Association Between Folate and Health Outcomes: An Umbrella Review of Meta-Analyses

Yacong Bo 1†, Yongjian Zhu 2†, Yuchang Tao 3, Xue Li 1,4, Desheng Zhai 1, Yongjun Bu 1, Zhongxiao Wan 3, Ling Wang 3, Yuming Wang 5 and Zengli Yu 1*  

1 School of Public Health, Xinxiang Medical University, Xinxiang, China, 2 Department of Cardiology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China, 3 School of Public Health, Zhengzhou University, Zhengzhou, China, 4 Centre for Population Health Sciences, University of Edinburgh, Edinburgh, United Kingdom, 5 Department of Administration, Henan University People’s Hospital, Zhengzhou, China

Background: There is no study that has systematically investigated the breadth and validity of the associations of folate and multiple health outcomes. We aimed to evaluate the quantity, validity, and credibility of evidence regarding associations between folate and multiple health outcomes by using umbrella review of meta-analysis.

Methods: We searched the MEDLINE, EMBASE, and Cochrane Library databases from inception to May 20, 2018, to identify potential meta-analyses that examined the association of folate with any health outcome. For each included meta-analysis, we estimated the summary effect size and their 95% confidence interval using the DerSimonian and Laird random-effects model. We used the AMSTAR 2 (A Measurement Tool to Assess Systematic Reviews) to assess methodological quality and the GRADE (Grading of Recommendations, Assessment, Development, and Evaluation working group classification) to assess the quality of evidence for each outcome included in the umbrella review.

Results: Overall, 108 articles reporting 133 meta-analyses of observational studies and 154 meta-analyses of randomized controlled trials (RCTs) were included in the study. Among them, 108 unique exposure–outcome–population triplets (referred to as unique meta-analyses hereafter) of RCTs and 87 unique meta-analyses of observational studies were reanalyzed. Beneficial effects of folate were observed in the all-cause mortality rate and in a number of chronic diseases, including several birth/pregnancy outcomes, several cancers, cardiovascular disease and metabolic-related outcomes, neurological conditions, and several other diseases. However, adverse effects of folate were observed for prostate cancer, colorectal adenomatous lesions, asthma or wheezing, and wheezing as an isolated symptom and depression.

Conclusions: Current evidence allows for the conclusion that folate is associated with decreased risk of all-cause mortality and a wide range of chronic diseases. However, folate may be associated with an increased risk of prostate cancer. Further research is warranted to improve the certainty of the estimates.

Keywords: folate, meta-analysis, umbrella review, multiple health outcomes, chronic diseases
INTRODUCTION

Folate, which mediates the transfer of one-carbon units in methylation and biosynthesis of nucleotides, has been well-established to play important roles in the processes of DNA synthesis, stability, repair, and methylation (1). It has been known for more than 2 decades that folic acid supplements during a woman’s pregnancy can reduce the risk of neural tube malformation. Since then, numerous studies have investigated the effects of folate on a wide range of health outcomes, including the all-cause and cause-specific mortality, cancer outcomes, cardiovascular disease (CVD), diabetes, neurocognitive disorders, and pregnancy and birth outcomes. It is also noteworthy that folate supplement has been becoming popular worldwide, although evidence regarding the associations between folate and various health outcomes is still inconclusive.

Given this, a systematic assessment of the credibility of the published evidence will provide important implications for folate in both clinical practice and public health. Previous original or meta-analysis studies of the health effects of folate usually focused on a single health outcome (e.g., neural tube malformation). We therefore carried out the current umbrella review of existing published data on the associations between folate exposure and diverse health outcomes. In addition, we aimed to describe the magnitude, direction, and significance of the suggested associations; evaluate the potential biases; and identify which studies produced the highest-quality evidence.

METHODS

Structure of Umbrella Review

The umbrella review method, which synthesizes information from meta-analyses of both observational studies and randomized controlled trials (RCTs) on multiple health outcomes associated with a particular exposure, could provide an instructive panorama for public health interventions (2, 3). We conducted this umbrella review of folate and multiple health outcomes by systematically searching for meta-analyses in which folate was part or all of the exposure of interest. Meanwhile, we excluded those systematic reviews without meta-analyses.

Search Strategy

The MEDLINE, EMBASE, and Cochrane Library databases were searched from inception to May 20, 2018 to identify meta-analyses that examined the association between folate and any health outcome. The detailed search strategies are presented in Supplementary Table 1. The titles, abstracts, and full texts of potentially eligible articles were screened by two researchers independently. Disagreements were arbitrated by a third researcher.

Eligibility Criteria

Articles with meta-analyses were included if they met the following inclusion criteria:

1. Meta-analyses of either observational (i.e., cohort, case-control, and cross-sectional studies) or interventional studies (i.e., RCTs)
2. Evaluating the association of folate (folate intake, folate supplementation, and folate concentration) with any health outcome
3. The included population aged 18 years or older
4. Published in peer-reviewed journals in English.

We excluded meta-analyses that evaluated the effects of genetic polymorphisms related to folate metabolism on health outcomes, animal research, and laboratory studies. If an article presented separate meta-analysis for more than one health outcome, we included each of these separately. For meta-analyses of observational studies, if more than one meta-analysis addressed the same research question, the one with the largest number of prospective cohort studies was included.

Data Extraction

Two investigators independently extracted information from eligible meta-analyses. For each meta-analysis, the following information was extracted: first author’s last name, year of publication, number of studies included, populations, health outcomes of interest, study designs, exposure of folate, effect sizes (odds ratio (OR), risk ratio (RR), hazard ratio (HR), or mean difference (MD)), and the corresponding 95% confidence intervals (CIs), and types of effect model used in the meta-analysis (fixed or random). In addition, we also extracted number of cases and controls (for case–control studies), events and participant/person-years (for cohort studies), or number of subjects in interventional and control groups (for RCTs). For each original study included in each meta-analysis, the following data were extracted for further reevaluation: the effect estimates (OR, RR, HR, or MD) with 95% CI, number of cases, total number of participants, and study design.

Data Analysis

Summary effects and 95% CIs for each meta-analysis were reanalyzed by using a DerSimonian and Laird random-effects model to be consistent with the method widely used in the included meta-analyses. For any associations with \( p < 0.05 \), the following metrics were further estimated: the 95% prediction interval to evaluate the uncertainty for the effect that would be expected in a new original study (4, 5); the between-study heterogeneity (defined as significant for \( I^2 \geq 50\% \) and \( p < 0.05 \)); the excess significance test to assess whether the observed number (O) of studies with significant results (positive studies) was larger than the expected number (E) (6); and the presence of small-study effect by using Egger regression asymmetry test (significance threshold \( p < 0.10 \)) (7).

For overlapping outcomes that were examined both in meta-analyses of RCTs and those of observational studies, we examined whether the observed direction and statistical significance were consistent between the two study types.

Assessment of Methodological Quality

We used the updated AMSTAR 2 (A Measurement Tool to Assess Systematic Reviews) to evaluate the methodological quality of the
included meta-analyses. Compared with the original AMSTAR tool, the AMSTAR 2 emphasizes the risk-of-bias assessment in study design and heterogeneity and is a reliable and valid tool for quality assessment of meta-analyses of both interventional and observational research (8). The AMSTAR 2 includes 16 items for evaluating the methodological quality of systematic reviews/meta-analyses, with each item scoring 0 or 1. The methodological quality of each individual meta-analysis was then classified as high, moderate, low, or critically low accordingly.

Credibility of the Evidence
The Grading of Recommendations, Assessment, Development, and Evaluation working group classification (GRADE) was used to assess the quality of evidence for those meta-analyses included in the umbrella review (9, 10). The GRADE categorizes evidence from systematic reviews and meta-analyses into the levels of high, moderate, low, or very low. In the GRADE approach, RCTs start as high-quality evidence, and observational studies start as low-quality evidence. Other factors may then upgrade or downgrade the quality level. For example, unexplained heterogeneity or high probability of publication bias may downgrade the quality of evidence, whereas a large effect or dose-response gradient may upgrade it. Two reviewers independently assessed the included studies, and a third reviewer settled disagreements.

RESULTS

Literature Review
The flow of study selection is presented in Figure 1. We initially identified 1,975 unduplicated articles. After considering the inclusion and exclusion criteria, 108 articles were finally included in the study. Among them, 133 meta-analyses of observational studies were reported in 62 articles (11–72), and 154 meta-analyses of RCTs were reported in 51 articles (13, 20, 28, 47, 62, 73–118). Another five articles reported both meta-analyses with observational studies and RCTs (13, 20, 28, 47, 62). As a result, a total of 195 unique health outcomes classified into eight health fields (i.e., all-cause and cause-specific mortality rates, cancer outcomes, cardiovascular outcomes, birth outcomes, pregnancy outcomes, neurocognitive disorders, and other outcomes) were reported (Supplementary Figure 1).

Meta-Analysis of Observational Studies
As shown in Supplementary Table 2, the median number of meta-analyses with observational studies included in each outcome was 7 (range, 2–36), and the median numbers of participants/case numbers were 43,063 (range, 635–59,514,473) and 3,463 (range, 11–147,424), respectively. Twenty-one outcomes were reported in more than one meta-analysis.

After excluding 46 duplicated meta-analyses, we further analyzed 87 unique exposure-outcome-population triplets (referred to as unique meta-analyses hereafter) of observational studies with a wide range of outcomes (Supplementary Table 3): all-cause and cause-specific mortality (n = 3), birth outcomes (n = 28), cancer-related outcomes (n = 45), cardiovascular outcomes (n = 2), neurocognitive disorders (n = 5), pregnancy outcomes (n = 3), and other outcomes (n = 1). Figures 2, 3 show the summarized results of these 87 unique meta-analyses. Overall, 35 of the 87 (40.2%) meta-analyses reported significantly pooled results (p < 0.05).

Of these 87 unique meta-analyses, 10 (11.5%) were with statistical significance of p < 10^{-6}, 7 (8.0%) had a 95% prediction interval excluding the null, 60 (69.0%) had more than 1,000 cases (or more than 20,000 participants for continuous outcomes), 16 (18.4%) had neither evidence of excess significance bias (p > 0.10) nor small-study effects (p > 0.10), and 46 (46.0%) had no large heterogeneity ($I^2 < 50\%$ and $p > 0.05$).

Supplementary Table 4 provides a breakdown of the AMSTAR 2 scores for the meta-analyses representing each outcome. None of the 87 meta-analyses was rated at the high methodological level, and 3 (3.4%) were rated as moderate, leaving 31 (35.6%) as low and 53 (60.9%) as critically low. Regarding the GRADE classification for evidence level, 4 of the 87 meta-analyses (4.6%) were rated as high-quality evidence for the corresponding outcomes, 21 (24.1%) were rated as moderate, 12 (13.8%) were rated as low, and 50 (57.5%) were rated as very low quality (Supplementary Table 5).

Data Synthesis for High- or Moderate-Quality Meta-Analysis of Observational Studies
Among the 25 meta-analyses with high or moderate GRADE classification, we found that folate intake was associated with lower risks of low birth weight (during preconception), esophageal adenocarcinoma, gastric cancer, head and neck squamous cell carcinoma, pancreatic cancer, coronary heart disease, and serrated colorectal polyps (among adults undergoing endoscopic investigation of the colorectal), but it did not show a significant association with low birth weight (during post-conception pregnancy), colorectal cancer, lung cancer, and Parkinson disease. Folate supplementation was associated with lower risks of non-syndromic cleft lip with or without cleft palate and small for gestational age, but it did not show a significant association with non-syndromic cleft palate, wheezing, acute lymphoblastic leukemia, and gestational hypertension/preeclampsia. A higher level of circulating folate was associated with lower risks of cervical cancer, colorectal adenoma, and Alzheimer disease, but it did not show a significant association with lung cancer and coronary heart disease (Supplementary Figure 2). Interestingly, we found that circulating folate did not show a significant association with prostate cancer, but higher serum folate was associated with increased risk of prostate cancer.

Meta-Analyses of RCTs
As shown in Supplementary Table 6, the median number of meta-analyses of RCTs included in each outcome was 5.5 (range, 2–25), and the median numbers of participants and cases were 3,113 (range, 28–82,723) and 653 (range, 3–39,923), respectively. More than one meta-analysis was reported for 17 outcomes.

After removing 46 duplicated meta-analyses, we further analyzed 108 unique meta-analyses of RCTs for the associations of folate with all-cause and cause-specific mortality (n = 2),
FIGURE 1 | Flowchart of selection of studies for inclusion in umbrella review on folate and health.
birth outcomes ($n = 17$), cancer-related outcomes ($n = 14$), cardiovascular outcomes ($n = 29$), diabetes-related outcomes ($n = 9$), endothelial function ($n = 5$), neurocognitive disorders ($n = 5$), pregnancy outcomes ($n = 8$), and other outcomes ($n = 19$). The summarized results of these 108 unique meta-analyses are presented in Figures 4, 5. Overall, 31 (28.7%) meta-analyses showed nominally significant pooled results ($p < 0.05$). Among the 31 meta-analyses, 6 were for birth outcomes, 3 were for cardiovascular outcomes, 5 were for diabetes-related outcomes, 1 was for neurocognitive disorders, 3 were for pregnancy outcomes, and 11 were for other outcomes, suggesting that folate supplementation was associated with a decreased risk of these aforementioned diseases. However, two meta-analyses for cancer-related outcomes reported pooled results with $p$-values lower than 0.05, suggesting that folate supplementation was associated with increased risks of colorectal adenomatous lesion and prostate cancer.

As shown in Supplementary Table 7, 25 of the 108 meta-analyses (23.1%) showed statistical significance ($p < 0.01$); the 95% prediction interval excluded the null in 6 (5.6%), 14 (13.0%) had more than 1,000 cases (or more than 20,000 participants for continuous outcomes), 8 (7.4%) had no evidence of excess significance bias ($p > 0.10$) or small-study effects ($p > 0.10$), and 49 (45.4%) showed no great heterogeneity ($I^2 < 50\%$ and $p > 0.05$).

Supplementary Table 8 presents a breakdown of the AMSTAR 2 scores for the meta-analyses representing each outcome. None of the 108 meta-analyses was rated at a high
Bo et al. Folate and Health Outcomes

FIGURE 3 | Summary random-effects estimates of all-cause and cause-specific mortality, birth outcomes, pregnancy outcomes, neurocognitive disorders, and other outcomes reported in meta-analyses of observational studies.

| Outcomes          | exposure                        | Population | No of participants | No of cases | Risk Ratio (95% CI) |
|-------------------|---------------------------------|------------|--------------------|-------------|---------------------|
| Cancer outcomes   |                                 |            |                    |             |                     |
| Bladder cancer    | Total folate intake             | General    | 498372             | 6280        | 0.88 (0.78, 1.01)   |
| Breast cancer (case–control) | Blood folate | General    | 1337               | 545         | 0.58 (0.32, 1.05)   |
| Breast cancer (cohort) | Blood folate | General    | 8677               | 3815        | 1.08 (0.87, 1.35)   |
| Breast cancer (case–control, 100μg/d increase) | dietary folate intake | Postmenopausal women | 1636566    | 24083       | 0.99 (0.92, 1.07)   |
| Breast cancer (cohort, 100μg/d increase) | dietary folate intake | Postmenopausal women | 4893       | 2259        | 0.92 (0.83, 1.03)   |
| Breast cancer (case–control, 100μg/d increase) | dietary folate intake | Premenopausal women | 162033    | 7130        | 1.01 (0.98, 1.05)   |
| Breast cancer (cohort, 100μg/d increase) | dietary folate intake | Premenopausal women | 6566     | 2419        | 0.87 (0.78, 0.97)   |
| Breast cancer (cohort, 100μg/d increase) | dietary folate intake | Premenopausal women | 155926   | 1828        | 1.00 (0.97, 1.04)   |
| Breast cancer | Folate supplement               | General    | 86647              | 2506        | 1.07 (0.95, 1.21)   |
| Breast cancer | total folate intake             | General    | 543650             | 22134       | 0.98 (0.90, 1.07)   |
| Cervical cancer (Deficient vs. normal) | Serum folate | General    | 2382               | 873         | 1.95 (1.34, 3.30)   |
| Cervical polypsis | Folate intake and serum folate | General    | 3089               | 1706        | 0.60 (0.41, 0.88)   |
| Coloectal adenoma (Lowest vs highest) | Circulating folate | General    | 17831              | 4812        | 1.23 (1.09, 1.39)   |
| Coloectal adenoma | circulating folate              | General    | 10516              | 3477        | 1.01 (0.87, 1.17)   |
| Coloectal cancer (case–control) | dietary folate intake | General    | 18992              | 8328        | 0.87 (0.74, 1.02)   |
| Coloectal cancer (cohort) | dietary folate intake | General    | 471924             | 6633        | 0.92 (0.81, 1.05)   |
| Coloectal cancer | Folate supplement               | General    | 169160             | 3783        | 0.87 (0.74, 1.01)   |
| Coloectal cancer | Folate supplement               | General    | 4517               | 638         | 0.71 (0.53, 0.96)   |
| Coloectal cancer | RBC folate                      | General    | 7908               | 3008        | 1.04 (0.84, 1.29)   |
| Coloectal cancer | total folate intake             | General    | 1996786            | 23147       | 0.88 (0.81, 0.95)   |
| Endometrial cancer | total folate intake             | General    | 270542             | 6151        | 0.89 (0.76, 1.05)   |
| Esophageal adenocarcinoma | dietary folate intake | General    | 1769               | 501         | 0.50 (0.49, 0.65)   |
| Esophageal cancer | Serum folate                    | General    | 36343              | 5489        | 0.70 (0.31, 1.59)   |
| Esophageal cancer | dietary folate intake           | General    | 4840400            | 2036        | 0.66 (0.52, 0.83)   |
| Esophageal cancer | Folate intake and serum folate  | General    | 557646             | 5442        | 0.59 (0.49, 0.71)   |
| Gastric cancer | dietary folate intake           | General    | 206689             | 4414        | 0.94 (0.76, 1.13)   |
| Gastric cancer | Folate intake and serum folate  | General    | 857918             | 6810        | 0.92 (0.71, 0.84)   |
| Head and neck squamous cell carcinoma | Folate intake and serum folate | General    | 14992              | 4090        | 0.53 (0.41, 0.68)   |
| Lung Cancer | dietary folate intake           | General    | 5087676            | 4681        | 0.92 (0.84, 1.01)   |
| Lung Cancer | Serum folate                    | General    | 6068               | 1482        | 0.78 (0.60, 1.03)   |
| Lung Cancer | total folate intake             | General    | 105288             | 4390        | 0.74 (0.65, 0.84)   |
| Ovarian cancer | dietary folate intake           | General    | 227859             | 5677        | 0.88 (0.75, 1.05)   |
| Pancreatic cancer | dietary folate intake           | General    | 295776             | 2459        | 0.66 (0.49, 0.89)   |
| Pancreatic cancer | Folate intake and serum folate  | General    | 997922             | 3067        | 0.76 (0.50, 0.96)   |
| Pancreatic cancer | Folate supplement               | General    | 235389             | 1175        | 1.08 (0.82, 1.41)   |
| Prostate cancer | Blood folate                    | General    | 133232             | 6122        | 1.43 (1.06, 1.93)   |
| Prostate cancer (10 mmol/L increase) | Circulating folate | General    | 9353               | 2958        | 1.13 (0.95, 1.38)   |
| Prostate cancer | dietary folate intake           | General    | 120349             | 14290       | 0.98 (0.90, 1.07)   |
| Prostate cancer | Serum folate                    | General    | 36243              | 54809       | 1.21 (1.05, 1.39)   |
| Prostate cancer | total folate intake             | General    | 93781              | 7114        | 0.99 (0.82, 1.19)   |
| Renal Cell Cancer | Folate supplement               | General    | 374901             | 2723        | 0.89 (0.77, 1.02)   |
| Acute lymphoblastic leukemia | Folate supplement | 1 month before pregnancy | 2042 | 490 | 1.06 (0.77, 1.46) |
| Cardiovascular outcomes |                                 |            |                    |             |                     |
| Coronary heart disease | Blood folate | General    | 14533              | 1936        | 0.74 (0.53, 1.02)   |
| Coronary heart disease | dietary folate intake           | General    | 221009             | 2682        | 0.69 (0.60, 0.80)   |

**NOTE:** Weights are from random effects analysis

Methodological level, and 14 (13.0%) were rated as moderate, leaving 36 (33.3%) as low and 58 (53.7%) as critically low. In terms of evidence quality for each outcome, 10 of the 108 meta-analyses (9.3%) were rated as high, 24 (22.2%) were rated as moderate, 22 (20.4%) were rated as low, and 52 (48.1%) were rated as very low quality by the GRADE classification (Supplementary Table 9).

**Data Synthesis for High- or Moderate-Quality Meta-Analyses of RCTs**

Among the 34 meta-analyses with high or moderate GRADE classification, we found that folate supplementation was associated with decreased risk of elective termination of pregnancy for fetal anomalies; megaloblastic anemia; neural tube defects; CVD (among those with preexisting diseases); liver toxicity (patients receiving methotrexate); gestational hypertension/preeclampsia; low predelivery serum folate; decreased scores on the Hamilton Depression Rating Scale and levels of plasma homocysteine (both among patients with type 2 diabetes and the general population); increased levels of birth weight, red blood cell folate, and serum/plasma folate; and increased risk of prostate cancer (among those with preexisting diseases). However, we did not find any significant association between folate supplementation and the all-cause mortality rate (among those with preexisting diseases), cancer mortality rate (among those with preexisting diseases), low birth weight, preterm birth, stillbirths/neonatal deaths, cancer incidence (among those with preexisting diseases), colorectal...
Comparison Findings in Meta-Analysis of Observational Studies and Those of RCTs

One hundred eighty (92.3%) unique meta-analyses examined only observational studies (n = 77) or RCTs (n = 103), so the evidence from those meta-analyses could not be compared between observational and randomized studies.

Five outcomes from 15 meta-analyses were investigated by meta-analyses of both observational studies (n = 10) and RCTs (n = 5) (Supplementary Table 10): cleft palate, neural tube defects, recurrence of neural tube defects, colorectal cancer, and gestational hypertension/pre-eclampsia. Between meta-analyses of observational studies and those of RCTs, the direction of the association/effect and level of statistical significance were concordant for cleft palate, neural tube defects, and the effects of different folate exposure [dietary folate intake (both case–control and cohort studies), red blood cell folate, circulating folate, and...
Bo et al. Folate and Health Outcomes

FIGURE 5 | Summary random-effects estimates of cancer outcomes, cardiovascular outcomes, and diabetes-related outcomes reported in meta-analyses of randomized controlled trials.

| Outcomes                      | exposure                        | Population                  | No of participants | No of cases | Risk Ratio (95%-CI) |
|-------------------------------|---------------------------------|-----------------------------|---------------------|-------------|---------------------|
| Cancer outcomes               |                                 |                             |                     |             |                     |
| advanced colorectal lesion    | Folate supplementation          | Patients with an adenoma history | 1922               | 202         | 1.07 (0.82, 1.39)   |
| Breast cancer                 | Folate supplementation          | Preexisting diseases        | 19800              | 203         | 0.84 (0.63, 1.12)   |
| Cancer incidence              | Folate supplementation          | Preexisting diseases        | 19663              | 371         | 1.05 (0.97, 1.13)   |
| Colorectal adenoma            | Folate supplementation          | Preexisting diseases        | 2652               | 168         | 3.32 (0.88, 1.97)   |
| Colorectal adenoma recurrence | Folate supplementation          | General                      | 1486               | 64          | 1.08 (0.87, 1.33)   |
| Colorectal adenomatous lesion | Folate supplementation          | for up to 3 years           | General            | 33495       | 381                 | 1.00 (0.82, 1.22)   |
| Colorectal adenomatous lesion | Folate supplementation          | for over 3 years            | General            | 6736        | 383                 | 1.34 (1.06, 1.70)   |
| Colorectal cancer             | Folate supplementation          | General                      | 34598              | 381         | 1.00 (0.82, 1.22)   |
| Hematological cancers         | Folate supplementation          | Preexisting diseases        | NA                 | NA          | 1.16 (0.55, 2.43)   |
| Hematological malignancy      | Folate supplementation          | Preexisting diseases        | 23570              | 170         | 0.70 (0.24, 1.99)   |
| Lung Cancer                   | Folate supplementation          | Preexisting diseases        | NA                 | NA          | 1.07 (0.88, 1.29)   |
| Melanoma                      | Folate supplementation          | Preexisting diseases        | 19128              | 38          | 0.51 (0.24, 1.10)   |
| Prostate cancer               | Folate supplementation          | Preexisting diseases        | 23738              | 432         | 1.24 (0.14, 1.49)   |

Cardiovascular disease

| Cardiovascular disease        | Folate supplementation          | Preexisting diseases        | 74346              | 979         | 0.94 (0.90, 0.99)   |
| Cardiovascular events         | Folate supplementation          | Preexisting diseases        | 57592              | 9531        | 0.98 (0.93, 1.03)   |
| Carotid intima-media thickness| Folate supplementation          | Preexisting diseases        | 2052               | NA          | 0.92 (0.89, 0.96)   |
| Coronary-syndrom              | Folate supplementation          | Preexisting diseases        | 19950              | 3146        | 0.98 (0.93, 1.14)   |
| Coronary artery bypass grafting| Folate supplementation          | General                      | 10703              | 811         | 0.90 (0.78, 1.03)   |
| Coronary heart disease        | Folate supplementation          | Preexisting diseases        | 78192              | 5899        | 1.04 (0.99, 1.09)   |
| Coronary restenosis           | Folate supplementation          | General                      | 936                | 224         | 0.52 (0.32, 0.85)   |
| Coronary revascularization    | Folate supplementation          | General                      | 27418              | 2573        | 0.99 (0.89, 1.11)   |
| Diastolic blood pressure      | Folate supplementation          | Coronary artery disease     | 237               | NA          | 1.17 (0.92, 1.47)   |
| Diastolic blood pressure      | Folate supplementation          | Patients with metabolic diseases | 262            | NA          | 0.34 (0.06, 1.96)   |
| End-diastolic diameter        | Folate supplementation          | Coronary artery disease     | 237               | NA          | 0.95 (0.69, 1.31)   |
| HDL–cholesterol               | Folate supplementation          | Patients with metabolic diseases | 492            | NA          | 1.08 (0.52, 2.23)   |
| Heart rate                    | Folate supplementation          | Coronary artery disease     | 237               | NA          | 0.49 (0.01, 16.60)  |
| LUS–cholesterol               | Folate supplementation          | Patients with metabolic diseases | 432            | NA          | 0.78 (0.37, 1.64)   |
| Myocardial infarction          | Folate supplementation          | Preexisting diseases        | 2917               | 39923       | 0.99 (0.93, 1.07)   |
| Pneumococcal pneumonia        | Folate supplementation          | Preexisting diseases        | 10703              | 1952        | 0.97 (0.94, 1.01)   |
| Primary cardiovascular clinical end point| Folate supplementation | Preexisting diseases | 19497              | 7265        | 1.02 (0.95, 1.09)   |
| Revascularization             | Folate supplementation          | General                      | 29314              | 2979        | 1.06 (0.99, 1.13)   |
| Revascularization             | Folate supplementation          | Preexisting diseases        | 38668              | 2939        | 1.10 (0.96, 1.26)   |
| Stroke                        | Folate supplementation          | Preexisting diseases        | 62773              | 3108        | 0.88 (0.51, 1.57)   |
| Systolic blood pressure       | Folate supplementation          | Coronary artery disease     | 237               | NA          | 0.14 (0.00, 246.78) |
| Systolic blood pressure       | Folate supplementation          | Patients with metabolic diseases | 262            | NA          | 0.21 (0.04, 1.18)   |
| Total cholesterol             | Folate supplementation          | Patients with metabolic diseases | 492            | NA          | 1.03 (0.06, 1.87)   |
| Triglycerides                 | Folate supplementation          | Patients with metabolic diseases | 492            | NA          | 1.03 (0.06, 1.87)   |
| VLDL–cholesterol              | Folate supplementation          | Patients with metabolic diseases | 71             | NA          | 1.15 (0.64, 2.19)   |

Diabetes related outcomes

| Diabetes related outcomes     | Folate supplementation          | Metabolic Diseases          | 521                | NA          | 0.58 (0.32, 1.04)   |
| Diabetes related outcomes     | Folate supplementation          | Metabolic Diseases          | 16708              | NA          | 0.76 (0.59, 0.97)   |
| Diabetes related outcomes     | Folate supplementation          | Metabolic Diseases          | 288                | NA          | 0.59 (0.33, 1.04)   |
| Diabetes related outcomes     | Folate supplementation          | General                     | 313                | NA          | 0.74 (0.41, 1.34)   |
| Diabetes related outcomes     | Folate supplementation          | Type 2 Diabetes             | 142                | NA          | 0.51 (0.14, 1.89)   |
| Diabetes related outcomes     | Folate supplementation          | Metabolic Diseases          | 494                | NA          | 0.15 (0.04, 0.51)   |
| Diabetes related outcomes     | Folate supplementation          | General                     | 435                | NA          | 0.22 (0.10, 0.51)   |
| Diabetes related outcomes     | Folate supplementation          | Metabolic Diseases          | 463                | NA          | 0.10 (0.03, 0.36)   |
| Diabetes related outcomes     | Folate supplementation          | General                     | 380                | NA          | 0.03 (0.00, 0.33)   |

NOTE: Weights are from random effects analysis

In this study, we first provided an overview and appraisal of the relationships between folate exposure and a wide range of health outcomes. We found that folate is more often associated with benefit than harm for a range of health outcomes across multiple measures of exposure, including folate intake, folate supplementation, and folate concentration. Overall, we observed the beneficial effects of folate intake/level/supplementation on all-cause mortality and a number of chronic diseases, including cancers, CVD, and metabolic-related outcomes, as well as several birth outcomes. However, adverse effects of supplemented/serum folate were observed on prostate cancer, colorectal adenomatous lesion, asthma or wheezing, and wheezing as an isolated symptom.

The beneficial effects of folate on the aforementioned health outcomes might be explained by a number of plausible mechanisms. First, folate is the cofactor for methionine synthase,
which catalyzes the conversion of homocysteine, and folate levels are therefore inversely associated with homocysteine levels (119, 120). Hyperhomocysteinemia has been found to be associated with higher risks of some birth/pregnancy outcomes (121–123), cancers (124–126), CVD (127), and neurological conditions (128, 129). Second, the polymorphisms of 5,10-methylenetetrahydrofolate reductase, which are critical junctions in the folate-metabolizing pathway via their role of guiding folate metabolites to the DNA methylation pathway and away from the DNA synthesis pathway, may modulate the susceptibility of subjects to several birth/pregnancy outcomes (130–132), cancers (133, 134), CVD (135), and neurological conditions (136).

The evidence on the association of folate with the all-cause mortality rate in the general population remains controversial. Several studies have demonstrated that folate supplementation could reduce the risk of CVD-related death, which might be attributable to serum homocysteine reduction (137). In contrast, Ebbing et al. reported that folate treatment was associated with increased risks of cancer outcomes and all-cause mortality in patients with ischemic heart disease (138). Excess folic acid intake may stimulate the growth of established neoplasms in experimental animals (139). As such, establishing the appropriate range of folate dosage might be crucial to balance the benefits against the risks and allow us to more accurately study the associations of folate with all-cause or cause-related mortality.

Other than the reasons mentioned above, the associations between folate and cancers may also be explained by two further mechanisms: (1) folate deficiency may induce complete transformation of deoxyuridylate monophosphate to deoxynucleosidyl monophosphate, which induces mis-incorporation of uracil into DNA and leads to chromosomal breaks and mutations (140, 141); and/or (2) folate deficiency may cause abnormal methylation of DNA, leading to alterations in expression of critical protooncogenes and tumor suppressor genes (142, 143). Experiments in vivo on mice and dogs have suggested that increased folate intake altered DNA methylation and in turn reduced the risks of cancers (144, 145).

Studies have suggested that folate can also prevent and reverse endothelial dysfunction (146, 147), which is an important risk factor for CVD (148, 149). Folate may improve the bioavailability of nitric oxide (NO) by increasing endothelial NO synthase coupling and NO production and by directly scavenging superoxide radicals (150, 151). By enhancing NO bioavailability, folate may improve endothelial function, thereby preventing or reversing the progression of CVD (147).

In contrast to its many beneficial effects, we observed adverse effects of folate on prostate cancer, colorectal adenomatous lesion, asthma or wheezing, and wheezing as an isolated symptom. For the adverse effect of folate on increased risk of prostate cancer/asthma, we found that the significant associations were both driven by one individual study. And the removal of these two influential studies from the respective meta-analysis resulted in non-significant results. However, the assessment of New Castle–Ottawa scale suggested that both of these two studies were with low risks of bias (i.e., scored 7–9 out of 10, data not shown). We thus speculate that the inconsistent findings across the included studies may be ascribed to the heterogeneity of population and study design. Further meta-analyses with larger sample size are warranted to verify these associations. For the association between folate and increased risk of colorectal adenomatous lesion, the most likely explanation is that undiscovered early precursor lesions might have existed in the mucosa of these patients, and folate could have accelerated the proliferation and growth of these paraneoplastic lesions.

We found high-quality evidence that folate supplementation was associated with a lower risk of several birth/pregnancy outcomes (neural tube defects, megaloblastic anemia, elective termination of pregnancy for fetal anomalies, small for gestational age, non-syndromic cleft lip with or without cleft palate, gestational hypertension/preeclampsia, and low pre-delivery serum folate), decreased scores on the Hamilton Depression Rating Scale (in a population with depressive disorder) and levels of plasma homocysteine, and increased serum/plasma folate. Although the meta-analyses of these outcomes might still be subject to potential biases, such as those without a preregistered protocol and the presence of high heterogeneity (for outcomes of small for gestational age and serum/plasma folate), our results are encouraging enough to verify the recommendation that women of child-bearing age should take folate supplementation to prevent adverse birth/pregnancy outcomes.

We found moderate-quality evidence from meta-analyses of observational studies that serum folate was associated with a higher risk of prostate cancer, which is consistent with the high-quality evidence from meta-analyses of RCT that folate supplementation was associated with increased risk of prostate cancer. The potential mechanism of folate in the development of this cancer is unclear. In vitro models using human prostate tissue have shown enhanced proliferation of tumor cells under conditions of elevated folate concentrations (152). Elsewhere, mice with transgenic adenoma of mouse prostate (TRAMP) that were fed a folate-depleted diet had lower cellular proliferation than mice with TRAMP fed a normal or high-folate diet (153).

In this umbrella review, the specific trends of relationships between folate and increased risks of neurocognitive disorders (such as cognitive impairment, Alzheimer disease, and depression) were also observed. The proposed mechanisms through which folate affects these diseases include suppression of DNA methylation and reduction of tetrahydrobiopterin levels, hyperhomocysteinemia, and excessive mis-incorporation of uracil into DNA (154). In contrast to the evidence that folate supplementation reduced the risk of stroke, the effect of folate on improving cognitive function or slowing cognitive decline in healthy or cognitively impaired older individuals was inconclusive (155). Prospective studies are strongly warranted to cover this knowledge gap.

**Strengths and Limitations**

This umbrella review has several strengths. First, we are the first to summarize the evidence for the associations between folate intake/levels and a wide range of health-related outcomes by incorporating information from published
REFERENCES

1. Plumpire I, Mash SP, Ly A, Aufreiter S, Sohn KJ, Croxford R, et al. High concentrations of folate and unmetabolized folic acid in a cohort of pregnant Canadian women and umbilical cord blood. Am J Clin Nutr. (2015) 102:848–57. doi: 10.3945/ajcn.115.110783

2. Aromataris E, Fernandez R, Godfrey CM, Holly C, Khalil H, Tungpunkom P. Summarizing systematic reviews: methodological development, conduct and reporting of an umbrella review approach. Int J Evid Based Healthc. (2015) 13:132–40. doi: 10.1097/XER.0000000000000055

3. Kalliala I, Markozannes G, Gunter MJ, Paraskevaidis E, Gabra H, Mitra A, et al. Obesity and gynaecological and obstetric conditions: umbrella review of the literature. BMJ. (2017) 359:j4511. doi: 10.1136/bmj.j4511

4. Jackson D, Bowden J. A re-evaluation of the ‘quantile approximation method’ for random effects meta-analysis. Stat Med. (2009) 28:338–48. doi: 10.1002/sim.3487

5. Higgins JP. Commentary: heterogeneity in meta-analysis should be expected and appropriately quantified. Int J Epidemiol. (2008) 37:1158-60. doi: 10.1093/ije/dyn204

6. Ioannidis JP, Trikalinos TA. An exploratory test for an excess of significant findings. Clin Trials. (2007) 4:245–53. doi: 10.1177/1740774507079441

7. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. (1997) 315:629–34. doi: 10.1136/bmj.315.7109.629

8. Shea BJ, Reeves BC, Wells G, Thuku M, Hamed C, Moran J, et al. AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. BMJ. (2017) 358:j4008. doi: 10.1136/bmj.j4008

9. Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, et al. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. J Clin Epidemiol. (2011) 64:383–94. doi: 10.1016/j.jclinepi.2010.04.026

10. Bailshem H, Helfand M, Schunemann HJ, Oxman AD, Kunz R, Brozek J, et al. GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol. (2011) 64:401-6. doi: 10.1016/j.jclinepi.2010.07.015

11. Badovinac RL, Werler MM, Williams PL, Kelsey KT, Hayes C. Folic acid-containing supplement consumption during pregnancy and risk for oral clefts: a meta-analysis. Birth Defects Res A Clin Mol Teratol. (2007) 79:8–15. doi: 10.1002/bdra.20315

12. Bailie L, Loughrey MB, Coleman HG. Lifestyle risk factors for serrated colorectal polyps: a systematic review and meta-analysis. Gastroenterology. (2017) 152:92–104. doi: 10.1053/j.gastro.2016.09.003

13. Blencowe H, Cousens S, Modell B, Lawn J. Folic acid to reduce neonatal mortality from neural tube disorders. Int J Epidemiol. (2010) 39(Suppl. 1):i110–21. doi: 10.1093/ije/dyp028

14. Burr NE, Hull MA, Subramanian V. Folic acid supplementation may reduce colorectal cancer risk in patients with inflammatory bowel disease: a systematic review and meta-analysis. J Clin Gastroenterol. (2017) 51:247–53. doi: 10.1097/MCG.0000000000000498

15. Chen P, Li C, Li X, Li J, Chu R, Wang H. Higher dietary folate intake reduces the breast cancer risk: a systematic review and meta-analysis. Br J Cancer. (2014) 110:2327–38. doi: 10.1038/bjc.2014.155

meta-analyses of observational studies or RCTs. Second, we used systematic methods that included a robust search strategy of three scientific literature databases and independent study selection and extraction by two investigators. When possible, we repeated each meta-analysis with a standardized approach that included the use of random-effects analysis and produced measures of heterogeneity and publication bias to allow better comparison across outcomes. We also used standard approaches to assess the quality of methods (AMSTAR 2) and the quality of evidence (GRADE) of the included meta-analyses.

Our study should also be interpreted cautiously with several limitations. First, the credibility assessment method was based on established tools for observational evidence, which are susceptible to bias and uncertainty. Another limitation of the umbrella review approach is the use of existing meta-analyses. Meta-analyses are known to have important limitations, such as limited coverage of the literature search, quality of included studies, and selective outcome reporting.

CONCLUSIONS

Our umbrella review found high- and moderate-quality evidence for the effect of folate on health outcomes such as mortality, cancers, CVD, and metabolic-related outcomes, as well as several birth outcomes. Therefore, our results support the current recommendation of daily folate supplementation for preventing adverse birth/pregnancy outcomes, cardiovascular and metabolic disease, and other disease. Further RCTs with large sample sizes are warranted to confirm these observed findings and to study the concentration–response relationships between folate exposure and health outcomes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

ZY was the project lead for the current study. YBo and YZ searched databases and screened the articles. YBo and YT extracted the data. YBo, XL, and YZ conducted statistical analysis. YBo wrote the manuscript. DZ, YBu, ZW, LW, and ZY reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the National Natural Science Foundation of China No. 21577119. The funder was not involved in the design of the study and collection, analysis and interpretation of data, and in writing the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2020.550753/full#supplementary-material
16. Chuang SC, Rota M, Gunter MJ, Zeleniuch-Jacquety A, Eussen SJ, Vollset SE, et al. Quantifying the dose-response relationship between circulating folate concentrations and colorectal cancer in cohort studies: a meta-analysis based on a flexible meta-regression model. *Am J Epidemiol.* (2013) 178:1028–39. doi: 10.1093/aje/kwt083

17. Collin SM, Metcalfe C, Refsum H, Lewis SJ, Zuccolo L, Smith GD, et al. Circulating folate, vitamin B12, homocysteine, vitamin B12 transport proteins, and risk of prostate cancer: a case-control study, systematic review, and meta-analysis. *Cancer Epidemiol Biomarkers Prev.* (2010) 19:1632–42. doi: 10.1158/1055-9966.EPI-10-0180

18. Crider KS, Kordower JM, Qi YP, Mulinare J, Dowling NF, Berry RJ. Prenatal folate and risk of asthma in children: a systematic review and meta-analysis. *Am J Clin Nutr.* (2009) 98:1272–81. doi: 10.3945/ajcn.105.065623

19. Dai WM, Yang B, Choi YH, Wang YQ, Zhao M, Chen L, et al. Association between folate intake, serum folate levels and the risk of lung cancer: a systematic review and meta-analysis. *Clin Med J.* (2013) 126:957–64. doi: 10.3760/cmj.issn.0366-6999.20130391

20. Dean SV, Lassi ZS, Iman AM, Bhutta ZA. Preconception care: nutritional risks and interventions. *Reprod Health.* (2014) 11(Suppl. 3):S3. doi: 10.1186/1742-4755-11-S3-S3

21. Du L, Wang Y, Zhang H, Zhang H, Gao Y. Folate intake and the risk of endometrial cancer: a meta-analysis. *Oncotarget.* (2016) 7:85176–84. doi: 10.18632/oncotarget.13211

22. Fan C, Yu S, Zhang S, Ding X, Su J, Cheng Z. Association between folate intake and risk of head and neck squamous cell carcinoma: an overall and dose-response PRISMA meta-analysis. *Medicine.* (2017) 96:e8182. doi: 10.1097/MD.0000000000001882

23. Feng Y, Yang S, Chen R, Tong X, Wu Z, Mo X. Maternal folate acid supplementation and the risk of congenital heart defects in offspring: a meta-analysis of epidemiological observational studies. *Sci Rep.* (2015) 5:8506. doi: 10.1038/srep08506

24. Gibbsey S, Lightfoot T, Sheldon T. Is low folate a risk factor for depression? A meta-analysis and exploration of heterogeneity. *J Epidemiol Commun Health.* (2006) 60:93–100. doi: 10.1136/jech.2006.050385

25. He H, Shui B. Folate intake and risk of bladder cancer: a meta-analysis of epidemiological studies. *Int J Food Sci Nutr.* (2014) 65:286–92. doi: 10.3109/09637486.2013.86641

26. Heine-Brockhoff RC, Winkels RM, Renkema JM, Kraag L, van Orsten-Luiten AC, Tigchelaar EF, et al. Dietary supplement use and colorectal cancer risk: a systematic review and meta-analyses of prospective cohort studies. *Int J Cancer.* (2015) 136:2388–401. doi: 10.1002/ijc.29277

27. Hoddgates VA, Morris RK, Francis A, Gardosi J, Ismail KM. Effectiveness of folic acid supplementation in pregnancy on reducing the risk of small-for-gestational age neonates: a population study, systematic review and meta-analysis. *BJOG.* (2015) 122:478–90. doi: 10.1111/1471-0528.13202

28. Hua X, Zhang J, Guo Y, Shen M, Gaudet L, Janoudi G, et al. Effect of folic acid supplementation during pregnancy on gestational hypertension/preeclampsia: a systematic review and meta-analysis. *Hypertens Pregnancy.* (2016) 35:447–60. doi: 10.1080/10641955.2016.1183673

29. Imdad A, Yakooob MY, Bhutta ZA. The effect of folic acid, protein energy and multiple micronutrient supplements in pregnancy on stillbirths. *BMJ Public Health.* (2011) 11(Suppl. 3):S4. doi: 10.1136/1471-2588-11-S3-S4

30. Johnson CY, Little J. Folate intake markers of folate status and oral clefts: is the evidence converging? *Int J Epidemiol.* (2008) 37:1041–58. doi: 10.1093/ije/dyn998

31. Kennedy DA, Stern SJ, Moretti M, Matok I, Sarkar M, Nickel C, et al. Folate intake and the risk of colorectal cancer: a systematic review and meta-analysis. *Cancer Epidemiol.* (2011) 35:2–10. doi: 10.1016/j.canep.2010.11.004

32. Kim DH, Smith-Warner SA, Spiegelman D, Yaun SS, Colditz GA, Freudenberg JI, et al. Pooled analyses of 13 prospective cohort studies on folate intake and colon cancer. *Cancer Causes Control.* (2010) 21:1919–30. doi: 10.1007/s10552-010-9620-8

33. Larsson SC, Giovannucci E, Wolk A. Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis. *Gastroenterology.* (2006) 131:1271–83. doi: 10.1053/j.gastro.2006.08.010

34. Larsson SC, Giovannucci E, Wolk A. Folate and risk of breast cancer: a meta-analysis. *JNCI.* (2007) 99:64–76. doi: 10.1093/jnci/djk006

35. Lewis SJ, Harbord RM, Harris R, Smith GD. Meta-analyses of observational and genetic association studies of folate intakes or levels and breast cancer risk. *J Nutr Cancer.* (2006) 56:1607–22. doi: 10.1007/s12027-006-9127-3

36. Mao B, Li Y, Zhang Z, Chen C, Ding C, et al. One-carbon metabolic factors and risk of renal cell cancer: a meta-analysis. *PLoS ONE.* (2015) 10:e0141762. doi: 10.1371/journal.pone.0141762

37. Michelakos T, Kousoulis AA, Katsiaris K, Dessypris N, Anastasiou A, Katsiaris KA, et al. Serum folate and B12 levels in association with cognitive impairment among seniors: results from the VELESTINO study in Greece and meta-analysis. *J Aging Health.* (2015) 23:589–616. doi: 10.1177/08982643134834288

38. Millacqua N, Pardo R, Cifuentes L, Suzao J. Effects of folic acid fortification on orofacial clefts prevalence: a meta-analysis. *Public Health Nutr.* (2017) 20:2260–8. doi: 10.1017/S1368946217000878

39. Milne E, Royle JA, Miller M, Bower C, de Klerk NH, Bailey HD, et al. Maternal folate and other vitamin supplementation during pregnancy and risk of acute lymphoblastic leukaemia in the offspring. *Int J Cancer.* (2010) 126:2690–9. doi: 10.1002/ijc.24969

40. Moaazenn S, Dolatkhah R, Tabrizi JS, Shaarabi J, Alizadeh BZ, de Bock GH, et al. Folic acid intake and folate status and colorectal cancer risk: a systematic review and meta-analysis. *Clin Nutr.* (2017) 36(6 Pt A):1926–34. doi: 10.1016/j.clnu.2017.10.010

41. Myers SK, Ju W, Kim SC, Kim H. Vitamin or antioxidant intake (or serum level) and risk of cervical neoplasm: a meta-analysis. *Biol J.* (2011) 118:1285–91. doi: 10.1111/j.1471-0528.2011.03032.x

42. Park YM, Youn J, Cho CH, Kim SH, Lee JE. Circulating folate levels and colorectal adenoma: a case-control study and a meta-analysis. *Nutr Res Pract.* (2017) 11:419–29. doi: 10.4162/nrp.2017.11.5.419

43. Petridou E, Kousoulis AA, Michalakos T, Papanicolaou P, Dessypris N, Papadopoulos FC, et al. Folate intake and intake and risk and breast cancer: a meta-analysis and dose-response meta-analysis. *Cancer Prev. Res.* (2017) 10:e0141762. doi: 10.11737/journal.pone.0141762

44. Price AJ, Travis RC, Appleby PN, Albanes D, Barriecate Gurrea A, Bjoerge T, et al. Circulating folate and vitamin B12 and risk of prostate cancer: a collaborative analysis of individual participant data from six cohorts including 6875 cases and 8104 controls. *Eur Urol.* 79:941–51. doi: 10.1016/j.euro.2016.05.024

45. Sanjoaquin MA, Allen N, Coutu E, Roddam AW, Key TJ. Folate intake and colorectal cancer risk: a meta-analytical approach. *Int J Cancer.* (2005) 113:825–8. doi: 10.1002/ijc.20648
53. Shen L, Ji HF. Associations between homocysteine, folic acid, Vitamin B12 and Alzheimer’s disease: insights from meta-analyses. J Alzheimers Dis. (2015) 46:777–90. doi: 10.3233/JAD-150140

54. Tio M, Andrici J, Cox MR, Eslick GD. Folate intake and the risk of upper gastrointestinal cancers: a systematic review and meta-analysis. J Gastroenterol Hepatol. (2014) 29:250–8. doi: 10.1111/jgh.12446

55. Tio M, Andrici J, Cox MR, Eslick GD. Folate intake and the risk of prostate cancer: a systematic review and meta-analysis. Prostate Cancer Prostatic Dis. (2014) 17:213–9. doi: 10.1039/p41416c00391z

56. Tio M, Andrici J, Eslick GD. Folate intake and the risk of prostate cancer: a systematic review and meta-analysis. Breast Cancer Res Treat. (2014) 145:513–24. doi: 10.1007/s10549-014-2969-8

57. Tio M, Andrici J, Cox MR, Eslick GD. Folate intake and the risk of prostate cancer: a meta-analysis of prospective studies. J Clin Nutr. (2017) 8:51. doi: 10.1161/jcn.12329-017-0170-8

58. Wang R, Zheng Y, Huang JY, Zhang AQ, Zhou YH, Wang JN. Folate intake, serum folate levels, and prostate cancer risk: a meta-analysis of prospective studies. BMC Public Health. (2014) 14:1326. doi: 10.1186/1471-2458-14-1326

59. Wang T, Zhang HP, Zhang X, Liang ZA, Ji YL, Wang G. Is folate status a risk factor for asthma or other allergic diseases? Allergy Asthma Immunol Res. (2014) 6:261–8. doi: 10.4168/aai.2014.6.4.261

60. Wang ZM, Zhou B, Nie ZL, Gao W, Wang YS, Zhao H, et al. Folate and risk of coronary heart disease: a meta-analysis of prospective studies. Nutr Metab Cardiovasc Dis. (2012) 22:890–9. doi: 10.1016/j.numecd.2011.04.011

61. Wien TN, Pike E, Wisloff T, Staff A, Smeland S, Klempt M. Cancer risk with folic acid supplements: a systematic review and meta-analysis. BMJ Open. (2012) 2:e000653. doi: 10.1136/bmjopen-2011-000653

62. Xu A, Cao X, Lu Y, Li H, Zhu Q, Chen X, et al. A meta-analysis of the relationship between maternal folic acid supplementation and the risk of congenital heart defects. Int Heart J. (2016) 57:725–728. doi: 10.1536/ihj.16-054

63. Yang X, Chen H, Du Y, Wang W, Wang Z. Periconceptional folic acid fortification for the risk of gestational hypertension and pre-eclampsia: a meta-analysis of prospective studies. Matern Child Nutr. (2012) 16:669–79. doi: 10.1111/j.1233-7229.101209

64. Zhang Q, Wang Y, Xin X, Zhang Y, Liu D, Peng Z, et al. Effect of folic acid supplementation on preterm delivery and small for gestational age births: a systematic review and meta-analysis. Reprod Toxicol. (2017) 67:35–41. doi: 10.1016/j.reprotox.2016.11.012

65. Zhang YE, Shi WW, Gao HF, Zhou L, Hou AJ, Zhou YH. Folate intake and the risk of breast cancer: a dose-response meta-analysis of prospective studies. PLoS ONE. (2014) 9:e100044. doi: 10.1371/journal.pone.0100044

66. Zhang YF, Zhou L, Zhang HW, Hou AJ, Gao HF, Zhou YH. Association between folate intake and the risk of lung cancer: a dose-response meta-analysis of prospective studies. PLoS ONE. (2014) 9:e93465. doi: 10.1371/journal.pone.0093465

67. Zhao Y, Guo C, Hu H, Zheng L, Ma J, Jiang L, et al. Folate intake, serum folate levels and esophageal cancer risk: an overall and dose-response meta-analysis. Oncotarget. (2017) 8:10458–69. doi: 10.18632/oncotarget.14432

68. Zhou X, Meng Y. Association between serum folate level and cervical cancer: a meta-analysis. Arch Gynecol Obstet. (2016) 293:871–7. doi: 10.1007/s00404-015-3852-5

69. Yi N, Du J, Yin X, Lu M. Folate intake, serum folate, and risk of esophageal cancer: a systematic review and dose-response meta-analysis. Eur J Cancer Prev. (2018) 27:173–80. doi: 10.1097/CEJ.0000000000000441

70. Jahanbin A, Shadkam E, Miri HH, Shirazi AS, Abbasi M. Maternal folic acid supplementation and the risk of oral clefts in offspring. J Craniofac Surg. (2018) 29:e534–41. doi: 10.1097/SCS.0000000000004888

71. Yang L, Jiang L, Bi M, Jia X, Wang Y, He C, et al. High dose of maternal folic acid supplementation is associated to infant asthma. Food Chem Toxicol. (2015) 75:88–93. doi: 10.1016/j.fct.2014.11.006

72. Homocysteine Lowering Trials’ Collaboration. Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. Am J Clin Nutr. (2005) 82:806–12. doi: 10.1093/ajcn/82.4.806

73. Miller ER, 3rd, Jurusich S, Pastor-Barriuso R, Bazzano LA, Appel LJ, Guallar E. Meta-analysis of folic acid supplementation trials on risk of cardiovascular disease and risk interaction with baseline homocysteine levels. Am J Cardiol. (2010) 106:517–27. doi: 10.1016/j.amjcard.2010.03.064
109. Wald DS, Kasturiratne A, Simmonds M. Effect of folic acid, with or without other B vitamins, on cognitive decline: meta-analysis of randomized controlled trials. *Am J Med.* (2010) 123:522–7.e2. doi: 10.1016/j.amjmed.2010.01.017

110. Wang X, Qin X, Demirtas H, Li J, Mao G, Hsuo Y, et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet.* (2007) 369:876–82. doi: 10.1016/S0140-6736(07)60854-X

111. Yang HT, Lee M, Hong KS, Orsiagbele B, Saver JL. Efficacy of folic acid supplementation in cardiovascular disease prevention: an updated meta-analysis of randomized controlled trials. *Eur J Intern Med.* (2012) 23:745–54. doi: 10.1016/j.ejim.2012.07.004

112. Yi X, Zhou Y, Jiang D, Li X, Guo Y, Jiang X. Efficacy of folic acid supplementation on endothelial function and plasma homocysteine concentration in coronary artery disease: a meta-analysis of randomized controlled trials. *Exp Ther Med.* (2014) 7:1100–10. doi: 10.3892/etm.2014.1553

113. Zhao M, Wu G, Li Y, Wang X, Hou FF, Xu X, et al. Meta-analysis of folic acid efficacy trials in stroke prevention: insight into effect modifiers. *Neurology.* (2017) 88:1830–8. doi: 10.1212/WNL.000000000003909

114. Zhou YH, Tang JY, Wu MJ, Lu J, Wei X, Qin YN, et al. Effect of folic acid supplementation on cardiovascular outcomes: a systematic review and meta-analysis. *PloS ONE.* (2011) 6:e25142. doi: 10.1371/journal.pone.0025142

115. Roberts E, Carter B, Young AH. Caveat emptor: folic in unipolar depressive illness, a systematic review and meta-analysis. *J Psychopharmacol.* (2018) 32:377–384. doi: 10.1177/0269881117756060

116. Akbari M, Tabrizi R, Lankarani KB, Heydari ST, Karamali M, Khashanian M, et al. The effects of folic acid supplementation on diabetes biomarkers among patients with metabolic diseases: a systematic review and meta-analysis of randomized controlled trials. *Horm Metab Res.* (2018) 50:93–105. doi: 10.1055/s-0043-125148

117. Zhao JV, Schooling CM, Zhao JX. The effects of folic acid supplementation on glucose metabolism and risk of type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials. *Ann Epidemiol.* (2018) 28:249–57.e1. doi: 10.1016/j.annepidem.2018.02.001

118. Hsu CY, Chiu SW, Hong KS, Saver JL, Wu YL, Lee JE, et al. Folic acid in stroke prevention in countries without mandatory folic acid food fortification: a meta-analysis of randomized controlled trials. *J Stroke.* (2018) 20:99–109. doi: 10.5853/jos.2017.01522

119. Javadi L, Pourghassem Gargari B, Salekzamani S, Yousefzadeh R. Folate and homocysteine levels and their association with dietary intakes in Iranian patients infected with *Helicobacter pylori*: a case-control study. *Acta Med Iran.* (2015) 53:162–7.

120. Serapinas D, Breikiekie A, Bartkeviciute A, Bandzveiciene R, Silkunas M, Bartkeviciene D. The importance of folate, vitamins B6 and B12 for the lowering of homocysteine concentrations for patients with recurrent pregnancy loss and MTHFR mutations. *Reprod Toxicol.* (2017) 72:159–3. doi: 10.1016/j.reprotox.2017.07.001

121. Limpach A, Dalton M, Riles G, Gadson P. Homocysteine inhibits retinoic acid synthesis: a mechanism for homocysteine-induced congenital defects. *Exp Cell Res.* (2000) 260:166–74. doi: 10.1006/excr.2000.5000

122. Smedts HP, van Uitert EM, Valkenburg O, Laven JS, Eijkemans MJ, et al. The effects of folate supplementation on pregnancy outcomes in survivors of colorectal adenomas and cancer: a systematic review of randomised controlled trials. *Am J Clin Nutr.* (2016) 7:505–15. doi: 10.3945/ajcn.2016.012342

123. Chiang FF, Wang HM, Lan YC, Yang MH, Huang SC, Huang YC. High homocysteine is associated with increased risk of colorectal cancer independently of oxidative stress and antioxidant capacities. *Clin Nutr.* (2014) 33:1054–60. doi: 10.1016/j.clnu.2013.11.007

124. Lin J, Lee IM, Song Y, Cook NR, Selhub J, Manson JE, et al. A demonstration of the maternal lipid profile is associated with an elevated risk of congenital heart disease in the offspring. *Nutr Metab Cardiovasc Dis.* (2012) 22:477–85. doi: 10.1016/j.numecd.2010.07.016

125. Gaiday AN, Tussupkaliyev AB, Bermagambetova SK, Zhumagulova SS, Sarsembayeva LK, Dossimbetova MB, et al. Effect of homocystine on glucose metabolism and risk of type 2 diabetes: systematic review and meta-analysis of randomized controlled trials. *J Psychopharmacol.* (2016) 30:27–39. doi: 10.1177/0269881115619303

126. Vollset SE, Clarke R, Lewinton S, Ebbing M, Halsey J, Lonn E, et al. Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: meta-analyses of data on 50,000 individuals. *Lancet.* (2013) 381:1029–36. doi: 10.1016/S0140-6736(12)62001-7

127. van Dijk M, Pot GK. The effects of nutritional interventions on recurrence in survivors of colorectal adenomas and cancer: a systematic review of randomised controlled trials. *Eur J Cancer Prev.* (2016) 25:566–73. doi: 10.1038/ejcp.2015.210

128. Taylor MJ, Carney SM, Goodwin GM, Geddes JR. Folate for depressive disorders: systematic review and meta-analysis of randomized controlled trials. *J Psychopharmacol.* (2004) 18:251–6. doi: 10.1097/01.jpp.000009426030

129. Tian T, Yang QK, Cui JQ, Zhou LL, Zhou XL. Folic acid supplementation for stroke prevention in patients with cardiovascular disease. *Am J Med Sci.* (2017) 354:379–87. doi: 10.1097/AMJ.0000000000000593

130. van Dijk M, Pot GK. The effects of nutritional interventions on recurrence in survivors of colorectal adenomas and cancer: a systematic review of randomised controlled trials. *Eur J Cancer Prev.* (2016) 25:566–73. doi: 10.1038/ejcp.2015.210
127. Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. *Nutr J.* (2015) 14:6. doi: 10.1186/1475-2891-14-6

128. Clarke R, Bennett D, Parish S, Lewington S, Skeaff M, Eussen S, et al. Effects of homocysteine lowering with B vitamins on cognitive aging: meta-analysis of 11 trials with cognitive data on 22,000 individuals. *Am J Clin Nutr.* (2014) 100:657–66. doi: 10.3945/ajcn.113.076349

129. Wu H, Zhu P, Geng X, Liu Z, Cui L, Gao Z, et al. Genetic polymorphism of MTHFR C677T with preterm birth and low birth weight susceptibility: a meta-analysis. *Arch Gynecol Obstet.* (2017) 295:1105–18. doi: 10.1007/s00404-017-4322-2

130. Yin M, Dong L, Zheng J, Zhang H, Liu J, Xu Z. Meta-analysis of the association between MTHFR polymorphism and the risk of congenital heart defects. *Ann Hum Genet.* (2012) 76:9–16. doi: 10.1111/j.1469-1809.2011.00687.x

131. Bo et al. Folate and Health Outcomes. *JAMA.* (2009) 302:2119–26. doi: 10.1001/jama.2009.1622

132. Clarke R, Bennett D, Parish S, Lewington S, Skeaff M, Eussen S, et al. Effects of homocysteine lowering with B vitamins on cognitive aging: meta-analysis of 11 trials with cognitive data on 22,000 individuals. *Am J Clin Nutr.* (2014) 100:657–66. doi: 10.3945/ajcn.113.076349

133. Yi K, Yang L, Lan Z, Xi M. The association between MTHFR polymorphism and Alzheimer disease: evidence for a causal link from mendelian randomization. *Alzheimers Dis.* (2016) 52:747–56. doi: 10.3233/JAD-150977

134. Hu Q, Teng W, Li J, Hao F, Wang N. Homocysteine and Alzheimer’s disease: role of folate in cognitive decline and Alzheimer’s disease risk: a meta-analysis. *Mol Neurobiol* (2017) 54:1173–86. doi: 10.1007/s12035-016-9722-8

135. Sonawane K, Zhu Y, Chan W, Aguilar D, Deshmukh AA, Suarez-Almazor ME. Association between maternal methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphism and birth defects and adverse pregnancy outcomes. *Prenat Diag.* (2018) 39:3–9. doi: 10.1002/pd.5396

136. Liu W, Li Y, Li R, Han X, Ma Y, Liu B, et al. Association of MTHFR A1298C polymorphism with breast cancer and/or ovarian cancer risk: a system review and meta-analysis. *Arch Gynecol Obstet.* (2016) 294:579–88. doi: 10.1007/s00404-016-4037-6

137. Konukoglu D, Uzon H. Endothelial dysfunction and hypertension. *Adv Exp Med Biol.* (2017) 956:511–40. doi: 10.1007/5584_2016_90

138. Staniewicz AE, Kenney WL. Role of folate in nitric oxide bioavailability and vascular endothelial function. *Nutr Rev.* (2017) 75:61–70. doi: 10.1093/nutrit/nux053

139. Ebbing M, Bønaa KH, Nygård O, Arnesen E, Ueland PM, Nordrehaug JE, et al. Cancer incidence and mortality after treatment with folic acid and vitamin B12. *British J Nutr.* (2011) 105:1097–1106. doi: 10.1017/S0007114511002101

140. Staniewicz AE, Alexander LM, Kenney WL. Folic acid supplementation improves microvascular function in older adults through nitric oxide-dependent mechanisms. *Clin Sci.* (2015) 129:159–67. doi: 10.1042/CS20140821

141. Petersen LF, Brockton NT, Bakkar A, Liu S, Wen J, Weljie AM, et al. Elevated physiological levels of folic acid can increase in vitro growth and invasiveness of prostate cancer cells. *BJU Int.* (2012) 109:788–95. doi: 10.1111/j.1464-410X.2011.10437.x

142. Dhiman VK, et al. Dietary folate deficiency blocks prostate cancer progression in the TRAMP model. *Cancer Prev Res.* (2011) 4:1825–34. doi: 10.1158/1940-6207.CAPR-11-0140

143. Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. *J Nutr.* (2000) 130:129–32. doi: 10.1093/jn/130.2.129

144. Xiao SD, Meng XJ, Shi Y, Hu YB, Zhu SS, Wang CW. Interventional study of high dose folic acid in gastric carcinogenesis in beagles. *Gui.* (2002) 50:61–4. doi: 10.1136/gut.50.1.61

145. Moat SJ, Lang D, McDowell IF, Clarke ZL, Madhavan AK, Lewis MJ, et al. Folic acid increases global DNA methylation and reduces inflammation to prevent Helicobacter-associated gastric cancer in mice. *Gastroenterology.* (2012) 142:824–833.e7. doi: 10.1053/j.gastro.2011.12.058

146. Xiao SD, Meng XJ, Shi Y, Hu YB, Zhu SS, Wang CW. Interventional study of high dose folic acid in gastric carcinogenesis in beagles. *Gui.* (2002) 50:61–4. doi: 10.1136/gut.50.1.61

147. Staniewicz AE, Alexander LM, Kenney WL. Folic acid supplementation improves microvascular function in older adults through nitric oxide-dependent mechanisms. *Clin Sci.* (2015) 129:159–67. doi: 10.1042/CS20140821

148. Petersen LF, Brockton NT, Bakkar A, Liu S, Wen J, Weljie AM, et al. Elevated physiological levels of folic acid can increase in vitro growth and invasiveness of prostate cancer cells. *BJU Int.* (2012) 109:788–95. doi: 10.1111/j.1464-410X.2011.10437.x

149. Konukoglu D, Uzon H. Endothelial dysfunction and hypertension. *Adv Exp Med Biol.* (2017) 956:511–40. doi: 10.1007/5584_2016_90

150. Sun HJ, Hou B, Wang X, Zhu XX, Li KX, Qiu LY. Endothelial dysfunction and cardiometabolic diseases: role of long non-coding RNAs. *Life Sci.* (2016) 167:6–11. doi: 10.1016/j.lfs.2016.11.005

151. Staniewicz AE, Alexander LM, Kenney WL. Folic acid supplementation improves microvascular function in older adults through nitric oxide-dependent mechanisms. *Clin Sci.* (2015) 129:159–67. doi: 10.1042/CS20140821

152. Petersen LF, Brockton NT, Bakkar A, Liu S, Wen J, Weljie AM, et al. Elevated physiological levels of folic acid can increase in vitro growth and invasiveness of prostate cancer cells. *BJU Int.* (2012) 109:788–95. doi: 10.1111/j.1464-410X.2011.10437.x

153. Staniewicz AE, Alexander LM, Kenney WL. Folic acid supplementation improves microvascular function in older adults through nitric oxide-dependent mechanisms. *Clin Sci.* (2015) 129:159–67. doi: 10.1042/CS20140821

154. Vlachos GS, Scarmeas N. Dietary interventions in mild cognitive impairment and dementia. *Dialogues Clin Neurosci.* (2019) 21:69–82. doi: 10.31887/DNC.2019.21.1/nscarmeas

155. Araújo JR, Martel F, Borges N, Araújo JM, Keating E, Folates and aging: role in mild cognitive impairment, dementia and depression. *Ageing Res Rev.* (2015) 22:9–19. doi: 10.1016/j.arr.2015.04.005

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Bo, Zhu, Tao, Li, Zhai, Bu, Wan, Wang, Wang and Yu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.