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Choline and betaine intake is inversely associated with breast cancer risk: A two-stage case-control study in China

Cai-Xia Zhang,[1,6,7] Mei-Xia Pan,[2,7] Bin Li,[1] Lian Wang,[1] Xiong-Fei Mo,[3] Yu-Ming Chen,[1] Fang-Yu Lin[4] and Suzanne C. Ho[5,6]

[1]Department of Medical Statistics and Epidemiology, School of Public Health, Sun Yat-sen University, Guangzhou; [2]Department of Clinical Nutrition, Guangdong General Hospital (Guangdong Academy of Medical Sciences), Guangzhou; [3]Department of Vascular Surgery, [4]Nursing Department, the First Affiliated Hospital of Sun Yat-sen University, Guangzhou; [5]Centre of Research and Promotion of Women's Health, School of Public Health and Primary Care, The Chinese University of Hong Kong, Hong Kong, China

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Few epidemiological studies have evaluated the association of choline and betaine intake with breast cancer risk and the results remain inconsistent. This study aimed to assess the relationship between dietary intake of choline and betaine and the risk of breast cancer among Chinese women. A two-stage case-control study was conducted, with 807 cases and 807 age- (5-year interval) and residence (rural/urban)-matched controls. A validated food frequency questionnaire was used to assess dietary intake by face-to-face interview. An unconditional logistic regression model was used to calculate multivariate-adjusted odds ratios (OR) and 95% confidence intervals (CI). A significant inverse association was found between dietary choline and betaine consumption and breast cancer risk. The adjusted OR for the highest quartile of intake compared with the lowest were 0.40 (95% CI = 0.28–0.57, P_{trend} < 0.001) for total choline intake, 0.58 (95% CI = 0.42–0.80, P_{trend} < 0.001) for betaine intake and 0.38 (0.27–0.53, P_{trend} < 0.001) for choline plus betaine intake, respectively. Intakes of individual choline compouds, choline from glycerophosphocholine, phosphocholine, phosphatidylcholine, sphingomyelin and free choline were also negatively associated with breast cancer risk. The inverse association between choline intake and breast cancer risk was primarily confined to participants with low folate level (<242 g/day), with an OR (95% CI) of 0.46 (0.23–0.91) comparing the fourth quartile with the first quartile of choline intake (P_{trend} = 0.005). The present study suggests that consumption of choline and betaine is inversely associated with the risk of breast cancer. The association of choline intake with breast cancer risk is probably modified by folate intake. (*Cancer Sci* 2013; 104: 250–258)

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.[1] DNA methylation depends on the availability of methyl groups from S-adenosylmethionine, the universal methyl donor. Folate can donate a methyl group to homocysteine to create methionine and ultimately to generate S-adenosylmethionine. Choline and betaine are important human nutrients obtained from the diet from a variety of foods.[2,3] Like folate, choline is a necessary source of methyl groups for methyl group transfer. Choline can be oxidized to betaine and is the immediate precursor of betaine, which serves as a methyl group donor in a reaction converting homocysteine to methionine, and ensures the supply of S-adenosylmethionine for many methylation reactions.[1,4]

The relationship between dietary folate intake and breast cancer risk has been examined in several epidemiological studies and many studies have found that folate intake was inversely associated with breast cancer risk.[5–7] Therefore, it is plausible that choline and betaine intake is also associated with breast cancer risk. So far, few epidemiological studies have examined the association of dietary intakes of choline and betaine with cancer risk,[8–10] although choline and betaine are important methyl donors. Only three studies examining the relationship between choline and betaine intake and breast cancer risk have been published and the results are inconsistent.[11–13] No association was observed in two of the three previous studies on this topic. Thus, more research is needed to clarify this issue.

The objective of the present study was to evaluate the associations between dietary choline and betaine intakes and breast cancer risk among Chinese women in Guangdong province. As homocysteine can be remethylated to methionine by accepting a methyl group from either betaine or folate, methylation of homocysteine by choline and betaine and by folate are highly interrelated.[4,14] We therefore evaluated whether the relationship between choline and betaine intake and breast cancer risk was modified by folate intake.

Materials and Methods

Study subjects. A two-stage case-control study was conducted to recruit breast cancer cases and controls. Detailed study methods of the first-stage case-control study design are reported elsewhere.[15,16] Briefly, a hospital-based case-control study was conducted in Guangdong province, China. Potential case subjects were recruited from patients admitted to the three teaching and general hospitals in the areas being studied. Eligible cases were 25–70-year-old natives of Guangdong province or those who have lived in Guangdong for at least 5 years, with incident, primary, histologically confirmed breast cancer diagnosed within 3 months prior to the interview. Women were excluded if they could not understand or speak Mandarin/Cantonese or had a prior history of breast cancer or other cancers. During June 2007 to August 2008, a total of 438 (96%) cases out of 455 eligible cases were successfully interviewed. Control subjects were patients with no history of cancer who were admitted to the same hospitals during the same time period as the case subjects. They were frequency matched by age (5-year interval) and residence (rural/urban) to the case patients. The controls were also natives of Guangdong province or those who had lived in Guangdong for at least 5 years. They were

[6]To whom correspondence should be addressed.
E-mails: zhangcx3@mail.sysu.edu.cn; suzanneho@cuhk.edu.hk
[7]These authors contributed equally to this work.

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Table 1. Comparison of breast cancer cases and controls on general characteristics among Chinese women

Characteristics	Stage 1			Stage 2			Total		
	Cases (*n* = 438)	Controls (*n* = 438)	*P*	Cases (*n* = 369)	Controls (*n* = 369)	*P*	Cases (*n* = 807)	Controls (*n* = 807)	*P*
Age (mean ± SD) (years)	47.04 ± 9.53	47.14 ± 9.58	0.875	48.18 ± 9.44	48.85 ± 9.40	0.334	47.56 ± 9.50	47.93 ± 9.53	0.445
Marital status, *n* (%)									
Married	409 (93.4)	413 (94.3)	0.574	342 (92.7)	341 (92.4)	0.889	751 (93.1)	754 (93.4)	0.766
Educational level, *n* (%)			0.401			0.014			0.158
Primary school or below	106 (24.2)	127 (29.0)		101 (27.4)	77 (20.9)		207 (25.7)	204 (25.3)	
Junior high school	127 (29.0)	116 (26.5)		89 (24.1)	83 (22.5)		216 (26.8)	199 (24.7)	
Senior high school/secondary technical school	109 (24.9)	109 (24.9)		94 (25.5)	133 (36.0)		203 (25.2)	242 (30.0)	
College or above	96 (21.9)	86 (19.6)		85 (23.0)	76 (20.6)		181 (22.4)	162 (20.1)	
Occupation, *n* (%)			0.157			0.022			0.005
Administrator/other white collar worker	190 (43.4)	166 (37.9)		151 (40.9)	126 (34.1)		341 (42.3)	292 (36.2)	
Blue collar worker	97 (22.1)	118 (26.9)		98 (26.6)	132 (35.8)		195 (24.2)	250 (31.0)	
Farmer/other	151 (34.5)	154 (35.2)		120 (32.5)	111 (30.1)		271 (33.6)	265 (32.8)	
Household income, *n* (%)			0.751			0.224			0.623
<2000 yuan/month	157 (35.8)	161 (36.8)		114 (30.9)	95 (25.7)		271 (33.6)	256 (31.7)	
2001–5000 yuan/month	152 (34.7)	149 (34.0)		115 (31.2)	140 (37.9)		267 (33.1)	289 (35.8)	
5001–8000 yuan/month	68 (15.5)	59 (13.5)		90 (24.4)	88 (23.8)		158 (19.6)	147 (18.2)	
>8001 yuan/month	61 (13.9)	69 (15.8)		50 (13.6)	46 (12.5)		111 (13.8)	115 (14.3)	
Body mass index (mean ± SD)	22.92 ± 3.33	22.46 ± 3.05	0.038	22.99 ± 3.43	22.48 ± 2.97	0.034	22.95 ± 3.38	22.47 ± 3.02	0.003
Regular smoker, *n* (%)	7 (1.6)	2 (0.4)	0.094	4 (1.1)	3 (0.8)	0.704	11 (1.4)	5 (0.6)	0.132
Passive smoker from husband, *n* (%)	202 (46.1)	170 (38.8)	0.029	194 (52.6)	118 (32.0)	<0.001	396 (49.1)	288 (35.7)	<0.001
Regular drinker, *n* (%)	12 (2.7)	10 (2.3)	0.666	29 (7.9)	11 (3.0)	0.003	42 (5.2)	21 (2.6)	0.007
Physical activity (exercise for health), *n* (%)			0.016			<0.001			<0.001
Never	170 (38.8)	139 (31.7)		289 (78.3)	103 (27.9)		459 (56.9)	242 (30.0)	
Occasional	46 (10.5)	35 (8.0)		19 (5.1)	137 (37.1)		65 (8.1)	172 (21.3)	
≥1 times/week	222 (50.7)	264 (60.3)		61 (16.5)	129 (35.0)		283 (35.1)	393 (48.7)	
Age at menarche (mean ± SD) (years)	14.82 ± 1.88	15.11 ± 1.84	0.019	14.46 ± 1.81	14.79 ± 1.80	0.012	14.65 ± 1.86	14.96 ± 1.83	0.001
Nulliparous, *n* (%)	17 (3.9)	19 (4.3)	0.734	13 (3.5)	15 (4.1)	0.700	30 (3.7)	34 (4.2)	0.610
No. live births (mean ± SD)†	1.98 ± 1.12	2.03 ± 1.20	0.532	1.94 ± 1.09	1.77 ± 1.08	0.046	1.96 ± 1.10	1.91 ± 1.15	0.412
Age at first live birth, *n* (%)†			0.050			0.098			0.016
≤19 years	11 (2.6)	11 (2.6)		13 (3.6)	10 (2.8)		24 (3.1)	21 (2.7)	
20–24 years	139 (33.0)	170 (40.6)		116 (32.6)	139 (39.3)		255 (32.8)	309 (40.0)	
25–29 years	227 (53.9)	201 (48.0)		170 (47.8)	167 (47.2)		397 (51.1)	368 (47.6)	
≥30 years	44 (10.5)	37 (8.8)		57 (16.0)	38 (10.7)		101 (13.0)	75 (9.7)	

Table 1 (continued)

Characteristics	Stage 1			Stage 2			Total		
	Cases (n = 438)	Controls (n = 438)	P	Cases (n = 369)	Controls (n = 369)	P	Cases (n = 807)	Controls (n = 807)	P
Months of breast feeding, n (%)†			0.034			0.077			0.375
Never	49 (11.6)	26 (6.2)		38 (10.3)	46 (13.0)		87 (11.2)	72 (9.3)	
1–3	21 (5.0)	22 (5.3)		23 (6.2)	22 (6.2)		44 (5.7)	44 (5.7)	
4–11	95 (22.6)	108 (25.8)		83 (22.5)	103 (29.1)		178 (22.9)	211 (27.3)	
12–23	118 (285.0)	102 (24.3)		87 (23.6)	92 (26.0)		205 (26.4)	194 (25.1)	
≥24	138 (32.8)	161 (38.4)		125 (33.9)	91 (25.7)		263 (33.8)	252 (32.6)	
Age at menopause (mean ± SD) (years)‡	49.33 ± 3.96	49.06 ± 3.93	0.579	50.12 ± 4.08	49.56 ± 3.84	0.251	49.71 ± 4.03	49.32 ± 3.89	0.253
Menopausal status, n (%)									
Premenopausal	306 (69.9)	295 (67.4)	0.423	243 (65.9)	218 (59.1)	0.057	549 (68.0)	513 (63.6)	0.059
Postmenopausal	132 (30.1)	143 (32.6)		126 (34.1)	151 (40.9)		258 (32.0)	294 (36.4)	
Mother/sister/daughter with breast cancer, n (%)	17 (3.9)	4 (0.9)	0.004	15 (4.1)	4 (1.1)	0.011	32 (4.0)	8 (1.0)	<0.001
Ever used an oral contraceptive, n (%)	27 (6.2)	21 (4.8)	0.373	30 (8.1)	20 (5.4)	0.143	57 (7.1)	41 (5.1)	0.095
Total energy intake (mean ± SD) (kcal/day)	1464 ± 430	1504 ± 429	0.160	1473 ± 403	1627 ± 461	<0.001	1468 ± 418	1560 ± 448	<0.001
Total choline intake (mean ± SD) (mg/day)	141.36 ± 63.46	164.97 ± 72.39	<0.001	153.32 ± 76.68	182.93 ± 79.12	<0.001	146.83 ± 70.02	173.18 ± 76.02	<0.001
Betaine intake (mean ± SD) (mg/day)	275.93 ± 261.44	326.91 ± 279.04	0.005	219.62 ± 151.84	304.35 ± 222.43	<0.001	250.18 ± 219.94	316.60 ± 254.81	<0.001
Folate intake (mean ± SD) (μg/day)	207.41 ± 72.51	240.08 ± 85.04	<0.001	214.06 ± 81.46	269.13 ± 98.99	<0.001	210.45 ± 76.75	253.36 ± 92.76	<0.001

†Among parous women. ‡Among menopausal women.

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Table 2. Comparison of five main individual choline compound intakes between breast cancer cases and controls

Choline compound	Cases (n = 807)					Controls (n = 807)					P†
	Mean	SD	P_{25}	P_{50}	P_{75}	Mean	SD	P_{25}	P_{50}	P_{75}	
Free choline (mg/day)	32.5	15.1	21.8	29.3	40.0	39.0	16.9	27.1	36.2	47.7	<0.001
Choline from glycerophosphocholine (mg/day)	12.6	9.2	6.8	9.5	15.1	16.0	10.8	8.3	12.6	20.9	<0.001
Choline from phosphocholine (mg/day)	12.4	7.8	7.2	10.7	15.5	15.1	8.5	9.5	13.7	18.9	<0.001
Choline from phosphatidylcholine (mg/day)	84.9	46.0	52.9	74.0	106.8	98.0	49.0	60.9	91.8	125.4	<0.001
Choline from sphingomyelin (mg/day)	4.5	3.3	2.2	3.8	6.0	5.4	3.9	2.6	4.6	7.4	<0.001

†Wilcoxon rank-sum test comparing median consumption levels between cases and controls. P_{25}, P_{50} and P_{75} represent the 25th, 50th and 75th percentile, respectively. SD, standard deviation.

Table 3. Top five food sources of total choline, betaine and folate among control subjects

Total choline		Betaine		Folate	
Food	Percentage	Food	Percentage	Food	Percentage
Eggs	24.57	Spinach	74.24	Rice	18.22
Chicken	20.53	Pasta	16.38	Chinese cabbage	10.04
Broccoli	7.61	White bread	3.93	Spinach	8.04
Spinach	4.82	Wheat bread	2.85	Eggs	7.71
Whole milk	3.82	Potatoes	1.05	Soybean milk	4.91

selected from the departments of Ophthalmology, Plastic and Reconstructive Surgery, Vascular Surgery, Ear-Nose-Throat, and Orthopedics and Microsurgery. In total, 448 controls were identified; 10 (2%) controls invited to take part in the study during their hospital stay refused to be interviewed.

To improve the statistical power of the study, we recruited a second set of cases and controls. The second-stage study is an ongoing case-control study beginning in September 2011. Between September 2011 and August 2012, 369 cases and 369 controls were recruited. These two studies shared the same diagnostic standard and the same inclusion and exclusion criteria. The distribution of conditions among the control subjects is as follows: glaucoma/uveitis/keratitis/pterygium/dacryocystitis/optic neuritis (376; 46.6%); sudden deafness/acute bacterial/viral otitis media/sinusitis/deviation of nasal septum /tonsillitis (328; 40.7%); varicose veins (43; 5.3%); traumatic skeletal disorders/osteoarthritis/degenerate joint disease (27; 3.4%); orthopedics (22; 2.7%); trifacial neuralgia (9; 1.1%); and acute appendicitis (2; 0.2%).

The procedures and protocols of the present study were approved by The Ethical Committee of School of Public Health, Sun Yat-sen University, and the Ethical Committee of the Chinese University of Hong Kong. Written informed consent was obtained from all participants.

Assessment of dietary intake. Dietary intake was assessed through an in-person interview by using an 81-item food frequency questionnaire (FFQ)[17] covering the habitual diet of participants during the preceding 12 months. Participants also reported their recent dietary changes and use of nutritional supplements. Food photographs with usual portion size were used to help participants quantify the portions consumed. During the interview, each woman was asked to report their usual frequency of consumption as the number of times per day, per week, per month, per year or never during the previous year before the time of diagnosis for cases or interview for controls and the average amount of food eaten each time. Information on the frequency of intake and portion size was used to calculate the amount of each food item in grams consumed on average per day. Daily dietary nutrient intakes including choline and betaine and other nutrients were calculated based on the Chinese Food Composition Table[18] and values published by Zeisel *et al.*[19,20] Total dietary intakes of energy, individual compound sources of choline (free choline, glycerophosphocholine, phosphocholine, phosphatidylcholine and sphingomyelin), betaine and folate were calculated by summing the product of the frequency of consumption, usual portion consumed and nutrient content of each food item. Total choline intake was calculated as the sum of choline intake from glycerophosphocholine, phosphocholine, phosphatidylcholine, sphingomyelin and free choline.

Development and validation of the FFQ have previously been described.[5,17] Briefly, 61 female subjects recruited from the community in Guangzhou city completed 3-day dietary records at intervals of 2 months during a 12-month period, and two FFQ administered 1 year apart. The correlation coefficients comparing the second FFQ and 18-day dietary records were 0.34 for choline, 0.26 for betaine, 0.35 for folate, 0.48 for glycerophosphocholine, 0.44 for phosphocholine, 0.23 for phosphatidylcholine and 0.36 for sphingomyelin and free choline, respectively. The correlation coefficients between the two FFQ of choline, betaine, folate, glycerophosphocholine, phosphocholine, phosphatidylcholine, sphingomyelin, free choline were 0.59, 0.44, 0.60, 0.67, 0.64, 0.56, 0.54 and 0.58, respectively.

Data collection. A structured questionnaire was used to collect information on socio-demographic indicators such as age, educational level, household income, occupation and marital status, anthropometrics including current weight and height, menstrual and reproductive history, family history of breast cancer, physical activity over the past 12 months, smoking and alcohol consumption habits, and prior disease history. Relevant medical information, medical diagnosis and histological findings were abstracted from hospital medical records. Body mass index (BMI) was computed by dividing weight (kg) by height squared (m^2). Regular smoking was defined as smoking at least one cigarette per day for more than six consecutive months. Regular drinking was defined as alcohol drinking at least once per week during the past year.

Statistical analyses. All data analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Chi-squared or t tests were used to test differences in socio-demographic and

Table 4. Odds ratios (OR) and 95% confidence intervals (CI) of breast cancer according to quartiles of choline and betaine intake in stage 1, stage 2 and the combined study

Methyl donors	Stage 1				Stage 2				Total			
	Intake	No. cases/controls	Crude OR (95% CI)	Adjusted OR (95% CI)†	Intake	No. cases/controls	Crude OR (95% CI)	Adjusted OR (95% CI)†	Intake	No. cases/controls	Crude OR (95% CI)	Adjusted OR (95% CI)†
Total choline (mg/day)	<111.64	162/109	1	1	<123.66	157/92	1	1	<117.65	321/201	1	1
	111.64–155.55	125/110	0.70 (0.50–0.99)	0.71 (0.49–1.02)	123.66–172.89	94/93	0.70 (0.47–1.04)	0.83 (0.50–1.36)	117.65–163.62	227/202	0.70 (0.54–0.91)	0.70 (0.53–0.93)
	155.55–207.86	86/110	0.42 (0.29–0.61)	0.49 (0.33–0.72)	172.89–232.24	67/92	0.45 (0.30–0.68)	0.58 (0.31–1.10)	163.62–217.47	141/203	0.44 (0.33–0.57)	0.47 (0.34–0.64)
	>207.86	65/109	0.41 (0.28–0.62)	0.35 (0.22–0.54)	>232.24	51/92	0.32 (0.21–0.49)	0.51 (0.30–0.87)	>217.47	118/201	0.37 (0.28–0.49)	0.40 (0.28–0.57)
	P_{trend}		<0.001	<0.001	P_{trend}		<0.001	0.022	P_{trend}		<0.001	<0.001
Betaine (mg/day)	<132.41	123/109	1	1	<154.75	146/92	1	1	<140.14	260/200	1	1
	132.41–239.18	126/110	1.00 (0.70–1.43)	1.10 (0.75–1.60)	154.75–257.51	115/93	0.85 (0.58–1.25)	0.98 (0.61–1.58)	140.14–246.87	248/204	0.94 (0.72–1.21)	1.13 (0.84–1.50)
	239.18–440.90	117/110	0.88 (0.61–1.29)	0.97 (0.66–1.43)	257.51–385.28	65/92	0.51 (0.34–0.76)	0.56 (0.33–0.95)	246.87–408.04	178/201	0.68 (0.52–0.90)	0.83 (0.61–1.12)
	>440.90	72/109	0.63 (0.44–0.92)	0.60 (0.39–0.92)	>385.28	43/92	0.28 (0.17–0.45)	0.45 (0.25–0.80)	>408.04	121/202	0.46 (0.34–0.62)	0.58 (0.42–0.80)
	P_{trend}		0.015	0.021	P_{trend}		<0.001	0.002	P_{trend}		<0.001	<0.001
Total choline + betaine (mg/day)	<279.40	144/109	1	1	<314.00	172/92	1	1	<297.57	320/201	1	1
	279.40–407.11	116/110	0.92 (0.64–1.33)	0.83 (0.56–1.22)	314.00–440.16	93/93	0.50 (0.34–0.74)	0.70 (0.43–1.13)	297.57–422.50	204/202	0.63 (0.49–0.82)	0.74 (0.56–1.00)
	407.11–627.38	115/110	0.76 (0.53–1.08)	0.81 (0.55–1.18)	440.16–600.19	59/92	0.30 (0.20–0.44)	0.45 (0.26–0.78)	422.50–615.63	176/203	0.54 (0.42–0.71)	0.67 (0.50–0.92)
	>627.38	63/109	0.43 (0.29–0.63)	0.43 (0.28–0.66)	>600.19	45/92	0.24 (0.15–0.38)	0.40 (0.22–0.71)	>615.63	107/201	0.33 (0.25–0.45)	0.38 (0.27–0.53)
	P_{trend}		<0.001	0.001	P_{trend}		<0.001	<0.001	P_{trend}		<0.001	<0.001

†Odds ratios were adjusted for occupation, body mass index, age at menarche, live births and age at first live birth, mother/sister/daughter with breast cancer, passive smoking, alcohol consumption, physical activity, total energy intake and study stage.

reproductive factors between the case and control subjects. Total and individual choline and betaine were grouped into quartiles based on their distributions in the control subjects for stage 1 and stage 2 separately, and combined, with the lowest quartile serving as the reference. Unconditional logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) of each quartile with the lowest quartile group serving as the reference after adjusting for the potential confounding variables. Potential confounders adjusted for in multivariate models included occupation (categorical, administrator/other white collar worker, blue collar worker and farmer/other), BMI (continuous), age at menarche, live births and age at first live birth ($\leq$19, 20–24, 25–29, $\geq$30, nulliparous), family history of breast cancer in a first-degree relative (yes/no), passive smoking from husband (yes/no), alcohol consumption (yes/no), physical activity (categorical, never, occasional and $\geq$1 time per week) and study stage (stage 1 or 2). Total energy intake was adjusted using the residual method. [21] Tests for trend were performed by entering the categorical variables as continuous variables in the models.

We previously observed that dietary folate intake was inversely associated with breast cancer risk.[5] Because both folate and choline (through betaine) are involved in methyl-group metabolism as methyl-group donors, analysis stratified by folate intake values above and below the median (<242 *vs* $\geq$242 μg/day) was conducted to evaluate the potential modifying effect on choline and betaine intake and breast cancer risk. Because pre- and postmenopausal breast cancer probably has a separate disease etiology, we further investigated associations of choline and betaine consumption with breast cancer risk by menopausal status. Likelihood ratio tests comparing models with and without multiplicative interaction terms were used to assess the potential effect measure modification of the association between choline and betaine intakes and breast cancer risk by folate intakes and menopause status. Alcohol is a known folate antagonist.[22,23] It might also interfere with choline or betaine absorption. Analysis of non-drinkers was performed to evaluate the association of choline or betaine intake with breast cancer risk. All statistical tests were based on two-tailed probability values with P values of $\leq$0.05 interpreted as statistically significant.

Results

The socio-demographic and established breast cancer risk factors of the two-stage study subjects are shown in Table 1. Overall, the participant characteristics in two-stage studies were highly comparable. In both the first- and second-stage study, compared with controls, cases had an earlier age at menarche, older age at first live birth, higher BMI and consumed more alcohol. Cases were more likely to have a history of breast cancer in a first-degree relative, a history of passive smoking from husband, and were less likely to be physically active than controls. No significant differences were found between the case and control subjects in educational level, marital status, household income or reproductive factors, including nulliparous, number of live births, age at menopause and use of oral contraceptive.

The mean ($\pm$SD) intakes were 173 $\pm$ 76 mg/day for total choline, 317 $\pm$ 255 mg/day for betaine and 490 $\pm$ 291 mg/day for choline plus betaine among the control subjects (Table 1). More than half (57.8%) the choline intake came from phosphatidylcholine, followed by free choline (22.1%), glycerophosphocholine (8.6%), phosphocholine (8.4%) and sphingomyelin (3.1%) (Table 2). Compared with controls, cases tended to have lower intakes of total choline, individual choline compounds and betaine.

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Table 5. Odds ratios (OR) and 95% confidence intervals (CI) of breast cancer according to quartiles of five main choline-containing compound intake in stage 1, stage 2 and the combined study

Choline compound	Stage 1				Stage 2				Total			
	Intake	No. cases/ controls	Crude OR (95% CI)	Adjusted OR (95% CI)†	Intake	No. cases/ controls	Crude OR (95% CI)	Adjusted OR (95% CI)†	Intake	No. cases/ controls	Crude OR (95% CI)	Adjusted OR (95% CI)†
Free choline	<25.74	167/109	1	1	<28.42	169/92	1	1	<27.12	342/201	1	1
(mg/day)	25.74–35.29	128/110	0.76 (0.54–1.08)	0.72 (0.49–1.04)	28.42–37.34	86/93	0.50 (0.34–0.74)	0.65 (0.40–1.05)	27.12–36.20	209/203	0.60 (0.47–0.78)	0.68 (0.51–0.90)
	35.29–46.99	84/110	0.50 (0.34–0.72)	0.46 (0.31–0.69)	37.34–48.70	60/92	0.36 (0.24–0.54)	0.47 (0.27–0.82)	36.20–47.68	144/203	0.42 (0.32–0.55)	0.46 (0.33–0.62)
	>46.99	59/109	0.35 (0.24–0.53)	0.30 (0.19–0.49)	>48.70	54/92	0.32 (0.21–0.49)	0.46 (0.25–0.84)	>47.68	112/200	0.33 (0.25–0.44)	0.38 (0.26–0.54)
	P_{trend}		<0.001	<0.001	P_{trend}		<0.001	0.004	P_{trend}		<0.001	<0.001
Choline from	<8.18	159/109	1	1	<8.35	153/92	1	1	<8.29	314/202	1	1
glycerophosphocholine	8.18–12.05	112/110	0.70 (0.49–1.00)	0.72 (0.49–1.06)	8.35–13.22	114/92	0.74 (0.51–1.09)	1.16 (0.70–1.92)	8.29–12.58	216/202	0.69 (0.53–0.89)	0.82 (0.61–1.10)
(mg/day)	12.05–20.33	93/110	0.58 (0.40–0.84)	0.60 (0.40–0.90)	13.22–22.09	60/93	0.39 (0.26–0.59)	0.46 (0.26–0.81)	12.58–20.93	161/200	0.52 (0.39–0.68)	0.64 (0.47–0.89)
	>20.33	74/109	0.46 (0.32–0.68)	0.45 (0.29–0.70)	>22.09	42/92	0.28 (0.18–0.43)	0.36 (0.19–0.70)	>20.93	116/203	0.37 (0.28–0.49)	0.44 (0.31–0.63)
	P_{trend}		<0.001	<0.001	P_{trend}		<0.001	<0.001	P_{trend}		<0.001	<0.001
Choline from	<9.18	179/109	1	1	<9.71	155/92	1	1	<9.50	336/202	1	1
phosphocholine	9.18–13.65	117/110	0.65 (0.46–0.92)	0.63 (0.44–0.91)	9.71–13.79	83/92	0.54 (0.36–0.79)	0.65 (0.39–1.07)	9.50–13.66	190/202	0.56 (0.43–0.74)	0.61 (0.46–0.81)
(mg/day)	13.65–18.72	78/110	0.43 (0.30–0.63)	0.41 (0.27–0.60)	13.79–19.06	75/93	0.48 (0.32–0.71)	0.72 (0.44–1.20)	13.66–18.87	162/204	0.48 (0.36–0.63)	0.49 (0.36–0.66)
	>18.72	64/109	0.36 (0.24–0.53)	0.34 (0.23–0.52)	>19.06	56/92	0.36 (0.24–0.55)	0.59 (0.33–1.06)	>18.87	119/199	0.36 (0.27–0.48)	0.40 (0.29–0.56)
	P_{trend}		<0.001	<0.001	P_{trend}		<0.001	0.051	P_{trend}		<0.001	<0.001
Choline from	<57.73	139/110	1	1	<65.67	141/92	1	1	<60.92	272/201	1	1
phosphatidylcholine	57.73–84.34	136/109	0.99 (0.69–1.41)	0.91 (0.63–1.32)	65.67–100.41	113/92	0.80 (0.55–1.17)	0.96 (0.59–1.56)	60.92–91.78	259/203	0.94 (0.73–1.22)	0.95 (0.72–1.27)
(mg/day)	84.34–120.21	91/110	0.66 (0.45–0.95)	0.62 (0.41–0.92)	100.41–137.34	48/93	0.34 (0.22–0.52)	0.38 (0.21–0.67)	91.78–125.39	127/202	0.46 (0.35–0.62)	0.52 (0.38–0.72)
	>120.21	72/109	0.52 (0.35–0.77)	0.48 (0.31–0.74)	>137.34	67/92	0.48 (0.32–0.72)	0.78 (0.44–1.38)	>125.39	149/201	0.55 (0.41–0.72)	0.61 (0.44–0.85)
	P_{trend}		<0.001	<0.001	P_{trend}		<0.001	0.069	P_{trend}		<0.001	<0.001
Choline from	<2.36	130/109	1	1	<3.26	142/92	1	1	<2.58	253/202	1	1
sphingomyelin	2.36–4.14	116/110	0.88 (0.61–1.27)	0.84 (0.58–1.22)	3.26–5.26	101/93	0.70 (0.48–1.03)	1.09 (0.65–1.84)	2.58–4.59	239/201	0.95 (0.73–1.24)	0.94 (0.70–1.26)
(mg/day)	4.14–6.45	102/112	0.76 (0.53–1.10)	0.74 (0.50–1.10)	5.26–8.33	94/92	0.66 (0.45–0.98)	0.65 (0.39–1.08)	4.59–7.39	193/203	0.76 (0.58–1.00)	0.88 (0.64–1.19)
	>6.45	90/107	0.70 (0.48–1.03)	0.71 (0.46–1.08)	>8.33	32/92	0.22 (0.14–0.36)	0.27 (0.14–0.54)	>7.39	122/201	0.48 (0.36–0.65)	0.56 (0.40–0.80)
	P_{trend}		0.049	0.081	P_{trend}		<0.001	0.014	P_{trend}		<0.001	0.003

†Odds ratios were adjusted for occupation, body mass index, age at menarche, live births and age at first live birth, mother/sister/daughter with breast cancer, passive smoking, alcohol consumption, physical activity, total energy intake and study stage.

The main food sources of total choline, betaine and folate among control subjects are listed in Table 3. Spinach contributed 74.24% of the betaine intake, whereas the other main sources of betaine were grain products. Eggs, chicken and whole milk were the main food sources of total choline. Rice, Chinese cabbage and spinach were the main food sources of folate. The correlation coefficients were 0.487 between folate and choline, 0.730 between folate and betaine and 0.366 between choline and betaine.

Associations between quartiles of intakes of dietary choline and betaine and breast cancer risk are presented in Table 4. Intakes of total choline and betaine were inversely related to breast cancer risk in a dose-response manner in stage 1, stage 2 and the combined study ($P_{trend} < 0.001$). In the combined subjects from the two-stage study, the OR for the highest quartile of intake in comparison with the lowest were 0.37 (95% CI = 0.28–0.49, $P_{trend} < 0.001$) for total choline intake and 0.46 (95% CI = 0.34–0.62, $P_{trend} < 0.001$) for betaine intake, respectively. Further adjustment for potential confounding by the major risk factors for breast cancer did not materially change the results.

Because the conversion of choline to betaine is irreversible when it donates a methyl group to homocysteine, we also examined the combined intakes of choline and betaine and an inverse association with breast cancer risk was also observed. The adjusted OR (95% CI) for the fourth versus the first quartile was 0.38 (0.27–0.53) ($P_{trend} < 0.001$).

Intakes of individual compound sources of choline, choline from glycerophosphocholine, phosphocholine, phosphatidylcholine, sphingomyelin and free choline were similarly inversely associated with breast cancer risk in the separate analysis in the stage 1 and 2 study and the combined study. Compared with the lowest quartile, the adjusted OR (95% CI) of breast cancer for the highest quartile was 0.44 (0.31–0.63) for choline from glycerophosphocholine, 0.40 (0.29–0.56) for choline from phosphocholine, 0.61 (0.44–0.85) for choline from phosphatidylcholine, 0.56 (0.40–0.80) for choline from sphingomyelin and 0.38 (0.26–0.54) for free choline in the combined subjects from the two-stage study, respectively (Table 5).

Stratified analyses by levels of folate intake showed the inverse association between choline or choline plus betaine intake with breast cancer risk only among women with low folate level (OR for the highest *vs* lowest quartile 0.46 [95% CI = 0.23–0.91, $P_{trend} = 0.005$] and 0.46 [95% CI = 0.26–0.81, $P_{trend} = 0.011$]), but not among those with a high folate intake. However, the interaction effect between consumption of folate and choline was not statistically significant (P for interaction = 0.437). Betaine intake was inversely associated with breast cancer risk at the low level of folate intake but the association was not statistically significant (Table 6). We also examined menopausal status as a potential effect modifier but found similar inverse associations across the strata (Table 7). Sensitivity analyses excluding women with alcohol intake yielded similar results of the association of choline and betaine intake and breast cancer risk (data not shown).

Discussion

This two-stage case-control study examined the association between choline and betaine intake and breast cancer risk among Chinese women. Our results found that higher intakes of choline and betaine were associated with a decreased risk of breast cancer. Choline intakes from individual sources were also inversely associated with breast cancer risk. The negative association between choline intake and breast cancer risk was modified by level of folate intake.

Studies examining the associations between choline and betaine intake and breast cancer risk have yielded inconsistent results. The Nurses' Health Study II, conducted in the USA,

Table 6. Associations between quartiles of choline and betaine intake and breast cancer risk according to folate intake

Methyl donors	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
Total choline					
Folate <242 μg/day					
No. cases/controls	310/182	186/128	82/68	20/25	0.005
Adjusted OR (95% CI)†	1	0.76 (0.55–1.04)	0.65 (0.43–0.99)	0.46 (0.23–0.91)	
Folate ≥242 μg/day					
No. cases/controls	11/19	41/74	59/135	98/176	0.970
Adjusted OR (95% CI)†	1	0.68 (0.27–1.72)	0.56 (0.23–1.36)	0.76 (0.32–1.84)	
$P_{interaction}$			0.437		
Betaine					
Folate <242 μg/day					
No. cases/controls	239/149	191/117	118/88	50/49	0.130
Adjusted OR (95% CI)†	1	1.11 (0.80–1.55)	0.88 (0.60–1.28)	0.68 (0.42–1.09)	
Folate ≥242 μg/day					
No. cases/controls	21/51	57/87	60/113	71/153	0.756
Adjusted OR (95% CI)†	1	1.71 (0.86–3.38)	1.38 (0.71–2.70)	1.19 (0.62–2.29)	
$P_{interaction}$			0.670		
Total choline + betaine					
Folate <242 μg/day					
No. cases/controls	307/170	160/126	102/75	29/32	
Adjusted OR (95% CI)†	1	0.74 (0.53–1.02)	0.80 (0.54–1.18)	0.46 (0.26–0.81)	0.011
Folate ≥242 μg/day					
No. cases/controls	13/31	44/76	74/128	78/169	
Adjusted OR (95% CI)†	1	1.50 (0.66–3.46)	1.35 (0.61–2.98)	1.06 (0.48–2.31)	0.424
$P_{interaction}$			0. 694		

†Odds ratios were adjusted for occupation, body mass index, age at menarche, live births and age at first live birth, mother/sister/daughter with breast cancer, passive smoking, alcohol consumption, physical activity, total energy intake and study stage. CI, confidence interval; OR, odds ratio.

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was the first study on choline and betaine intake and breast cancer risk.[11] This study showed no evidence that higher intakes of choline and betaine reduce the risk of pre-menopausal breast cancer, with an adjusted relative risk (RR) (95% CI) of 0.86 (0.57–1.30) for choline (P_{trend} = 0.67) and 0.85 (0.54–1.33) for betaine (P_{trend} = 0.55) comparing the top and bottom quintiles. Cho *et al.*[12] also reported that no association was found between choline or betaine intake and the risk of postmenopausal breast cancer in the Nurses' Health Study; the RR (95% CI) were 1.10 (0.99–1.22, P_{trend} = 0.14) for choline and 0.98 (0.89–1.09, P_{trend} = 0.96) for betaine comparing the highest and lowest quintiles. However, one case-control study of 1508 breast cancer cases and 1556 controls found an inverse association between choline intake and breast cancer risk; the OR was 0.76 (95% CI = 0.58–1.00) comparing the top quintile with the bottom quintile.[13] Inconsistent results were also found on the relationships between choline and betaine intake and other cancers. No associations were found between intake of choline and betaine and the risk of ovarian cancer and colorectal cancer.[9,10] In contrast, a nested case-control study reported that an elevated plasma concentration of choline was associated with an increased risk of prostate cancer,[8] most likely because plasma choline levels can reflect dietary intake, genetic influences and other factors. Becasue of the limited studies, the protective effects of choline and betaine intake on breast cancer risk observed in both pre- and postmenopausal women in the present study need to be confirmed by further research in other populations.

Because phosphatidylcholine and sphingomyelin are lipid soluble, whereas free choline and other sources of choline including phosphocholine and glycerophosphocholine are water soluble, the bioavailability of different choline sources might differ. However, analyses of individual choline compounds revealed that intakes from different sources were all associated with a reduced risk of breast cancer.

In the present study, the inverse association between choline intake and breast cancer risk appeared to be particularly strong among those with a lower level of folate intake. Our data support the contention that individuals with a low folate intake might benefit from the intake of choline. This concurs with studies in animals and humans indicating the use of choline for methyl transfer reactions in the absence of folate. Animal studies reported a reduction in hepatic choline content in rats fed a folate-deficient diet compared with controls.[24] A folate-depletion study conducted in men and women also showed that the need for choline is significantly increased during folate deficiency.[25] A modifying effect of folate intake on choline was also found in the Framingham Offspring Study.[26] This study showed that the inverse association between choline intake and homocysteine levels was limited to participants with low levels of folate intake ($\leq$250 μg/day).[26] However, the Nurses' Health Study found no association between choline intake and breast cancer risk when stratified by levels of folate intake.[12] Half of the women in this cohort had adequate folate intake ($\geq$400 μg/day), thus choline might not be as important in a population with low folate intake.

Dietary choline is obtained primarily from animal sources, whereas betaine is largely derived from plant sources, as is folate.[19] As folate and betaine in human diets mainly come from plant foods and share the same food sources, participants with a lower intake level of folate might simultaneously have a lower betaine intake level. This might explain the less significant effect of dietaty folate on the association of betaine intake with breast cancer risk, as observed in the present study.

In China, the recommended intake for choline is 450 mg /day[27] and no recommended daily intake is set for betaine. The mean dietary choline intake in the control group of the present study was 173 mg/day, which shows the potential deficiency of choline in the current study population.

Table 7. Associations between quartiles of choline and betaine intake and breast cancer risk according to menopausal status

Methyl donors	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p_{trend}
Total choline					
Pre-menopause					
No. cases/controls	220/128	159/123	88/144	82/118	
Adjusted OR (95% CI)†	1	0.76 (0.54–1.08)	0.40 (0.27–0.60)	0.47 (0.30–0.72)	<0.001
Postmenopause					
No. cases/controls	101/73	68/79	53/59	36/83	
Adjusted OR (95% CI)†	1	0.60 (0.36–1.01)	0.67 (0.38–1.19)	0.32 (0.17–0.63)	0.003
$P_{interaction}$			0.945		
Betaine					
Pre-menopause					
No. cases/controls	170/121	166/125	125/134	88/133	
Adjusted OR (95% CI)†	1	1.17 (0.82–1.69)	0.83 (0.57–1.20)	0.60 (0.40–0.89)	0.004
Postmenopause					
No. cases/controls	90/79	82/79	53/67	33/69	
Adjusted OR (95% CI)†	1	1.03 (0.63–1.68)	0.83 (0.48–1.44)	0.50 (0.27–0.92)	0.031
$P_{interaction}$			0.830		
Total choline + betaine					
Pre-menopause					
No. cases/controls	210/127	143/125	121/130	75/131	
Adjusted OR (95% CI)†	1	0.82 (0.57–1.18)	0.73 (0.50–1.07)	0.40 (0.27–0.61)	<0.001
Postmenopause					
No. cases/controls	110/74	61/77	55/73	32/70	
Adjusted OR (95% CI)†	1	0.63 (0.38–1.06)	0.61 (0.35–1.05)	0.32 (0.17–0.62)	0.001
$P_{interaction}$			0.711		

†Odds ratios were adjusted for occupation, body mass index, age at menarche, live births and age at first live birth, mother/sister/daughter with breast cancer, passive smoking, alcohol consumption, physical activity, total energy intake, and study stage. CI, confidence interval; OR, odds ratio.

The present study had some limitations. Selection bias is a potential limitation in hospital-based case-control studies. The socio-demographic characteristics and dietary habits of cases and controls consecutively recruited from the studied hospitals might differ from that of the general population or patients admitted into other hospitals. However, a substantially high participation rate (96% and 98% for cases and controls, respectively) weighs against the selection bias in the present study. The use of hospital-based controls with conditions potentially related to diet is also a major concern. To reduce this bias, an attempt was made to recruit controls from several disease conditions with no apparent association with a dietary cause. Recall bias is a particular problem inherent in case-control studies. We tried to interview patients as soon as a diagnosis was made or before the operation to minimize this bias. We also provided photographs with usual intake portions of foods to help participants quantify the amount of food consumed. In addition, misclassification of choline and betaine intake is unavoidable due to measurement error in the food frequency questionnaire. However, any measurement error would most likely attenuate the true association between breast cancer risk and diet intake.

In summary, the present study found that intakes of dietary choline and betaine were inversely associated with breast cancer risk. The inverse association of dietary choline intake with breast cancer risk was manifested primarily in participants with low folate intakes.

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Disclosure Statement

The authors have no conflict of interest.

References

1 Kim YI. Folate and carcinogenesis: evidence, mechanisms, and implications. *J Nutr Biochem* 1999; **10**: 66–88.
2 Craig SA. Betaine in human nutrition. *Am J Clin Nutr* 2004; **80**: 539–49.
3 Zeisel SH. Choline: critical role during fetal development and dietary requirements in adults. *Annu Rev Nutr* 2006; **26**: 229–50.
4 Zeisel SH, Blusztajn JK. Choline and human nutrition. *Annu Rev Nutr* 1994; **14**: 269–96.
5 Zhang CX, Ho SC, Chen YM, Lin FY, Fu JH, Cheng SZ. Dietary folate, vitamin B6, vitamin B12 and methionine intake and risk of breast cancer by estrogen and progesterone receptor status. *Br J Nutr* 2011; **106**: 936–43.
6 Ericson U, Sonestedt E, Gullberg B, Olsson H, Wirfält E. High folate intake is associated with lower breast cancer incidence in postmenopausal women in the Malmö Diet and Cancer cohort. *Am J Clin Nutr* 2007; **86**: 434–43.
7 Larsson SC, Giovannucci E, Wolk A. Folate and risk of breast cancer: a meta-analysis. *J Natl Cancer Inst* 2007; **99**: 64–76.
8 Johansson M, Van Guelpen B, Vollset SE *et al.* One-carbon metabolism and prostate cancer risk: prospective investigation of seven circulating B vitamins and metabolites. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1538–43.
9 Kotsopoulos J, Hankinson SE, Tworoger SS. Dietary betaine and choline intake are not associated with risk of epithelial ovarian cancer. *Eur J Clin Nutr* 2010; **64**: 111–4.
10 Lee JE, Giovannucci E, Fuchs CS, Willett WC, Zeisel SH, Cho E. Choline and betaine intake and the risk of colorectal cancer in men. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 884–7.
11 Cho E, Holmes M, Hankinson SE, Willett WC. Nutrients involved in one-carbon metabolism and risk of breast cancer among premenopausal women. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 2787–90.
12 Cho E, Holmes MD, Hankinson SE, Willett WC. Choline and betaine intake and risk of breast cancer among post-menopausal women. *Br J Cancer* 2010; **102**: 489–94.
13 Xu X, Gammon MD, Zeisel SH *et al.* Choline metabolism and risk of breast cancer in a population-based study. *FASEB J* 2008; **22**: 2045–52.
14 Niculescu MD, Zeisel SH. Diet, methyl donors and DNA methylation: interactions between dietary folate, methionine and choline. *J Nutr* 2002; **132**: 2333S–5S.
15 Zhang CX, Ho SC, Chen YM, Fu JH, Cheng SZ, Lin FY. Greater vegetable and fruit intake is associated with a lower risk of breast cancer among Chinese women. *Int J Cancer* 2009; **125**: 181–8.
16 Zhang CX, Ho SC, Lin FY, Cheng SZ, Fu JH, Chen YM. Soy product and isoflavone intake and breast cancer risk defined by hormone receptor status. *Cancer Sci* 2010; **101**: 501–7.
17 Zhang CX, Ho SC. Validity and reproducibility of a food frequency questionnaire among Chinese women in Guangdong province. *Asia Pac J Clin Nutr* 2009; **18**: 240–50.
18 Yang YX, Wang GY, Pan XC. *China Food Composition*. Beijing: Peking University Medical Press, 2002.
19 Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. *J Nutr* 2003; **133**: 1302–7.
20 Zeisel SH, Mar MH, Howe JC, Holden JM. Erratum: concentrations of choline-containing compounds and betaine in common foods. *J Nutr* 2003; **133**: 2918–9.
21 Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997; **65**: 1220S–8S; discussion 9S–31S.
22 Hillman RS, Steinberg SE. The effects of alcohol on folate metabolism. *Annu Rev Med* 1982; **33**: 345–54.
23 Weir DG, McGing PG, Scott JM. Folate metabolism, the enterohepatic circulation and alcohol. *Biochem Pharmacol* 1985; **34**: 1–7.
24 Kim YI, Miller JW, da Costa KA *et al.* Severe folate deficiency causes secondary depletion of choline and phosphocholine in rat liver. *J Nutr* 1994; **124**: 2197–203.
25 Jacob RA, Jenden DJ, Allman-Farinelli MA, Swendseid ME. Folate nutriture alters choline status of women and men fed low choline diets. *J Nutr* 1999; **129**: 712–7.
26 Cho E, Zeisel SH, Jacques P *et al.* Dietary choline and betaine assessed by food-frequency questionnaire in relation to plasma total homocysteine concentration in the Framingham Offspring Study. *Am J Clin Nutr* 2006; **83**: 905–11.
27 Chinese Nutrition Society. *Chinese Dietary Reference Intake*, 1st edn. Beijing: China Light Industry Press, 2000.

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