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Systematic review of adverse health outcomes associated with high serum or red blood cell folate concentrations

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ABSTRACT

Background To examine the relationship between reported high serum or red blood cell (RBC) folate status and adverse health outcomes.

Methods We systematically searched PubMed/Medline and EMBASE (to May 2013), with no limits by study type, country or population, to identify studies reporting high folate concentrations in association with adverse health outcomes. Two reviewers screened studies and extracted data. Study quality was assessed.

Results We included 51 articles, representing 46 studies and 71 847 participants. Quantiles were used by 96% of studies to identify high folate concentrations. Eighty-three percent of serum folate and 50% of RBC folate studies reported a high folate cutoff that corresponded with a clinically normal concentration. Increasing values of reported high folate concentration did not demonstrate a consistent association with risk of adverse health outcomes. Overall, reported high folate concentrations appeared to be associated with a decreased risk of adverse health outcomes, though substantial methodological heterogeneity precluded complex analyses.

Conclusions Our interpretation was complicated by methodological variability. High folate cutoffs varied and often corresponded with normal or desirable blood concentrations. In general, a negative association appeared to exist between reported high folate status and adverse health outcomes. Consistent methods and definitions are needed to examine high folate status and ultimately inform public health interventions.

Keywords health, high folate status, red blood cell folate, serum folate, systematic review

Background

Folate is the generic term for both naturally occurring folates in food and folic acid. Folic acid is the most oxidized, monoglutamate form that is used in dietary supplements and fortified foods.[1] Consuming synthetic folic acid—from supplements and fortified food—is more effective in quickly elevating blood concentrations and tissue folate stores than intake of folate from natural sources.[2,3] There is a well-established link between folate status and the occurrence of neural tube defects (NTDs).[4,5] The Institute of Medicine guidelines recommend that women of childbearing age consume 0.4 mg/day of folic acid from fortified foods, supplements or both, in addition to natural food sources of folate.[3]

The neural tube is formed in the first 4 weeks post-conception, and since >50% of pregnancies are unplanned, the opportunity to reduce NTD risk may be limited for women who do not consume folic acid preconceptually.[6–8] Thus, >50 countries have reported policies for folic acid fortification of the food supply; however, regulations may not be implemented in all countries.[9–11] These policies have had a positive impact on NTD births. In Canada, for example, folic acid fortification was implemented in 1998, and there has

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since been a documented 46% reduction in the prevalence of NTDs.[12]

Increased blood folate concentrations have been documented in countries where the population is exposed to folic acid through fortification of the food supply and common usage of folic acid supplements.[13,14] For folate, the Tolerable Upper Intake Level (UL)—the highest intake of a nutrient thought to pose no adverse health effects in health individuals—is set at 1000 μg/day of synthetic folic acid.[3] This is based on the lowest observed adverse effect level (5000 μg/day), a dose that could potentially mask neurological symptoms of vitamin B12 deficiency and an uncertainty factor of 5, which accounts for the limited evidence and uncertainty in the process of defining a UL.[3] Though many positive associations have been made between safe doses of folic acid and health outcomes,[10] reports of a potential link between high synthetic folic acid intake and adverse health effects, such as an increased risk of cancer in those with pre-existing neoplasms, have resulted in significant media attention and controversy.[15–17] Further, the relationship between high folate concentrations in the blood, or high folate status, with adverse outcomes is poorly understood. Folate status research is challenged by a lack of consensus on a high folate cutoff[14] and a growing lack of confidence in the validity of folate measurement methods, in particular red blood cell (RBC) folate concentrations, which can vary depending on the assay method used, for example by 30–40% when measured by Bio-Rad radioassay versus microbiologic assay.[18–20] The aim of this research was to conduct a systematic review to examine the relationship between reported high serum or RBC folate status and adverse health outcomes.

Methods

This systematic review followed a protocol registered with PROSPERO CRD42013004622.

Study inclusion and exclusion criteria

We sought to identify studies examining the association between reported high folate concentrations in the blood and adverse health outcomes in humans. A broad range of adverse health outcomes—various cancers, cognitive issues, cardiovascular and offspring health outcomes—were chosen for inclusion *a priori* by the study authors (see Supplementary data, Table S1 for details). These adverse health outcomes represent those that, according to the literature, have a potential association with blood folate concentrations or folic acid intake.[17,21–27] For example, outcomes in the general population included neoplasms[17] (e.g. gastrointestinal, brain, breast), cardiovascular diseases[21] and cognition disorders.[22] To examine adverse outcomes in offspring, we included congenital abnormalities,[23–25] neoplasms[26] (e.g. central nervous system, trophoblastic) and diabetes mellitus.[26] We excluded studies that examined folic acid intake (as opposed to blood concentrations); did not report a specific high folate cutoff or did not refer to the concentration of folate being studied as high or elevated; were published in languages other than English; or were not primary studies.

Search strategy

We conducted a comprehensive search of the literature to identify relevant studies of high folate concentrations and adverse health outcomes. A search was performed in May 2013 using PubMed/Medline and EMBASE. The search strategy was conceptualized by C.K.C. with the assistance of an external consultant. No filters were applied to limit the retrieval by study type, publication years, country or population. The search was limited to English language and human research studies. In addition to the search of electronic databases, reference lists of relevant articles were reviewed and four key content experts were contacted and asked to identify the most influential papers examining the association between blood folate concentrations and adverse health outcomes. Previous reviews and meta-analyses were also used to identify articles. Upon completion of screening, a simplification of the MEDLINE search was validated as able to identify the included studies with a sensitivity of 0.92. The search was updated 18 August 2014 using the simplified Boolean search, limited to records created since April 2013. As well, PubMed related article searches were performed based on the three newest and three largest included studies for each of cancer, cognitive, offspring and child health and cardiovascular outcome. Search strategies for the original searches and update are presented in Supplementary data, Table S2.

Data extraction and analyses

Two reviewers screened all eligible titles and abstracts (C.K.C. and B.W.). Articles identified by either author were retrieved for full-text review. Articles were excluded when the exposure or outcome of interest was not included; folate status was measured inconsistently within the study (e.g. the participants were grouped either by radiobinding or microbiologic assay); high folate status was not stated or included in the analyses or the study did not include human subjects. Where required, folate concentration was converted to nmol/l from ng/ml using a conversion factor of 2.265. For each study, we extracted the following information: study year, country, study design, sample size, folate concentration characteristics (measurement method, fasting status, definition of high folate concentration) and effect size. The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach was used to

guide the review.[28–34] Quality of evidence for each study was assessed for risk of bias, indirectness of evidence, and inconsistency and imprecision of results. One reviewer (C.K.C.) assessed the quality of evidence for all studies. Meta-analyses were planned for data that were sufficiently homogeneous in terms of statistical, clinical and methodological characteristics using Review Manager Software 5.0 (The Cochrane Collaboration, Copenhagen, Denmark). Otherwise, narrative syntheses were conducted for remaining studies. *A priori* comparisons for subgroup analyses were planned as follows: by serum or RBC folate; by population subgroup (e.g. age, sex); by adverse health outcome and by study risk of bias assessment. Following the review, it was determined that serum and RBC folate concentrations should be examined separately by reported value for high folate status within assay method.

Results

After de-duplication, the preliminary search of electronic databases, reference lists and expert contributions identified 2994 potentially relevant records (Fig. 1). After a preliminary review of titles and abstracts, 120 were selected for a full-text review. Of these, 51 articles, representing 46 studies, met the inclusion criteria (50 observational studies [22 case–control; 28 cohort] and 1 RCT). Of the 51 studies included, a total of 13 studies utilized the same cohort: 8 cancer (4 from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention [ATBC] Study[35–38]; 2 from the Nurses Health Study[39,40]; and 2 from the European Prospective Investigation into Cancer and Nutrition [EPIC])[41,42]; 3 cardiovascular disease (Kuopio Ischaemic Heart Disease Risk Factor [KIHD])[43–45]; and the NHANES I Epidemiologic Follow-up Study [NEFUS] was used to examine cancer in one study and cardiovascular disease in another study.[46,47] All participants were included in the determination of the final sample size. Though cases were identified as distinct health outcomes in these studies, there may be some duplication in controls that could lead to the population being overestimated.

The final studies included 71 847 participants in four broad adverse outcome categories (cancer: 24 observational studies [$n = 23\,002$]; cognition: 1 RCT [$n = 369$] and 7 observational studies [$n = 12\,254$]; offspring and child health: 9 observational studies [$n = 20\,063$]; and cardiovascular and kidney disease: 10 observational studies [$n = 16\,159$]). Key characteristics of these studies can be found in Supplementary data, Tables S3–S6.

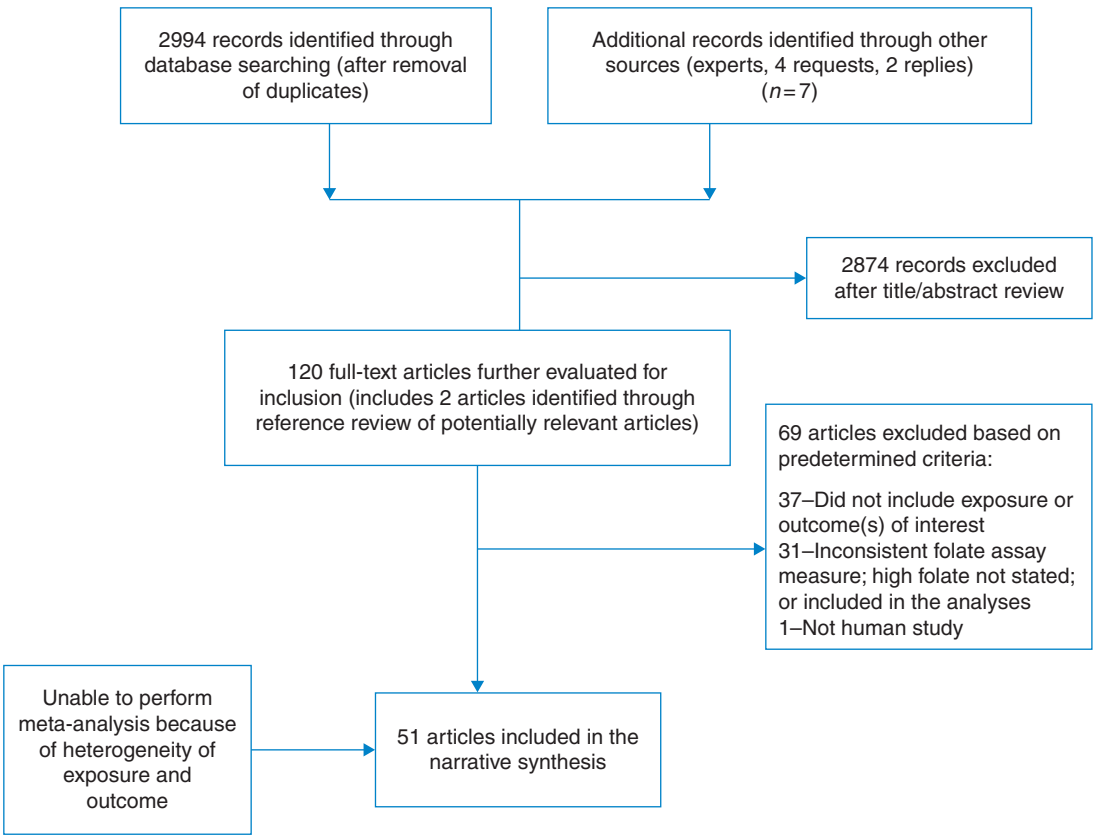

Fig. 1 Flowchart of study selection process.

The included studies reported results from 27 different countries. Seven of these studies involved population data from the USA following fortification of the food supply with folic acid. The largest study was a birth cohort involving 8742 pregnant women (the Netherlands),[48] while the smallest was a cohort, also from the Netherlands ($N = 77$).[49] The oldest study involved data collected in 1970–72 (Canada)[50] and the most recent study involved data from 2010–2012 (China).[51]

In the included articles, 9 studies used immunoassay,[48,51–58] 21 used radioimmunoassay,[22,35–40,43–45,59–69] 11 used microbiologic assay[41,42,47,50,70,71–76] and 3 used another method[77–79] to study serum folate. For RBC folate, one study used immunoassay,[49] four used radioimmunoassay[27,64,69,80] and four used microbiologic assay.[72,74,75,81] Ninety-one percent of studies—that examined serum folate—reported a high serum folate cutoff[35–48,50,52–68,71–74,76–79,82,83] that was within the range of values considered clinically normal, or possibly deficient, using macrocytic anemia as a metabolic indicator (7–45 nmol/l).[3] There is no established normal range for RBC folate, but 33% of the studies examining RBC folate reporting a high cutoff[64,74,80] concentrations were close to 305 nmol/l, the deficiency cutoff established by the Institute of Medicine.[3]

Considering the bias that may be introduced due to variation in folate measurement methods, including unknown fasting status and variability in the reported cutoff for high folate concentration, we examined significant findings by creating categories for serum and RBC folate within each adverse health outcome (Table 1). We then established four broad folate assay method groups: immunoassay, radioimmunoassay, microbiologic assay and other assay methods. Regardless of folate assay method, no consistent association was observed between increasing values of reported high folate concentration cutoffs and adverse health outcomes. For reported high serum folate, one study measured by immunoassay demonstrated an increased risk of prostate cancer[52] and another found an increased risk of atopic dermatitis in offspring.[48] Four studies, measuring high folate cutoff by immunoassay, demonstrated a decreased risk of cancer (colorectal,[53,54] breast,[56] esophageal squamous cell carcinoma[51]). Those measured by radioimmunoassay showed a decreased risk of cancer (pancreatic,[38] colorectal[39]); high-risk human papillomavirus,[60] cardiovascular disease[43–45]; allergy, atopy and wheeze[62]; respiratory tract infections at 6 months and atopic dermatitis at 24 months in the offspring[82]; NTD[63] in the offspring; and cognitive issues.[65] Increased risk of colorectal cancer[59] was observed in one study. At the highest cutoff reported (>59 nmol/l) in this group, an increased risk of cognitive impairment was observed in those with concurrently low vitamin B_{12} status.[22] Reported high serum folate, measured by microbiologic assay, showed a persistent decrease in adverse health outcome risk at all reported high folate cutoffs for lung cancer,[41] cardiovascular disease,[47,50] cognitive impairment[73] and cleft lip/palate in offspring,[75] though one study showed an increase in cardiovascular disease risk for those older than 55 years of age.[47] Other measurement methods for reported high serum folate cutoff showed a decreased risk of cognitive impairment,[78] prostate cancer[77] and anencephaly[79] in offspring of women with the methylenetetrahydrofolate reductase (MTHFR) TT genotype, though an increased risk in stroke risk was found in one of these studies.[78] There were five studies with significant results in the RBC folate category, three measured by radioimmunoassay,[27,64,80] one by microbiologic assay[81] and one by immunoassay.[49] The highest reported RBC folate cutoff (≥1853 nmol/l), which demonstrated a significant association, was measured by microbiologic assay and was positively associated with the risk of advanced hepatocellular carcinoma.[81] Another, measured by immunoassay, reported an association between high RBC folate (≥1813 nmol/l) and decreased embryonic size.[49] For those measured by radioimmunoassay, one demonstrated an increase in prevalence of insulin resistance and central adiposity at a cutoff of ≥1269 nmol/l,[27] while the other two reported high folate cutoffs (≥320 and ≥544 nmol/l) with a decrease in colorectal cancer[80] and NTD birth in those with high homocysteine,[64] respectively.

Meta-analyses and subgroup analyses were not possible for any outcomes due to substantial heterogeneity in the measurement methods for serum or RBC folate; population under study; definition of high folate status; adverse health outcome examined; and study quality (Table 2). For example, when reporting on the relationship between high folate concentration and colorectal cancer, one study may report the relationship with a folate concentration defined as the upper quartile, whereas another may examine folate concentrations above the mean. Further, some would report serum folate measured by Bio-Rad radioassay while another would report serum folate measured by microbiologic assay. In addition, some studies reported on data for males or females only, while others reported only overall estimates. As a result, we were unable to determine a summary measure for a high folate concentration cutoff to determine common point estimates and associated measures of error for the studies included in this review.

Discussion

Main findings of this study

This review indicates that, overall, reported high folate concentrations appear to be related to a decreased risk of adverse health outcomes without consistent association observed

Table 1 Risk of adverse health outcome by folate assay method and reported high folate concentration cutoff

Citation	*Assay method*	*High folate definition*			*Change in risk with highest folate concentration versus lowest folate concentration*		
	Health outcome	*Fasted (Y/N)*	*Value (nmol/l)*[a]	*Rationale*	*Adverse health outcome*	*Direction*	*Risk (95% CI)*
Cancer serum folate							
52	Immunoassay (Immulite analyser)	N	≥11.0	Lower bound of highest tertile	Prostate	↑	OR 5.82 (3.12 to 10.88) $P = 0.0001$
53	Immunoassay (ADVIA Centaur)	Y	≥18.1	Stratified after grouping by gender and serum folate concentration	Colorectal	↓	RR 0.49 (0.32 to 0.66) in males RR 0.77 (0.50 to 1.05) in females
56	Competitive immunoassay	Not stated	>20.4	Lower bound of highest quartile	Breast	↓	OR 0.23 (0.09 to 0.54) $P < 0.01$
54	Immunoassay	N	≥31.0	Lower bound of highest quartile	Colorectal	↓	OR 0.52 (0.27 to 0.97) $P = 0.04$
51	Enzyme-linked immunoassay (ELISA) kit	Y	>61.0	Lower bound of highest quartile	Esophageal squamous cell carcinoma	↓	OR 0.11 (0.04 to 0.33) $P < 0.05$
38	Bio-Rad radioassay	Y	>10.1	Lower bound of highest tertile	Pancreas	↓	OR 0.45 (0.24 to 0.82) $P = 0.009$
59	Radioimmunoassay	Not stated	≥12.5	Above the median	Colorectal	↑	OR 4.9 (1.4 to 17.7) $P = 0.01$
60	Competitive radiobinding assay	Not stated	>13.6	Lower bound of highest tertile	High-risk human papillomavirus	↓	OR 0.26 (0.08 to 0.89) $P = 0.03$
39	Bio-Rad radioassay	Not stated	≥34.2	Median of highest quintile	Colorectal cancer-specific mortality	↓	0.42 (0.20 to 0.88) $P = 0.01$
					Overall mortality	↓	0.46 (0.24 to 0.88) $P = 0.02$
41	Microbiologic assay	Not stated	≥22.5	Lower bound of highest quartile	Lung	↓	Lung OR 0.68 (0.51 to 0.90), P (for trend) = 0.001
77	pABG equivalents following oxidation and mild acid	N	≥17.5	Lower bound of the highest quintile	Prostate	↑	≥50 years of age OR 1.40 (1.07 to 1.84), P-trend = 0.02
Cognition serum folate							
65	Bio-Rad radioassay	Not stated	≥23.8	Lower bound of highest quartile	Reading	↓	Mean difference ± SE 3.05 ± 1.24, $P < 0.05$
					Block design	↓	0.64 ± 0.28, $P < 0.05$
67	Bio-Rad radioassay	Not stated	>44.8	Lower bound of highest tertile	Depression	↓	OR 0.52 (0.35 to 0.76), $P < 0.05$

22	Bio-Rad radioassay	Not stated	>59.0	80th percentile of the distribution of seniors	Cognition	↑	Anemia High folate/low B_{12} OR 4.8 (2.3 to 10.4)
						↓	Cognitive impairment High folate/normal B_{12} OR 0.5 (0.2 to 0.96)
						↑	High folate/low B_{12} OR 4.9 (2.6 to 9.2)
73	Microbiologic assay	N	≥30.0	No rationale given	Cognition		Q3 as reference anemia
			≥60.0			↓	OR 2.02 (1.46 to 2.80)
			≥42.1	Lowest bound of the highest tertile		↓	Cognitive impairment OR 1.55 (1.28 to 1.87)
78	Lab method varied since no central lab	Not stated	>14.0	Lower bound of highest quartile	Mild cognitive impairment	↓	Q3 as reference OR 3.1 (1.3 to 8.1) $P = 0.007$
					Dementia	↓	OR 3.8 (1.3 to 11.2) $P = 0.018$
					Stroke	↑	$b2 = 0.22$ $P = 0.007$
					Dementia	↓	Q1 64.6%, Q4 45.7% $P < 0.0001$
					Alzheimer's disease	↓	Q1 41.5%, Q4 31.3% $P = 0.01$
					Other types of dementia	↑	Q1 4.3%, Q4 1.5% $P = 0.05$
Health in offspring serum folate							
48	Immunoassay	N	≥23.2	Lower bound of highest quartile	Atopic dermatitis	↑	OR 1.18 (1.05 to 1.33) $P < 0.05$
62	Bio-Rad radioassay	Not stated	≥40.8	Lower bound of highest quintile	Total IgE	↓	OR 0.70 (0.53 to 0.92)
					Atopy	↓	OR 0.69 (0.57 to 0.85)
					Wheeze past 12 months	↓	OR 0.60 (0.44 to 0.82)
63	Bio-Rad radioassay	Not stated	>44.4	Lower bound of highest quartile	NTD	↓	OR 0.2 (0.08 to 0.62) $P = 0.002$
82	SimulTRAC-SNB radioassay kit	Y	≥21.5	Lower bound of median value in mid-pregnancy	Respiratory tract infections at 6 months	↓	aOR 0.50 (0.28 to 0.91)
					Atopic dermatitis at 24 months	↓	aOR 0.52 (0.31 to 0.88)
79	Ionic capture using an IMx analyser	Y	>31.9	Lower bound of highest tertile	Anencephaly	↓	MTHFR TT genotype OR 0.05 (0.01 to 0.37)

Continued

Table 1 Continued

Citation	*Assay method*	*High folate definition*			*Change in risk with highest folate concentration versus lowest folate concentration*		
	Health outcome	*Fasted (Y/N)*	*Value (nmol/l)*[a]	*Rationale*	*Adverse health outcome*	*Direction*	*Risk (95% CI)*
75	Microbiologic assay	Not stated	≥74.1	Lower bound of highest quartile (controls)	Isolated CL/P	↓	OR 0.34 (0.21 to 0.56) $P < 0.001$
					Isolated CP	↓	OR 0.35 (0.18 to 0.68) $P = 0.002$
					Combined CL/P + CP	↓	OR 0.35 (0.23 to 0.53) $P < 0.001$
Cardiovascular serum folate							
43	Bio-Rad radioassay	Not stated	≥11.2	Lower bound of highest tertile	All strokes	↓	Hazard rate ratios 0.35 (0.14 to 0.87) $P = 0.046$
44	Bio-Rad radioassay	Y	>11.3	Lower bound of highest tertile	Acute coronary events	↓	0.39 (0.18 to 0.83) $P = 0.016$
45	Bio-Rad radioassay	Y	>11.3	Lower bound of highest tertile	Acute coronary events	↓	Q3 as reference (versus Q1/Q2) RR 0.03 (0.10 to 0.84) $P = 0.023$
68	Radioimmunoassay	Y	>16.8	Lower bound of highest tertile	Retinal vein occlusion	↓	Q3 as reference (versus Q1) OR 5.41 (3.08 to 9.51)
50	Microbiologic assay	Not stated	>13.6	Lower bound of highest quartile	Fatal coronary heart disease	↓	Q4 as reference Rate ratio 1.69 (1.10 to 2.61)
47	Microbiologic assay	Not stated	≥21.8	Lower bound of highest quartile	Acute coronary events		Q4 as reference Age adjusted
						↓	35–55 years of age RR 2.4 (1.1 to 5.2)
						↑	≥55 years of age RR 0.5 (0.3 to 0.8)
Cancer RBC folate							
80	Bio-Rad radioassay	Not stated	≥320	Lower bound of highest tertile	Colorectal	↓	Q3 as reference OR 3.05 (1.34 to 6.96) *P*-trend = 0.006
81	Microbiologic assay (*Lactobacillus casei*)	Y	≥1853	Lower bound of upper tertile	Advanced HCC stages (III and IV)	↑	Q3 HR 2.05 (1.11 to 3.78)
Health in offspring RBC folate							

49	Electrochemiluminescence immunoassay	N	≥1813	Lower bound of upper quartile	Crown-rump length length	↑	Q3 as reference −0.29 (−0.49 to −0.09)
					Embryonic size	↑	Q4 versus Q3, 1.1 mm (23.5%) and 4.5 mm (7.4%) smaller at 6 and 12 weeks gestations, respectively
64	Bio-Rad radioassay	Not stated	≥544	Lowest bound of second quintile	NTD	↓	Low Hcy/high folate as reference High Hcy/High folate OR 2.9 (1.1 to 7.4)
27	Radioassay	Y	≥1269	Lowest bound of highest quartile	HOMA-IR	↑	Median (intraquartile range) Q1: 0.52 (0.28 to 0.81) Q2: 0.65 (0.36 to 0.92) Q3: 0.71 (0.39 to 1.12) Q4: 0.85 (0.51 to 1.27), *P*-trend < 0.001
					Fat mass	↑	Mean (SD) Q1: 3.0 (1.0) Q2: 3.1 (0.9) Q3: 3.2 (1.2) Q4: 3.4 (1.1)

pABG, p-aminobenzoylglutamate; OR, odds ratio; RR, relative risk; CI, confidence interval; SD, standard deviation; HOMA-IR, homeostasis model assessment insulin resistance; MTHFR, methylenetetrahydrofolate reductase; IgE, immunoglobulin E; Hcy, homocysteine; NTD, neural tube defect; CP, cleft palate; CL/CP, cleft lip/cleft palate; HCC, hepatocellular carcinoma.
[a]Folate concentration converted to nmol/l from ng/ml (2.265×) when needed.

Table 2 Association between reported high folate concentrations and adverse health outcomes: quality assessment

Outcome	*Quality assessment*							*No. of participants*[a]	*Quality*
	No. of studies	*Design*	*Risk of bias*	*Inconsistency*	*Indirectness*	*Imprecision*	*Other considerations*		
Cancer	24	Observational study[b]	Somewhat serious risk of bias[c]	Some inconsistency evident[d,e]	No serious indirectness	No serious imprecision[f]	None	23 002 (7601 cases/9769 controls and 5632 cohort)	Low
Adverse cognitive outcome	1	RCT	Somewhat serious risk of bias[c–g]	Not applicable	No serious indirectness	No serious imprecision[h]	None	369	Moderate
Adverse cognitive outcome	7	Observational study[i]	Somewhat serious risk of bias[c]	Some inconsistency evident[c]	No serious indirectness	No serious imprecision	None	12 254	Low
Adverse offspring outcome	9	Observational study[j]	Somewhat serious risk of bias[c]	Some inconsistency evident[c]	No serious indirectness	No serious imprecision[h,k]	None	20 063 (655 cases/974 controls and 18 472 cohort)	Low
Adverse cardiovascular outcome	10	Observational study[l]	Somewhat serious risk of bias[c]	Some inconsistency evident[c]	No serious indirectness	No serious imprecision	None	16 159 (393 cases/451 controls and 15 315 cohort)	Very low

Quality of evidence assessed using the Grades of Recommendation, Assessment, Development and Evaluation (GRADE) approach.

[a]Nine studies demonstrated duplication in use of cohorts (four cancer (Alpha-Tocopherol, Beta-Carotene Cancer Prevention [ATBC] Study),[35–38] three cardiovascular disease (Kuopio Ischaemic Heart Disease Risk Factor [KIHD]),[43–45] and the NHANES I Epidemiologic Follow-up Study [NEFUS] was used to examine cancer in one study and cardiovascular disease in another study.[46,47] All participants were included in the final sample size calculation; therefore, the population may be overestimated.

[b]Includes 16 case–control[35–38,40–42,51,52,54–56,59,61,71,77] and 8 cohort[39,46,53,60,70,72,80,81] studies.

[c]Measurement (assay method) of RBC folate variable,[27,64,69,72,74,75,80,81] no indication of fasting status for serum folate measurement[22,39–41,43,46,49,50,55,56,59,60,62–65,67,72,76,78,83] and variable definition of reported high folate concentration.

[d]Results related to prostate cancer were variable.[52,70,77]

[e]One study showed positive association between study definition of high folate concentrations and colorectal tumor[59] or hepatocellular carcinoma.[81]

[f]Two studies had small sample sizes;[59,70] two studies had wide confidence intervals.[52,56,59,60,80]

[g]Blinding not clearly described.[74]

[h]Wide confidence intervals.[63,79]

[i]Includes seven cohort studies.[22,65–67,73,74,78,83]

[j]Includes four case–control[63,64,75,79] and five cohort[27,48,49,62,82] studies.

[k]Small sample size for cases.[49,79]

[l]Includes 2 case–control[68,69] and 8 cohort[43–45,47,50,57,58,76] studies.

with increasing concentrations. However, high-quality evidence describing the association between reported high folate concentrations and adverse health outcomes is scarce. The majority of reported high folate cutoffs could be considered indicative of a clinically normal folate status and not necessarily representative of elevated values. Further, variability was observed in several factors, including participant fasting status; country fortification policies; folate assay methodology; and the cutoff selected to define high folate status. As a result, the evidence was insufficient to infer any solid conclusions. Consistent methods are needed to compare the results of health research related to high folate status.

What is already known on this topic

The health outcomes observed in this review of folate status were similar to those that have been identified in the folic acid intake literature. For example, folic acid intake has been associated with many positive health outcomes, including a decreased risk of NTD and other congenital anomalies, including cardiovascular defects, oral cleft, urinary tract abnormalities, congenital hydrocephalus and limb defects.[23–26] Safe doses of folic acid, as defined by the dietary reference intakes, have also been implicated in lower risk of colorectal cancer—and other cancers such as breast, lung and prostate—in the general population.[3,10] A meta-analyses involving 26 RCTs ($n = 58\,804$) did not support an association between folic acid supplementation and decreased risk of cardiovascular disease, but a potential link was made to a decreasing trend in stroke risk.[21] Though the many benefits of folic acid intake have been studied rigorously, the safety of higher doses of folic acid has been questioned and there is on-going debate about the association with negative health outcomes.[10,84–89] For example, folic acid may mask vitamin B_{12} deficiency when consumed at unsafe levels ($\geq$5 mg/day).[85] This is of particular concern in the elderly population; however, it has been suggested that current recommended intakes of folic acid pose a minimal threat of masking or exacerbating neuropathies.[10] The influence of folic acid consumed during pregnancy on DNA methylation has been implicated in epigenetic changes that could alter the way genes are expressed—without changing the DNA sequence—potentially leading to adverse outcomes in the offspring, including childhood wheeze or asthma.[87,88] However, a 2012 cohort study did not support an association between folic acid supplementation in early pregnancy and the development of asthma in offspring at 6 years of age.[89] Conflicting evidence has also emerged following evidence of a possible association between folic acid intake and increased risk of cancer in individuals with pre-existing neoplasms,[86] though several subsequent systematic reviews and meta-analyses did not demonstrate a relationship between folic acid supplement intake and most cancers.[15–17] For example, a 2013 meta-analysis included individual participant datasets from 13 trials ($n = 49\,969$) that compared folic acid supplementation (in amounts ranging from 0.5 to 5 mg/day, with one trial using 40 mg/day) with placebo, had a duration of at least 1 year, included at least 500 participants and recorded data on cancer incidence.[17] In the folic acid-supplemented participants, no short-term effects on overall or site-specific cancer incidence were demonstrated.

What this study adds

Though several of the adverse health outcomes that have been associated with folic acid intake were also identified in this systematic review of folate concentrations, there were few that supported an association with increased risk at high folate concentration cutoffs. However, since the majority of reported high folate concentration cutoffs are considered to be normal values, it is difficult to determine the clinical relevance of these findings. Overall, the studies captured in this review demonstrated mixed results. The majority of the included studies used quantiles of the study population—referring to the upper quantile(s) as high—to examine folate concentrations categorically in the absence of a consensus definition of high folate status. For example, based on the captured studies, it appears that reported high folate status is negatively associated with colorectal cancer prevalence.[35,39,53,54,59,80] However, there were marked inconsistencies among the colorectal cancer studies captured in our review, including variable or unreported fasting status; different folate measurement method (an immunoassay or a radioimmunoassay); and cutoffs for the reported high serum folate status that ranged from 11.8 to 34.2 nmol/l based on different quantiles (e.g. above the mean, upper tertile, upper quartile or upper quintile) and notably in a normal serum folate range. Thus, any overarching conclusion is limited by these inconsistencies. This methodological heterogeneity complicated any comparisons and precluded conclusions from being drawn regarding the relationship between folate status and adverse health outcomes.

A consistent cutoff for high folate status—defined in association with a clinical outcome—was not observed in this review. Several papers have proposed folate concentration cutoffs that do not represent upper quantiles, but these do not report adverse health outcomes thus were not captured by our search strategy.[14,90,91] For example, Fazili *et al.*[90] assigned a cutoff of 45 nmol/l to define high serum folate status as this value represented the upper end of the calibration curve, as well as an observed increase in folic acid concentrations at greater than ~50 nmol/l total folate. Another example is our examination of the Canadian Health Measures

Survey data, in which the cutoff was determined based on the 97th percentile of Bio-Rad 1999–2004 NHANES, as recent national Canadian data were unavailable.[14]

Further methodological variability was observed in folate measurement. The majority of studies in our review used serum folate status, which is considered a sensitive indicator of folic acid intake. This was difficult to interpret since fasting status was often not reported. RBCs build folate stores during erythropoeisis that are retained through the 120-day life span of the RBC; thus, RBC folate concentrations are a common measure of long-term folate status.[92] Few studies in our review reported RBC folate status cutoffs. Further, significant interassay differences exist in folate measurement, which can impair the ability to define high folate concentrations and compare serum and RBC folate measurement methods.[18–20] These differences exist due to multiple complications; for example, reduced folates are unstable at extreme pH values and when exposed to elevated temperatures, oxygen or light. Fazili *et al.*[18] conducted a methods comparison study analyzing whole blood hemolysates, from an American and a European blood bank, to examine differences in total folate concentrations measured by liquid chromatography–tandem mass spectrometry (LC/MS/MS), microbiologic assay (using *Lactobacillus Rhamnosus*) and Bio-Rad radioassay. The microbiologic assay was comparable to the LC/MS/MS within $\pm$ 10%, but the values were 45% lower for Bio-Rad assay in whole blood samples. Similarly, considerably lower results (29%) were observed with Bio-Rad assay, in comparison to LC/MS/MS, for serum folate, potentially due to under recovery of certain folate forms. It is notable that polymorphisms in the enzyme *MTHFR C677T* can impair folate metabolism and decrease circulating folate by 10–25% in those with the homozygous TT variant. This could impact laboratory measures of RBC folate.[18,93] However, MTHFR genotype was not accounted for consistently in the studies included in our review, therefore was not considered in the present examination. Standardization of folate measurement methodology would allow for high folate status to be more reliably defined and, subsequently, for studies to be more readily compared.

Limitations of this study

Strengths of this review included a comprehensive search strategy; and *a priori* inclusion and exclusion criteria and analyses. We included both serum and RBC folate concentrations, and a range of health outcomes that have previously been associated with high folate supplement intake. The review was large in scope and included numerous health outcomes and measurement methods. The quality of the captured studies was a limitation of this review. The majority of studies included in this review were observational studies; thus, causation cannot be inferred and results should be interpreted with caution. A detailed meta-analysis would have allowed us to estimate the overall effect sizes for each outcome. However, due to the heterogeneity of the data, it was impossible to complete such an analysis. Many studies grouped their variables into quantiles, and although it was still possible to ascertain information regarding the association between folate concentrations and health outcomes, it made it impossible to compare the information across studies. We did not attempt to determine a grouping by 'high folate concentration' since cutoffs were arbitrary and had the potential to lead to false conclusions.

Conclusions

The scientific value of this review is the demonstrated variance in how researchers are studying and defining high folate status, which affects the ability to interpret associations with adverse health outcomes. Further, these variable definitions can be misleading as high folate is often indicative of a clinically normal concentration; thus, if higher values had been selected as being 'high', different conclusions could be drawn. Our results demonstrate that there is no consistent relationship between increasing folate concentrations and the adverse health outcomes we examined. This review provides evidence that standard methods are needed to examine the intersection of folate status and adverse health outcomes. Further, using a consistent, evidence-based cutoff to define high folate status may enhance the ability to examine associations with adverse health outcomes and compare study results. This would provide clinicians with direction for assessing patient folate status and ultimately inform public health interventions and clinical guidelines for folic acid.

Authors' contributions

C.K.C., M.S.T. and D.L.O. conceptualized and designed the search strategy with input from M.S. M.S. reviewed and oversaw the implementation of the search strategy. C.K.C. and B.W. reviewed the data and conducted all aspects of data capture. All authors assisted in the interpretation of the results. C.K.C. drafted the manuscript, and all authors provided critical review and feedback. All authors read and approved the final manuscript.

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Supplementary data

Supplementary data are available at *PUBMED* online.

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