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Choline intake and risk of lethal prostate cancer: incidence and survival[1–3]

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ABSTRACT

Background: Meat, milk, and eggs have been inconsistently associated with the risk of advanced prostate cancer. These foods are sources of choline—a nutrient that may affect prostate cancer progression through cell membrane function and one-carbon metabolism. No study has examined dietary choline and the risk of lethal prostate cancer.

Objective: Our objective was to examine whether dietary choline, choline-containing compounds, and betaine (a choline metabolite) increase the risk of lethal prostate cancer.

Design: We prospectively examined the intake of these nutrients and the risk of lethal prostate cancer among 47,896 men in the Health Professionals Follow-Up Study. In a case-only survival analysis, we examined the postdiagnostic intake of these nutrients and the risk of lethal prostate cancer among 4282 men with an initial diagnosis of nonmetastatic disease during follow-up. Diet was assessed with a validated questionnaire 6 times during 22 y of follow-up.

Results: In the incidence analysis, we observed 695 lethal prostate cancers during 879,627 person-years. Men in the highest quintile of choline intake had a 70% increased risk of lethal prostate cancer (HR: 1.70; 95% CI: 1.18, 2.45; *P*-trend = 0.005). In the case-only survival analysis, we observed 271 lethal cases during 33,679 person-years. Postdiagnostic choline intake was not statistically significantly associated with the risk of lethal prostate cancer (HR for quintile 5 compared with quintile 1: 1.69; 95% CI: 0.93, 3.09; *P*-trend = 0.20).

Conclusion: Of the 47,896 men in our study population, choline intake was associated with an increased risk of lethal prostate cancer. *Am J Clin Nutr* 2012;96:855–63.

INTRODUCTION

Red meat and dairy products have been inconsistently associated with an increased risk of advanced prostate cancer (1–3). In addition, we recently reported that whole egg intake was positively associated with the risk of lethal prostate cancer (4), and the postdiagnostic intakes of whole eggs and poultry with skin were associated with increased risks of prostate cancer progression (5). Meat, milk, whole eggs, and poultry are all dietary sources of choline—an essential nutrient with many roles, including cell membrane structure and function, one-carbon metabolism, and neurotransmitter synthesis. Choline is highly concentrated in prostate cancer cells, and blood concentrations of choline have been associated with an increased risk of prostate cancer (6, 7). Thus, it is possible that the previously reported relations between meat, milk, and eggs and advanced prostate cancer were attributable in part to the choline content of these foods. However, no study has examined dietary choline in relation to the risk of lethal prostate cancer or prostate cancer survival.

Therefore, we examined dietary choline in relation to the risk of lethal prostate cancer among men in the Health Professionals Follow-Up Study. Secondarily, we examined the intake of the 5 choline-containing compounds and betaine, a choline metabolite, in relation to the risk of lethal prostate cancer. We also examined the postdiagnostic intake of these nutrients in relation to the risk of lethal prostate cancer among men with an initial diagnosis of nonmetastatic disease. We focused on the risk of lethal prostate cancer in all analyses because prostate cancer has both indolent and lethal forms, and the outcome of lethal disease has clear clinical and public health relevance. On the basis of our recent findings for eggs and the observation that higher concentrations of plasma choline were associated with an increased risk of prostate cancer (4, 7), we hypothesized that higher dietary choline would be associated with an increased risk of lethal prostate cancer.

SUBJECTS AND METHODS

Study population

The Health Professionals Follow-Up Study is a prospective cohort study initiated in 1986 in the United States among 51,529 male health professionals aged 40–75 y. Participants completed a questionnaire at baseline on medical diagnoses, medication use, physical activity, body weight, family history, and smoking and a semiquantitative food-frequency questionnaire (FFQ). Medical diagnoses, medications, physical activity, body weight, and smoking data were updated every 2 y, and dietary data were updated every 4 y. The average response rate for the question-

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naires exceeded 90% (8). The Institutional Review Board of the Harvard School of Public Health approved this study.

Dietary assessment

The FFQ asked participants to report their usual intake of ~140 foods and beverages over the past year. A common serving size was specified for each item (eg, one egg including yolk), and frequency options ranged from never or less than once per month to ≥6/d. The FFQ also asked about multivitamin and supplement use. Nutrient composition values for each food were obtained from the USDA (9, 10). We multiplied a weight based on the nutrient composition of the specified portion size of each food by the frequency of its use and summed across foods to calculate total nutrient intakes. Total dietary choline was calculated by summing free choline and choline from glycerophosphocholine, phosphocholine, phosphatidylcholine, and sphingomyelin.

Nutrient-composition data for choline were not available when the FFQ was validated in 1986 (11). However, dietary choline measured by the FFQ was inversely associated with plasma homocysteine (12). Homocysteine is converted to methionine when it acquires a methyl group from a methyl donor, such as choline (via betaine) or folate; therefore, one would expect dietary choline to be inversely associated with homocysteine concentrations, particularly among persons with low dietary folate. Indeed, participants in the fifth quintile of dietary choline had a geometric mean total plasma homocysteine of 9.8 (95% CI: 9.5, 10.2) compared with 10.6 (95% CI: 10.2, 11.0) in the lowest quintile (P-trend < 0.0001), independent of folate and vitamins B-6 and B-12. Furthermore, this association was strongest among men, persons with low dietary folate, and persons with high alcohol intake. An association between choline assessed via the FFQ and plasma homocysteine concentration, independent of other predictors, suggests that the FFQ can accurately rank individuals according to their choline intake.

Outcome assessment and follow-up

Participants were asked every 2 y whether they received a diagnosis of prostate cancer. After report of a prostate cancer diagnosis, we requested the participants' permission to obtain medical records and pathology reports to confirm the diagnosis. Study investigators abstracted information on the date of diagnosis, clinical T-stage, Gleason score, prostate specific antigen, metastases, and treatments from the medical records. Prostate cancer–specific follow-up questionnaires were mailed biannually to collect additional information on treatment and disease progression. Mortality data were obtained via mail, telephone, and review of the National Death Index; we ascertain >98% of deaths using these methods (13). An Endpoints Committee of 4 study physicians determined cause of death from death certificates and medical records. If prostate cancer metastasis was present and no more plausible cause of death was mentioned, the death was attributed to prostate cancer. Our primary outcome was lethal prostate cancer, defined as distant organ metastases from prostate cancer or prostate cancer death.

Inclusion and exclusion criteria

We limited the analysis to participants who adequately completed the baseline FFQ (eg, 800–4200 kcal/d and missing <70 food items) and who were free of cancer diagnosis (except nonmelanoma skin cancer) in 1986, which left 47,896 men eligible for the incidence analysis. For the case-only survival analysis, in addition to the above criteria, participants must have had a diagnosis of nonmetastatic prostate cancer during follow-up and could not have missing data on their clinical stage at diagnosis or primary treatment, which left 4282 men with nonmetastatic prostate cancer eligible for follow-up for lethal outcomes.

Statistical analysis

All statistical analyses were performed by using SAS version 9.2 (SAS Institute), and results with a 2-sided P value <0.05 were considered statistically significant.

Incidence of lethal prostate cancer

We used Cox proportional hazards regression to examine the associations between intake of choline, choline-containing compounds, and betaine and risk of lethal prostate cancer. Person-time was calculated from the time of return of the baseline questionnaire until diagnosis of prostate cancer, death from other cause, or end of follow-up (31 January 2008), whichever occurred first. We used calendar time in 2-y intervals as the time scale and stratified by age in months.

Cumulative average intakes of choline, choline-containing compounds, and betaine were calculated from all FFQs before diagnosis of prostate cancer to reduce measurement error in the long-term diet (eg, the 1986 FFQ was used for person-time accrued between 1986 and 1990; the average of the 1986 and 1990 FFQs was used for person-time accrued between 1990 and 1994, etc) (14). We categorized the nutrient intakes into quintiles and modeled them by using indicator variables. We modeled the median intake of each quintile as a continuous term to test for linear trend.

All nutrients were adjusted for energy by using the nutrient-residual method (14), and we addressed potential confounding by adjusting for factors that have been previously associated with lethal prostate cancer (2, 3, 15–19). Model 1 included age (mo; continuous), time period (2-y intervals), and energy (kcal/d; quintiles). Model 2 included the covariates in model 1 plus BMI (in kg/m^2; <25, 25–29.9, ≥30, or missing), smoking (never, former, current, or missing), and vigorous physical activity (metabolic equivalent task-hours/wk; quartiles). Model 3 included the covariates in model 2 plus quintiles of intakes of calcium, cholesterol, zinc, coffee, saturated fat, lycopene, phosphorus, and protein. These foods and nutrients were selected because they are risk factors for lethal prostate cancer or are present in foods that contain choline and were retained in the model because they changed the point estimate of one or more of the exposures of interest by ≥10%. We also considered adjustment for race, history of diabetes, prostate specific antigen screening, and intakes of folate, polyunsaturated fat, monounsaturated fat, and vitamins D and E; however, none of these changed the effect estimates by ≥10%; therefore, they were omitted from the multivariate models. In addition, to examine whether the observed associations for choline were a marker of one or more choline-containing foods, we examined multivariate models with the following foods added one at a time: whole eggs, skim milk, beef or lamb as a main dish, chicken or turkey without skin, hamburger, other fish, chicken or turkey with skin, beef or lamb as a sandwich or mixed dish, beer, potatoes, and dark-meat

fish. We also examined models with total red meat, total milk, total poultry, and total fish. These foods were chosen because they were among the top 10 contributors to choline intake on at least one of the FFQs administered between 1986 and 2006.

Additionally, we examined whether age (continuous), calendar time (2-y intervals, continuous), smoking (current or not current), or BMI (<25 or ≥25) modified the relation between choline intake and the risk of lethal prostate cancer by including a cross-product term between the potential effect modifier and choline intake (modeling the median of each quintile as an ordinal score) in our multivariate model and using a likelihood ratio test to test for evidence of effect modification.

Last, we examined time lags ranging from 4–8 y to 16–20 y (eg, for a 16–20-y lag, we applied the 1986 FFQ to person-time accrued between 2002 and 2006 and the average of the 1986 and 1990 FFQ to person-time accrued between 2006 and the end of follow-up). We also repeated the analyses censoring men at the date of lethal prostate cancer (eg, date of diagnosis of distant organ metastases or death from prostate cancer), death from another cause, or end of follow-up. In this secondary analysis, we examined cumulative updated choline intake from baseline until the date of lethal event or censoring and applied a 2–6-y time lag because men with undiagnosed metastatic disease may change their diet as a result of their illness (eg, the 1986 FFQ was applied to person-time accrued between 1988 and 1992, etc).

Case-only survival analysis

We used Cox proportional hazards regression to examine the relations between postdiagnostic choline, choline-containing compounds, and betaine intake and risk of lethal prostate cancer. Person-time was calculated from the date of prostate cancer diagnosis to prostate cancer death or diagnosis of distant organ metastases, death from another cause, or end of follow-up (31 January 2008), whichever occurred first. We calculated cumulative average intake of choline, choline-containing compounds, and betaine from the FFQ preceding diagnosis up to the end of follow-up. The FFQ preceding diagnosis was used to classify the participants' exposure from the date of diagnosis until the next available FFQ because men with a diagnosis of prostate cancer did not change their diet more or less on average compared with men without a diagnosis of prostate cancer during the same time period.

We considered all of the confounders mentioned above (*see* Incidence of lethal prostate cancer) and age at diagnosis, Gleason score, prostate specific antigen at diagnosis, clinical T-stage, and primary treatment. Model 1 included age at diagnosis (y; continuous), energy (kcal/d; quintiles), time period (2-y intervals), and time since diagnosis (y; continuous). Model 2 included the covariates in model 1 plus primary treatment (radical prostatectomy, radiation therapy, hormonal therapy, or other/active surveillance), Gleason score (≤6, 7, or ≥8), clinical T-stage (T1, T2, or T3), BMI (<25, 25–29.9, ≥30, or missing), smoking (never, former, or current), and vigorous physical activity (metabolic equivalent task-hours/wk; quartiles). Model 3 included the covariates in model 2 plus quintile ranks of intakes of calcium, cholesterol, coffee, saturated fat, phosphorus, and polyunsaturated fat. Additionally, we examined models that adjusted for prediagnostic intake of the nutrient of interest based on the 1986 FFQ.

In addition, we examined whether the association between postdiagnostic choline intake and lethal prostate cancer was

TABLE 1
Baseline age-standardized characteristics of 47,896 male health professionals, by choline intake

	Quintile of choline					
	1	2	3	4	5	*P* value[1]
Median choline intake (mg/d)	305	351	385	425	509	—
Age (y)	52.2 ± 9.9[2]	53.4 ± 9.8	53.8 ± 9.7	54.7 ± 9.6	55.7 ± 9.4	<0.001
BMI (kg/m^2)	25.2 ± 3.2	25.4 ± 3.2	25.5 ± 3.3	25.7 ± 3.5	25.8 ± 3.5	<0.001
Vigorous physical activity (MET-h[3]/wk)	12.6 ± 24.8	12.6 ± 22.9	12.6 ± 25.0	12.5 ± 26.4	13.4 ± 30.7	0.08
White (%)	90	91	91	91	91	0.008
Current smokers (%)	8	9	9	10	12	<0.001
Family history of prostate cancer (%)	13	12	13	13	13	0.89
Pearson correlation between intake of choline and other nutrients at baseline[4]						
Cholesterol	0.62	—	—	—	—	—
Saturated fat	0.04	—	—	—	—	—
Polyunsaturated fat	−0.04	—	—	—	—	—
Protein	0.53	—	—	—	—	—
Calcium	0.30	—	—	—	—	—
Phosphorus	0.43	—	—	—	—	—
Zinc	0.29	—	—	—	—	—
Lycopene	0.01	—	—	—	—	—

[1] Calculated from a logistic model that examined categorical choline as the dependent variable and the characteristic of interest as the independent variable, adjusted for age.
[2] Mean ± SD (all such values).
[3] MET-h, metabolic equivalent task-hours.
[4] All nutrients were adjusted for energy by using the nutrient-residual method, and all *P* values were <0.001 except for the correlation coefficient between lycopene and choline ($P = 0.20$).

modified by age at diagnosis (<69 or ≥69 y), Gleason grade (<7 or ≥7), BMI (<25 or ≥25), or smoking (current or not current) by creating a cross-product term between choline intake (ordinal score) and the potential effect modifier and used likelihood ratio tests to test for evidence of effect modification.

RESULTS

Incidence of lethal prostate cancer

At baseline, men in the fifth quintile of choline were older (56 compared with 52 y), had a higher BMI (25.8 compared with 25.2), and were more likely to be current smokers (12% compared with 8%) compared with men in the lowest quintile of choline intake (**Table 1**). After adjustment for energy, choline intake was correlated with intake of cholesterol (r = 0.62), protein (r = 0.53), phosphorus (r = 0.43), calcium (r = 0.30), and zinc (r = 0.29).

The top 5 foods contributing to choline based on data from the 2006 FFQ were whole eggs, beef as a main dish, skim milk, reduced-fat milk, and poultry without skin; these foods accounted for 27% of choline intake (**Table 2**). Nearly 50% of choline was consumed in the form of phosphatidylcholine, followed by free choline (24%), glycerophosphocholine (17%), sphingomyelin (5%), and phosphocholine (4%). The fat-soluble choline-containing compounds (eg, phosphatidylcholine and sphingomyelin) came primarily from whole eggs, beef, poultry, and pork. In contrast, a wide variety of foods contributed to intake of the water-soluble choline-containing compounds (eg, free choline, glycerophoshocholine, and phosphocholine), including coffee, potatoes, skim milk, beer, reduced-fat milk, bananas, fish, broccoli, poultry, and soymilk. Cold cereal, pasta, cooked spinach, dark bread, and pizza accounted for 49% of betaine intake.

We observed 695 events of lethal prostate cancer during 879,627 person-years. In multivariate analyses, men in the highest quintile of choline intake had a 70% increased risk of lethal prostate cancer compared with men in the lowest quintile (HR: 1.70; 95% CI: 1.18, 2.45; P-trend = 0.005) (**Table 3**). This relation was not appreciably changed after adjustment for important food contributors to choline intake (eg, whole eggs, skim milk, beef or lamb as a main dish, and chicken or turkey without skin). In addition, intakes of free choline and glycerophosphocholine were positively associated with risk of lethal prostate cancer, but the relations between phosphatidylcholine, sphingomyelin, and phosphocholine intake and lethal prostate cancer were not statistically significant. Betaine intake was not associated with risk of lethal prostate cancer.

We observed no evidence of effect modification between age, calendar time, smoking, or BMI and choline intake in relation to the risk of lethal prostate cancer (data not shown). In addition, when we examined various time lags, the results became stronger. For example, when applying a 16–20-y lag (69 events), men in the fifth quintile of choline intake had a risk of lethal prostate cancer >3-fold that of men in the lowest quintile (HR: 3.28; 95% CI: 1.09, 9.84; P-trend = 0.05). Our results were also stronger when we followed men to the date of lethal event instead of initial date of diagnosis (HR for quintile 5 compared with quintile 1: 2.80; 95% CI: 1.55, 5.04; P-trend < 0.001).

TABLE 2
Food sources of choline, choline-containing compounds, and betaine in the Health Professionals Follow-Up Study, based on the food-frequency questionnaire administered in 2006

Food source	Proportion
Choline	
Whole eggs	10.4
Beef or lamb as a main dish	5.9
Skim milk	4.2
Reduced-fat milk	3.6
Poultry without skin	3.3
Phosphatidylcholine (49% of choline)	
Whole eggs	19.8
Beef or lamb as a main dish	9.6
Poultry without skin	4.5
Beef, pork, or lamb as a sandwich or mixed dish	4.4
Pork, main dish	4.1
Sphingomyelin (5% of choline)	
Beef or lamb as a main dish	12.4
Poultry without skin	12.1
Whole eggs	8.7
Poultry, sandwich, or frozen dinner	7.5
Beef, pork, or lamb as a sandwich or mixed dish	5.1
Free choline (24% of choline)	
Coffee	5.7
Potatoes	3.9
Skim milk	3.2
Beer	3.2
Reduced-fat milk	3.1
Glycerophosphocholine (17% of choline)	
Skim milk	15.6
Reduced-fat milk	12.5
Bananas	4.3
Dark fish	4.2
Other fish	3.8
Phosphocholine (4% of choline)	
Skim milk	10.6
Broccoli	9.7
Reduced-fat milk	8.5
Poultry without skin	3.6
Soymilk	2.9
Betaine	
Cold cereal	19.6
Pasta	12.3
Cooked spinach	6.4
Dark bread	5.3
Pizza	5.2

Case-only survival analysis

Sociodemographic characteristics varied similarly across quintiles of postdiagnostic choline intake in comparison with men in the entire cohort (**Table 4**). Additionally, the distribution of clinical factors was similar across quintiles of postdiagnostic choline intake; however; men who consumed the most choline after diagnosis were somewhat less likely to have a Gleason sum of 7 compared with men who consumed the least amount of choline.

We observed 271 lethal events during 33,679 person-years. Postdiagnostic choline intake was not statistically significantly associated with risk of lethal prostate cancer (HR: 1.69; 95% CI: 0.93, 3.09; P-trend = 0.20) (**Table 5**), and this relation was unchanged when we included red meat, milk, poultry, whole eggs, or fish in the multivariate model. However, the association became stronger after adjustment for prediagnostic intake of

TABLE 3
Relative hazard of lethal prostate cancer among 47,896 male health professionals by intake of choline, choline-containing compounds, and betaine

	Quintile of intake					
	1	2	3	4	5	*P*-trend[1]
Choline						
Events	105	123	140	155	172	
Model 1, HR (95% CI)[2]	1.0	1.12 (0.86, 1.46)	1.16 (0.90, 1.50)	1.24 (0.96, 1.59)	1.36 (1.06, 1.74)	0.009
Model 2, HR (95% CI)[3]	1.0	1.12 (0.86, 1.46)	1.17 (0.90, 1.51)	1.25 (0.97, 1.60)	1.38 (1.08, 1.77)	0.006
Model 3, HR (95% CI)[4]	1.0	1.24 (0.93, 1.65)	1.38 (1.01, 1.87)	1.55 (1.11, 2.16)	1.70 (1.18, 2.45)	0.005
Fat-soluble choline-containing compounds						
Phosphatidylcholine						
Events	118	135	134	140	168	
Model 1, HR (95% CI)[2]	1.0	1.17 (0.91, 1.50)	1.12 (0.87, 1.44)	1.15 (0.89, 1.47)	1.33 (1.05, 1.69)	0.03
Model 2, HR (95% CI)[3]	1.0	1.17 (0.91, 1.50)	1.12 (0.87, 1.44)	1.14 (0.89, 1.47)	1.34 (1.05, 1.70)	0.03
Model 3, HR (95% CI)[4]	1.0	1.28 (0.97, 1.70)	1.32 (0.95, 1.82)	1.37 (0.96, 1.96)	1.46 (1.00, 2.13)	0.10
Sphingomyelin						
Events	133	148	158	119	137	
Model 1, HR (95% CI)[2]	1.0	1.16 (0.91, 1.47)	1.25 (0.99, 1.58)	0.95 (0.74, 1.22)	1.19 (0.93, 1.52)	0.53
Model 2, HR (95% CI)[3]	1.0	1.15 (0.90, 1.46)	1.24 (0.98, 1.57)	0.95 (0.73, 1.22)	1.20 (0.93, 1.53)	0.53
Model 3, HR (95% CI)[4]	1.0	1.23 (0.93, 1.62)	1.35 (0.98, 1.87)	1.03 (0.71, 1.51)	1.28 (0.82, 2.00)	0.57
Water-soluble choline-containing compounds						
Free choline						
Events	120	122	161	136	156	
Model 1, HR (95% CI)[2]	1.0	0.81 (0.63, 1.05)	1.06 (0.83, 1.35)	0.87 (0.68, 1.12)	1.08 (0.85, 1.37)	0.32
Model 2, HR (95% CI)[3]	1.0	0.82 (0.64, 1.06)	1.08 (0.85, 1.38)	0.89 (0.69, 1.15)	1.13 (0.88, 1.44)	0.17
Model 3, HR (95% CI)[4]	1.0	0.91 (0.70, 1.19)	1.29 (0.99, 1.69)	1.14 (0.85, 1.53)	1.49 (1.10, 2.01)	0.003
Glycerophosphocholine						
Events	96	124	164	141	170	
Model 1, HR (95% CI)[2]	1.0	1.14 (0.87, 1.49)	1.42 (1.10, 1.84)	1.18 (0.91, 1.54)	1.37 (1.06, 1.77)	0.03
Model 2, HR (95% CI)[3]	1.0	1.13 (0.86, 1.49)	1.43 (1.10, 1.84)	1.18 (0.91, 1.54)	1.38 (1.07, 1.78)	0.03
Model 3, HR (95% CI)[4]	1.0	1.20 (0.90, 1.58)	1.55 (1.16, 2.06)	1.32 (0.96, 1.80)	1.59 (1.12, 2.25)	0.02
Phosphocholine						
Events	95	128	168	146	158	
Model 1, HR (95% CI)[2]	1.0	1.22 (0.93, 1.60)	1.44 (1.11, 1.86)	1.22 (0.94, 1.59)	1.20 (0.93, 1.55)	0.39
Model 2, HR (95% CI)[3]	1.0	1.23 (0.94, 1.61)	1.45 (1.12, 1.88)	1.25 (0.96, 1.62)	1.23 (0.95, 1.60)	0.28
Model 3, HR (95% CI)[4]	1.0	1.31 (0.98, 1.75)	1.60 (1.18, 2.16)	1.38 (0.99, 1.93)	1.34 (0.92, 1.96)	0.26
Betaine						
Events	131	134	135	157	138	
Model 1, HR (95% CI)[2]	1.0	1.02 (0.80, 1.31)	1.02 (0.80, 1.31)	1.16 (0.91, 1.46)	0.99 (0.78, 1.27)	0.85
Model 2, HR (95% CI)[3]	1.0	1.02 (0.80, 1.31)	1.03 (0.80, 1.31)	1.17 (0.92, 1.48)	1.01 (0.79, 1.29)	0.73
Model 3, HR (95% CI)[4]	1.0	1.07 (0.83, 1.37)	1.08 (0.84, 1.39)	1.22 (0.95, 1.57)	1.02 (0.78, 1.33)	0.78

[1] Calculated by modeling the median of each quintile as a continuous term.

[2] Cox proportional hazards regression model adjusted for age (mo, continuous), time period (2-y intervals), and energy (kcal/d, quintiles).

[3] Cox proportional hazards regression model adjusted for the variables in model 1 plus BMI (in kg/m^2; <25, 25–29.9, ≥30, or missing), smoking (never, former, current, or missing), and vigorous activity (metabolic equivalent task-hours/wk; quartiles).

[4] Cox proportional hazards regression model adjusted for the variables in model 2 plus quintile intakes of calcium, cholesterol, zinc, coffee, saturated fat, lycopene, phosphorus, and protein.

choline. Men in the fifth quintile of postdiagnostic choline had a risk of lethal prostate cancer nearly 2-fold that of men in the lowest quintile (HR: 1.98; 95% CI: 1.06, 3.70; *P*-trend = 0.08). Postdiagnostic intakes of the choline-containing compounds were not associated with risk of progression to lethal prostate cancer. Smoking, BMI, Gleason score, and age at diagnosis did not modify the association between postdiagnostic choline intake and risk of lethal prostate cancer (data not shown).

DISCUSSION

In this novel prospective analysis, we observed a positive association between intake of choline and risk of lethal prostate cancer. We attempted to account for the association between choline intake and risk of lethal prostate cancer by adjusting for nutrients found in animal products that may also affect risk of prostate cancer, such as cholesterol, fatty acids, protein, zinc, phosphorus, and vitamin D. Furthermore, we examined the association between choline intake and the risk of lethal prostate cancer adjusting for the top food contributors to choline (eg, whole eggs, beef or lamb as a main dish, skim milk, and chicken or turkey without skin), because several of these foods have been positively associated with the risk of prostate cancer in our study population (4, 20). The positive relation between choline intake and risk of lethal prostate cancer remained in all of these models. Yet, we cannot exclude the possibility that unmeasured factors in

TABLE 4
Age-standardized characteristics of 4282 male health professionals with a diagnosis of nonmetastatic prostate cancer, by choline intake[1]

	Quintile of choline					
Characteristic	1	2	3	4	5	*P* value[2]
Median choline intake at diagnosis (mg/d)	297	341	371	405	471	
Age at diagnosis (y)	69.0 ± 7.3[3]	68.9 ± 7.2	69.1 ± 7.2	69.1 ± 6.8	69.7 ± 7.0	0.03
BMI at diagnosis (kg/m^2)	25.4 ± 3.0	25.5 ± 3.3	25.9 ± 3.6	26.1 ± 3.4	26.2 ± 3.4	<0.001
Vigorous physical activity at diagnosis (MET-h/wk)	12.3 ± 22.4	13.5 ± 25.1	12.0 ± 21.9	13.2 ± 24.0	12.0 ± 20.4	0.78
White (%)	91	94	94	92	94	0.08
Current smoker at diagnosis (%)	4	2	5	6	5	0.005
Family history of prostate cancer (%)	20	20	23	23	21	0.27
Clinical T-stage (%)						0.75
T1	59	58	57	59	59	
T2	38	38	39	36	38	
T3a	3	4	4	5	3	
Gleason sum (%)						0.05
2–6	52	50	52	52	53	
7	34	36	32	30	30	
8–10	9	9	9	11	11	
Missing	6	5	6	6	6	
PSA (%)						0.22
<4 ng/mL	12	12	12	10	9	
4–9.9 ng/mL	56	55	55	57	57	
10–19.9 ng/mL	17	16	17	18	19	
≥20 ng/mL	8	8	10	8	8	
Missing	6	8	6	7	6	
Treatment (%)						0.30
Radical prostatectomy	49	50	48	49	44	
Radiation therapy	37	37	38	36	41	
Hormonal therapy	4	4	5	6	4	
Other/active surveillance	10	9	9	10	10	

[1] MET-h, metabolic equivalent task-hours; PSA, prostate specific antigen.
[2] Calculated from a logistic model that examined categorical choline as the dependent variable and the characteristic of interest as the independent variable, adjusted for age at diagnosis.
[3] Mean ± SD (all such values).

animal foods or persons who consume animal foods accounted for the association we observed between choline intake and risk of lethal prostate cancer.

No previous studies have examined dietary choline in relation to the risk of prostate cancer. A nested case-control study among Swedish men reported that a doubling in plasma choline was associated with a 46% increased risk of prostate cancer (OR: 1.46; 95% CI: 1.04, 2.05; *P*-trend = 0.03) (7). Nearly 77% of the cases were detected because of clinical symptoms, which supports the hypothesis that a high choline intake may increase the risk of advanced or aggressive prostate cancer or prostate cancer progression. Plasma choline is a relatively sensitive marker of supplemental choline intake, but less is known regarding its use as a biomarker of long-term dietary intake (21).

Biologic mechanisms linking higher choline intake to an increased risk of lethal prostate cancer are unknown. Additionally, it is not known whether dietary choline is correlated with, or affects, choline concentrations in the prostate. However, choline metabolism is clearly altered in prostate cancer, with greater concentrations of choline-containing compounds in malignant than in normal cells (6). Because of the selective and high uptake of circulating choline by prostate cancer cells, radiolabeled choline is used to identify prostate cancer recurrence and metastases (22, 23), and patients with high-grade prostate cancers have higher concentrations of choline-containing compounds than do those with low-grade prostate cancers (24).

One potential mechanism mediating a relation between choline and lethal prostate cancer may involve choline's role in cell membrane structure and function. Choline is converted to phosphatidylcholine—a phospholipid necessary for cell membranes—and choline kinase, the enzyme that catalyzes the first and rate-limiting step in this conversion, is overexpressed in many human cancers, including prostate cancer (25). In addition, the release of choline metabolites and the turnover of phosphatidylcholine differ between prostate cancer cells and normal cells (26), and treatment of prostate cancer cell lines with chemotherapeutic agents decreases the concentration of choline and choline-containing compounds (27, 28). Furthermore, the alternative pathway for synthesis of phosphatidylcholine catalyzed by phosphatidylethanolamine-*N*-methyltransferase may also affect carcinogenesis (29, 30).

Another potential mechanism may involve choline's role as a source of methyl groups for DNA methylation and synthesis. Choline is irreversibly converted to betaine in the liver, which donates a methyl group to homocysteine to form methionine (31). Methionine is a precursor to *S*-adenosylmethionine—an important methyl donor for DNA methylation and synthesis. A secondary analysis of a randomized trial reported a positive

TABLE 5
Relative hazard of lethal prostate cancer among 4282 men with an initial diagnosis of nonmetastatic prostate cancer by postdiagnostic intake of choline, choline-containing compounds, and betaine

	Quintile of intake					
	1	2	3	4	5	*P*-trend[1]
Choline						
Events (*n*)	36	63	52	59	61	
Model 1, HR (95% CI)[2]	1.0	1.58 (1.04, 2.38)	1.30 (0.85, 1.99)	1.51 (1.00, 2.29)	1.68 (1.11, 2.54)	0.04
Model 2, HR (95% CI)[3]	1.0	1.61 (1.06, 2.44)	1.26 (0.82, 1.94)	1.33 (0.87, 2.03)	1.55 (1.02, 2.36)	0.16
Model 3, HR (95% CI)[4]	1.0	1.64 (1.06, 2.54)	1.30 (0.80, 2.13)	1.40 (0.82, 2.41)	1.69 (0.93, 3.09)	0.20
Fat-soluble choline-containing compounds						
Phosphatidylcholine						
Events (*n*)	43	52	61	50	65	
Model 1, HR (95% CI)[2]	1.0	1.15 (0.76, 1.72)	1.27 (0.86, 1.88)	1.10 (0.73, 1.65)	1.59 (1.07, 2.34)	0.03
Model 2, HR (95% CI)[3]	1.0	1.11 (0.74, 1.68)	1.27 (0.85, 1.89)	1.01 (0.67, 1.53)	1.39 (0.94, 2.07)	0.16
Model 3, HR (95% CI)[4]	1.0	1.12 (0.74, 1.71)	1.31 (0.84, 2.06)	1.07 (0.62, 1.83)	1.54 (0.84, 2.81)	0.16
Sphingomyelin						
Events (*n*)	43	49	50	76	53	
Model 1, HR (95% CI)[2]	1.0	1.00 (0.66, 1.51)	0.99 (0.66, 1.49)	1.59 (1.09, 2.32)	1.28 (0.86, 1.93)	0.05
Model 2, HR (95% CI)[3]	1.0	1.02 (0.68, 1.55)	1.01 (0.67, 1.52)	1.57 (1.07, 2.30)	1.23 (0.81, 1.85)	0.10
Model 3, HR (95% CI)[4]	1.0	0.98 (0.64, 1.51)	0.96 (0.60, 1.52)	1.48 (0.93, 2.37)	1.13 (0.64, 1.98)	0.46
Water-soluble choline-containing compounds						
Free choline						
Events (*n*)	47	54	65	58	47	
Model 1, HR (95% CI)[2]	1.0	1.07 (0.72, 1.59)	1.29 (0.88, 1.88)	1.11 (0.75, 1.63)	0.98 (0.65, 1.46)	0.87
Model 2, HR (95% CI)[3]	1.0	1.08 (0.73, 1.61)	1.21 (0.82, 1.77)	1.09 (0.73, 1.61)	0.96 (0.64, 1.45)	0.78
Model 3, HR (95% CI)[4]	1.0	1.16 (0.77, 1.75)	1.35 (0.86, 2.07)	1.26 (0.79, 2.00)	1.20 (0.72, 1.99)	0.58
Glycerophosphocholine						
Events (*n*)	38	59	54	66	54	
Model 1, HR (95% CI)[2]	1.0	1.33 (0.88, 2.01)	1.17 (0.77, 1.77)	1.51 (1.01, 2.25)	1.18 (0.78, 1.80)	0.54
Model 2, HR (95% CI)[3]	1.0	1.38 (0.91, 2.09)	1.29 (0.85, 1.96)	1.59 (1.06, 2.37)	1.27 (0.83, 1.93)	0.38
Model 3, HR (95% CI)[4]	1.0	1.36 (0.89, 2.06)	1.23 (0.79, 1.92)	1.46 (0.91, 2.33)	1.14 (0.65, 2.01)	0.87
Phosphocholine						
Events (*n*)	39	57	62	60	53	
Model 1, HR (95% CI)[2]	1.0	1.32 (0.88, 1.99)	1.45 (0.97, 2.17)	1.34 (0.89, 2.02)	1.22 (0.81, 1.85)	0.52
Model 2, HR (95% CI)[3]	1.0	1.35 (0.89, 2.04)	1.39 (0.92, 2.08)	1.34 (0.88, 2.01)	1.24 (0.81, 1.90)	0.50
Model 3, HR (95% CI)[4]	1.0	1.38 (0.90, 2.11)	1.40 (0.90, 2.18)	1.34 (0.81, 2.20)	1.27 (0.71, 2.28)	0.60
Betaine						
Events (*n*)	43	63	53	60	52	
Model 1, HR (95% CI)[2]	1.0	1.23 (0.83, 1.81)	1.04 (0.69, 1.56)	1.09 (0.73, 1.62)	1.09 (0.72, 1.63)	0.98
Model 2, HR (95% CI)[3]	1.0	1.15 (0.77, 1.70)	1.03 (0.68, 1.55)	1.07 (0.71, 1.59)	1.09 (0.72, 1.64)	0.86
Model 3, HR (95% CI)[4]	1.0	1.16 (0.78, 1.73)	1.07 (0.70, 1.62)	1.12 (0.74, 1.69)	1.08 (0.70, 1.67)	0.92

[1] Calculated by modeling the median of each quintile as a continuous term.

[2] Cox proportional hazards regression model adjusted for age at diagnosis (y, continuous), energy (kcal/d, quintiles), time period (2-y intervals), and time since diagnosis (y, continuous).

[3] Cox proportional hazards regression model adjusted for the variables in model 1 plus treatment (radical prostatectomy, radiation therapy, hormonal therapy, or other/active surveillance), Gleason sum (≤6, 7, ≥8, or missing), clinical T-stage (T1, T2, or T3), BMI (in kg/m^2; <25, 25–29.9, ≥30, or missing), smoking (never, former, or current), and vigorous activity (metabolic equivalent task-hours/wk; quartile rank).

[4] Cox proportional hazards regression model adjusted for the variables in model 2 plus quintile ranks of intakes of calcium, coffee, phosphorus, saturated fat, cholesterol, and polyunsaturated fat.

association between folate supplementation and prostate cancer risk, which supports a potential adverse effect of methyl donors on prostate cancer progression (32). However, the lack of association between betaine intake and risk of lethal prostate cancer does not support this potential mechanism.

Last, dietary choline is converted by gut bacteria to trimethylamine, which is converted to trimethylamine oxide in the liver. Mice treated with choline or trimethylamine oxide have increased activation of macrophages and atherosclerotic lesions (33). Although purely speculative, increased trimethylamine oxide from high dietary choline may increase inflammation, and this may promote progression of prostate cancer to lethal disease.

Choline is an essential nutrient; therefore, it must be consumed in the diet for optimal health. Low concentrations of choline are associated with the development of fatty liver and liver damage (34, 35). In addition, animal data suggest that choline intake may be beneficial to cognitive function and memory (36–39), and cytidinediphosphocholine—the intermediate produced in the conversion of choline to phosphatidylcholine—has been associated with improved memory in elderly persons in short-term randomized

controlled trials (40). Thus, future studies need to examine whether the benefits of choline intake outweigh the potential risks among men.

The limitations of our study included the lack of validation of our FFQ's estimate of choline intake, our limited statistical power to examine the independent effects of the choline-containing compounds, restricted generalizability, and the potential for unmeasured confounding. Our study population was a homogeneous group of well-educated white men; thus, our results may not be generalizable to populations with different racial or socioeconomic distributions. Additionally, we attempted to thoroughly control for nutrients and foods and lifestyle, clinical, and sociodemographic factors, which have been shown to be associated with lethal prostate cancer and with nutrients present in animal products that could potentially be associated with prostate cancer. Yet, this was not a randomized controlled trial; therefore, we cannot be certain that the associations we observed were the results of intake of choline or some other nutrients or factors associated with the consumption of choline. The strengths of our study include the large number of events of lethal prostate cancer, our detailed and repeated assessments of diet and covariate data, and the completeness and length of our follow-up.

In conclusion, dietary choline may increase the risk of lethal prostate cancer. Future studies replicating these novel findings in large independent populations and studies examining the relation of dietary choline to choline concentrations in the prostate and the effects of dietary choline on normal and malignant prostate cells would be of interest.

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