original article

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Dietary and supplemental intake of one-carbon nutrients and the risk of type I and type II endometrial cancer: a prospective cohort study

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Background: Type I and II endometrial cancer are biologically and clinically distinct, with type II cancers having a high frequency of p53 mutations and an association with chromosomal instability. This raises the hypothesis that one-carbon nutrients (folate, methionine, and the enzymic cofactors vitamins B2, B6, and B12), which mediate chromosomal stability and DNA methylation, may be protective for type II but not type I endometrial cancer.
Methods: Using a prospective cohort of 23 356 postmenopausal women followed 20 years, we estimated the relative risks (RRs) of type I (N = 471) and II (N = 71) endometrial cancers according to intake of one-carbon nutrients, adjusting for confounders.
Results: No associations were observed between dietary or supplemental intake of any one-carbon nutrient and risk of type I cancer. For type II cancer, positive associations were due to supplemental, rather than dietary, intake of these nutrients: supplemental folate (RR = 1.80 for >228.6 versus 0 μg/day; P trend = 0.027) and vitamins B2 (RR = 1.94 for >1.70 versus 0 mg/day; P trend = 0.011), B6 (RR = 2.08 for >2.00 versus 0 mg/day; P trend = 0.012), and B12 (RR = 2.10 for >3.43 versus 0 μg/day; P trend = 0.0060).
Conclusion: Contrary to our hypothesis, use of supplements containing folate and vitamins B2, B6, and B12 was associated with an increased risk of type II endometrial cancer.
Key words: one-carbon nutrients, diet, endometrial cancer, prospective study

introduction

In the United States, endometrial cancer is the most common malignancy of the female reproductive tract [1]. Epidemiologic research has identified several risk factors for the development of this malignancy, such as obesity, unopposed estrogen exposure, and diabetes [2–5]. However, currently known risk factors do not completely explain endometrial cancer risk. Recently, attention has focused on dietary intake in order to identify food and nutrients that might be associated with an increased or decreased risk of developing endometrial cancer [6–11].

Alterations in one-carbon metabolism have been hypothesized to be carcinogenic, in part mediated by chromosomal instability and altered DNA methylation. Negri et al. [12] suggested an inverse association between folate intake and the risk of this malignancy, which was replicated in a case–control study by Xu et al. [13]. Conversely, others have reported no association between folate intake and endometrial cancer risk [14, 15].

One possible reason for the discrepancies observed in the literature is the failure to stratify endometrial cancer on the basis of the two main histological categories: type I endometrioid and type II non-endometrioid [16]. This pathological classification reflects important differences in terms of clinical characteristics, molecular abnormalities and prognosis. From a molecular point of view, type II endometrial cancer is often associated with *p53* mutations, which commonly lead to DNA derangement, chromosomal instability and a more aggressive clinical behavior [17]. Women affected by type II cancers have a higher incidence of advanced-stage disease at diagnosis, a marked tendency to recurrence, and consequently a worse prognosis [18]. Further, uterine carcinosarcoma and type II endometrial cancers share similar molecular characteristics [19] and have similar clinical outcomes [20]. Conversely, *PTEN* (phosphatase and tensin homolog) mutations are an early event in the vast majority of type I cancers, along with other

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commonly mutated genes in type I including *Bcl-2, KRAS*, and *β-catenin*. Alterations of *p53* have been reported in a small proportion of type I tumors and, when they occur, they are usually a late event [17].

Based on this literature, we hypothesized that low intake of nutrients in the one-carbon metabolism pathway, such as folate and methionine, as well as the related enzymic cofactors (vitamins B2, B6, and B12), might be specifically associated with an increased risk of type II endometrial cancer, since this subtype is most strongly associated with altered DNA methylation and possible chromosomal instability.

methods

study population and dietary assessment

This study was reviewed and approved by the human subjects review board at the University of Minnesota. A detailed description of the Iowa Women's Health Study (IWHS) has been previously published [21]. Briefly, 41 836 older women, age 55–69 years, were enrolled in 1986. Participants completed a self-administered survey that included a semiquantitative food-frequency questionnaire (FFQ), which was adapted from a 126-item instrument developed by Willett et al. [22]. For each food, a commonly used portion size or unit was specified, and respondents were asked how often on average over the last year they had consumed that amount of each food item. Respondents were also asked if they used multivitamins (including frequency of use) as well as selected supplements including any regular use of folic acid, B-complex vitamins, and vitamin B6. For the latter supplements, dose and duration of use were not collected.

The daily intake of total energy and selected nutrients was calculated by multiplying the frequency of consumption of each unit of food by the nutrient content of the specified portions, using the Harvard Food Composition Database, which is based on U.S. Department of Agriculture data [23]. Three types of nutrient variables were considered: intake from diet only, intake from supplements only, and combined dietary and supplemental intake.

The FFQ was found to be reliable and valid in this population [24]. The reproducibility of this FFQ instrument given 6 months apart as measured by Pearson's correlation coefficient (r) was quite high for folate ($r = 0.76$) and vitamins B2 ($r = 0.83$), B6 ($r = 0.88$), and B12 ($r = 0.73$). With respect to validity, energy-adjusted intakes assessed by the FFQ estimate and five 24-h recalls were moderately correlated for folate ($r = 0.43$) and more strongly correlated for vitamins B2 ($r = 0.93$), B6 ($r = 0.69$), and B12 ($r = 0.76$).

cohort follow-up and case ascertainment

Vital status of cohort members was ascertained via follow-up surveys (1987, 1989, 1992, 1997 and 2004) and yearly linkage with Iowa death certificates and the National Death Index through 2005. Migration out of Iowa has been low for this cohort, and we have estimated that <1% of the cohort has been lost to follow-up [25].

Incident endometrial cancer cases were identified through annual linkage with the Iowa Cancer Registry, a Surveillance, Epidemiology and End Results (SEER) program member. The Iowa Cancer Registry collects cancer data including primary site and morphology. All pathology data were derived from pathology reports of the diagnosing pathologist, and there was no central review. Topographic and morphologic data were coded according to the International Classification of Diseases for Oncology (ICD-O), 2nd [26] or 3rd [27] edition. Using these codes, we classified endometrial cancer into type I or type II (Table 1), based on prior literature [16–20] and systematic review of the ICD-O codes by the authors (SU and AM).

data analysis

Women with history of cancer before baseline, except nonmelanoma skin cancer ($n = 3830$), hysterectomy before baseline ($n = 14\,350$), extreme dietary intake (<600 or >5000 kcal/day), or incomplete FFQs (≥30 blank food items) ($n = 3096$) or who were not postmenopausal at baseline ($n = 569$) were excluded from the present analysis, yielding a final sample size of 23 356 study participants. Each woman accumulated person-years of follow-up from baseline to the date of endometrial cancer diagnosis, move from Iowa, death, or administrative censoring on 31 December 2005.

Data were descriptively summarized using means and standard deviations for continuous variables and frequencies and percents for categorical variables. We assessed pairwise associations of the one-carbon nutrient variables with each other using Spearman correlation coefficients. Relative risks (RRs) and 95% confidence intervals (CIs) examining the association between exposure variables and risk of endometrial cancer were estimated using Cox proportional hazards regression models, modeling age as the time variable [28]. Separate analyses were carried out for type I and type II endometrial cancer. For each, the outcome variable of interest was endometrial cancer of that specific type, and women with endometrial cancer not of that type were censored at the time of diagnosis. Nutrient variables were categorized into approximate quartiles based on their distribution of consumption of all women in the analytic cohort at study baseline. Tests for trend were carried out by ordering the intake quartiles from lowest to highest and including the resulting variable as a 1 degree-of-freedom linear term in the Cox regression models.

We formally determined whether risk ratios for the exposure variables differed by type of endometrial cancer using a competing risk form of Cox proportional hazards regression [29]. This approach allowed us to specifically model and test the ordered interaction between a given risk factor (modeled as a covariate) and endometrial cancer type (included as a stratum variable). Following the analyses of categorized intake variables, we re-assessed associations using the actual continuously distributed nutrient values via penalized smoothing splines or P-splines [30]. Briefly, this is a nonparametric modeling approach that is a generalization of polynomial splines. It allowed us to examine the unrestricted association of the nutrient variables with endometrial cancer, without regard to functional form. All models described above initially accounted for total energy and other potential confounding (Table 2). Total energy was modeled as a continuous covariate in the Cox model and was included to adjust for systematic over- and underreporting of food intake [31]. Subsequent analyses assessed the independent associations of the nutrient variables with risk of endometrial cancer by simultaneously including each in one multivariate Cox model, along with the potential confounding variables listed above. All statistical tests were two sided, and analyses were carried out using the SAS (SAS Institute Inc., Cary, NC) and S-Plus (Insightful, Seattle, WA) software systems.

results

During the 20-year follow-up period, we identified a total of 542 incident cases, including 471 cases of type I endometrial cancer and 71 cases of type II endometrial cancer (including carcinosarcoma). The mean age at diagnosis of type I endometrial cancer was 71.8 years (range 57.2–89.6), while the mean age at diagnosis of type II endometrial cancer was 72.8 years (range 60.2–89.3).

Table 2 describes the correlation of folate intake with key endometrial cancer risk factors. Higher intake was associated with greater intake of total energy, lower body mass index, greater use of hormone replacement therapy, less current

Table 1. Definition of type I and II endometrial cancer

Morphology code	Morphology	Type I endometrial cancer		Type II endometrial cancer	
		n	%	*n*	%
8000	Neoplasm, malignant	1	0.2		
8010	Carcinoma, NOS	3	0.6		
8041	Small-cell carcinoma, NOS	1	0.2		
8050	Papillary carcinoma, NOS			1	1.4
8140	Adenocarcinoma, NOS	343	72.8		
8210	Adenocarcinoma in adenomatous polyp	1	0.2		
8260	Papillary adenocarcinoma			13	18.3
8262	Villous adenocarcinoma	1	0.2		
8263	Adenocarcinoma in tubulovillous adenoma	2	0.4		
8310	Clear cell adenocarcinoma, NOS			7	9.9
8323	Mixed cell adenocarcinoma			1	1.4
8380	Endometrioid adenocarcinoma, NOS	91	19.3		
8441	Serous cystadenocarcinoma, NOS			5	7.0
8460	Papillary serous cystadenocarcinoma			27	38.0
8480	Mucinous adenocarcinoma	2	0.4		
8560	Adenosquamous carcinoma	13	2.8		
8570	Adenocarcinoma with squamous metaplasia	13	2.8		
8950	Mullerian mixed tumor			10	14.1
8951	Mesodermal mixed tumor			1	1.4
8980	Carcinosarcoma, NOS			6	8.5
Total		471	100	71	100

NOS, not otherwise specified.

Table 2. Distribution of baseline (1986) factors by level of folate intake

Variable	Total folate intake (diet and supplements)			
	Quartile 1 (<250.2 μg/day), *N* = 5840	Quartile 2 (250.2–348.6 μg/day), *N* = 5838	Quartile 3 (348.7–560.9 μg/day), *N* = 5839	Quartile 4 (>560.9 μg/day), *N* = 5839
Age (years), mean ± SD	61.9 ± 4.2	62.2 ± 4.2	62.2 ± 4.1	62.3 ± 4.2
Body mass index (kg/m^2), mean ± SD	27.0 ± 5.1	27.0 ± 5.2	26.9 ± 5.2	26.4 ± 4.9
Waist-to-hip ratio, mean ± SD	0.84 ± 0.08	0.84 ± 0.09	0.84 ± 0.08	0.83 ± 0.08
Total energy (kcal/day), mean ± SD	1394 ± 397	1796 ± 446	2094 ± 634	1955 ± 683
Smoking (pack-years), mean ± SD	11.6 ± 19.1	9.4 ± 18.0	8.5 ± 16.9	8.9 ± 17.3
Adult-onset diabetes (%)	4.7	6.2	6.0	5.8
History of hypertension (%)	35	35	34	34
Age at menarche >12 years (%)	59	59	58	59
Age at menopause >50 years (%)	59	62	64	65
Ever-used hormone replacement therapy (%)	24	25	26	31
Smoking history (%)				
Current	20	15	14	13
Former	19	19	18	21
Never	61	66	68	66
Any alcohol use (%)	42	47	48	47

SD, standard deviation.

smoking, and slightly greater use of alcohol, although absolute differences were very modest. There was little association of folate intake with age at study baseline, waist-to-hip ratio, prevalence of adult-onset diabetes, history of hypertension, age at menarche, and age at menopause.

Table 3 summarizes the results for one-carbon nutrients and risk of type I and II endometrial cancer. There was no association of total, dietary, or supplemental intake of folate or vitamins B2 and B12 with risk of type I endometrial cancer. There was also no association of total or supplemental intake of vitamins B6 with risk of type I endometrial cancer, while there were suggestive but not statistically significant inverse trends for dietary intake of vitamin B6 (RR = 0.79 for highest versus lowest quartile of intake, 95% CI 0.55–1.13, *P* trend = 0.25)

Table 3. Nutrients implicated in the one-carbon metabolism and the risk of type I and type II endometrial cancer

Nutrient		Level of intake	Person-years	Type I			Type II		
				Events	RR[a] (95% CI)	*P*	Events	RR[a] (95% CI)	*P*
Folate (μg/day)	Total	43.5–250.1	94 093	115	1.00 (reference)	0.59	18	1.00 (reference)	0.09
		250.2–348.6	93 944	107	0.85 (0.64–1.13)		15	0.93 (0.45–1.95)	
		348.7–560.9	93 772	128	1.08 (0.81–1.44)		13	0.97 (0.44–2.12)	
		>560.9	92 110	121	1.00 (0.76–1.32)		25	1.71 (0.87–3.35)	
	Food only	43.5–225.1	93 312	114	1.00 (reference)	0.74	18	1.00 (reference)	0.76
		225.2–293.8	93 668	118	1.00 (0.76–1.31)		23	1.48 (0.75–2.9)	
		293.9–373.6	93 589	119	0.93 (0.70–1.25)		14	1.05 (0.47–2.3)	
		>373.6	93 350	120	1.09 (0.79–1.52)		16	1.34 (0.55–3.23)	
	Supplements only	0	264 898	319	1.00 (reference)	0.56	42	1.00 (reference)	**0.027**
		0.1–228.6	22 056	44	1.61 (1.15–2.26)		4	1.33 (0.47–3.72)	
		>228.6	86 965	108	1.00 (0.80–1.25)		25	1.80 (1.07–3.02)	
Vitamin B6 (mg/day)	Total	0.21–1.71	93 923	121	1.00 (reference)	0.97	14	1.00 (reference)	**0.012**
		1.72–2.39	95 272	102	0.72 (0.54–0.95)		16	1.40 (0.64–3.04)	
		2.40–3.80	93 217	133	1.06 (0.80–1.40)		16	1.71 (0.78–3.75)	
		>3.80	91 507	115	0.88 (0.66–1.17)		25	2.42 (1.17–4.99)	
	Food only	0.21–1.49	94 596	128	1.00 (reference)	0.25	20	1.00 (reference)	0.60
		1.50–1.92	93 141	107	0.74 (0.56–0.97)		18	1.11 (0.55–2.24)	
		1.93–2.41	93 346	122	0.80 (0.59–1.07)		19	1.27 (0.59–2.75)	
		>2.41	92 836	114	0.79 (0.55–1.13)		14	1.23 (0.47–3.24)	
	Supplements only	0	243 670	290	1.00 (reference)	0.40	37	1.00 (reference)	**0.012**
		0.01–2.00	71 382	106	1.23 (0.97–1.55)		15	1.53 (0.83–2.81)	
		>2.00	58 866	75	1.01 (0.77–1.31)		19	2.08 (1.16–3.73)	
Vitamin B12 (μg/day)	Total	0.03–5.78	94 026	115	1.00 (reference)	0.72	15	1.00 (reference)	0.21
		5.79–11.10	94 527	116	0.97 (0.74–1.28)		17	1.36 (0.65–2.85)	
		11.11–16.66	93 132	115	0.98 (0.75–1.30)		21	1.56 (0.77–3.17)	
		>16.66	92 235	125	1.05 (0.79–1.39)		18	1.58 (0.75–3.33)	
	Food only	0.03–4.76	93 066	114	1.00 (reference)	0.84	22	1.00 (reference)	0.33
		4.77–7.70	94 720	111	0.92 (0.70–1.23)		11	0.61 (0.28–1.36)	
		7.71–14.42	92 832	128	1.09 (0.83–1.44)		19	1.15 (0.58–2.25)	
		>14.42	93 302	118	0.97 (0.71–1.31)		19	1.19 (0.57–2.48)	
	Supplements only	0	247 271	293	1.00 (reference)	0.25	37	1.00 (reference)	**0.0060**
		0.01–3.43	35 940	57	1.28 (0.95–1.74)		6	1.27 (0.53–3.04)	
		>3.43	90 708	121	1.10 (0.88–1.36)		28	2.10 (1.26–3.50)	
Vitamin B2 (mg/day)	Total	0.23–1.61	94 423	112	1.00 (reference)	0.50	13	1.00 (reference)	**0.026**
		1.62–2.34	93 864	113	1.03 (0.78–1.36)		18	1.84 (0.85–3.96)	
		2.35–3.57	93 977	125	1.14 (0.85–1.51)		18	2.12 (0.97–4.63)	
		>3.57	91 655	121	1.08 (0.81–1.44)		22	2.41 (1.13–5.13)	
	Food only	0.23–1.40	95 486	118	1.00 (reference)	0.98	17	1.00 (reference)	0.25
		1.41–1.84	91 324	116	0.99 (0.75–1.3)		19	1.42 (0.69–2.95)	
		1.85–2.39	93 880	114	0.96 (0.71–1.29)		22	2.04 (0.95–4.37)	
		>2.39	93 228	123	1.02 (0.71–1.45)		13	1.47 (0.56–3.88)	
	Supplements only	0	246 663	293	1.00 (reference)	0.18	37	1.00 (reference)	**0.011**
		0.01–1.70	47 026	65	1.09 (0.82–1.45)		11	1.73 (0.88–3.41)	
		>1.70	80 230	113	1.16 (0.93–1.46)		23	1.94 (1.12–3.34)	
Methionine (g/day)	Food only	0.18–1.36	93 314	115	1.00 (reference)	0.13	22	1.00 (reference)	0.36
		1.37–1.76	92 627	131	1.03 (0.79–1.36)		17	1.10 (0.55–2.23)	
		1.77–2.22	94 121	108	0.78 (0.57–1.06)		12	0.91 (0.39–2.13)	
		>2.22	93 857	117	0.82 (0.55–1.20)		20	1.89 (0.70–5.09)	
Multivitamins	Any use	No	250 324	300	1.00 (reference)	0.30	38	1.00 (reference)	**0.022**
		Yes	118 545	164	1.11 (0.91–1.36)		31	1.78 (1.09–2.92)	

[a]RRs and 95% CIs from Cox proportional hazards regression analysis, adjusted for age, total energy, body mass index, waist-to-hip ratio, diabetes, hypertension, age at menarche, age at menopause, use of hormone replacement therapy, smoking status, pack-years of smoking, and alcohol use. *P* value is a test for trend, assessing the dose–response effect of the variable of interest with risk of endometrial cancer.

RR, relative risk; CI, confidence interval. Significant *P* values (<0.05) are reported in bold.

and methionine (RR = 0.82 for highest versus lowest quartile of intake, 95% CI 0.55–1.20, *P* trend = 0.13). Multivitamin use was not associated with type I endometrial cancer.

In contrast to type I endometrial cancer, greater intake (highest versus lowest quartile) of total folate (RR = 1.71, 95% CI 0.87–3.35; *P* trend = 0.09), vitamin B2 (RR = 2.41, 95% CI 1.13–5.13; *P* trend = 0.026), and vitamin B6 (RR = 2.42, 95% CI 1.17–4.99; *P* trend = 0.012) were positively associated with risk of type II endometrial cancer. Methionine (only available from dietary intake) was not associated with risk of type II endometrial cancer (*P* trend = 0.36), although the top quartile of intake showed an excess risk (RR = 1.89, 95% CI 0.70–5.09) that was not statistically significant. Figure 1 shows the multivariate-adjusted associations of total folate and vitamins B2 and B6 with type I versus type II endometrial cancer using smoothing splines. These splines show the lack of association with type I endometrial cancer, a J-shaped association for folate with type II endometrial cancer, and mainly linear and positive associations for vitamins B2 and B6 with type II endometrial cancer. In formal testing for any difference in the association of the one-carbon nutrients between type I and type II endometrial cancer, we found strong evidence for differences for total vitamin B6 intake (*P* = 0.021), suggestive evidence for total vitamin B2 (*P* = 0.072), and limited evidence for total folate (*P* = 0.17).

Further inspection of these associations for type II endometrial cancer showed that they were mainly driven by supplemental versus dietary intake. Compared with no supplement use, there were positive associations with higher supplemental intake of folate (RR = 1.80 for >228.6 μg/day, 95% CI 1.07–3.02; *P* trend = 0.027) and vitamins B2 (RR = 1.94 for >1.70 mg/day, 95% CI 1.12–3.34; *P* trend = 0.011), B6 (RR = 2.08 for >2.00 mg/day, 95% CI 1.16–3.73; *P* trend = 0.012), as well as vitamin B12 (RR = 2.10 for >3.43 μg/day, 95% CI 1.26–3.50; *P* trend = 0.0060). Multivitamin use was associated with an increased risk of type II endometrial cancer (RR = 1.78, 95% CI 1.09–2.92; *P* trend = 0.022). The difference in risk ratios for type I versus type II endometrial cancer was statistically significant for intakes of supplemental vitamins B12 (*P* = 0.036) and B6 (*P* = 0.046), while there was suggestive evidence for intakes of supplemental folate (*P* = 0.068) and supplemental vitamin B2 (*P* = 0.067).

When the results for folate were stratified by era of diagnosis (1986–1996, 1997–2005; pre- and post-folate fortification in the United States), the associations reported in Table 3 were consistent with the overall results (data not shown).

Intake of total one-carbon nutrients was modestly to strongly correlated (range of r's: 0.37–0.89), and intake of these nutrients as supplements was very strongly correlated (range of r's: 0.82–0.97). We were able to fit a multivariate model for risk of type II endometrial cancer that simultaneously included all the one-carbon nutrients (as total intake) and the other endometrial cancer risk factors, and the association with vitamin B6 intake showed the clearest positive association (RRs = 1.00, 1.35, 1.56, 2.47), while the association with methionine intake attenuated (RRs = 1.00, 0.94, 0.73, 1.52), vitamin B2 intake became unstable (RRs = 1.00, 2.13, 1.90, 1.31), and intakes of folate (RRs = 1.00, 0.68, 0.56, 0.90) and vitamin B12 (RRs = 1.00, 0.89, 0.98, 0.82) were no longer associated with risk. The high correlations among intake of supplements precluded us from fitting a similar model.

discussion

Contrary to our initial hypothesis, we found that higher total intake of folate and vitamins B2 and B6 was associated with an increased risk of type II endometrial cancer, and this was mainly due to positive associations with the use of supplemental intake of these nutrients. Supplemental vitamin B12 intake was also associated with type II endometrial cancer risk. These results were not confounded by a wide variety of endometrial cancer risk factors, including body mass index, estrogen use, smoking, and alcohol use. In contrast, we found that one-carbon nutrients from the diet or supplements were not associated with type I endometrial cancer.

An accumulating body of evidence suggests that folate and B-vitamins may play a role in the etiology of several malignancies, including cancers of the colon, rectum, breast, pancreas, lung, and prostate [32–38]. A majority of the available reports indicate an inverse association between folate status and the risk of these malignancies [32–35]. Several experimental studies have substantiated these epidemiologic observations: the anticancer properties of folate appear to be due at least in part to its role in the synthesis of nitrogenous bases and in the mechanism of DNA replication and repair. Moreover, folate is crucial for the one-carbon metabolism, and an adequate folate status is essential for the production of *S*-adenosylmethionine, the donor of methyl groups involved in the methylation of DNA [39]. A normal pattern of DNA methylation is at the basis of gene silencing or expression and has been described to be substantially associated with a reduced risk of cancer [40].

However, the role of folate and perhaps other one-carbon nutrients in carcinogenesis may be more complex than it was initially believed [41]. Animal experiments suggest that modest supplementation of folic acid can be protective against the development of cancer, whereas higher intakes may facilitate tumor growth [42]. This dichotomous effect could be explained by the observation that the synthesis of nucleotides is essential not only for DNA repair processes but also for extensive DNA derangement [43]. Moreover, supplements contain folic acid that is more bioavailable than the form of folate in food [44] and thus they could be more potent in promoting cell replication. In this context, there have been provocative findings from epidemiologic studies that have observed a direct association between folic acid supplementation and the risk of cancer [36, 37]. An emerging view suggests that a very low folate status may be related to a higher risk of cancer; normal intake of folate is related to a modest protective effect, and higher levels of this nutrient may promote cancer [45].

Our finding of an association that is specific for folic acid supplementation and type II but not type I endometrial cancer could be due to the different characteristics of these two tumor subtypes. In support of this hypothesis, folate receptors have been shown to be preferentially overexpressed in cells of high-grade non-endometrioid cancers [43]. Our study is one of the few investigating the association between folate intake and

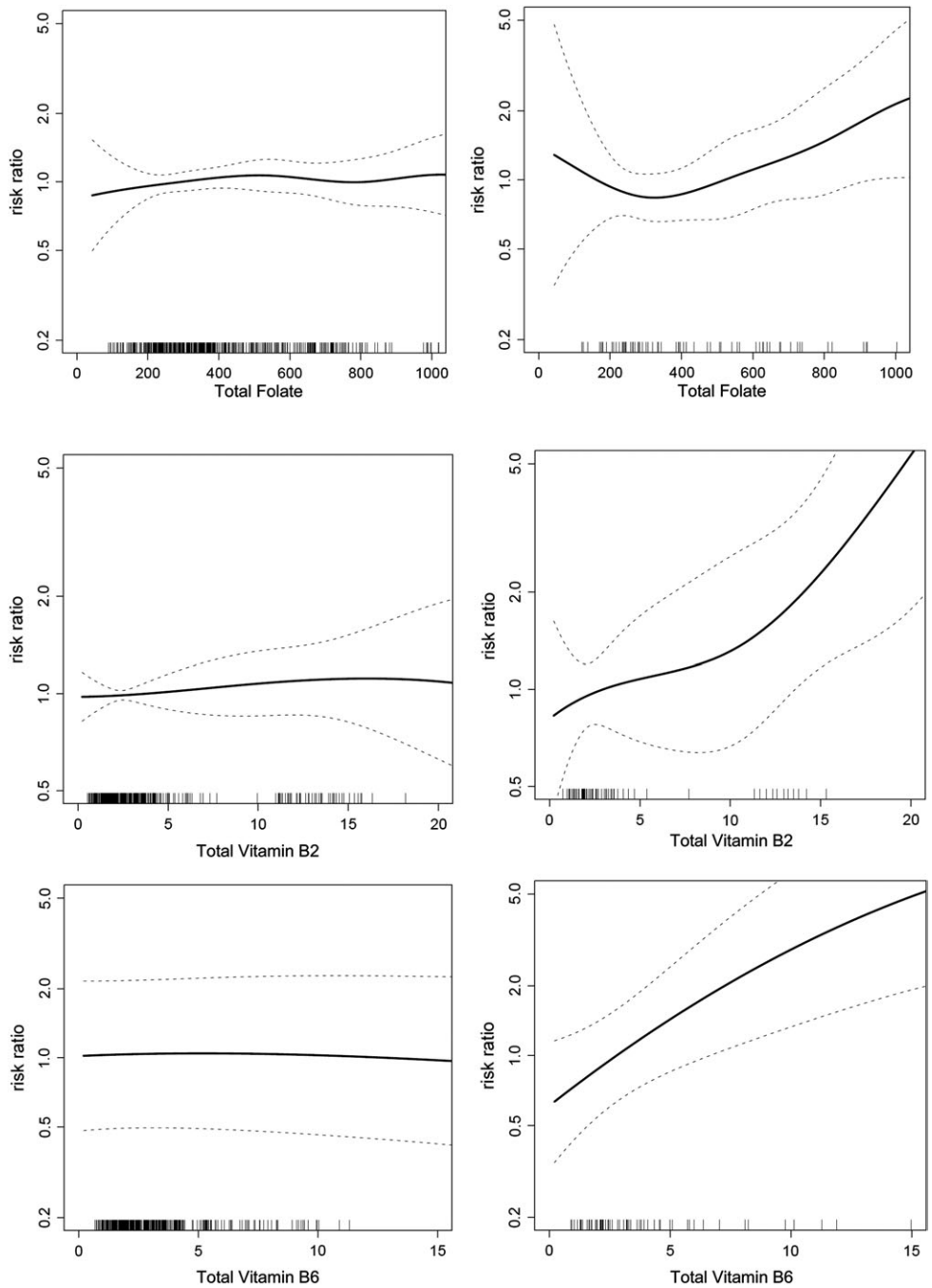

Figure 1. Multivariate-adjusted association of total folate with risk of type I (upper left) versus type II (upper right), vitamin B2 with risk of type I (middle left) versus type II (middle right), and total vitamin B6 with risk of type I (lower left) versus type II (lower right) endometrial cancer risk. Relative risks modeled using P-splines (solid lines) and 95% confidence intervals (dashed lines).

endometrial cancer and the only to evaluate type I versus type II endometrial cancer. Our results for type II endometrial cancer are consistent with animal studies, which found that folate has a complex, nonlinear and, perhaps, dual relationship with carcinogenesis [42].

Another interesting finding of the present study is that multivitamin supplements in general, and B-vitamin supplements in particular, appear to increase the risk of type II endometrial cancer. The possible explanation for this observation is that B-vitamins have a synergistic effect in the one-carbon metabolism and in the synthesis of nucleotides, and they are implicated along with folic acid in the above-mentioned processes. Finally, our data suggest that an assessment of potential negative health consequences of vitamin supplement use needs to be more fully considered.

Our study has several key strengths, including the prospective cohort study design from a defined population, comprehensive dietary assessment that was found to be valid and reliable [24], identification of cancer cases using a SEER cancer registry, and minimal loss to follow-up. We were also

able to adjust for a variety of potential confounding factors. A limitation of the present study is that we do not have data about the basal folate status of the women or regular updates of their diet during follow-up. We do note that women who participated in both the 1986 baseline and the 2004 follow-up showed an increase in the prevalence of the use of folate supplements from 1.2% to 6.9% [46]. Furthermore, the introduction of the fortification of grain products in the mid-1990s could potentially bias our observations, particularly if these agents act late in the carcinogenic process. However, even when analyzing only the cases of type II endometrial cancer stratified on diagnosis through 1996 versus after 1996, the associations with folic acid supplementation and endometrial cancer risk were present for both eras. The cases in this study were not reviewed by a central pathologist but rather relied on coding from original pathology reports by SEER abstractors. Given that this is an initial and unexpected observation, our findings require replication. Finally, the cohort consists of an older population of women from the upper Midwest United States, and the results may not generalize to other populations.

In conclusion, use of supplements containing folate, vitamins B2, B6, and B12 was associated with increased risk of type II but not type I endometrial cancer. While mandatory folate fortification of enriched grain products at the population level has lead to a decline in the incidence of neural tube defects [47], this and other recent studies raise concerns about potential negative health effects of excess levels of folic acid [41] and thus need to be fully evaluated.

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disclosure

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