family histories of cystinuria, primary hyperoxaluria, and Dent's disease were also excluded. Patients taking diuretics, citrate, topiramate, calcitriol, allopurinol, and steroids were excluded.

Study procedures

Research nutritionists administered three 24-hour dietary recalls to participants. Dietary recalls were administered over the phone, began immediately after study enrollment, and were performed on 2 weekdays and 1 weekend day. The recalls were collected using Nutrition Data System for Research, a computer-based software application[13] that uses a multiple pass interview to assess dietary intake and estimate nutrient and mineral content of food and beverages, and has been validated among adolescents.[14, 15]

Participants provided a voided urine specimen at enrollment in which zinc, pH, calcium, oxalate, citrate, and creatinine were measured. Urine chemistries were analyzed by ARUP and CHOP core laboratories.

Statistical Analysis

A sample size of 30, with 15 participants in each group, provides a power of 80% (with two-sided α=5%) to detect a difference of 0.78 mg/day in dietary zinc between study groups.[9] We increased planned enrollment to 60 participants to account for the effect of dietary phytate on zinc bioavailability, which was not considered in prior studies.

Paired t-tests or rank-sum tests were used to compare continuous variables between groups according to the distribution of the data. Conditional logistic regression models with robust standard errors, which accounted for the matched design, were used to estimate the association between daily dietary zinc intake (mg) and incident calcium stones. Models were adjusted for daily intake of phytate, sodium, total protein, calcium, and oxalate. We also estimated the change in the odds of incident calcium stones per unit increase in the interquartile range (IQR) for the dietary intakes in our study sample. Linear regression was used to estimate the relationship between dietary zinc and urine zinc, adjusting for urine creatinine and dietary phytate and calcium. An interaction term between dietary zinc and case/control status was included to determine if the relationship between zinc intake and urinary zinc excretion differed between cases and controls. All dietary recalls were included in the regression models. Analyses were performed in R v3.2.2 and SAS 9.4. Tests were two-sided, and $p<0.05$ was the threshold for statistical significance.

Sensitivity analyses

Matched pairs with solitary extreme dietary intake values were excluded to determine if non-representative meals influenced the results. Second, analyses were restricted to participants with stones containing >50% CaOx to determine if the results were sensitive to stone composition.

Results

Dietary intake and incident calcium-based kidney stones

Thirty participants in each group were enrolled (Figure, Table 1). Mean daily zinc intake was lower among cases (8.1mg) than controls (10mg; p=0.03; Table 2). Mean daily phytate intake was lower among cases than controls (p<0.001). Sodium, protein, calcium, and fluid intake were similar. Oxalate intake was similar except for one recall for a control participant who had an oxalate intake over three times the maximum observed for any other participant in either group. The main sources of zinc were red meat, zinc-fortified cereal, and pizza. Most phytate intake came from breakfast cereals, chips, seeds, and nuts. Controls ate more cereal (source of zinc and phytate) and pizza (source of zinc) than cases.

There were statistically significant associations between the amount of daily zinc, phytate, and oxalate intake and incident calcium stones, adjusting for sodium, protein, and calcium intake (Table 3a). A 1mg increase in zinc intake was associated with 13% lower odds of incident calcium stones (OR, 0.87; 95% CI, 0.75-0.99). There was a 58% lower odds of incident calcium stones for patients at the 75th percentile of zinc intake compared to those at the 25th percentile (OR, 0.42; 95% CI, 0.18-0.97), which was a 6mg difference in our study sample. Every 423mg higher daily phytate intake, the IQR of phytate in our study sample, was associated with a 51% lower odds of stones (OR, 0.49; 95% CI, 0.25-0.95). Every 73mg greater daily oxalate intake, the IQR of oxalate in our study sample, was associated with a 30% lower odds of stones (OR, 0.70; 95% CI, 0.51-0.97). No statistically significant associations between other minerals or nutrients and incident stones were observed.

Urine zinc

Urine zinc was 32.5μg/dL and 50μg/dL among cases and controls respectively, but this difference was not statistically significant (p=0.28). Adjusting for urine creatinine and dietary phytate and calcium, there was an estimated 4.5μg/dL increase in urine zinc per 1mg increase in dietary zinc (p=0.009). There was some evidence of a smaller increase in urine zinc in cases than controls, as demonstrated by the moderately large coefficient estimate for the interaction term that approached statistical significance (Table 4). There were no statistically significant associations between dietary zinc and urine calcium, citrate, or oxalate.

Sensitivity analyses

After excluding the matched pair with the outlying oxalate observation, the magnitude and statistical significance of the association between lower dietary zinc and stones increased and the associations of oxalate and phytate were no longer statistically significant (Table 3b). Including only the 25 participants with CaOx stones and their matched pairs did not change the association between zinc and incident stones (OR, 0.36; 95% CI, 0.13-0.98).

Discussion

The increasing incidence of nephrolithiasis among adolescents and the lack of identifiable causes for this rise necessitate a better understanding of risk factors for developing kidney stone disease during childhood. Despite many *in vitro* experiments that demonstrate zinc inhibits calcium crystallization, this study is, to our knowledge, the first to examine the relationship between dietary zinc intake and incident calcium kidney stones among either children or adults. We observed a strong association between higher

dietary zinc and lower odds of incident calcium kidney stones among adolescents. In multiple sensitivity analyses, the odds of calcium stones were consistently 60% lower among patients with daily zinc intake at the 75th percentile compared to those at the 25th percentile.

Daily intake of zinc is necessary to maintain cellular metabolism, adequate bone density, and normal growth during childhood. The recommended daily intake of zinc for boys and girls aged 14-18 years is 11mg and 9mg, respectively.[16] In our study, mean daily zinc intake of boys and girls with stones was 2mg and 1.2mg lower, respectively, than these requirements. Sources of zinc were similar between the groups, which indicates that quantity rather than type of zinc-containing food accounts for the association between zinc and stones. These findings suggest that insufficient zinc intake may be a risk factor for developing calcium stones during childhood.

The most likely explanation for the inverse association between dietary zinc and incident calcium stones is the inhibitory effect of zinc on CaP crystallization. Zinc promotes more soluble CaP phases, decreases the size of CaP crystals, and inhibits the transformation of brushite to hydroxyapatite.[8, 17] This inhibition of CaP crystallization and transformation is important to both CaP and CaOx stone formation. Idiopathic CaOx stones form on Randall's plaques, which are deposits of hydroxyapatite that begin in the basement membrane of the thin loops of Henle.[18, 19] One hypothesis for the association we observed is that zinc inhibits early CaP crystallization in the kidney and that this proximal inhibition prevents growth of CaP stones and the initial formation of Randall's plaques. Further anatomic and experimental studies are needed to determine the effect of zinc on the early stages of calcium stone formation.

Prior studies demonstrated conflicting results about the direction of the association between zinc and nephrolithiasis. Rapid stone formation was reported in an adult woman with zinc toxicity.[20] Additionally, an analysis of NHANES found that adults older than 30 years with zinc intake >15mg/day (which far exceeds recommended intake for adults) had a 70% increased odds of nephrolithiasis compared to those who consumed less than 7 mg/day.[9] However, zinc intake was 1mg lower among 18-29 year olds with a history of stones than among 18-29 year olds without stones. One explanation for these discrepant results is that both excess and insufficient zinc promotes stone formation. It is also possible that the greater importance of adequate zinc intake during adolescence might account for the different findings between younger and older patients. Multiple prior studies have found that higher zinc intake is associated with greater bone mineral content and density among children and young adults.[21, 22] Taken in this context of mineral metabolism, our results support the idea that zinc may be an important regulator of abnormal mineralization during periods of growth. Further mechanistic studies are needed to elucidate the biological effects of dietary zinc on calcium stone formation and its potential relationship to abnormal bone homeostasis.[23, 24]

We also found an association between higher dietary phytate and a lower odds of incident calcium nephrolithiasis, which is consistent with findings among young women.[25] Phytate, which is the principal storage form of phosphate in plants, strongly binds zinc in the gut and reduces its bioavailability. Additionally, phytate inhibits hydroxyapatite and CaOx crystallization[26] and may have an independent inhibitory effect on stone formation.[27] Nevertheless, our results should be considered preliminary since the association between phytate and calcium stones became statistically insignificant after

excluding the participant with high dietary oxalate. We also detected an inverse relationship between dietary oxalate and calcium nephrolithiasis. This association is likely spurious since it was driven by one control with extremely high oxalate intake and there is no biologic plausibility for this association. Sodium intake was over 3gm/day in cases and controls and was not associated with incident stones. This result is similar to findings among men and older women,[1, 28] but differs from the increased risk associated with high sodium intake among younger women.[25] There were no differences in fluid intake between cases and controls. Although it is possible that high sodium intake and low fluid intake are not as important risk factors among adolescents, it is more likely that participants with newly diagnosed stones decreased sodium and increased fluid intake prior to dietary assessment as these common recommendations for stone prevention are readily accessible.[29]

There was a strong positive relationship between dietary and urine zinc, which is consistent with prior studies of adult women.[30] Our results suggest that spot urine zinc levels reflect dietary zinc intake among adolescents with and without nephrolithiasis. However, it remains unknown whether dietary or urine zinc reflects zinc concentration and activity at the level of the renal papillae, which is the presumed site of activity for stone inhibition. There was also weak evidence that adolescents with calcium stones excrete less zinc in the urine for each increase in dietary zinc intake. The possibility of lower urinary zinc excretion among adolescents with calcium nephrolithiasis merits further study.

There are limitations in causal inference related to the case-control design. The ideal study would have measured dietary intake prior to stone diagnosis. However,

prospective cohort studies of children that measure diet and kidney stones do not exist. Kidney stones are also rare during childhood, which necessitated a case-control study to examine for these associations. Despite this limitation, it is unlikely that cases knowingly or inadvertently decreased zinc intake after stone diagnosis. Neither modifying dietary zinc nor decreasing protein intake (the primary source of zinc) is currently recommended for children with stones. Additionally, the dietary recalls and urine samples were obtained prior to counseling patients about modifying diet to prevent kidney stone recurrence. Finally, cases were consecutively screened and enrolled, and this study was conducted within an enumerated source population, which minimizes selection bias. Second, controls did not undergo imaging, raising the possibility that they may have had asymptomatic kidney stones. This is unlikely considering <1% of people have nephrolithiasis by 18 years.[3] Third, we did not assess supplemental zinc intake. However, zinc supplementation is not prescribed routinely during childhood and is not related to kidney stone status. It is likely that any zinc supplement use, should it exist, would be similar between groups. Fourth, we measured spot rather than 24-hour urine chemistries. Future studies of dietary zinc should examine 24-hour zinc excretion. Finally, although residual confounding is possible, it is unlikely that other dietary factors would negate the strong association between higher dietary zinc and reduced risk of stones among adolescents.

In conclusion, insufficient dietary intake of zinc was independently associated with incident calcium kidney stone disease in this population of adolescents.

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Figure Legend

Flowchart of the Study Population

Tables

Table 1: Characteristics of 30 Adolescents with Incident Calcium Kidney Stones and 30 Matched Healthy Controls

Characteristic	Case (n=30)	Control (n=30)
Median age in years (IQR)	15.0 (14.0, 16.0)	15.0 (14.0, 16.0)
Sex		
Male (%)	10 (33)	10 (33)
Female (%)	20 (67)	20 (67)
Race (n,%)		
Caucasian	29 (97)	29 (97)
African-American	1 (3)	1 (3)
Ethnicity (n,%)		
Hispanic	3 (10)	1 (3)
Non-Hispanic	27 (90)	29 (97)
BMI (IQR)	21.3 (20.3, 29.3)	20.3 (18.4, 23.3)
Stone Composition		
≥50% calcium oxalate	25 (83)	N/A
≥50% calcium phosphate	5 (7)	N/A

Table 2: Unadjusted Average Dietary Intake from three 24-hour dietary recalls of Adolescents with Incident Calcium Kidney Stones and Healthy Controls

Daily Intake	**Cases**	**Controls**	**p-value**
Zinc (mg) **Males*** **Females**	**8.2 (3.1)** **9.1** **7.8**	**10.0 (4.1)** **12.9** **8.6**	0.03
Phytate (mg)	444 (173)	601 (255)	< 0.001
Total Protein (g)	63.5 (20.2)	67.9 (21.4)	0.23
Animal Protein (g)	41.2 (17.6)	41.5 (15.8)	0.83
Vegetable Protein (g)	22.3 (5.6)	26.5 (9.5)	0.03
Calcium (mg)	1,041 (363.8)	1,120 (421)	0.36
Sodium (mg)	3,138 (839)	3,091 (926)	0.80
Oxalic Acid (mg)	97.7 (69.9, 128.6)	110.9 (87.5, 148.7)	0.06
Fructose (g)	20.1 (13.0)	22.7 (11.7)	0.33
Water (mL)	2,166 (679)	1,915 (674)	0.46
Energy (kcal)	1,749 (433.0)	1,875 (542)	0.32

Data are presented as mean (SD) or median (interquartile range) unless otherwise indicated. Variables with median (interquartile range) have non-normal distributions.
*The recommended daily zinc intake for boys and girls aged 14-18 years is 11mg and 9mg, respectively

Table 3: Adjusted Odds Ratios for Incident Calcium Kidney Stones According to Dietary Intake a) before and b) after removing the matched pair with outlying oxalate intake

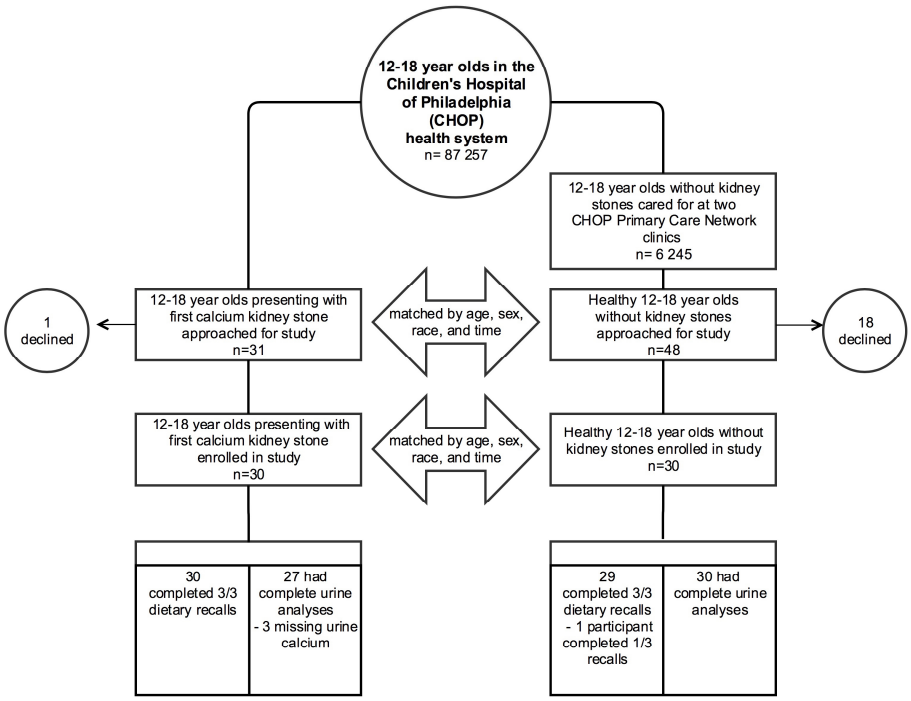
a) Nutrient	**OR**	**95% CI**	**p-value**
Zinc (mg)	0.87	0.75-0.99	0.04
Phytate (mg)	0.99	0.99-1.00	0.03
Sodium (mg)	1.00	1.00-1.001	0.18
Protein (g)	1.01	0.99-1.03	0.38
Calcium (mg)	1.00	0.99-1.001	0.84
Oxalate (mg)	0.99	0.99-1.00	0.03
b) Nutrient	**OR**	**95% CI**	**p-value**
Zinc (mg)	0.84	0.73-0.97	0.02
Phytate (mg)	0.99	0.997-1	0.05
Sodium (mg)	1	1-1.001	0.19
Protein (g)	1.01	0.99-1.03	0.26
Calcium (mg)	1	0.99-1.00	0.73
Oxalate (mg)	1.00	0.99-1.00	0.11

Models included all three dietary recalls for each subject and were adjusted for phytate, sodium, total protein, calcium, and oxalate intake.

Table 4: Estimates and 95% confidence intervals from a linear regression model examining the association between urine zinc (μg/dL) and zinc intake (mg).

Nutrient	**β**	**95% CI**	**p-value**
Zinc (mg)	4.46	1.23-7.70	0.009
Case:zinc intake	-3.58	-7.47-0.30	0.08
Phytate (mg)	-0.01	-0.05-0.02	0.49
Calcium (mg)	0.003	-0.03-0.02	0.82

Models included all three dietary recalls for each subject and were adjusted for dietary phytate and calcium and urine creatinine (mg/dL).



Abbreviations

NHANES	National Health and Nutrition Examination Survey
CaOx	calcium oxalate
CaP	calcium phosphate