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A prospective study of one-carbon metabolism biomarkers and risk of renal cell carcinoma

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Abstract

Objective—Previous studies have found associations between one-carbon metabolism factors and risk of several cancers, but little is known regarding renal cell carcinoma (RCC). We conducted a nested case-control study within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, a prospective study of Finnish male smokers aged 50–69 at baseline.

Methods—Prediagnostic folate, vitamin B₆, vitamin B₁₂, cysteine, riboflavin and homocysteine concentrations were measured in fasting serum from 224 incident RCC cases and 224 controls (matched on age and date of serum collection). Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs), adjusted for potential confounders.

Results—Serum folate tended to be inversely associated with RCC; compared to the first quartile, the odds ratios (95% CI) for subsequent quartiles were 0.62 (0.35–1.08), 0.52 (0.29–0.93), and 0.67 (0.37–1.20) (P-trend = 0.19). When modeled as a threshold effect, subjects in the lowest serum folate quartile (≤ 6.64 nmol/L), which corresponds to deficient folate status, had a significant increased RCC risk (OR = 1.68, 95% CI 1.06–2.65) compared to those with higher serum folate. The other one-carbon metabolism biomarkers were not associated with RCC.

Conclusions—This study in male smokers suggests that deficient folate status may increase risk of RCC, but confirmation is needed in other epidemiologic studies that include women and non-smokers.

Keywords

folate; renal cell carcinoma; biological markers; nested case-control study; B vitamins

Introduction

The incidence rate of kidney cancer has been increasing in the United States and worldwide over the past three decades [1,2]. More than 85% of kidney cancers are classified as renal cell

carcinoma (RCC), the etiology of which is not well understood [3]. Smoking, obesity and hypertension have been directly associated with risk of RCC, but a large proportion of cases are not accounted for by these factors [3-6]. Limited or inconsistent evidence exists for other exposures, including diet. Individual cohort studies have yielded inconsistent evidence [7-11], but a pooled analysis of 13 prospective studies found a protective effect for higher fruit and vegetable intake [12]. Evidence is limited regarding associations with intake of individual nutrients. Specifically, little is known about the role of nutrients related to one-carbon metabolism and RCC risk.

One-carbon metabolism refers to a system of interdependent metabolic pathways that allow the transfer of one-carbon groups, and which are essential for biosynthetic reactions such as methylation and DNA synthesis [13]. The primary component of the system is folate, as the one-carbon transfer reactions involve interconversion between several forms of this nutrient. Other nutrients important in one-carbon metabolism include the B-vitamins B₆, B₁₂ and riboflavin (B₂), each of which acts as an essential cofactor for one or more enzymes that catalyze one-carbon transfer reactions [14]. Vitamin B₁₂ is a cofactor for the reaction by which homocysteine accepts a methyl group from 5-methyltetrahydrofolate to form methionine, which is a precursor to S-adenosylmethionine (SAM). SAM serves as the primary methyl group donor in over 100 methylation reactions in the body, including the methylation of DNA [15, 16]. Vitamin B₆ participates in the conversion of tetrahydrofolate to 5,10-methylenetetrahydrofolate, which is required for the synthesis of thymidylate and a precursor of purine synthesis. 5,10-methylenetetrahydrofolate can also be reduced by methylenetetrahydrofolate reductase (MTHFR) to form 5-methyl tetrahydrofolate for the methylation pathway in a reaction requiring a form of riboflavin (FAD) [14]. Cysteine is tangentially involved in one-carbon metabolism and potentially important for cancer risk as well because homocysteine can be converted to cysteine in a reaction requiring vitamin B₆. Cysteine can then be converted to glutathione, which is an important cofactor in detoxification reactions [17].

A major function of one-carbon metabolism is to supply the methyl groups required for DNA synthesis and DNA methylation. Imbalances in one-carbon metabolism could result in genomic instability and changes in the expression of oncogenes or tumor suppressor genes due to deficiencies in nucleotide availability or aberrant DNA methylation [18,19]. One-carbon metabolism is a complex set of interdependent reactions, so it is important to examine multiple components of the system. Deficiencies in any of the one-carbon-related factors could cause an imbalance in the system and potentially contribute to carcinogenesis. Previous studies have suggested associations between one-carbon metabolism factors and risk of several cancers, including colorectal, pancreatic and esophageal [20-23], but little is known regarding renal cancer. A candidate-gene study found a significant association between single nucleotide polymorphisms in the one-carbon metabolism enzymes MTHFR and thymidylate synthase (TYMS) and RCC risk [24]. The purpose of our study was to investigate the association between serum biomarkers of one-carbon metabolism (folate, vitamin B₆, vitamin B₁₂, riboflavin, cysteine, and homocysteine) and risk of renal cell carcinoma in a cohort of male smokers.

Materials and Methods

Study population

We conducted a nested case-control study within the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study, which was conducted in Finland as a joint project between the U.S. National Cancer Institute and the National Public Health Institute of Finland. The ATBC study was a randomized, double-blind, placebo-controlled trial designed to investigate whether daily supplementation with 50 mg alpha-tocopherol, 20 mg beta-carotene, or both

could reduce the risk of lung and other cancers. The study population included 29,133 male smokers (at least five cigarettes per day) from southwestern Finland between the ages of 50 and 69 years. The trial was initiated in 1985, recruitment was completed in 1988, and supplementation ended in 1993 (median time on treatment 6.1 years) [25]. Follow-up continued after cessation of treatment. The study was approved by the institutional review boards of the U.S. National Cancer Institute and the National Public Health Institute of Finland, and written informed consent was obtained from each participant before randomization.

Selection of cases and controls

Cases (n = 229) were diagnosed with incident renal cell carcinoma between study baseline and April, 2002 and identified by linkage with the Finnish Cancer Registry, which provides almost 100% case ascertainment [26]. Cases were defined as subjects with RCC (ICD-9 code 189.0) as their first cancer, and did not include those with cancers of the renal pelvis (189.1) and ureter (189.2), because these cancers have a different pathophysiology and epidemiology [3]. Matched controls (1:1) were selected from study participants who were alive and free of cancer at the time the cases were identified. Controls were matched on age at randomization (+/-5 years) and date of blood draw (+/-30 days). Matched pairs where the case had missing values for the serum measurements (n = 5) were excluded from the study. Thus the analysis included 224 RCC cases and 224 matched controls.

Biomarker measurements and questionnaire data

All men enrolled in the study provided fasting serum samples at a baseline visit, prior to randomization, and these samples were stored at -70°C for future analyses. Serum folate and vitamin B₁₂ concentrations were determined by radioassay (Bio-Rad Laboratories, Richmond, CA, USA). Pyridoxal 5'-phosphate, the principal active form of vitamin B₆, was analyzed using the tyrosine decarboxylase apoenzyme method [27]. A high performance liquid chromatography (HPLC) method was used to measure cysteine and homocysteine concentrations [28], and riboflavin was measured using HPLC with fluorimetric detection [29]. Cases and matched controls were placed adjacently but in random order within analysis batches, and each batch also contained 6 blinded quality control samples derived from a pool of control serum. The within-batch coefficients of variation were calculated using nested components of variance analysis on logarithmically transformed quality control data [30]. The coefficients of variation were 6.2% for the folate analysis, 4.5% for vitamin B₆, 7.1% for vitamin B₁₂, 6.3% for cysteine, 4.8% for riboflavin, and 6.2% for homocysteine.

Each subject completed questionnaires on general background, health, smoking and physical activity histories and a separate detailed dietary history questionnaire at a baseline visit [25]. The diet questionnaire included 276 food items and mixed dishes, as well as 122 pictures to assist in portion size estimation. The questionnaire, intended to determine intake over the previous 12 months, was developed specifically for the ATBC study and was found to have good validity and reliability [31]. Data on supplement use were obtained from an open ended question regarding use of any vitamin or trace element preparation in the past two weeks, and a query of the name of the preparation and the daily dose. Subjects' height, weight and blood pressure were also measured by trained staff at the pre-randomization baseline visit.

Statistical analysis

Baseline characteristics of cases and matched controls were compared using McNemar's test for categorical variables and Wilcoxon signed rank tests for continuous variables. Spearman's rank order coefficients estimated the correlation of one-carbon biomarkers and nutrient intakes among control subjects. Odds ratios (ORs) and 95% confidence intervals (CIs) for the association between one-carbon metabolism biomarkers and risk of renal cell carcinoma were calculated using age-adjusted and multivariable-adjusted conditional logistic regression

models. Age as five year intervals was a matching criterion, but we also included age as a continuous variable in the models. Separate regression models were created for each biomarker, and quartiles were formed for each based on the distribution among the control subjects. Indicator variables were created for each quartile and the lowest quartile was used as the reference category. Tests for linear trend were conducted by creating a continuous variable in which each quartile was assigned the median concentration among controls in that quartile. Intakes of foods and individual nutrients were adjusted for total energy intake using the residual method [32].

Potential confounders were evaluated using forward and backward selection methods and multivariable models were developed by individually adding or subtracting covariates in the models. Variables associated with both the disease and the exposure that changed the risk estimates by >10% using both methods were considered confounders. Confounders differed among the models for different biomarkers, but included BMI (kg/m²), smoking (cigarettes per day and years smoked), fat, protein, methionine, and energy intake, and leisure-time physical activity. Serum folate was included as a covariate in the models for the other one-carbon metabolism biomarkers. Alcohol intake was not found to be a confounder, and inclusion of alcohol in the multivariable models did not change the results. Age, height, intervention group, education, high blood pressure, history of diabetes, and dietary intake of the one-carbon nutrients, fruit, vegetables, red meat and fish were not confounders and therefore were not included in any of the final models.

Interactions were assessed by adding cross-product terms to the multivariable models, and the statistical significance of interactions was determined by likelihood ratio tests. Cross-product terms were formed based on both dichotomous categorical exposures (split at the median among controls) and continuous exposures. Interactions that were significant using both categorical and continuous exposures were identified for further testing of joint effects (conditional regression) and stratified analyses (unconditional regression adjusted for matching factors). The effect of the combination of the six one-carbon biomarkers was assessed by comparing the RCC risk of subjects above the median in all biomarker concentrations (opposite for homocysteine) to that of subjects with below-median concentrations for any or all biomarkers. Sensitivity analyses including only cases with at least 2 years of follow-up were performed to minimize potential bias due to reverse causation from undiagnosed prevalent disease. Statistical analyses were performed using SAS software version 9.1.3 (SAS Institute, Inc., Cary, NC) and all p-values were two-sided.

Results

The baseline characteristics of cases and controls are shown in Table 1. Compared with controls, RCC cases were taller ($p = 0.01$) and smoked more cigarettes per day ($p < 0.01$). Cases also consumed fewer calories ($p = 0.04$) and less alcohol ($p = 0.03$), and participated in a lower amount of moderate or heavy leisure-time physical activity ($p = 0.01$). Non-significant differences included longer duration of smoking, lower dietary folate intake and lower serum folate concentration for cases compared with controls.

A number of the one-carbon biomarkers were significantly correlated among the control subjects (Table 2). Serum folate was correlated with all the biomarkers, with positive correlations for all but homocysteine. Homocysteine was correlated with cysteine and negatively correlated with folate and vitamin B₁₂. Dietary intakes of the one-carbon nutrients, adjusted for energy intake, were weakly but statistically significantly correlated with the corresponding biomarker. Spearman correlation coefficients were 0.15 for folate, 0.23 for vitamin B₆, 0.12 for vitamin B₁₂, 0.15 for cysteine, and 0.22 for riboflavin. Serum homocysteine was not significantly correlated with dietary folate ($r = -0.07$).

We observed a non-significant inverse association between serum folate and RCC risk (Table 3). There was no evidence of a dose-response trend across quartiles, but rather a similar decreased risk for each quartile compared to the lowest quartile. An analysis comparing the lowest quartile of serum folate to the top three quartiles combined yielded a significantly increased risk of RCC for the lowest quartile (OR = 1.68, 95% CI 1.06-2.65), suggesting a threshold effect near a serum folate concentration of 6.6 nmol/L. To determine the robustness of this effect, we conducted a sensitivity analysis by varying the threshold. When the cutoff for serum folate was moved by 10% from 6.6 nmol/L down to 5.9 nmol/L or up to 7.3 nmol/L, the odds ratios were 1.66 (0.98-2.80) and 1.16 (0.76-1.78), respectively. Cut-points above 6.8 nmol/L consistently yielded lower odds ratios, suggesting that the increased risk applies only to the lowest concentrations of serum folate. We observed no significant association between RCC risk and any of the other one-carbon biomarkers examined (Table 3). Evaluation of threshold effects similar to that observed for folate did not yield significant associations for the other biomarkers. There were no meaningful interactions observed for any of the one-carbon biomarkers with smoking habits, BMI, height, or alcohol consumption (P-interaction > 0.05 for all).

In an analysis that combined all the biomarkers, a comparison of subjects with the hypothesized lowest risk profile (i.e. high concentrations of all the biomarkers except homocysteine) with those that did not meet this criteria yielded a reduced risk for the low-risk-profile group (OR = 0.24, 95% CI 0.05-1.13). The association did not reach statistical significance due to low numbers in the low-risk-profile group (n = 11). Given a lack of *a priori* evidence for protective effects of cysteine, its indirect role in one-carbon metabolism, and the relatively high correlation between homocysteine and cysteine in our study, we also performed the combined biomarker analysis excluding cysteine from the risk profile. This increased the numbers in the low-risk group (n = 22), but a significant effect was not observed (OR = 0.62, 95% CI 0.26-1.49).

Analyses that excluded the 28 cases that occurred during the first 2 years of follow-up found no evidence of reverse causation due to existing prevalent disease. The multivariable odds ratios for serum folate quartiles, compared to the first quartile, were 0.57 (0.31-1.04), 0.55 (0.29-1.02) and 0.58 (0.30-1.14, p-trend = 0.12) respectively. For the threshold analysis comparing the first quartile to the other three quartiles combined, the association remained significant, with an odds ratio of 1.77 (1.07-2.93). The only nutrient for which the exclusion of early cases made an impact on the results was cysteine, which demonstrated a stronger but still non-significant inverse association. Compared with the first quartile, the multivariable odds ratios for the subsequent cysteine quartiles were 0.56 (0.24-1.35), 0.59 (0.21-1.70) and 0.38 (0.13-1.16, p-trend = 0.10).

Discussion

We conducted a nested case-control study within the ATBC Study to examine the association between one-carbon metabolism biomarkers and risk of renal cell carcinoma. To our knowledge, this study is the first to examine biomarkers of one-carbon metabolism in association with RCC. We observed a significant increased risk of RCC for men with low folate status, defined as serum folate concentrations near or below 6.6 nmol/L and corresponding to the lowest quartile of serum folate in our population. A possible inverse association was observed for serum folate when analyzed by quartiles, but there was no evidence of a linear trend. There was no evidence of an association with RCC for vitamin B₆, vitamin B₁₂, cysteine, riboflavin or homocysteine.

The apparent threshold effect for folate occurred near 6.6 nmol/L, which corresponds to the concentration associated with clinical deficiency and risk of megaloblastic anemia [33]. A

sensitivity analysis indicated that the effect near 6.6 nmol/L is a threshold and not simply an artifact of our quartile-based analysis. A threshold effect is biologically plausible given the hypothesized mechanism of action, which is inadequate methylation and/or DNA synthesis due to reduced activity of the one-carbon metabolism pathway. A certain minimum level of folate may be necessary to allow the one-carbon transfer required for maintenance of adequate DNA synthesis and/or methylation. Inadequate folate availability could disrupt these processes and increase cancer risk. Increasing the available folate above the minimum required level may not result in a further decrease in risk because an adequate level of one-carbon transfer has already been attained. If this is the case, the lack of a linear trend across quartiles of serum folate is not surprising. It is increasingly being recognized that the effects of folate exposure on carcinogenesis are complicated and likely vary by both dosage and timing. The idea of a nonlinear threshold relationship with increasing folate beyond an optimum exposure has been previously discussed, particularly in the context of colorectal cancer [34,35]. Although we observed an increased risk of RCC among subjects with the lowest concentrations of serum folate, we did not observe any association between serum homocysteine and RCC risk. The lack of association is noteworthy because elevated serum homocysteine can be an indicator of folate deficiency, although other metabolic pathways also influence homocysteine concentrations and the correlation between serum homocysteine and serum folate was modest (-0.28) in our study.

Some cohort studies, but not all, have found associations between higher fruit and vegetable intake and reduced risk of RCC [7-11]. A recent pooled analysis of 13 prospective studies, including the ATBC study, found a significant protective effect of increased fruit and vegetable consumption [12]. Fruits and vegetables are primary sources of dietary folate, particularly in studies conducted prior to folic acid fortification or in non-fortified populations such as Finland [36-38], so the observed associations may support a role of folate intake. However, fruits and vegetables are also sources of numerous other components thought to impact health and carcinogenesis, so other non-folate explanations for the associations are possible. A recent study that examined only the ATBC population did not find an association between fruit and vegetable consumption and RCC risk [11], possibly due to lower power relative to the pooled analysis or lower intake of fruits and vegetables compared to other prospective studies [12]. A role for one-carbon metabolism in RCC risk is supported by a candidate gene study that reported significant associations between single nucleotide polymorphisms in one-carbon metabolism genes and RCC risk [24]. The MTHFR gene is a key regulator of one-carbon metabolism, and a variant allele known to decrease MTHFR activity [39] was associated with increased risk of RCC in a population from Central and Eastern Europe. Additional polymorphisms in the TYMS gene were also associated with RCC risk, particularly among people with low folate intake.

A major strength of our study is that it is prospective, with all data being collected prior to disease diagnosis. The use of serum biomarkers for the one-carbon factors may be a better indication of absorbed and biologically active dose compared to self-reported nutrient intake. Serum folate tends to reflect short-term folate balance [40], but still has good predictive value as an indicator of systemic folate status [33,41]. The prospective nature of the exposure assessment minimizes the potential of biomarker concentrations being altered by existing cancers (reverse causation). Recall bias is also minimized because information on risk factors and diet were collected at baseline, and biased control selection is not an issue since cases and controls were selected from the same study cohort. Our study also included a long follow-up time, allowing identification of a relatively large number of RCC cases and examination of the effect of biomarker concentrations over an extended period. Finland does not have mandatory fortification of grain products with folic acid, so our study population included subjects with low folate status that would rarely be seen in fortified populations. The mean ($\pm$ standard error) serum folate concentration for subjects in our study was 8.6 ($\pm$ 0.2) nmol/L, compared to 26.9 ($\pm$ 0.5) nmol/L in a United States population after fortification [38]. Finally, we were able to

investigate the impact of multiple biomarkers of one-carbon metabolism rather than just folate alone.

Our study was conducted in a cohort of male Finnish smokers, and therefore may not be generalizable to other populations. Smoking is associated with a modest increased risk of RCC [3], and smokers have been found to have lower serum and red blood cell folate concentrations than non-smokers [42]. The reduced folate status of smokers may be due to lower intake of folate as well as greater body requirements due to the effects of tobacco smoke [42-44]. Folate intake in the ATBC cohort was relatively low, with 75% of control subjects having an intake below the U.S. Dietary Reference Intake of 400 ug/day. Although we adjusted for smoking in all models (both number of cigarettes smoked per day and years of smoking), the possibility of residual confounding remains. However, there was no significant interaction for the folate association by smoking dose or duration, and an analysis restricted to subjects who reported smoking 20 or more cigarettes per day showed similar associations between folate and RCC risk (data not shown). In addition, adjustment for smoking cessation during the ATBC trial did not change the risk estimates for serum folate and RCC. The role of folate and one-carbon metabolism in RCC risk needs to be further examined in populations including nonsmokers and women. However, studies conducted in populations with folic acid fortification of grain products may not be able to examine effects of deficient folate status. Further limitations of our study include the fact that assessment of the biomarkers in this study occurred at a single point in time, so they may not have captured lifetime exposure and may have changed over the course of the long follow-up period. Finally, serum biomarkers may not correlate with tissue-specific concentrations in the kidney.

In conclusion, we found that low serum folate concentrations were associated with a non-significant increased renal cell carcinoma risk. Analysis of a threshold effect near the level of folate deficiency showed a moderate and statistically significant increased risk of RCC. There was no evidence of a dose-response trend across quartiles, so the association with serum folate appears to be restricted to concentrations considered deficient. Given the mandatory fortification of grain products with folic acid in the United States and the resulting increase in population folate status (< 1% of the U.S. population has deficient concentrations of serum folate) [45], folate deficiency is unlikely to play a large role in RCC risk in the United States and other fortified populations. However, folate may be a risk factor in other parts of the world where folic acid fortification is not widespread and deficient status is more prevalent. We found no associations for the other one-carbon biomarkers examined (vitamin B₆, vitamin B₁₂, cysteine, riboflavin and homocysteine). A role for one-carbon metabolism in renal cancer development is biologically plausible, but epidemiologic evidence regarding such a role is limited. Further studies are needed to more clearly elucidate the relationship between one-carbon metabolism and renal cell carcinoma, particularly for folate intake and status in other populations.

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Table 1

Baseline characteristics (medians and interquartile ranges, or percents) for renal cell carcinoma cases and controls in male Finnish smokers

Characteristic	Cases (n= 224)	Controls (n= 224)	P-value ^a
Age at randomization (y)	57 (54-61)	57 (54-61)	0.09
Height (cm)	175 (171-178)	174 (168-178)	0.01
BMI (kg/m ²)	26.7 (24.5-29.4)	25.9 (24.0-28.6)	0.13
Systolic blood pressure (mmHg)	142 (132-158)	142 (130-158)	0.54
Diastolic blood pressure (mmHg)	90 (82-96)	88 (82-94)	0.16
Education %(n)			
Elementary or less	79.5 (178)	78.6 (176)	0.82
Smoking			
Cigarettes per day ^b	20 (15-25)	20 (14-20)	<0.01
Years smoked	38 (32-42)	37 (31-41)	0.06
History of diabetes %(n)	4.0 (9)	1.8 (4)	0.27
Occupational activity %(n)			
Moderate/Heavy	25.5 (57)	20.5 (46)	0.20
Leisure physical activity %(n)			
Moderate/Heavy	54.5 (122)	67.0 (150)	0.01
Serum biomarkers			
Folate (nmol/L)	7.7 (6.1-9.9)	8.2 (6.6-10.2)	0.06
Vitamin B ₆ (pmol/ml)	24.5 (16.8-37.2)	25.1 (16.9-36.3)	0.44
Vitamin B ₁₂ (pmol/L)	349 (272-440)	353 (286-433)	0.43
Cysteine (nmol/ml)	301 (258-344)	300 (261-349)	0.36
Riboflavin (pmol/ml)	8.7 (6.4-11.9)	8.7 (6.8-11.7)	0.81
Homocysteine (nmol/ml)	13.2 (11.1-15.7)	13.2 (11.0-15.6)	0.78
Dietary intake/day ^c			
Energy (kcal)	2502 (2105-2946)	2684 (2170-3105)	0.03
Folate (μg)	332 (271-383)	333 (283-411)	0.07
Vitamin B ₆ (mg)	2.4 (2.0-2.9)	2.5 (2.0-2.9)	0.42
Vitamin B ₁₂ (μg)	10.4 (7.3-13.0)	10.2 (7.9-12.7)	0.53
Riboflavin (mg)	2.8 (2.2-3.4)	2.8 (2.4-3.5)	0.25
Methionine (mg)	1978 (1589-2369)	1980 (1675-2331)	0.64
Protein (g)	90 (75-108)	92 (77-108)	0.49
Fat (g)	115 (93-140)	119 (95-144)	0.28
Fruit (g)	177 (84-279)	189 (104-310)	0.48
Vegetables (g)	677 (535-829)	732 (563-887)	0.25
Red meat (g)	62.9 (48.1-85.8)	60.5 (46.3-79.2)	0.70
Alcohol (g/day)	7.4 (1.4-18.5)	11.2 (2.9-23.7)	0.03
Supplement use %(n) ^d			
Folate	4.5 (10)	6.7 (15)	0.32

Characteristic	Cases (n= 224)	Controls (n= 224)	P-value ^a
Vitamin B ₆	11.6 (26)	13.0 (29)	0.69
Vitamin B ₁₂	6.3 (14)	8.0 (18)	0.48
Riboflavin	8.9 (20)	11.6 (26)	0.38

^a Wilcoxon signed rank test for continuous variables, McNemar's test for categorical variables

^b Medians are equivalent for cases and controls (1 pack per day) but groups are significantly different by Wilcoxon signed rank test

^c 210 cases, 208 controls for dietary intake and foods; unadjusted intake, excluding supplements

^d Supplement use includes single nutrient and combination supplements

Table 2

Spearman correlation coefficients for serum biomarkers among controls in male Finnish smokers.

	Folate	Vitamin B ₆	Vitamin B ₁₂	Cysteine	Riboflavin	Homocysteine
Folate (nmol/L)	1.00	0.27*	0.26*	0.15*	0.27*	-0.28*
Vitamin B ₆ (pmol/ml)		1.00	0.12	0.08	0.27*	-0.06
Vitamin B ₁₂ (pmol/L)			1.00	-0.01	0.29*	-0.19*
Cysteine (nmol/ml)				1.00	0.11	0.35*
Riboflavin (pmol/ml)					1.00	-0.10
Homocysteine (nmol/ml)						1.00

* P-value < 0.01

Table 3

Age- and multivariable-adjusted odds ratios for one-carbon metabolism biomarkers and renal cell carcinoma in male Finnish smokers.

Biomarker concentration ^a	No. of cases (n=224)	No. of controls (n=224)	Age-adjusted		Multivariable-adjusted ^b	
			OR ^c	95% CI ^c	OR	95% CI
Folate (nmol/L)						
≤6.64	81	56	1.00	(referent)	1.00	(referent)
6.65 – 8.18	46	56	0.60	(0.36-1.00)	0.62	(0.35-1.08)
8.19 – 10.19	47	56	0.57	(0.33-0.99)	0.52	(0.29-0.93)
>10.19	50	56	0.61	(0.36-1.04)	0.67	(0.37-1.20)
P for trend				0.10		0.19
>6.64 ^d	143	168	1.00	(referent)	1.00	(referent)
≤6.64	81	56	1.68	(1.10-2.55)	1.68	(1.06-2.65)
Vitamin B ₆ (pmol/ml)						
≤16.91	57	56	1.00	(referent)	1.00	(referent)
16.92 – 25.12	58	56	1.03	(0.59-1.79)	1.25	(0.69-2.26)
25.13 – 36.27	49	56	0.90	(0.52-1.57)	1.15	(0.62-2.13)
>36.27	60	56	1.08	(0.63-1.85)	1.33	(0.73-2.42)
P for trend				0.76		0.43
Vitamin B ₁₂ (pmol/L)						
≤286.41	67	56	1.00	(referent)	1.00	(referent)
286.42 – 353.07	46	56	0.65	(0.38-1.13)	0.63	(0.34-1.16)
353.08 – 433.38	53	56	0.76	(0.44-1.33)	0.93	(0.49-1.76)
>433.38	58	56	0.86	(0.50-1.48)	0.87	(0.46-1.63)
P for trend				0.80		0.98
Cysteine (nmol/ml)						
≤260.85	60	56	1.00	(referent)	1.00	(referent)
260.86 – 299.98	51	56	0.77	(0.37-1.59)	0.73	(0.33-1.61)
299.99 – 349.19	61	56	0.90	(0.39-2.11)	0.84	(0.33-2.15)
>349.19	52	56	0.78	(0.33-1.85)	0.65	(0.24-1.77)
P for trend				0.66		0.43
Riboflavin (pmol/ml)						

Biomarker concentration ^a	No. of cases (n=224)	No. of controls (n=224)	Age-adjusted		Multivariable-adjusted ^b	
			OR ^c	95% CI ^c	OR	95% CI
≤6.78	66	56	1.00	(referent)	1.00	(referent)
6.79 – 8.71	46	56	0.65	(0.36-1.16)	0.54	(0.29-1.02)
8.72 – 11.73	52	56	0.81	(0.48-1.37)	0.69	(0.39-1.23)
>11.73	60	56	0.92	(0.55-1.56)	0.76	(0.42-1.40)
P for trend				0.90		0.68
Homocysteine (nmol/ml)						
≤11.03	54	56	1.00	(referent)	1.00	(referent)
11.04 – 13.21	58	56	1.04	(0.59-1.82)	1.13	(0.62-2.06)
13.22 – 15.55	56	56	0.98	(0.55-1.74)	0.93	(0.50-1.74)
>15.55	56	56	1.02	(0.59-1.75)	0.92	(0.51-1.66)
P for trend				0.99		0.60

^aQuartiles based on serum concentration in control subjects

^bAll models adjusted for age (continuous), BMI (continuous; weight(kg)/height (m)²) and smoking (number of cigarettes and years smoked). Folate additionally adjusted for protein (g/day) and fat (g/day). Vitamin B6, riboflavin and homocysteine additionally adjusted for serum folate (quartile). Vitamin B12 additionally adjusted for protein, leisure-time physical activity (moderate/heavy or not) and serum folate. Cysteine additionally adjusted for methionine (quartile of adjusted intake) and serum folate.

^cOR, odds ratio; CI, confidence interval

^dThreshold analysis