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The Bioavailability of Various Oral Forms of Folate Supplementation in Healthy Populations and Animal Models: A Systematic Review

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Abstract

Background and aims: Folate is an essential nutrient required for many different functions in the body. It is particularly important for DNA synthesis, immune functions, and during pregnancy. Folate supplements are commonly prescribed by health professionals for a number of different conditions, however, the absorption of the different derivatives remains unclear. The aim of this review was to assess the bioavailability of various forms of folate supplements in healthy populations and animal models.

Methods: A systematic literature review was conducted of original research, which assessed the bioavailability of different oral forms of folate in healthy adults or animal models. The following databases were searched: PubMed (U.S. National Library of Medicine), ProQuest Medical Collection (ProQuest) and ScienceDirect (Elsevier) up to March 30, 2017. The inclusion criteria consisted of both animal and human research, no disease state or condition, and assessed levels after an intervention of a folate derivative.

Results: A total of 23 studies out of 5226 met the full inclusion criteria. Of these, 4 were animal studies and 19 were human studies. There was variation in supplement forms used with the most commonly tested being folic acid followed by 5-methylenetetrahydrofolate (5-MTHF). Dosages ranged from 25 μg up to 200 mg. Only three studies found a statistically significant difference in folate bioavailability when evaluating different supplement forms. These studies found 5-MTHF to be more effective at increasing folate levels in participants.

Conclusions: This review has found a number of methodological limitations and conflicting results. Only three out of the 23 studies assessed found a statistically significant difference between different supplemental forms of folate. Quality absorption studies assessing the bioavailability of oral folate supplements are crucial if clinicians are to make effective evidence-based recommendations. More research is required for greater clarification regarding the bioavailability of these supplements.

Keywords: absorption, folic acid, folinic acid, 5-methyltetrahydrofolate, bioavailability

Introduction

There are several different forms of oral folate supplements available to clinicians and the general public. Numerous studies have assessed the efficacy of folate supplements for different conditions and disease states[1–3]; however, relatively little literature has been published on the absorption and bioavailability of different folate supplements. To date, there has been conflicting information generated on the internet and by supplementation companies, which has led to uncertainty regarding the most effective oral folate supplement for healthy individuals. Folate is an essential nutrient required for many different functions in the body and is particularly important for DNA synthesis, immune functions, and during pregnancy. This article will review the current literature focusing on the bioavailability of various oral forms of folate supplements in healthy populations and animal models.

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Background

Folate is the generic term for a B group vitamin, which functions as a carbon donor in the synthesis of amino acids, purines, and pyrimidine bases required for DNA synthesis.[4] It also functions as a methyl donor in the production of methylcobalamin and methionine.[4] It is found in a wide range of foods, including whole grains, legumes, and green leafy vegetables.[5] Folate supplements have shown to be effective for a number of conditions, including Alzheimer's disease,[6] sleep problems,[7] and depression.[8] It is often prescribed alongside medications, such as methotrexate, to reduce unwanted and harmful side effects of the medication.[9]

Under the Australian New Zealand Food Standards Code, Australian millers have been required to add folic acid to wheat flour used for bread making since September 2009. The flour has to contain 2–3 mg of folic acid per kg. This equates to three slices of bread providing approximately half of the recommended daily intake of folate.[10] However, not all countries have implemented this fortification.[11] To assess the efficacy of this intervention, a retrospective analysis of serum and red blood cell (RBC) folate samples collected between 2007 and 2010 were analyzed. A total of 20,592 blood samples were evaluated and a 31% increase in mean serum folate and a 22% increase in mean RBC folate level was observed highlighting the success of the fortification policy in improving folate status.[10] Given the importance of this policy, understanding if any differences exist between supplemental forms of folate is crucial. Especially when considering other influencing factors such as enzyme activity.

A percentage of the population is unable to properly metabolize folate because they have defects with the methylenetetrahydrofolate reductase (MTHFR) enzyme. MTHFR is the rate-limiting enzyme in the methylation cycle, and it is encoded by the MTHFR gene.[12] This enzyme is responsible for converting 5,10-methylene tetrahydrofolate into 5-methyltetrahydrofolate by adding methyl groups to make folate bioavailable to the body.[12] Individuals who have impaired MTHFR activity have a reduced ability to convert 5,10-MTHF into 5-methyltetrahydrofolate.[13] Due to this reduced activity they may have difficulty processing folic acid from supplements and fortified foods. Other independent factors to consider include intestinal absorption and transport of folate. The reduced folate carrier and the proton-coupled folate transporter involved in mediating folate transport across the epithelia and into systemic tissues contribute to folate homeostasis.[14]

There are several variations when it comes to MTHFR mutations depending on which genes are passed on from each parent. Currently, 34 mutations have been identified with the MTHFR gene, which are associated with enzymatic deficiency.[12] A 2003 study, which assessed the MTHFR status of 7000 newborns over 16 areas worldwide, found that between 60% and 70% of individuals will have at least one of these polymorphisms.[15]

The folate vitamers found in whole foods typically occur as reduced methyl and formyl polyglutamate forms.[4] This is different from the structure of synthetic forms used in supplements and food fortification. Due to the chemical differences in structure, there is a difference in bioavailability. In 1998, The Institute of Medicine introduced the use of dietary folate equivalents to adjust for the variations in bioavailability of food folate and synthetic forms.[16] Several different forms are used in dietary supplements. Folic acid, folinic acid, and 5-methyltetrahydrofolate are the most commonly available oral vitamin supplements available worldwide. However, there is limited research assessing their bioavailability. This review aims to critically appraise the current evidence on oral folate bioavailability and discuss the key findings from the current literature.

Methodology

A protocol was developed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols (PRISMA-P) 2015 statement.[17]

Search strategies and inclusion criteria

A literature search was conducted in the following databases: PubMed (U.S. National Library of Medicine), ProQuest Medical Collection (ProQuest) and ScienceDirect (Elsevier). All authors contributed to the development of search terms and inclusion/exclusion criteria. Search terms were divided in two groups and combined within the search. Group 1: folate OR folic acid OR folinic acid OR 5 MTHF OR 5-methyltetrahydrofolate OR Tetrahydrofolate. Group 2: absorption OR bioavailability OR pharmacokinetics OR oral OR pharmacodynamics. Original research, which assessed the bioavailability of different oral forms of folate in healthy adults or animal models, were included in the review published up to the 30th March 2017.

Study selection and data extraction

The initial search identified 5226 articles. After removal of 128 duplicates, articles were screened by title and by abstract. The remaining articles were then screened by full text resulting in 23 articles, which met the full inclusion criteria to be assessed in this review. Screening was performed by J.B. and citations were stored and filed in EndNote X7. Articles were excluded from the review for the following reasons; articles were not in English; articles were not related to the topic; not original research; studies which examined folate in disease states; trials assessing folate from whole foods or fortified products; studies testing efficacy of folate supplements in conjunction with medications; or studies looking at correcting a folate deficiency. The article selection process is outlined in Figure 1.

Assessment of risk of bias and data summary table

Each article was critically appraised for methodological consistency using the Joanna Briggs Institute Critical Appraisal tool for Systematic Reviews. The Checklist for Quasi-Experimental Studies was used for thirteen of the studies and the Checklist for Randomized Control Trials (RCTs) was used for six of the studies.[18] The critical appraisal tools assessed the 19 human studies included in this literature review. Overall, the appraisal found reliable methodology and no articles were excluded from the review. The results for quasi-experimental studies are displayed in Table 1 and the results for RCTs are displayed in Table 2. During this process, data were extracted from the final articles and summarized in Tables 3 and 4.

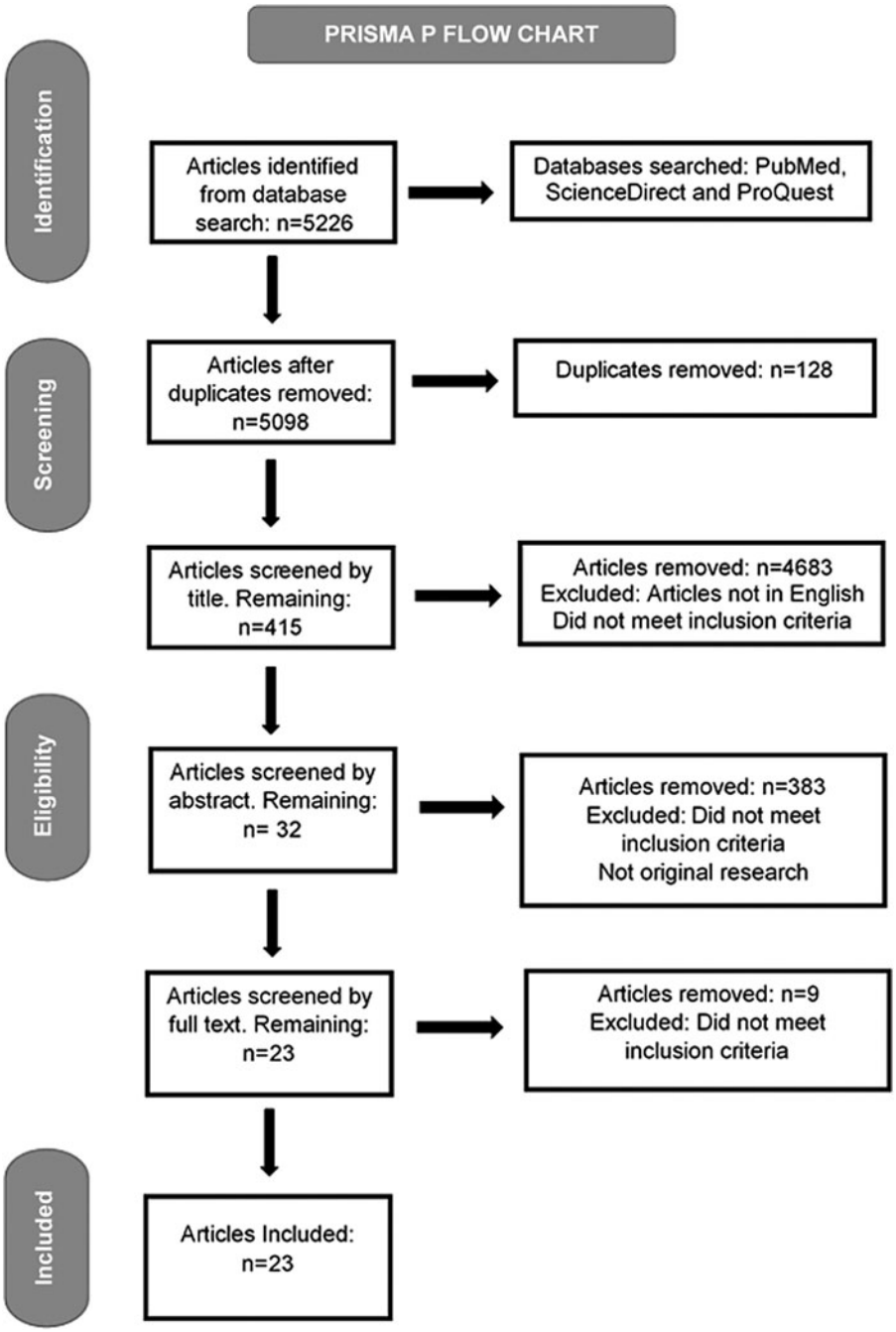

FIG. 1. PRISMA-P flow diagram. PRISMA-P, Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols.

Limitations of This Review

This review has several limitations of its own, which need to be considered. Only articles available in English were included, which may have resulted in important research being omitted from the review. Another consideration is publication bias. Only published trials available on the preselected databases were available to be reviewed, which may have skewed the findings. All *in vitro*, animal and human studies, which met the inclusion criteria, were assessed in this review. Differences exist between clinical and animal studies and supplementation preparations vary accordingly. This should be considered when interpreting the results from this review.

Results

A total of 23 studies out of the original 5226 articles identified fit the full inclusion criteria and were appraised in this review. All studies provided quantitative data with 4 trials[19–22] using animal models and 19 studies[23–41] conducting human trials.

The studies varied in length from 8 h to 24 weeks. Twelve studies[19,20,22,24–26,30,33,37,38,40,41] administered the intervention as an individual dose and measured outcomes at various intervals over the next 8–24 h. Seven[23–26,37,40,41] of those studies included a washout period of at least 1 week between the first intervention and the second. Three studies[25,26,33] also included a saturation period, where participants were predosed with folate before beginning the trial. Two

Table 1. Critical Appraisal Table: Human Trials

The Joanna Briggs Institute Critical Appraisal Tool for Systematic Reviews: Checklist for Quasi-Experimental Studies

Authors/date	*(1)*	*(2)*	*(3)*	*(4)*	*(5)*	*(6)*	*(7)*	*(8)*	*(9)*	*Total*
McGuire et al. (1986)[23]	Y	N/A	N/A	N	Y	Y	N/A	Y	Y	5
Guelen et al. (1988)[24]	Y	Y	Y	N	Y	Y	Y	Y	Y	8
Gregory et al. (1991)[25]	Y	Y	Y	N	Y	Y	Y	Y	Y	8
Gregory et al. (1992)[26]	Y	Y	Y	N	Y	Y	Y	Y	Y	8
Truswell and Kounnavong (1997)[27]	Y	Y	Y	N	Y	Y	Y	Y	Y	8
Baggott and Tamura (1999)[28]	Y	Y	Y	N	Y	Y	Y	Y	Y	8
Litynski et al. (2002)[29]	Y	Y	Y	N	Y	Y	Y	Y	Y	8
Kok et al. (2004)[30]	Y	Y	Y	N	Y	Y	Y	Y	Y	8
Pentieva et al. (2004)[33]	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
Melse-Boonstra et al. (2006)[36]	Y	Y	Y	N	Y	Y	Y	Y	Y	8
Hartman-Craven et al. (2009)[38]	N	Y	Y	N	Y	Y	Y	Y	Y	7
Maki et al. (2012)[40]	N	Y	Y	N	Y	Y	Y	Y	Y	7
Lakoff et al. (2014)[41]	Y	N/A	N/A	N	Y	Y	N/A	Y	Y	5

Key: Y, yes; N, no; N/A, not applicable.
(1) Is it clear in the study what is the ''cause'' and what is the ''effect''?
(2) Were the participants included in any comparisons similar?
(3) Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?
(4) Was there a control group?
(5) Were there multiple measurements of the outcome both pre and post the intervention/exposure?
(6) Was follow-up complete, and if not, was follow-up adequately reported and strategies to deal with loss to follow-up employed?
(7) Were the outcomes of participants included in any comparisons measured in the same way?
(8) Were outcomes measured in a reliable way?
(9) Was appropriate statistical analysis used?

studies[31,32] included a run-in period with a placebo for 5 weeks to get participants accustomed to taking supplements.

The main outcome measure utilized by 17 of the human trials[23,24,27–41] included plasma or serum folate. Two studies[25,26] measured 24-h urine. Five studies[27,31,32,35,41] measured RBC folate and five trials[29,31,32,34,39] also included homocysteine as an outcome measure. A summary is provided in Table 5 below. All studies measured key outcomes at baseline level. The animal studies also measured tissue samples at the conclusion of the trials.

In the human studies, five studies[23,25,26,33,37] tested only males and four[27,35,38,40] tested only females. Three studies[31,32,34] failed to clearly specify the gender of their participants, and the remaining seven studies[24,28–30,36,39,41] included both sexes.

Table 2. Critical Appraisal Table

The Joanna Briggs Institute Critical Appraisal Tool for Systematic Reviews: Checklist for Randomized Controlled Trials

Authors/date	*(1)*	*(2)*	*(3)*	*(4)*	*(5)*	*(6)*	*(7)*	*(8)*	*(9)*	*(10)*	*(11)*	*(12)*	*(13)*	*Total*
Melse-Boonstra et al. (2004a)[31]	UC	UC	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	11
Melse-Boonstra et al. (2004b)[32]	UC	UC	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	11
De Meer et al. (2005)[34]	UC	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	12
Lamers et al. (2006)[35]	UC	UC	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	11
Verlinde et al. (2008)[37]	UC	Y	Y	Y	UC	UC	Y	Y	Y	Y	Y	Y	Y	10
Obeid et al. (2011)[39]	Y	UC	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	12

Key: Y, yes; UC, unclear.
(1) Was true randomization used for assignment of participants to treatment groups?
(2) Was allocation to treatment groups concealed?
(3) Were treatment groups similar at the baseline?
(4) Were participants blind to treatment assignment?
(5) Were those delivering treatment blind to treatment assignment?
(6) Were outcomes assessors blind to treatment assignment?
(7) Were treatments groups treated identically other than the intervention of interest?
(8) Was follow-up complete, and if not, were strategies to address incomplete follow-up utilized?
(9) Were participants analyzed in the groups to which they were randomized?
(10) Were outcomes measured in the same way for treatment groups?
(11) Were outcomes measured in a reliable way?
(12) Was appropriate statistical analysis used?
(13) Was the trial design appropriate and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?
RCT, randomized control trial.

Table 3. Data Summary Table on Animal Trials

Animal studies											
							Themes				
Authors	*Year*	*Study design and duration*	*Participants*	*Intervention/dosage*	*Outcome measures*	*Main findings*	*1*	*2*	*3*	*4*	*5*
Bhandari and Gregory[19]	1992	Animal model; 8-day duration.	9 Male rats.	Folic acid, 5-tetrahydrofolate, 5-formyltetrahydrofolate; dosage: 50 pmol/100 g body weight. Or 0.02 μg each.	Urine and faeces collected at 4, 8, 12 and 24 h and 2, 3, 4, 5, 6, 7, and 8 days after treatment dose. Tissue sample collected after 8 days.	No significant difference was observed between supplement groups.	*				
Kudo et al.[20]	1995	Animal model; duration: once off dosage.	Female Wistar rats.	Folic acid, folic acid with pyrimethamine.	Tissue samples.	Absorbed pteroylglutamic acid is not converted into reduced folates in the liver due to pyrimethamine.	*				
Pérez-conesa et al.[21]	2009	Animal model; duration: 4 weeks.	36 Male rats.	Folic acid (1000 μg/L), (*6S*)-5-methyltetrahydrofolate (1041.91 μg/L).	Plasma folate, RBC folate and liver tissue folate taken at the end of 4 weeks.	Rats fed (*6S*)-5-methyltetrahydrofolate displayed significantly higher folate levels than those in the folic acid group.	*				
Miraglia et al.[22]	2016	Animal model; duration: 8 h.	18 Male rats.	Folic acid, (*6S*) 5-methyltetrahydrofolate calcium salt, (*6S*) 5-methyltetrahydrofolate glucosamine salt; dosage: 70 μg/kg of body weight.	Plasma folate taken at 0.5, 1, 2, 4, and 8 h.	(*6S*) 5-methyltetrahydrofolate glucosamine salt showed the greatest bioavailability in rats.	*				

*Signifies the article contains this theme.
RBC, red blood cell.

Table 4. Data Summary Table on Human Trials

Human studies							*Themes*				
Authors	*Year*	*Study design and duration*	*Participants*	*Intervention/dosage*	*Outcome measures*	*Main findings*	*(1)*	*(2)*	*(3)*	*(4)*	*(5)*
McGuire et al.[23]	1986	Randomized crossover study. Duration: 24 h (supplement administration and pathology testing) followed by a 1-week washout period and another 24-h supplement administration and pathology testing.	30 Healthy males. Twenty-four in a four-way crossover dose-proportionality study and six in a high-dose study.	5-Formyltetrahydrofolate. Dosage: 20, 40, 60, 80, and 100 mg in the four-way crossover trial. Two hundred milligrams in the high-dose study.	Serum folate taken at 20, 40, and 60 min, 1.5, 2, 3, 4, 7, 12, and 24. 24-H urine testing.	Incrementally increased dosages of Folinic acid failed to increase circulating folate levels proportional to dosage.	*				
Guelen et al.[24]	1988	Crossover intervention trial. Duration: 24-h supplement administration and testing period followed by 1-week washout and another 24-h supplement administration and testing period.	12 Adults, 3 female and 9 men, 23–38 years of age.	Rescuvolin (folinic acid calcium salt, charge No. 83F24B). Dosage:15 mg. Ledervorin (folinic acid calcium salt, charge No. 3L24/46118. Dosage 15 mg.	Plasma folate taken through fingertip puncture at 0.5, 1, 1.5, 2, 3, 4, 8, 10, and 24 h.	No significant differences in bioavailability in these the two Folinic acid preparations.		*			
Gregory et al.[25]	1991	Quasi-experimental. Duration: 2-week saturation phase before a 3-day experimental phase (Folic acid) followed by a 3-week washout period and then another 3-day intervention phase with (Pteroylhexaglutamate).	7 Adult men.	300 μg Folic acid, 700 μg Pteroylhexaglutamate. A 2-week saturation period of 2 mg of folic acid before the experimental period. Dosage: Single oral dose of either supplement.	48-H urine analysis.	Pteroylhexaglutamate is an available source of folate in humans, but shows substantially less bioavailability compared with folic acid.		*			
Gregory et al.[26]	1992	Quasi-experimental. Duration: 2-week saturation phase before a 3-day experimental phase followed by a 3-week washout period and then another 3-day intervention phase.	7 Adult men.	300 μg Folic acid, 300 μg Tetrahydrofolate, 320 μg 5-formyltetrahydrofolate, 320 μg 10-formyltetrahydrofolate, 320 μg 5-methyltetrahydrofolate. A 2-week saturation period of 2 mg of folic acid before the experimental period. Dosage: Single oral dose.	48-H urinary folate.	Differences were observed between the bioavailability of monoglutamyl folates.		*			
Truswell and Kounnavong[27]	1997	Quasi-experimental study design consisting of three experiments. Total duration: 6 weeks.	20 Healthy women.	Folic acid (varying dosages). Experiment 1: 100 μg and then 1000 μg of folic acid. Experiment 2: 500 μg and then 1500 μg of folic acid. Experiment 3: 1000 μg and then 2000 μg of folic acid.	Serum folate was tested in experiment 1 and 2. In experiment 3 RBC folate was also tested.	Clear increases in serum folate levels were observed in relation to every increased dosage of folic acid.	*				
Baggott and Tamura[28]	1999	Quasi-experimental study design. Three experiments with a total 2-week duration.	5 Healthy subjects (2 females, 3 males) 27–58 years of age.	1.2 mg (*6R*)-5-formyltetrahydrofolate, 1.2 mg (*6S*)-5-formyltetrahydrofolate, 1.2 mg (*6S*)-5,10-methenyltetrahydrofolate. Experiment 1: all five subjects took 1.2 mg of either (*6R*)-5-formyltetrahydrofolate or (*6S*)-5-formyltetrahydrofolate in 10 mL of water. Experiment 2: three participants were given 1.2 mg of (*6S*)-5,10-methenyltetrahydrofolate in 10 mL of 5.0 m*M* citric acid. Experiment 3: One male participant was given 6.4 mg of either (*6R*)-5-formyltetrahydrofolate or (*6S*)-5-formyltetrahydrofolate.	Plasma folate taken at 1, 2, and 4 h after dose. Urinary Folate was collected for 2 weeks after the last dose.	The orally administered unnatural isomers are biologically active in humans.			*		

(*continued*)

TABLE 4. (CONTINUED)

Human studies

Authors	*Year*	*Study design and duration*	*Participants*	*Intervention/dosage*	*Outcome measures*	*Main findings*	*Themes* (1)	(2)	(3)	(4)	(5)
Litynski et al.[29]	2002	Quasi-experimental. Treatment duration 7 weeks. Follow-up at 24 weeks.	40 Healthy adults (32 males and 8 females).	400 μg (*6RS*) 5-methyltetrahydrofolate, 400 μg folic acid. Single dose per day.	Plasma homocysteine and total plasma folate. Measured at 3 and 7 weeks of treatments and 24 weeks after stopping treatment.	5-Methyltetrahydrofolate showed comparable efficacy in reducing homocysteine as folic acid.		*	*		*
Kok et al.[30]	2004	Quasi-experimental single-dose study. Duration: 8 h.	5 Healthy adults (1 female and 4 males).	5.6 mg folic acid in capsules, 5.3 mg folic acid as a drink. Dosage: once off treatment.	Plasma taken at 20, 40, 60, 90, 120, 150, 180, 240, 300, 360, and 480 min.	No significant differences observed in absorption time, first-pass effect, and elimination rate between interventions.				*	
Melse-Boonstra et al.[31]	2004	RCT. Duration: a 5-week run-in period with a placebo followed by 12 weeks of treatment.	180 Healthy adults 50–75 years of age.	350 μg Monoglutamyl folic acid, 350 μg heptaglutamyl folic acid, placebo.	Serum folate, RBC folate, and homocysteine taken at week 12.	There were no significant differences in bioavailability between treatment groups.		*	*		*
Melse-Boonstra et al.[32]	2004	Randomized parallel trial. Duration: a 5-week run-in period with a placebo followed by 12 weeks of treatment.	180 Healthy adults aged 50–75 years.	350 μg Monoglutamyl folic acid, 350 μg heptaglutamyl folic acid, placebo.	Serum folate, RBC folate, and homocysteine taken at weeks 2 and 12.	Mean serum and RBC folate concentrations increased less in the polyglutamyl folic acid group than in the mono-glutamyl folic acid group.		*	*		
Pentieva et al.[33]	2004	A double-blind, crossover study. Duration: A 1-week saturation period with 5 mg Folic acid. Followed by 2 folic acid free days. Then the treatment period of 3 weeks.	13 Healthy men.	500 μg folic acid, 500 μg (*6S*)-5-methyltetrahydrofolate, Placebo. All participants received each of the interventions in a random order (one off dose) at 1-week intervals.	Plasma folate taken at 0.5, 1, 1.5, 2, and 2.5 h (before midmorning snack); 3 and 5 h (before lunch); 7 h (before afternoon snack); and 10 h.	(*6S*)-5-MTHF and folic acid display equivalent short-term bioavailability.		*			
De Meer et al.[34]	2005	Randomized double-blind intervention study. Duration: 5 weeks.	12 Young (<30 years) and 12 middle-aged (>50 years) healthy volunteers.	400 μg folic acid, 400 μg (*6S*)-5-methyltetrahydrofolate.	Total folate, serum, homocysteine, and methylmalonic acid.	The folate turnover in young adults increased after folic acid supplementation comparative to (*6S*)-5-methyltetrahydrofolate, however, no differences were observed in middle aged adults.		*	*		
Melse-Boonstra et al.[36]	2006	Quasi-experimental study design. Duration: 28 days.	20 Healthy adults 20–50 years of age (5 men and 15 women).	55 μg Hexaglutamyl folic acid, 25 μg mono-glutamyl folic acid.	Plasma folate taken on day 1, 2, 4, 8, 14, and 28 of the intervention period. Tested again at 1 and 4 weeks after treatment ended.	Multiple dosing of low amounts of labeled folic acid is accurate for measuring the bioavailability of folic acid compounds.		*			
Lamers et al.[35]	2006	Double-blind, randomized, placebo-controlled intervention trial. Duration: 24 weeks.	144 Healthy women 19–33 years of age.	400 μg folic acid, 416 μg (*6S*)-5-MTHF, 208 μg (*6S*)-5-MTHF, placebo.	RBC folate and plasma folate tested at weeks 4, 8, 12, 16, 20, and 24.	(*6S*)-5-MTHF is more effective than folic acid at improving folate status. Preconception folic acid supplementation should be extended to >4 weeks.	*	*			
Verlinde et al.[37]	2008	Crossover placebo-controlled intervention study. Duration: 8 weeks. Single dosage of each supplement with varying washout periods between interventions.	9 Healthy adult men.	343 μg (*6S*)-5-methyltetrahydrofolic acid, 343 μg (*6S*)-5-methyltetrahydrofolic acid plus 289.4 mg of L-ascorbic acid, 343 μg (*6S*)-5-methyltetrahydrofolic acid plus 973.8 mg of L-ascorbic acid. Placebo. Participants received all interventions in a random order as a single dose.	Serum folate tested on each intervention day after 0.5, 1, 1.5, and 2 h (before mid-morning snack), after 2.5, 3, and 4 h (before lunch), after 6 h (before afternoon snack) and after 8 h.	Coadministration of L-ascorbic acid with 5-methyltetra-hydrofolate significantly improved serum folate levels in healthy men.		*			

(*continued*)

TABLE 4. (CONTINUED)

Human studies

Authors	*Year*	*Study design and duration*	*Participants*	*Intervention/dosage*	*Outcome measures*	*Main findings*	*Themes* (1)	(2)	(3)	(4)	(5)
Hartman-Craven et al.[38]	2009	Randomized, three-period, two-intervention crossover study. Duration: 8 h.	18 Healthy pregnant women (24–32 weeks gestation).	Powdered supplement—contained 30 mg of iron and 600 μg folic acid. Tablet supplement—contained 27 mg iron and 1000 μg folic acid. Dosage: once off treatment.	Hemoglobin, serum ferritin sTfR, serum iron, and plasma folate taken 1, 2, 3, 4, and 8 h after supplement ingestion.	There were no significant differences in the absorption of folic acid between the powdered and tablet supplement.	*			*	
Obeid et al.[39]	2011	Double-blind, randomized, placebo-controlled intervention trial. Duration: 3 weeks.	74 Elderly adults (median age of 82) 63 women and 11 men.	Supplement containing 5 mg of folic acid, 40 mg of vitamin B6, and 2 mg of cyanocobalamin, placebo. Dosage: 1 capsule per day.	Total Homocysteine, cystathionine, methylmalonic acid, total serum cobalamin, S-Adenosyl-L-homocysteine (SAH), S-Adenosyl methionine (SAM), betaine, choline, dimethylglycine, and plasma folate tested on day 3 and at 3 weeks.	In older adults, folic acid is mostly converted to THF and 5-MTHF. Supplementation lowered homocysteine concentrations but caused an increase in unmetabolized folic acid measured in plasma.			*		
Maki et al.[40]	2012	Randomized crossover study. Duration: 1-day treatment period followed by 1-week washout and then another 1-day treatment period.	16 Healthy women.	1000 μg folic acid through two tablets, 1000 μg folic acid in a multivitamin soft gel capsule. Dosage: once off treatment.	Serum Folate taken at 1, 2, 3. 4, 6, and 8 h after intervention dose.	Both interventions displayed similar bioavailability.				*	
Lakoff et al.[41]	2014	Quasi-experimental study design. Duration: 1-day intervention period (capsules) followed by a 4-week washout period followed by the second 1-day intervention (intravenous injection).	9 Healthy adults (6 males and 3 females).	400 μg (*6S*)-5-formyltetrahydrofolic acid (in a ph-sensitive enteric caplet), 400 μg (*6S*)-5-formyltetrahydrofolic acid (as an intravenous injection). Dosage: once off dosage.	RBC folate, total plasma folate, vitamin B12 and B6. Blood samples collected hourly after intervention.	Folate is absorbed across the colon in humans with an undisturbed microbiome.				*	

The units of measure nmol and μmol have been converted into μg and mg for consistency. All calculations were performed by N.A.

Themes: (1) Comparing different dosages of folate. (2) Comparing different forms of folate. (3) Assessing the efficacy of lowering homocysteine. (4) Comparing different delivery systems, for example, soft gel versus tablet. (5) Considered populations with MTHFR gene mutation.

*Signifies the article contains this theme.

5-MTHF, 5-methylenetetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; RBS, red blood cell.

Table 5. Outcome Measures

No. of trials	*Outcome measure*
17	Plasma or serum folate
2	Urinary folate
5	RBC folate
5	Homocysteine

RBC, red blood cell.

Four studies[31,32,34,39] were conducted on older adults (>50 years) with one study[34] comparing an intervention in a group of older adults to a group of younger adults. One study[38] was conducted on healthy pregnant women with no signs of pre-existing disease conditions.

The number of participants included in the 19 human trials varied greatly with the average number of participants being 42. The boxplot in Figure 2 displays the minimum (5), Q1 (9), median (16), Q3 (35) and the range (175) of participants.

A number of different themes were present throughout the studies. Three trials[23,27,35] tested varying different dosages of folate to see the effect on outcome measures. Twelve studies[19,21,22,26,28,29,31–36] compared two or more different forms of folate against each other. One study tested a soft gel capsule versus a tablet[40] and another looked at a powdered formula verses a tablet.[38] Two studies[29,31] included individuals with MTHFR gene mutations.

There was also great variation in the forms of folate supplements used. The most frequently tested supplement was folic acid with 15 trials[19–22,25–27,29,30,33–35,38–40] using this form. The second most common was (*6S*)-5-methyltetrahydrofolate with eight trials[21,22,26,29,33–35,37] assessing its efficacy. Table 6 highlights the different supplements used.

The dosages used in the human trials varied greatly from only 25 μg up to 200 mg. Of the 12 studies,[19,21,22,26,28,29,31–36] which compared 2 or more different forms of folate, 9 studies[19,26,28,29,31–34,36] showed no significant differences between the groups and three studies[21,22,35] showed 5-methylenetetrahydrofolate (5-MTHF) to be the most bioavailable.

Discussion

Health practitioners may recommend folate supplements for a number of different conditions. Currently, the oral folate supplements available include folic acid, folinic acid, and 5-MTHF. The most bioavailable form in healthy populations remains unclear. Identifying the most effective oral form of folate will facilitate advancements in this field and may assist in improving patient health and outcomes.

This is the first review to explore the bioavailability of different oral forms of folate supplementation in a healthy population. The review highlighted that only 12 studies[19,21,22,26,28,29,31–36] have compared two or more different forms of folate supplementation. The literature from this review did not find a substantial difference between the bioavailability of one or more forms. Only three studies[21,22,35] found a statistically significant difference between supplements and reporting 5-MTHF to be more bioavailable.

However, these findings are subject to a number of limitations. Only two studies[31,32] tested RBC folate as an outcome measure. These studies had a duration of 12 weeks making RBC folate an appropriate choice for these longer clinical trials.[42] RBC folate concentrations respond slowly to changes in folate intake due to the 120-day lifespan of RBCs, which accumulate folate only during erythropoiesis. This makes RBC folate a more reliable indicator of long-term folate status due to being less sensitive to fluctuations in dietary intake than plasma or serum folate.[43] The other seven studies,[19,26,28,29,33,34,36] which assessed two or more different forms of folate ranged from 3 days to 7 weeks and measured plasma or serum folate concentrations at multiple intervals. This is an appropriate and reliable outcome measure for the duration of those trials.[44] However, it has been documented that shorter trial durations require a larger sample size to detect the same treatment effect.[45] An important limitation to be noted is the effect dietary folate intake may have on the results. None of the studies included in this review monitored dietary folate intake in the days before or during the trial period with the exception of the animal studies which were fed controlled diets.[19–22] Due to the sensitivity of plasma and serum folate to fluctuations in dietary folate intake, monitoring dietary folate intake for the duration of the trials would give a clearer indication of the interventions' effect and whether or not changes in dietary intake affected the results. The average number of participants from the studies reviewed was 15. This is another important limitation and may partially explain the lack of statistical significance observed in these trials.

The review uncovered that only one study[26] has directly compared folic acid, folinic acid, and 5-MTHF. The study design was a 3-day quasi-experimental trial including seven male participants. There was no control group or placebo. The study measured 24-h urinary folate levels and observed the excretion rates of each supplement. The results of the study indicate relative differences in the excretion of folates, but suggest that the intestinal absorption was similar among folate groups. Slight differences were observed between treatment groups with folic acid appearing somewhat more

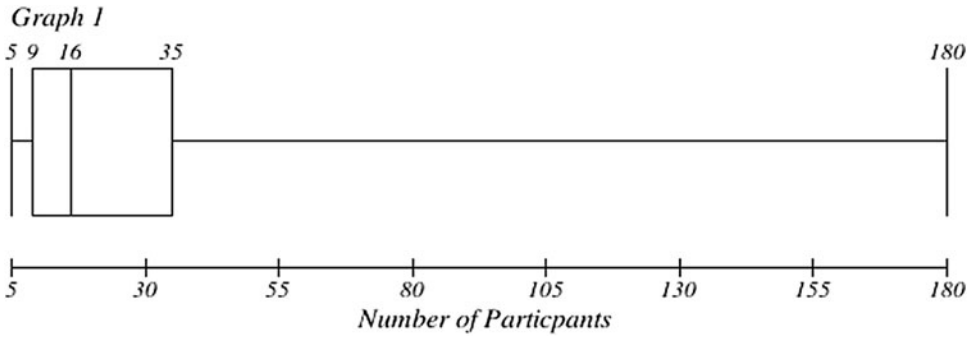

FIG. 2. Box plot of number of participants.

TABLE 6. FOLATE SUPPLEMENTS USED

	Folic acid	*5-Tetra-hydrofolate*	*5-Formyltetra-hydrofolate*	*(6S)-5-methyltetra-hydrofolate*	*10-Formyltetra-hydrofolate*	*(6S)-5,10-methenyltetra-hydrofolate*	*Monoglutamyl folic acid*	*Heptaglutamyl folic acid*	*Hexaglutamyl folic acid*
Bhandari and Gregory[19]	*	*	*						
Kudo et al.[20]	*								
Pérez-conesa et al.[21]	*			*					
Miraglia et al.[22]	*			*					
McGuire et al.[23]			*						
Guelen et al.[24]			*						
Gregory et al.[25]	*								
Gregory et al.[26]	*	*	*	*	*				
Truswell and Kounnayong[27]	*								
Baggott and Tamura[28]			*			*			
Litynski et al.[29]	*			*					
Kok et al.[30]	*								
Melse-Boonstra et al.[31]							*	*	
Melse-Boonstra et al.[32]							*	*	
Pentieva et al.[33]	*			*					
De Meer et al.[34]	*			*					
Melse-Boonstra et al.[36]							*		*
Lamers et al.[35]	*			*					
Verlinde et al.[37]				*					
Hartman-Craven et al.[38]	*								
Obeid et al.[39]	*								
Maki et al.[40]	*								
Lakoff et al.[41]			*						

*Indicates that the article measures this form of folate.

bioavailable. This finding, while preliminary, suggests that differences in bioavailability exist for each supplement form. As folic acid, folinic acid, and 5-MTHF are the most common forms of folate available to health practitioners, assessing effectiveness is an important clinical question and larger clinical trials are needed to observe any true differences in outcome measures.

Two trials[23,27] which tested varying dosages of folate had conflicting results. McGuire et al.[23] found that incrementally increased dosages of folinic acid failed to increase circulating folate levels proportional to dosage. In contrast, Truswell and Kounnavong[27] observed clear increases in serum folate levels in relation to each increased dosage of folic acid. There are several possible explanations for this result. For example, McGuire et al.[23] conducted a four-way crossover trial, where participants were given an individual dose and had blood taken at various intervals over 24 h. Truswell and Kounnavong[27] had participants take one lower dose of folic acid every day for 3 weeks followed by a higher dose of folic acid for another 3 weeks. The differences in study design could be a possible explanation for the variations observed. These results could also suggest that folic acid and folinic acid have different bioavailability at different dosages and warrants further investigation.

Two of the studies[29,31] included in this review considered individuals with MTHFR gene mutations. Both studies observed similar bioavailability between folate derivatives. Melse-Boonstra et al.[31] assessed 180 participants in a 12-week, randomized, double-blind, placebo-controlled trial. Two MTHFR polymorphisms were observed in the participants, 161 with the *CC* genotype and 19 with the *CT* genotype. The results concluded that the bioavailability of polyglutamyl folic acid relative to that of monoglutamyl folic acid did not differ significantly between genotypes. The second study found similar results. Litynski et al.[29] conducted a seven-week quasi-experimental trial in 40 healthy adults. Of these, 20 were wild type and 20 homozygous for the 677C → T polymorphism. The trial found that 5-MTHF displayed similar efficacy in reducing homocysteine as folic acid. Interestingly, a prolonged effect 6 months after ceasing treatment was observed with 5-MTHF in the homozygous participants. This raises important questions surrounding the processing and turnover time of folate in homozygous participants and more research is required to better understand the mechanisms involved.

This review highlights the lack of studies evaluating the bioavailability of folate oral supplements. The discrepancies among the results for dose-dependent studies warrant further experimental investigation. The data from the trials comparing different forms of folate must be interpreted with caution due to the small sample sizes and short trial duration.

Conclusion

The aim of this review was to assess the bioavailability of various forms of folate supplements in healthy populations and animal models. This is an area of great importance as folate supplements are prescribed for a number of different health conditions and disease states. Choosing the most bioavailable form may improve treatment efficacy and patient results. This is the first review to assess the current literature on supplemental folate bioavailability in a healthy population. The review has uncovered some conflicting results and several methodological limitations. In particular, there is need for more research directly assessing the most common forms of folate supplements available to clinicians and the general public so that they can make informed choices. This will have implications for both clinical interventions and patient outcomes.

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Author Disclosure Statement

There are no conflicts of interest and no competing financial interests exist.

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