

Serum betaine but not choline is inversely associated with breast cancer risk: a case–control study in China

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Abstract

Purpose Choline and betaine are important for DNA methylation and synthesis, and may affect tumor carcinogenesis. To our knowledge, no previous study has examined the association between serum choline and betaine and breast cancer risk. This study aimed to examine whether serum choline and betaine were inversely associated with breast cancer risk among Chinese women.

Methods This hospital-based case–control study consecutively recruited 510 breast cancer cases and 518 frequency-matched (age and residence) controls, and blood samples were available for 500 cases and 500 controls. Serum choline and betaine were assayed by high-performance liquid chromatography–tandem mass spectrometry. Multiple unconditional logistic regression was used to estimate odds ratios (ORs) and 95 % confidence intervals (CIs).

Results An inverse association with breast cancer risk was observed for serum betaine (fourth vs first quartile adjusted OR 0.68, 95 % CI 0.47–0.97) and for the ratio of serum betaine to choline (fourth vs first quartile adjusted OR 0.70,

95 % CI 0.48–1.00), but not for serum choline (fourth vs first quartile adjusted OR 0.80, 95 % CI 0.56–1.15). Serum betaine was inversely associated with breast cancer risk in subjects with below-median dietary folate intake (fourth vs first quartile adjusted OR 0.48, 95 % CI 0.30–0.77).

Conclusions This study suggested that serum betaine but not choline was inversely associated with breast cancer risk. This result needed to be further confirmed by the prospective studies.

Keywords Serum choline · Serum betaine · Breast cancer risk · Case–control study

Introduction

One-carbon metabolism is a network of biological reactions that transfer methyl groups from one compound to another. Betaine and its precursor choline, like folate, involved in one-carbon metabolism are essential for the methylation and synthesis of DNA [1–3]. A low status of choline and betaine, which may increase the possibility of DNA damage and gene mutations caused by aberrant methylation, is potentially related to carcinogenesis [4, 5]. Although an inverse association between levels of circulating folate and risk of breast cancer has been found in many [6–8] except a few studies [9, 10], less is known about the association between circulating choline and betaine and breast cancer risk.

Recently, some studies have examined the association of circulating choline and betaine with some types of cancers such as colorectal cancer [11–13] and prostate cancer [14, 15]. Three large prospective studies have found a reduction in colorectal cancer risk associated with plasma betaine [11–13], and one of them found the

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protective role of choline in colorectal cancer [13]. However, the Northern Sweden Health and Disease Cohort study reported an increased risk of prostate cancer risk associated with elevated plasma levels of choline but not with plasma betaine [15]. In a Norway population, null association was found between plasma betaine and prostate cancer [14].

So far, no previous study has assessed the relationship between circulating choline and betaine and breast cancer risk. The aim of this study was to examine the association between serum concentrations of choline and betaine and breast cancer risk by utilizing a hospital-based case–control study among Chinese women. The association between breast cancer risk and ratio of betaine to choline was also explored because the ratio is considered as a better predictor of disturbed metabolism [16]. Stratified analyses by menopause status were conducted due to the regulatory role of estrogen in endogenous biosynthesis of choline [17]. We also evaluated whether the associations between serum choline and betaine and breast cancer risk were modified by status of dietary folate.

Materials and methods

Study population

A detailed description of this ongoing case–control study has been addressed previously [18]. In brief, cases were recruited from two hospitals in Guangzhou, China, from September 2011 to November 2014 which partly came from the second-stage case–control study [18]. Eligible cases were newly (no more than 3 months prior to the interview), histologically confirmed female breast cancer patients, aged 25–70 years, and who were Guangdong natives or have lived in Guangdong for at least 5 years. Women were excluded if they simultaneously had a history of any other cancers. All cases were confirmed by the physician and medical records. A total of 510 cases out of 526 eligible cases were successfully interviewed, and the blood samples of 500 cases were available.

Control subjects free of cancer at the time of admitting to the hospital were selected from the departments of Ophthalmology, Plastic and Reconstructive Surgery and Vascular Surgery in the same hospitals. They were frequency matched to controls on age (5 years interval) and residence (rural/urban). Control subjects mainly consist of patients who have suffered chronic otitis media, sudden deafness, chronic sinusitis, varicose veins, vocal cord polyp, nasal polyps, cataract, fungal sinusitis and tonsillitis. In total, 518 controls out of 530 eligible controls were successfully interviewed (97.7 % participation rate), and 500 blood samples were available.

All participants were informed of the requirements of the study and provided written consent. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments and was approved by the Ethical Committee of School of Public Health, Sun Yat-sen University.

Data collection

Trained interviewers interviewed participants on demographic, lifestyle behaviors and reproductive information via a standardized questionnaire. A validated 81-item food frequency questionnaire (FFQ) was used for assessing dietary information [19]. Nutrient intake per day was calculated by multiplying the frequency of each food consumption and the nutrient content in the corresponding portion size. Nutrient values in foods were obtained from The Chinese Food Composition Table [20]. Information on weight and height was obtained through nursing measurement on the first day after admission to hospital. Body mass index (BMI) was calculated by dividing weight (kg) by height (m^2). Physical activity was designated as leisure-time physical activity and was categorized as never, occasionally and often (more than once a week). Regular smoking was defined as smoking at least one cigarette per day for more than six consecutive months. Passive smoking was defined as non-smokers who reported being exposed to the smoke exhaled by smokers at least 15 min per day in a week. Regular drinking was defined as alcohol drinking at least once per week over the past year. Menopausal status was defined as at least 12 months since the last menstrual cycle. Women were considered premenopausal if they were currently menstruating, or if they were not menstruating because of hysterectomy and younger than 50 years old. Women were defined as postmenopausal if they either had undergone a natural menopause, or surgery to remove both ovaries and they were older than 50 years old. We also recorded relevant medical information, medical diagnosis by reviewing hospital medical records.

Sample collection, storage and processing

Approximately 5 mL blood sample of each participant was obtained in the second day after patients were admitted to the hospital, which is prior to any drug treatment or examination. All of the blood samples were collected at least 8 h after the participant had last eaten. On arrival at laboratory, the samples were centrifuged at 3000 rpm for 10 min at 4 °C, and the supernatants were then aliquoted into eight parts of 200 μ L. All serum samples were stored at -80 °C in continuously alarmed and monitored refrigerator. Samples were averagely stored 23.2 months for cases and 22.4 months for controls before measurement.

Measurement of serum choline and betaine

Concentrations of serum choline and betaine were determined by high-performance liquid chromatography–tandem mass spectrometry (HPLC/MS–MS) as described by Holm et al. [21]. Briefly, thirty microliters of either the serum or standards was mixed with 90 μ L acetonitrile containing 10 μ M internal standards and were subsequently centrifuged at 13,000 $\times g$ for 10 min to precipitate the proteins. The supernatants were collected and analyzed by HPLC/MS–MS. D9-choline was used as internal standards. A normal-phase silica column was used to separate choline and betaine. All serum samples were handled identically and shipped in the same batch. Serum samples of cases and controls were matched on the time of blood collection, and matched pairs were in the same analytical run. We also randomly interspersed 25 pairs of duplicate control samples to assess laboratory precision. The mean coefficients of variation were 5.4 % for choline and 7.4 % for betaine. All assays were conducted by the researchers who were blinded to the case–control status of the samples.

Statistical analysis

Since distributions of serum choline and betaine concentrations tended to be right-skewed, differences were assessed by Wilcoxon tests, and other normally distributed covariates were tested with *t* test. Discrete variables were evaluated by χ^2 test. Spearman correlation analysis was used to assess the correlations of serum betaine and dietary betaine as well as correlations of serum choline and dietary choline among control subjects.

Concentrations of serum choline and betaine and ratio of betaine to choline were categorized into quartiles based on the distribution among the control subjects. Unconditional logistic regression was applied to estimate odds ratios (ORs) and corresponding 95 % confidence intervals (CIs) for breast cancer risk in relation to serum choline and betaine, using the lowest quartile as a reference group. The following variables were adjusted in multivariate models: education (categorical, primary school or below, junior high school, senior high school/secondary technical school, college or above), income (yuan/month, categorical, <2000, 2001–5000, 5001–8000, >8001), smoking (yes/no), passive smoking (yes/no), drinking (yes/no), physical activity (categorical, never, occasional and ≥ 1 time per week), first-degree relative with cancer (yes/no) and BMI (continuous). Tests for trend were undertaken by assuming categorized variable as continuous variable and entered the variable into a logistic regression model. Stratified analyses by menopause status and median values of dietary folate

intake (209 μ g/day) among controls were also evaluated. Likelihood ratio tests comparing models with and without multiplicative interaction were used to evaluate effect modification by menopause status and folate intakes. Since multivitamin supplement users were generally more health conscious and have a healthier lifestyle [22] and renal diseases may have an effect on serum levels of choline and betaine [23, 24], sensitivity analysis was done by excluding multivitamin users or individuals suffering nephrolithiasis. In this study, significance was defined as $P < 0.05$, and all statistical tests were two-tailed. Statistical analyses were conducted by SPSS version 20.0.

Results

The characteristics of the study subjects are presented in Table 1. Compared with the controls, the cases had a higher BMI, higher percentage of passive smoking and drinking, lower education level, lower income and fewer physical activity, and were more likely to have a family history of breast cancer in their first-degree relatives. No significant differences were observed between case and control subjects with regard to age, occupation, active smoking, age at menarche, parity, age at first live birth, months of breast-feeding, menopausal status, age at menopause, hormone replacement therapy use and oral contraceptive use. Dietary choline and folate intake were lower among cases compared to controls, whereas dietary intake of energy and betaine were similar. We observed significantly lower median levels of serum betaine among cases than controls, but no difference of serum choline and the ratio of serum betaine to choline were found. Serum concentrations of choline and betaine were positively correlated with dietary intake of these two nutrients (Spearman correlation coefficients 0.11 for choline and 0.23 for betaine, respectively). Spearman correlation coefficient between serum betaine and choline values was 0.20.

Table 2 shows the associations between serum choline and betaine and breast cancer risk. Serum betaine and the ratio of betaine to choline were found to be inversely associated with breast cancer risk after controlling for various confounders. The adjusted ORs (95 % CI) were 0.68 (0.47–0.97) for serum betaine and 0.70 (0.48–1.00) for the ratio of betaine to choline comparing the highest with the lowest quartile. However, serum choline was not associated with breast cancer risk (fourth vs first quartile OR 0.80, 95 % CI 0.56–1.15).

As shown in Table 3, dietary folate intake modified the association between serum betaine and breast cancer risk ($P_{\text{interaction}} = 0.04$). A decreased breast cancer risk was associated with elevated serum betaine only among women

Table 1 Characteristics of breast cancer cases and matched controls

Variables ^a	Cases (<i>n</i> = 500)	Controls (<i>n</i> = 500)	<i>P</i>
Age, years (mean ± SD)	47.6 ± 9.3	47.8 ± 9.6	0.74
Marital status, <i>n</i> (%)			
Married	472 (94.4)	467 (93.4)	0.51
Unmarried/divorced/widowed	28 (5.6)	33 (6.6)	
Education level, <i>n</i> (%)			
Primary school or below	119 (23.8)	129 (25.8)	0.03
Junior high school	152 (30.4)	115 (23.0)	
Senior high school/secondary technical school	122 (24.4)	119 (23.8)	
College or above	107 (21.4)	137 (27.4)	
Occupation, <i>n</i> (%)			
Administrator/other white-collar worker	114 (22.8)	114 (22.8)	0.93
Blue-collar worker	140 (28.0)	135 (27.0)	
Farmer/other	246 (49.2)	251 (50.2)	
Income (yuan/month), <i>n</i> (%)			
<2000	35 (7.0)	20 (4.0)	<0.01
2001–5000	153 (30.6)	118 (23.6)	
5001–8000	184 (36.8)	192 (38.4)	
>8001	128 (25.6)	170 (34.0)	
Physical activity (exercise for health), <i>n</i> (%)			
Never	313 (62.6)	278 (55.6)	0.02
Occasionally	97 (19.4)	96 (19.2)	
Often (≥1 time/week)	90 (18.0)	126 (25.2)	
Body mass index, kg/m ² (mean ± SD)	23.1 ± 3.3	22.5 ± 3.2	0.01
Regular smoker, <i>n</i> (%)	5 (1.0)	5 (1.0)	1.00
Passive smoking, <i>n</i> (%)	362 (73.1)	232 (46.9)	<0.01
Regular drinker, <i>n</i> (%)	46 (9.2)	26 (5.2)	0.02
Age at menarche, years (mean ± SD)	14.9 ± 1.9	14.5 ± 1.7	0.36
Age at first live birth, years (mean ± SD) ^b	24.4 ± 6.4	24.4 ± 6.1	0.99
Age at menopause, years (mean ± SD) ^c	49.8 ± 4.3	49.3 ± 3.8	0.21
Menopausal status, <i>n</i> (%)			
Premenopausal	332 (66.4)	325 (65.0)	0.64
Postmenopausal	168 (33.6)	175 (35.0)	
First-degree relative with cancer, <i>n</i> (%)	72 (14.4)	45 (9.0)	0.01
Hormone replacement therapy use, <i>n</i> (%)	3 (0.6)	2 (0.4)	0.65
Parity, <i>n</i> (%)			
0	23 (4.6)	22 (4.4)	0.94
1–2	374 (74.8)	379 (75.8)	
≥3	103 (20.6)	99 (19.8)	
Months of breast-feeding (mean ± SD) ^d	21.9 ± 24.7	19.3 ± 18.0	0.09
Ever used an oral contraceptive, <i>n</i> (%)	36 (7.2)	23 (4.6)	0.08
Dietary intake			
Total energy, kcal/day (mean ± SD)	1399.4 ± 376.5	1413.0 ± 351.4	0.55
Choline intake, mg/day (mean ± SD)	142.1 ± 56.9	160.1 ± 58.8	<0.01
Betaine intake, mg/day (mean ± SD)	219.5 ± 141.5	229.2 ± 133.9	0.27
Folate intake, μg/day (mean ± SD)	199.8 ± 57.8	216.4 ± 62.1	<0.01

Table 1 continued

Variables ^a	Cases (<i>n</i> = 500)	Controls (<i>n</i> = 500)	<i>P</i>
Serum concentrations			
Choline, $\mu\text{mol/L}$ (median, P_{25} – P_{75})	12.9 (9.7–16.7)	13.2 (10.0–17.7)	0.68
Betaine, $\mu\text{mol/L}$ (median, P_{25} – P_{75})	38.0 (30.3–47.8)	40.7 (31.8–51.7)	0.01
Serum betaine/choline ratio (mean \pm SD)	3.4 \pm 1.75	3.4 \pm 1.45	0.93

^a Continuous variables were analyzed by *t* tests or Wilcoxon tests. Categorical variables were analyzed by χ^2 tests

^b Among women who have had a live birth

^c Among menopausal women

^d Among women who had breast-fed

Table 2 Unconditional logistic regression analyses with corresponding odds ratios and 95 % confidence intervals for breast cancer, according to quartiles of serum concentrations or the betaine/choline ratio

Serum concentration	Quartiles ^a	Cases/controls	Crude OR (95 % CI)	Adjusted OR (95 % CI) ^b
Betaine ($\mu\text{mol/L}$)	1 (<31.9)	156/125	1.00	1.00
	2 (31.9–<40.7)	136/125	0.87 (0.62–1.21)	0.94 (0.66–1.32)
	3 (40.7–<51.7)	108/125	0.70 (0.49–0.99)	0.69 (0.49–0.99)
	4 (\geq 51.7)	100/125	0.64 (0.45–0.91)	0.68 (0.47–0.97)
	<i>P</i> _{trend}		0.01	<0.01
Choline ($\mu\text{mol/L}$)	1 (<10.0)	146/125	1.00	1.00
	2 (10.0–<13.1)	143/125	1.00 (0.71–1.40)	1.00 (0.71–1.41)
	3 (13.1–<17.7)	98/125	0.69 (0.48–0.98)	0.70 (0.49–1.03)
	4 (\geq 17.7)	113/125	0.81 (0.57–1.14)	0.80 (0.56–1.15)
	<i>P</i> _{trend}		0.07	0.07
The ratio of betaine to choline	1 (<2.3)	144/125	1.00	1.00
	2 (2.3–<3.2)	134/125	0.93 (0.66–1.31)	0.92 (0.65–1.32)
	3 (3.2–<4.3)	124/125	0.86 (0.61–1.22)	0.86 (0.60–1.23)
	4 (\geq 4.3)	98/125	0.68 (0.48–0.97)	0.70 (0.48–1.00)
	<i>P</i> _{trend}		0.04	0.06

^a Quartiles are based on the distribution of serum concentrations or the ratio of betaine to choline among controls

^b Adjusted for education, income, physical activity, smoking, passive smoking, first-degree relative with cancer, energy intake and body mass index

with below-median level of dietary folate intake (<209 $\mu\text{g/day}$). However, the interaction effect between dietary folate intake with serum choline was not statistically significant ($P_{\text{interaction}} = 0.46$). Menopause status did not modify the association of serum choline and betaine with breast cancer risk ($P_{\text{interaction}} = 0.28$ and 0.77 , respectively). Sensitivity analyses excluding participants with casual multivitamin supplement (13.8 % of cases and 17.2 % of controls) yielded similar results (data not shown). We also excluded participants complicated with nephrolithiasis (3.2 % of cases and 3.0 % of controls), and results were not altered notably (data not shown).

Discussion

To the best of our knowledge, this is the first study to examine serum levels of choline and betaine and breast cancer risk. The results showed that the elevated concentrations of serum betaine and ratio of serum betaine to choline were associated with the reduced risk of breast cancer. The inverse association between serum betaine and breast cancer risk was only observed among individuals with lower dietary folate intake.

To our knowledge, no previous study has examined circulating choline and betaine and breast cancer risk.

Table 3 Associations between quartiles of serum concentrations choline and betaine and breast cancer risk according to menopausal status and dietary folate intake

Methyl donors	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}	$P_{\text{interaction}}$
Choline						
Premenopause						0.28
No. cases/controls	101/93	99/82	58/76	74/74		
Adjusted OR (95 % CI) ^a	1	1.06 (0.69–1.62)	0.66 (0.41–1.05)	0.92 (0.59–1.44)	0.37	
Postmenopause						
No. cases/controls	45/34	44/43	38/48	41/50		
Adjusted OR (95 % CI) ^a	1	0.78 (0.42–1.46)	0.60 (0.32–1.13)	0.61 (0.33–1.15)	0.08	
Dietary folate (<209 µg/day)						0.46
No. cases/controls	92/57	90/58	57/66	74/69		
Adjusted OR (95 % CI) ^a	1	0.88 (0.54–1.43)	0.48 (0.29–0.79)	0.62 (0.39–1.00)	0.01	
Dietary folate (≥209 µg/day)						
No. cases/controls	54/70	53/67	39/58	41/55		
Adjusted OR (95 % CI) ^a	1	1.01 (0.59–1.72)	0.85 (0.48–1.49)	0.90 (0.51–1.59)	0.59	
Betaine						
Premenopause						0.77
No. cases/controls	109/88	98/83	67/83	58/71		
Adjusted OR (95 % CI) ^a	1	0.93 (0.61–1.43)	0.64 (0.41–0.99)	0.64 (0.40–1.03)	0.03	
Postmenopause						
No. cases/controls	47/37	38/43	41/41	42/54		
Adjusted OR (95 % CI) ^a	1	0.72 (0.38–1.36)	0.78 (0.42–1.46)	0.60 (0.33–1.09)	0.21	
Dietary folate (<209 µg/day)						0.04
No. cases/controls	103/62	90/57	62/56	58/75		
Adjusted OR (95 % CI) ^a	1	1.04 (0.65–1.67)	0.66 (0.40–1.08)	0.48 (0.30–0.77)	<0.01	
Dietary folate (≥209 µg/day)						
No. cases/controls	53/63	46/69	46/68	42/50		
Adjusted OR (95 % CI) ^a	1	0.69 (0.40–1.20)	0.77 (0.44–1.32)	0.88 (0.50–1.57)	0.70	

^a Adjusted for education, income, physical activity, smoking, passive smoking, first-degree relative with cancer, energy intake and body mass index

However, some studies have explored the association of serum choline and betaine with colorectal cancer or prostate cancer risk [11–15]. Our finding of the inverse association between serum betaine and breast cancer risk corroborated with the results from all of three studies focused on colorectal cancer [11–13], but not with studies on prostate cancer [14, 15]. In detail, the European Prospective Investigation into Cancer and Nutrition (EPIC) study found the protective role of circulating betaine in colorectal cancer among individuals whose plasma folate concentrations below 11.3 nmol/L (fifth vs first quintile OR 0.71; 95 % CI 0.50–1.00; $P_{\text{trend}} = 0.02$) [13], and The Women's Health Initiative Observational Study reported a stronger overall protective effect of circulating betaine in colorectal cancer (fourth vs first quartile OR 0.68; 95 % CI 0.47–0.99; $P_{\text{trend}} = 0.01$) [11]. de Vogel et al. [12] also found an inverse association of plasma betaine with distal colorectal adenomas in the Norwegian Colorectal Cancer Prevention (NORCCAP) study (fourth vs first quartile OR 0.74; 95 %

CI 0.54–1.02; $P_{\text{trend}} = 0.03$). However, the study conducted in Norway [14] and another in Sweden [15] found null association of circulating betaine with prostate cancer.

With regard to circulating choline, four [11–13, 15] of five studies [11–15] mentioned above have explored association of circulating choline with cancer risk and produced controversial results. The Women's Health Initiative Observational Study [11] and the study in Sweden [15] reported a positive association of plasma choline with colorectal or prostate cancer. By contrast, the EPIC study found that plasma choline was inversely associated with colorectal cancer risk [13]. The NORCCAP study showed null association with distal colorectal cancer risk [12], a finding that is consistent with our study.

In the present study, the inverse association of serum betaine with breast cancer risk was only limited to the individuals with lower dietary folate intake. The median intake of dietary folate among control subjects in our study was 209 µg/day, which is far lower than the recommended

nutrient intake for folate (400 $\mu\text{g/day}$) in China [20]. The results support the notion that relatively insufficient status of dietary folate resulted in an increased importance of alternative methyl donors such as betaine [2, 25]. Betaine intersects with folate in the reaction that donates methyl group to homocysteine to form methionine. In a study of 500 healthy subjects, plasma betaine is a strong predictor of fasting total homocysteine only in subjects with low serum folate and a strong interaction between folate and betaine has also been observed [26], which strongly suggests the betaine's importance of serving as a methyl donor under conditions of impaired folate status.

The ratio of serum betaine to choline captures the divergent associations of choline and betaine with metabolic disturbances stems from phosphatidylethanolamine N-methyltransferase (PEMT) pathway [16]. An upregulated PEMT pathway resulted in increasing use of betaine as a methyl donor and enhanced the production of choline therefore yielding a lower ratio of serum betaine to choline. However, we did not observe a stronger association between the ratio of betaine and choline with breast cancer risk than betaine alone as found in the Women's Health Initiative Observational Study [11].

Dietary choline is obtained primarily from animal sources, such as eggs, chickens and whole milk, whereas betaine is largely derived from plant sources, such as spinach and grain products. In the present study, the top five contributors of dietary choline were eggs (28.8 %), broccoli (21.7 %), chicken (5.6 %), potatoes (3.3 %) and whole milk (3.0 %), whereas the top five sources of dietary betaine were spinach (62.2 %), pasta (24.4 %), white bread (5.9 %), potatoes (2.3 %) and wheat bread (2.2 %). Some previous epidemiological studies have assessed the relationship between choline and betaine intake and breast cancer risk, but the results were inconsistent. The Nurses' Health Study observed no significant inverse association between choline or betaine intake and breast cancer risk [27, 28]. However, two case-control studies, the Long Island Breast Cancer Study Project and our previous two-stage case-control study conducted in China, found a significant reduction in breast cancer risk with a higher choline intake [18, 29].

Choline is essential for the generation of S-adenosylmethionine, the chief methyl donor for most methylation reactions. Choline is oxidized to betaine, and betaine further serves as a substrate for the betaine-homocysteine methyltransferase to form methionine and eventually into the S-adenosylmethionine. Inadequate methyl availability could lead to increased oxidative stress, aberrant DNA methylation and insufficient nucleotide synthesis [1, 4], all of which are involved in carcinogenesis. In a study including 136 breast cancer cases, DNA methylation of breast tumors was significantly less than that of adjacent as well as normal parenchyma [30]. However, choline, also as a

cell membrane phospholipid, is required to generate cell membranes in proliferation of tumor once a tumor is initiated. Elevated level of choline kinase, an enzyme converting choline to phosphocholine for the subsequent generation of membrane phospholipids, was found in human breast cancer [31]. Complex biological metabolism and dual effects of choline on cancer development may interpret divergent associations of circulating choline with cancer risk. The inconsistent results also seem to suggest that different cancers may have different underlying etiology. Blood levels of one-carbon metabolites modify DNA methylation levels in blood cells, which could be used as a surrogate to explore mechanisms of carcinogenesis [32]. Nevertheless, altered choline phospholipid metabolite profile with elevation of phosphocholine and choline metabolites has been found in breast cancer cells [33]. Whether this change further influences the blood levels of choline metabolites is still not clear, and further research is warranted to elucidate the issues.

Plasma betaine seems to be under strict metabolic control. On repeated sampling over 10 h, 10 days and 3 years, the plasma betaine concentration was found to be consistent [34]. Therefore, there was a small intra-individual variation in plasma betaine concentration. Plasma choline was also relatively consistent. It has been indicated that normal diets cause only small elevations in plasma choline [35]. Two studies demonstrated a moderate (10 %) increase in plasma choline 1 h after a typical meal [21, 36]. One study including ten healthy humans showed that plasma choline averagely diminished 18 % after 1 week of fasting [37].

The main strength of the present study is that choline and betaine nutrition was assessed by direct measurements. High participation rate (96.9 % for cases and 97.7 % for controls) as well as high proportion of blood donors (98.0 % for cases and 96.5 % for controls) in our study minimized the selection bias as much as possible. All samples were stored in freezers at $-80\text{ }^{\circ}\text{C}$ with a relative short period of storage time, thus eliminating preanalytical bias [38]. In addition, extensive collections of multiple risk factors allowed us to assess the potential effect modifications.

There are some limitations of this study. Diagnosis of breast cancer may lead to stressful state of cases and would further cause less food consumption, but all blood samples were collected in the second day after the patients were admitted to the hospital, when histological results were usually unavailable. What is more, a single measurement may not directly reflect the long-term dietary intake or body stores of nutrients. We cannot exclude the possibility that onset and development of breast cancer could itself have an effect on the levels of serum choline and betaine due to the retrospective study design.

In conclusion, this study suggested that serum betaine was inversely associated with breast cancer risk. Our

preliminary results should be interpreted with caution and need to be further confirmed by large prospective studies.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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