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Nutrients related to one-carbon metabolism and risk of renal cell cancer

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Abstract

Purpose—Folate, vitamins B6 and B12, methionine, choline, and betaine are nutrients related to one-carbon metabolism and have been hypothesized to decrease cancer risk. Few studies have evaluated dietary intakes of these nutrients in relation to renal cell cancer (RCC).

Methods—We conducted prospective follow-up studies of women in the Nurses' Health Study and men in the Health Professionals Follow-up Study. Diet was assessed repeatedly using a validated semi-quantitative food-frequency questionnaire in both studies.

Results—During follow-up of 24 years among 77,208 women (918,891 person-years) and 22 years among 47,886 men (1,731,752 person-years), we accrued 436 cases of RCC (225 women and 211 men). Intakes of folate, vitamins B6 and B12, methionine, and betaine were not found to be related to RCC risk. Higher intake of free choline, but not other forms of choline, was associated with reduced RCC risk. The results were similar in men and women.

Conclusions—We found little evidence that higher intakes of nutrients related to one-carbon metabolism lower RCC risk. One-carbon metabolism may have little influence on renal carcinogenesis.

Keywords

one-carbon metabolism; renal cell cancer; folate; prospective study

INTRODUCTION

Kidney cancer is the sixth leading malignancy among men and the eighth among women, with a projected total of 64,770 new cases and 13,570 deaths due to kidney cancer in the U.S. in 2012 [1]. Renal cell cancer (RCC), also known as adenocarcinoma of the kidney, is the most common type of kidney cancer and accounts for 90–95% of malignancy arising from the kidney [2]. Kidney cancer is one of the six cancer sites on an increasing trend in the U.S. [3], with incidence rates rising in most racial and ethnic groups. Although incidental detection through increased abdominal imaging might have contributed to the rising incidence figures, the increase in incidence of all stages of RCC suggests that other environmental factors may also be responsible [4].

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One-carbon metabolism constitutes a network of biochemical reactions that transfer one-carbon (methyl) groups from one compound to another [5]. Both folate and betaine donate methyl groups to homocysteine and produce methionine, which subsequently transfer a methyl-group to S-adenosylmethionine, a universal methyl-group donor for methylation of DNA and RNA. One-carbon metabolism can influence gene stability and expression and is essential for nucleotide synthesis. Disruption of the metabolism may cause chromosome breaks and perturbation of DNA repair [6,7].

Besides folate, betaine, and methionine, other nutrients including vitamins B6 and B12 and choline influence one-carbon metabolism. A few studies have investigated folate intake in relation to RCC risk but found no association [8,9]. To our knowledge, no study has investigated intakes of other nutrients related to one-carbon metabolism in relation to risk of RCC. We therefore investigated intakes of such nutrients in relation to RCC risk in two prospective studies of men and women.

MATERIALS AND METHODS

Study population

The Nurses' Health Study (NHS) started with 121,700 female nurses aged 30–55 years in 1976 [10]. The Health Professionals Follow-up Study (HPFS) started with 51,529 male health professionals aged 40–75 years in 1986. In both cohorts, follow-up questionnaires have been sent biennially to update information on lifestyle factors and to document new diagnoses of major illnesses including RCC. Deaths have been documented by reports from family members, the postal service, and a search of the National Death Index. More than 98% of deaths were documented through these sources [11].

In the NHS, we started follow-up for the current analysis in 1984, when an expanded food-frequency questionnaire (FFQ) was first used to assess dietary intake. In the HPFS, we started follow-up in 1986, the start of the study. In both studies, we excluded participants who did not answer the FFQ and those who had a history of cancer (except nonmelanoma skin cancer) at baseline. Participants who had diagnosed cancers other than RCC were censored at the time of diagnosis. The follow-up rates have been over 90% in both cohorts [12].

The studies were approved by the Institutional Review Boards of the Brigham and Women' Hospital and Harvard School of Public Health.

Assessment of diet

In 1980, an FFQ with about 60 food items was sent to the participants in the NHS. An expanded FFQ with about 130 food items was sent in 1984, 1986, and every 4 years thereafter in the NHS and in 1986 and every 4 years thereafter in the HPFS. Participants answered how often, on average, they had consumed each type of food during the past year. A serving size was specified for each food in the FFQ. The questionnaire contained nine intake frequency choices, ranging from never or less than once per month to six or more times per day.

Participants in the cohorts were also asked their current use and dose of multivitamins and vitamin supplements biennially. Nutrient intake was calculated using data from the U.S. Department of Agriculture [13,14] or other sources [15,16]. As the United States Food and Drug Administration (FDA) mandated fortification of enriched grain-based foods with folic acid beginning in 1998, we took that into account in our assessment of folate intake. Total intake included both dietary and supplemental sources. Dietary intake included intake contribution from food only. Total choline intake was calculated as the sum of choline

intake from free choline, phosphocholine, glycerophosphocholine, phosphatidylcholine (lecithin), and sphingomyelin. The regression-residual method was used to adjust nutrient intakes for total energy intake [17]. After evaluating values of the nutrients across different FFQs, in 1984 we started follow-up in the NHS, when more comprehensive FFQs began to be used and nutrient intake values became more comparable across time.

Intakes of folate and vitamins B6 and B12 measured by FFQ have been validated. The Pearson correlation coefficients with two one-week diet records were 0.77 for total folate, 0.85 for total vitamin B6, and 0.56 for total vitamin B12 in the HPFS [18]. The correlation for total vitamin B6 in the NHS was 0.58[19]. Also, folate intake predicted folate levels in red blood cells (r=0.55 in the NHS and 0.56 in the HPFS)[20]. Similarly, vitamin B6 intake predicted plasma pyridoxal phosphate (PLP) levels (r=0.52 in the NHS and 0.54 in the HPFS)(X Zhang; personal communication). Intakes of choline and betaine measured by our FFQ predicted plasma total homocysteine levels in a cohort study of men and women in the Framingham Offspring Study; for the lowest and highest quintiles of choline intake, the multivariate geometric means for homocysteine were 10.6 and 9.8 μmol/L (P for trend <0.0001), respectively[21]. For the lowest and highest quintiles of betaine intake, the corresponding geometric means were 10.4 and 10.1 μmol/L (P for trend 0.05), respectively. Although slightly weaker, an inverse association similar to that found in the Framingham Offspring Study was identified between choline plus betaine intake and plasma homocysteine levels in the NHS; compared with those in the lowest quintile, individuals in the highest quintile of choline-plus-betaine intake had 8% lower levels of homocysteine[22].

Intakes of total energy, alcohol, fruits, and vegetables were calculated from the FFQ and were available to adjust for as covariates.

Assessment of other risk factors for RCC

We have collected information on body weight, smoking (including number of cigarettes smoked), recreational physical activity, and history of hypertension and diabetes biennially in the two cohorts. We confirmed that diagnosis of hypertension was reliably reported [23]. Body mass index (BMI; kg/m^2) was calculated using self-reported height and weight. Women were also asked about number of children (parity).

Identification of cases

We inquired about the diagnosis of cancer on each biennial questionnaire, and also asked participants (or next-of-kin for those who died) who reported a diagnosis of kidney cancer for permission to access their medical and pathological records. Study physicians blinded to participants' questionnaire information reviewed medical and pathological records to confirm the diagnosis and to accrue information on histological subtype. Based on the WHO classification [24], as RCC cases we included RCC, clear cell, papillary, chromophobe, collecting duct RCC, and RCC not otherwise classified. Self-reported kidney cancer was confirmed in 78% of the NHS cases and 81% of the HPFS cases.

Statistical analysis

Because diet may affect renal carcinogenesis over an extended time period, for our primary analysis, we calculated cumulative averaged nutrient intakes using repeated dietary data to best represent long-term dietary intake. Cumulative averaged intake can also decrease measurement error in assessing diet [25]. For example, in women, 1984 intake was used for 1984–1986 follow-up, and the average of 1984 and 1986 intake was used for 1986–1990 follow-up, and so on. Because remote or recent dietary intake may also affect renal carcinogenesis, baseline and most recent intake were each investigated in secondary analyses.

Participants contributed person-time from the date of return of the baseline FFQ until the date of RCC diagnosis, death, or end of follow-up (June 2008 for NHS and January 2008 for HPFS), whichever came first. Participants were grouped based on dietary intake quintiles. Relative risks (RRs) of RCC (and 95% confidence invervals [CIs]) for quintiles of nutrients were calculated. Cox proportional hazards regression was used to adjust for risk factors for RCC [26]. To adjust for confounding by age, calendar time, and any possible two-way interactions between these two time scales, we stratified the analysis jointly by age in months at start of follow-up and calendar year of the current questionnaire cycle. In multivariable analysis, we additionally adjusted for known and emerging risk factors for RCC including BMI (continuous), smoking status (never, past, current), history of hypertension (yes/no), history of diabetes (yes/no), and intakes of total energy, fruits, vegetables, and alcohol (all continuous). In women, we also adjusted for parity (nulliparous, 1–2, 3, 4, or 5+ children). SAS PROC PHREG was used, and the Anderson-Gill data structure [27] was employed for efficient handling of time-varying covariates. Tests for trend across quintiles of intake were conducted using the median within each quintile as a continuous variable [28]. To test whether the association between nutrients and RCC risk was modified by age, smoking, BMI, history of hypertension, alcohol intake, and total folate intake, cross-product terms for the level of an interaction variable and each nutrient intake were included in multivariable models. The P value for the test for interaction was calculated from a Wald test. All P values are two-sided.

Separate analyses were conducted for each cohort. Then we tested for heterogeneity between studies and used a random effects model to pool the RRs from the cohorts [29].

RESULTS

During follow-up of 24 years among 77,208 women (1,731,752 person-years) and 22 years among 47,886 men (918,891 person-years), we documented 436 cases of RCC (225 women and 211 men). The mean age of diagnosis of RCC was 65.6 years in both women and men (standard deviation= 7.9 years for women and 8.1 years for men, respectively).

Participants with higher total folate intake were less likely to be current smokers in both NHS and HPFS and alcohol drinkers in HPFS (Table 1). They were also more likely to have higher intakes of betaine, vitamins B6 and 12, and choline. The results on characteristics of study participants by quintiles of choline, betaine, vitamin B6, vitamin B12, and methionine are presented in Supplementary Tables 1–5. The results on characteristics of study participants by case status are presented in Supplementary Table 6.

Intakes of folate, vitamins B6 and 12, and methionine were not related to RCC risk (Table 2). The pooled multivariate RRs comparing top versus bottom quintiles were 0.84 (95% CI 0.60–1.18; P value, test for trend= 0.52) for total folate, 0.88 (95% CI 0.65–1.19; P value, test for trend= 0.55) for total vitamin B6, 1.24 (95% CI 0.90–1.70; P value, test for trend= 0.61) for total vitamin B12, and 1.29 (95% CI 0.93–1.78; P value, test for trend= 0.10) for methionine. The results were similar in men and women.

Intakes of choline and betaine were not related to risk of RCC (Table 3). When we investigated individual forms of choline, free choline intake was inversely associated with RCC risk. The pooled multivariate RR for top versus bottom quintiles of free choline intake was 0.59 (95% CI 0.38–0.92; P value, test for trend= 0.19). The associations were similar in women and men and significant in women (RR=0.47 [95% CI 0.28–0.79], P value, test for trend= 0.002). Other forms of choline were not related to risk of RCC. The results were similar regardless of whether we evaluated baseline or most recent intake (data not shown).

The results were similar when we evaluated clear cell RCC (n=151 in women and 131 in men), which is the major histologic subtype of RCC, vs. non-clear cell RCC (n=74 in women and 80 in men; data not shown).

Because major food contributors of free choline intake in the cohort studies were coffee, milk, and beer, we adjusted for each of these foods one at a time in the multivariate model for free choline intake to evaluate whether the inverse association was due to specific food item(s). We carried out this work in the NHS only because the association between free choline intake and RCC risk was not significant in the HPFS. In the NHS, a similar inverse association between free choline intake and RCC risk both persisted and remained significant (data not shown).

The inverse association between free choline intake and RCC risk was not modified by age (<65, ≥ 65 years), BMI (<27.5, ≥27.5 kg/m^2), smoking status (never, ever), history of hypertension (no, yes), alcohol intake (non-drinkers, <15, ≥15g/d), total folate intake (<300, 300–400, ≥400ug/d), or postmenopausal hormone use (women only) (data not shown).

DISCUSSION

In these prospective studies of women and men, dietary intakes of folate, vitamins B6 and B12, methionine, choline, and betaine were not associated with a reduced risk of RCC.

Our findings on folate intake are consistent with previous case-control studies from Italy [8] and US[9]. On the other hand, a nested case-control study among male smokers examined prediagnostic levels of serum folate and vitamins B6 and B12 in relation to RCC risk and found that lower folate levels were associated with elevated RCC risk [30].

We are not aware of any epidemiologic studies investigating other nutrients related to one-carbon metabolism besides folate in relation to RCC risk. The association we found with free choline intake is somewhat puzzling, given that none of the other nutrients involved in one-carbon metabolism and no other types of choline were associated with reduced RCC risk. Few studies have investigated choline intake and cancer risk in general, because choline intake databases have been available only since 2003. Some studies on choline intake found that higher intake was related to increased risk of colorectal adenoma[31] and breast cancer[32], while other studies found no association with breast[33], colorectal[34], or ovarian[35] cancers. We are not aware of any specific associations between free choline intake and risk of other cancer sites. Major food contributors of free choline intake include beverages such as coffee, beer, and milk in our cohort studies. The association between intakes of coffee, beer, and milk and RCC risk were examined in our previous studies [36]; there was a suggestive inverse association with intakes of beer (pooled RR=0.68 [95% CI 0.38–1.23] for drinkers vs. non-drinkers) and coffee (pooled RR=0.84 [95% CI 0.54–1.30] for highest vs. lowest intake), but not with milk (RR=1.09 [95% CI 0.39–2.99] for highest vs. lowest intake). The inverse association we found with free choline might be related to some other components in these foods. However, when we adjusted for each food item, the inverse association for free choline was maintained. It is hard to interpret the fact that just one form of choline (but not others) was associated with risk. Free choline is one of the water-soluble forms of choline; others include phosphocholine and glycerophosphocholine. On the other hand, sphingomyelin and phosphatidylcholine are fat-soluble forms. In the study where choline intake was related to increased risk of colorectal adenomas, a fat-soluble form of choline was associated with risk [31]. As a limitation, because we evaluated multiple nutrients, the inverse association we found with free choline may be due to chance.

Our study has several strengths. Because of its prospective design, recall and selection biases were avoided. Our study also provided a unique opportunity to evaluate nutrient

intakes and RCC risk prospectively in a relatively large cohort. By taking advantage of repeated assessments of dietary intake, we were able to use cumulative averaged nutrient intake in our primary analysis, which might have reduced measurement error in diet [25]. We have collected information on a wide range of potential confounders prospectively and adjusted for them in multivariate analyses.

In conclusion, we found little evidence that higher intakes of the nutrients related to one-carbon metabolism decrease risk of RCC. The inverse association we found between free choline intake and RCC risk needs to be replicated in other studies and investigated further.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Age-standardized characteristics of study participants by quintile of energy-adjusted total folate intake in the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) in 1994[a]

	Intake Quintiles					
	NHS			HPFS		
	Q1	Q3	Q5	Q1	Q3	Q5
Intake of folate, median, μg/d [b]	219	383	767	249	440	884
No. of individuals	13084	13070	13139	6754	6799	6821
Age, mean, y	61	61	61	62	62	62
Body mass index, mean, kg/m^2	27	26	26	26	26	25
Current smokers, %	19	11	10	9	5	4
Past smokers, %	40	43	46	47	46	48
History of hypertension, %	37	37	38	32	32	32
History of type 2 diabetes, %	7	7	7	5	5	5
Parity, mean [b]	3	3	3	-	-	-
Dietary intake, mean						
Total betaine, mg/d [c]	92	115	119	114	143	147
Total vitamin B6, mg/d[c]	5	7	16	4	6	19
Total vitamin B12, μg/d [c]	6	9	17	7	11	21
Total choline, mg/d [c]	290	313	317	352	370	372
Total methionine, mg/d[c]	2	2	2	2	2	2
Fruit, servings/d	2	3	3	2	3	3
Vegetables, servings/d	3	4	3	2	4	4
Alcohol, g/d	6	6	6	12	11	10

[a] Except for the data on mean of age, all data are standardized to the age distribution of each cohort in 1994. For simplicity, only data for the first, third, and fifth quintiles are shown.

[b] Among parous women.

[c] Nutrient values are energy-adjusted intake.

Table 2

Relative risks (RRs) and 95% confidence interval (CIs) of renal cell cancer by quintiles of intake of cumulative averaged energy-adjusted folate, vitamins B6 and B12, and methionine in the Nurses' Health Study (NHS), 1984–2008 and Health Professionals Follow-Up Study (HPFS), 1986–2008[a]

	Intake Quintiles									
Nutrient	**1**		**2**		**3**		**4**		**5**	P_{trend}[b]
Total folate										
NHS										
Median (μg/d)	224.7	305.0		388.3		495.8		670.0		
No. of cases	39	45		36		56		49		
Age-adjusted RR (95% CI)	1	0.90	(0.58 – 1.39)	0.65	(0.41 – 1.02)	0.89	(0.58 – 1.37)	0.78	(0.50 – 1.21)	0.47
Multivariate RR (95% CI)	1	0.92	(0.59 – 1.44)	0.66	(0.41 – 1.06)	0.93	(0.59 – 1.45)	0.83	(0.52 – 1.34)	0.73
HPFS										
Median (μg/d)	274.0	365.0		465.8		617.2		860.0		
No. of cases	48	54		20		49		40		
Age-adjusted RR (95% CI)	1	1.05	(0.70 – 1.56)	0.37	(0.22 – 0.63)	0.87	(0.57 – 1.32)	0.71	(0.46 – 1.10)	0.18
Multivariate RR (95% CI)	1	1.16	(0.77 – 1.75)	0.44	(0.25 – 0.77)	1.03	(0.66 – 1.61)	0.85	(0.53 – 1.36)	0.62
Pooled multivariate RR (95% CI)	1	1.04	(0.77 – 1.41)	0.55	(0.37 – 0.81)	0.98	(0.71 – 1.34)	0.84	(0.60 – 1.18)	0.52
Folate from food only										
NHS										
Median (μg/d)	201.6	249.5		286.0		325.2		389.8		
No. of cases	33	50		48		41		53		
Age-adjusted RR (95% CI)	1	1.19	(0.76 – 1.85)	1.02	(0.65 – 1.60)	0.80	(0.50 – 1.28)	0.98	(0.62 – 1.54)	0.44
Multivariate RR (95% CI)	1	1.20	(0.76 – 1.89)	1.03	(0.63 – 1.67)	0.81	(0.48 – 1.37)	1.01	(0.57 – 1.77)	0.63
HPFS										
Median (μg/d)	254.7	316.5		363.9		415.8		506.5		
No. of cases	44	39		46		40		42		
Age-adjusted RR (95% CI)	1	0.80	(0.51 – 1.24)	0.92	(0.60 – 1.41)	0.80	(0.52 – 1.24)	0.82	(0.53 – 1.26)	0.44
Multivariate RR (95% CI)	1	0.92	(0.59 – 1.45)	1.18	(0.75 – 1.85)	1.09	(0.67 – 1.79)	1.27	(0.75 – 2.15)	0.30
Pooled multivariate RR (95% CI)	1	1.05	(0.76 – 1.45)	1.11	(0.79 – 1.54)	0.95	(0.66 – 1.36)	1.14	(0.77 – 1.67)	0.59
Total vitamin B6										
NHS										
Median (mg/d)	1.5	2.0		2.7		4.1		19.9		

Nutrient	Intake Quintiles 1	2		3		4		5		P_{trend}[b]
No. of cases	44	43		49		32		57		
Age-adjusted RR (95% CI)	1	0.78	(0.51 – 1.19)	0.78	(0.51 – 1.18)	0.53	(0.33 – 0.83)	0.87	(0.58 – 1.31)	0.49
Multivariate RR (95% CI)	1	0.78	(0.50 – 1.20)	0.79	(0.51 – 1.21)	0.54	(0.33 – 0.86)	0.90	(0.59 – 1.37)	0.38
HPFS										
Median (mg/d)	1.8	2.4		3.1		4.7		19.1		
No. of cases	50	40		43		36		42		
Age-adjusted RR (95% CI)	1	0.77	(0.51 – 1.18)	0.75	(0.49 – 1.14)	0.62	(0.40 – 0.96)	0.75	(0.49 – 1.13)	0.61
Multivariate RR (95% CI)	1	0.86	(0.55 – 1.32)	0.86	(0.55 – 1.33)	0.70	(0.45 – 1.11)	0.86	(0.56 – 1.33)	0.91
Pooled multivariate RR (95% CI)	1	0.82	(0.60 – 1.11)	0.82	(0.61 – 1.12)	0.62	(0.44 – 0.86)	0.88	(0.65 – 1.19)	0.55
Vitamin B6 from food only										
NHS										
Median (mg/d)	1.4	1.6		1.8		1.9		2.2		
No. of cases	29	51		50		47		48		
Age-adjusted RR (95% CI)	1	1.44	(0.91 – 2.28)	1.32	(0.83 – 2.09)	1.15	(0.72 – 1.84)	1.14	(0.71 – 1.82)	0.84
Multivariate RR (95% CI)	1	1.44	(0.90 – 2.30)	1.31	(0.81 – 2.12)	1.13	(0.68 – 1.88)	1.11	(0.64 – 1.90)	0.72
HPFS										
Median (mg/d)	1.7	2.0		2.2		2.5		3.0		
No. of cases	50	42		30		43		46		
Age-adjusted RR (95% CI)	1	0.80	(0.52 – 1.21)	0.55	(0.35 – 0.87)	0.75	(0.50 – 1.14)	0.79	(0.52 – 1.19)	0.35
Multivariate RR (95% CI)	1	0.88	(0.58 – 1.35)	0.64	(0.40 – 1.04)	0.94	(0.59 – 1.49)	1.03	(0.63 – 1.67)	0.71
Pooled multivariate RR (95% CI)	1	1.12	(0.69 – 1.80)	0.92	(0.46 – 1.84)	1.02	(0.73 – 1.44)	1.06	(0.74 – 1.52)	0.92
Total vitamin B12										
NHS										
Median (μg/d)	4.7	7.0		9.3		12.3		19.3		
No. of cases	34	40		43		48		60		
Age-adjusted RR (95% CI)	1	1.04	(0.65 – 1.64)	1.03	(0.65 – 1.62)	1.12	(0.72 – 1.74)	1.24	(0.80 – 1.91)	0.24
Multivariate RR (95% CI)	1	1.06	(0.67 – 1.68)	1.03	(0.65 – 1.62)	1.10	(0.70 – 1.72)	1.25	(0.81 – 1.93)	0.25
HPFS										
Median (μg/d)	5.5	8.0		10.9		15.0		25.5		
No. of cases	31	51		45		40		44		

Nutrient	Intake Quintiles 1	2		3		4		5		P_{trend}[b]
Age-adjusted RR (95% CI)	1	1.64	(1.04 – 2.57)	1.35	(0.85 – 2.14)	1.17	(0.73 – 1.88)	1.25	(0.78 – 2.00)	0.88
Multivariate RR (95% CI)	1	1.65	(1.05 – 2.59)	1.33	(0.84 – 2.12)	1.18	(0.74 – 1.90)	1.22	(0.76 – 1.96)	0.81
Pooled multivariate RR (95% CI)	1	1.32	(0.86 – 2.05)	1.17	(0.84 – 1.61)	1.14	(0.82 – 1.58)	1.24	(0.90 – 1.70)	0.61
Vitamin B12 from food only										
NHS										
Median (μg/d)	3.8	4.9		6.1		7.7		10.7		
No. of cases	38	45		41		45		56		
Age-adjusted RR (95% CI)	1	1.11	(0.72 – 1.70)	0.94	(0.61 – 1.47)	1.00	(0.65 – 1.54)	1.46	(0.96 – 2.22)	0.07
Multivariate RR (95% CI)	1	1.06	(0.68 – 1.63)	0.91	(0.58 – 1.42)	0.92	(0.60 – 1.43)	1.30	(0.85 – 1.97)	0.21
HPFS										
Median (μg/d)	4.5	6.1		7.4		9.2		14.1		
No. of cases	24	45		49	2	50		43		
Age-adjusted RR (95% CI)	1	1.83	(1.11 – 3.01)	2.05	(1.25 – 3.35)	2.00	(1.22 – 3.26)	1.64	(0.99 – 2.73)	0.33
Multivariate RR (95% CI)	1	1.77	(1.07 – 2.91)	1.92	(1.17 – 3.15)	1.85	(1.13 – 3.04)	1.50	(0.90 – 2.50)	0.59
Pooled multivariate RR (95% CI)	1	1.34	(0.81 – 2.23)	1.31	(0.63 – 2.74)	1.29	(0.65 – 2.56)	1.37	(0.99 – 1.90)	0.24
Total methionine										
NHS										
Median (g/d)	1.4	1.6		1.7		1.9		2.1		
No. of cases	37	42		59		41		46		
Age-adjusted RR (95% CI)	1	1.09	(0.70 – 1.70)	1.52	(1.01 – 2.30)	1.15	(0.73 – 1.79)	1.36	(0.88 – 2.10)	0.19
Multivariate RR (95% CI)	1	1.06	(0.68 – 1.65)	1.40	(0.92 – 2.13)	1.01	(0.64 – 1.59)	1.10	(0.69 – 1.76)	0.82
HPFS										
Median (g/d)	1.7	1.9	(1.8 – 2.0)	2.1	(2.0 – 2.2)	2.3	(2.2 – 2.4)	2.6	(2.4 – 6.9)	
No. of cases	36	36	1	37		47		55		
Age-adjusted RR (95% CI)	1	1.00	(0.63 – 1.59)	1.04	(0.65 – 1.65)	1.22	(0.79 – 1.90)	1.54	(1.01 – 2.36)	0.02
Multivariate RR (95% CI)	1	0.97	(0.61 – 1.55)	1.03	(0.64 – 1.64)	1.17	(0.75 – 1.85)	1.49	(0.95 – 2.32)	0.04
Pooled multivariate RR (95% CI)	1	1.02	(0.74 – 1.40)	1.22	(0.89 – 1.67)	1.09	(0.79 – 1.50)	1.29	(0.93 – 1.78)	0.10

[a]Multivariate models were adjusted for age (months), smoking status (never, past, current), body mass index (continuous), history of hypertension (yes/no), history of diabetes (yes/no), physical activity (quintiles), fruit intake (continuous), vegetable intake (continuous), and alcohol intake (continuous) in NHS and HPFS and parity (nulliparous, 1–2, 3, 4, 5+ children) in NHS.

[b]P-value, test for trend

Table 3

Relative risks (RRs) and 95% confidence intervals (CIs) of renal cell cancer by quintiles of cumulative averaged energy-adjusted choline and betaine intake in the Nurses' Health Study (NHS), 1984–2008 and Health Professionals Follow-Up Study (HPFS), 1986–2008[a]

	Intake Quintiles									
Nutrient	**1**		**2**		**3**		**4**		**5**	P_{trend}[b]
Total choline										
NHS										
Median (mg/d)	267.1	300.8		325.1		351.9		399.8		
No. of cases	41	57		45		48		34		
Age-adjusted RR (95% CI)	1	1.36	(0.91 – 2.03)	1.10	(0.72 – 1.68)	1.20	(0.79 – 1.83)	0.94	(0.59 – 1.49)	0.59
Multivariate RR (95% CI)	1	1.33	(0.88 – 1.99)	1.04	(0.67 – 1.60)	1.07	(0.70 – 1.66)	0.78	(0.48 – 1.27)	0.17
HPFS										
Median (mg/d)	308.3	348.0		376.9		409.5		471.7		
No. of cases	43	39		54		28		47		
Age-adjusted RR (95% CI)	1	0.88	(0.57 – 1.37)	1.19	(0.79 – 1.78)	0.60	(0.37 – 0.97)	0.92	(0.60 – 1.41)	0.43
Multivariate RR (95% CI)	1	0.89	(0.58 – 1.38)	1.22	(0.81 – 1.84)	0.62	(0.38 – 1.01)	0.96	(0.62 – 1.48)	0.55
Pooled multivariate RR (95% CI)	1	1.10	(0.75 – 1.62)	1.13	(0.84 – 1.52)	0.83	(0.49 – 1.41)	0.87	(0.63 – 1.21)	0.19
Free choline										
NHS										
Median (mg/d)	55.1	63.3		69.1		75.3		85.5		
No. of cases	47	58		39		49		32		
Age-adjusted RR (95% CI)	1	1.07	(0.73 – 1.58)	0.69	(0.45 – 1.05)	0.83	(0.55 – 1.24)	0.54	(0.34 – 0.85)	0.003
Multivariate RR (95% CI)	1	1.06	(0.71 – 1.57)	0.66	(0.42 – 1.03)	0.75	(0.49 – 1.17)	0.47	(0.28 – 0.79)	0.002
HPFS										
Median (mg/d)	67.6	78.3		86.1		94.7		111.3		
No. of cases	49	38		46		46		32		
Age-adjusted RR (95% CI)	1	0.72	(0.47 – 1.12)	0.86	(0.57 – 1.31)	0.84	(0.56 – 1.27)	0.57	(0.36 – 0.90)	0.04
Multivariate RR (95% CI)	1	0.78	(0.50 – 1.21)	0.99	(0.64 – 1.52)	1.02	(0.65 – 1.60)	0.74	(0.44 – 1.25)	0.49
Pooled multivariate RR (95% CI)	1	0.92	(0.69 – 1.24)	0.81	(0.54 – 1.20)	0.87	(0.64 – 1.20)	0.59	(0.38 – 0.92)	0.19
Glycerophosphocholine										
NHS										
Median (mg/d)	33.2	42.2		49.9		59.0		75.3		

Nutrient	Intake Quintiles 1	2		3		4		5		P_{trend}[b]
No. of cases	40	51		57		37		40		
Age-adjusted RR (95% CI)	1	1.11	(0.73 – 1.69)	1.18	(0.79 – 1.78)	0.75	(0.48 – 1.18)	0.81	(0.52 – 1.26)	0.09
Multivariate RR (95% CI)	1	1.13	(0.74 – 1.71)	1.19	(0.79 – 1.80)	0.76	(0.48 – 1.19)	0.80	(0.51 – 1.25)	0.08
HPFS										
Median (mg/d)	39.4	49.9		58.6		69.5		90.8		
No. of cases	33	48		42		41		47		
Age-adjusted RR (95% CI)	1	1.33	(0.85 – 2.09)	1.13	(0.71 – 1.79)	1.08	(0.68 – 1.72)	1.22	(0.78 – 1.91)	0.72
Multivariate RR (95% CI)	1	1.42	(0.90 – 2.23)	1.24	(0.78 – 1.97)	1.19	(0.74 – 1.90)	1.30	(0.82 – 2.05)	0.56
Pooled multivariate RR (95% CI)	1	1.25	(0.92 – 1.70)	1.21	(0.89 – 1.65)	0.94	(0.61 – 1.47)	1.02	(0.63 – 1.64)	0.61
Phosphocholine										
NHS										
Median (mg/d)	9.5	11.9		13.7		15.8		19.1		
No. of cases	46	41		51		48		39		
Age-adjusted RR (95% CI)	1	0.82	(0.53 – 1.25)	0.96	(0.64 – 1.43)	0.88	(0.58 – 1.32)	0.72	(0.47 – 1.10)	0.19
Multivariate RR (95% CI)	1	0.77	(0.50 – 1.19)	0.89	(0.59 – 1.36)	0.79	(0.51 – 1.23)	0.60	(0.36 – 0.99)	0.071
HPFS										
Median (mg/d)	10.9	13.6		15.7		18.1		22.1		
No. of cases	42	48		41		39		41		
Age-adjusted RR (95% CI)	1	1.09	(0.72 – 1.66)	0.87	(0.56 – 1.35)	0.83	(0.54 – 1.30)	0.84	(0.55 – 1.31)	0.24
Multivariate RR (95% CI)	1	1.16	(0.75 – 1.78)	0.97	(0.61 – 1.54)	0.95	(0.59 – 1.54)	0.99	(0.60 – 1.64)	0.73
Pooled multivariate RR (95% CI)	1	0.95	(0.63 – 1.41)	0.93	(0.68 – 1.27)	0.86	(0.62 – 1.19)	0.77	(0.47 – 1.26)	0.20
Phosphatidylcholine										
NHS										
Median (mg/d)	129.0	151.7		168.8		188.2		225.7		
No. of cases	37	46		52		55		35		
Age-adjusted RR (95% CI)	11	1.28	(0.83 – 1.98)	1.57	(1.03 – 2.40)	1.81	(1.19 – 2.76)	1.29	(0.80 – 2.06)	0.14
Multivariate RR (95% CI)		1.24	(0.80 – 1.93)	1.45	(0.94 – 2.23)	1.60	(1.04 – 2.46)	1.07	(0.66 – 1.75)	0.63
HPFS										
Median (mg/d)	139.6	166.5		187.4		212.0		262.0		
No. of cases	37	44		45		38		47		

	Intake Quintiles									
Nutrient	**1**		**2**		**3**		**4**		**5**	P_{trend}[b]
Age-adjusted RR (95% CI)	1	1.18	(0.76 – 1.83)	1.22	(0.79 – 1.89)	1.00	(0.64 – 1.58)	1.18	(0.76 – 1.84)	0.69
Multivariate RR (95% CI)	1	1.19	(0.76 – 1.84)	1.18	(0.76 – 1.84)	0.96	(0.60 – 1.52)	1.13	(0.72 – 1.77)	0.92
Pooled multivariate RR (95% CI)	1	1.21	(0.89 – 1.66)	1.31	(0.96 – 1.79)	1.25	(0.75 – 2.05)	1.10	(0.79 – 1.54)	0.69
Sphingomyelin										
NHS										
Median (mg/d)	14.2	16.9	(15.8 – 17.8)	18.8		20.9		24.7		
No. of cases	41	40	/ 345922	49		52		43		
Age-adjusted RR (95% CI)	1	0.99	(0.64 – 1.53)	1.32	(0.87 – 2.00)	1.50	(0.99 – 2.27)	1.47	(0.94 – 2.27)	0.02
Multivariate RR (95% CI)	1	0.95	(0.61 – 1.47)	1.21	(0.79 – 1.84)	1.31	(0.85 – 2.00)	1.18	(0.75 – 1.87)	0.26
HPFS										
Median (mg/d)	14.8	18.3			20.7		23.5		28.2	
No. of cases	42	38			32		46		53	
Age-adjusted RR (95% CI)	1	0.90	(0.58 – 1.41)	0.78	(0.49 – 1.24)	1.13	(0.74 – 1.73)	1.26	(0.83 – 1.90)	0.14
Multivariate RR (95% CI)	1	0.86	(0.55 – 1.34)	0.71	(0.45 – 1.13)	1.02	(0.67 – 1.57)	1.07	(0.70 – 1.64)	0.47
Pooled multivariate RR (95% CI)	1	0.90	(0.66 – 1.24)	0.93	(0.56 – 1.57)	1.16	(0.85 – 1.56)	1.12	(0.82 – 1.53)	0.21
Total betaine										
NHS										
Median (mg/d)	70.5	85.6			97.7		112.0		138.4	
No. of cases	43	42			50		54		36	
Age-adjusted RR (95% CI)	1	0.86	(0.56 – 1.31)	0.98	(0.65 – 1.48)	1.06	(0.71 – 1.59)	0.74	(0.47 – 1.15)	0.34
Multivariate RR (95% CI)	1	0.84	(0.55 – 1.29)	0.98	(0.65 – 1.49)	1.09	(0.72 – 1.65)	0.78	(0.49 – 1.24)	0.57
HPFS										
Median (mg/d)	84.9	106.2			124.0		145.5		186.4	
No. of cases	41	41			50		34		45	
Age-adjusted RR (95% CI)	1	1.03	(0.66 – 1.61)	1.24	(0.81 – 1.90)	0.83	(0.52 – 1.31)	1.10	(0.72 – 1.70)	0.94
Multivariate RR (95% CI)	1	1.09	(0.70 – 1.70)	1.35	(0.88 – 2.08)	0.95	(0.59 – 1.52)	1.30	(0.83 – 2.02)	0.40
Pooled multivariate RR (95% CI)	1	0.95	(0.70 – 1.30)	1.15	(0.84 – 1.57)	1.03	(0.75 – 1.40)	1.01	(0.62 – 1.65)	0.69

[a] Multivariate models were adjusted for the same variables as Table 2.

[b] P-value, test for trend