Published in final edited form as:
Eur J Clin Nutr. 2010 January ; 64(1): 111–114. doi:10.1038/ejcn.2009.109.

Dietary betaine and choline intake are not associated with risk of epithelial ovarian cancer

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Abstract

Evidence suggests that nutrients involved in one-carbon metabolism are implicated in ovarian cancer etiology. No studies have evaluated the role of choline, and its metabolite, betaine. We prospectively examined the relationship between intake of these nutrients and ovarian cancer risk among 159,957 participants from the Nurses' Health Study (NHS) and NHSII. Average nutrient intake was assessed every 2–4 years beginning in 1984 (NHS) and 1991 (NHSII). With up to 22 years of follow-up per cohort, there were 526 incident cases of ovarian cancer diagnosed. There were no associations between total choline, betaine, and choline plus betaine intake and ovarian cancer risk (e.g., relative risk, top vs bottom quintile of choline=0.98; 95%CI 0.73–1.31; *P*trend=0.81). Results did not vary by alcohol consumption, folate intake, or following exclusion of cases diagnosed during the 4-year period following dietary assessment. These data provide little evidence for a role of these nutrients in ovarian cancer etiology.

Keywords

ovarian cancer; betaine; choline

Introduction

The intake of folate and nutrients involved in one-carbon metabolism has been implicated in the etiology of cancer due to their role in mediating the transfer of moieties required for DNA methylation and *de novo* purine and thymidylate synthesis (Kim 1999). For ovarian cancer, prospective studies have suggested an inverse relationship for dietary folate intake and risk (Tworoger et al 2006); whereas, case-control studies have been null (Pelucchi et al 2005). To our knowledge, no studies have evaluated other methyl donors including choline, and its primary metabolite betaine, in ovarian cancer. Among their diverse biological roles, both nutrients are a major source of methyl groups and ensure the supply of *S*-adenosylmethionine (SAM) for many methylation reactions (Kim 1999). DNA methylation is an important epigenetic determinant of gene expression, maintenance of DNA integrity and stability, chromatin modifications, and development of mutations - all events implicated in carcinogenesis (Kim 1999). Because few modifiable risk factors for ovarian cancer exist, it is important to clarify the role of dietary factors. Thus, we prospectively examined whether

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Conflict of interest: The authors declare no conflict of interest.

betaine and choline intake were associated with the risk of developing epithelial ovarian cancer using data collected from 2 large cohort studies, the Nurses' Health Study (NHS) and NHSII.

Materials and Methods

The NHS cohort was established in 1976 among 121,700 female nurses, ages 30–55 years, and the NHSII was established in 1989 among 116,430 female nurses, ages 25–42 years. All women completed an initial questionnaire, and have been followed biennially to update exposure status and disease diagnoses. Diet was assessed through a validated self-administered, semi-quantitative food frequency questionnaire (FFQ) (Willett et al 1985). Intake was assessed in 1984, 1986, 1990, 1994, 1998 and 2002 (NHS), and 1991, 1995, 1999, and 2003 (NHSII). Nutrient intake was calculated using U.S. Department of Agriculture food composition data (U.S. Department of Agriculture 2004) and other sources (Zeisel et al 2003). We calculated the cumulative average intake of total choline and betaine (from food and supplements), adjusting for total energy intake using the nutrient residual method (Willett and Stampfer 1986). The women were classified into quintiles of cumulative average intakes.

Incident cases of epithelial ovarian cancer were reported on biennial questionnaires or identified via death certificate, and confirmed via medical record review by a pathologist. We excluded women at baseline (1984 NHS/1991 NHSII) if they did not complete the 1984 (NHS) or 1991 (NHSII) FFQ, had implausible dietary intakes, reported a previous diagnosis of cancer, or had a history of bilateral oophorectomy or pelvic irradiation. Participants contributed person-time from baseline until the date of ovarian cancer diagnosis, report of other cancer (except nonmelanoma skin cancer), death, or June 1, 2006 (NHS) or June 1, 2005 (NHS II), whichever occurred sooner.

Cases and person-time were assigned to the appropriate intake category. Cox proportional hazards models with age in months and 2-year questionnaire cycle as the time scale were used to estimate relative risks (RRs) and 95% confidence intervals (CIs), adjusting for putative ovarian cancer risk factors. Trend tests were conducted by modeling quintile median intake levels and calculating the Wald statistic. Data analyses were conducted separately for each cohort and then pooled using a random effects model to test for heterogeneity (DerSimonian and Laird 1986). *P*-values were based on 2-sided tests and considered significant at <0.05. Analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

Results

During 2,134,156 person-years for the NHS and NHSII combined, we observed 526 incident cases of ovarian cancer. Ovarian cancer risk factors were similar across quintiles of intake, except for BMI and caffeine intake, which tended to be higher among women in the fifth quintile (Table 1).

Total dietary intake of betaine, choline, and betaine plus choline, were not associated with the risk of ovarian cancer (Table 2). The RRs and 95%CIs for the top versus bottom quintile were 0.98 (0.73–1.31), 0.98 (0.74–1.31) and 0.99 (0.75–1.32), respectively. The results were similar in the lagged analysis which excluded cases diagnosed 4 years after dietary intake assessment. The associations did not vary by alcohol consumption (<5 vs ≥ 5g/day) or dietary folate intake (<400 vs ≥ 400 μg/day) (data not shown).

Discussion

In this first prospective evaluation of the relationship between choline and betaine intake and risk of ovarian cancer, no associations were observed and no effect modification was noted by alcohol consumption, a known folate antagonist, or dietary folate status.

Choline can be made *de novo* or obtained through dietary sources (Zeisel and Blusztajn 1994). Adequate intake is necessary for phospholipid synthesis in cell membranes, trans-membrane signaling, and lipid-cholesterol transport and metabolism (Zeisel and Blusztajn 1994). Betaine is an osmolyte that protects cells and proteins from environmental stress. Because homocysteine can be remethylated into methionine by accepting a methyl group from folate or betaine, these pathways are highly interrelated, and deficiency of one nutrient is compensated by the other. Most women in this cohort had adequate intake of folate (Tworoger et al 2006), thus choline and betaine may not be as important as for a population with low folate intake. A key difference between the folate and choline pathways is that betaine-mediated methylation of homocysteine predominates in the liver or kidneys; whereas, methylation by folate occurs in all cells throughout the body (Niculescu and Zeisel 2002). Given that little evidence links aberrant methylation patterns in the etiology of ovarian carcinogenesis, it is likely that epigenetic modification, particularly in the liver or kidneys, is not implicated in the development of this disease.

A major strength is the prospective nature of the NHS/NHSII, with repeated assessment of dietary and risk factor information, allowing adequate control for confounding. A limitation is that mean intake among the women in this study was somewhat low (~327 mg/day), and the variation may not have been large enough to detect an association.

In conclusion, we found no evidence for an association between choline or betaine intake and risk of ovarian cancer. It is critical to continue to investigate a role of diet in the prevention of ovarian cancer given the poor prognosis associated with this disease.

Acknowledgments

The authors thank the NHS and NHSII participants for their dedication to these studies and their contribution to this research. This research was supported by Research Grants P01 CA87969, R01 CA50385, and P50 CA105009 from the National Cancer Institute. J.K. is a Research Fellow of the Canadian Cancer Society supported through an award from the National Cancer Institute of Canada.

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Table 1

Age and age-standardized[a] characteristics of participants in the Nurses' Health Study (NHS) in 1994 and the Nurses' Health Study II (NHSII) in 1995 by study population and by the highest and lowest quintile of choline intake.

	NHS		NHSII	
	Choline (n = 49,108)		Choline (n =74,453)	
Quintile	1	5	1	5
Sample size	8794	9838	14,251	14,966
Means				
Age (years)	59.1	61.5	39.5	40.9
Age at menarche (years)	12.6	12.3	12.5	12.3
Parity (among parous women)	3.2	3.1	2.2	2.2
Duration of oral contraceptive use (among users, months)	24.1	25.4	50.3	51.2
Current body mass index (kg/m^2)	25.4	27.7	25.0	26.6
Height (meters)	1.6	1.6	1.7	1.7
Caloric intake (kcal/day)	1685	1693	1771	1778
Caffeine intake (mg/day)	264	329	195	287
Alcohol consumption (g/day)	6.0	5.5	2.7	3.6
Percentages				
Parous	94%	93%	75%	79%
Oral contraceptive use	48%	51%	85%	85%
Premenopausal[b]	13%	12%	95%	95%
Postmenopausal[b]	87%	88%	2%	3%
Family history of ovarian cancer[c]	3%	3%	2%	2%
History of tubal ligation	21%	21%	22%	24%
Hysterectomy	20%	24%	6%	7%
Current postmenopausal hormone user (among postmenopausal women)	35%	39%	65%	61%

[a] All factors except age were age-standardized in five-year intervals for each cohort. Results for quintiles of betaine are comparable (data not shown).

[b] The percentages for premenopausal and postmenopausal do not add to 100% due to women with unknown menopausal status.

[c] Mother or sister had ovarian cancer according to participant's response on questionnaire; family history was evaluated using data from 1992 for NHS because it was not available in previous cycles.

Table 2

Pooled relative risks (RR) and 95% confidence intervals (CI) of ovarian cancer according to quintile of choline and betaine intake in the Nurses' Health Study (NHS) and NHSII[a]

	Quintile[b]					
	1 (lowest)	**2**	**3**	**4**	**5 (highest)**	***P* trend[c]**
Total choline (mg/day)						
Cases (n)	87	98	121	110	110	
Person-years	400,830	405,555	413,596	433,495	480,681	
Multivariate RR (95%CI) [d]	1.00 (ref)	1.05 (0.79–1.41)	1.27 (0.96–1.68)	1.10 (0.82–1.46)	0.98 (0.73–1.31)	0.79
Lagged RR (95%CI)[e]	1.00 (ref)	1.02 (0.74–1.39)	1.24 (0.92–1.67)	0.85 (0.62–1.18)	0.96 (0.71–1.30)	0.49
Total betaine (mg/day)						
Person-years	434,793	426,722	417,769	416,778	438,093	
Cases (n)	96	106	118	103	103	
Multivariate RR (95%CI) [d]	1.00 (ref)	1.06 (0.80–1.41)	1.19 (0.90–1.56)	1.05 (0.79–1.39)	0.98 (0.74–1.31)	0.51
Lagged RR (95%CI)[e]	1.00 (ref)	1.17 (0.87–1.56)	1.26 (0.94–1.70)	1.04 (0.76–1.41)	0.99 (0.73–1.33)	0.39
Total betaine + choline (mg/day)						
Cases (n)	90	95	122	104	115	
Person-years	407,586	408,559	414,557	425,112	478,343	
Multivariate OR (95%CI) [d]	1.00 (ref)	0.99 (0.74–1.32)	1.21 (0.92–1.60)	1.01 (0.76–1.34)	0.99 (0.75–1.32)	0.76
Lagged RR (95%CI)[e]	1.00 (ref)	0.97 (0.72–1.32)	0.99 (0.73–1.34)	0.91 (0.67–1.23)	0.91 (0.68–1.22)	0.38

[a] No heterogeneity in the risk estimates between the NHS and NHSII cohorts (*P* heterogeneity = 0.76, 0.59, and 0.96, for total choline, total betaine, and total choline+betaine, respectively).

[b] The quintile cutpoints the NHS were: betaine: <70.5, 70.5-<85.7, 85.7-<101.9, 101.9-<127.3, ≥127.3 mg/day; choline: <249.5, 249.5-<278.5, 278.5-<304.9, 304.9-<338.8, ≥338.8 mg/day; total betaine+choline: <339.8, 339.8-<376.2, 376.2-<409.6, 409.6-<453.8, ≥453.8 mg/day. In the NHSII, quintile cutpoints were: betaine: <80.6, 80.6-<97.6, 97.6-<114.4, 114.4-<138.9, ≥138.9 mg/day, choline: <269.7, 269.7-<301.3, 301.3-<330.3, 330.3-<367.3, ≥367.3 mg/day, total betaine+choline: <371.6, 371.6-<411.2, 411.2-<446.2, 446.2-<491.7, ≥491.7 mg/day. These were based on the distribution in the control subjects.

[c] *P* trend values were determined using the quintile median intake as a continuous variable.

[d] Multivariate analyses adjusted for age at menarche (continuous), parity (continuous), duration of oral contraceptive use (continuous), tubal ligation (yes, no), height (< 1.6, 1.6-<1.65, 1.65-<1.7, 1.7-<1.75, and ≥ 1.75 meters), family history of breast or ovarian cancer (yes, no), caffeine intake (mg/day), hysterectomy (yes, no), postmenopausal hormone use/menopausal status (premenopausal, postmenopausal/never PMH use, postmenopausal/past PMH use, postmenopausal/current PMH use, postmenopausal/missing PMH use, and missing/unknown menopausal status), caloric intake (kcal/day), alcohol consumption (g/day), and BMI (kg/m^2).

[e] Excluding cases diagnosed during the first four years of follow-up after dietary intake assessment.