Research Article

Cancer Epidemiology, Biomarkers & Prevention

Plasma Levels of Nitrate and Risk of Prostate Cancer: A Prospective Study

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Abstract

Background: Nitrate and nitrite supplements have recently been shown to improve cardiovascular health, but there is concern that these supplements could contribute to the development of cancer. Previous small, cross-sectional studies reported positive associations between circulating nitrate/nitrite levels and cancer. Prospective studies examining the association between plasma nitrate and cancer, especially prostate cancer, are lacking.

Methods: We conducted a nested case–control study within the Health Professionals Follow-up Study. Baseline blood samples were collected in 1994, and incident cases of prostate cancer were identified from 1997 to 2005. Baseline plasma levels of nitrate were measured in the 630 cases and 630 matched controls.

Results: We have found that baseline levels of plasma nitrate were not associated with risk of prostate cancer. Compared to quintile 1, the relative risk from quintiles 2 to 5 were 1.13 [95% confidence interval (CI), 0.78–1.63], 0.93 (95% CI, 0.63–1.38), 0.95 (95% CI, 0.65–1.39), and 0.99 (95% CI, 0.68–1.48); P_{trend} was 0.9 after adjustment of multivariate risk factors. When analyses were restricted to men fasting more than 6 hours, the trend was similar. Furthermore, plasma nitrate seemed to be inversely associated with advanced-stage prostate cancer. The relative risk across extreme quartiles was 0.44 (95% CI, 0.17–1.12; $P_{trend} = 0.07$) for the whole dataset and 0.30 (95% CI, 0.09–0.99; $P_{trend} = 0.05$) for the fasting dataset.

Conclusions: In summary, we did not find an increased risk of prostate cancer associated with higher plasma nitrate levels. A potential protective association between nitrate and aggressive forms of prostate cancer requires confirmation.

Impact: Nitrate–nitrite–nitric oxide pathway has emerged as a new therapeutic pathway for chronic diseases. The results of this study certainly merit replications in other prospective studies. *Cancer Epidemiol Biomarkers Prev; 22(7); 1210–8. ©2013 AACR.*

Introduction

In the past, nitrate and nitrite were generally considered to be harmful to humans because nitrate can be converted to nitrite, which can form nitrosamine, a potential carcinogen (1, 2). However, research conducted in the past decade has suggested a more complex view of the role of nitrate and nitrite in health. Nitrate can be converted to nitrite and, subsequently, nitrite converted to nitric oxide; nitric oxide can also be converted back to nitrite, and then to nitrate as shown in Fig. 1 (3–5). The nitrate–nitrite–nitric oxide pathway is generated *in vivo* by bacteria and enzymes. Nitrate can be converted to nitrite by commensal bacteria mainly in the mouth but also in the gastrointestinal tract (6–8). The conversion from nitrite to nitric oxide occurs primarily in tissues (9) via several enzymes such as xanthine oxidoreductase (10), mitochondrial enzymes (11), and deoxygenated hemoglobin (4). Alternatively, nitric oxide is produced from L-arginine by the enzymatic action of nitric oxide synthase (NOS). *In vivo*, the NOS pathway is the major pathway for generating nitric oxide. When the NOS pathway is dysfunctional, nitrate through conversion to nitrite, has been postulated as an alternative source for nitric oxide generation. Vegetables contribute 80% of nitrate intake.

Nitric oxide is recognized as a signaling molecule and a critical regulator of various physiologic functions, including vascular homeostasis, neurotransmission, and host defense (12, 13). Emerging evidence shows that nitrate or nitrite supplements, by serving as precursors for nitric oxide, are beneficial for blood circulation, and may help regulate blood pressure and prevent cardiovascular disease (14–18). In regards to cancer risk, most emphasis regarding nitrate has been as a possible precursor to

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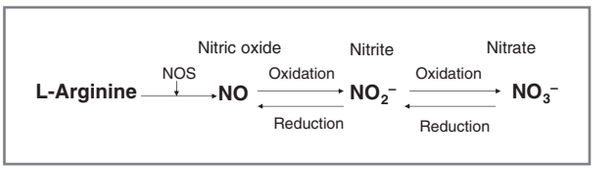

Figure 1. NOS denotes nitric oxide synthase.

nitrosamines. However, the recent evidence of important signaling functions of nitric oxide suggests potentially more complex relationships to cancer risk, although mechanisms remain largely speculative at this time. Several small-scale case–control studies have shown that nitrite or nitrate levels were positively associated with risk of lung cancer (19), gastric cancer (20), and advanced laryngeal cancer (21). In these studies, blood samples were collected at the time of cancer identification; therefore, whether high nitrate levels are a cause or a result of cancer cannot be determined. Nonetheless, these results suggest that nitrate and nitrite deserve further scrutiny in regards to cancer risk in a prospective setting to exclude reverse causation.

We used a prospective study design (blood was collected before prostate cancer is diagnosed) to determine whether the baseline plasma nitrate level is associated with risk of prostate cancer. Because risk factors for advanced-stage prostate cancer may differ from those for total prostate cancer (22), and because advanced-stage prostate cancer is the more important clinical manifestation, we also examined advanced-stage prostate cancer, prostate cancer with higher Gleason score, or non–stage 1 prostate cancer as endpoints.

Materials and Methods

The study population for the prospective, nested case–control study

We conducted a prospective, nested case–control study within the Health Professionals Follow-up Study (HPFS; ref. 23). The HPFS cohort study is an ongoing prospective cohort study of 51,529 men. The study began in 1986, and biennial questionnaires on anthropometric variables, medical conditions, and lifestyle factors were administered every 2 years afterward. A validated semiquantitative food frequency questionnaire (24) was also administered every 4 years since 1986. Within the HPFS, a blood cohort was initiated between 1993 and 1995; this subcohort consisted of 18,018 participants who provided blood specimens. For each report of a diagnosis of prostate cancer, we requested written permission to obtain the medical and pathology reports. Study investigators, who were blinded to the information from the questionnaires, confirmed the diagnosis, and extracted stage at diagnosis and the Gleason grade. prostate cancer with T_1 were not excluded for total analyses and but excluded in subgroup analyses. Between 1997 and 2005, we identified 630 cases of prostate cancer (95% cases were identified after July 1999). The follow-up time to mortality was until May 2010.

Each case was matched with one control by the date of birth (±1 year), the time of the blood draw (midnight to before 9 am, 9 am to before noon, noon to before 4 pm, and 4 pm to before midnight), the season of the blood draw (winter, spring, summer, and fall), and the year of the blood draw. The eligibility criteria for a control was to be alive and free of cancer at the date that the matched case was diagnosed and to have had a prostate-specific antigen (PSA) test after the date of the blood draw and before the matched case was identified. This study was approved by the Human Subject Committee at the Harvard School of Public Health and the Institute of Review Board at the University of Cincinnati (Cincinnati, Ohio).

The assessment of plasma nitrate levels. The original method (Griess assay) to assess plasma nitrate was documented by Wishnok and colleagues (25). This assay gives the total amount of nitrite and nitrate. Measurement of nitrate is through nitrate reduction to nitrite via enzymatic conversion by nitrite reductase as the Griess reaction *per se* only measures nitrite. However, nitrite is not very stable, and can be quickly oxidized to nitrate after blood collection if the samples are not processed immediately. In the HPFS cohort study, the blood samples were processed 24 to 48 hours after the blood was collected; therefore, the nitrite was already converted to nitrate during these periods. In addition, using colorimetric detection, the total nitrite and nitrate levels were observed to be in the μmol/L range, whereas the nitrite level was expected to be in nmol/L range. Therefore, the amount of nitrate and nitrite that we measured reflects the nitrate levels.

The assay variability, the stability of measurement of nitrate in whole blood samples stored on ice for up to 48 hours, and within-person reproducibility of measurement of nitrate. (i) The coefficient of variation for nitrate measurement in Dr. Wu's laboratory was 7.4%, indicating fairly low laboratory error and assay variability. (ii) The HPFS blood samples were stored on ice until they were processed between 24 and 48 hours after the blood was drawn. We designed a pilot study to mimic the HPFS collection procedures. We found that delays in processing up to 48 hours seemed to have minimal influence on the measurement of nitrate. Nitrate levels in samples processed after 24 and 48 hours were not significantly different from levels in samples processed immediately ($P = 0.2$ and 0.5, respectively). The overall intra-class correlation (ICC) across all 3 time points was 0.89 (26). (iii) The within-person reproducibility over 1-year period was 0.49 after an adjustment for the fasting length, and 0.48 without an adjustment for the fasting length (not shown in the table). These were documented in detail in Wang and colleagues (26).

Statistical analysis

The determinants of plasma nitrate levels. Although most of circulating nitrate originates from diet (especially vegetables), the determinants of plasma nitrate levels are not well known. The factors that are strongly associated with both nitrate levels and prostate cancer can potentially confound the association between nitrate levels and

prostate cancer risk. For example, some dietary factors such as lycopene have been shown to be inversely associated with prostate cancer in some large cohort studies (27); therefore, it is important to know whether the nitrate level is associated with lycopene. Fasting levels of nitrate are not associated with an acute nitrate intake but can be indirectly influenced by the usual intake of nitrate. To examine the potential selected factors including dietary factors that may strongly influence the nitrate levels, we conducted multivariate linear regression analyses among the controls in the nested case–control set. We analyzed the association between the dietary and lifestyle factors (collected in 1994) in relation to plasma levels of nitrate (blood was collected in 1994). We use linear regression models with robust variance estimate. The variance estimate allows for valid inference without the normal distribution assumption in the dependent variable (28, 29).

Case–control analyses. Conditional logistic regression was used for case–control analyses. Unconditional logistic regression was used for subgroup analyses (different fasting strata) to keep all of the controls to enhance power. Nitrate was classified into quintiles, with the cutpoints defined by the distribution of the controls. We tested the results in an unadjusted model and several multivariate-adjusted models. In multivariable model 1, we included body mass index (BMI), vigorous physical activity, family history of prostate cancer, history of smoking and hypertension and diabetes, total intake of energy, alcohol intake, and hours since last meal. We added potential dietary risk factors, including intake of nitrate, lycopene, vitamin E, vegetables, red and processed meat, and history of vasectomy, to models 2 and 3. None of the factors added to models 2 and 3 changed the association; therefore, we selected model 1 for further analyses. P_{trend} were obtained from a continuous variable of nitrate, which was created using the median of each quintile.

Nitrate and stage of prostate cancer. To examine the association between the nitrate levels and more aggressive prostate cancer, we examined separately: high-grade (Gleason score $\geq$ 7), non–stage 1 prostate cancer, and advanced-stage prostate cancer that includes T3a–T4, N1, or M1, metastatic prostate cancer at diagnosis or during follow-up and fatal prostate cancer. We compared the above cases with controls using unconditional logistic regression. We classified nitrate into quartiles because of fewer cases in these datasets.

Results

Baseline characteristics

As shown in Table 1, the baseline characteristics were not different between the total prostate cancer cases and the controls except that advanced-stage prostate cancer has higher percentage of elderly men (over 70 years old), lower levels of plasma nitrate, lower levels of lycopene, and vitamin E intakes but higher levels of vegetable intake and red and processed meat than controls. According to the multivariable-adjusted analyses (Table 2), we found that several dietary and lifestyle factors were potentially associated with plasma nitrate levels including history of smoking (positively), intake of red and processed meat (inversely), intake of vegetables (positively), and intake of lycopene (positively). These factors were either statistically ($P \leq 0.05$) or marginally significant ($P \leq 0.1$) associated with plasma nitrate levels. Dietary nitrate was marginally, positively associated with plasma nitrate in whole dataset but not in fasting dataset. The associations of risk factors with plasma nitrate were quantitatively different between the entire set and those limiting to fasting. Of note, although some of these associations were marginally significant to significant, the overall magnitude size on plasma nitrate was small. In addition, the top nitrate-contributing food sources in HPFS collected from 1994 diet questionnaires were shown in Table 3. The sum of the contribution from vegetables equals to 85% of nitrate intake in HPFS cohort (Table 3); whereas, nitrate intake from meat (processed meat, beef, pork, hamburger, and bacon) were less than 5% (not shown).

Case–control analyses

As shown in Table 4, no clear linear trend was noted between plasma nitrate and risk of total prostate cancer (P_{trend} were not significant). Of note, moderate levels of nitrate (Q4) were inversely associated with prostate cancer, suggestive of a U-shape pattern. These associations were only significant when the analyses were limited to the men who had fasted more than 6 to 8 hours. The results from fasting more than 6 hours were not materially different from the results from fasting more than 8 hours; therefore, we restricted our further analyses to those who had fasted more than 6 hours. As shown in Table 4, the unadjusted and multivariate-adjusted analyses (from models 1 to 3) showed similar trends. Furthermore, smoking is a risk factor for prostate cancer and is also positively associated with plasma nitrate, especially in whole dataset. To minimize residual confounding by smoking, we limited our analyses to nonsmokers, the overall results did not materially changed for both whole and fasting dataset. Moreover, we did not find any significant association between nitrate intake and prostate cancer in the all the above models (data not shown).

Nitrate and stage of prostate cancer

We next examined plasma nitrate level in relation to high-grade prostate cancer, non–stage 1 prostate cancer, and advanced-stage prostate cancer in the whole dataset and the fasting over 6 hours dataset. Based on the results from the unconditional logistic regression (Table 5), we found that, for high-grade prostate cancer and non–stage 1 prostate cancer, no overall linear trend was observed although a U-shape pattern was suggested. The positive associations reached significance at quartile 3 in both whole and fasting datasets for non–stage 1 prostate cancer. Importantly, nitrate levels were inversely associated

Table 1. Baseline characteristics of study population by case–control status in HPFS study

	Total prostate cancer ($n = 630$)	Advanced-stage prostate cancer ($n = 48$)	Controls ($n = 630$)
Plasma nitrate (μmol/L)	37.71 (29.39–51.47)[a]	33.43 (27.80–43.91)	39.01 (30.10–49.77)
Age group			
<55	111 (17.62%)	5 (10.42%)	105 (16.67%)
56–60	93 (14.76%)	3 (6.25%)	98 (15.56%)
61–65	156 (24.76%)	8 (16.67%)	155 (24.60%)
66–70	133 (21.11%)	12 (25.00%)	133 (21.11%)
>70	137 (21.75%)	20 (41.67%)	139 (20.06%)
Hypertension			
No	462 (73.33%)	36 (75.00%)	437 (69.37%)
Yes	168 (26.67%)	12 (25.00%)	193 (30.63%)
Family history of prostate cancer			
No	561 (89.05%)	42 (87.50%)	574 (91.11%)
Yes	69 (10.95%)	6 (12.50%)	56 (8.89%)
Vasectomy			
No	538 (85.40%)	40 (83.33%)	522 (82.86%)
Yes	92 (14.60%)	8 (16.67%)	108 (17.14%)
Race			
Caucasian	597 (94.76%)	45 (93.75%)	583 (92.54%)
African-American	2 (0.32%)	0 (0%)	0 (0%)
Other ethnic group	31 (4.92%)	3 (6.25%)	47 (7.46%)
Smoking status			
Nonsmoker	305 (48.41%)	19 (39.58.06%)	266 (42.22%)
Past smoker	277 (43.97%)	25 (52.08%)	302 (47.94%)
Unknown	25 (3.97%)	2 (4.17%)	44 (6.98%)
Current smoker	23 (3.65%)	2 (4.17%)	18 (2.86%)
BMI (kg/cm^2)	25.25 (23.50–27.40)	25.10 (23.10–26.95)	25.40 (23.60–27.40)
Vitamin E intake (mg/d)	19.60 (10.20–179.50)	15.30 (11.50–94.10)	21.00 (10.00–191.30)
Lycopene intake (μg/d)	6587 (4422–9659)	6303 (4258–9977)	6466 (4320–9770)
Red and processed meat intake (serving/d)	0.75 (0.43–1.17)	0.92 (0.56–1.18)	0.76 (0.43–1.22)
Vegetables (serving/d)	3.1 (2.3–4.5)	3.5 (2.3–5.2)	3.1 (2.2–4.3)

[a]Numbers in parenthesis indicate interquartile range for medians and percentages for frequencies.

with advanced-stage prostate cancer ($P_{trend} = 0.07$ in whole data; $P_{trend} = 0.05$ in fasting dataset). Although we had relatively few cases diagnosed early in follow-up (blood samples taken in 1993–1995 and 95% of cases diagnosed after July 1999), to further exclude the possibility of reverse causation, we exclude all the cases and matched controls identified in the first 5 years after blood draw. The overall trends were similar to the main analysis. For the whole dataset (total advanced cases = 40), $P_{trend} = 0.1$ and for the fasting dataset (total advanced cases = 26), $P_{trend} = 0.2$.

We conduct the analyses among nonsmokers and observed similar associations. The inverse association between nitrate and advanced-stage prostate cancer was even stronger in the fasting dataset, the RR between extreme quartiles was 0.27 (95% CI, 0.07–0.81) and the $P_{trend} = 0.03$. Finally, no significant association was observed between nitrate intake and different stages of prostate cancer (data not shown).

Discussion

To our knowledge, these are the first prospective data of plasma nitrate levels and prostate cancer risk, or risk of any cancer. We found no evidence of an increased risk of any prostate cancer subgroup with increasing nitrate levels. For total prostate cancer, there was no evidence of a linear relationship, although a lower risk was observed in quintiles 4. Interestingly, we found a decreased risk of advanced-stage prostate cancer with increasing nitrate level. Overall, our results are reassuring that higher plasma nitrate levels do not increase risk of any form of prostate cancer, and in fact, suggest that higher levels may be associated with lower risk of more aggressive prostate cancer. These findings are appealing,

Table 2. Multivariable-adjusted associations between dietary and lifestyle factors in 1994 and plasma nitrate levels (blood was collected in 1994) among controls in HPFS* prostate cancer case–control study

		(387 controls fasting over 6 hours)	(630 controls)
Dietary and lifestyle factors in 1994		**β estimate (*P* value)**	**β estimate (*P* value)**
Age (years)	>63 vs. <63	2.79 (0.25)	**3.96 (0.007)**
Smoking status	Never	Ref	Ref
	Past	4.52 (0.27)	**2.85 (0.07)**
	Current	11.80 (0.12)	**13.01 (0.01)**
History of hypertension	No	Ref	Ref
	Yes	1.66 (0.48)	2.28 (0.16)
Physical activity (mets/d)	<15	Ref	Ref
	15–25	4.17 (0.14)	**5.24 (0.01)**
	25–40	2.72 (0.30)	2.58 (0.19)
	40–60	**9.11 (0.03)**	**3.45 (0.09)**
	≥60	3.41 (0.19)	**5.37 (0.008)**
BMI (kg/m^2)	BMI < 23	Ref	Ref
	23–25	−3.47 (0.30)	−1.10 (0.62)
	≥25	−1.73 (0.60)	−2.10 (0.32)
Alcohol intake (g/d)	0	Ref	Ref
	0–10	−1.37 (0.66)	−1.32 (0.54)
	10–25	−2.29 (0.46)	−2.68 (0.25)
	>25	−0.87 (0.79)	−1.11 (0.65)
Red and processed meat intake (serving/d)	Tertile 1	Ref	Ref
	Tertile 2	−2.67 (0.33)	**−5.20 (0.007)**
	Tertile 3	**−5.75 (0.04)**	**−5.37 (0.007)**
Lycopene intake (μg/d)	Tertile 1	Ref	Ref
	Tertile 2	1.80 (0.44)	0.14 (0.94)
	Tertile 3	**5.83 (0.04)**	**4.27 (0.03)**
Vitamin E intake (mg/d)	Tertile 1	Ref	Ref
	Tertile 2	2.50 (0.34)	0.27 (0.88)
	Tertile 3	1.83 (0.45)	0.59 (0.74)
Nitrate intake (mg/d)	Tertile 1	Ref	Ref
	Tertile 2	2.16 (0.28)	2.06 (0.19)
	Tertile 3	1.44 (0.49)	**3.18 (0.1)**
Vegetable intake (serving/d)	Tertile 1	Ref	Ref
	Tertile 2	**4.22 (0.09)**	**3.37 (0.04)**
	Tertile 3	**5.89 (0.1)**	**6.96 (0.0003)**

NOTE: Vegetables and nitrate were not adjusted simultaneously. The above risk factors except vegetables were adjusted simultaneously.

but given the relatively small sample size, the results are marginally significant and need to be replicated.

Plasma nitrate comes from 2 sources. Nitrate can form endogenously from nitric oxide produced through the NOS pathway (as shown in Fig. 1) or from dietary intake, mainly vegetables. Approximately 80% of nitrate intake derives from vegetable consumption (6). A plate of green leafy vegetables such as spinach contains more nitrate than is formed endogenously from all NOS pathways in a day (30). Our study shows that vegetable intake seems to have a stronger association with plasma nitrate than total nitrate intake. The possible reasons for this is that other factors besides the absolute nitrate intake affect plasma nitrate levels and the nitrate levels in foods are so variable that databases are not accurate.

Our study has several strengths, including the prospective nature with a time lag between blood collection and event, the relatively large sample size, measure of other covariates, and validated measure of plasma nitrate. The ICC for within-person over 1-year period suggests that a single measure of nitrate provides useful, albeit imperfect measure of long-term status. We matched for PSA screening, so confounding by PSA screening was not likely a confounding factor in our study. A potential limitation of our study is that most were White, and as for any observational study, causality cannot be definitively proven. However, confounding by known and suspected risk

Table 3. Top 85% of nitrate-contributing food sources[a] in 1994 in the Health Professionals Follow-up Study

Food name	% Contributing to nitrate
Lettuce (Iceberg lettuce or head lettuce)	25.11
Romaine lettuce or leaf lettuce	13.4
Kale, chard greens, or mustard greens	7.58
Cooked spinach	6.69
Celery	6.12
Broccoli	5.84
Potatoes	3.81
Raw spinach	3.68
Cabbage	2.49
Tomato sauces	2.48
String beans	1.79
Cauliflower	1.78
Onions	1.75
Tomato	1.65
Raw carrots	1.31

[a]Calculated from semiquantitative food frequency questionnaire collected in 1994 in the Health Professionals Follow-up Study.

factors seemed to be minimal. The relative homogeneity by race and socioeconomic status is advantageous in excluding residual confounding, but studies in other populations in diverse settings would be important to assess the generalizability of the findings.

The mechanisms by which nitrate may prevent advanced-stage prostate cancer have not been extensively investigated experimentally. The conversion of nitrate to nitrite to nitric oxide occurs under hypoxic and/or acidic conditions when the NOS pathway is dysfunctional (15, 31–35). The L-arginine NOS pathway is oxygen dependent, whereas the nitrate–nitrite–nitric oxide pathway (Fig. 1) is gradually activated when oxygen is depleted. Nitrate and nitrite can be considered relatively inert *in vivo* until they are converted back to nitric oxide. Lundberg and colleagues have stated "Plasma levels of nitrate can be considered as a 'Prodrug' of nitrite and NO with a slow release-profile" (36). Zweier and Modin showed that, during ischemic conditions, sufficient acidosis develops, permitting nitric oxide generation from endogenously stored nitrite (37, 38). Thus, nitrate may affect prostate cancer tissues where a low pH/hypoxic condition exist, and the major NOS system does not function properly. Nitrite derived nitric oxide can modulate inflammation (18) and the antitumor effect of nitrite was observed in the cell culture model recently, and the effects paralleled the formation of nitric oxide (39).

There are historical concerns about nitrate and nitrite, as nitrite can form nitrosamine, which is considered to be carcinogenic (1). Nitrate-derived nitrite can be reduced to nitric oxide as shown in Fig. 1; however, nitrate-derived nitrite can also form nitrosamine in presence of amines and amides. If the formation of carcinogenic nitrosamine had been the major metabolic pathway for nitrite, we would have observed a positive association between plasma nitrate and prostate cancer, which was not found in this study. As displayed in Tables 2 and 3, vegetables are positively associated with plasma nitrate levels and the major food sources for nitrate intake in HPFS cohort. Many antioxidants and phenol compounds in vegetables are suppressors of nitrosamine formation (40, 41). A recent updated review indicated that carcinogenic nitrosamine formation (N-nitrosation) requires conditions beyond those usually found in normal metabolism (42). Therefore, whether *in vivo* nitrosamine formation is determined by circulating levels of nitrate, the sources of nitrate, inflammatory status (43), or a combination of factors are needed to be to determined in future studies. All previous studies that have examined plasma or serum nitrate levels in relation to cancer risk have been based on a cross-sectional study design, which cannot determine whether increased nitrate is caused by prostate cancer or can cause prostate cancer (19–21). In cancer patients, systemic levels of oxidation are increased, and thus can also increase the oxidation of nitric oxide, leading more nitrate generation. Therefore, a prospective study in which blood is collected before the cases are identified is essential to avoid reverse causation. Furthermore, all of those studies are in small scale, less than 50 for both cases and controls, and thus confounding factors may not be entirely adjusted for those studies.

Until now, a few prospective studies have examined dietary nitrate intake with risk of cancer (44–50). The results were not consistent. For instance, the positive association was observed between nitrate intake and thyroid cancer for women only in the Iowa Women Health Study (47) but not in National Institutes of Health-American Association of Retired Persons (NIH-AARP) Diet and Health Study (48). The trend is only significant with high nitrate intake from animal source, especially processed meat for thyroid cancer in Shanghai Women's Health Study (49) and for renal cell carcinoma in NIH-AARP study (50). No association was observed between nitrate intake and incidence of ovarian cancer (45) and pancreatic cancer (46) in the NIH-AARP study. These findings need to be confirmed with the measurement of plasma nitrate levels, which is more closely associated with nitrate's physiological levels and function. As indicated in our study, nitrate intake was not associated with fasting levels of plasma nitrate, which better reflects usual dietary nitrate intake than nonfasting plasma nitrate. In addition to substantial measurement error from dietary studies, other factors besides nitrate intake also can influence plasma nitrate levels, as suggested from our study. We found that processed meat were inversely associated with plasma nitrate levels. Therefore, whether the positive association between nitrate from processed meat and thyroid cancer in Shanghai Women's Health Study (49)

Table 4. Plasma levels of nitrate in relation to risk of prostate cancer in the HPFS prospective nested case–control study (1994–2005)

	Conditional logistic regression (for whole dataset, 630 cases and 630 controls) RR across quintiles (95% CI)					
	Q1	Q2	Q3	Q4	Q5	P_{trend}
Unadjusted[a]	ref	1.07 (0.76–1.50)	0.85 (0.60–1.22)	0.88 (0.62–1.25)	1.01 (0.71–1.43)	0.9
Multivariate model 1[b]	ref	1.13 (0.78–1.63)	0.93 (0.63–1.38)	0.95 (0.65–1.39)	0.99 (0.68–1.48)	0.9
Multivariate model 2[c]	ref	1.07 (0.74–1.56)	0.91 (0.61–1.34)	0.98 (0.66–1.45)	0.98 (0.65–1.46)	0.9
Multivariate model 3[d]	ref	1.11 (.0.76–1.61)	0.90 (0.61–1.34)	0.89 (0.61–1.32)	0.97 (0.65–1.44)	0.9
	Unconditional logistic regression (for fasting over 6 hr dataset, 382 cases and 387 controls) RR across quintiles (95% CI)					
	Q1	Q2	Q3	Q4	Q5	P_{trend}
Unadjusted	Ref	0.96 (0.63–1.48)	**0.63 (0.39–1.00)**	**0.50 (0.31–0.81)**	0.94 (0.61–1.47)	0.6
Multivariate model 1	Ref	1.05 (0.66–1.65)	0.62 (0.38–1.03)	**0.53 (0.32–0.88)**	1.06 (0.65–1.73)	0.9
Multivariate model 2	Ref	1.03 (0.65–1.64)	0.62 (0.37–1.02)	**0.54 (0.32–0.91)**	1.06 (0.65–1.75)	0.9
Multivariate model 3	Ref	1.06 (0.67–1.82)	0.62 (0.37–1.02)	**0.51 (0.31–0.86)**	0.96 (0.60–1.53)	0.9

[a]Unadjusted model includes matching factors only (age, time of blood draw, season of blood draw, and year of blood draw).
[b]Model 1: additionally adjusted for family history of prostate cancer, history of smoking (never, past, and current), and hypertension, body mass index (categories), history of diabetes, vigorous physical activity (categories), total calorie intake (quintiles), and hours since last meal.
[c]Model 2: included covariates in model 1 and additionally adjusted for intake of nitrate (quintiles), lycopene (quintiles), vitamin intake E (quintiles), history of vasectomy.
[d]Model 3: included covariates in model 1 and additionally adjusted for intake of vegetables (tertiles), and red and processed meat (tertiles), and history of vasectomy.

was due to lower levels of plasma nitrate or high intakes of nitrate cannot be determined. Based on our results, which need to be confirmed, current databases of dietary nitrate are unlikely to be informative in determining the effect of nitrate levels on cancer risk because they may not be adequately predictive of circulating levels of nitrate.

Smoking is a risk factor for prostate cancer, especially for aggressive prostate cancer (51). In this article, we found a positive association between current smoking and plasma nitrate in whole dataset (statistically significant) and fasting dataset (marginally significant). Thus, smoking can be potentially a strong confounder and

Table 5. Baseline plasma levels of nitrate in relation to stages of prostate cancer in the HPFS prospective nested case–control study (1994–2005) using samples collected in whole dataset or after 6 hours since last meal

	RR across quartiles (95CI%CI)[a]				
	Q1	Q2	Q3	Q4	P_{trend}
Gleason score ≥7 (245 cases, whole dataset)	ref	1.02 (0.67–1.57)	**0.55 (0.34–0.89)**	1.05 (0.68–1.64)	0.9
Gleason score ≥7 (153 cases, fasting over 6 hours)	ref	0.94 (0.53–1.69)	0.54 (0.28–1.03)	0.99 (0.54–1.82)	0.7
Non–stage 1 cases (T stage ≥2a, 415, whole dataset)	ref	1.08 (0.76–1.56)	**0.64 (0.43–0.94)**	0.93 (0.64–1.35)	0.3
Non–stage 1 cases (T stage ≥2a, 255 cases, fasting over 6 hours)	ref	0.88 (0.55–1.42)	**0.47 (0.28–0.81)**	0.75 (0.46–1.24)	0.2
Advanced stage[b] (48 cases, whole dataset)	ref	0.60 (0.27–1.46)	0.47 (0.19–1.22)	0.44 (0.17–1.12)	**0.07**
Advanced stage (36 cases, fasting over 6 hours)	ref	0.42 (0.14–1.27)	0.44 (0.14–1.41)	**0.30 (0.09–0.99)**	**0.05**

[a]Adjusted for age, time of blood draw, season of blood draw and year of blood draw, family history of prostate cancer, history of smoking (never, past, and current), and hypertension, body mass index (categories), history of diabetes, vigorous physical activity (categories), total calorie intake (quintiles), and hours since last meal.
[b]Advanced stage including lethal and fatal prostate cancer, metastatic prostate cancer, and clinical stage equal to or higher than T3a.

attenuate the reverse association between nitrate and prostate cancer toward the null. In our analyses, we not only adjusted for smoking status and but also examined the results among nonsmokers. The overall trend did not materially change. The percentage of current smokers in HPFS cohort is only 3% to 4% and thus they may not confound the results dramatically. However, in a cohort with a higher percentage of smokers, stratified analyses among smokers and nonsmokers may be recommended.

In summary, we did not find an increased risk of prostate cancer associated with higher plasma nitrate levels, and in fact, found a potential protective association for aggressive forms of prostate cancer. These findings, if confirmed, would support further studies examining the potential role of nitrates, from dietary sources, as a potential strategy to reduce cardiovascular disease, vasodilation, and potentially cancer. Mechanistic studies are also warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: T. Wu, Y. Wang, S.-M. Ho, E. Giovannucci
Development of methodology: T. Wu, Y. Wang, E. Giovannucci
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Wu, E. Giovannucci
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Wu, Y. Wang, E. Giovannucci
Writing, review, and/or revision of the manuscript: T. Wu, S.-M. Ho, E. Giovannucci
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Wu
Study supervision: T. Wu, E. Giovannucci

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