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Circulating concentrations of B group vitamins and urothelial cell carcinoma

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B-group vitamins, as components of the one carbon metabolism pathway, are involved in DNA synthesis, repair and methylation. Our aim was to investigate associations between circulating plasma levels of B vitamins and urothelial cell carcinoma (UCC). We conducted a nested case–control study of UCC within the Melbourne Collaborative Cohort Study. B vitamins were measured in pre-diagnostic plasma samples. Conditional logistic regression was used to estimate odds ratios (OR) for UCC risk associated with circulating B vitamins in 363 matched cases and controls. In a case-only analysis (N = 390), hazard ratios (HR) for overall survival associated with plasma B vitamins were estimated using Cox regression. There were no strong associations between UCC risk and pre-diagnostic levels of plasma B vitamins. No heterogeneity in UCC risk was observed by subtype (invasive or superficial), sex, smoking status or alcohol intake. There was no heterogeneity by country of birth for most B vitamins, except for folate (p-homogeneity = 0.03). In UCC cases, there were no strong associations between plasma B vitamins and overall survival. We found no associations between pre-diagnostic plasma concentrations of B-group vitamins and UCC risk or survival.

Key words: B vitamins, urothelial cell carcinoma, bladder cancer, Melbourne Collaborative Cohort Study

Abbreviations: BMI: body mass index; CI: confidence interval; CIS: Carcinoma *in Situ*; DAG: directed acyclic graph; EPIC: European Prospective Investigation into Cancer and Nutrition; FFQ: food frequency questionaire; FMN: flavin mononucleotide; HR: hazard ratio; ICD: International Classification of Diseases; MCCS: Melbourne Collaborative Cohort Study; MDS: Mediterranean diet score; OR: odds ratio; Q: quartile; UCC: urothelial cell carcinoma; VCR: Victorian Cancer Registry; WCRF: World Cancer Research Fund

Additional Supporting Information may be found in the online version of this article.

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Introduction

Urothelial cell carcinoma (UCC), of which about 90% originates in the bladder, is one of the most common urological cancers, and is the fourth most commonly diagnosed cancer in males in developed countries.[1,2] More than half of all cases are superficial but recurrence is common, constituting a substantial healthcare burden.[3] Bladder cancer survival is low, especially for women, for whom the 5-year relative survival in 2009–2013 in Australia was 46%, compared to 55% for men.[4] Apart from age and sex, the most consistent risk factors are cigarette smoking and occupational exposure to carcinogens such as aromatic amines and polycyclic hydrocarbons which are excreted in the urine.[2,5] Similarly, dietary factors could be involved in the aetiology of UCC given that the urothelium is also exposed to diet-related carcinogens during excretion.

B-group vitamins such as B2, B6, B9 and B12 are involved in the one-carbon metabolism pathway and play a major role in DNA synthesis, repair and methylation.[6] The association between B vitamin intakes and risk of cancers other than UCC has been reported previously from the Melbourne Collaborative Cohort Study (MCCS)[7–10] but the literature related to UCC risk is limited. The World Cancer Research Fund

What's new?

B vitamins are involved in DNA synthesis, repair, and methylation, and therefore they might affect cancer risk. While they appear to be protective against some cancers, data on the effect of B-vitamins on bladder cancer have been inconsistent. In this prospective study, the authors examined levels of circulating B vitamins in patients with urothelial-cell carcinoma (UCC) and matched controls. However, they found no association between pre-diagnostic concentrations of B vitamins and UCC risk or survival.

(WCRF) second expert report concluded that there was only limited evidence to suggest that milk (which is high in vitamin B2) protects against bladder cancer[11] but the WCRF Continuous Update Project on diet and bladder cancer based on fewer but more recent studies reported that the evidence was too limited to confirm an association.[12] Both reports concluded there was insufficient evidence for an association between folate and bladder cancer. Of the few studies that have examined associations between B-vitamins and UCC risk, the majority used dietary data collected with food frequency questionnaires (FFQs).[13–16] A Spanish case–control study reported inverse associations between B2, B6, B9 and B12 intakes and bladder cancer.[14] A recent meta-analysis reported an inverse association between dietary folate and bladder cancer but this finding was restricted to case–control studies and not present in cohort studies,[15] consistent with the null association we found in a recent MCCS analysis of FFQ-derived dietary intakes of B vitamins.[17] Dietary measures have limitations for assessing the association between these nutrients and UCC. Plasma measures of B-group vitamins may provide a better exposure assessment than reported dietary intakes as the bioavailability of B-group vitamins may be compromised by some drugs, smoking and alcohol intake,[18] gut flora and absorption characteristics (e.g. chronic gastritis[19]), liver function or individual genetic factors.[20,21] However, we identified only one study that investigated the association of blood measures of B-group vitamins with UCC.[22] This hospital-based case–control study only investigated folate and reported an inverse association between circulating levels of folate and UCC risk.

While most of the evidence has focussed on occurrence of cancer, higher vitamin B6 concentration has been reported to be inversely associated with survival from prostate cancer and renal cell carcinoma.[23,24] This suggests that circulating levels of B-group vitamins may be informative for both the risk of and survival from UCC.

The primary aim of our study was to use the MCCS to investigate prospectively the association between pre-diagnostic concentrations of circulating B-group vitamins and UCC risk, and for those who develop UCC, the association between pre-diagnostic plasma B vitamins and survival. Secondary aims were to examine whether associations with risk varied by disease subtype, and in participant subgroups whose nutritional status may be compromised according to smoking status, alcohol consumption and country of birth.

Material and Methods

Study population

The MCCS is a prospective cohort study of 41,513 women and men aged 27 to 75 years (99% were between 40 and 69 years) when recruited between 1990 and 1994.[25] Italian and Greek migrants were over-sampled to extend the range of lifestyle exposures. Participants were recruited *via* the electoral rolls (registration to vote is compulsory for adults in Australia), advertisements, and community announcements in local media (e.g. television, radio, and newspapers). Comprehensive lists of Italian and Greek surnames in the phone book and electoral rolls were also used to target southern European migrants. The Cancer Council Victoria's Human Research Ethics Committee approved the study protocol. Participants gave written consent to participate and for the investigators to obtain access to their medical records. Vital status and cause of death information were obtained *via* linkage to the National Death Index of Australia.

The nested case–control study

We conducted a case–control study nested within the MCCS (Supporting Information Fig. S1). Participants were excluded if they had a diagnosis of UCC or a cancer with an unknown primary site when the blood sample was collected, or if they had missing data for any of the matching variables. Incident cases were identified from first diagnoses of UCC (morphology codes 8120, 8122, 8130 and 8131 of the International Classification of Diseases, ICD-0-3) to the Victorian Cancer Registry (VCR) or the Australian Cancer Database (for cases diagnosed outside Victoria) to 31 December 2012. Controls were selected by incidence density sampling using age as the time axis and matched to cases on year of birth, country of birth, sex, type of blood sample (buffy coat, Guthrie card or lymphocyte) and sample collection period (baseline (study entry) or follow-up). Although, all blood samples used for measurements of B vitamins were collected at study entry (1990–94) before UCC diagnosis, this nested case–control sample was originally set up to investigate associations between DNA methylation and UCC risk, which required matching on blood sample type.[26]

Participants with a primary diagnosis of UCC (excluding ICD-0-3 C529 and those classified with uncertain behaviour) were included as cases. Disease subtypes were defined according to behaviour, with invasive UCC including any tumour that had penetrated or invaded the basement membrane (pT1-4). Superficial UCC included papillary transitional/

urothelial cell neoplasm of low malignant potential or Carcinoma *in Situ* (CIS) that was completely confined within the epithelium (pTa-pTcis).

We excluded participants with missing data for any of the confounders (N = 14). For the conditional logistic regression analyses we excluded any cases or controls without a matched pair.

Biochemical analyses

After drawing the blood, plasma fractions were stored in liquid nitrogen tanks at −196 °C until shipment to the laboratory for analysis. Plasma samples were sent on dry ice to the Bevital A/S laboratory (http://www.bevital.no) in Bergen, Norway for measurements of plasma concentrations of vitamin B2 (riboflavin plus flavin mononucleotide (FMN)), vitamin B3 (nicotinamide), vitamin B6 (pyridoxal 5′-phosphate, its active form), vitamin B9 (folate, predominantly 5-methyltetrahydrofolate) and vitamin B12 (cobalamin). Cotinine was also measured as an indicator of recent smoking behaviour. Concentrations of vitamin B2, B3, B6, and cotinine were determined by mass spectrometry-based methods (liquid chromatography coupled to tandem mass spectrometry)[27] and microbiological methods were used to determine concentrations of B9 (*Lactobacillus casei*)[28] and B12 (*Lactobacillus leich mannii*).[29]

Assessment of other risk factors

A structured interview schedule was used to obtain information on potential risk factors including age, sex, country of birth, alcohol consumption, and smoking status. Height and weight were measured, and body mass index was calculated from these (BMI = weight (kg)/height $(m)^2$). Participants completed a food frequency questionnaire that was developed from a study of weighed food records in 810 Melburnians of similar demographics to the cohort.[30] Nutrient intakes were calculated using mean sex-specific portion sizes from the weighed food records.[30] The Mediterranean Diet Score (MDS) was calculated using intakes of vegetables, fruit, cereals, legumes, fish, dairy, red meat, olive oil and alcohol[31] and used as a measure of diet quality.

Statistical analysis

Quartile (Q) cut-points for each circulating B vitamin were calculated from the distribution in controls and applied to the whole sample. Conditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between UCC risk and plasma B vitamin concentrations relative to the lowest quartile. Log-linear trends were estimated using the base 2 logarithm ($\log_2$) of each B vitamin as a continuous variable; the estimates from these models can be interpreted as ORs for a doubling in the B vitamin concentration.

Potential confounders were identified from a Directed Acyclic Graph (DAG)[32] based on existing literature regarding risk factors for UCC. All models were adjusted for smoking status (never, current, former), cotinine level (0 nmol/l; >0 to ≤85 nmol/l; >85 to <1,000 nmol/l; ≥1,000 nmol/l), alcohol intake (none, low intake:1-39 g/day (men), 1-19 g/day (women); moderate/high intake: ≥40 g/day (men), ≥20 g/day (women)), BMI (continuous), country of birth (Australia/New Zealand/United Kingdom/other, Greece and Italy), and MDS (score from 0 to 9). Effect modification was assessed by testing two-way interaction terms (using the likelihood ratio test) between each B vitamin and sex, country of birth, smoking status, alcohol intake and time between blood collection and diagnosis (≤10 years and > 10 years).

Interactions were fitted between UCC subtype (invasive or superficial) and each B vitamin and results of UCC risk and plasma B vitamins by UCC subtype from these models are also presented. As some of the B vitamin concentrations were correlated, we also performed a principal component analysis of the correlation matrix of the log-transformed (base 2) B vitamins (as they were positively skewed), followed by orthogonal (varimax) rotation of the components obtained from the principal component analysis[33,34] to identify any distinct combinations of these measures. These components were then included in a conditional logistic regression model to estimate ORs and 95% CIs for the association between each principal component analysis-defined combination of B vitamins and UCC risk.

For the survival analysis, Cox proportional hazards regression models were fitted to data from UCC cases, using time since diagnosis as the timescale, to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for overall survival. Person-time began at date of UCC diagnosis and ended at the earliest of date of death, date left Australia or 31 December 2014 (the date at which death data was complete). Models were adjusted for country of birth, sex, smoking status, cotinine level, alcohol intake, BMI, age at UCC diagnosis and MDS score (0–3; 4–6; 7–9).

To investigate departure from linearity in the relationship between plasma B vitamins and overall UCC risk and survival in UCC cases we included a quadratic term for each plasma B vitamin and used the likelihood ratio test to test for a quadratic trend. We performed sensitivity analyses (1) restricted to participants who were not using multi-vitamins at the time of blood collection; too few people were using multivitamins to assess associations in users, and (2) excluding participants with a cancer diagnosis at any site prior to blood collection. Tests based on Schoenfeld residuals showed no evidence of violation of the proportional hazards assumption except for the MDS so we stratified the Cox models on this variable. All statistical tests were two sided. Statistical analyses were performed using Stata/MP 14.2® (Stata Corporation, College Station, TX).

Results

B vitamins were measured in pre-diagnostic plasma samples for 790 participants (Supporting Information Fig. S1). After excluding 14 participants who had missing data for any of the

Table 1. Participant characteristics at blood collection (baseline)

	Cases (n = 363)	Controls (n = 363)
Age[1]	61 (7)	61 (7)
BMI[1] kg/m^2	27.3 (3.7)	27.1 (3.6)
Riboflavin[2] nmol/L	21 [16, 32]	23 [17, 34]
FMN[2] (flavin mononucleotide) nmol/L	6.5 [5.2, 8.6]	7.3 [5.2, 9.2]
Total B2[2,3] nmol/L	29 [22, 40]	30 [23, 43]
Vitamin B3[2] (Nicotinamide) nmol/L	440 [335, 598]	462 [324, 599]
Vitamin B6[2] (Pyridoxal 5′-phosphate) nmol/L	41 [30, 58]	42 [31, 59]
Vitamin B9[2] (Folate) nmol/L	13 [10, 17]	13 [9, 17]
Vitamin B12[2] (Cobalamin) pmol/L	404 [328, 489]	388 [312, 485]
Invasive cases, N (%)	159 (43.8)	
Smoking status, N (%)		
Never	114 (31.4)	162 (44.6)
Former	185 (51.0)	158 (43.5)
Current	64 (17.6)	43 (11.8)
Cotinine level, N (%)		
0 nmol/L	158 (43.5)	179 (49.3)
>0 and 85 nmol/L	136 (37.5)	133 (36.6)
>85 and < 1,000 nmol/L	19 (5.2)	22 (6.1)
≥1,000 nmol/L	50 (13.8)	29 (8.0)
Ethanol intake, N (%)		
None	99 (27.3)	84 (23.1)
Low	204 (56.2)	227 (62.5)
Moderate/high	60 (16.5)	52 (14.3)

[1]Mean (SD).
[2]Median [25th, 75th percentile].
[3]Total B2 = Riboflavin + FMN.

confounders (alcohol intake, N = 2; smoking status, N = 1; MDS, N = 2) or for any of the plasma B vitamins (N = 12) there were 390 cases and 386 controls. For the analysis of UCC risk (conditional logistic regression), we further excluded 50 participants (27 cases and 23 controls) without a matched pair, leaving 726 available participants (363 cases and 363 controls). Cases with complete data (N = 390) were used for the analysis of UCC survival.

Participant characteristics at the time of blood collection are given in Table 1. Cases had slightly lower levels of total B2, riboflavin, FMN and B3, and higher B12 and were more likely to be current or former smokers. Pearson correlation coefficients between the base 2 log transformed B vitamin measures are shown in Table 2. Total B2 is the sum of riboflavin and FMN, and as expected showed high to moderate correlations with riboflavin (r = 0.98) and FMN (r = 0.54). Riboflavin and FMN were moderately correlated (r = 0.36). Moderate correlations were also observed for vitamin B6 and riboflavin (r = 0.35), total B2 (r = 0.35) and B9 (r = 0.25), all other correlations were weak.

Table 2. Pearson correlation coefficients for plasma B vitamins[1]

	Riboflavin	FMN	Total B2	B3	B6	B9
FMN	0.36					
Total B2[2]	0.98	0.54				
B3	0.07	−0.07	0.05			
B6	0.35	0.15	0.35	0.09		
B9	0.12	0.04	0.11	−0.13	0.25	
B12	0.14	−0.02	0.12	0.02	0.13	0.06

p > 0.05 to p < 0.30 between B3 and each of B2, FMN & total B2 and between FMN & B9. p > 0.60 between B12 and both FMN & B9. All other p < 0.04.
[1]Correlations between $\log_2$ of plasma concentrations.
[2]Total B2 = Riboflavin + FMN.

Table 3 shows the ORs for UCC risk in relation to plasma B vitamin levels, overall and by UCC subtype. There was no evidence of a quadratic trend for any of the B vitamins (p > 0.09). There was a weak inverse association between UCC risk and total B2 ($OR_{Q4\ vs\ Q1}$ = 0.66 (0.42, 1.05); p_{trend} = 0.11) with a 16% reduction in UCC risk for a doubling of plasma B2. No associations were seen between UCC risk and other B vitamins, nor were there differences by subtype except for vitamin B9 where there was a weak positive association with superficial disease (p = 0.04) but no association for invasive disease (p-homogeneity = 0.06). There were no differences in the ORs for UCC risk and any of the B vitamins by sex, smoking status, alcohol intake, time between blood collection and diagnosis or country of birth, except for

Table 3. Odds ratios (ORs) and 95% confidence intervals (CIs) for UCC risk in relation to circulating B vitamins

	All UCC		Invasive		Superficial		
	Cases	OR[1] (95% CI)	Cases	OR[2] (95% CI)	Cases	OR[2] (95% CI)	P[3]
Riboflavin nmol/L							
Q1 (‹16.7)	112	1.00	57	1.00	55	1.00	
Q2 (16.7–23.7)	107	0.99 (0.64, 1.51)	45	0.78 (0.40, 1.50)	62	1.18 (0.67, 2.06)	
Q3 (23.8–34.7)	63	0.54 (0.33, 0.86)	24	0.43 (0.21, 0.87)	39	0.65 (0.34, 1.24)	
Q4 (›34.7)	81	0.74 (0.47, 1.17)	33	0.59 (0.29, 1.18)	48	0.90 (0.49, 1.65)	
Linear model	363	0.86 (0.71, 1.04)	159	0.83 (0.63, 1.11)	204	0.88 (0.68, 1.15)	0.77
p-trend[4]		0.12		0.21		0.35	
FMN nmol/L							
Q1 (‹5.2)	92	1.00	35	1.00	57	1.00	
Q2 (5.2–7.3)	128	1.32 (0.90, 1.94)	44	1.20 (0.64, 2.25)	84	1.45 (0.88, 2.40)	
Q3 (7.3–9.2)	67	0.72 (0.47, 1.10)	36	1.06 (0.56, 2.02)	31	0.55 (0.30, 1.00)	
Q4 (›9.2)	76	0.86 (0.55, 1.34)	44	1.47 (0.73, 2.93)	32	0.53 (0.28, 0.99)	
Linear model	363	0.86 (0.69, 1.06)	159	1.02 (0.73, 1.42)	204	0.76 (0.56, 1.01)	0.19
p-trend[4]		0.16		0.92		0.06	
Total B2[5] nmol/L							
Q1 (‹23.4)	118	1.00	56	1.00	62	1.00	
Q2 (23.4–31.0)	97	0.83 (0.54, 1.25)	44	0.84 (0.45, 1.59)	53	0.81 (0.47, 1.42)	
Q3 (31.1–43.1)	71	0.64 (0.41, 1.01)	23	0.43 (0.21, 0.86)	48	0.88 (0.48, 1.60)	
Q4 (›43.1)	77	0.66 (0.42, 1.05)	36	0.61 (0.30, 1.22)	41	0.72 (0.39, 1.33)	
Linear model	363	0.84 (0.67, 1.04)	159	0.84 (0.61, 1.16)	204	0.83 (0.62, 1.11)	0.96
p-trend[4]		0.11		0.29		0.21	
B3 nmol/L							
Q1 (‹329)	87	1.00	43	1.00	44	1.00	
Q2 (329–468)	112	1.19 (0.78, 1.80)	51	1.50 (0.80, 2.78)	61	1.00 (0.56, 1.76)	
Q3 (469–602)	77	0.94 (0.60, 1.46)	29	0.81 (0.41, 1.58)	48	1.02 (0.56, 1.85)	
Q4 (›602)	87	0.98 (0.61, 1.56)	36	0.82 (0.39, 1.70)	51	1.08 (0.58, 2.00)	
Linear model	363	1.01 (0.80, 1.28)	159	0.97 (0.69, 1.37)	204	1.05 (0.76, 1.43)	0.75
p-trend[4]		0.93		0.86		0.78	
B6 nmol/L							
Q1 (‹31.3)	104	1.00	52	1.00	52	1.00	
Q2 (31.3–42.6)	87	1.03 (0.67, 1.58)	33	0.64 (0.33, 1.25)	54	1.45 (0.83, 2.51)	
Q3 (42.7–59.3)	88	1.00 (0.64, 1.56)	40	0.79 (0.41, 1.52)	48	1.14 (0.63, 2.08)	
Q4 (›59.3)	84	1.02 (0.65, 1.62)	34	0.92 (0.45, 1.87)	50	1.09 (0.61, 1.94)	
Linear model	363	1.03 (0.87, 1.22)	159	0.98 (0.75, 1.29)	204	1.06 (0.86, 1.30)	0.66
p-trend[4]		0.73		0.90		0.59	
B9 nmol/L							
Q1 (‹9.3)	84	1.00	45	1.00	39	1.00	
Q2 (9.3–12.9)	96	1.15 (0.74, 1.78)	40	0.72 (0.36, 1.45)	56	1.54 (0.87, 2.72)	
Q3 (12.9–17.0)	92	1.18 (0.78, 1.79)	35	0.61 (0.32, 1.18)	57	1.92 (1.09, 3.38)	
Q4 (›17.0)	91	1.27 (0.81, 2.00)	39	0.77 (0.37, 1.59)	52	1.73 (0.96, 3.12)	
Linear model	363	1.13 (0.91, 1.41)	159	0.89 (0.63, 1.25)	204	1.35 (1.01, 1.81)	0.06
p-trend[4]		0.27		0.49		0.04	
B12 pmol/L							
Q1 (‹313)	78	1.00	36	1.00	42	1.00	
Q2 (313–392)	93	1.15 (0.75, 1.78)	45	0.87 (0.45, 1.71)	48	1.42 (0.79, 2.55)	
Q3 (393–485)	98	1.24 (0.78, 1.96)	41	0.77 (0.38, 1.58)	57	1.78 (0.97, 3.28)	
Q4 (›485)	94	1.22 (0.79, 1.90)	37	1.16 (0.56, 2.41)	57	1.25 (0.72, 2.18)	
Linear model	363	1.07 (0.83, 1.36)	159	1.10 (0.66, 1.84)	204	1.06 (0.80, 1.40)	0.88
p-trend[4]		0.61		0.71		0.70	

[1]Adjusted for smoking status, alcohol intake, cotinine level, BMI and MDS, conditioning on individual case set.
[2]Adjusted for smoking status, alcohol intake, cotinine level, BMI and MDS and includes an interaction between UCC subtype and each B vitamin, conditioning on individual case set.
[3]*p*-value for homogeneity from Likelihood Ratio Test.
[4]Assessed by $\log_2$ of plasma concentrations.
[5]Total B2 = Riboflavin + FMN.

Table 4. Odds ratios[1] (ORs) and 95% confidence intervals (CIs) for UCC risk in relation to circulating B vitamins by demographic and lifestyle factors, and time since blood collection

	Riboflavin	FMN	Total B2[2]	B3	B6	B9	B12
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Sex							
Men	0.87 (0.69, 1.09)	0.78 (0.61, 1.01)	0.83 (0.65, 1.07)	1.04 (0.78, 1.38)	1.03 (0.84, 1.27)	1.06 (0.82, 1.39)	1.12 (0.84, 1.48)
Women	0.84 (0.58, 1.21)	1.17 (0.73, 1.86)	0.85 (0.56, 1.28)	0.96 (0.63, 1.44)	1.03 (0.78, 1.34)	1.28 (0.88, 1.87)	0.91 (0.54, 1.52)
p-homogeneity[3]	0.89	0.14	0.94	0.74	0.97	0.42	0.49
Country of birth							
Australia/NZ/UK	0.91 (0.73, 1.14)	0.76 (0.57, 1.02)	0.88 (0.68, 1.13)	1.01 (0.76, 1.34)	1.04 (0.87, 1.24)	0.96 (0.74, 1.25)	0.98 (0.70, 1.38)
Italy	0.69 (0.41, 1.16)	1.10 (0.73, 1.67)	0.73 (0.42, 1.29)	0.92 (0.54, 1.56)	1.12 (0.63, 1.99)	2.12 (1.21, 3.70)	1.08 (0.71, 1.64)
Greece	0.79 (0.47, 1.31)	0.85 (0.48, 1.52)	0.75 (0.42, 1.34)	1.19 (0.63, 2.24)	0.87 (0.49, 1.53)	1.12 (0.58, 2.14)	1.48 (0.70, 3.11)
p-homogeneity[3]	0.58	0.35	0.79	0.83	0.79	0.03	0.61
Smoking							
Never	0.91 (0.68, 1.21)	1.16 (0.78, 1.71)	0.92 (0.66, 1.28)	1.14 (0.79, 1.65)	1.05 (0.83, 1.34)	1.20 (0.83, 1.74)	1.10 (0.75, 1.61)
Former	0.80 (0.62, 1.05)	0.67 (0.49, 0.92)	0.75 (0.56, 1.02)	0.90 (0.65, 1.24)	1.02 (0.80, 1.31)	1.01 (0.75, 1.36)	1.10 (0.78, 1.56)
Current	0.95 (0.56, 1.62)	1.10 (0.56, 2.15)	0.96 (0.52, 1.77)	1.16 (0.61, 2.21)	0.98 (0.64, 1.50)	1.50 (0.85, 2.63)	0.71 (0.26, 1.96)
p-homogeneity[3]	0.76	0.07	0.59	0.58	0.95	0.45	0.72
Alcohol							
None	0.84 (0.58, 1.21)	0.91 (0.61, 1.36)	0.82 (0.54, 1.24)	0.99 (0.64, 1.52)	0.97 (0.75, 1.27)	0.93 (0.64, 1.36)	1.39 (0.85, 2.29)
Low	0.89 (0.70, 1.13)	0.79 (0.59, 1.06)	0.85 (0.65, 1.11)	1.04 (0.76, 1.44)	1.09 (0.87, 1.36)	1.21 (0.90, 1.62)	1.03 (0.73, 1.47)
Moderate/High	0.78 (0.48, 1.26)	1.06 (0.62, 1.83)	0.81 (0.47, 1.40)	0.96 (0.57, 1.60)	0.98 (0.62, 1.55)	1.34 (0.79, 2.30)	0.78 (0.44, 1.41)
p-homogeneity[3]	0.88	0.62	0.98	0.95	0.79	0.43	0.31
Time between blood collection and diagnosis							
≤ 10 years	0.88 (0.66, 1.16)	0.97 (0.71, 1.34)	0.90 (0.66, 1.23)	0.96 (0.66, 1.40)	1.09 (0.87, 1.38)	1.19 (0.85, 1.68)	0.99 (0.70, 1.41)
> 10 years	0.85 (0.65, 1.09)	0.77 (0.58, 1.04)	0.78 (0.58, 1.05)	1.04 (0.77, 1.40)	0.97 (0.78, 1.22)	1.09 (0.82, 1.45)	1.14 (0.81, 1.61)
p-homogeneity[3]	0.85	0.30	0.50	0.74	0.47	0.70	0.58

[1]Assessed by $\log_2$ of plasma concentrations; adjusted for smoking status, alcohol intake, cotinine level, BMI and MDS including an interaction between each B vitamin and each demographic or lifestyle factor or time between blood collection and diagnosis, conditioning on individual case set.
[2]Total B2 = Riboflavin + FMN.
[3]*p*-value from Likelihood Ratio Test.

Table 5. Hazard ratios[1] (HRs) and 95% CIs for risk of all-cause mortality for UCC cases in relation to circulating B vitamins

	All UCC	
	Deaths	**HR (95% CI)**
Riboflavin nmol/L		
Q1 (‹16.7)	53	1.00
Q2 (16.7–23.7)	52	1.19 (0.80, 1.77)
Q3 (23.8–34.7)	36	1.10 (0.69, 1.76)
Q4 (›34.7)	32	1.08 (0.68, 1.73)
Linear model	173	1.07 (0.88, 1.31)
p-trend[2]		0.48
FMN nmol/L		
Q1 (‹5.2)	43	1.00
Q2 (5.2–7.3)	56	0.89 (0.59, 1.35)
Q3 (7.3–9.2)	37	0.99 (0.62, 1.57)
Q4 (›9.2)	37	1.03 (0.65, 1.63)
Linear model	173	0.93 (0.73, 1.17)
p-trend[2]		0.53
Total B2[3] nmol/L		
Q1 (‹23.4)	51	1.00
Q2 (23.4–31.0)	55	1.41 (0.94, 2.13)
Q3 (31.1–43.1)	36	1.10 (0.69, 1.75)
Q4 (›43.1)	31	1.16 (0.72, 1.87)
Linear model	173	1.06 (0.84, 1.33)
p-trend[2]		0.64
B3 nmol/L		
Q1 (‹329)	42	1.00
Q2 (329–468)	62	0.93 (0.62, 1.40)
Q3 (469–602)	30	0.64 (0.39, 1.04)
Q4 (›602)	39	0.84 (0.54, 1.32)
Linear model	173	0.93 (0.73, 1.18)
p-trend[2]		0.54
B6 nmol/L		
Q1 (‹31.3)	55	1.00
Q2 (31.3–42.6)	45	0.92 (0.60, 1.39)
Q3 (42.7–59.3)	41	0.69 (0.44, 1.07)
Q4 (›59.3)	32	0.73 (0.45, 1.18)
Linear model	173	0.87 (0.72, 1.06)
p-trend[2]		0.17
B9 nmol/L		
Q1 (‹9.3)	41	1.00
Q2 (9.3–12.9)	49	1.38 (0.88, 2.17)
Q3 (12.9–17.0)	43	1.28 (0.82, 2.02)
Q4 (›17.0)	40	0.82 (0.50, 1.33)
Linear model	173	1.04 (0.84, 1.27)
p-trend[2]		0.74
B12 pmol/L		
Q1 (‹313)	40	1.00
Q2 (313–392)	46	0.85 (0.55, 1.30)
Q3 (393–485)	42	0.65 (0.41, 1.04)
Q4 (›485)	45	0.93 (0.59, 1.45)
Linear model	173	0.98 (0.75, 1.27)
p-trend[2]		0.86

[1]Adjusted for smoking status, alcohol intake, cotinine level, BMI, MDS, country of birth, sex and age at UCC diagnosis.
[2]Assessed by $\log_2$ of plasma concentrations.
[3]Total B2 = Riboflavin + FMN.

vitamin B9 by country of birth (p-homogeneity = 0.03); participants of Italian origin had an increased risk of UCC (OR = 2.12 (1.21, 3.70) for a doubling of plasma B9) and no association was found for other participants (Table 4).

After the principal components analysis we used the varimax rotation which derived 3 components characterised by (i) high loadings on B2, B6 and B9 (ii) high loadings on B3 and low loadings on B9 and (iii) high loadings on B12. These components explained 72% of the total variance, but none were associated with UCC risk (data not shown).

A total of 390 cases, 173 of whom died, were included in the survival analyses (Table 5). There was no evidence of a quadratic trend for any of the B vitamins ($p > 0.16$). There were no associations between any of the plasma B vitamins and overall survival in the UCC cases.

Consistent results for UCC risk and overall survival were observed after restricting to participants who were not taking multivitamins at time of blood collection (N = 292 matched case–control pairs and N = 346 cases for the UCC risk and survival analyses respectively) and after excluding anyone who had been diagnosed with cancer at any site prior to blood collection (N = 335 matched case–control pairs and N = 373 cases for the UCC risk and survival analyses respectively).

Discussion

We did not find strong evidence that the risk of UCC was associated with plasma concentrations of B-group vitamins. Smoking, alcohol, or country of birth did not appear to modify associations with UCC risk. There was also no evidence that B-group vitamins were differently associated with risk of superficial or invasive tumours. No association between plasma B vitamins and overall survival of UCC cases was observed.

Our study had the advantage of assessing plasma concentrations of B-group vitamins prior to diagnosis, avoiding any effects related to UCC such as treatment, physiological changes due to the disease process or behavioural changes due to illness. Another strength of our study was the availability of a wide range of potential confounders to take into consideration in the analyses, including cotinine, as an additional control for smoking exposure.

Weaknesses of our study included having only a single blood measure for analysis. However, vitamins B2, B6, B9 and

B12 had good within-person reproducibility (ICCs range from 0.61 to 0.87) in plasma from healthy women in the Nurses' Health Study collected on two occasions 1–2 years apart.[35] Other biomarkers of B vitamin status such as homocysteine and methylmalonic acid were unavailable.

The European Prospective Investigation into Cancer and Nutrition (EPIC) study reported inverse associations between plasma B6 and the risk of breast,[36] gastric,[37] colorectal[38] and lung cancer[39] and a positive association for prostate cancer.[40] Inverse associations between plasma B6 and renal cell carcinoma risk and survival have also been reported by EPIC and the MCCS.[24] We found no evidence for an association of B6 with UCC incidence or survival.

The EPIC study reported a positive association between folate and lung cancer in smokers.[39] A Swedish cohort study reported elevated ORs for all quintiles of plasma concentrations of folate and risk of colorectal cancer (CRC) relative to the lowest quintile, with the middle quintile associated with the highest risk.[41] We did not see any association of folate (B9) with UCC except in the Italian-born subgroup. This positive association we observed is not consistent with our hypotheses and could be due to chance.

The EPIC study also reported inverse associations between circulating vitamin B2 and risk of breast,[36] gastric[37] and colorectal[38] cancers; a positive association with prostate cancer[40] and no association with renal cell carcinoma[24] or lung cancer.[39] A recent meta-analysis reported inverse associations between serum vitamin B2 and other nutrients from the one carbon metabolism pathway (B6, B9 and B12) with renal cell cancer risk.[42] We found a weak inverse association between vitamin B2 and UCC risk. Animal studies have shown that vitamin B2 has anti-carcinogenic properties, such as detoxifying pathogens that can potentially affect the integrity of the epithelium at body sites like the lungs and oesophagus, but its role in risk of developing UCC in humans remains unclear.[43,44]

Dietary studies from the MCCS[7–10] have examined associations between B vitamin intakes and risk for several cancers but have only found inconsistent or weak associations. We also found no association between dietary intakes of B vitamins and UCC in our prospective cohort.[17] These findings are in contrast to a large Spanish case–control study[14] which reported protective associations with bladder cancer risk for B2, B6, B9 and B12 intakes. Risk of recall bias and reverse causation are a potential limitation of retrospective case–control studies.[45] A recent meta-analysis reported an inverse association between folate intake and UCC risk in case–control studies, but not in cohort studies.[15] In addition to the problems of measuring dietary intakes, individuals vary in their ability to absorb B vitamins and their metabolism of these, hence dietary intake may not accurately reflect the bioavailability of B vitamins.[20]

Our results do not support associations between any of the B-group vitamins we investigated with either UCC risk or survival.

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