

Estimated urine pH and bladder cancer risk in a cohort of male smokers (Finland)[★]

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Abstract

Objective: Low urine pH may be an important risk factor for bladder cancer, although few studies have evaluated this association. We examined the relationship between estimated renal net acid excretion (NAE), an indirect measure of urine pH based on nutrient intake and anthropometry, and bladder cancer risk in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study.

Methods: At baseline, 27,096 male smokers 50–69 years old completed a dietary questionnaire that assessed usual frequency of consumption and portion sizes for the previous 12 months, had height and weight measured, and provided a history of smoking. A total of 446 incident bladder cancer cases were identified during up to 17.4 years of follow-up.

Results: In multivariate proportional hazards models, the relative risk (RR) for bladder cancer was 1.15 (95% confidence interval (CI)=0.86–1.55) for individuals in the highest (*i.e.*, most acidic) *versus* the lowest (*i.e.*, least acidic) NAE quintile ($p=0.38$). Among men who smoked for more than 45 years, there was a suggestion of increased risk with higher NAE levels (RR = 1.72, 95% CI = 0.96–3.10, $p=0.08$).

Conclusions: These findings do not indicate that urine pH is a major risk factor for bladder cancer, although certain subsets of individuals may be at increased risk.

Introduction

Low urine pH is associated with uric acid stone formation and other urothelial disorders [1], and may also be an important risk factor for bladder cancer. In several animal and human studies, acidic urine has been associated with elevated levels of free (unconjugated) aromatic amines in urine and arylamine-DNA adducts in bladder epithelium [2–7]. For example, among

workers occupationally exposed to benzidine in India, there was a strong inverse relationship between urine pH and the proportion of free benzidine and associated metabolites in urine samples ($p < 0.0001$) [7]. In the same study, workers with urine pH < 6.0 had ten fold higher DNA adduct levels in exfoliated urothelial cells compared to those with urine pH ≥ 7.0 , after controlling for internal dose ($p = 0.0037$).

N-glucuronide conjugates of aromatic amines, which are inactive and intended for excretion via the kidney, are readily hydrolyzed to their active free parent amines in acidic environments [2, 8]. In experimental studies, for example, the half-lives of *N*-glucuronides of benzidine were reported to be 5 min at pH 5.3, 25 min at pH 6.3, and 140 min at pH 7.4 [9]. Glucuronides of 4-aminobiphenyl, an aromatic amine present in cigarette

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smoke, exhibited half-lives of 11 min at pH 5.5 and 185 min at pH 7.4 in a separate study [2]. Further metabolic activation by prostaglandin H synthase, which is expressed at relatively high levels in the bladder, enables free aromatic amines to covalently bind to urothelial DNA [10]. Since aromatic amines are prevalent in tobacco smoke and certain industrial settings, low urine pH may be a particularly important risk factor for bladder cancer among those individuals who smoke and/or work in high-risk occupations.

Systemic acid–base homeostasis is maintained in large part by renal excretion of excess hydrogen ions in urine. Diet composition is a major determinant of urine pH, with fruits and vegetables considered alkalinizing foods owing to their high potassium content [11], and fish, cheeses, meat and meat products considered acidifying foods due to their high protein content [12]. Body weight also influences urine pH through its effect on the secretion of endogenously produced organic acids [13]. A model has been developed that utilizes dietary intake and body weight to estimate an individual's total renal net acid excretion (NAE). This model involves taking the difference between the sum of the major urinary nonbicarbonate anions (sulfate, phosphate, and organic acids) and the sum of the urinary mineral cations (potassium, magnesium, and calcium), accounting for differences in intestinal absorption rates. Positive NAE values reflect an excess acid load, which increases urine acidity (*i.e.*, lowers urine pH). NAE estimated in this manner has been shown to correlate reasonably well with urine pH measured from 24-h urine samples in healthy adults ($r = -0.50$ in the Observing Protein and Energy Nutrition (OPEN) study and $r = -0.83$ in a German study) [14, 15], and may therefore be a useful surrogate measure in studies that do not have urine specimens available for analysis.

We investigated the relationship between estimated renal NAE and bladder cancer risk in a large cohort study conducted in male smokers in Finland. To date, this is the first prospective study to evaluate whether low urine pH, as estimated by NAE, enhances the risk of bladder cancer.

Materials and methods

Study population

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study was a randomized, double-blinded, placebo-controlled, 2×2 factorial design, primary chemoprevention trial that tested whether daily supplementation with β -carotene (20 mg) and/or vitamin E

(50 mg *dl*-alpha-tocopherol) reduced the incidence of lung and other cancers. Details regarding the study design, methods, participant characteristics, and compliance have been reported previously [16]. Briefly, 29,133 participants meeting all eligibility criteria (male residents of southwestern Finland aged 50–69 years who smoked five or more cigarettes per day) were successfully randomized into the trial between 1985 and 1988. Reasons for exclusion included a history of cancer (other than nonmelanoma skin cancer or carcinoma *in situ*) or other diseases/conditions that might limit participation in a long-term intervention trial, use of vitamin E, vitamin A, or β -carotene supplements in excess of predefined amounts, and treatment with anticoagulants. The trial ended on 30 April 1993, with passive case ascertainment continuing thereafter. The present analysis is based on 27,096 cohort subjects with complete baseline dietary and anthropometric information. Person-years of observation were calculated from the date of randomization to the date of bladder cancer diagnosis, death, or 30 April 2002. The institutional review boards of both the National Public Health Institute of Finland and the US National Cancer Institute approved the study, and written, informed consent was obtained from each participant prior to randomization.

Case ascertainment

Incident cases of bladder cancer (ICD-9 code 188 and 233.7) were ascertained through the Finnish Cancer Registry (FCR) and the Register of Causes of Death; the FCR alone provides almost 100% case ascertainment nationwide [17]. Cancers of the renal pelvis, ureter, or urethra were not included in the present analysis. Upon identification of a case, all relevant medical records were obtained and reviewed independently by one or two study physicians. A total of 473 bladder cancer cases were identified during up to 17.4 years of follow-up (median = 14.2 years). Of these, 27 had incomplete baseline dietary data, leaving 446 cases for analysis.

Data collection

Prior to randomization, all subjects were asked to provide detailed demographic, medical, smoking, and occupational information, and to complete a dietary questionnaire. Height and weight were measured by specially trained, registered nurses. The food use questionnaire inquired about the usual frequency of consumption and portion sizes of 276 common food items/mixed dishes and beverages during the past year. A color picture booklet was provided to each participant in order to assist with portion size estimation.

Daily nutrient intakes were calculated using the food composition database of the National Public Health Institute in Finland. The dietary questionnaire was developed specifically for use in the ATBC trial, and was validated against food consumption records in a pilot study conducted in middle-aged Finnish men [18]. Energy-adjusted correlation coefficients (corrected for attenuation) were 0.65, 0.63, 0.71, and 0.73 for protein, potassium, magnesium, and calcium, respectively [18].

Estimation of urine pH with renal NAE

Renal NAE is conventionally determined from urine analyses as the sum of the titratable acid and ammonium minus bicarbonate. However, NAE can also be determined indirectly using a validated formula based entirely on nutrient intake and anthropometric information [15]. This model involves taking the difference between the sum of the remaining important urinary anions (sulfate, phosphate, and organic acids (OA)) and the sum of the mineral cations (potassium, magnesium, and calcium) [15]. Sodium and chloride are omitted from this model because they have equal intestinal absorption rates, which implies that ingested salt does not impact renal NAE [19]. In addition, chloride data are frequently missing or incomplete in food composition tables. The following equations were utilized to estimate NAE:

NAE = PRAL + OA, where

$$\begin{aligned} \text{PRAL (mEq/d)} &= [0.49 \times \text{SO}_4(\text{g/d})] + \\ & [0.037 \times \text{PO}_4(\text{mg/d})] - [0.021 \times \text{K}(\text{mg/d})] - \\ & [0.026 \times \text{Mg}(\text{mg/d})] - [0.013 \times \text{Ca}(\text{mg/d})], \text{ and} \\ \text{OA} &= \text{body weight}(\text{kg}) \times 0.66 \end{aligned}$$

Potential renal acid load (PRAL) is a measure of the acid- or base-forming potential of commonly consumed foods and beverages, with negative values reflecting an excess of base-forming potential and positive values indicating an excess of acid-forming potential. Specific intestinal absorption rates of contributing nutrients, ionic valences for calcium and magnesium ($\times 2$), and grade of dissociation of phosphate at pH 7.4 ($\times 1.8$) are accounted for when converting daily nutrient intakes to milliequivalents (mEq) (corresponding to 1 mol H^+ or OH^-) [15]. Urinary excretion of sulfate is estimated from total protein intake since metabolism of methionine and cysteine provides the majority of organic sulfur. In this study, protein was assumed to have an average content of 2.4% methionine and 2.0% cysteine (as in [15]).

Basal OA excretion is a diet-independent determinant of NAE, and is predominantly influenced by body surface area. A simplified estimate based on individual body weight can also be used to approximate daily OA excretion [15].

Statistical analysis

Estimated renal NAE was divided into quintiles based on the distribution in the entire cohort; this approach does not assume a linear relationship between the predictor and the outcome, and allows for a more intuitive interpretation of results when the unit of measurement is complex. Cox proportional hazards models were utilized to estimate relative risks (RR) and 95% confidence intervals (CI) for each quintile relative to the referent category (quintile 1; least acidic urine). Tests for linear trend were carried out by taking the median values of each quintile and modeling as a continuous variable. A base model was specified a priori and included energy intake (kcal), age, number of cigarettes smoked daily, and number of years of smoking as continuous variables, along with α -tocopherol and β -carotene intervention group assignment. Addition of other putative confounders, including body mass index (BMI; kg/m^2), total fluid intake (from coffee, tea, milk, juice, soft drinks, beer, wine, and liquor; note that we did not have information on tap water consumption), education level (primary, high school, vocational, university), place of residence (small town, large town), pack-years of smoking, smoking inhalation (never/seldom, often/always), and smoking cessation (defined as having quit for three consecutive visits (i.e., 1 year) during the trial), to the base model did not alter risk estimates by more than 10%.

Effect modification by smoking duration (≤ 35 , 35–45, > 45 years), smoking dose (< 20 , 20–30, > 30 cigarettes per day), total fluid intake (< 1394 , 1394–1856, > 1856 ml per day), age (≤ 57 , > 57 years), BMI (≤ 25 , > 25 kg/m^2), and intervention group (α -tocopherol, no α -tocopherol, β -carotene, no β -carotene) was evaluated in stratified analyses (smoking dose and BMI were categorized according to pre-specified cut points, whereas other continuous study factors were divided into tertile or median split categories based on the distribution among the entire cohort), and by adding the relevant cross-product term to main effects models. In order to conserve power, NAE was divided into tertiles (rather than quintiles) in all stratified analyses.

At baseline, a small proportion of participants reported consuming calcium, magnesium, and/or potassium supplements. To address this, we ran multivariate models with and without adjustment for

supplement use, excluded supplement users altogether, and integrated baseline use of calcium, magnesium, and potassium supplements with dietary intakes of these nutrients. None of these procedures altered the association between NAE and bladder cancer risk. There was no departure from the underlying proportional hazards assumption (p -value > 0.05). All analyses were conducted with SAS (version 8.2, SAS Institute Inc., Cary, NC), and statistical tests were two-tailed with significance levels set at $p < 0.05$.

Results

Age-adjusted baseline characteristics by quintile of NAE are presented in Table 1. In general, men in higher NAE quintiles (*i.e.*, those with more acidic urine) weighed

more, smoked fewer years, were more likely to live in an urban area, and were more highly educated. In terms of dietary intake, those with higher NAE values consumed more calories, protein-rich foods (meats and cheeses), phosphorus, and calcium and less fluid, fruits and vegetables, and potassium compared to men in lower quintiles. The median NAE value among bladder cancer cases (48.8) was not appreciably different from the corresponding value in noncases (49.8) ($p > 0.05$).

Individuals in the highest quintile of renal NAE had a similar risk of bladder cancer compared to those in the referent category in both age-adjusted and multivariate models (Table 2). Results obtained from a reduced multivariate model adjusted only for age and duration of smoking – the two major confounders of the NAE-bladder cancer association – were comparable to those presented in Table 2 (data not shown). Analysis of the

Table 1. Age-adjusted means and proportions of baseline characteristics by quintile of estimated renal net acid excretion (NAE) in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study

	Quintile of NAE				
	1 (Least acidic)	2	3	4	5 (Most acidic)
Participants (<i>n</i>)	5419	5419	5420	5419	5419
Age (years)	57.4	57.5	57.4	57.2	56.4
BMI ^a (kg/m ²)	23.8	25.1	26.0	27.3	29.2
Weight (kg)	70.5	74.8	78.4	82.9	90.0
Smoking history					
Years smoked regularly	36.5	36.1	35.9	35.5	35.7
Cigarettes/day	20.5	20.2	20.2	20.4	20.9
Smoking inhalation (%)					
Never/seldom	8.0	9.0	8.7	9.3	9.2
Often/Always	92.0	91.0	91.3	90.7	90.8
Smoking cessation ^b (%)	14.2	15.9	16.2	16.7	17.2
Urban residence (%)	39.8	42.3	43.8	43.7	42.6
Education level (%)					
Primary school	66.7	64.6	65.1	63.2	63.3
High school	7.0	8.1	7.3	7.8	8.1
Vocational	21.8	22.6	23.2	24.2	24.1
University	4.3	4.6	4.3	4.8	4.3
Daily dietary intake					
Energy (kcal)	2682	2652	2724	2831	3185
Fluid ^c (ml)	1849	1679	1657	1655	1686
Fruits and vegetables (g)	265	236	225	221	219
Fish, meat/meat products ^d (g)	175	179	189	202	246
Cheeses (g)	16.8	19.4	22.5	26.4	40.7
Protein ^e (g)	94.5	95.5	99.2	105.1	121.9
Phosphorus (mg)	2032	2019	2076	2181	2483
Potassium (mg)	5328	4858	4754	4763	4959
Magnesium (mg)	499	470	468	476	508
Calcium (mg)	1312	1298	1339	1412	1626

^a BMI = body mass index.

^b Stopped smoking for at least one year during the trial.

^c Includes coffee, tea, milk, juice, soft drinks, beer, wine, and liquor.

^d Includes fish and shellfish, poultry, and red meat (beef, pork, sausages and other cold cuts, and inner organs and blood).

^e Total protein intake was utilized to estimate urinary excretion of sulfate.

Table 2. Relative risks (RR) and 95% confidence intervals (CI) for bladder cancer according to quintiles of estimated renal net acid excretion (NAE) in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, 1985–2002

	Quintile of NAE					<i>p</i> trend
	1 (Least acidic)	2	3	4	5 (Most acidic)	
Median NAE ^a	30.5	41.9	49.7	57.9	71.4	
Median PRAL ^b	-16.8	-7.3	-1.7	3.5	13.7	
Median OA ^c	46.2	49.1	51.5	54.5	58.9	
No. of cases	94	90	84	86	92	
Person-years	68,363	67,613	68,109	67,804	67,446	
Age-adjusted RR	1.00	0.96	0.89	0.95	1.10	0.55
95% CI	Ref.	0.72–1.28	0.66–1.20	0.71–1.27	0.83–1.47	
Multivariate RR ^d	1.00	0.97	0.91	0.98	1.15	0.38
95% CI	Ref.	0.72–1.29	0.68–1.22	0.73–1.31	0.86–1.55	

^a NAE = PRAL + OA.

^b Potential renal acid load; PRAL = phosphate + sulfate – potassium – calcium – magnesium.

^c Organic acids; OA = body weight × 0.66.

^d Adjusted for energy intake (kcal), age, number of years of smoking, cigarettes/day, and intervention assignment.

uppermost ranges of NAE (*i.e.*, those men with the most highly acidic urine) did not reveal any associations with bladder cancer risk (RR for men >95th percentile compared to those ≤50th percentile = 1.17, 95% CI = 0.74–1.86, *p* = 0.34). Since the relationship between calcium intake and renal calcium excretion is typically very weak, and because its inclusion has been shown to

underestimate NAE [20], we removed calcium intake from NAE and re-estimated its association with bladder cancer risk. The corresponding risk estimates were slightly stronger than those obtained using the original NAE model that included calcium intake (for highest *versus* lowest NAE quintile, RR = 1.29, 95% CI = 0.95–1.77, *p* = 0.19).

Table 3. Multivariate relative risks (RR) and 95% confidence intervals (CI) for bladder cancer according to tertiles of estimated renal net acid excretion (NAE), stratified by selected factors (Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, 1985–2002)

	Tertile of NAE								<i>p</i> trend
	1 (Least acidic)		2			3 (Most acidic)			
	No. of cases	RR ^a	No. of cases	RR ^a	95% CI	No. of Cases	RR ^a	95% CI	
<i>Years of smoking</i>									
≤ 35	45	1.00	35	0.73	0.47–1.14	42	0.87	0.56–1.33	0.53
35–45	90	1.00	88	1.02	0.76–1.37	81	1.03	0.76–1.40	0.84
> 45	23	1.00	17	0.79	0.42–1.48	25	1.72	0.96–3.10	0.08
<i>Cigarettes/day</i>									
< 20	60	1.00	46	0.78	0.53–1.14	47	0.96	0.65–1.42	0.79
20–30	69	1.00	74	1.12	0.80–1.55	62	1.05	0.74–1.49	0.78
> 30	29	1.00	20	0.67	0.38–1.19	39	1.23	0.75–2.02	0.34
<i>Daily fluid intake^b (ml)</i>									
< 1394	50	1.00	60	1.00	0.68–1.45	54	1.01	0.68–1.62	0.98
1394–1856	43	1.00	45	1.05	0.69–1.60	51	1.35	0.89–2.07	0.16
> 1856	65	1.00	35	0.70	0.47–1.06	43	0.91	0.61–1.36	0.54
<i>Age (y)</i>									
≤ 57	61	1.00	52	0.88	0.60–1.27	62	0.99	0.69–1.42	0.98
> 57	97	1.00	88	0.92	0.69–1.23	86	1.10	0.82–1.49	0.54
<i>BMI^c (kg/m²)</i>									
≤ 25	97	1.00	47	0.79	0.56–1.13	26	1.00	0.63–1.56	0.62
> 25	61	1.00	93	0.94	0.68–1.30	122	1.07	0.78–1.46	0.57

^a Adjusted for energy intake (kcal), age, number of years of smoking, cigarettes/day, and intervention assignment.

^b Includes coffee, tea, milk, juice, soft drinks, beer, wine, and liquor.

^c BMI = body mass index.

In stratified analyses, a suggestive association between high NAE and bladder cancer risk was apparent among longer-term smokers (Table 3). Smoking dose, fluid intake, age, BMI, and trial intervention group had no material effect on the NAE-bladder cancer association (p for interaction > 0.05).

Discussion

There was no significant overall association between estimated urine pH and bladder cancer risk in this prospective cohort of male smokers. A modest, yet statistically nonsignificant increase in risk was, however, observed for the highest NAE levels (*i.e.*, most acidic urine) among men who smoked for 45 years or more.

To date, only one study has been published that directly evaluated whether urine pH influences bladder cancer risk. In a hospital-based case-control study conducted in Japan, there was no apparent association between acidic urine (as measured with a test tape) and bladder cancer risk in the entire study population (odds ratio (OR) = 0.87, 95% CI: 0.39–1.93) or in a subset of smokers (OR = 0.74, 95% CI not reported) [21]. This study was limited by its retrospective design, however, with urine specimens obtained after bladder cancer diagnosis. In addition, the authors did not specify whether risk estimates were adjusted for potentially important confounding variables. In contrast to the findings from the Japanese study, preliminary results from a large hospital-based case-control study in Spain suggest that individuals with “consistently acidic” urine (defined as urine pH readings < 6.0 on multiple consecutive days measured by subjects on urine samples collected at their homes) have an increased risk of bladder cancer [22].

Individuals exposed to aromatic amines for extended periods of time are likely to be most susceptible to low urinary pH, which may explain the elevated risk we observed with higher NAE levels in men who smoked for 45 years or more. Although the stratum-specific sample sizes were small, which could have resulted in unstable risk estimates, this elevation in risk may be real; however, this association needs to be confirmed in larger studies. We observed no significant increase in bladder cancer risk with higher NAE levels among the heaviest smokers (> 30 cigarettes per day). Although this may seem incongruent with the aforementioned results, it should be noted that smoking duration is a stronger predictor of bladder cancer risk than amount smoked per day [23, 24]. We did not have adequate information about past occupational exposures relevant to bladder cancer, such as employment in rubber, dye, or chemical industries, and therefore could not evaluate whether

higher NAE was a risk factor in these subgroups. Although there have been substantial reductions in occupational exposures to aromatic amines since their identification as human bladder carcinogens, exposures prior to the implementation of regulations could have been important.

There are several possible reasons why we might have missed an association between renal NAE and bladder cancer risk. We relied on questionnaire-based information to determine NAE, and measurement errors incurred during nutrient intake estimation likely attenuated any true association. In addition, renal NAE itself is only an approximation of actual urine pH, which further increases the likelihood of random misclassification of individuals. We were unable to perform a calibration study of NAE because we did not have urine specimens available for analysis; therefore, we could not correct our risk estimates for deattenuation due to within-person variability in urine pH. This is a potential source of error, as demonstrated in a previous validation study that reported observed and deattenuated correlation coefficients between NAE and urine pH values of -0.39 and -0.50 , respectively [14]. However, it should be noted that the unadjusted correlation coefficient for NAE and urine pH is similar to those found in other dietary questionnaire validation studies. Narrow intake distributions of foods and nutrients that contribute to NAE could have also impeded our ability to detect modest increases in risk. Finally, inaccuracies in the prediction of urine pH may have occurred in our study because we had no information regarding other factors that are known to directly influence urine pH, including bacteriuria and the use of specific medical drugs [25, 26].

We did not have information on several important modifiers of the association between urine pH and bladder cancer, including water consumption and frequency of urination. For example, DNA-adduct formation was shown to depend largely on frequency of urination in an animal study, with longer intervals between voiding facilitating the hydrolysis of acid-labile *N*-glucuronides and reabsorption of potentially reactive carcinogens across the urothelium [5]. The harmful effect of acidic urine on bladder cancer risk may therefore be limited to those individuals with greater voiding intervals, and future studies need to address this important issue.

The strengths of this study include its prospective design, the relatively large number of bladder cancer cases available for analysis, and use of a validated food use questionnaire to capture usual nutrient intakes. In addition, smokers constitute an important group within

which to explore the effects of renal NAE on bladder cancer risk, given that over 50% of the disease is attributable to smoking [27].

In summary, we did not observe an overall association between low estimated urine pH (*i.e.*, higher NAE values) and bladder cancer in Finnish male smokers, although our results suggest that acidic urine may increase risk in specific subsets of individuals, including longer-term smokers. Further evaluation of this association is warranted in other studies, particularly those that are able to account for factors such as voiding frequency that affect exposure of the bladder epithelium to carcinogens.

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