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Risk Factors for Cancer

Dietary intake of nutrients involved in folate-mediated one-carbon metabolism and risk for endometrial cancer

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Abstract

Background: Studies disagree as to whether intakes of folate-mediated one-carbon metabolism nutrients are associated with endometrial cancer.
Methods: Using data from the large, prospective NIH-AARP Diet and Health Study, we used Cox proportional hazards models to evaluate endometrial cancer risk associated with calorie-adjusted dietary intake of several B vitamins and methionine. All models accounted for age, race, body mass index (BMI), smoking, oral-contraceptive use, menopausal hormone therapy use and caloric intake. We estimated associations by time from baseline ($\leq$3 or $>$3 years) and stratified models by BMI ($<$25 or $\geq$25 kg/m^2). During 16 years of follow-up, we identified 2329 endometrial cancer cases among 114 414 participants.
Results: After adjustment for confounding, we observed increased risk for endometrial cancer with greater consumption of dietary total folate, natural folate, B2, B6 and B12 [hazard ratios (HRs) ranging from 1.14 to 1.24 for the highest quintile (Q5) vs the lowest (Q1)]. Higher intakes of total folate, natural folate, B6 and B12 continued to be associated with increased risk when limiting follow-up to $>$3 years from baseline. We observed risks for the highest intakes of B2 [Q5 vs Q1: HR 1.27 95% confidence interval (CI) 1.07–1.50], B12 (Q5 vs Q1: HR 1.38 CI 1.17–1.63) and methionine (Q5 vs Q1: HR 1.26 CI 1.07–1.48) among women who were overweight/obese, but not among normal/underweight women.
Conclusions: Our findings indicate that one-carbon metabolism plays a role in endometrial carcinogenesis and exploration of this role in tissue and cellular biology studies is warranted.

Key words: diet, folate-mediated one-carbon metabolism, endometrial cancer, prospective cohort study

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Key Messages

- High dietary intakes of certain B vitamins were associated with modest increased risk for endometrial cancer.
- Greater dietary intakes of vitamins B2 and B12 and methionine were associated with increased risk among women with body mass indices of $\geq$25 kg/m^2, but not among those with lower BMIs.
- Multivitamin use and use of folic-acid supplements were not associated with endometrial cancer risk.

Introduction

Endometrial cancer is the most common cancer of the female reproductive tract in the USA.[1] Incidence and mortality rates have been steadily increasing during the past decade[1]; researchers estimate incidence rates for endometrial cancer in the USA will increase by 55% between 2010 and 2030.[2] Major risk factors for endometrial cancer include older age, obesity and oestrogen use that is unopposed by progesterone.

Folate-mediated one-carbon metabolism refers to a group of biologic pathways that are important for methylation and DNA synthesis and may also influence oxidative stress—all of which impact the regulation of cellular division.[3,4] Methionine, folate (vitamin B9) and other B vitamins play key roles in one-carbon metabolism. Several studies evaluated the associations between dietary and/or supplemental intake of these nutrients and risk for endometrial cancer, but small sample sizes preclude exploring important effect modification in most.[5–9] Results from the prospective Iowa Women's Health Study suggest B vitamin supplements increased risk of 'type II' endometrial cancers.[5] Both the Nurse's Health Study and a prospective study from Canada do not identify associations with these nutrients and the effect estimates for the highest intakes of folate in these studies are in the opposite direction.[6,7] To our knowledge, no studies report estimates (i) stratified by time from baseline (i.e. by follow-up time/time to diagnosis) to assess potential confounding by subclinical disease or (ii) stratified by obesity to comment on potential effect modification. These studies also focus on reporting associations for total dietary folate and folic-acid supplement use, but they do not separate the potentially independent effects of synthetic folate (folic acid from fortification) and naturally occurring folates from within the diet. Whereas synthetic folate is not useable by cells, there is increasing research interest in excess circulating folic acid serving as a potential reservoir for useable folates in cancer cells [i.e. after conversion by dihydrofolate reductase (DHFR)].

Using data from the large, prospective NIH-AARP Diet and Health Study, we estimated risks for endometrial cancer associated with dietary intake of nutrients involved in one-carbon metabolism, including total folate and both natural and synthetic folate. We also evaluated modification of these associations by time from baseline, body mass index (BMI) at enrolment, multivitamin use, alcohol use and cancer characteristics (e.g. histology, grade, stage).

Materials and methods

Study cohort

The NIH-AARP Diet and Health Study is a large prospective cohort that enrolled participants in 1995–96.[10] The National Cancer Institute Special Studies Institutional Review Board approved the study. AARP members aged 50–71 years and living in California, Florida, Louisiana, New Jersey, North Carolina, Pennsylvania or Atlanta, Georgia, or Detroit, Michigan were mailed a baseline questionnaire; 566 398 members completed the questionnaire and provided informed consent. We excluded participants who completed questionnaires by proxy ($n = 15\,760$), were men ($n = 325\,171$), had a history of cancer other than nonmelanoma skin cancer ($n = 23\,998$), were identified as having cancer through death reports only ($n = 1430$), showed disagreement between reported sex across information sources ($n = 136$) or indicated their menses stopped due to chemotherapy or radiation ($n = 157$). We additionally excluded participants who had a hysterectomy or were missing this information ($n = 84\,321$), whose cancers were non-epithelial/unknown histology ($n = 78$) and whose caloric intake was implausible ($n = 933$; details on outlier removal are below). Women excluded due to hysterectomy/missing hysterectomy information were similar to our final analytic population with respect to demographic and health history characteristics, supplement use and intake of the nutrients we evaluated; the only exception was, as expected, a higher prevalence of current menopausal hormone therapy use among women excluded due to hysterectomy (61%).

Participants provided information on their diet, demographics, and health and lifestyle history on the baseline questionnaire. Anic and colleagues previously described details of the food-frequency questionnaire (FFQ) and

estimation of the Healthy Eating Index-2010, which we used for adjustment in sensitivity analyses.[11] The FFQ was validated with 24-h dietary recalls within a subset of participants.[12] Food-group equivalents were constructed with the United States Department of Agriculture's (USDA) Continuing Survey of Food Intakes by Individuals. Using methods described by Subar and associates, nutrient variables were created using this information, the USDA Survey Nutrient Database and the Nutrition Data System for Research.[13] We evaluated dietary folate intake using estimates of the nutrient composition of foods from both before and after mandatory fortification (1998 in the USA).[14]

The Healthy Eating Index-2010 (HEI-2010), which is based on the Dietary Guidelines for Americans 2010, assessed 12 components of the diet; a higher score indicates better diet quality or conformance with guidelines.[15] Participants were also asked about the frequency with which they used multivitamins ('stress-tab type', 'therapeutic or theragran type' and 'one-a-day type'). They also reported any use of folic-acid supplements more than once per month, during the last year.

Our exposures of interest were intake of: dietary folate—including total folate, synthetic folate (i.e. folic acid) and naturally occurring folate—as well as intake of methionine and vitamins B2, B3, B6 and B12. All exposures reflect intake from the diet and not supplements. As enrolment for this study occurred while mandatory folic-acid fortification in the USA was implemented,[14] total folate was estimated in two ways for each woman, from the same food-frequency questionnaire: first, using estimates of synthetic-folate content in foods after US fortification ('total folate') and also using estimates from before mandatory fortification ('pre-fortification total folate'). Synthetic-folate intake from the diet was estimated from post-fortification food content.

Cohort follow-up and case ascertainment

Cancer cases were ascertained through linkage with state cancer registries in the enrolment states plus those in Arizona, Texas and Nevada. Vital status was obtained after linkage to the Social Security Administration Death Master File, the National Death Index Plus and the registries. Women were followed from their enrolment date until the earliest of the following: date of diagnosis of invasive epithelial endometrial cancer (ICD-O-3 sites C54.0-C54.9) or date of censoring [death, the end of study follow-up (31 December 2011) or loss to follow-up].

Statistical analyses

We used the multivariate nutrient density approach to adjust for caloric intake, which involves standardizing the exposures to caloric intake and additionally adjusting for intake in the models.[16] First, we removed outliers for caloric intake by log-transforming kilocalories per day and obtaining the population interquartile range (IQR); we excluded observations more than two times the IQR below the 25th or above the 75th percentile values. We then divided continuous measures of dietary intake for each nutrient by caloric intake to provide the energy-adjusted exposure, which was categorized into quintiles; the lowest quintile served as the reference. After comparing to models using the middle quintile for each exposure as a reference, we deemed that using the first quintile was appropriate. We evaluated correlations between the continuous calorie-adjusted nutrient exposures via Spearman rank tests. Using chi-square and Kruskal-Wallis tests, we compared population characteristics across quintiles of total post-fortification dietary folate intake in Table 1.

We used Cox proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations between nutrient intake in quintiles and risk of endometrial cancer. The time scale for analysis was age. Follow-up started at baseline age and ended with age at diagnosis or censoring. Left censoring was accommodated and we additionally adjusted for baseline age to minimize the impact of confounding by age at enrolment.[17] The exact method was used to handle ties. These were complete case analyses; observations with missing data were dropped from regression models, but missingness was less than 5% for all covariates. We report *p*-values for trends across quintiles from chi-square tests when the quintile intake variables were treated as continuous measures. We selected potential confounders a priori using knowledge of the literature and directed acyclic graphs. Models were adjusted for age, race, BMI, smoking status, history of oral-contraceptive use, history of menopausal hormone therapy use and caloric intake. These factors and the other modifiers we examined were categorized as presented in Table 1.

For sensitivity analyses, we first adjusted the main nutrient–cancer models for the HEI-2010 score. In secondary analyses, we compared associations by time from baseline (i.e. follow-up ended and/or cancer diagnosis occurred within $\leq$3 years of enrolment or >3 years) with extended Cox models as described by Kleinbaum and Klein (specifically, with heaviside functions/interactions).[18] We computed *p*-values for heterogeneity by comparing models with and without the interactions between follow-up time and each exposure via a likelihood ratio test.

We also examined effect modification by baseline BMI (categorized as <25 or $\geq$25 kg/m^2), multivitamin use and alcohol use at baseline via stratification. We generated heterogeneity *p*-values using likelihood ratio tests to compare

Table 1. Characteristics of the analytic population ($n = 114\,414$) by quintile of total intake of post-fortification dietary folate: the NIH-AARP Diet and Health Study (enrolled 1995–96, followed until 2011)

	Total dietary folate[a]										*P*
	Q1 $n = 22\,882$ median (mcg): 254.6 IQR: [186.8–340.7]		Q2 22 883 311.2 [238.9–400.8]		Q3 22 883 344.9 [268.7–437.5]		Q4 22 883 381.1 [298.9–482.0]		Q5 22 883 457.2 [351.6–591.8]		
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	
Entry age (years)											<0.001
Median [IQR]	61.3 [56.4–65.8]		62.1 [57.2–66.3]		62.2 [57.3–66.5]		62.4 [57.4–66.6]		62.3 [57.3–66.5]		
Race											<0.001
White	20 903	92.5	20 984	92.7	20 891	92.4	20 650	91.4	20 151	89.4	
Black	1219	5.4	1031	4.6	1008	4.5	1000	4.4	1016	4.5	
Other	473	2.1	613	2.7	723	3.2	937	4.2	1373	6.1	
BMI (kg/m2)											<0.001
<25	9602	43.4	9565	43.1	10 041	45.2	10 692	48.1	11 632	52.7	
25 to <30	6877	31.1	7092	32.0	7177	32.3	7066	31.8	6706	30.4	
≥30	5653	25.5	5534	24.9	5022	22.6	4467	20.1	3743	17.0	
Overweight/obese											<0.001
Yes (BMI ≥ 25)	12 530	56.6	12 626	56.9	12 199	54.9	11 533	51.9	10 449	47.3	
Education											<0.001
Did not complete high school	1636	7.4	1302	5.9	1176	5.3	1017	4.6	1003	4.5	
High school	6833	30.8	6096	27.4	5648	25.3	5167	23.2	4464	20.1	
Some college/post-high school	8017	36.1	7913	35.5	7831	35.1	7736	34.8	7466	33.6	
College graduate	2999	13.5	3554	15.9	3721	16.7	3847	17.3	4094	18.5	
Post-graduate	2724	12.3	3410	15.3	3949	17.7	4466	20.1	5166	23.3	
Smoking status											<0.001
Non-smoker	8202	36.9	10 025	45.0	10 589	47.6	10 660	47.9	10 716	48.4	
Former smoker	7960	35.8	8706	39.1	8938	40.2	9279	41.7	9492	42.9	
Current smoker	6062	27.3	3534	15.9	2705	12.2	2309	10.4	1933	8.7	
Current alcohol use											<0.001
Non-drinker	6110	26.8	6061	26.6	6076	26.7	6182	27.1	6851	30.1	
Drinker	16 679	73.2	16 754	73.4	16 720	73.4	16 616	72.9	15 931	69.9	
Total caloric intake (kilocalories)											
Median [IQR]	1610 [1201–2144]		1528 [1174–1970]		1472 [1147–1865]		1416 [1112–1790]		1331 [1033–1707]		<0.001
Healthy Eating Index											<0.001
Median [IQR]	58.6 [50.9–65.5]		66.3 [59.9–72.1]		69.7 [63.7–75.1]		72.3 [66.5–77.3]		74.0 [68.8–78.7]		
Multivitamin use											<0.001
Yes	12 526	54.7	13 535	59.2	14 087	61.6	14 324	62.6	14 205	62.1	
Menopausal status											0.02
Post-menopausal	21 244	93.3	21 335	93.7	21 378	93.9	21 354	93.7	21 406	94.0	
Menopausal hormone therapy use											<0.001
Never	14 663	64.2	13 724	60.1	13 419	58.7	13 254	58.0	13 410	58.7	
Current	6475	28.4	7332	32.1	7655	33.5	7801	34.2	7669	33.6	
Former	1705	7.5	1797	7.9	1775	7.8	1791	7.8	1762	7.7	
Prior oral-contraceptive use											<0.001
Never/<1 year	12 895	56.8	13 529	59.5	13 399	59.0	13 649	60.0	13 915	61.3	
1–4 years	3 938	17.3	4008	17.6	4091	18.0	4016	17.7	4029	17.8	
5–9 years	3172	14.0	2787	12.3	2927	12.9	2830	12.5	2645	11.7	
≥10 years	2714	12.0	2411	10.6	2309	10.2	2238	9.8	2115	9.3	

(Continued)

Table 1. Continued

	Total dietary folate[a]										*P*
	Q1 $n = 22\,882$ median (mcg): 254.6 IQR: [186.8–340.7]		Q2 22 883 311.2 [238.9–400.8]		Q3 22 883 344.9 [268.7–437.5]		Q4 22 883 381.1 [298.9–482.0]		Q5 22 883 457.2 [351.6–591.8]		
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	
Endometrial cancer											0.15
Non-cases	22 465	98.2	22 403	97.9	22 398	97.9	22 412	97.9	22 407	97.9	
Cases	417	1.8	480	2.1	485	2.1	471	2.1	476	2.1	
Tumour characteristics (cases only)[b]											0.84
Tumour 'type'											
Type I ($n = 1939$)	354	84.9	402	83.8	397	81.9	394	83.7	392	82.4	
Type II ($n = 176$)	31	7.4	37	7.7	41	8.5	29	6.2	38	8.0	
Other ($n = 214$)	32	7.7	41	8.5	47	9.7	48	10.2	46	9.7	
Type I histology (excludes mucinous)											0.87
Endometrioid ($n = 1306$)	241	69.7	266	67.5	267	68.6	274	70.1	258	67.0	
Adenocarcinoma ($n = 599$)	105	30.4	128	32.5	122	31.4	117	29.9	127	33.0	
Type I grade											0.78
I/II (Low) ($n = 1549$)	282	83.7	317	84.1	319	85.3	311	84.1	320	86.7	
III/IV (High) ($n = 278$)	55	16.3	60	15.9	55	14.7	59	16.0	49	13.3	
Type I preliminary stage at diagnosis											0.02
Localized ($n = 1108$)	195	82.6	220	80.6	239	82.4	218	78.4	236	89.1	
Regional or distant ($n = 234$)	41	17.4	53	19.4	51	17.6	60	21.6	29	10.9	

mcg, micrograms; Q1–Q5, quintiles; IQR, interquartile range; *n*, number; BMI, body mass index.

[a]Non-calorie-adjusted medians and IQRs are presented for interpretation, but quintiles were derived from calorie-adjusted dietary folate intake (not including supplements).

[b]Type I cancers included endometrioid ($n = 1306$) and mucinous cancers ($n = 34$), as well as adenocarcinomas ($n = 599$). Type II included serous ($n = 141$) and clear cell cancers ($n = 35$). Carcinosarcomas ($n = 108$) and other epithelial tumours ($n = 106$) were categorized as 'other histology'. Comparisons across histology, grade and stage were limited to those with 'type I' tumours.

Tabular percentages are proportions of the population not missing information for given covariates. Missingness was less than 5% for all covariates, except for tumour stage and grade (within the type I cancer cases, 6 and 31% were missing, respectively). Characteristics are compared across quintiles of total folate intake by Kruskal-Wallis tests (continuous variables) or Chi-square tests (categorical variables).

models with and without interaction terms for each nutrient exposure and these modifiers of interest. We did not evaluate methionine associations by multivitamin use, as it is not a typical component. The specific nutrient components of the multivitamins were not directly queried; as such, we had limited information on the amount of B vitamins consumed via supplements. We categorized multivitamin use as any or none in the past year for secondary analyses. Only $n = 188$ cases occurred among women reporting folic-acid supplementation; we therefore stratified secondary analyses by multivitamin use, rather than folic-acid use, but we also examined associations between endometrial cancer and both multivitamin and folic-acid use (categorized as any vs none; adjusted for the potential confounders plus total dietary folate and the Healthy Eating Index).

We used competing-risks Cox proportional hazards regression to evaluate statistical heterogeneity in associations by tumour characteristics, including cancer 'type'.[19,20] Cases classified as 'type I' included women with endometrioid ($n = 1306$) and mucinous cancers ($n = 34$), as well as adenocarcinomas ($n = 599$). 'Type II' cases included women with serous ($n = 141$) and clear cell cancers ($n = 35$). Carcinosarcomas ($n = 108$) and other epithelial tumours ($n = 106$) were categorized as 'other histology' and not included in the analyses of effect modification by cancer characteristics. We first compared type I and type II endometrial cancers and then further divided type I cancers into endometrioid and adenocarcinoma tumours (excluding all other cases and mucinous tumours). To assess whether estimates differed across the histologic types, we

used a likelihood ratio test to compare (i) a model in which the exposure associations varied by histology with (ii) a model in which these associations did not vary. Parameters for the confounders (listed above) varied by histology in both models. To evaluate associations with disease severity, we also used these models to make comparisons across grade (grade I/II or grade III/IV) and stage (localized or regional/distant) among women with type I tumours, because type II cancers are almost universally diagnosed at later stages/higher grades and sample sizes were limited. We also compared associations between tumour type for multivitamin and folic-acid use (with adjustment as described above for these exposures). Tests of significance were two-sided and used an alpha of 0.05. We conducted statistical analyses with SAS 9.3 (SAS Institute Inc., Cary, North Carolina).

Results

During 16 years of follow-up, we identified 2329 endometrial cancer cases among 114 414 participants. Women consuming the highest amounts of total folate [quintile 5 (Q5)] were more likely to be older, non-White, have completed more years of education, have used supplements and have a higher HEI-2010 score compared with participants with the lowest total folate intake (Q1; Table 1). Participants with the highest intake were less likely to be current smokers, drank less alcohol per day and had lower BMI. Women with higher intake reported using menopausal hormone therapy at baseline more frequently, but were less likely to have used oral contraceptives for 5 or more years compared with those consuming the lowest amounts of total folate.

The overall median and IQRs for nutrient intakes in our population after removing outliers for caloric consumption were: total folate (345.5 and 356.3–457.7 mcg), natural folate (237.9 and 169.9–315.2 mcg), synthetic folate (102.3 and 67.5–149.5 mcg), pre-fortification total folate (262.4 and 190.3–354.5 mcg), vitamins B2 (1.5 and 1.1–2.0 mg), B3 (17.4 and 13.2–22.6 mg), B6 (1.6 and 1.2–2.1 mg), B12 (3.5 mcg and 2.3–5.0 mcg) and methionine (1.2 and 0.9–1.6 g). For total folate, median intakes ranged from 254.6 (186.8–340.7) mcg in quintile 1 to 457.2 (351.6–591.8) mcg in quintile 5 (where quintiles were created from calorie-adjusted intakes). The median intakes for the highest quintiles of the other exposures were: natural folate (341.2 mcg), synthetic folate (172.6 mcg), pre-fortification total folate (372.9 mcg), vitamins B2 (2.0 mg), B3 (20.5 mg), B6 (2.0 mg), B12 (6.0 mcg) and methionine (1.6 g). With energy adjustment, our measures of pre- and post-fortification total folate intake were strongly correlated (rho = 0.92; not tabulated). Natural-folate intake was not strongly correlated with synthetic folate, B12 or methionine intakes (rho = –0.03 to 0.03); it also displayed modest correlation with B2 (rho = 0.27) and B6 (rho = 0.56) and a strong correlation with pre-fortification total folate (rho = 0.86). Correlations between the non-folate nutrients ranged from rho = 0.25 to rho = 0.62.

Greater consumption of total folate calculated with both pre- and post-fortification estimates [quintile 5 (Q5) vs quintile 1 (Q1): HR 1.17, CI 1.02, 1.34 for post-fortification], vitamins B2 (Q5 vs Q1: HR 1.14, CI 0.99, 1.31), B6 (Q5 vs Q1: HR 1.24, CI 1.08, 1.42) and B12 (Q5 vs Q1: HR 1.17, CI 1.03, 1.35) was associated with modestly elevated risk for endometrial cancer (Table 2). Trends of increasing risk across quintiles were most visible for B2 and B6 intakes (p-trend 0.04 and 0.01, respectively). Compared with those with the lowest intakes, all amounts of natural folate from the diet were associated with modestly increased endometrial cancer risk (i.e. all quintiles). We did not identify associations with intake of synthetic folate or methionine. Effect magnitudes across exposures were similar after additional adjustment for HEI-2010 score (not tabulated).

Compared with our overall, unstratified models, we noted similar risk magnitudes for the highest intake of total folate, regardless of time from baseline (Table 2). Endometrial cancer risks increased across quintiles of natural-folate intake when limiting follow-up time to $\leq$3 years from baseline and were consistently elevated across quintiles when follow-up was >3 years from baseline. Similarly, the highest intake of B6 was associated with increased risk for endometrial cancer regardless of time from baseline ($\leq$3 years from baseline, Q5 vs Q1: HR 1.38, CI 1.10, 1.73 and >3 years, Q5 vs Q1: HR 1.20, CI 1.03, 1.39). The highest intake of B12 was associated with endometrial cancer risk >3 years from baseline and our findings suggest a similar association for methionine. We noted consistent effect magnitudes between the time periods for other nutrients.

We identified potential modification of our nutrient–cancer associations by BMI, particularly for intakes of B2, B12 and methionine (Table 3). The highest intakes of all three nutrients suggested increased risks for endometrial cancer among women who were overweight or obese (BMI $\geq$ 25), but not among women with lower BMI. The increased risks among the overall population that we observed for natural-folate intake seemed to be explained by associations among women with BMI <25, but a dose–response was not apparent.

In general, associations between one-carbon metabolism nutrient intakes and endometrial cancer risk were not heterogeneous by tumour 'type', histology, grade or stage (Table 4). Increased risks associated with natural-folate

Table 2. Intake of nutrients involved in folate-mediated one-carbon metabolism and time to diagnosis of endometrial cancer: overall associations and those stratified by time from baseline

	Overall		*P* trend[a]	Time from baseline				*P* interaction[b]
				≤3 years non-cases: 4111 cases: 458		>3 years non-cases: 107 974 cases: 1871		
	HR	95% CI		HR	95% CI	HR	95% CI	
Total folate (post-fortification)			0.05					0.74
Q1		Reference			Reference		Reference	
Q2	1.09	(0.96–1.25)		0.95	(0.74–1.22)	1.13	(0.98–1.31)	
Q3	1.09	(0.95–1.25)		1.06	(0.83–1.34)	1.10	(0.95–1.27)	
Q4	1.10	(0.96–1.26)		1.12	(0.88–1.42)	1.09	(0.94–1.27)	
Q5	1.17	(1.02–1.34)		1.12	(0.88–1.43)	1.18	(1.02–1.37)	
Natural folate			0.02					0.26
Q1		Reference			Reference		Reference	
Q2	1.22	(1.06–1.40)		0.99	(0.77–1.28)	1.28	(1.11–1.48)	
Q3	1.18	(1.03–1.35)		1.15	(0.91–1.47)	1.19	(1.02–1.37)	
Q4	1.20	(1.05–1.38)		1.06	(0.83–1.36)	1.24	(1.07–1.44)	
Q5	1.23	(1.07–1.42)		1.27	(1.00–1.62)	1.22	(1.05–1.41)	
Synthetic folate (folic acid)			0.98					0.74
Q1		Reference			Reference		Reference	
Q2	1.13	(0.99–1.29)		1.08	(0.85–1.37)	1.14	(0.99–1.32)	
Q3	1.10	(0.96–1.26)		1.15	(0.91–1.45)	1.09	(0.94–1.25)	
Q4	1.06	(0.92–1.22)		0.95	(0.74–1.22)	1.09	(0.94–1.26)	
Q5	1.04	(0.90–1.20)		1.10	(0.86–1.40)	1.03	(0.88–1.19)	
Pre-fortification total folate[c]			0.04					0.97
Q1		Reference			Reference		Reference	
Q2	1.06	(0.92–1.21)		1.00	(0.78–1.28)	1.07	(0.93–1.24)	
Q3	1.11	(0.97–1.28)		1.06	(0.93–1.35)	1.13	(0.98–1.30)	
Q4	1.14	(0.99–1.31)		1.11	(0.87–1.41)	1.15	(0.99–1.33)	
Q5	1.13	(0.99–1.30)		1.14	(0.89–1.45)	1.13	(0.98–1.32)	
Vitamin B2			0.04					0.08
Q1		Reference			Reference		Reference	
Q2	1.04	(0.90–1.20)		0.74	(0.56–0.98)	1.12	(0.96–1.29)	
Q3	1.10	(0.96–1.26)		1.06	(0.83–1.34)	1.11	(0.96–1.29)	
Q4	1.11	(0.97–1.27)		1.10	(0.87–1.39)	1.11	(0.96–1.29)	
Q5	1.14	(0.99–1.31)		1.11	(0.88–1.41)	1.15	(0.99–1.33)	
Vitamin B3			0.04					0.01
Q1		Reference			Reference		Reference	
Q2	1.09	(0.95–1.24)		0.82	(0.63–1.06)	1.16	(1.01–1.33)	
Q3	1.03	(0.90–1.18)		1.20	(0.96–1.49)	0.99	(0.86–1.15)	
Q4	0.97	(0.85–1.11)		0.83	(0.64–1.07)	1.01	(0.88–1.17)	
Q5	0.90	(0.78–1.04)		0.83	(0.64–1.07)	0.92	(0.79–1.07)	
Vitamin B6			0.01					0.04
Q1		Reference			Reference		Reference	
Q2	1.09	(0.95–1.25)		0.84	(0.64–1.10)	1.16	(1.00–1.34)	
Q3	1.13	(0.99–1.30)		1.25	(0.99–1.57)	1.10	(0.95–1.28)	
Q4	1.10	(0.96–1.27)		0.99	(0.77–1.28)	1.13	(0.97–1.31)	
Q5	1.24	(1.08–1.42)		1.38	(1.10–1.73)	1.20	(1.03–1.39)	
Vitamin B12			0.01					0.05
Q1		Reference			Reference		Reference	
Q2	1.04	(0.90–1.19)		0.80	(0.61–1.06)	1.10	(0.95–1.28)	
Q3	1.03	(0.89–1.18)		1.13	(0.89–1.43)	1.00	(0.86–1.16)	
Q4	1.14	(0.99–1.30)		0.99	(0.77–1.26)	1.18	(1.02–1.36)	
Q5	1.17	(1.03–1.35)		1.04	(0.82–1.32)	1.21	(1.05–1.40)	

(Continued)

Table 2. Continued

	Overall		*P* trend[a]	Time from baseline				*P* interaction[b]
				≤3 years non-cases: 4111 cases: 458		>3 years non-cases: 107 974 cases: 1871		
	HR	95% CI		HR	95% CI	HR	95% CI	
Methionine			0.23					0.35
Q1		Reference			Reference		Reference	
Q2	1.02	(0.88–1.17)		0.90	(0.69–1.17)	1.05	(0.90–1.22)	
Q3	1.07	(0.93–1.22)		1.07	(0.84–1.36)	1.06	(0.92–1.23)	
Q4	1.04	(0.91–1.19)		1.12	(0.88–1.41)	1.02	(0.88–1.18)	
Q5	1.09	(0.95–1.24)		0.93	(0.73–1.19)	1.13	(0.98–1.30)	

HR, hazard ratio; CI, confidence interval; Q1–Q5, calorie-adjusted quintiles of intake for each nutrient (supplements not included); Q1 serves as the reference in all models.

All models adjusted for: age, race, body mass index, smoking status, oral-contraceptive use, menopausal hormone therapy use, caloric intake.

[a]*P* trend calculated by treating each quintile variable as a continuous exposure.

[b]*P* interaction from likelihood ratio tests comparing models with and without interaction terms for time from baseline and each dietary-intake exposure.

[c]Total dietary folate intake using estimates of food nutritional content before US fortification (1996–98).

intake were indicated across tumour type, histology and stage; we noted risks that were higher in magnitude for type II vs type I tumours and for low-grade vs high-grade tumours, but estimates were imprecise and precluded drawing conclusions about heterogeneity. Across quintiles of synthetic-folate intake, risks were greater in magnitude for adenocarcinomas than for endometrioid tumours. The increased risks we saw for the highest intakes of B6 were apparent regardless of histology and stage among type I cancers; increased risk was apparent for type I tumours but not type II (Q5 vs Q1, type I: HR 1.29, CI 1.11, 1.50; Q5 vs Q1, type II: HR 0.69, CI 0.42, 1.13; p-het-0.21). Multivitamin and folic-acid supplement use were not associated with type I or type II cancers (not tabulated).

Similar to the findings among the entire analytic population, we estimated modest increased risks for endometrial cancer with the highest quintiles of total folate, natural folate, B2, B6 and B12 among women who did not report multivitamin use (Supplementary Table 1, available as Supplementary data at *IJE* online). Our data suggest increased risks among multivitamin users, in terms of effect magnitude, but estimates were attenuated and imprecise. Multivitamin use itself was not associated with endometrial cancer risk after adjustment (HR 0.97, CI 0.86, 1.05; not tabulated), nor was folic-acid supplementation (HR 0.95, CI 0.81, 1.10). We noted elevated endometrial cancer risks among women who did not report drinking alcohol at baseline and attenuated associations among those reporting alcohol use (Supplementary Table 1, available as Supplementary data at *IJE* online). Specifically, women who did not use alcohol and consumed the highest levels of total folate (pre- and post-fortification), natural folate, B2 and methionine had increased risks compared with women reporting alcohol use.

Discussion

We identified modestly elevated risks for endometrial cancer associated with greater dietary intake of several key nutrients involved in folate-mediated one-carbon metabolism—in particular, total folate, natural folate, B2, B6 and B12. The risks associated with B2 and B12 were driven by women with BMI ≥25; we also observed endometrial cancer risks associated with methionine intake among this group. We noted that higher intakes of natural folate and B6 were associated with endometrial cancer risk ≤3 years from baseline; this risk may reflect excess nutrient intake that is potentially being used by rapidly dividing, hyperplastic, precancerous tissues (i.e. confounding by subclinical disease). However, higher intakes of natural folate, B6, B12 and possibly methionine were also associated with risk >3 years from baseline—indicating a potential role for these nutrients in the early development of endometrial cancers.

The prevailing biologic rationale for exploring the role of one-carbon metabolism in many diseases is that these metabolic pathways are crucial for methylation and DNA synthesis (see Laanpere *et al.*[3] and Newman *et al.*[21] for reviews). The B vitamins involved in these pathways also serve as enzymatic co-factors in other important reactions. Biologically active forms of vitamin B2 are co-factors for enzymes involved not only in one-carbon metabolism (e.g. methylenetetrahydofolate reductase), but also beta oxidation, electron transport and cholesterol biosynthesis.[22] Vitamin B6 plays a role as cofactor in many inflammatory

Table 3. Intake of nutrients involved in folate-mediated one-carbon metabolism and time to diagnosis of endometrial cancer: associations stratified by body mass index (BMI) at enrolment in the NIH-AARP Diet and Health Study

	BMI				*P* interaction[b]
	<25 non-cases: 50 881 cases: 651		≥25 non-cases: 57 717 cases: 1620		
	HR[a]	95% CI	HR[a]	95% CI	
Total folate (post-fortification)					0.71
Q1		Reference		Reference	
Q2	1.06	(0.81–1.40)	1.10	(0.94–1.28)	
Q3	1.13	(0.87–1.48)	1.04	(0.89–1.22)	
Q4	1.09	(0.83–1.42)	1.06	(0.90–1.25)	
Q5	1.05	(0.80–1.37)	1.15	(0.98–1.36)	
Natural folate					0.28
Q1		Reference		Reference	
Q2	1.33	(1.01–1.76)	1.16	(0.99–1.36)	
Q3	1.29	(0.98–1.71)	1.12	(0.95–1.31)	
Q4	1.39	(1.06–1.83)	1.10	(0.93–1.29)	
Q5	1.23	(0.94–1.63)	1.18	(1.00–1.39)	
Synthetic folate (folic acid)					0.96
Q1		Reference		Reference	
Q2	1.14	(0.88–1.48)	1.12	(0.95–1.31)	
Q3	1.06	(0.82–1.38)	1.11	(0.95–1.30)	
Q4	1.06	(0.82–1.38)	1.03	(0.88–1.22)	
Q5	0.97	(0.75–1.25)	1.04	(0.88–1.22)	
Pre-fortification total folate[c]					0.70
Q1		Reference		Reference	
Q2	0.98	(0.74–1.29)	1.05	(0.90–1.23)	
Q3	1.10	(0.84–1.44)	1.07	(0.92–1.26)	
Q4	1.12	(0.86–1.46)	1.07	(0.91–1.26)	
Q5	0.99	(0.76–1.30)	1.12	(0.95–1.32)	
Vitamin B2					0.40
Q1		Reference		Reference	
Q2	0.96	(0.75–1.23)	1.08	(0.91–1.28)	
Q3	1.00	(0.78–1.28)	1.17	(0.99–1.39)	
Q4	0.89	(0.69–1.14)	1.24	(1.05–1.46)	
Q5	0.92	(0.72–1.18)	1.27	(1.07–1.50)	
Vitamin B3					0.32
Q1		Reference		Reference	
Q2	1.09	(0.85–1.40)	1.09	(0.94–1.28)	
Q3	1.14	(0.89–1.46)	0.99	(0.84–1.16)	
Q4	1.05	(0.82–1.36)	0.94	(0.80–1.11)	
Q5	0.83	(0.63–1.08)	0.92	(0.78–1.08)	
Vitamin B6					0.78
Q1		Reference		Reference	
Q2	1.11	(0.84–1.45)	1.08	(0.92–1.27)	
Q3	1.20	(0.92–1.57)	1.08	(0.92–1.27)	
Q4	1.06	(0.81–1.39)	1.08	(0.92–1.27)	
Q5	1.16	(0.89–1.51)	1.22	(1.03–1.44)	
Vitamin B12					0.11
Q1		Reference		Reference	
Q2	0.99	(0.78–1.24)	1.10	(0.93–1.32)	
Q3	0.84	(0.65–1.07)	1.17	(0.99–1.40)	
Q4	0.95	(0.75–1.21)	1.30	(1.10–1.54)	
Q5	0.92	(0.72–1.19)	1.38	(1.17–1.63)	

(Continued)

Table 3. Continued

	BMI				*P* interaction[b]
	<25 non-cases: 50 881 cases: 651		≥25 non-cases: 57 717 cases: 1620		
	HR[a]	95% CI	HR[a]	95% CI	
Methionine					0.04
Q1	Reference		Reference		
Q2	0.96	(0.76–1.22)	1.05	(0.88–1.25)	
Q3	1.07	(0.84–1.34)	1.12	(0.94–1.32)	
Q4	0.77	(0.59–1.00)	1.22	(1.04–1.45)	
Q5	0.89	(0.69–1.14)	1.26	(1.07–1.48)	

BMI, body mass index, calculated as kg/m^2; HR, hazard ratio; CI, confidence interval; Q1–Q5, calorie-adjusted quintiles of intake for each nutrient (supplements not included); Q1 serves as the reference in all models.

[a]Models adjusted for: age, race, smoking status, oral-contraceptive use, menopausal hormone therapy use, caloric intake.

[b]*P* interaction from likelihood ratio tests comparing models with and without interaction terms for dichotomous BMI and each dietary-intake exposure.

[c]Total dietary folate intake using estimates of food nutritional content before US fortification (1996–98).

pathways including homocysteine and tryptophan/kynurenine metabolism[23]; the latter may influence endometrial cancer prognosis.[24,25] Methionine is an essential amino acid that is crucial for methylation reactions and research suggests that many tumours may be methionine-dependent.[26] We noted increased risk for endometrial cancer with higher intake of methionine among women with BMI ≥ 25; this implies that methionine restriction may be beneficial for these women, who would likely be at increased risk for endometrial hyperplasia (a precursor to many endometrial cancers).

Research among reproductive-aged women shows that both greater dietary folic-acid intake and total serum folate are associated with higher progesterone levels.[27,28] Exposures to oestrogens unopposed by progestogens across the life course is considered a major risk factor for endometrial cancer,[29] so this demonstrates a potential for reduced endometrial cancer risk with increased intake of folates. However, cells undergoing rapid cellular division would likely require greater amounts of nutrients like folate to accommodate the demands for DNA synthesis. In the context of colorectal cancer, it appears that folate may both reduce or increase risk for the disease, depending on the presence of polyps.[30,31] For endometrial cancer, dietary exposures may differentially influence carcinogenesis if subclinical disease or cancer precursors are present (i.e. endometrial hyperplasia). Histopathological studies of endometrial cancers note staining for folate receptor α, but the extent to which this expression correlates with stage, grade or histology is uncertain.[32–34]

Synthetic folate/folic acid (a monoglutamate) is used to fortify grain products and, unlike the polyglutamate forms of folate found naturally in foods, it can cross the intestinal mucosa without additional enzymatic processing if concentrations are high (e.g. after supplementation).[35–37] Ultimately, the absorption of both natural folate and folic acid depends on factors such as the amount and type of foods consumed, pH and medication or alcohol use.[35] To be used within cells, folic acid must be converted to biologically useable forms of folate (dihydrofolate or tetrahydrofolate) by DHFR and, because this conversion is slow, it is argued that a reservoir of unmetabolized folic acid can potentially be created in circulation.[35–37] Whereas the biologic implications of excess folic acid are uncertain,[35,38] it is possible that a reservoir of folic acid could be converted to useable folates within cancer cells (e.g. increased DHFR activity). We note only one study of DHFR in the context of endometrial cancer, where DHFR expression was increased in endometrial cells after administration of tamoxifen.[39]

We assessed both synthetic and natural folates from the diet, in addition to modification by multivitamin use. We predominately observed associations between endometrial cancer and total and natural folate, but identified potential risk heterogeneity for synthetic-folate intake between endometrioid tumours and adenocarcinomas. However, the latter is a catch-all classification that likely represents a complex mix of tumour histologies. The primary food sources for naturally occurring folates are those foods thought to be part of 'healthy' diets (e.g. leafy greens, legumes). Therefore, it may seem contradictory that we identified risks with high intake of natural folates—but high levels of natural folates would be immediately useable to cells, whereas excess synthetic acid would require additional processing by DHFR.

Interestingly, the endometrial cancer risks associated with the highest intakes of total folate, natural folate, B2, B6 and B12 were stronger among women who did not report multivitamin use than among those with the highest dietary intakes who did report use—which is reassuring if

Table 4. Intake of nutrients involved in folate-mediated one-carbon metabolism and time to diagnosis of endometrial cancer: associations by tumour characteristics in the NIH-AARP Diet and Health Study

		Tumour 'type'			Histology			Grade			Stage		
	Q	Type I cases $n = 1939$ HR (95% CI)[a]	Type II cases $n = 176$ HR (95% CI)[a]	P^b	Endometrioid cases $n = 1306$ HR (95% CI)[a]	Adenocarcinoma cases $n = 599$ HR (95% CI)[a]	P^b	Low-grade cases $n = 1549$ HR (95% CI)[a]	High-grade cases $n = 278$ HR (95% CI)[a]	P^b	Localized cases $n = 1108$ HR (95% CI)[a]	Regional/distant cases $n = 234$ HR (95% CI)[a]	P^b
Total folate	1	Reference	Reference	0.71	Reference	Reference	0.64	Reference	Reference	0.57	Reference	Reference	0.02
	2	1.09 (0.94–1.27)	1.07 (0.66–1.75)		1.04 (0.87–1.24)	1.24 (0.95–1.63)		1.07 (0.90–1.26)	1.13 (0.77–1.65)		1.10 (0.90–1.34)	1.19 (0.78–1.81)	
	3	1.07 (0.92–1.24)	1.16 (0.72–1.87)		1.05 (0.87–1.25)	1.14 (0.86–1.50)		1.06 (0.89–1.25)	1.06 (0.71–1.56)		1.16 (0.95–1.42)	1.10 (0.72–1.70)	
	4	1.10 (0.94–1.27)	0.82 (0.49–1.40)		1.10 (0.91–1.31)	1.16 (0.88–1.54)		1.07 (0.90–1.27)	1.18 (0.80–1.73)		1.12 (0.91–1.37)	1.37 (0.91–2.06)	
	5	1.15 (0.99–1.34)	1.13 (0.69–1.83)		1.08 (0.89–1.30)	1.35 (1.02–1.78)		1.17 (0.99–1.39)	0.92 (0.60–1.39)		1.26 (1.03–1.55)	0.69 (0.42–1.14)	
Natural folate	1	Reference	Reference	0.41	Reference	Reference	0.95	Reference	Reference	0.30	Reference	Reference	0.42
	2	1.21 (1.04–1.40)	1.52 (0.92–2.53)		1.25 (1.04–1.50)	1.16 (0.89–1.52)		1.28 (1.09–1.52)	0.84 (0.58–1.23)		1.34 (1.09–1.63)	1.27 (0.82–1.97)	
	3	1.14 (0.98–1.33)	1.41 (0.85–2.35)		1.19 (0.99–1.43)	1.05 (0.80–1.38)		1.17 (0.99–1.39)	0.84 (0.58–1.23)		1.24 (1.01–1.51)	1.38 (0.89–2.13)	
	4	1.19 (1.03–1.39)	0.99 (0.57–1.71)		1.24 (1.03–1.49)	1.12 (0.86–1.47)		1.23 (1.04–1.46)	0.86 (0.59–1.26)		1.29 (1.06–1.59)	1.33 (0.85–2.06)	
	5	1.17 (1.01–1.37)	1.50 (0.91–2.48)		1.20 (0.99–1.45)	1.17 (0.89–1.54)		1.23 (1.04–1.47)	0.87 (0.59–1.28)		1.35 (1.09–1.66)	0.96 (0.59–1.54)	
Synthetic folate	1	Reference	Reference	0.93	Reference	Reference	0.27	Reference	Reference	0.09	Reference	Reference	0.95
	2	1.13 (0.98–1.32)	1.07 (0.67–1.73)		1.12 (0.93–1.33)	1.17 (0.88–1.55)		1.05 (0.89–1.24)	1.28 (0.86–1.90)		1.18 (0.97–1.45)	1.12 (0.74–1.69)	
	3	1.10 (0.94–1.27)	0.92 (0.56–1.52)		1.01 (0.84–1.21)	1.37 (1.04–1.80)		1.08 (0.91–1.27)	1.08 (0.71–1.63)		1.23 (1.01–1.50)	1.08 (0.71–1.65)	
	4	1.08 (0.92–1.25)	0.99 (0.61–1.61)		1.02 (0.85–1.22)	1.25 (0.94–1.65)		0.98 (0.83–1.16)	1.53 (1.04–2.25)		1.07 (0.88–1.32)	0.99 (0.64–1.52)	
	5	1.09 (0.93–1.27)	0.88 (0.53–1.45)		1.01 (0.84–1.22)	1.31 (0.99–1.74)		1.09 (0.92–1.28)	1.02 (0.67–1.56)		1.16 (0.95–1.43)	0.96 (0.62–1.48)	
Pre-fortification total folate[c]	1	Reference	Reference	0.74	Reference	Reference	0.12	Reference	Reference	0.54	Reference	Reference	0.44
	2	1.06 (0.91–1.23)	0.91 (0.55–1.51)		1.10 (0.91–1.32)	0.99 (0.76–1.29)		1.06 (0.90–1.25)	1.06 (0.72–1.55)		1.08 (0.88–1.31)	1.49 (0.96–2.30)	
	3	1.09 (0.94–1.27)	1.16 (0.73–1.84)		1.13 (0.94–1.36)	1.04 (0.79–1.35)		1.12 (0.95–1.32)	0.95 (0.64–1.40)		1.17 (0.96–1.42)	1.40 (0.90–2.18)	
	4	1.12 (0.96–1.30)	0.93 (0.57–1.51)		1.25 (1.04–1.50)	0.89 (0.67–1.17)		1.12 (0.94–1.32)	1.12 (0.76–1.65)		1.20 (0.98–1.46)	1.27 (0.80–2.01)	
	5	1.15 (0.99–1.34)	0.90 (0.55–1.48)		1.13 (0.94–1.37)	1.22 (0.93–1.59)		1.20 (1.01–1.42)	0.89 (0.59–1.35)		1.22 (0.99–1.49)	1.11 (0.69–1.79)	
Vitamin B2	1	Reference	Reference	0.87	Reference	Reference	0.88	Reference	Reference	0.01	Reference	Reference	0.75
	2	1.04 (0.89–1.21)	0.97 (0.59–1.58)		1.12 (0.92–1.35)	0.94 (0.71–1.24)		0.92 (0.77–1.09)	1.64 (1.12–2.41)		0.91 (0.74–1.11)	1.19 (0.76–1.86)	
	3	1.11 (0.95–1.29)	0.87 (0.52–1.43)		1.16 (0.96–1.40)	1.05 (0.80–1.37)		1.11 (0.94–1.31)	1.05 (0.70–1.59)		1.02 (0.83–1.24)	1.23 (0.79–1.91)	
	4	1.16 (1.00–1.35)	0.93 (0.57–1.53)		1.21 (1.00–1.46)	1.09 (0.83–1.42)		1.15 (0.98–1.36)	1.07 (0.71–1.63)		1.08 (0.89–1.31)	1.17 (0.75–1.84)	
	5	1.17 (1.00–1.36)	0.98 (0.60–1.60)		1.23 (1.02–1.48)	1.06 (0.81–1.40)		1.13 (0.95–1.33)	1.15 (0.76–1.75)		1.04 (0.86–1.27)	1.37 (0.89–2.12)	
Vitamin B3	1	Reference	Reference	0.43	Reference	Reference	0.33	Reference	Reference	0.06	Reference	Reference	0.50
	2	1.10 (0.95–1.27)	1.03 (0.66–1.62)		1.23 (1.03–1.47)	0.90 (0.69–1.18)		1.01 (0.86–1.19)	1.39 (0.95–2.02)		1.10 (0.90–1.33)	0.73 (0.48–1.12)	
	3	1.05 (0.91–1.22)	0.89 (0.56–1.41)		1.14 (0.95–1.37)	0.95 (0.73–1.23)		1.04 (0.88–1.22)	0.93 (0.62–1.40)		1.08 (0.89–1.31)	0.83 (0.55–1.26)	
	4	0.98 (0.85–1.14)	0.69 (0.42–1.14)		1.04 (0.87–1.26)	0.92 (0.71–1.20)		0.90 (0.76–1.06)	1.22 (0.83–1.78)		1.01 (0.83–1.23)	0.90 (0.60–1.36)	
	5	0.93 (0.80–1.09)	0.59 (0.35–1.02)		0.96 (0.80–1.17)	0.93 (0.71–1.22)		0.91 (0.77–1.08)	0.77 (0.50–1.20)		0.94 (0.77–1.16)	0.80 (0.52–1.23)	

(Continued)

Table 4. Continued

		Tumour 'type'			Histology			Grade			Stage		
	Q	Type I cases $n = 1939$ HR (95% CI)[a]	Type II cases $n = 176$ HR (95% CI)[a]	P^b	Endometrioid cases $n = 1306$ HR (95% CI)[a]	Adenocarcinoma cases $n = 599$ HR (95% CI)[a]	P^b	Low-grade cases $n = 1549$ HR (95% CI)[a]	High-grade cases $n = 278$ HR (95% CI)[a]	P^b	Localized cases $n = 1108$ HR (95% CI)[a]	Regional/distant cases $n = 234$ HR (95% CI)[a]	P^b
Vitamin B6	1	Reference	Reference	0.21	Reference	Reference	0.44	Reference	Reference	0.68	Reference	Reference	0.62
	2	1.11 (0.96–1.29)	0.75 (0.46–1.22)		1.17 (0.97–1.41)	1.05 (0.80–1.37)		1.14 (0.96–1.35)	1.02 (0.69–1.50)		1.07 (0.87–1.31)	1.31 (0.84–2.03)	
	3	1.12 (0.97–1.31)	0.83 (0.52–1.32)		1.20 (0.99–1.44)	1.03 (0.79–1.36)		1.16 (0.98–1.38)	1.00 (0.68–1.49)		1.18 (0.96–1.44)	1.13 (0.71–1.77)	
	4	1.09 (0.93–1.27)	0.87 (0.55–1.38)		1.18 (0.98–1.43)	0.95 (0.72–1.26)		1.09 (0.92–1.30)	0.98 (0.65–1.45)		1.20 (0.98–1.47)	1.05 (0.66–1.68)	
	5	1.29 (1.11–1.50)	0.69 (0.42–1.13)		1.27 (1.05–1.54)	1.34 (1.03–1.75)		1.35 (1.14–1.60)	0.98 (0.65–1.47)		1.29 (1.05–1.58)	1.46 (0.94–2.28)	
Vitamin B12	1	Reference	Reference	0.88	Reference	Reference	0.60	Reference	Reference	0.71	Reference	Reference	0.61
	2	1.06 (0.91–1.24)	0.90 (0.55–1.49)		1.17 (0.97–1.41)	0.89 (0.67–1.17)		1.06 (0.89–1.26)	1.08 (0.73–1.59)		1.01 (0.83–1.24)	1.05 (0.66–1.68)	
	3	1.02 (0.88–1.20)	0.99 (0.61–1.62)		1.07 (0.88–1.29)	0.98 (0.75–1.29)		1.03 (0.87–1.23)	1.00 (0.67–1.48)		1.00 (0.82–1.22)	1.21 (0.78–1.89)	
	4	1.17 (1.01–1.36)	0.93 (0.57–1.53)		1.23 (1.02–1.48)	1.09 (0.84–1.41)		1.20 (1.02–1.42)	0.96 (0.65–1.42)		1.16 (0.95–1.41)	1.16 (0.74–1.82)	
	5	1.20 (1.04–1.39)	1.19 (0.74–1.91)		1.24 (1.03–1.50)	1.09 (0.84–1.42)		1.21 (1.02–1.43)	1.02 (0.69–1.50)		1.11 (0.91–1.35)	1.49 (0.97–2.29)	
Methionine	1	Reference	Reference	0.42	Reference	Reference	0.80	Reference	Reference	0.49	Reference	Reference	0.94
	2	1.07 (0.92–1.25)	0.74 (0.46–1.20)		1.12 (0.92–1.35)	1.01 (0.76–1.33)		1.12 (0.94–1.33)	0.89 (0.60–1.30)		1.10 (0.90–1.35)	1.05 (0.68–1.63)	
	3	1.09 (0.93–1.26)	0.78 (0.49–1.26)		1.09 (0.90–1.32)	1.11 (0.85–1.45)		1.12 (0.94–1.33)	0.81 (0.54–1.19)		1.13 (0.92–1.38)	0.98 (0.63–1.53)	
	4	1.09 (0.93–1.26)	0.67 (0.41–1.10)		1.13 (0.94–1.36)	1.06 (0.81–1.38)		1.13 (0.95–1.34)	0.89 (0.61–1.30)		1.06 (0.87–1.30)	1.13 (0.74–1.73)	
	5	1.11 (0.95–1.29)	0.85 (0.53–1.35)		1.17 (0.98–1.41)	1.00 (0.76–1.31)		1.15 (0.97–1.36)	0.79 (0.54–1.16)		1.09 (0.89–1.33)	1.06 (0.69–1.63)	

Q, quintile (Q1 serves as the reference in all models); *n*, number; HR, hazard ratio; CI, confidence interval; Q1–Q5, calorie-adjusted quintiles of intake for each nutrient (supplements not included); Q1 serves as the reference in all models.

[a]All models adjusted for: age, race, body mass index, smoking status, oral-contraceptive use, menopausal hormone therapy use, caloric intake.

[b]*P* for heterogeneity from likelihood ratio tests (see manuscript for details). Comparisons made by tumour type: type I (endometrioid, adenocarcinoma, mucinous) vs type II (serous and clear cell); by histology within type I cancers (all other histologies excluded): endometrioid vs adenocarcinomas; by grade within type I cancers (all other histologies excluded): low-grade (grade I and II) vs high-grade (grade III and IV); and by stage within type I cancers (all other histologies excluded): localized vs regional/distant. Women without endometrial cancer were included in all analyses ($n = 112\,085$).

[c]Total dietary folate intake using estimates of food nutritional content before US fortification (1996–98).

one views the latter group as being at risk for greatly exceeding recommended daily intakes. In our study, there was no association between multivitamin use itself or folic-acid supplementation and endometrial cancer. A recent meta-analysis noted a slight increase in any cancer risk when considering folic-acid supplementation specifically (risk ratio 1.06, CI 0.99, 1.11); however, the effect estimates for endometrial cancer from this analysis were imprecise, as data from only 59 cases were available.[40]

There are several prospective studies with which to compare our findings. In the Iowa Women's Health Study, Uccella and colleagues reported elevated risk for type II tumours ($n = 71$) with greater intake of several B vitamins, but this was driven by supplements rather than dietary intake.[5] In our study, we did not find evidence for heterogeneity by tumour type, with the possible exception of increased risks for type I tumours with high B6 intake. This highlights the limitation in evaluating endometrial cancer risks by 'type'. Biologic interpretation within the confines of this outdated classification system is largely uninformative, given our growing knowledge about the molecular heterogeneity of endometrial cancers. We saw potential evidence for stronger risk magnitudes with natural folate in the risk for low-grade tumours but, in general, we consistently observed increased risks for total and natural folate across cancer characteristics.

Our effect magnitudes for total folate from the diet are consistent with those for total folate (including supplements) reported by researchers for the Nurses' Health Study[6]—though the authors concluded there were no associations due to imprecise estimates ($n = 788$ cases). In a prospective Canadian study, Kabat and colleagues did not identify associations between dietary intake of one-carbon metabolism nutrients (not including supplements) and endometrial cancer risk ($n = 426$ cases).[7] They report consistent observations after excluding cases diagnosed within the first 5 years of follow-up, but do not report on heterogeneity of effects between the time periods.

Ultimately, findings across studies are difficult to compare due to differences in study design and underlying population structure (e.g. the proportion of participants who are post-menopausal or obese). Ours is the largest study to explore dietary intake of one-carbon metabolism nutrients and endometrial cancer risk, but there are several limitations. A major limitation of our analysis is the lack of information on exact doses of nutrients from supplements; similarly to many studies, participants reported the frequency of multivitamin use and the types they usually take, but daily total intake of nutrients typically included in multivitamins can only be approximated. Rather than estimating total intake for all multivitamin users based on typical multivitamin composition, we chose an analogous approach of examining associations for dietary intake between multivitamin users and non-users. This is arguably the most appropriate approach when the chemical composition or biological activity of vitamins in food vs supplements differs and associations with 'total intake' could have confusing biological interpretations (e.g. folic acid and natural folate). Our study population was also homogeneous (predominately composed of White women), which limits generalizability. It is important to note that the quintile cut-points in our analysis were study-specific but, as we mainly identified risks with the highest quintiles of intake for all nutrients evaluated, this likely indicates excess intake.

Our findings provide mechanistic insight and directions for future research. We evaluated both natural and synthetic folate from the diet as well as effect modification by supplement use. Unlike other studies, we commented on important modification by time from baseline, BMI and tumour characteristics. Our results suggest that several nutrients may increase endometrial cancer risk regardless of time from baseline or cancer prognosis (e.g. grade, stage)—likely ruling out confounding by undiagnosed cancers/pre-cancers or a large proportion of advanced-stage cancers—but specific information on endometrial hyperplasia or atrophy was unavailable to fully investigate this. Future studies must consider these analyses of effect modification, as women with elevated BMI may have a unique hyper-inflammatory milieu and metabolism that modifies the influence of diet on cancer risk. Furthermore, exploring effects by time from baseline and by cancer characteristics other than 'type' is necessary to parse out the influence of confounding by disease characteristics within a given population. If our observations are replicated and with further basic science studies, we may be able to determine whether dietary interventions merit investigation as a method to reduce incidence of endometrial hyperplasia and cancer among women at risk for these conditions.

Supplementary data

Supplementary data are available at IJE online.

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