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A pilot trial examining the absorption of oral forms of folate supplementation in a healthy population: A randomised control trial

Jessica Bayes [a,*], Nitish Agrawal [b], Janet Schloss [b]

[a] Endeavour College of Natural Health, Melbourne, Victoria, Australia
[b] Endeavour College of Natural Health, Brisbane, Queensland, Australia

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ABSTRACT

Background & aims: Folate supplements are commonly prescribed by health professionals for a number of different conditions, however, the absorption of the different derivatives remains unclear. This pilot trial aims to assess the absorption of various forms of folate supplements in a healthy population.
Methods: A four-week, single blinded, randomised control trial was conducted on 30 healthy individuals. The intervention included supplementation with 500mcg of either folic acid, folinic acid or 5-Methyltetrahydrofolate (5-MTHF). Fifteen participants were allocated to the intervention and fifteen to the control group for comparison. At baseline (week 0) a case report was completed and pathology tests for serum folate, B12 and MTHFR polymorphisms were conducted. Follow up serum folate blood pathology tests were assessed at week 2 and week 4 along with additional food diaries.
Results: Of the cohort, 87% (n = 26) were found to have a MTHFR polymorphism with both A1298C (n = 12) and C677 T (n = 18) being tested. Only13% (n = 4) of participants had no mutation. The different MTHFR mutations were observed across both the control group and all of the intervention groups. The mean (±SD) baseline folate was 33.7 nmol/L ± 7.55 (Reference range: >9.0). Participants who had both A1298C and C677 T polymorphism had lower baseline folate with a mean (±SD) of 29 nmol/L ± 8.75. During the four weeks an overall increase in mean serum folate was observed in both the folinic acid and 5-MTHF group. The folinic acid intervention saw a mean (±SD) increase of 15.3 nmol/L ± 3.56 and 5-MTHF saw a mean (±SD) increase of 9.1 nmol/L ± 1.67, however a decrease in mean serum folate was detected in the folic acid group. In the folinic acid and 5-MTHF groups, serum folate increases were observed in individuals irrespective of their MTHFR status.
Conclusions: This research has provided a foundation for further work investigating folate absorption. The results of the trial suggest that folinic acid has the best absorption, however, it may not have the best bioavailability. More research is required for greater clarification regarding the absorption and bioavailability of these supplements.

1. Introduction

Folate is the generic term for a B group vitamin found in a wide range of foods including whole grains, legumes and green leafy vegetables [1]). There are three main forms of folate used in nutraceutical supplementation worldwide. These arefolic acid, folinic acid and 5-Methyltetrahydrofolate (5-MTHF) [2]. 5-MTHF is the predominant folate form that undergoes human metabolism and is distributed into peripheral tissues via reduced folate carrier-1, the carrier protein has a very poor affinity for folic acid and is specific for reduced folates [3]. Under normal conditions of dietary intake, folate turns-over slowly, with a half-life of elimination over 100 days [4]. Studies assessing the absorption of these three commonly prescribed supplements is lacking. This project is the first to explore the absorption of these commonly available forms of folate supplements in a healthy population and to describe any differences observed between them. The project results offer a foundation for further work in this area and may guide clinician's decisions when selecting folate supplements for treating their patients

2. Background

Folate plays a critical role in purine nucleotide and thymidylate synthesis and is crucial for the *de novo* production of RNA and DNA

* Corresponding author at: Australian Research Centre in Complementary and Integrative Medicine, University of Technology Sydney, Sydney, Australia
E-mail address: Jessica.bayes@yahoo.com (J. Bayes).

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[5]. Folate is also necessary for vitamin B12 dependent synthesis of methionine, from which S-adenosylmethionine is formed [5]. Dietary folates predominantly exist in a polyglutamate form, which have to be hydrolyzed to monoglutamates in order to be transported [6]. The principal folate found in the blood is 5-MTHF which is transported into the cell by means of carrier- or receptor-mediated transport [5].

A number of different factors can influence folate status such as genetic variations in the MTHFR gene which affects folate metabolism [7]. Between 60–70% of individuals will have at least one polymorphism with 8.5% of the population being homozygous for two of the most common variants, A1298C and C677T [8]. There is a large reduction in enzymatic activity in vitro for the TT genotype with approximately 75% reduction being observed [9]. This leads to a decreased production of 5-MTHF and is associated with elevated plasma homocysteine levels [9].

3. Methodology

A single-blind pilot clinical trial was conducted on thirty healthy individuals to investigate the absorption of three different forms of folate: folic acid, folinic acid and L-5-MTHF. Ethics approval was granted by Endeavour College of Natural Health: Office of Research and registered with the Australian New Zealand Clinical Trials Registry (Trial Id: ACTRN12617001033336). The trial was four weeks in duration with data collected at baseline (week 0), week 2 and week 4. The intervention included supplementation with 500mcg of one of the folate derivatives. Fifteen people were allocated to the intervention (n = 15) and fifteen to the control group (n = 15) for comparison via a computer-generated randomisation sequence. Informed consent was obtained after the participant had time to read the participant information sheet, ask questions and were fully aware of the requirements of the trial and their involvement. Written consent was then obtained and witnessed by a researcher before any requirements of the trial were undertaken. A copy of the consent was given to the participant and the researchers kept a copy of the signed written consent.

All participants were blinded to the oral form of folic acid administered. Researchers were not blinded to the random allocation although the researcher conducting the research was blinded to intervention allocation. Prior to randomisation, participants had their base line blood pathology tests conducted which included vitamin B12, serum folate and MTHFR testing as well as a case report and food diary. Blood samples were collected via venepuncture and conducted at Dorevitch pathology labs in Melbourne, Victoria. Additional blood tests were taken at two and four weeks with the main outcome assessed being the serum folate levels. Food diaries were also recorded throughout the 4 weeks for all participants.

The intervention consisted of folic acid (Blackmores), folinic acid and 5-MTHF (Bioceuticals) capsules. All supplement bottles arrived with no labels. The neutral labels were attached to the bottles listing the supplements as intervention A, B or C. There was no branding on the labels. The label contained the trial name, HREC approval number, intervention group, instructions for use and contact details of the researchers. Only the chief investigator was unblinded. Participants were instructed to take one capsule per day with breakfast after initial baseline blood pathology test. Each capsule provides 500mcg of folate. Participants in the supplement groups were asked to complete a supplement diary which recorded their supplement use and to bring their supplement diary along with their remaining capsules to follow up appointments so unused capsules could be counted to check compliance.

3.1. Sample

The sample was based on a healthy population. The inclusion criteria included participants who were non-pregnant, healthy individuals aged between 18 and 50 years old. All participants were advised not to take any oral supplements for 2 weeks prior to testing. The participants were excluded if they were pregnant or lactating, had a diagnosed malabsorption conditions such as coeliac disease or had a major diagnosed mental condition such as schizophrenia. Participants were also excluded if they were found to be folate deficient after initial baseline testing. Folate deficiency was determined as a reading of <9.0 nmol/L by Dorevitch Pathology. Participants were also excluded if they were taking medications which interfere with folate absorption such as anticonvulsants, metformin, antacids, non-steroidal anti-inflammatory drugs (NSAIDs) and the oral contraceptive pill (OCP).

A total of 32 patients were screened. One participant declined to participate due to travel restrictions and another was excluded due to folate deficiency. Thirty participants were recruited into the trail and allocated into groups via a computer-generated block randomisation sequence. Randomisation was not conducted due to weight, gender or other demographics. All basic descriptive information was collected and can be seen in Table 1. The fifteen included in the intervention arm were divided into three groups with five participants per group. Fig. 1 displays a consort diagram outlining this process. The participants were recruited through Endeavour College of Natural Health Melbourne Campus and included both students and staff members. Recruitment was through an advertisement poster and email.

3.2. Statistical analysis

Statistical analysis of the data was conducted at the conclusion of the trial with STATA Data Analysis and Statistical Software. Descriptive statistics were utilised to give the population details of the participants and were organised and displayed into graphs and charts. Data analysis used a variety of statistical testing options to analyse the data including using Pearson's chi-square test, fishers exact test, continuous unpaired sample *t*-tests with welch's approximation, unpaired *t*-tests and Analysis of Variance (ANOVA). and repeated measures ANOVA with Bonferroni correction.

Table 1
Participant Demographics.

Participants	Demographics
Gender	Female 83.3% (n = 25) Male 16.6% (n = 5)
Age	Mean ± SD 25.5 ± 6.47
Emotional Disorders	16.6% (n = 5)
Skin Conditions	13.3% (n = 4)
Asthma	13.3% (n = 4)
Respiratory Conditions	6.7% (n = 2)
High Cholesterol	6.7% (n = 2)
Sexually transmitted diseases	3.3% (n = 1)
Bone or Joint issues	13.3% (n = 4)
Blood Pressure disorders	6.7% (n = 2)
Migraines	10% (n = 3)
Caffeine/week	Mean ± SD 10.7 ± 8.99
Alcohol/week	Mean ± SD 2.1 ± 2.22
Smoking/day	Mean ± SD 0.2 ± 1.27
Chicken/pork/week	Mean ± SD 2.0 ± 1.47
Fish/week	Mean ± SD 1.4 ± 1.40
Red meat/week	Mean ± SD 1.8 ± 1.34
Current vegan	13% (n = 4)
Past vegan	3% (n = 1)
Current vegetarian	3% (n = 1)
Past vegetarian	23% (n = 7)
Current pescatarian	7% (n = 2)
Past pescatarian	3% (n = 1)

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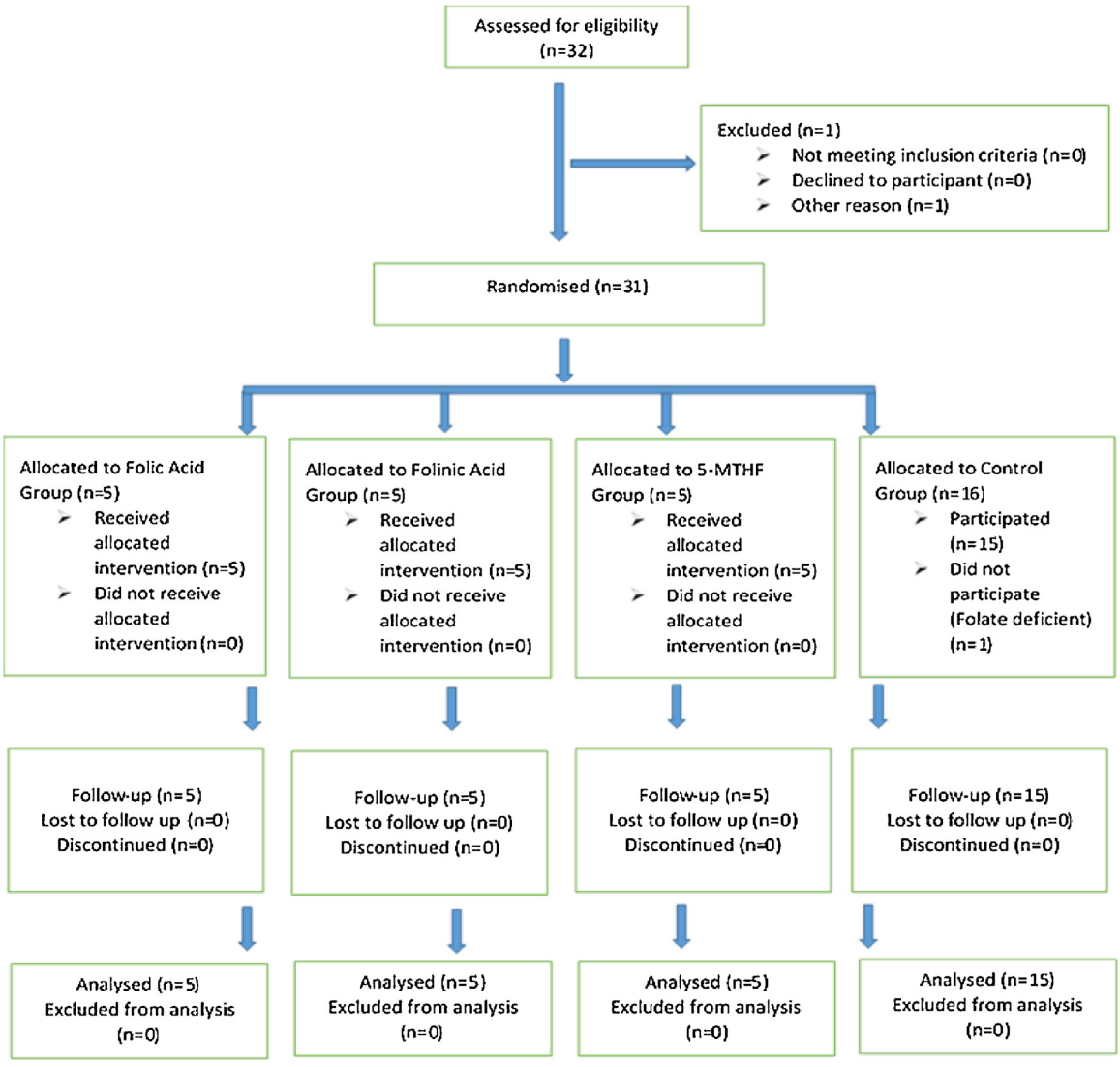

Fig. 1. Consort Diagram.

Pearson's chi-square and fishers exact tests were used to assess associations between categorical variables [10]. Variables such as gender, dietary and lifestyle habits, disease demographics, allergies and dietary supplement use were analysed and the results grouped into tables. ANOVA and t-tests were used to assess whether the means of each group were statistically different from each other [11]. ANOVA was used to show the differences between the three intervention groups with time as the repeated value. This provided the 95% Confidence Interval (CI) and the p value. Results were considered statistically significant when $p=<0.05$ [12].

Histograms were created to assess the distribution of the data sets. Continuous unpaired unequal sample *t*-tests were then performed using Welch's approximation to assess the differences between the intervention and control group at baseline, week 2 and week 4. This provided the mean, standard error (Std Err), 95% CI and p values which have been organised into a table. The main limitation to the analysis is the data size. The sample size is small which may impact statistical significance and outcomes.

4. Demographic analysis

A total of 32 individuals were screened for eligibility for the trial. One individual could not participate due to travel restrictions and one participant had to be excluded after the baseline pathology results indicated that they were folate deficient. This participant was contacted and informed of the results. This left 30 participants who were enrolled into the study with 15 in the control group and 15 in the intervention groups.

Of the 30 participants enrolled in the trial 83% were female and 17% male. The mean (±SD) age of participants was 25.5 SD ± 6.47. The baseline case report indicated that 17% of participants presented with emotional problems, 13% have skin conditions, asthma or blood pressure related issues and 10% suffer from migraines. Table 1 displays the demographics of the participants. Pearsons Chi-square test and Fishers exact test were calculated to demonstrate the distribution of variables across the cohort. The results are displayed in Table 2.

The Pearson's chi-square and fishers exact tests found no significant relationships between the variables.

Dietary habits analysis found the average caffeine consumption to be 11 units per week and alcohol consumption to be 2 standard drinks per week. 97% of people in the trial did not smoke cigarettes. ANOVA tests were completed to compare the folate intake in the diet with caffeine per week, alcohol per week and smoking per day.

4.1. Serum folate analysis

The primary outcome measure of the trail was serum folate which was measured at baseline (week 0), week 2 and week 4 for

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Table 2
Pearson's chi-square and Fishers exact tests comparing each variable across all groups.

Variable	Pearson's chi-square		Fishers exact
Disease or illness	1.36	Pr = 0.22	0.26
Allergies	1.36	Pr = 0.71	0.76
Alcohol/week	2.30	Pr – 0.51	0.71
Smoking/day	1.03	Pr = 0.79	1.00
Caffeine/week	5.48	Pr = 0.48	0.54
Dietary supplement use	10.42	Pr = 0.31	0.17
Gender	3.12	Pr = 0.37	0.67

all participants. The mean baseline folate was 33.7. Participants who had both A1298C and C677T polymorphism had lower baseline folate with a mean of 29. Table 3 shows the serum results across all groups. Statistically significant differences in serum folate were seen between all intervention groups (p = 0.0113; 95% CI 2–5.32) overall and at week 2 (p = 0.0005; 95% CI −19.88 to −6.2) and week 4 (p = 0.0003; 95% CI −17.52 to −5.92). At week 4, the folinic acid group observed mean increase of 15.3 nmol/L ± 3.56 (±SD) of serum folate. The 5-MTHF saw a mean increase in serum folate of 9.1 nmol/L ± 1.67 (±SD). A decrease in mean serum folate at week 4 was detected in the folic acid group after an initial peak in week 2. Fig. 2 displays these changes. The serum folate in the control group remained relatively stable showing a mean increase of only 1.8. The intervention groups combined displayed a mean increase of 6.6. Fig. 3 displays these changes.

Histograms were created to assess the distribution of the data sets. Data was found to be skewed so continuous unpaired unequal sample t-tests were then performed using Welch's approximation to assess the differences between the intervention and control group at baseline, week 2 and week 4. The results are displayed in Table 4.

A repeated measures analysis of variance on ranks with Bonferroni correction was conducted to assess differences between the three intervention groups with time used as the repeated measure. A statistically significant difference was observed p = 0.0113 (95%: CI 2–5.32) indicating that absorption

Table 4
Continuous unpaired sample *t*-test for serum folate results comparing all treatment groups vs. control.

Time	Mean	Std Err	95% CI	P value
Baseline	−3.9	3.85	−11.77 – 3.97	0.32
Week 2	−13.04	3.34	−19.88 to −6.2	0.0005
Week 4	−11.72	2.8	−17.52 to −5.92	0.0003

of the different folate derivatives is statistically significant over 4 weeks. A pair wise test for the three treatment groups was also conducted displayed in Table 5. It shows no statistical difference between the groups at baseline, week 2 or week 4.

4.2. MTHFR status

Of the cohort, 87% (n = 26) were found to have a MTHFR polymorphism and only 13% (n = 4) had no mutation. The different MTHFR mutations were observed across both the control group and all of the intervention groups. The C677 T heterozygous polymorphism was indicated in the folinic acid group (n = 2), folic acid (n = 3), 5-MTHF (n = 2) and the control group (n = 3). The C677 T homozygous polymorphism was indicated in the folinic acid group (n = 1) and the control group (n = 3). The A1298C heterozygous polymorphism was indicated in the folic acid group (n = 1) and control group (n = 3). The A1298C homozygous polymorphism was indicated in the 5-MTHF group (n = 1) and the control group (n = 2). Participants who were heterozygous for both C677 T and A1298C were indicated in the folinic acid group (n = 1), the 5-MTHF group (n = 2) and the control group (n = 2). Participants with no polymorphisms were indicated in the folinic acid group (n = 1), folic acid group (n = 1) and the control group (n = 2).The mean (±SD) baseline folate was 33.7 nmol/L ± 7.55 (Reference range: >9.0). Participants who had both A1298C and C677 T polymorphism had lower baseline folate with a mean (±SD) of 29 nmol/L ± 8.75. A serum folate increase was observed in individuals irrespective of their MTHFR status. Fig. 4 shows the breakdown of the MTHFR mutations across the 4 groups.

Table 3
Folate Results.

Group	No. of participants	Baseline (week 0)	Week 2	Week 4
Folic Acid	5	43.2 ± 11.78	54 ± 7.05	39.9 ± 6.77
Folinic Acid	5	36.6 ± 11.31	53.7 ± 4.52	51.9 ± 6.02
5-MTHF	5	34.2 ± 10.57	42.2 ± 11.45	43.3 ± 8.20
Control	15	32.9 ± 10.46	31.6 ± 8.72	30.2 ± 8.21
ANOVA *analysis*	*P = 0.0113 (95%: CI2–5.32)*			

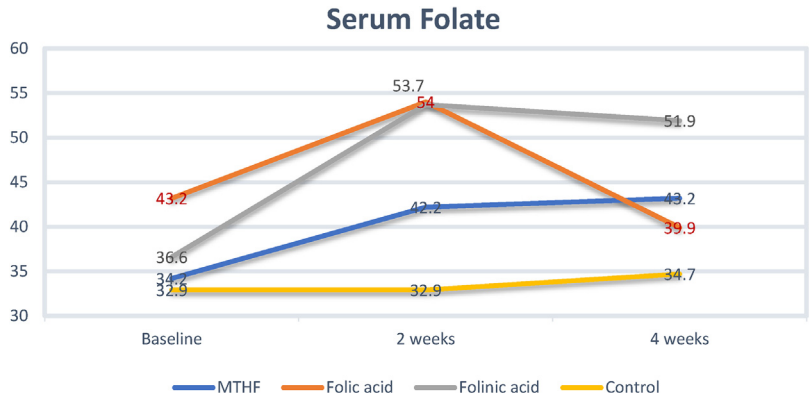

Fig. 2. Serum Folate Results.

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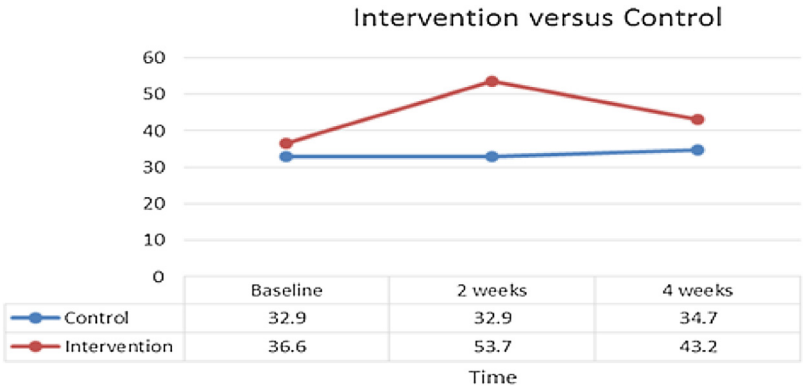

Fig. 3. Intervention vs Control Serum Folate.

Table 5
Unpaired sample *t*-test for serum folate results comparing the three treatment groups.

Time	Mean Diff	Std Err	95% CI	P value
Baseline				
Folinic acid v MTHF	1.92	6.85	−13.37 to 17.21	0.785
Folinic acid v folic acid	−3.54	7.30	−19.82 to 12.74	0.638
MTHF v Folic acid	−5.46	7.008	−21.12 to 10.2	0.454
Week 2				
Folinic acid v MTHF	10.54	5.5	−3.031 to 21.11	0.105
Folinic acid v folic acid	0.125	4.064	−9.77 to 10.02	0.976
MTHF v Folic acid	10.41	6.219	−3.848 to 24.67	0.131
Week 4				
Folinic acid v MTHF	−5.76	4.55	−16.05 to 4.53	0.237
Folinic acid v folic acid	7.34	4.05	−1.71 to 16.39	0.1008
MTHF v Folic acid	1.58	4.76	−9.09 to 12.25	0.747

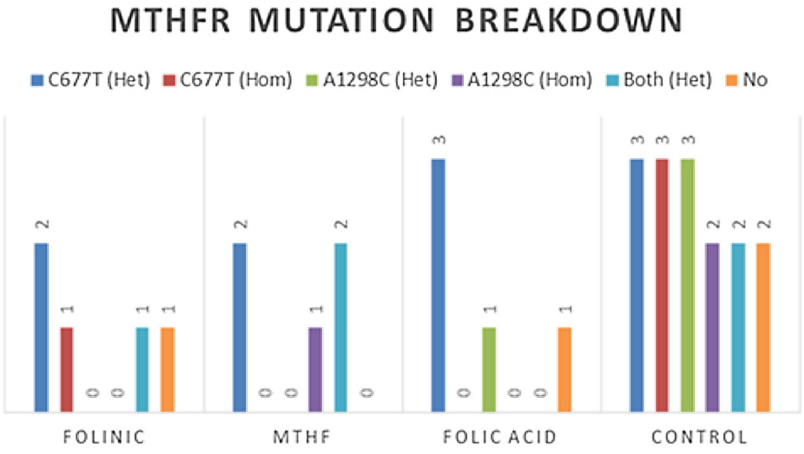

Fig. 4. Distribution of MTHFR Polymorphisms across the groups.

4.3. Supplement compliance

A supplement diary was given to each participant in the intervention groups so they could indicate when their supplements were taken. Compliance was high across all supplement groups with the average number of supplements taken totalling 29/30. 60% of participants took all 30 of their capsules with the minimum number taken being 25/30. All participants brought in their unused supplement to check compliance in addition to their participant dairies to check compliance.

4.4. Dietary folate analysis

A 24-hour food recall was taken for each participant at baseline, week 2 and week 4. The folate content of these food recalls was evaluated with the nutrition software program FoodZone, (Computer software, Sydney, Australia). The dietary folate intake among participants for the duration of the trial averaged 349mcg with 57% of participants failing to reach the recommended daily intake of 400mcg over the four-week trial. Variations were also observed in folate intake for individual participants with the average range of

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152mcg noted. Pearson's chi-squared analysis was conducted using STATA to determine if there were any correlations between dietary folate intake and serum folate results and week 2 and week 4. No statistical significance was observed between the results.

5. Discussion

The primary research question was examining if there is a variation in absorption from different forms of oral folate supplements in a healthy population. From the results, increases in serum folate were observed in supplementation groups and no significant changes were observed among the controls ($p = 0.32$). Statistically significant differences were observed between the folate groups and the control at week 2 ($p = 0.0005$) and week 4 ($p = 0.0003$) per Table 4. Both the folinic acid and 5-MTHF groups saw an increase in serum folate with folinic acid showing greatest efficacy or absorption. ANOVA analysis showed statistically significant differences between the three supplement groups ($p = 0.0113$). However, the ANOVA analysis of all four time points was not statistically significant ($p = 0.8820$). Folinic acid saw a mean increase of 15.3 and 5-MTHF saw a mean increase of 9.1 over the four weeks of supplementation. Interestingly, the folic acid group had an increase after two weeks of supplementation but then decreased after four weeks in serum folate (mean decrease of 3.3 from week 2). This could be due to a number of different reasons. It is possible that a decrease was observed due to folic acid being effectively taken up by cells and thus not appearing in the serum. Further testing of red cell folate may have revealed this theory, however the test is now unavailable in Australia.

Serum folate increased irrespective of MTHFR status, however lower baseline serum folate was observed in participants with both polymorphisms. It has been previously theorised that individuals with MTHFR polymorphisms require the form 5-MTHF in supplements as they are unable to manufacturer 5-MTHF in certain cells as the MTHFR enzyme is reduced [13]. There is variation in function of the MTHFR enzyme between the different polymorphisms, with certain variants seeing a 70% loss of function. A similar distribution of these polymorphisms was observed across each group. Despite the reduced function, the greatest increases in serum folate were observed in the folinic acid group over the four weeks, which requires the MTHFR enzyme to be changed to 5-MTHF in certain cells.

This raises a number of questions. It is possible that folinic acid is well absorbed but not effectively transported into cells leading to continued raised folate levels in the serum. Folic acid is absorbed at around the same rate as folinic acid as seen at the two week mark but as the levels in serum drop by the fourth week, it may indicate that the folic acid is transported into cells rather than staying in the serum. As there were only small numbers in this trial, only speculation can occur with larger trials required.

The 5 MTHF was slow in increasing in serum folate levels. Certain theories can be postulated in relation to result. It is possible that 5 MTHF is not converted back to the simple folate form (THF) which is transported around in serum, hence why it did not increase in the serum as much as folinic and folic acid. Moreover, it is possible that it could be taken up by cells as the whole complex (5 MTHF) but further studies are required.

The decrease in serum folate observed at week 4 in the folic acid group may suggest that it is being taken up by cells rather than staying in the serum. This can be seen by the same rate of serum folate at week two as folinic acid, but then the serum folate drops in the folic acid group but not in the folinic acid group. As postulated, 5-MTHF increased serum folate but at a much lower rate over the four weeks which may indicate that the 5-MTHF complex is being taken up in cells or transported as 5-MTHF rather than be converted to THF or folate in serum. This was the first trial to compare the absorption of folic acid, folinic acid and 5-MTHF in healthy individuals. These results are surprising as they go against commonly held beliefs and recommendations surrounding folate supplements in individuals with MTHFR polymorphisms. Larger studies are required to confirm these results.

Previous studies have also shown that individuals with MTHFR polymorphisms have increased plasma homocysteine levels. Previous studies have reported that approximately 60–70% of the population have an MTHFR polymorphism [8]. Our trial observed polymorphisms in 87% of participants, which is much higher than previous estimates. This may be explained by the small number of participants which could influence sampling variance. The reduced function of the MTHFR enzyme leads to decreased levels of 5-MTHF, which is required to convert homocysteine to methionine [14]. The results from our trial showed that both folinic acid and 5-MTHF increased plasma folate. It would be useful to determine if either supplement causes a change in homocysteine levels in participants with MTHFR polymorphisms.

The serum folate results were compared to the dietary folate intake to see if any correlations occurred. No statistically significant differences between dietary folate intake and serum folate results at week 2 and week 4 were observed. This indicates that changes in serum folate were due to the supplementation only and not dietary folate intake. The major source of dietary folate observed from the dietary analysis was from bread and avocado. The data on dietary folate intake only represents the content found in those foods and doesn't take into account cooking techniques or individual GIT function which can affect how much folate actually gets absorbed. Folate-binding proteins present in foods can also effect bioavailability by protecting dietary folates from capture by intestinal bacteria thereby increasing the efficiency of folate absorption [15]. Other interactions include intestinal pH which can potentially modify conjugase activity, the presence of folate antagonists and factors that alter the rate of gastric emptying [15].

Intestinal absorption and transport of folate should also be considered. The involvement of the reduced folate carrier (RFC) and the proton-coupled folate transporter (PCFT) were outside the scope of the study, however their involvement in mediating folate transport across the epithelia and into systemic tissues should not be overlooked [5]. These folate transporters contribute to folate homeostasis and their function could be tested alongside MTHFR in further folate absorption trials.

The primary study outcome was to describe which oral form of folate changes levels of folate in the blood. A recently published systematic review assessed the bioavailability of different forms of folate in healthy populations [16]. Only three of the 23 studies assessed found a statistically significant difference between different supplement forms of folate and concluded that 5-MTHF may be more bioavailable. Several methodological limitations and conflicting results were observed in the review and the need for further research was emphasised. The current trial results also observed 5-MTHF to be effective at raising serum folate. Previous studies have also shown folic acid supplementation to be effective in raising serum folate levels [17]. However, the trial did not support this finding. A possible explanation could be the short duration of the study. Another possibility could be that only serum folate was measured and not red blood cell (RBC) folate. RBC folate was not offered by the pathology lab however.

5.1. Future direction and recommendations

This research has provided a foundation for further work investigating folate absorption. The results of the trial suggest that folinic acid has the best absorption, however, it may not have the best bioavailability. Future research including testing of the red blood cell (RBC) folate may assist in confirming folate bioavail-

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ability. This is of particular importance due to folic acid being the form used in fortification and is an important public health initiative.

Researching the absorption of different folate derivatives in other conditions and disease states such as in depression or cancer patients would also be beneficial. As there is an increased requirement for folate during pregnancy, further research in this specific demographic is needed. Additional trials assessing individuals with MTHFR polymorphisms and their response to folate supplementation on a larger scale would similarly be beneficial.

In order to investigate these results further, larger scale trials on a healthy population base are needed. Trials which also test RBC folate and homocysteine alongside serum folate and MTHFR are recommended. It is advised that these trials have a minimum three-month duration in order for the RBC folate absorption to be stable and reliable.

6. Significance & conclusion

This pilot study is the first to directly compare the absorption of three widely available forms of folate supplements in a healthy population. It uncovered a number of key findings. Firstly, the analysis suggests that folinic acid may be the best absorbed supplement over four weeks. However, given there was no evidence that the serum folate was being absorbed into tissues following folinic acid supplementation, further analysis is required to determine if it is well utilised by the body. Secondly, the study observed a decrease in serum folic acid after an initial peak in week 2 for individuals taking the folate supplement. This raises speculation as to whether folate is biochemically more efficient in being utilised by the body and thus not present in high amounts in the serum. Finally, the results also demonstrate that individuals with MTHFR polymorphisms may not be limited to only 5-MTHF when selecting folate supplements, as increases in serum folate were observed irrespective of MTHFR status.

This research presents novel and clinical relevant insights into the absorption of folate supplements in individuals with MTHFR polymorphisms and gives rise to questions surrounding absorption verses utilisation. Additionally, this trial draws attention to the need for further research in folate absorption. In order for clinicians to effectively prescribe and treat patients, understanding which forms of folate are not only well absorbed, but also effectively utilised, is of great importance.

Statement of authorship

JS designed the study; JB recruited and randomised the subjects and set up the trial through Dorevitch pathology; JS and NA supervised the study; JB obtained the funding; JS organised the folate supplements, JB and JS analysed the data; JB drafted the manuscript with edits contributed by JS and NA.

Conflicts of interest

The authors declare no conflict of interest regarding the publication of this paper.

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