The bioavailability of various oral forms of folate supplementation in healthy populations and animal models: A systematic Review

Jessica Bayes[a], Dr Nitish Agrawal[b] and Dr Janet Schloss[b]

[a] Endeavour College of Natural Health, 368 Elizabeth St, Melbourne, VIC, 3000, Australia

[b] Endeavour College of Natural Health, 2/269 Wickham St, Brisbane City, QLD, 4006, Australia

Corresponding author details: Jessica Bayes

Email: Jessica.bayes@yahoo.com

Phone: 0401962947

Dr Nitish Agrawal

Email: nitish.agrawal@endeavour.edu.au

Dr Janet Schloss

Email: janet.schloss@endeavour.edu.au

ABSTRACT

Background & 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 (US National Library of Medicine), ProQuest Medical Collection (ProQuest) and ScienceDirect (Elsevier) up to 30th March 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, four 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-MTHF. Dosages ranged from 25µg up to 200mg. 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 is 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 paper will review the current literature focusing on the bioavailability of various oral forms of folate supplements in healthy populations and animal models.

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 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 2-3mg 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 RBC folate samples collected between 2007 and 2010 were analysed. 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 are unable to properly metabolise 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 – methyl tetrahydrofolate 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-Methylenetetrahydrofolate 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 (RFC) and the proton-coupled folate transporter (PCFT) are 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 60–70% 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 in order 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 world-wide. However, there is limited research assessing their bioavailability. This review aims to critically apprise 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 (US 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 papers. 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 JB 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.

INSERT FIGURE #1

Assessment of Risk of Bias and Data Summary Table:

Each paper 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 Randomised Control Trials 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 papers were excluded from the review. The results for quasi-experimental studies are

displayed in Table 1 and the results for randomised control trials (RCTs) are displayed in Table 2. During this process data was extracted from the final articles and summarised in Tables 3 and 4.

INSERT TABLE #1

INSERT TABLE #2

INSERT TABLE #3

INSERT TABLE #4

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 pre-selected data-bases 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 papers 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 hours 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 hours. 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 pre-dosed with folate prior to beginning the trial. Two 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 utilised by 17 of the human trials [23,24,27-41] included plasma or serum folate. Two studies [25,26] measured 24 hour 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.

INSERT TABLE #5

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.

Four studies [31,32,34,39] were conducted on older adults (>50yrs) 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.

INSERT FIGURE #2

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 vs 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 8 trials [21,22,26,29,33-35,37] assessing its efficacy. Table 6 highlights the different supplements used.

INSERT TABLE #6

The dosages used in the human trials varied greatly from only 25µg up to 200mg. Of the twelve studies [19,21,22,26,28,29,31-36] which compared two or more different forms of folate, nine studies [19,26,28,29,31-34,36] showed no significant differences between the groups and three studies [21,22,35] showed 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 twelve 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. ~~The most significant was the short duration of the trials.~~ Only t~~T~~wo studies [31,32] ~~which~~ tested RBC folate as an outcome measure ~~only had a duration of 12 weeks.~~ . These studies had a duration of 12 weeks making RBC folate ~~is generally considered more~~ an appropriate choice for these ~~for~~ longer clinical trials [42]. Red blood cell folate concentrations respond slowly to changes in folate intake due to the 120 day lifespan of red blood cells 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]. ~~Therefore, RBC levels in a 12 week study may not produce meaningful results. A longer duration of 4 months is recommended to observe reliable changes in folate status as a result of the intervention.~~ 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 prior to 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 three day quasi-experimental trial including seven male participants. There was no control group or placebo. The study measured 24 hour 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 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 & 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 4-way crossover trial where participants were given an individual dose and had blood taken at various intervals over 24 hours. Truswell & 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 suggests 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, randomised, 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 dependant studies warrants 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

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