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Meta-analyses

Association of choline and betaine levels with cancer incidence and survival: A meta-analysis

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SUMMARY

Background & aims: Evidences suggest possible link between betaine and choline, methyl group donors, and cancer progression. We examined the association between choline and betaine levels and cancer incidence and survival in a meta-analysis of observational studies.

Methods: We identified observational studies examining the association between choline and/or betaine levels from diet or blood and cancer incidence and survival by searching the PubMed and Web of Science databases for studies published up to Jan, 2018. After applying the selection criteria, 28 observational studies (9 case-control, 1 cross-sectional, and 18 cohort studies) were included. Relative risks (RRs) and 95% confidence intervals (CIs) were extracted, and combined RRs were calculated using random-effects models.

Results: Choline levels were not associated with cancer incidence in a meta-analysis of cohort studies. Betaine levels reduced the risk of cancer incidence in a meta-analysis of cohort studies; combined relative risks (RRs) (95% CIs) comparing the top with the bottom categories were 0.93 (0.87–0.99). When we analyzed separately according to exposure assessment method, combined RRs (95% CIs) comparing the top with the bottom categories of betaine levels were 0.87 (95% CI: 0.78–0.95) for dietary betaine and 0.88 (95% CI: 0.77–0.99) for blood levels of betaine. There were no significant associations with cancer survivorship of choline or betaine levels.

Conclusions: We concluded that high betaine levels were associated with lower risk of the cancer incidence, especially for colorectal cancer.

1. Introduction

Choline, which is essential for integrity of cell membrane structure, signaling functions of cells, and synthesis of neurotransmitters, is available as free choline, phosphocholine, glycerophosphocholine, sphingomyelin, or phosphatidylcholine [1]. Furthermore, choline is a precursor of betaine and acetylcholine, which is an important neurotransmitter in the processes of memory storage, muscle control, and various other functions [1]. Along with folate and other B vitamins, choline and betaine take part in one-carbon metabolism, which constitutes a network of integrated biochemical pathways transferring one-carbon (methyl) groups [2]. Betaine and folate are methyl-group donor for homocysteine that is converted to methionine with assistance from betaine homocysteine methyltransferase (BHMT) and methionine synthase (MTH). Methionine transfers a methyl-group to S-adenosylmethionine (SAM) which donates methyl group to DNA and RNA [3]. DNA methylation, which is a genetic alteration of gene function that occurs without changes to the sequence of DNA, is an important determinant in the development of cancerous cells, and has been implicated in gene expression, conservation of DNA integrity and firmness, chromatin alteration, and mutation [4].

Epidemiological evidence regarding the role of dietary choline and betaine in cancer development and progression has accumulated in last ten years following the advent of a food composition database for choline and betaine in 2003 [5,6]. Given the lack of comprehensive systematic reviews of the role of choline and betaine in cancer development, we investigated the associations

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between choline or betaine and cancer incidence and survival by performing a meta-analysis of observational epidemiological studies.

2. Materials and methods

We followed the Meta-analysis Of Observational Studies in Epidemiology guidelines [7].

2.1. Search strategy

Searching the PubMed and Web of Science databases for studies published up to January 22, 2018, epidemiological studies investigating the association between choline and/or betaine and cancer were identified. A single author (J Youn) performed the systematic search and another author (JE Lee) checked the extracted studies. We used the following search terms: "choline or betaine or one-carbon metabolite or one-carbon metabolism" and "intake or diet or serum or plasma or circulating" and "cancer or carcinoma or neoplasia or adenoma" in PubMed; and "choline or betaine" and "intake or diet or serum or plasma or circulating" and "cancer or carcinoma or neoplasia or adenoma" in Web of Science. We used a filter function of PubMed and limited the search to "Human" in species and "English" in languages. For Web of Science, we refined the searches to "Articles" in document types and "English" in languages. We also checked the reference lists from all retrieved journals in order to include additional relevant studies.

2.2. Inclusion criteria

Studies were included in our meta-analysis if they met the following criteria: 1) the exposure of interest was choline and/or betaine, which was assessed using dietary or biochemical analyses; 2) the endpoint of interest was cancer incidence or mortality; 3) relative risk (RR) estimates and 95% confidence intervals (CIs) were provided for the associations between choline and/or betaine levels and incidence and/or mortality of any cancer; 4) the study was designed as an observational study; and 5) the study was published in English.

2.3. Data extraction

We extracted the following information from each study: the first author's full last name and initial of the first name; publication year; country of the study population; study design; study period; sex of study population; outcomes (cancer types or deaths); the number of cases and controls for case-control studies, and the incidence and total person-years for cohort studies; estimate parameters (RRs, odds ratio or hazard ratio); 95% CIs; ranges and/or medians of each category; type of exposure assessment (dietary or biochemical); and covariates adjusted in multivariate models. The quality of each study was assessed using selected items of "strengthening the reporting of observational studies in epidemiology" [8]. For case-control and cross-sectional studies, we assessed adjustment for confounding factors, the sources and methods of case ascertainment, control selection, recall bias, and assessment of exposures, outcomes and covariates. For cohort studies, we assessed whether important confounding factors were adjusted for, how loss to follow-up was addressed, and how endpoint was measured.

2.4. Statistical analysis

The combined RRs and 95% CIs for the top choline or betaine categories, as compared with the bottom categories, were calculated using the random effects model [9]. Weight was given to individual studies depending on the inverse proportion of variances. Heterogeneity among studies was examined using the I^2 statistic, where $I^2 = 100\% \times (Q - df)/Q$, where Q is Cochran's heterogeneity statistic and df is the degrees of freedom [10,11]. A sensitivity analysis was performed to identify the influence of the RRs by excluding studies has heterogeneous results, such as Chinese studies. Furthermore, a subgroup analysis and meta-regression analysis [12,13] using the natural logarithm of the RR from each study were conducted to evaluate whether the associations between choline or betaine and cancer differed according to the type of exposure assessment, sex, geographic region (Australia, Europe, and the US and China and Singapore), or study design (case-control and cohort). When reported [14–22], or when investigators provided estimates by folate levels [17,19,20,23–25], whether the association between choline or betaine and cancer differed according to folate intake (low and high) was investigated.

To assess the dose-response association between choline or betaine levels and cancer incidence, we estimated the RR per 50 mg/d increase for choline or betaine intake and the RR per 5 μmol/L increase for circulating choline or betaine levels by regressing the log RRs using generalized least squares [26]. Authors were contacted, or our previous studies were reviewed, to obtain information regarding the number of cases or person-years according to categories, or the median of each category, which are required for a dose-response analysis [19,23–25,27–31]. We used the median of each category in the dose-response analysis. We assumed that the level had the same amplitude as the neighboring categories if the bottom or top category was open-ended. We examined the publication bias using a funnel plot and Egger's test [32]. $p < 0.10$ was considered indicative of departure from no publication bias. Statistical analyses were performed with the STATA 15 statistical software (Stata corp. College Station, TX, USA). Two-sided p-values of <0.05 were considered statistically significant.

3. Results

3.1. Publication identification

A total of 1,526 papers were extracted from PubMed and Web of Science, of which 1,495 were excluded for the following reasons: 1,343 did not examine the association between choline and/or betaine and cancer; 131 were reviews; two were books; eight were case reports; and eleven were editorial commentaries. Of the 31 articles that were retrieved, four case-control studies included the same population [33–36]. We included one [33] out of four for both cancer incidence and survival analyses for the following reasons: the study included [33] was published after the publications of two studies [34,35]; and used conventional logistic models whereas the other study reported betaine and specific types of choline in pathway based-hierarchical regression models [36]. There were three studies [14,37,38] which included partly overlapped populations from the first and second stage case-control studies. Out of these three studies, we included the estimates from the first stage case-control study in the prior published study [14] and the second stage case-control study in the more recent study [37]. When we conducted the dose-response and meta-regression analysis by folate levels, we included the first and second stage case-control studies in the previous study [14], where the estimates by folate levels were available. The other study [38] was excluded because the serum choline and betaine levels were measured after ascertaining the cases, that is, the study did not demonstrated cancer prevention. In summary, a total of 4 studies were excluded out of 31 articles. One additional study was identified from the references of

the retrieved papers. As a result, nine case-control studies [14–16,22,27,33,37,39,40], one cross-sectional study [41], and 16 cohort studies [17–21,23–25,28–31,42–45] were included for cancer incidence. Four prospective studies [30,33,46,47] were included for the analysis of cancer survival in the present meta-analysis. As a result, a total of 28 observational studies were included. A flowchart of the study inclusion process is shown in Fig. 1.

3.2. Study characteristics and quality assessment

Supplementary Tables S1 and S2 show the characteristics of the case-control, cross-sectional, and cohort studies that investigated cancer incidence. A total of 11,444 cases and 18,645 controls were included from nine case-control studies and one cross-sectional study (Supplementary Table S1), and 18,349 cancer incidences from 16 cohort studies (Supplementary Table S2). For cancer incidence (case-control, cross-sectional, and cohort studies), five studies were conducted in Europe, two in Australia, 11 in the US, and six in China and one in Singapore; 26 studies had examined colorectal adenoma (n = 2), or cancers of the breasts (n = 5), colorectum (n = 6), ovaries (n = 2), prostate (n = 3), esophagus (n = 1), lungs (n = 1), pancreas (n = 1), nasopharynx (n = 1), endometrium (n = 1), liver (n = 2), or renal cells (n = 1). Among the studies included for cancer incidence, the majority assessed choline and betaine intake using a food frequency questionnaire (FFQ), and one cross-sectional study [41], and seven nested case-control studies [18,19,21,31,42,44,45], assessed circulating levels of choline and/or betaine. The ranges of choline and betaine levels varied across studies. High intake of choline and low intake of betaine were observed in Western populations, as compared with Asian populations.

For cancer survival, four survival studies were included that reported 1,499 deaths among 7,170 cancer patients (Supplementary Table S3). One study was conducted in Mexico, one in Australia, and two in the US. Overall mortality was reported in survival studies investigating breast (n = 1) and ovarian (n = 1) cancer, and disease-specific mortality was reported in three studies of breast (n = 1), stomach (n = 1), and prostate (n = 1) cancer. All survivorship studies assessed FFQ-based choline and betaine intakes.

Among 20 studies that assessed dietary intake of choline and betaine, nine included both dietary and supplementary intake of choline and betaine [15,20,24,25,27,29,30,33,46], and eleven assessed dietary intake from food only [14,16,17,22,23,28,37,39,40,43,47].When we examined the quality of study, the quality of each cohort study including nested case-control study was overall good. All the studies adjusted for major confounding factors and reported the follow-up rate in their publications or used linkage to national data. For case-control and cross-sectional studies, all the studies described case ascertainment and control selection and adjusted for potential confounding factors, but there could be the possibility of selection or recall bias.

3.3. Associations of choline and betaine levels with cancer prevalence/incidence/survival

In a meta-analysis of case-control and prospective cohort studies, combined RRs (95% CIs) comparing the top with the bottom categories were 0.90 (0.76–1.04) for choline levels (Fig. 2), and 0.87 (0.81–0.94) for betaine levels (Fig. 3). When we examined the associations among case-control studies only, combined RRs (95% CIs) comparing the top with the bottom categories were 0.73 (0.55–0.91) for choline levels and 0.80 (0.70–0.91) for betaine levels. However, the associations became weaker when only

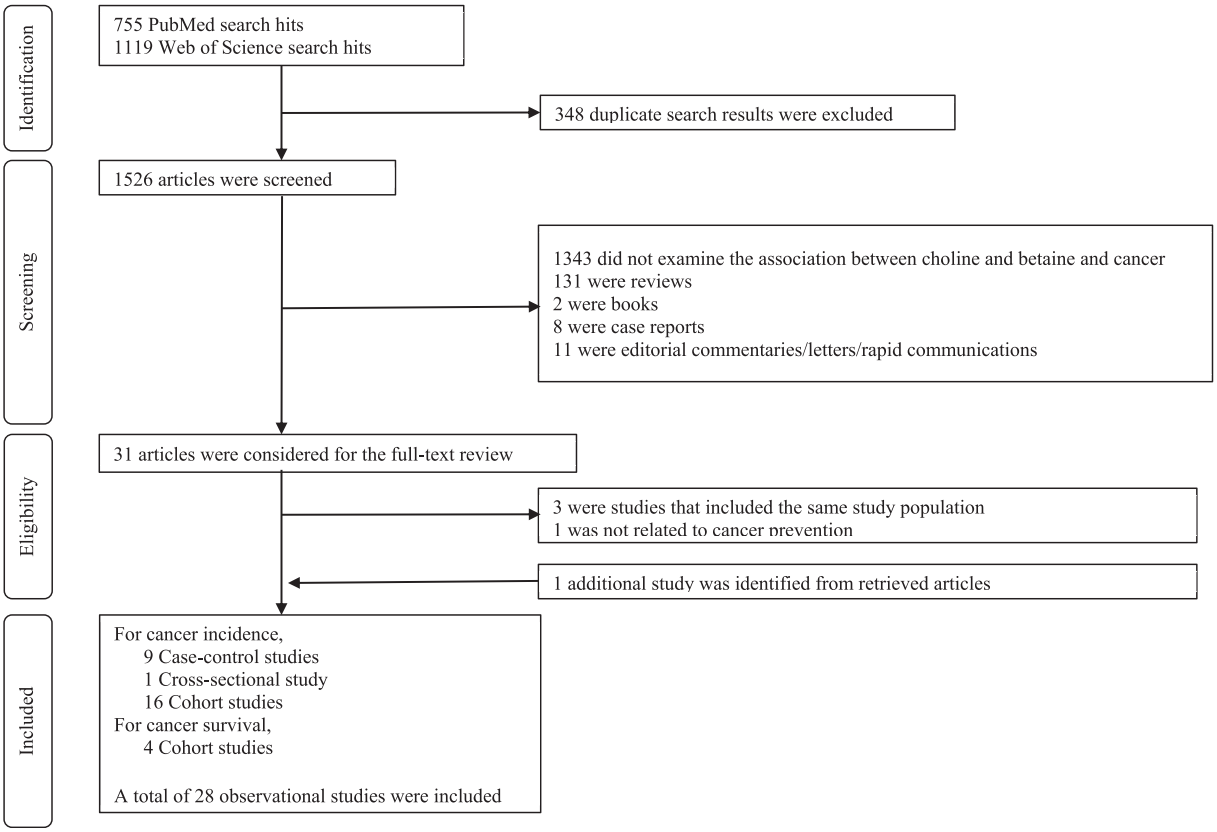

Fig. 1. Flow chart of publication selection for the meta-analysis of the association between choline and betaine levels and cancer.

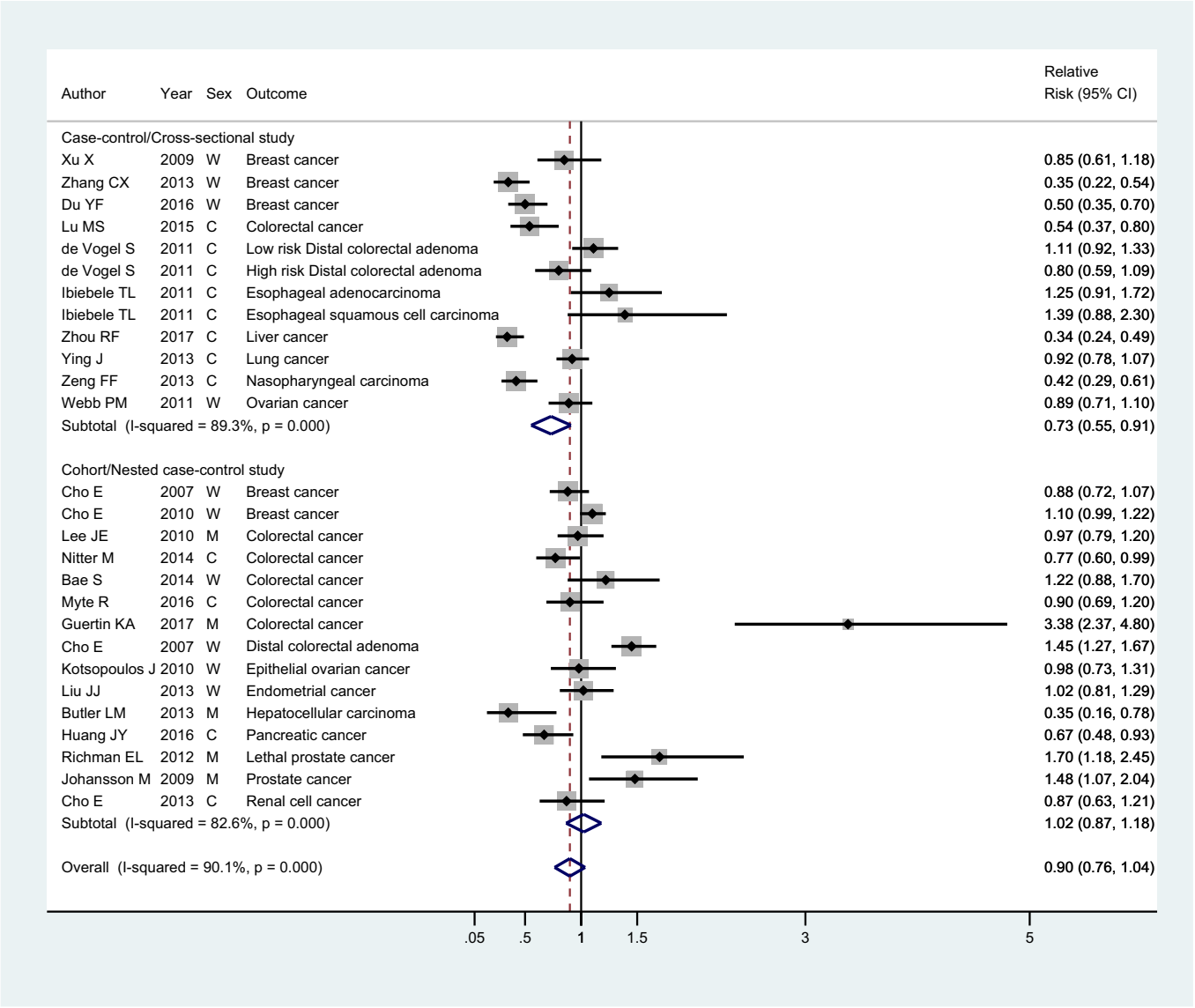

Fig. 2. Forest plot for top versus bottom categories of choline levels in relation to cancer incidence by study design. M, W, and C represent men, women, and combined sex, respectively. The black circles indicate the study specific relative risk (RR)s and the horizontal lines indicate the 95% CIs. The gray squares represent the study specific weights, which are inverse of the variance. The dash line indicates the overall combined RR and the diamonds indicate the combined RRs and the 95% CIs for the combined RRs. p for difference between case-control/cross-sectional study and cohort/nested case-control study was 0.01.

prospective cohort studies were included: combined RRs (95% CIs) were 1.02 (0.87–1.18) for choline levels and 0.93 (0.87–0.99) for betaine levels. A high degree of heterogeneity was observed in a meta-analysis of case-control or cohort studies for the association between choline levels and cancer risk. Similarly, a meta-analysis of case-control studies showed significant heterogeneity for betaine. Because we observed that the estimates from Asian population studies tended to be lower than those from Australia, Europe, and the US population studies, we conducted the sensitivity analysis. When Chinese studies were excluded, the heterogeneity among case-control studies was reduced, as compared with the analysis where all studies were included (I^2 for heterogeneity after exclusion = 27.8 for choline and I^2 = 53.3 for betaine).

We conducted a dose-response analysis of choline or betaine intake and risk of cancer by including 16 studies for choline [14–17,20,22–25,27–30,33,39,43] or betaine [14–17,20,22–25,27,29,30,33,39,43] and circulating levels of choline or betaine by including 5 studies [18,19,42,44,45] for choline and 6 studies [18,19,21,42,44,45] for betaine. The combined RRs (95% CIs) for a 50 mg/d increment in choline intake were 0.94 (0.90–0.97) in a meta-analysis of case-control studies, and 1.00 (0.99–1.02) in a meta-analysis of cohort studies. For a 50 mg/d increment in betaine intake, the combined RRs (95% CIs) were 0.96 (0.94–0.99) in a meta-analysis of case-control studies, and 0.99 (0.98–1.01) in a meta-analysis of cohort studies. The combined RR (95% CI) for a 5 μmol/L increment in circulating choline levels was 1.04 (0.88–1.24), and the combined RR (95% CI) for a 5 μmol/L increment in circulating betaine levels was 0.99 (0.98–1.01) in a meta-analysis.

When we examined the associations between choline or betaine and cancer according to cancer location, choline levels were positively associated with the risk of prostate cancer, but inversely associated with the risk of liver cancer (Fig. 4). Betaine levels were inversely associated with colorectal adenoma and colorectal cancer (Fig. 5). When the analysis was limited to cohort studies, the combined RR (95% CI) for prostate cancer comparing the top with

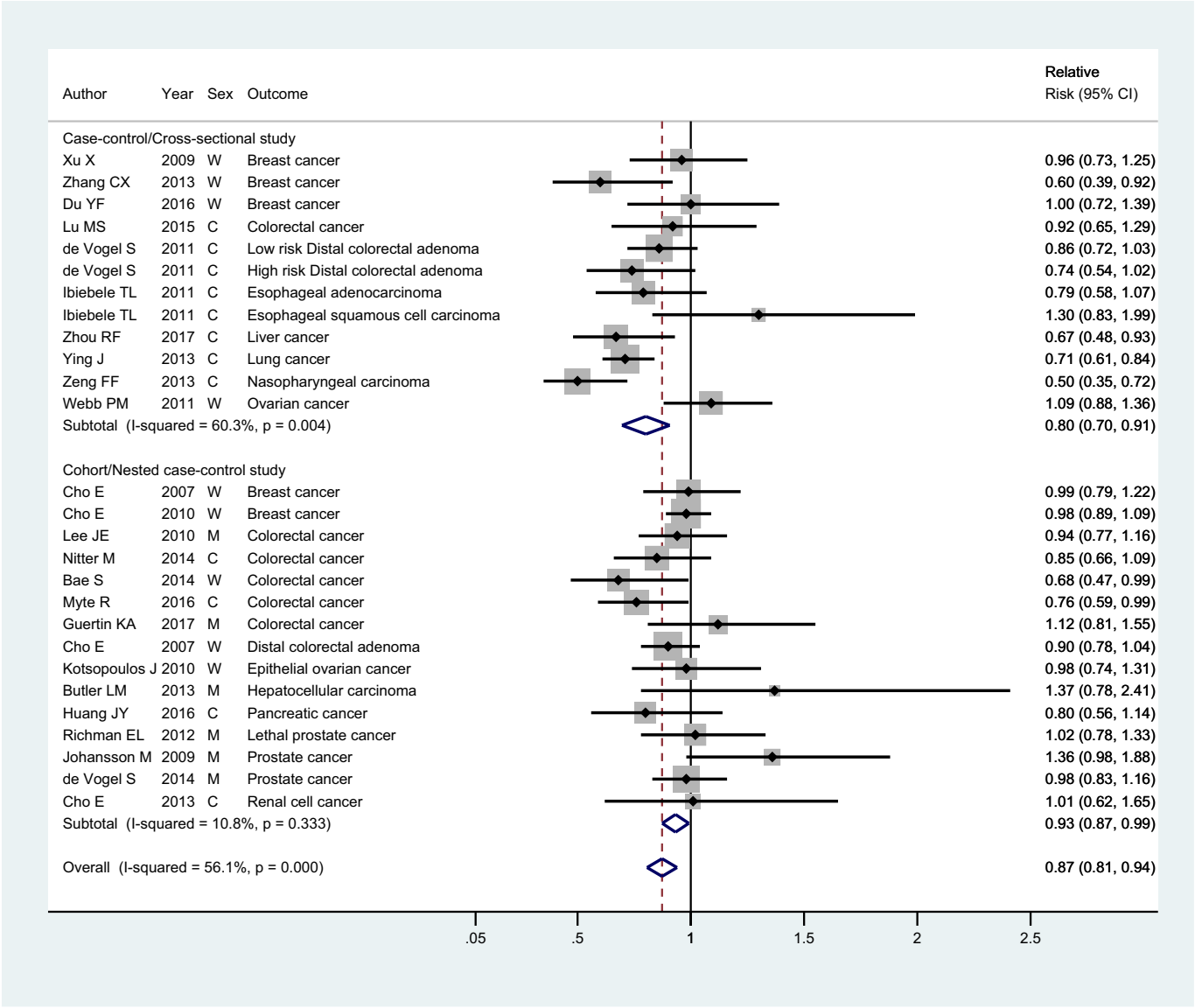

Fig. 3. Forest plot for top versus bottom categories of betaine levels in relation to cancer incidence by study design. M, W, and C represent men, women, and combined sex, respectively. The black circles indicate the study specific relative risk (RR)s and the horizontal lines indicate the 95% CIs. The gray squares represent the study specific weights, which are inverse of the variance. The dash line indicates the overall combined RR and the diamonds indicate the combined RRs and the 95% CIs for the combined RRs. p for difference between case-control/cross-sectional study and cohort/nested case-control study was 0.02.

the bottom category of choline levels was 1.56 (1.18–1.95) and the combined RR (95% CI) for colorectal cancer comparing the top with the bottom category of betaine levels was 0.85 (0.73–0.97).

For cancer survival, there were no associations between betaine or choline levels and overall or disease-specific deaths (Table 1).

3.4. Subgroup analysis

Subgroup analyses were performed to determine whether the associations between choline or betaine levels and cancer incidence differed according to study design (Figs. 2 and 3), folate levels, type of exposure assessment, sex, or geographic region (Table 2). There was no significant difference due to type of exposure assessment, or sex. According to folate levels, although the difference was not statistically significant, the associations were stronger when folate levels were low. According to study design, the combined estimate of case-control showed a stronger association than that of cohort studies (p for difference = 0.01 for choline; p for difference = 0.02 for betaine). When the analysis was limited to Chinese and Singapore studies, the combined RRs (95% CIs) comparing the top with the bottom categories were 0.44 (0.35–0.52) for choline levels and 0.74 (0.58–0.90) for betaine levels (p for difference between studies from Australia, Europe and the US and Chinese and Singapore studies < 0.001 for choline; p for difference = 0.05 for betaine). Since five of the Chinese studies were case-control studies and one Chinese and one Singapore studies were cohort studies, we examined whether this difference came from difference by study design by comparing the combined estimates of Western and Asian studies in cohort studies only; for choline, the combined RRs (95% CIs) were 1.09 (0.95–1.24) in a meta-analysis of studies from Australia, Europe and the US, and 0.53 (0.22–0.84) in a meta-analysis of Chinese and Singapore studies (p for difference = 0.01). For betaine, the combined RRs (95% CIs) were 0.94 (0.87–1.00) in a meta-analysis of studies from Australia, Europe and the US, and 0.95 (0.46–1.45) in a meta-analysis of Chinese and Singapore studies (p for

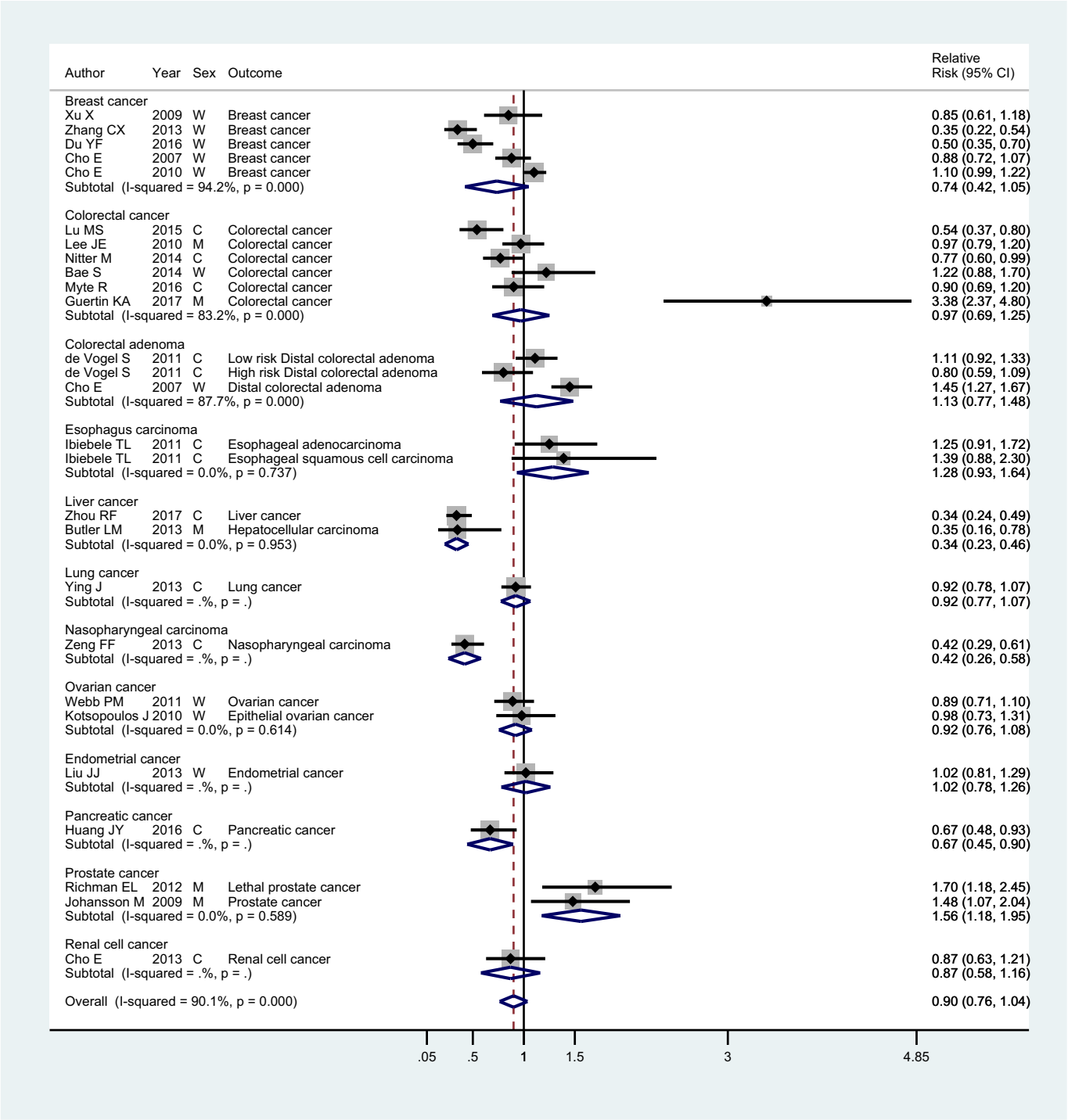

Fig. 4. Forest plot for top versus bottom categories of choline levels in relation to cancer incidence by cancer sites. M, W, and C represent men, women, and combined sex, respectively. The black circles indicate the study specific relative risk (RR)s and the horizontal lines indicate the 95% CIs. The gray squares represent the study specific weights, which are inverse of the variance. The dash line indicates the overall combined RR and the diamonds indicate the combined RRs and the 95% CIs for the combined RRs.

difference = 0.88). Folate levels, type of exposure assessment, sex did not modify the associations for either choline or betaine levels in a meta-analysis of cohort studies.

3.5. Publication bias

When we examined publication bias, there was a suggestion of publication bias for choline for case-control studies, but not for cohort studies; p values by Egger's test were 0.05 for choline and 0.59 for betaine in a meta-analysis of case-control studies; p values for publication bias were 0.63 for choline and 0.89 for betaine in a meta-analysis of cohort studies.

4. Discussion

Our meta-analysis indicated that there is insufficient evidence for a preventive role of choline against cancer development, but betaine reduced the cancer risk. In a meta-analysis of case-control

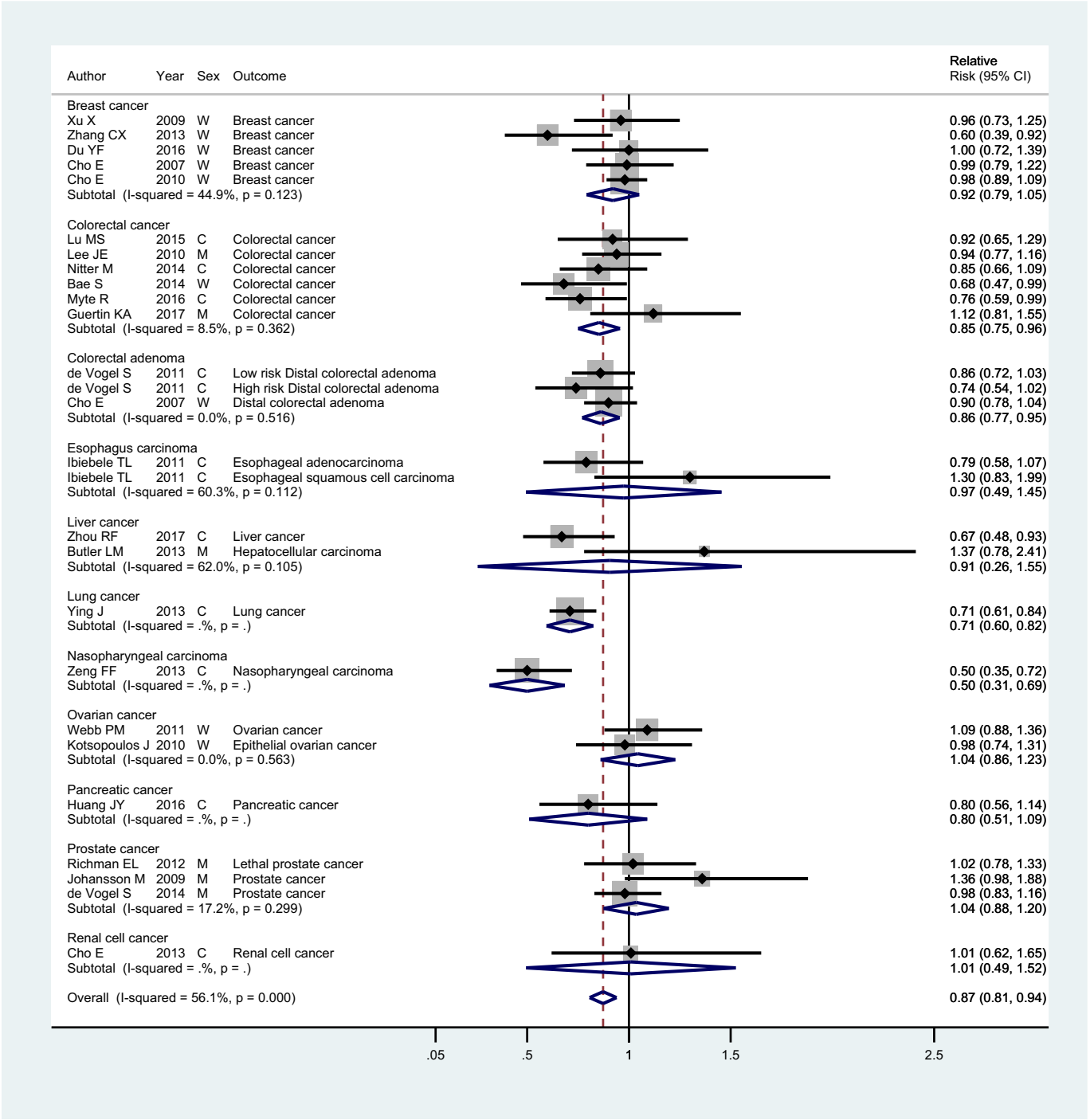

Fig. 5. Forest plot for top versus bottom categories of betaine levels in relation to cancer incidence by cancer sites. M, W, and C represent men, women, and combined sex, respectively. The black circles indicate the study specific relative risk (RR)s and the horizontal lines indicate the 95% CIs. The gray squares represent the study specific weights, which are inverse of the variance. The dash line indicates the overall combined RR and the diamonds indicate the combined RRs and the 95% CIs for the combined RRs.

Table 1
Combined RRs and 95% CIs comparing the top with the bottom categories for the association between choline or betaine intake and cancer survival.

	Number of studies	Combined RR (95% CI)	$p_{heterogeneity}$
Choline			
All-cause deaths	2 [33,46]	0.96 (0.78–1.14)	0.66
Disease-specific deaths	3 [30,33,47]	0.98 (0.52–1.43)	0.21
Betaine			
All-cause deaths	2 [33,46]	0.86 (0.42–1.30)	0.01
Disease-specific deaths	3 [30,33,47]	0.81 (0.55–1.08)	0.29

Abbreviations: RRs, relative risks; 95% CIs, 95% confidence intervals.

studies, inverse correlations were observed for choline and betaine in relation to cancer risk; however, a significant inverse association was observed for betaine, but not for choline in relation to cancer risk in cohort studies. When we examined the association in relation to specific cancer sites, the inverse association of betaine was more pronounced for colorectal neoplasia. The heterogeneity across choline studies was more apparent than a meta-analysis of betaine studies, particularly in a meta-analysis of cancer-specific results. No significant associations were identified between choline or betaine levels and mortality in survival studies.

Table 2
Combined RRs (95% CIs) comparing the top with the bottom categories of choline and betaine levels for cancer incidence according to study design, folate levels, exposure assessment method, sex, and geographic region of study.

	Choline			Betaine		
	No. of studies	RR (95% CI)	$P_{difference}$	No. of studies	RR (95% CI)	$P_{difference}$
Folate levels						
Low	11 [14–20,22–25]	0.70 (0.51–0.90)		11 [14,15,17–25]	0.81 (0.68–0.93)	
High	11 [14–20,22–25]	0.82 (0.59–1.05)	0.68	11 [14,15,17–25]	0.90 (0.82–0.99)	0.50
Exposure assessment						
FFQ	18 [14–17,20,22–25, 27–30,33,37,39,40,43]	0.86 (0.70–1.02)		17 [14–17,20,22–25,27,29,30, 33,37,39,40,43]	0.87 (0.78–0.95)	
Blood	7 [18,19,31,41,42,44,45]	1.02 (0.75–1.28)	0.17	8 [18,19,21,31,41,42,44,45]	0.88 (0.77–0.99)	0.72
Sex						
Men	8 [15,23,24,30,31,42,43,45]	1.03 (0.68–1.37)		9 [15,21,23,24,30,31,42,43,45]	1.00 (0.89–1.10)	
Women	13 [14,15,17,19,24,25, 27–29,33,37,43]	0.88 (0.69–1.07)	0.17	12 [14,15,17,19,24,25,27,29,33,37,43]	0.92 (0.84–0.99)	0.25
Geographic region						
Australia, Europe and the US	18 [17–20,23–25,27–31, 33,39–41,44,45]	1.04 (0.94–1.15)		18 [17–21,23–25,27,29–31, 33,39–41,44,45]	0.91 (0.84–0.97)	
China and Singapore	7 [14–16,22,37,42,43]	0.44 (0.35–0.52)	<0.001	7 [14–16,22,37,42,43]	0.74 (0.58–0.90)	0.05

RR, relative risk; 95% CI, 95% confidence interval; FFQ, food frequency questionnaire.

When the analysis was limited to Asian studies only [14–16,22,37,42,43], both choline and betaine levels were inversely associated with cancer development. Therefore, it is possible that the role of choline and betaine intake in cancer development is dependent on other nutritional conditions of populations, although this requires further analysis. In addition, as five of the Asian studies out of seven were case-control studies, further prospective studies, where selection or recall bias is less likely compared with case-control studies, are warranted.

Choline/betaine is a precursor of universal methyl group donor, SAM, and therefore may affect changes in DNA methylation [48]. Disturbance in DNA methylation is attributed to cancer development [49]. The reason why betaine, but not choline, showed the reduction in cancer risk in our study was not clear. Because of various functions of choline including methyl group donor, cell membrane signal transduction as well as cell membrane component, neurotransmitter, and hepatic secretor of very low-density lipoprotein (VLDL) [1], choline may not be specific enough to serve as a cancer preventive component. Although it is possible that betaine could prevent cancer development, we still cannot rule out the possibility of confounding factors related to plant-based food intake, because the major food sources of betaine are vegetables and whole grains including wheat bran, wheat germ, spinach, pretzel and wheat bread [5].

Folate and choline are competitively involved in donating methyl groups to homocysteine for DNA methylation via SAM [50]. An animal study of folate and choline demonstrated that the hepatic concentration of choline, and the *de novo* synthesis of choline, were reduced under folate deficient conditions [51]. Furthermore, a randomized clinical trial suggested that choline acted as a methyl donor upon depletion of folate [50]. The Framingham Offspring Study reported that, when folate intake was low (<250 μg/d), choline and betaine intake was inversely associated with the plasma concentration of total homocysteine, although there was no association when folate intake was high (>400 μg/d) [52]. Due to the potential interaction between choline or betaine and folate levels, the present study explored whether the association with cancer risk varied according to folate levels. Since a few previous studies were published by authors of this study, we were able to extract and re-analyze the original datasets and stratify according to folate levels [17,20,23–25]. However, we did not find significant difference, although we found that levels of choline and betaine were more inversely related to cancer when folate levels were low.

Previous meta-analysis included 11 studies published and concluded a lower cancer incidence with choline and betaine intake based on combined estimate of case-control and cohort studies [53]. In that meta-analysis of both case-control and cohort studies, the combined estimates were 0.82 (95% CI, 0.70 to 0.97) for choline and 0.86 (95% CI, 0.76 to 0.97) for betaine comparing the highest versus the lowest intake. We added six more studies including three studies [27,28,30] that were not included in a previous meta-analysis and three studied recently published [22,37,43]. We did not observe the inverse association for choline intakes in cohort studies. Although we observed an inverse association in a meta-analysis of case-control studies, potential bias, including recall or selection bias in case-control studies, might have precluded the conclusion that choline intake is beneficial for cancer prevention. Notably, a more apparent inverse association with choline and betaine levels was observed in a meta-analysis of Asian studies only, as compared with Western studies only; however, since five of the seven Asian studies were case-control studies, the evidence was insufficient to draw a definitive conclusion. As this meta-analysis included studies that were predominantly derived from Europe and the US, further studies on other populations are required. Our meta-analysis demonstrated that studies investigating the associations between choline or betaine levels and cancer risk and survival were heterogeneous, which suggested that other factors, such as other nutritional conditions and socioeconomic status, might have influenced the observed associations. Furthermore, the inclusion of different cancer types may have produced the heterogeneity.

5. Conclusions

In conclusion, the present meta-analysis suggested no association for choline, but inverse association for betaine levels with cancer incidence. For cancer survivorship, we did not find any associations, but it warrants further studies. We cannot rule out the possibility that the benefit of choline or betaine could differ according to other factors, including socioeconomic status or the intake of other nutrients. To better elucidate the role of choline and betaine in the development and progression of cancer, further prospective studies of diverse populations are needed.

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Author contributions

Youn J. performed the statistical analysis and drafted the manuscript; Cho E. reviewed the manuscript; Lee J.E. designed the study and drafted the manuscript.

Conflict of interest

None of the authors had any personal or financial conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clnu.2018.01.042.

References

[1] Zeisel SH, Blusztajn JK. Choline and human nutrition. Annu Rev Nutr 1994;14:269–96.
[2] Niculescu MD, Zeisel SH. Diet, methyl donors and DNA methylation: interactions between dietary folate, methionine and choline. J Nutr 2002;132:2333S–5S.
[3] Selhub J, Miller JW. The pathogenesis of homocysteinemia: interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. Am J Clin Nutr 1992;55:131–8.
[4] Kim Y-I. Folate and carcinogenesis: evidence, mechanisms, and implications. J Nutr Biochem 1999;10:66–88.
[5] Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. J Nutr 2003;133:1302–7.
[6] Patterson Kristine Y, Bhagwat Seema A, Williams Juhi R, Howe Juliette C, Holden JM. USDA database for the choline content of common foods release two. 2008.
[7] Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al., Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Group. Meta-analysis of observational studies in epidemiology: a proposal for reporting. JAMA 2000;283:2008–12.
[8] Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Prev Med 2007;45:247–51.
[9] DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.
[10] Higgins J, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ (Clinical Research Ed) 2003;327:557–60.
[11] Higgins J, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539–58.
[12] Stram DO. Meta-analysis of published data using a linear mixed-effects model. Biometrics 1996:536–44.
[13] Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. Am J Epidemiol 1992;135:1301–9.
[14] Zhang CX, Pan MX, Li B, Wang L, Mo XF, Chen YM, et al. Choline and betaine intake is inversely associated with breast cancer risk: a two-stage case-control study in China. Cancer Sci 2013;104:250–8.
[15] Lu MS, Fang YJ, Pan ZZ, Zhong X, Zheng MC, Chen YM, et al. Choline and betaine intake and colorectal cancer risk in Chinese population: a case-control study. PLoS One 2015;10, e0118661.
[16] Zeng FF, Xu CH, Liu YT, Fan YY, Lin XL, Lu YK, et al. Choline and betaine intakes are associated with reduced risk of nasopharyngeal carcinoma in adults: a case-control study. Br J Cancer 2013;110:808–16.
[17] 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.
[18] Nitter M, Norgård B, de Vogel S, Eussen S, Meyer K, Ulvik A, et al. Plasma methionine, choline, betaine, and dimethylglycine, in relation to colorectal cancer risk in the European prospective investigation into cancer and nutrition (EPIC). Ann Oncol 2014;25:1609–15.
[19] Bae S, Ulrich CM, Neuhouser ML, Malysheva O, Bailey LB, Xiao L, et al. Plasma choline metabolites and colorectal cancer risk in the Women's health initiative observational study. Cancer Res. 2014;74:7442–52.
[20] Cho E, Willett WC, Colditz GA, Fuchs CS, Wu K, Chan AT, et al. Dietary choline and betaine and the risk of distal colorectal adenoma in women. J Natl Cancer Inst 2007;99:1224–31.
[21] de Vogel S, Ulvik A, Meyer K, Ueland PM, Nygard O, Vollset SE, et al. Sarcosine and other metabolites along the choline oxidation pathway in relation to prostate cancer–a large nested case-control study within the JANUS cohort in Norway. Int J Cancer 2014;134:197–206.
[22] Zhou RF, Chen XL, Zhou ZG, Zhang YJ, Lan QY, Liao GC, et al. Higher dietary intakes of choline and betaine are associated with a lower risk of primary liver cancer: a case-control study. Sci Rep 2017;7:679.
[23] 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.
[24] Cho E, Giovannucci EL, Joh HK. Nutrients related to one-carbon metabolism and risk of renal cell cancer. Cancer Causes Control 2013;24:373–82.
[25] 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.
[26] Orsini N, Bellocco R, Greenland S. Generalized least squares for trend estimation of summarized dose-response data. Stata J 2006;6:40–57.
[27] Webb PM, Ibiebele TI, Hughes MC, Beesley J, van der Pols JC, Chen X, et al. Folate and related micronutrients, folate-metabolising genes and risk of ovarian cancer. Eur J Clin Nutr 2011;65:1133–40.
[28] Liu J, Hazra A, Giovannucci E, Hankinson S, Rosner B, De Vivo I. One-carbon metabolism factors and endometrial cancer risk. Br J Cancer 2013;108:183–7.
[29] 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.
[30] Richman EL, Kenfield SA, Stampfer MJ, Giovannucci EL, Zeisel SH, Willett WC, et al. Choline intake and risk of lethal prostate cancer: incidence and survival. Am J Clin Nutr 2012;96:855–63.
[31] Johansson M, Van Guelpen B, Vollset SE, Hultdin J, Bergh A, Key T, 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.
[32] Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ (Clinical Research Ed) 1997;315:629–34.
[33] Xu X, Gammon MD, Zeisel SH, Bradshaw PT, Wetmur JG, Teitelbaum SL, et al. High intakes of choline and betaine reduce breast cancer mortality in a population-based study. FASEB J 2009;23:4022–8.
[34] Xu X, Gammon MD, Wetmur JG, Bradshaw PT, Teitelbaum SL, Neugut AI, et al. B-vitamin intake, one-carbon metabolism, and survival in a population-based study of women with breast cancer. Cancer Epidemiol Biomarkers Prev 2008;17:2109–16.
[35] Xu X, Gammon MD, Zeisel SH, Lee YL, Wetmur JG, Teitelbaum SL, et al. Choline metabolism and risk of breast cancer in a population-based study. FASEB J 2008;22:2045–52.
[36] Bradshaw PT, Khankari NK, Teitelbaum SL, Xu X, Fink BN, Steck SE, et al. Nutrient pathways and breast cancer risk: the long Island breast cancer study project. Nutr Cancer 2013;65:345–54.
[37] Du Y-F, Luo W-P, Lin F-Y, Lian Z-Q, Mo X-F, Yan B, et al. Dietary choline and betaine intake, choline-metabolising genetic polymorphisms and breast cancer risk: a case–control study in China. Br J Nutr 2016;116:961–8.
[38] Du Y-F, Lin F-Y, Long W-Q, Luo W-P, Yan B, Xu M, et al. Serum betaine but not choline is inversely associated with breast cancer risk: a case–control study in China. Eur J Nutr 2016:1–9.
[39] Ibiebele TI, Hughes MC, Pandeya N, Zhao Z, Montgomery G, Hayward N, et al. High intake of folate from food sources is associated with reduced risk of esophageal cancer in an Australian population. J Nutr 2011;141:274–83.
[40] Ying J, Rahbar MH, Hallman DM, Hernandez LM, Spitz MR, Forman MR, et al. Associations between dietary intake of choline and betaine and lung cancer risk. PLoS One 2013;8, e54561.
[41] de Vogel S, Schneede J, Ueland PM, Vollset SE, Meyer K, Fredriksen A, et al. Biomarkers related to one-carbon metabolism as potential risk factors for distal colorectal adenomas. Cancer Epidemiol Biomarkers Prev 2011;20:1726–35.
[42] Butler LM, Arning E, Wang R, Bottiglieri T, Govindarajan S, Gao Y-T, et al. Prediagnostic levels of serum one-carbon metabolites and risk of hepatocellular carcinoma. Cancer Epidemiol Biomarkers Prev 2013;22:1884–93.
[43] Huang JY, Butler LM, Wang R, Jin A, Koh W-P, Yuan J-M. Dietary intake of one-carbon metabolism–related nutrients and pancreatic cancer risk: the Singapore Chinese Health Study. Cancer Epidemiol Biomarkers Prev 2016;25:417–24.
[44] Myte R, Gylling B, Schneede J, Ueland PM, Häggström J, Hultdin J, et al. Components of one-carbon metabolism other than folate and colorectal cancer risk. Epidemiology 2016;27:787–96.
[45] Guertin KA, Li XS, Graubard BI, Albanes D, Weinstein SJ, Goedert JJ, et al. Serum trimethylamine N-oxide, carnitine, choline and betaine in relation to colorectal cancer risk in the alpha tocopherol and beta carotene study. Cancer Epidemiol Biomarkers Prev 2017;26.
[46] Dixon SC, Ibiebele TI, Protani MM, Beesley J, Defazio A, Crandon AJ, et al. Dietary folate and related micronutrients, folate-metabolising genes, and ovarian cancer survival. Gynecol Oncol 2013;132:556–72.

[47] Galván-Portillo MV, Oñate-Ocaña LF, Pérez-Pérez GI, Chen J, Herrera-Goepfert R, Chihu-Amparan L, et al. Dietary folate and vitamin B12 intake before diagnosis decreases gastric cancer mortality risk among susceptible *MTHFR 677TT* carriers. Nutrition 2010;26:201–8.
[48] Zeisel S. Choline, other methyl-donors and epigenetics. Nutrients 2017;9.
[49] Laird PW, Jaenisch R. The role of DNA methylation in cancer genetic and epigenetics. Annu Rev Genet 1996;30:441–64.
[50] 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.
[51] Kim YI, Miller JW, da Costa KA, Nadeau M, Smith D, Selhub J, et al. Severe folate deficiency causes secondary depletion of choline and phosphocholine in rat liver. J Nutr 1994;124:2197–203.
[52] Cho E, Zeisel SH, Jacques P, Selhub J, Dougherty L, Colditz GA, 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.
[53] Sun S, Li X, Ren A, Du M, Du H, Shu Y, et al. Choline and betaine consumption lowers cancer risk: a meta-analysis of epidemiologic studies. Sci Rep 2016;6:35547.