Dietary Protein and Potassium, Diet–Dependent Net Acid Load, and Risk of Incident Kidney Stones

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Abstract

Background and objectives Protein and potassium intake and the resulting diet–dependent net acid load may affect kidney stone formation. It is not known whether protein type or net acid load is associated with risk of kidney stones.

Design, setting, participants, & measurements We prospectively examined intakes of protein (dairy, nondairy animal, and vegetable), potassium, and animal protein-to-potassium ratio (an estimate of net acid load) and risk of incident kidney stones in the Health Professionals Follow-Up Study (n=42,919), the Nurses' Health Study I (n=60,128), and the Nurses' Health Study II (n=90,629). Multivariable models were adjusted for age, body mass index, diet, and other factors. We also analyzed cross-sectional associations with 24-hour urine (n=6129).

Results During 3,108,264 person-years of follow-up, there were 6308 incident kidney stones. Dairy protein was associated with lower risk in the Nurses' Health Study II (hazard ratio for highest versus lowest quintile, 0.84; 95% confidence interval, 0.73 to 0.96; P value for trend <0.01). The hazard ratios for nondairy animal protein were 1.15 (95% confidence interval, 0.97 to 1.36; P value for trend =0.04) in the Health Professionals Follow-Up Study and 1.20 (95% confidence interval, 0.99 to 1.46; P value for trend =0.06) in the Nurses' Health Study I. Potassium intake was associated with lower risk in all three cohorts (hazard ratios from 0.44 [95% confidence interval, 0.36 to 0.53] to 0.67 [95% confidence interval, 0.57 to 0.78]; P values for trend <0.001). Animal protein-to-potassium ratio was associated with higher risk (P value for trend =0.004), even after adjustment for animal protein and potassium. Higher dietary potassium was associated with higher urine citrate, pH, and volume (P values for trend <0.002).

Conclusions Kidney stone risk may vary by protein type. Diets high in potassium or with a relative abundance of potassium compared with animal protein could represent a means of stone prevention.

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Introduction

Although protein intake is thought to be an important risk factor for kidney stone formation, evidence is limited. Higher animal protein intake may result in increased urinary excretion of calcium and uric acid and reduced citrate and urine pH, thus potentially favoring formation of calcium oxalate and uric acid stones (1,2). However, relatively few studies have examined associations between animal protein intake and risk of actual kidney stones. In one randomized trial, the risk of recurrence in calcium oxalate stone formers with lower animal protein intake was not significantly different from the control group with higher animal protein intake (3), but the study was limited by the high dropout rate (46%). Observational studies also have reported conflicting results; in particular, previous analyses of the association between animal protein and risk of stones in the Nurses' Health Study I (NHS I) and the Nurses' Health Study II (NHS II) cohorts showed no association (4,5), and a positive association in the Health Professionals Follow-Up Study (HPFS) was only in men with a body mass index (BMI) <25 kg/m^2 (6). To date, no data are available on the risk of stones associated with intakes of different types of animal protein (*i.e.*, dairy versus nondairy) or vegetable protein.

Data on the association between intake of potassium, a marker of dietary intake of organic anion, and risk of stones also are inconclusive. Higher dietary potassium intake would decrease urinary excretion of calcium, thus potentially protecting against stone formation (7). To date, no trial has examined the effect of a diet rich in fruits and vegetables (a major dietary source of potassium) on the risk of stones; however, potassium citrate has been shown to be effective in reducing recurrence in stone formers (8). Higher potassium intake has been associated with lower risk of kidney stones in the NHS I and the HPFS but not in the NHS II (4–6).

The balance between protein and potassium intake from diet also might be important with regard to risk of forming kidney stones. These two factors are major determinants of net endogenous acid production (NEAP), with acid production from sulfur–containing amino acids from protein counterbalanced by bicarbonate generation from alkali–containing potassium salts (9). Higher estimated NEAP values have been

associated with higher urinary calcium and lower citrate excretion (10,11), and cross-sectional studies have described higher dietary acid load among stone formers (12); however, the association between dietary net acid load and incident kidney stones has not been analyzed in longitudinal prospective studies.

We analyzed prospective associations between intake of different types of protein (vegetable, dairy, and nondairy animal), intake of potassium, and the interaction between animal protein and potassium intake and risk of incident kidney stones in three large cohort studies.

Materials and Methods

Study Populations

The HPFS was established in 1986 with the enrollment of 51,529 men who were health professionals (dentists, optometrists, osteopaths, pharmacists, podiatrists, and veterinarians) between the ages of 40 and 75 years old. The NHS I was established in 1976 with the enrollment of 121,700 women who were registered nurses between the ages of 30 and 55 years old, and the NHS II was established in 1989 with the enrollment of 116,430 women who were registered nurses between the ages of 25 and 42 years old. At enrollment, participants from each cohort completed a questionnaire with detailed information on diet, medical history, and medications. Participants subsequently were followed with biennial questionnaires including updated information and newly diagnosed diseases.

For this analysis, follow-up was started in 1986 for the HPFS and the NHS I and 1991 for the NHS II (the dates of the first detailed food frequency questionnaire [FFQ]) and went until the latest available information on confirmed incident stones: January of 2012 for the HPFS, May of 2006 for the NHS I, and May of 2011 for the NHS II. We only included participants without a history of kidney stones at baseline; furthermore, participants with a history of malignancy (except for nonmelanoma skin cancer) at baseline were excluded, and those who developed malignancies during follow-up were censored. For the NHS I, only participants who answered questionnaires in 1992 (the year of the first lifetime kidney stone history inquiry) or later were included.

Assessment of Diet

In 1986 (the HPFS and the NHS I) and 1991 (the NHS II), participants completed an FFQ asking about the average annual intake of >130 foods and 22 beverages; subsequently, the information was updated through FFQs every 4 years. Frequencies of consumption of each unit of food were used to compute intakes of nutrients using data from the US Department of Agriculture, except for oxalate content, which was measured by capillary electrophoresis in the majority of foods from the FFQ (13,14). All nutrients were energy adjusted to account for total amount of food eaten. FFQs were previously found to be reproducible and valid in the HPFS and the NHS I (15,16).

Dietary–dependent net acid load was estimated as the animal protein-to-potassium ratio (APKR), which previously was shown to be highly correlated with renal net acid excretion (r=0.90) (9). We also estimated NEAP from total protein and potassium intake using the equation in the work by Frassetto *et al.* (9), which is also highly correlated with renal net acid excretion (r=0.84).

Assessment of Kidney Stones

Participants who reported the occurrence of a kidney stone on the biennial questionnaire were asked to complete a supplementary questionnaire to establish the date of occurrence and accompanying symptoms. For this analysis, only kidney stones accompanied by pain and/or hematuria were considered; those participants reporting a new stone event without accompanying pain or hematuria were censored. Evaluation of medical records among 582 participants from the HPFS, 194 participants from the NHS I, and 858 participants from the NHS II who reported a kidney stone confirmed the diagnosis in 95%, 96%, and 98% of patients, respectively.

Assessment of Other Covariates

Information on age, BMI, history of diabetes, history of hypertension, use of thiazides, and amount of calcium supplements was obtained from the biennial questionnaire. Self-reported weight, used to calculate BMI, was validated in the HPFS and the NHS I cohorts (17).

Assessment of Urinary Composition

Twenty-four–hour urine samples were collected in three cycles. In the first cycle, participants were eligible if they were ≤70 years of age in the HPFS or ≤65 years of age in the NHS I and had no history of cancer or cardiovascular disease. In the second cycle, participants were eligible if they were ≤75 years of age and had no history of cancer (other than nonmelanoma skin cancer). The third cycle was performed only by the participants in the NHS II, with the following criteria: age ≤55 years old, white race, and no history of high BP, coronary heart disease, or cancer. Urine samples collected in the first two cycles were analyzed with the system provided by Mission Pharmacal (San Antonio, TX), whereas the samples collected in the third cycle were analyzed by the Litholink Corporation (Chicago, IL). Participants with a history of kidney stones were oversampled in the first two cycles.

Participants with possible over- or undercollections (defined as urinary creatinine excretion in the top or bottom 1% of the nonstone-formers distribution) were removed from the analysis. For participants who provided more than one collection, the first sample was analyzed.

Statistical Analyses

The analysis of kidney stone risk was prospective; dietary intakes were assessed before the incident kidney stone. For each cohort, participants were divided into quintiles of intakes, and time at risk was calculated as time from start of follow-up to an incident kidney stone, loss to follow-up, death, or censoring (whichever happened first). Cox proportional hazards regression models generated hazard ratios (HRs) and 95% confidence intervals (95% CIs) adjusted for age (continuous), BMI (13 categories), history of diabetes (yes versus no), history of hypertension (yes versus no), use of thiazides (yes versus no), supplemental calcium intake (four categories), and dietary intakes of fluid, calcium, sodium, fructose, oxalate, phytate (all quintiles), and

alcohol (four categories). Vegetable, nondairy animal, and dairy protein models were simultaneously adjusted for the other types of protein and potassium intake (all quintiles) but not for dietary calcium (because of high correlation between dietary calcium and dairy protein); APKR and NEAP models were further adjusted for their components: animal protein intake (APKR), all types of protein (NEAP), and potassium intake (APKR and NEAP). With the components included in the models, estimates for APKR and NEAP are interpreted as interaction terms. Information on exposures and covariates was updated every 4 years. Tests for linear trends were constructed by analyzing the median value for each quintile as a continuous variable. Effect modification by age (≤50 versus >50 years old), BMI (≤25 versus >25 kg/m^2), and use of thiazides was investigated through interaction terms for each type of protein, dietary potassium intake, and APKR.

Differences in urinary components across quintiles of exposure were analyzed with linear regression models using each urinary component as the dependent variable; adjusted for age, BMI, history of kidney stones, history of diabetes, history of hypertension, use of thiazides, supplemental calcium, study cohort, intake of fluid, urine creatinine, and sodium, and expressed as adjusted means, differences, and 95% CIs. When dietary potassium was used as the exposure, models were further adjusted for all types of protein; when protein intake and APKR were used as the exposure, models were also further adjusted for dietary potassium. For these analyses, we used information from the most recent questionnaire before the urine collection.

For each analysis, we evaluated between-cohort heterogeneity; pooled estimates obtained by random effects meta–analysis are presented whenever the *P* value for heterogeneity was >0.05.

Results

In total, 193,676 participants were included in the analysis of incident kidney stones (42,919 from the HPFS, 60,128 from the NHS I, and 90,629 from the NHS II). Baseline characteristics of the study participants are shown in Table 1 and Supplemental Table 1.

Association between Type of Protein and Risk of Stones

Over 3,108,264 person-years of follow-up, 6308 incident kidney stones were confirmed. The association between the type of protein and risk of kidney stones is presented in Tables 2 and 3 and Supplemental Table 2.

There was a significant inverse association between dairy protein intake and risk of stones in the NHS II (the multivariate-adjusted HR for the highest quintile compared with the lowest was 0.84; 95% CI, 0.73 to 0.96; *P* value for trend <0.01) but not in the HPFS (HR, 0.93; 95% CI, 0.78 to 1.10; *P* value for trend =0.59) or the NHS I (HR, 0.95; 95% CI, 0.76 to 1.17; *P* value for trend =0.89). The pooled analysis showed that the HR was 0.89 (95% CI, 0.81 to 0.98; *P* value for trend =0.06).

The HRs for nondairy animal protein intake were 1.15 in the HPFS (95% CI, 0.97 to 1.36; *P* value for trend =0.04), 1.20 in the NHS I (95% CI, 0.99 to 1.46; *P* value for trend =0.06), and 1.02 in the NHS II (95% CI, 0.90 to 1.17; *P* value for trend =0.64). The pooled analysis showed an HR of 1.10 (95% CI, 1.00 to 1.21; *P* value for trend =0.20). Because the higher risk was manifest predominantly in the highest quintile of nondairy animal protein, we also analyzed participants with intakes higher than those in the top quintile median. In this subanalysis, the HRs were 1.20 (95% CI, 0.99 to 1.46; *P* value for trend =0.03) for the HPFS, 1.28 (95% CI, 1.02 to 1.61; *P* value for trend =0.02) for the NHS I, and 0.97 (95% CI, 0.83 to 1.14; *P* value for trend =0.66) for the NHS II.

Table 1. Baseline characteristics of the study cohorts

Variable	HPFS, *n*=42,919	NHS I, *n*=60,128	NHS II, *n*=90,629
Age, yr	54.3 (9.8)	52.9 (7.1)	36.6 (4.6)
BMI, kg/m^2	25.5 (3.4)	25.2 (4.7)	24.6 (5.3)
History of diabetes, %	3	3	1
History of hypertension, %	21	24	6
Thiazide use, %	9	14	2
Calcium supplements use, %	23	57	34
Dietary calcium, mg/d	802 (306)	720 (253)	886 (305)
Supplemental calcium, mg/d	98 (262)	357 (429)	129 (273)
Magnesium, mg/d	355 (83)	300 (69)	316 (75)
Fructose, g/d	25.3 (11.4)	21.6 (9.0)	22.9 (11.0)
Oxalate, mg/d	144 (136)	119 (92)	135 (115)
Phytate, mg/d	943 (392)	708 (269)	783 (240)
Alcohol intake, g/d	11.5 (15.6)	6.2 (10.7)	3.1 (6.1)
Fluid intake, L/d	1.9 (0.8)	2.0 (0.7)	2.1 (0.8)
Dairy protein, g/d	15.9 (9.2)	15.0 (7.8)	18.6 (9.1)
Nondairy animal protein, g/d	51.5 (18.2)	47.1 (14.5)	49.1 (16.2)
Vegetable protein, g/d	24.8 (6.2)	20.2 (4.2)	22.5 (5.0)
Dietary potassium, mg/d	3389 (659)	3002 (562)	2933 (540)

Data are reported as means (SD) for continuous variables. HPFS, Health Professionals Follow-Up Study; NHS I, Nurses' Health Study I; NHS II, Nurses' Health Study II; BMI, body mass index.

Table 2. Quintiles of dairy protein intake and risk of kidney stones

Cohort	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* Value for Trend
HPFS						
Median, g/d	6.1	10.2	13.8	18.5	28.0	<0.001
Range	<8.4	8.4–12.0	12.1–15.9	16.0–22.2	>22.2	0.59
Patients	426	427	361	419	330	
Person-time	129,932	132,983	134,714	135,399	136,458	
Age-adjusted HR (95% CI)	1.00 (reference)	0.97 (0.85 to 1.11)	0.81 (0.71 to 0.94)	0.94 (0.82 to 1.08)	0.76 (0.66 to 0.88)	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	0.97 (0.84 to 1.11)	0.83 (0.71 to 0.96)	1.00 (0.87 to 1.16)	0.93 (0.78 to 1.10)	
NHS I						
Median, g/d	6.5	10.3	13.6	17.8	25.4	0.003
Range	<8.5	8.5–11.9	12.0–15.5	15.6–20.9	>20.9	0.89
Patients	282	281	275	275	218	
Person-time	195,814	202,303	203,431	205,077	203,817	
Age-adjusted HR (95% CI)	1.00 (reference)	0.96 (0.81 to 1.13)	0.93 (0.79 to 1.10)	0.93 (0.78 to 1.09)	0.76 (0.63 to 0.90)	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	0.99 (0.84 to 1.17)	1.01 (0.85 to 1.20)	1.06 (0.89 to 1.27)	0.95 (0.76 to 1.17)	
NHS II						
Median, g/d	8.4	13.0	17.0	22.2	30.7	<0.001
Range	<10.9	10.9–15.0	15.1–19.3	19.4–25.7	>25.7	<0.01
Patients	684	655	597	569	509	
Person-time	279,594	284,238	286,507	288,062	289,934	
Age-adjusted HR (95% CI)	1.00 (reference)	0.93 (0.83 to 1.03)	0.84 (0.75 to 0.93)	0.79 (0.70 to 0.88)	0.70 (0.62 to 0.78)	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	0.97 (0.87 to 1.08)	0.90 (0.80 to 1.01)	0.88 (0.78 to 0.99)	0.84 (0.73 to 0.96)	
Pooled cohorts						
Age-adjusted HR (95% CI)	1.00 (reference)	0.95 (0.88 to 1.02)	0.85 (0.78 to 0.92)	0.87 (0.77 to 0.99)	0.73 (0.67 to 0.79)	<0.001
Multivariate-adjusted HR (95% CI)	1.00 (reference)	0.97 (0.90 to 1.05)	0.90 (0.82 to 1.00)	0.97 (0.86 to 1.08)	0.89 (0.81 to 0.98)	0.06

Multivariate models were adjusted for age, body mass index, history of diabetes, history of hypertension, use of thiazides, supplemental calcium, and intakes of fluid, sodium, potassium, fructose, oxalate, phytate, alcohol, and all other sources of protein. Medians refer to the first time period; however, dietary variables were updated over the course of the study. HPFS, Health Professionals Follow-Up Study; HR, hazard ratio; 95% CI, 95% confidence interval; NHS I, Nurses' Health Study I; NHS II, Nurses' Health Study II.

Table 3. Quintiles of nondairy animal protein intake and risk of kidney stones

Cohort	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* Value for Trend
HPFS						
Median, g/d	31.4	42.6	50.3	58.6	72.5	0.004
Range	<38.9	38.9–47.2	47.3–55.4	55.5–66.2	>66.2	0.04
Patients	351	364	379	424	445	
Person-time	133,204	133,448	134,142	134,288	134,406	
Age-adjusted HR (95% CI)	1.00 (reference)	1.04 (0.90 to 1.20)	1.05 (0.91 to 1.22)	1.17 (1.01 to 1.35)	1.19 (1.04 to 1.37)	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	0.98 (0.84 to 1.14)	0.99 (0.85 to 1.15)	1.09 (0.93 to 1.28)	1.15 (0.97 to 1.36)	
NHS I						
Median, g/d	30.9	39.6	45.9	52.8	64.4	0.001
Range	<36.2	36.2–42.9	43.0–49.5	49.6–58.1	>58.1	0.06
Patients	229	253	259	268	322	
Person-time	202,112	203,103	203,627	202,284	199,316	
Age-adjusted HR (95% CI)	1.00 (reference)	1.10 (0.92 to 1.31)	1.09 (0.91 to 1.30)	1.12 (0.94 to 1.34)	1.34 (1.13 to 1.59)	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.04 (0.87 to 1.25)	1.02 (0.84 to 1.22)	1.03 (0.85 to 1.25)	1.20 (0.99 to 1.46)	
NHS II						
Median, g/d	30.9	41.3	48.3	55.8	68.5	0.01
Range	<37.3	37.3–45.1	45.2–52.7	52.8–62.7	>62.7	0.64
Patients	560	645	566	576	667	
Person-time	286,109	286,994	286,767	285,358	282,807	
Age-adjusted HR (95% CI)	1.00 (reference)	1.14 (1.02 to 1.28)	1.01 (0.90 to 1.13)	1.03 (0.92 to 1.16)	1.21 (1.09 to 1.36)	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.07 (0.95 to 1.20)	0.91 (0.80 to 1.03)	0.91 (0.80 to 1.03)	1.02 (0.90 to 1.17)	
Pooled cohorts						
Age-adjusted HR (95% CI)	1.00 (reference)	1.10 (1.02 to 1.19)	1.04 (0.96 to 1.12)	1.09 (1.00 to 1.18)	1.23 (1.14 to 1.33)	<0.001
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.04 (0.95 to 1.12)	0.95 (0.88 to 1.04)	1.00 (0.89 to 1.12)	1.10 (1.00 to 1.21)	0.20

Multivariate models were adjusted for age, body mass index, history of diabetes, history of hypertension, use of thiazides, supplemental calcium, and intakes of fluid, sodium, potassium, fructose, oxalate, phytate, alcohol, and all other types of protein. Medians refer to the first time period; however, dietary variables were updated over the course of the study. HPFS, Health Professionals Follow-Up Study; HR, hazard ratio; 95% CI, 95% confidence interval; NHS I, Nurses' Health Study I; NHS II, Nurses' Health Study II.

There was no significant association between intake of vegetable protein and risk of stones. There was no significant effect modification by age, BMI, or use of thiazides for any source of protein.

Association between Intake of Potassium and Risk of Stones

The association between dietary potassium intake and risk of kidney stones is presented in Table 4. After multivariable adjustment, there was a strong inverse association between potassium intake and risk of stones: the HR was 0.44 (95% CI, 0.36 to 0.53) for the HPFS, 0.57 (95% CI, 0.45 to 0.72) for the NHS I, and 0.67 (95% CI, 0.57 to 0.78) for the NHS II (all P values for trend <0.001). There was no significant effect modification by age, BMI, or use of thiazides.

Association between Dietary Net Acid Load and Risk of Stones

The association between estimated dietary net acid load and risk of kidney stones is presented in Table 5 and Supplemental Table 3. After multivariable adjustment including animal protein and potassium intake, there was a significant positive association between APKR and risk of stones: the pooled HR was 1.41 (95% CI, 1.18 to 1.68; P value for trend =0.004). A similar association was seen with NEAP (pooled HR, 1.22; 95% CI, 1.05 to 1.41; P value for trend <0.01). There was no significant effect modification by age, BMI, or use of thiazides.

Association between Type of Protein and Urinary Composition

The association between the type of protein and urinary composition is reported in Supplemental Tables 4 and 5. After multivariable adjustment, compared with those in the lowest quintile, those in the highest quintile of dairy protein intake had 24 (95% CI, 16 to 32) mg/d higher urine calcium, 1.4 (95% CI, 0.5 to 2.4) mg/d lower urine oxalate, 36 (95% CI, 11 to 60) mg/d higher urine citrate, and higher relative supersaturation for calcium phosphate. Those in the highest quintile of nondairy animal protein intake had 54 (95% CI, 30 to 78) mg/d lower urine citrate, 24 (95% CI, 13 to 35) mg/d higher urine uric acid, 0.13 (95% CI, 0.09 to 0.17) lower urine pH, and higher relative supersaturation for uric acid.

Association between Potassium Intake and Urinary Composition

The association between potassium intake and urinary composition is reported in Table 6. After multivariable adjustment including total fluid intake, those in the highest quintile had 1.6 (95% CI, 0.6 to 2.5) mg/d higher urine oxalate, 39 (95% CI, 13 to 65) mg/d higher urine citrate, 26 (95% CI, 14 to 38) mg/d higher urine uric acid, 0.15 (95% CI, 0.10 to 0.19) higher urine pH, 237 (95% CI, 173 to 299) ml/d higher urine volume, and significantly lower relative supersaturations for calcium oxalate and uric acid. There was no association between potassium intake and urinary excretion of calcium.

Association between Dietary Net Acid Load and Urinary Composition

The association between APKR and urinary composition is reported in Supplemental Table 6. After multivariable adjustment including main components of APKR, those in the highest quintile had 70 (95% CI, 22 to 118) mg/d lower urine citrate, 0.16 (95% CI, 0.07 to 0.23) lower urine pH, and 140 (95% CI, 23 to 256) ml/d lower urine volume.

Associations between dietary intakes and urine composition were similar among those participants with and without a history of kidney stones.

Discussion

We found that associations between vegetable, dairy, and nondairy animal protein intake and kidney stone risk were either null or modest and of borderline statistical significance. However, different types of protein may affect the risk of kidney stones differently: in particular, vegetable protein was not associated with risk of stones, dairy protein was associated with reduced risk in younger women, and nondairy animal protein may have been associated with modestly higher risk in men and older women. In contrast, higher dietary potassium was associated with a statistically significant and large reduction in kidney stone risk in all cohorts. APKR was associated with higher risk of stones, independent of the components of APKR (animal protein and potassium intake). The 24-hour urine composition also varied by type of protein. Higher dairy protein intake was associated with higher excretion of calcium and citrate but lower oxalate and uric acid. Higher nondairy animal protein, however, was associated with significantly lower citrate, higher uric acid, and a more acidic urine. Taken together, these findings suggest that dairy protein intake might have a mixed effect on urinary lithogenic factors. The higher urinary calcium might be because of the effect of casein on intestinal calcium absorption (18) and/or the higher content of calcium present in dairy products. The latter possibility may explain the lower excretion of oxalate with higher dairy protein intake, because higher calcium in the gut can bind with oxalate, thus reducing intestinal absorption of oxalate (19). However, nondairy animal protein may have a more marked prolithogenic effect on urine composition, likely to favor calcium stone formation (because of lower citrate) and uric acid stone formation (because of lower pH and higher uric acid). Another potential mechanism through which large intakes of animal protein could affect stone risk is the increased synthesis of oxalate secondary to metabolism of amino acids, such as hydroxyproline. However, studies with controlled dietary intakes of oxalate did not find any association between intake of animal protein and urinary oxalate excretion (20). Similarly, in our study, urinary oxalate was not higher among participants with larger intakes of animal protein.

A major finding in our study is the strong inverse association between higher dietary potassium intake, a marker of dietary intake of organic anion, and risk of stones: there was a 33%–56% lower risk of stones for those participants in the highest quintile of potassium intake. Previous analyses of these cohorts reported associations of smaller magnitude for the HPFS (6) and the NHS I (4) and no association for the NHS II (5). Our updated findings are likely caused by increased statistical power. Participants in the highest quintile of potassium intake had

Table 4. Quintiles of dietary potassium intake and risk of kidney stones

Cohort	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* Value for Trend
HPFS						
Median, g/d	2601	3016	3327	3667	4224	<0.001
Range	<2835	2835–3175	3176–3488	3489–3888	>3888	<0.001
Patients	567	459	368	332	237	
Person-time	128,870	134,246	135,483	135,420	135,469	
Age-adjusted HR (95% CI)	1.00 (reference)	0.78 (0.69 to 0.88)	0.64 (0.56 to 0.73)	0.60 (0.52 to 0.68)	0.44 (0.38 to 0.51)	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	0.78 (0.68 to 0.89)	0.64 (0.55 to 0.74)	0.60 (0.51 to 0.70)	0.44 (0.36 to 0.53)	
NHS I						
Median, g/d	2323	2694	2971	3261	3722	<0.001
Range	<2535	2535–2835	2836–3108	3109–3448	>3448	<0.001
Patients	393	287	245	211	195	
Person-time	196,777	203,677	204,510	204,711	200,767	
Age-adjusted HR (95% CI)	1.00 (reference)	0.71 (0.61 to 0.82)	0.61 (0.52 to 0.72)	0.53 (0.45 to 0.63)	0.50 (0.42 to 0.60)	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	0.74 (0.63 to 0.87)	0.66 (0.55 to 0.79)	0.58 (0.48 to 0.72)	0.57 (0.45 to 0.72)	
NHS II						
Median, g/d	2275	2649	2910	3180	3612	<0.001
Range	<2491	2491–2784	2785–3038	3039–3354	>3354	<0.001
Patients	815	662	595	533	409	
Person-time	282,413	285,735	287,785	286,885	285,518	
Age-adjusted HR (95% CI)	1.00 (reference)	0.81 (0.73 to 0.90)	0.72 (0.65 to 0.80)	0.65 (0.58 to 0.73)	0.50 (0.45 to 0.57)	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	0.91 (0.82 to 1.02)	0.87 (0.77 to 0.98)	0.83 (0.73 to 0.94)	0.67 (0.57 to 0.78)	

Multivariate models were adjusted for age, body mass index, history of diabetes, history of hypertension, use of thiazides, supplemental calcium, and intakes of fluid, calcium, sodium, fructose, oxalate, phytate, alcohol, and all other types of protein. Medians refer to the first time period; however, dietary variables were updated over the course of the study. Cohort-specific results were not pooled because of statistically significant test for heterogeneity. HPFS, Health Professionals Follow-Up Study; HR, hazard ratio; 95% CI, 95% confidence interval; NHS I, Nurses' Health Study I; NHS II, Nurses' Health Study II.

Table 5. Quintiles of animal protein-to-potassium ratio and risk of kidney stones

Cohort	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* Value for Trend
HPFS						
Median, g/mEq	0.46	0.59	0.70	0.81	1.01	<0.001
Range	<0.53	0.53–0.64	0.65–0.75	0.76–0.88	>0.88	<0.001
Patients	268	336	378	408	573	0.004
Person-time	131,584	132,719	134,206	135,099	135,879	
Age-adjusted HR (95% CI)	1.00 (reference)	1.21 (1.03 to 1.42)	1.30 (1.10 to 1.52)	1.35 (1.16 to 1.59)	1.84 (1.58 to 2.14)	
Model 1 multivariate–adjusted HR (95% CI)	1.00 (reference)	1.25 (1.06 to 1.48)	1.33 (1.13 to 1.57)	1.36 (1.14 to 1.60)	1.72 (1.45 to 2.04)	
Model 2 multivariate–adjusted HR (95% CI)	1.00 (reference)	1.19 (0.99 to 1.44)	1.26 (1.01 to 1.53)	1.28 (0.98 to 1.66)	1.63 (1.18 to 2.25)	
NHS I						
Median, g/mEq	0.46	0.57	0.67	0.77	0.94	<0.001
Range	<0.50	0.50–0.60	0.61–0.70	0.71–0.82	>0.82	0.004
Patients	205	213	253	301	359	<0.01
Person-time	201,541	201,865	202,107	202,490	202,439	
Age-adjusted HR (95% CI)	1.00 (reference)	1.01 (0.84 to 1.23)	1.21 (1.01 to 1.47)	1.44 (1.20 to 1.74)	1.72 (1.43 to 2.06)	
Model 1 multivariate–adjusted HR (95% CI)	1.00 (reference)	0.98 (0.80 to 1.19)	1.10 (0.90 to 1.35)	1.23 (1.00 to 1.50)	1.29 (1.04 to 1.61)	
Model 2 multivariate–adjusted HR (95% CI)	1.00 (reference)	1.06 (0.85 to 1.32)	1.26 (0.97 to 1.62)	1.45 (1.08 to 1.95)	1.59 (1.10 to 2.31)	
NHS II						
Median, g/mEq	0.49	0.64	0.74	0.86	1.07	<0.001
Range	<0.57	0.57–0.69	0.70–0.79	0.80–0.94	>0.94	0.16
Patients	507	549	616	639	703	0.16
Person-time	283,922	284,365	285,185	286,649	288,214	
Age-adjusted HR (95% CI)	1.00 (reference)	1.10 (0.97 to 1.24)	1.25 (1.11 to 1.41)	1.34 (1.19 to 1.51)	1.53 (1.35 to 1.72)	
Model 1 multivariate–adjusted HR (95% CI)	1.00 (reference)	1.04 (0.92 to 1.18)	1.11 (0.98 to 1.26)	1.11 (0.97 to 1.27)	1.12 (0.97 to 1.27)	
Model 2 multivariate–adjusted HR (95% CI)	1.00 (reference)	1.07 (0.93 to 1.24)	1.18 (1.00 to 1.40)	1.20 (0.98 to 1.46)	1.25 (0.97 to 1.60)	
Pooled cohorts						
Age-adjusted HR (95% CI)	1.00 (reference)	1.11 (1.02 to 1.21)	1.26 (1.15 to 1.37)	1.37 (1.25 to 1.49)	1.67 (1.48 to 1.88)	<0.001
Model 1 multivariate–adjusted HR (95% CI)	1.00 (reference)	1.08 (0.94 to 1.25)	1.18 (1.04 to 1.33)	1.21 (1.07 to 1.37)	1.35 (1.03 to 1.78)	0.02
Model 2 multivariate–adjusted HR (95% CI)	1.00 (reference)	1.10 (0.99 to 1.22)	1.21 (1.07 to 1.36)	1.26 (1.14 to 1.40)	1.41 (1.18 to 1.68)	0.004

Multivariate model 1 was adjusted for age, body mass index, history of diabetes, history of hypertension, use of thiazides, supplemental calcium, and intakes of fluid, calcium, sodium, fructose, oxalate, phytate, and alcohol. Multivariate model 2 was further adjusted for animal protein and potassium intake; in these models, animal protein-to-potassium ratios represent interaction terms. Medians refer to the first time period; however, dietary variables were updated over the course of the study. HPFS, Health Professionals Follow-Up Study; HR, hazard ratio; 95% CI, 95% confidence interval; NHS I, Nurses' Health Study I; NHS II, Nurses' Health Study II.

Table 6. Pooled adjusted mean values and 95% confidence intervals of 24-hour urine components by quintile of dietary potassium intake

Variable	Quintile 1, *n*=1227	Quintile 2, *n*=1226	Quintile 3, *n*=1224	Quintile 4, *n*=1226	Quintile 5, *n*=1226	*P* Value for Trend
Median, mg/d	2641	3052	3357	3691	4162	
Creatinine, g	1.3 (1.3 to 1.3)	1.3 (1.3 to 1.4)	1.3 (1.3 to 1.4)	1.4 (1.3 to 1.4)	1.3 (1.3 to 1.4)	0.12
Potassium, mEq	56.1 (54.7 to 57.5)	61.5 (60.1 to 62.8)	63.7 (62.4 to 65.0)	67.6 (66.2 to 68.9)	71.3 (69.9 to 72.7)	<0.001
Calcium, mg	189 (182 to 196)	194 (188 to 201)	195 (189 to 202)	192 (185 to 198)	189 (182 to 196)	0.84
Oxalate, mg	32.6 (31.8 to 33.4)	33.1 (32.4 to 33.9)	32.9 (32.1 to 33.6)	32.9 (32.3 to 33.7)	34.2 (33.4 to 35.0)	<0.01
Citrate, mg	675 (655 to 696)	681 (661 to 700)	703 (683 to 722)	696 (677 to 716)	714 (693 to 735)	0.002
Uric acid, mg	517 (507 to 526)	522 (513 to 531)	527 (518 to 536)	529 (520 to 538)	543 (533 to 552)	<0.001
Sodium, mEq	151 (147 to 155)	149 (147 to 152)	148 (144 to 152)	150 (146 to 154)	149 (145 to 153)	0.51
Magnesium, mg	104 (102 to 107)	107 (105 to 110)	109 (106 to 112)	110 (108 to 113)	112 (109 to 115)	<0.001
Phosphate, mg	846 (830 to 862)	866 (851 to 881)	850 (835 to 865)	873 (858 to 888)	867 (851 to 883)	0.03
Sulfate, mmol	18.2 (17.8 to 18.6)	18.6 (18.2 to 19.0)	18.7 (18.3 to 19.0)	19.1 (18.8 to 19.5)	19.4 (19.0 to 19.8)	<0.001
Ammonium, mmol	29.2 (28.2 to 30.3)	30.4 (29.5 to 31.3)	28.4 (27.6 to 29.3)	29.4 (28.5 to 30.3)	29.1 (28.1 to 30.0)	0.39
pH, U	5.95 (5.92 to 5.99)	6.00 (5.97 to 6.03)	6.04 (6.01 to 6.07)	6.05 (6.02 to 6.09)	6.10 (6.06 to 6.13)	<0.001
Volume, ml	1737 (1687 to 1788)	1853 (1805 to 1902)	1830 (1783 to 1877)	1878 (1829 to 1926)	1974 (1923 to 2025)	<0.001
SS CaOx						
Pharmacal	1.74 (1.58 to 1.91)	1.60 (1.44 to 1.77)	1.60 (1.43 to 1.76)	1.50 (1.34 to 1.66)	1.48 (1.32 to 1.65)	<0.001
Litholink	5.80 (5.46 to 6.13)	5.56 (5.26 to 5.86)	5.54 (5.24 to 5.83)	5.25 (4.95 to 5.54)	5.07 (4.76 to 5.39)	0.001
SS CaP						
Pharmacal	1.33 (1.14 to 1.51)	1.39 (1.21 to 1.57)	1.38 (1.20 to 1.56)	1.44 (1.26 to 1.62)	1.37 (1.19 to 1.56)	0.43
Litholink	1.09 (0.99 to 1.19)	1.07 (0.98 to 1.16)	1.14 (1.05 to 1.22)	1.07 (0.98 to 1.16)	1.06 (0.96 to 1.15)	0.21
SS UA						
Pharmacal	2.05 (1.80 to 2.30)	1.78 (1.53 to 2.02)	1.76 (1.51 to 2.00)	1.61 (1.37 to 1.85)	1.55 (1.30 to 1.80)	<0.001
Litholink	0.79 (0.72 to 0.86)	0.68 (0.62 to 0.75)	0.66 (0.60 to 0.73)	0.65 (0.59 to 0.72)	0.62 (0.55 to 0.69)	0.002

Models were adjusted for age, body mass index, history of kidney stones, history of diabetes, history of hypertension, total fluid intake, use of thiazides, supplemental calcium, intake of protein (dairy, nondairy animal, and vegetable), study cohort, urine creatinine, and sodium. Ammonium data were only available for the Nurses' Health Study II Litholink collection. Pharmacal results are reported as relative supersaturations, whereas Litholink results as supersaturations. Medians refer to the first time period; however, dietary variables were updated over the course of the study. SS CaOx, supersaturation calcium oxalate; SS CaP, supersaturation calcium phosphate (brushite); SS UA, supersaturation uric acid.

higher urinary levels of citrate, larger urine volumes, and higher urine pH, resulting in reduced relative supersaturations for calcium oxalate and uric acid, despite higher urinary excretion of oxalate. The larger urine volume, even after adjustment for fluid intake from beverages, might be explained by the high water content in fruits and vegetables.

We also found an association between higher levels of dietary net acid load, estimated by APKR and NEAP, and a higher risk of kidney stones. Such associations were significant, even after including the respective components of the ratios in the model. An imbalance between intakes of acid-generating and alkali-generating foods, reflected in higher APKR and NEAP values, respectively, could lead to a series of metabolic derangements, including higher urinary excretion of calcium (plausibly through bone buffering because of subclinical systemic acidosis [10]), reduced excretion of citrate (11), and reduced urine pH (which would increase the risk of uric acid stones). Of note, we found significantly lower excretion of citrate and lower urine pH for higher quintiles of APKR and a higher relative supersaturation for uric acid. The APKR results may partially account for our previous finding that a dietary approaches to stop hypertension–style diet, which includes higher intakes of fruits and vegetables and lower intakes of red and processed meats, is associated with a lower risk of kidney stones in the same cohorts (21).

Our study has limitations. We did not have information on stone composition for all participants and thus, could not determine whether type of protein or estimated dietary net acid load was associated with risk of selectively forming specific stone types. Also, our study participants are predominantly white, and our results may not apply to other populations.

In conclusion, the risk of kidney stone formation associated with protein intake may vary by protein type. In contrast with vegetable and dairy protein, nondairy animal protein may be associated with a slightly higher risk of kidney stones among men and older women. Higher potassium intake is strongly associated with a lower risk of stones, and a higher estimated net dietary acid load is associated with higher risk independent of potassium intake. Our data suggest that diets rich in fruits and vegetables as well as diets with a relative abundance of fruits and vegetables compared with animal protein may represent effective interventions to prevent kidney stone formation.

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Supplementary Table 1. Baseline characteristics by quintiles of animal protein to potassium ratio

	HPFS		NHS I		NHS II	
	Quintile 1	Quintile 5	Quintile 1	Quintile 5	Quintile 1	Quintile 5
APKR, g/mEq	0.43(0.09)	1.06(0.18)	0.44(0.08)	0.98(0.15)	0.45(0.11)	1.11(0.17)
Age, years	55.9(10.0)	53.4(9.6)	54.5(7.0)	51.8(7.1)	37.4(4.6)	36.0(4.7)
BMI, kg/mq	24.7(3.1)	26.0(3.5)	24.3(4.3)	26.0(5.2)	23.1(4.3)	25.4(5.8)
History of diabetes, %	2	3	2	4	1	1
History of hypertension, %	21	22	24	25	6	7
Thiazide use, %	8	9	14	14	2	2
Calcium supplements use, %	31	21	61	55	37	32
Dietary calcium, mg/day	796(289)	754(298)	681(222)	688(256)	823(261)	899(333)
Supplemental calcium, mg/day	144(317)	85.1(246)	402(453)	341(428)	165(317)	116(256)
Magnesium, mg/day	422(105)	322(70)	335(82)	276(63)	369(95)	294(68)
Fructose, g/day	35.6(14.5)	21.1(9.8)	28.3(10.9)	17.8(8.2)	30.6(12.7)	19.8(10.7)
Oxalate, mg/day	198(234)	121(109)	148(129)	103(81)	204(190)	109(88)
Phytate, mg/day	1,337(612)	774(274)	907(393)	592(200)	1,029(343)	682(182)
Alcohol intake, g/day	10.6(16.0)	10.8(14.9)	6.5(11.7)	5.9(10.6)	3.9(7.5)	2.5(5.3)
Fluid intake, L/day	2.0(0.8)	1.8(0.8)	2.1(0.7)	1.9(0.7)	2.2(0.9)	2.0(0.8)
Dairy protein, g/day	13.4(8.2)	15.7(9.4)	12.4(6.6)	15.3(8.5)	15.1(7.8)	19.8(10.0)
Non-dairy animal protein, g/day	30.4(12.4)	65.6(18.5)	31.9(9.8)	59.9(15.1)	28.7(12.5)	59.7(15.8)
Vegetable protein, g/day	32.3(8.6)	21.5(4.6)	24.3(5.2)	17.7(3.5)	29.1(7.2)	19.9(3.8)
Dietary potassium, mg/day	3,988(793)	3,056(534)	3,395(653)	2,696(480)	3,357(655)	2,696(464)

Values are means(SD) or percentages.

Supplementary Table 2. Quintiles of vegetable protein intake and risk of kidney stones

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	p-value for trend
HPFS						
Median (g/day)	18.8	21.6	24.2	27.1	32.3	
Range	< 20.0	20.0, 22.9	23.0, 25.5	25.6, 29.1	> 29.1	
Cases	408	454	417	363	321	
Person-time	132,151	133,570	134,896	134,499	134,370	
Age-adjusted HR	1.00 (Ref.)	1.11 (0.97, 1.26)	1.01 (0.88, 1.16)	0.90 (0.78, 1.04)	0.79 (0.68, 0.91)	< 0.001
Multivariate-adjusted HR	1.00 (Ref.)	1.18 (1.01, 1.37)	1.14 (0.97, 1.37)	1.11 (0.91, 1.35)	1.05 (0.84, 1.33)	0.91
NHS I						
Median (g/day)	15.3	18.1	20.0	22.0	25.4	
Range	< 16.9	16.9, 19.0	19.1, 20.9	21.0, 23.3	> 23.3	
Cases	325	274	253	260	219	
Person-time	197,621	203,135	203,844	204,229	201,612	
Age-adjusted HR	1.00 (Ref.)	0.81 (0.69, 0.95)	0.76 (0.64, 0.90)	0.78 (0.66, 0.92)	0.68 (0.57, 0.80)	< 0.001
Multivariate-adjusted HR	1.00 (Ref.)	0.86 (0.72, 1.03)	0.87 (0.71, 1.06)	0.96 (0.77, 1.19)	0.89 (0.69, 1.16)	0.69
NHS II						
Median (g/day)	16.7	19.9	22.0	24.4	28.4	
Range	< 18.5	18.5, 21.0	21.1, 23.2	23.3, 25.9	> 25.9	
Cases	723	616	630	542	503	
Person-time	283,167	285,398	287,122	286,960	286,209	
Age-adjusted HR	1.00 (Ref.)	0.85 (0.76, 0.95)	0.87 (0.78, 0.97)	0.75 (0.67, 0.84)	0.70 (0.63, 0.79)	< 0.001
Multivariate-adjusted HR	1.00 (Ref.)	0.94 (0.83, 1.07)	1.05 (0.91, 1.20)	0.98 (0.84, 1.14)	1.02 (0.85, 1.22)	0.64
Pooled cohorts						
Age-adjusted HR	1.00 (Ref.)	0.91 (0.76, 1.10)	0.88 (0.76, 1.02)	0.81 (0.72, 0.91)	0.72 (0.66, 0.78)	< 0.001
Multivariate-adjusted HR	1.00 (Ref.)	0.99 (0.83, 1.17)	1.02 (0.88, 1.18)	1.01 (0.93, 1.10)	1.00 (0.88, 1.13)	0.84

Multivariate models adjusted for age, BMI, history of diabetes, history of hypertension, use of thiazides, supplemental calcium, intakes of fluid, sodium, potassium, fructose, oxalate, phytate, alcohol and for all the other sources of protein. Medians refer to the first time period; however dietary variables were updated over the course of the study.

Supplementary Table 3. Quintiles of NEAP and risk of kidney stones

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	p-value for trend
HPFS						
Median (mEq/day)	30.0	38.3	44.6	51.6	63.8	
Range	< 34.5	34.5, 41.3	41.4, 47.7	47.8, 55.9	> 55.9	
Cases	257	319	359	452	576	
Person-time	132,320	132,966	133,850	135,554	135,985	
Age-adjusted HR	1.00 (Ref.)	1.18 (1.00, 1.39)	1.27 (1.08, 1.49)	1.53 (1.31, 1.79)	1.90 (1.63, 2.21)	< 0.001
Multivariate-adjusted HR (1)	1.00 (Ref.)	1.20 (1.02, 1.43)	1.29 (1.09, 1.53)	1.50 (1.26, 1.77)	1.74 (1.46, 2.08)	< 0.001
Multivariate-adjusted HR (2)	1.00 (Ref.)	1.09 (0.91, 1.31)	1.09 (0.89, 1.34)	1.18 (0.94, 1.49)	1.26 (0.96, 1.67)	0.09
NHS I						
Median (mEq/day)	28.0	35.4	41.1	47.6	58.3	
Range	< 31.5	31.5, 37.7	37.8, 43.5	43.6, 51.1	> 51.1	
Cases	193	222	223	321	372	
Person-time	201,927	202,014	202,137	202,072	202,291	
Age-adjusted HR	1.00 (Ref.)	1.13 (0.93, 1.37)	1.13 (0.93, 1.37)	1.63 (1.35, 1.98)	1.87 (1.55, 2.24)	< 0.001
Multivariate-adjusted HR (1)	1.00 (Ref.)	1.11 (0.91, 1.35)	1.07 (0.87, 1.31)	1.47 (1.20, 1.79)	1.55 (1.25, 1.92)	< 0.001
Multivariate-adjusted HR (2)	1.00 (Ref.)	1.03 (0.83, 1.27)	0.94 (0.73, 1.19)	1.19 (0.91, 1.56)	1.10 (0.79, 1.52)	0.42
NHS II						
Median (mEq/day)	32.4	41.2	47.7	55.2	68.1	
Range	< 37.2	37.2, 44.1	44.2, 50.8	50.9, 59.9	> 59.9	
Cases	507	500	629	644	734	
Person-time	284,628	284,794	284,936	286,295	287,683	
Age-adjusted HR	1.00 (Ref.)	1.00 (0.88, 1.13)	1.28 (1.14, 1.44)	1.35 (1.19, 1.51)	1.58 (1.40, 1.78)	< 0.001
Multivariate-adjusted HR (1)	1.00 (Ref.)	0.98 (0.86, 1.11)	1.20 (1.06, 1.35)	1.18 (1.04, 1.34)	1.26 (1.10, 1.44)	< 0.001
Multivariate-adjusted HR (2)	1.00 (Ref.)	0.96 (0.84, 1.11)	1.19 (1.02, 1.38)	1.17 (0.98, 1.39)	1.24 (1.00, 1.53)	0.04
Pooled cohorts						
Age-adjusted HR	1.00 (Ref.)	1.08 (0.97, 1.20)	1.25 (1.15, 1.36)	1.47 (1.31, 1.65)	1.75 (1.55, 1.99)	< 0.001
Multivariate-adjusted HR (1)	1.00 (Ref.)	1.08 (0.95, 1.23)	1.20 (1.09, 1.31)	1.36 (1.15, 1.60)	1.49 (1.21, 1.84)	< 0.001
Multivariate-adjusted HR (2)	1.00 (Ref.)	1.01 (0.92, 1.12)	1.09 (0.96, 1.24)	1.18 (1.07, 1.30)	1.22 (1.05, 1.41)	0.006

Multivariate model 1 adjusted for age, BMI, history of diabetes, history of hypertension, use of thiazides, supplemental calcium, intakes of fluid, calcium, sodium, fructose, oxalate, phytate and alcohol. Multivariate model 2 further adjusted for all types of protein and potassium intake; in these models NEAP values represent interaction terms. Medians refer to the first time period; however dietary variables were updated over the course of the study.

Supplementary Table 4. Pooled adjusted mean values and 95% confidence intervals of 24-h urine components by quintile of dairy protein

	Quintile 1 (n = 1,227)	Quintile 2 (n = 1,225)	Quintile 3 (n = 1,225)	Quintile 4 (n = 1,226)	Quintile 5 (n = 1,226)	p-value for trend
Median (g/day)	5.6	10.0	13.1	17.3	25.6	—
Creatinine, g	1.4 (1.3, 1.4)	1.3 (1.3, 1.4)	1.3 (1.3, 1.4)	1.3 (1.3, 1.3)	1.3 (1.3, 1.4)	0.09
Calcium, mg	180 (173, 187)	185 (178, 191)	195 (189, 202)	195 (188, 202)	204 (198, 211)	< 0.001
Oxalate, mg	34.1 (33.3, 34.9)	33.0 (32.3, 33.8)	33.0 (32.3, 33.8)	32.9 (32.2, 33.7)	32.7 (31.9, 33.5)	0.011
Citrate, mg	675 (655, 696)	674 (655, 694)	703 (683, 722)	706 (687, 726)	711 (690, 731)	0.002
Uric acid, mg	535 (526, 545)	526 (517, 535)	520 (511, 529)	533 (523, 542)	523 (514, 533)	0.17
Sodium, mEq	145 (141, 149)	151 (147, 154)	148 (144, 152)	152 (149, 156)	151 (147, 155)	0.021
Potassium, mEq	61.8 (60.4, 63.1)	63.5 (62.2, 64.8)	63.6 (62.3, 65.0)	65.2 (639, 66.2)	66.0 (64.6, 67.4)	< 0.001
Magnesium, mg	107 (104, 110)	106 (103, 108)	109 (106, 112)	111 (108, 113)	111 (108, 114)	0.001
Phosphate, mg	823 (807, 839)	847 (832, 862)	855 (840, 870)	878 (863, 892)	900 (884, 915)	< 0.001
Sulfate, mmol	18.0 (17.6, 18.4)	18.4 (18.1, 18.8)	18.7 (18.3, 19.0)	19.3 (19.0, 19.7)	19.6 (19.2, 20.0)	< 0.001
Ammonium, mmol	28.7 (27.7, 29.7)	29.5 (28.5, 30.4)	29.7 (28.8, 30.6)	29.3 (28.4, 30.2)	29.4 (28.4, 30.3)	0.56
pH, U	6.05 (6.02, 6.09)	6.02 (5.99, 6.05)	6.02 (5.99, 6.06)	6.03 (6.00, 6.07)	6.01 (5.98, 6.05)	0.10
Volume, mL	1,833 (1,783, 1,883)	1,842 (1,794, 1,890)	1,862 (1,814, 1,910)	1,905 (1,857, 1,953)	1,830 (1,780, 1,883)	0.80
SS CaOx						
- Pharmacal	1.62 (1.46, 1.79)	1.52 (1.36, 1.68)	1.61 (1.45, 1.77)	1.54 (1.38, 1.70)	1.63 (1.46, 1.79)	0.91
- Litholink	5.22 (4.89, 5.54)	5.37 (5.07, 5.68)	5.58 (5.28, 5.88)	5.39 (5.09, 5.69)	5.65 (5.34, 5.96)	0.08
SS CaP						
- Pharmacal	1.35 (1.17, 1.53)	1.31 (1.13, 1.49)	1.37 (1.19, 1.55)	1.37 (1.19, 1.55)	1.51 (1.32, 1.69)	0.016
- Litholink	1.02 (0.92, 1.11)	0.99 (0.90, 1.08)	1.11 (1.02, 1.20)	1.12 (1.03, 1.21)	1.18 (1.09, 1.27)	0.003
SS UA						
- Pharmacal	1.74 (1.50, 1.98)	1.74 (1.50, 1.98)	1.78 (1.54, 2.02)	1.73 (1.49, 1.97)	1.75 (1.51, 2.00)	0.89
- Litholink	0.66 (0.58, 0.73)	0.71 (0.65, 0.78)	0.67 (0.60, 0.73)	0.69 (0.62, 0.75)	0.68 (0.61, 0.75)	0.86

Models adjusted for age, BMI, history of kidney stones, history of diabetes, history of hypertension, total fluid intake, use of thiazides, supplemental calcium, intake of all the other types of protein, intake of potassium, study cohort, urine creatinine and sodium. Ammonium data only available for NHS II Litholink collection. CaOx, calcium oxalate; CaP, calcium phosphate (brushite); SS, supersaturation; UA, uric acid.

Pharmacal results are reported as relative supersaturations, whereas Litholink results as supersaturations. Medians refer to the first time period; however dietary variables were updated over the course of the study.

Supplementary Table 5. Pooled adjusted mean values and 95% confidence intervals of 24-h urine components by quintile of non-dairy animal protein

	Quintile 1 (n = 1,225)	Quintile 2 (n = 1,227)	Quintile 3 (n = 1,225)	Quintile 4 (n = 1,227)	Quintile 5 (n = 1,225)	p-value for trend
Median (g/day)	24.3	33.8	41.3	49.1	64.7	—
Creatinine, g	1.3 (1.3, 1.4)	1.3 (1.3, 1.4)	1.3 (1.3, 1.4)	1.3 (1.3, 1.4)	1.3 (1.3, 1.4)	0.26
Calcium, mg	187 (181, 194)	193 (187, 200)	192 (186, 198)	192 (185, 198)	195 (189, 202)	0.11
Oxalate, mg	33.3 (32.5, 34.1)	32.9 (32.2, 33.7)	32.9 (32.1, 33.6)	32.8 (32.1, 33.5)	33.8 (33.1, 34.6)	0.29
Citrate, mg	720 (700, 740)	696 (676, 716)	707 (687, 726)	680 (661, 699)	666 (646, 686)	< 0.001
Uric acid, mg	514 (505, 524)	525 (515, 534)	525 (515, 534)	537 (527, 546)	538 (528, 547)	< 0.001
Sodium, mEq	141 (138, 145)	147 (143, 151)	152 (148, 156)	150 (146, 154)	156 (153, 160)	< 0.001
Potassium, mEq	65.8 (64.4, 67.1)	64.6 (63.2, 65.9)	63.5 (62.2, 64.8)	63.3 (62.0, 64.6)	62.9 (61.6, 64.3)	< 0.001
Magnesium, mg	112 (109, 115)	109 (106, 111)	107 (104, 109)	107 (104, 109)	110 (107, 112)	0.12
Phosphate, mg	845 (829, 860)	856 (841, 87)	846 (832, 861)	872 (857, 886)	883 (868, 898)	< 0.001
Sulfate, mmol	17.8 (17.4, 18.2)	18.5 (18.1, 18.9)	18.6 (18.2, 18.9)	19.1 (18.8, 19.5)	20.1 (19.7, 20.4)	< 0.001
Ammonium, mmol	28.3 (27.4, 29.2)	29.3 (28.4, 30.2)	28.4 (27.5, 29.3)	30.2 (29.3, 31.1)	30.3 (29.4, 31.3)	< 0.001
pH, U	6.09 (6.05, 6.12)	6.05 (6.02, 6.09)	6.05 (6.02, 6.08)	6.00 (5.96, 6.03)	5.96 (5.92, 5.99)	< 0.001
Volume, mL	1,869 (1,820, 1,918)	1,839 (1,791, 1,888)	1,851 (1,803, 1,899)	1,840 (1,793, 1,887)	1,873 (1,825, 1,922)	0.89
SS CaOx						
- Pharmacal	1.57 (1.40, 1.73)	1.60 (1.43, 1.76)	1.55 (1.39, 1.71)	1.58 (1.42, 1.74)	1.63 (1.46, 1.79)	0.42
- Litholink	5.18 (4.88, 5.48)	5.42 (5.12, 5.71)	5.56 5.27, 5.84)	5.50 (5.20, 5.81)	5.56 (5.23, 5.48)	0.08
SS CaP						
- Pharmacal	1.47 (1.29, 1.65)	1.49 (1.31, 1.67)	1.33 (1.15, 1.50)	1.31 (1.13, 1.49)	1.31 (1.13, 1.49)	0.001
- Litholink	1.04 (0.95, 1.13)	1.10 (.01, 1.18)	1.16 (1.07, .24)	1.10 (1.01, 1.19)	1.02 (0.93, 1.11)	0.70
SS UA						
- Pharmacal	1.61 (1.37, 1.85)	1.60 (1.36, 1.84)	1.71 (1.47, 1.95)	1.87 (1.63, 2.11)	1.95 (1.71, 2.20)	< 0.001
- Litholink	0.61 (0.55, 0.68)	0.71 (0.65, 0.78)	0.65 (0.59, 0.72)	0.69 (0.62, 0.76)	0.74 (0.67, 0.81)	0.02

Models adjusted for age, BMI, history of kidney stones, history of diabetes, history of hypertension, total fluid intake, use of thiazides, supplemental calcium, intake of all the other types of protein, intake of potassium, study cohort, urine creatinine and sodium. Ammonium data only available for NHS II Litholink collection. CaOx, calcium oxalate; CaP, calcium phosphate (brushite); SS, supersaturation; UA, uric acid.

Pharmacal results are reported as relative supersaturations, whereas Litholink results as supersaturations. Medians refer to the first time period; however dietary variables were updated over the course of the study.

Supplementary Table 6. Pooled adjusted mean values and 95% confidence intervals of 24-h urine components by quintile of animal protein to potassium ratio

	Quintile 1 (n = 1,225)	Quintile 2 (n = 1,227)	Quintile 3 (n = 1,225)	Quintile 4 (n = 1,227)	Quintile 5 (n = 1,225)	p-value for trend
Median (g/mEq)	0.43	0.57	0.66	0.77	0.97	—
Creatinine, g	1.4 (1.3, 1.4)	1.3 (1.3, 1.4)	1.3 (1.3, 1.4)	1.3 (1.3, 1.4)	1.3 (1.3, 1.3)	0.19
Calcium, mg	192 (183, 202)	191 (184, 198)	192 (185, 198)	193 (186, 200)	192 (182, 201)	0.87
Oxalate, mg	33.7 (32.6, 34.8)	33.4 (32.6, 34.2)	32.7 (32.0, 33.5)	32.6 (31.8, 33.5)	33.5 (32.4, 34.6)	0.85
Citrate, mg	730 (701, 760)	715 (693, 737)	680 (660, 700)	691 (669, 713)	660 (632, 689)	0.003
Uric acid, mg	531 (517, 545)	529 (519, 539)	528 (519, 537)	523 (513, 534)	526 (513, 540)	0.53
Sodium, mEq	147 (141, 153)	150 (146, 154)	149 (145, 153)	149 (145, 153)	151 (146, 157)	0.46
Potassium, mEq	68.0 (66.0, 70.0)	66.5 (65.0, 68.0)	64.3 (62.9, 65.6)	62.6 (61.1, 64.1)	59.1 (57.2, 61.0)	< 0.001
Magnesium, mg	115 (111, 119)	110 (107, 113)	107 (104, 109)	106 (103, 109)	107 (103, 110)	0.041
Phosphate, mg	859 (836, 881)	861 (844, 877)	866 (851, 881)	854 (837, 871)	866 (844, 888)	0.84
Sulfate, mmol	18.7 (18.1, 19.2)	18.9 (18.5, 19.3)	18.9 (18.5, 19.3)	18.6 (18.2, 19.0)	19.0 (18.5, 19.5)	0.57
Ammonium, mmol	28.7 (27.2, 30.1)	28.5 (27.5, 29.5)	29.3 (28.5, 30.2)	29.3 (28.3, 30.2)	30.9 (29.4, 32.4)	0.06
pH, U	6.11 (6.06, 6.16)	6.07 (6.04, 6.11)	6.02 (5.99, 6.06)	5.99 (5.96, 6.03)	5.95 (5.90, 6.00)	< 0.001
Volume, mL	1,932 (1,859, 2,004)	1,888 (1,835, 1,941)	1,833 (1,785, 1,881)	1,838 (1,785, 1,891)	1,792 (1,722, 2,004)	0.05
SS CaOx						
- Pharmacal	1.62 (1.43, 1.82)	1.63 (1.46, 1.80)	1.56 (1.39, 1.72)	1.52 (.35, 1.68)	1.61 (1.43, 1.80)	0.99
- Litholink	4.96 (4.49, 5.44)	5.28 (4.96, 5.61)	5.65 (5.36, 5.95)	5.69 (5.35, 6.02)	5.66 (5.18, 6.15)	0.12
SS CaP						
- Pharmacal	1.45 (1.23, 1.67)	1.44 (1.25, 1.63)	1.44 (1.25, 1.62)	1.30 (1.12, 1.48)	1.29 (1.08, 1.49)	0.09
- Litholink	1.08 (0.94, 1.22)	1.15 (1.05, 1.24)	1.12 (1.04, 1.21)	1.07 (0.97, 1.17)	1.02 0.88, 1.16)	0.42
SS UA						
- Pharmacal	1.70 (1.41, 2.00)	1.67 (1.42, 1.93)	1.72 (1.47, 1.96)	1.77 (1.52, 2.02)	1.90 (1.62, 2.18)	0.11
- Litholink	0.53 (0.42, 0.63)	0.58 (0.51, 0.65)	0.70 (0.64, 0.77)	0.74 (0.66, 0.81)	0.85 (0.74, 0.95)	< 0.001

Models adjusted for age, BMI, history of kidney stones, history of diabetes, history of hypertension, total fluid intake, use of thiazides, supplemental calcium, intake of protein and potassium, study cohort, urine creatinine and sodium. Ammonium data only available for NHS II Litholink collection. CaOx, calcium oxalate; CaP, calcium phosphate (brushite); SS, supersaturation; UA, uric acid. Pharmacal results are

reported as relative supersaturations, whereas Litholink results as supersaturations. Medians refer to the first time period; however dietary variables were updated over the course of the study.