Folic Acid Supplementation in Postmenopausal Women with Hot Flushes: Phase III Randomised Double-Blind Placebo-Controlled Trial

Ayman Ewies[1], Ikhlaq Ahmed[2], Farook Alazzawi[3], Joan Pitkin[4], Pratima Gupta[5], Mojca Persic[6], Banchhita Sahu[7], Alaa El-Ghobashy[8], Lisa Barraclough[9], Jacqueline Woodman[10], Jaspreet Babrah[2], Sarah Bowdem[2], Deborah Stocken [11], Lucinda Billingham[2], Sudha Sundar[2], and Daniel Rea[2]

[1]Sandwell and West Birmingham Hospitals NHS Trust
[2]University of Birmingham Edgbaston Campus
[3]University Hospitals of Leicester NHS Trust
[4]Northwest University Healthcare NHS Trust
[5]University Hospitals Birmingham NHS Foundation Trust
[6]Royal Derby Hospital
[7]Princess Royal Hospital
[8]Royal Wolverhampton Hospitals NHS Trust
[9]The Christie NHS Foundation Trust
[10]University Hospital Coventry
[11]University of Leeds

July 24, 2020

Abstract

Objective: To assess whether folic acid supplementation ameliorates hot flushes. Design: Double-blind, placebo-controlled randomised trial. Setting: Nine hospitals in England. Population: Postmenopausal women experiencing [?]50 hot flushes weekly. Methods: Women (n=164) were randomly assigned in a 1:1 ratio to receive folic acid 5mg tablet or placebo daily for 12 weeks. Participants recorded frequency and severity of hot flushes in Sloan Diary daily and completed Greene Climacteric and Utian Quality of Life (UQoL) Scales at 4-weekly intervals. Main Outcome Measures: The change in daily Hot Flush Score at week-12 from randomisation based on Sloan Diary Composite Score B calculation. Results: Data of 143 (87%) women was available for the primary outcome. The mean change (SD) in Hot Flush Score at week-12 was -6.98 (10.30) and -4.57 (9.46) for folic acid and placebo group, respectively. The difference between groups in the mean change was -2.41 (95% CI: -5.68, 0.87), p=0.149 and in the adjusted mean change was -2.61 (95% CI: -5.72, 0.49) with p=0.098. There was an increased benefit in the folic acid group regarding changes in total and emotional UQoL scores at week-8 when compared with placebo. The difference in the mean change from baseline was 5.22 (95% CI: 1.16, 9.28) and 1.88 (95% CI: 0.23, 3.52) for total and emotional score, respectively. Conclusions: Folic acid had a greater benefit in reducing Hot Flush Score over 12 weeks in postmenopausal women when compared with placebo; however, the difference did not reach statistical significance. Definitive evidence of benefit requires a larger study.

Tweetable Abstract

Folic acid may ameliorate hot flushes in postmenopausal women but confirmation is required from a larger

study

Introduction

Hot flushes, the most characteristic menopausal symptom, are experienced by up to 75% of menopausal women, and in half of them symptoms are severe enough to seek medical advice.[1] They are associated with oestrogen deprivation; therefore, hormone replacement therapy (HRT) is the first treatment option.[2] However, the perception of the risks and benefits of HRT has changed since the publication of Women's Health Initiative Trial in 2002,[3] and many women are seeking alternatives because of the concerns over increased risks particularly breast cancer.[4, 5] The use of HRT in breast cancer survivors is not recommended because of the potential stimulation of residual cancer and the induction of new hormone-sensitive disease.[6,7] Furthermore, the improved longevity of breast cancer survivors, the increased use of aromatase inhibitors over tamoxifen, leading to profound oestrogen deprivation[8], and the limited success shown by the currently used non-hormonal alternatives[9] made it imperative to find a therapy that is effective and safe.

Hot flushes are possibly triggered within the hypothalamus by increased central noradrenergic activity leading to disturbances in the thermoregulatory centre,[10, 11] and/or activation of oestrogen withdrawal induced up-regulated 5-HT_{2A}receptors by mild internal or external stimuli resulting in a hyperthermic response.[12]Oestrogen replacement was shown to ameliorate hot flushes by interacting with monoamine neurotransmitters in the brain; noradrenaline and serotonin (5-hydroxytryptamine; 5-HT). It was found to significantly decrease plasma noradrenaline, increase plasma serotonin[13] and augment serotonergic activity[14] in postmenopausal women.

Folic acid is involved - via donation of a methyl group - in the monoamine neurotransmitters synthesis.[15] Studies reported that it reduced noradrenaline secretion[16, 17]and increased serotonin activity.[16, 18] Folic acid administered to mice produced an antidepressant-like effect mediated by an interaction with the noradrenergic receptors (α_1 and α_2) and serotonergic receptors (5-$HT_{1A}$and 5-$HT_{2A/2C}$).[16] Four small studies[19-22] reported that folic acid ameliorates hot flushes in postmenopausal women; however, they had substantial methodologic flaws.

We hypothesised that folic acid supplementation ameliorates hot flushes by the same mechanism as oestrogen replacement i.e. by interacting with monoamine neurotransmitters in the brain. It lowers noradrenaline and increases serotonin activities.[23] This randomised controlled trial (RCT) was designed to assess the efficacy of folic acid supplementation *versus* placebo to symptomatic postmenopausal women in terms of amelioration of hot flushes as the primary outcome measure, and to assess the efficacy on other menopausal symptoms and Quality of life (QoL) as secondary outcome measures.

Methods

Study Oversight

The trial is titled: Phase III randomised study of **FO** lic**A** cid supplementation in the management of **M** enopausal symptoms in cancer survivors and healthy postmenopausal women (FOAM Trial). It was co-sponsored by Sandwell and West Birmingham Hospitals NHS Trust and University of Birmingham, and was conducted under the auspices of the Cancer Research UK Clinical Trial Unit (CRCTU Ref No.: MX3009). Guys' and St Thomas' Hospital Pharmacy Manufacturing Unit was responsible for purchasing the trial drug from Actavis (Devon, UK, rebranded as Accord Healthcare in January 2017), and for manufacturing the placebo tablets. Study oversight and monitoring were provided by a trial steering committee and by an independent data and safety monitoring committee. The trial protocol and ethical approval are submitted as Supplementary Document 1 and 2, respectively.

Study Participants

The participants were recruited from 9 NHS hospitals across the UK via menopause, oncology or research clinics. Women also attended for screening by direct self-referral following advertisements placed within the participating hospitals, and local general practitioners' surgeries, pharmacies and libraries. Women were

eligible for enrolment in the study if they were 40 to 70 years of age, with normal baseline serum folate level (3.1 to 20.0μg/L), postmenopausal (either healthy, or breast or endometrial cancer survivors with iatrogenic onset of menopause) and experiencing [?]50 hot flushes per week as quantified from daily Sloan Diary[24] recordings for 7 days prior to randomisation (Supplementary Document 1, p 48). Menopausal status was defined as cessation of menstruation for 12 months or 6 weeks after surgical removal of ovaries. All participants provided written informed consent.

Participants were excluded from randomisation in the following circumstances: (1) Baseline serum folic acid level above the normal laboratory range, (2) Intestinal malabsorption e.g. celiac or Crohn's disease, (3) Chronic renal impairment, (4) Chronic conditions mimicking climacteric presentation e.g. poorly controlled hypertension, hyperglycaemia or thyroid instability, (5) Pernicious anaemia due to vitamin B12 deficiency, (6) Alcohol consumption > 14 units per week, (7) Pheochromocytoma or carcinoid syndrome, (8) Allergy to folic acid, (9) Participation in another clinical trial within 4 weeks prior to enrolment, and/or (10) Taking prohibited medications unless the participant was willing and it was safe to discontinue. In such cases, wash out periods were allowed before randomisation and were estimated based on the drug specifications published on MHRA website[25](Supplementary Document 1, p22).

Study Design and Drug Regimen

Participants were randomly assigned in a 1:1 ratio to receive tablets containing either folic acid 5mg or matched placebo to be taken orally once daily from the time of randomisation for 12 completed weeks. The appearance of the study medications was identical so that participants and researchers were unaware of the study-group assignments throughout the trial. Randomisation was performed centrally in a double-blinded manner via telephone to CRCTU, which allocated treatments using a computer minimisation technique with a random element that was developed by CRCTU. Randomisation was stratified by participant subgroup; healthy women *versus* breast or endometrial cancer survivors and body mass index (BMI) [?]30 *versus* >30. Participants were required to record frequency and severity of hot flushes on daily basis in a Sloan Diary[24] over 12 weeks while taking the study medications. Participants were also requested to complete Greene Climacteric Scale[26-28] and Utian QoL Scale[29] (Supplementary Document 1, p50-51) at entry and at weeks 4, 8 and 12. Blood samples were obtained for serum folate at trial entry and week 12.

Outcome Measures

The primary outcome measure was the change in daily Hot Flush Score at 12 weeks from randomisation based on the validated composite score B calculation[24] (Supplementary Document 1, p49). This was calculated based on frequency and severity as recorded by participants in Sloan Diaries. The secondary outcome measures were the changes at weeks 4, 8 and 12 from randomisation in the following (1) hot flushes frequency as calculated using the frequency score B, (2) hot flushes severity as calculated using the severity score B, (3) occurrence of a response (defined as a reduction in Hot Flush Score of [?]50%) as calculated using composite score B, (4) other menopausal symptoms as measured by the Greene Climacteric Scale, (5) longitudinal QoL data as measured by the Utian QoL Scale. The trial investigated the treatment effect on outcomes in specific prognostic subgroups of healthy women *versus* breast or endometrial cancer survivors and BMI [?]30*versus* >30.

Statistical Analysis

Sample size calculation: The null hypothesis being tested was that there is no difference in the mean change in composite score B at 12 weeks compared across the two treatment groups. Previous literature of 375 breast cancer women randomised to placebo reported a mean Hot Flush Score at randomisation of 15.7 (SD=11.7). A 3.6-point (˜25%) reduction in score was reported, and was expected in women randomised to placebo. The standard deviation of the change from baseline was reported as 7.1. A clinically relevant reduction is an additional [?]20% reduction with folic acid over and above the placebo effect which translates to [?]7-point (˜45%) reduction in Hot Flush Score at 12 weeks. To detect a true 3.4-point mean difference in the change in Hot Flush Score with folic acid compared to placebo, using two-sided type 1 error $\alpha = 0.05$ and 80% power and a within-group standard deviation of 7.1 for the change from baseline, 70 patients are

required per arm i.e. 140 in total. We planned to include 162 women in the study to account for a 15% rate of loss to follow-up. The statistical analysis plan is uploaded as Supplementary Document 3.

Analysis: All outcome measures were recorded longitudinally at screening, 4, 8 and 12 weeks. The primary analysis compared the treatment groups in terms of the primary outcome measure, change in daily Hot Flush Score at 12 weeks from randomisation, using a two-sample t-test. A significance level of $p < 0.05$ was used. The primary outcome measure was analysed using a linear regression model, which evaluated treatment effects adjusted by clinically relevant baseline covariates (number of hot flushes at screening and folate level at baseline) and stratification factors (healthy *versus* cancer as categorical and BMI as continuous). All outcomes were analysed using multi-level mixed effects models, where repeated measurements from baseline through to 12 weeks were analysed as random effects and clinically relevant baseline covariates and stratification factors were forced in to the model as fixed effects. Where the shape of the data appears to be quadratic over time (week), time has been used as a quadratic term in the model. The mean change in serum folate at week-12 from baseline was compared between the groups using a two-sample t-test.

A planned sensitivity analysis was performed which accounted for missing data via multiple imputation for the primary outcome analysis. Women were required to have data available for week 1 to be included. This analysis was performed using a regression-based imputation model using a bootstrap approach. For women with complete data up to a particular week, a multiple regression model was developed that included the outcome at that visit as the dependent variable and outcomes at previous visits, treatment, site, and stratification variables as independent variables. Models were constructed separately for subsequent visits. Missing value was imputed sequentially starting from week 2 to week 12. This was repeated 100 times, resulting in 100 complete analysis datasets. The analyses were performed separately and then combined in to one inference.[30] A sensitivity analysis using the Last Observation Carried Forward (LOCF) imputation procedure was also performed, which used the last observed value for a pariticipant to fill in missing values. The sensitivity analyses were unadjusted and adjusted as described above. Stata v16.0 was used for the analysis.

Results

A total of 1493 women were screened for eligibility from 9 July 2015 through 30 April 2019, and 164 of these women were randomly assigned to receive either folic acid 5mg tablets (n =83) or placebo (n =81). Since women were allowed self-referral, and given the strict inclusion and exclusion criteria, a high number of screened women (89%) were deemed ineligible for randomisation. For 105 (67%) randomised women, full compliance to the 12 weeks of allocated treatment was recorded, with only 13 (8%) women receiving no treatment and compliance was balanced across treatment arms (Figure 1). The percentage of women with available data for the primary outcome was 87% (143; 74 in folic acid and 69 in placebo). The characteristics of the participants at baseline were similar in the two groups as demonstrated in Table 1. The compliance data was collected at weeks 4, 8, and 12 and presented in Table 2.

Primary outcome: The mean Composite Hot Flush Score B decreased over time in both groups and the mean change at week 12 was -6.98 (10.30) and -4.57 (9.46) for folic acid and placebo group, respectively. The difference in the mean change between groups was -2.41 (95% CI: -5.68, 0.87) with t-test giving$p$ =0.149. From the adjusted linear regression model, the difference in the mean change was -2.61 (95% CI: -5.72, 0.49) with$p$ =0.098. There was no statistically significant difference between the two groups at other time points (Figure 2 and Table S1).

Secondary Outcomes: There was no statistically significant difference for severity score B, frequency score B or the number of responders at any time point (Figure S1, Table S1). A lower score for Greene Climacteric Scale represents an improvement in symptoms. The scores were similar for both groups and no statistically significant difference was found at any time point for any subscale score. A higher score equates to better QoL for Utian QoL Score. The scores were similar for both groups and no statistically significant difference was found at any time point for any subscale score except for the total score and emotional score at week 8. The mean changes from baseline in total score and emotional score were statistically significantly higher for

folic acid group when compared to placebo. The number of women with data available for the total score and emotional score at trial entry and week 8 was 151; 77 for folic acid group and 74 for placebo group. The mean change (SD) from baseline in total score was 0.88 (12.54) and -4.34 (12.69) for folic acid group and placebo group, respectively. The difference in the mean change was 5.22 (95% CI: 1.16, 9.28). The mean change (SD) for emotional score from baseline was 1.34 (5.11) and -0.54 (5.12) for the folic acid group and placebo group, respectively. The difference in the mean change was 1.88 (95% CI: 0.23, 3.52). The overall climacteric symptoms and QoL analysis are presented in Figure 2. The detailed analysis for all domains at various time points are presented in Figures S2 and S3 and Table S2. None of the primary or secondary outcomes provided a statistically significant result when analysed using Multilevel mixed-effects modelling (Table S3 and S4).

The mean change (±SD) in serum folate at week 12 was significantly higher in the folic acid group (11.06 ± 3.86) when compared with placebo group (0.66 ± 3.15), and the difference in the mean change was 10.39 (95% CI: 9.18, 11.61) with p <0.001 (Table S5).

Since there was no data for the primary outcome analysis for 21 women, a sensitivity analysis was performed. It was possible to impute data for a further 15 women thus increasing the total number to 158. The mean change (SD) in Hot Flush Score at week 12 was -6.79 (10.21) and -4.09 (9.82) for folic acid and placebo group, respectively. The difference in the unadjusted mean change was -2.69 (95% CI: -5.88, 0.50) with p =0.099. The difference in the adjusted mean change was -2.82 (95% CI: -5.87, 0.24) with p =0.071. The sensitivity analysis was repeated using the LOCF procedure which displayed similar results (Table S6).

The frequency of adverse events was similar in the two treatment groups. In total, 43 adverse events were observed in 19 women; 22 were observed in 12 women on folic acid and 21 were observed in 8 women on placebo. All these were reported as grade 1 except one as grade 2 and five as grade 3. All events resolved spontaneously. The causality of the treatment with these adverse events is hard to ascertain but were considered unlikely to be related. The details of events and grades are provided in Table S7.

Discussion

Main findings:

This RCT showed that folic acid gave greater benefit in reducing the Hot Flush Score in postmenopausal women when compared with placebo. However, the difference between the two groups did not reach statistical significance. There was no statistically significant difference in the secondary outcome measures except in the total and emotional Utian QoL scores at week 8 where a significant improvement was found in the folic acid group. However; this difference disappeared at week 12. This finding was not replicated in a multilevel mixed-effects model analysis.

Interpretation:

It was plausible to hypothesise that folic acid ameliorates hot flushes in postmenopausal women. Tetrahydrofolates; the metabolically active and tissue-usable forms of folic acid, are essential for the biosynthesis of the monoamine neurotransmitters serotonin and noradrenaline. 5-methyltetrahydrofolate, participates in re-methylation of the amino acid metabolite homocysteine, creating methionine. The downstream metabolite of methionine; S-adenosylmethionine must be present as a methyl donor for both the serotonin and catecholamine pathways to function properly. Without the participation of 5-methyltetrahydrofolate in this process, S-adenosylmethionine and neurotransmitter levels decrease in the cerebrospinal fluid. 5-methyltetrahydrofolate was also found to stabilise and enhance production of tetrahydrobiopterin, which is an essential nutrient cofactor in the biosynthesis of serotonin and noradrenaline.[15, 31-34] In addition, 5-methyltetrahydrofolate was shown to cause a significant reduction in the noradrenaline secretion to only 12.9% of control release, probably by duplicating the rate limiting behaviour of a synthetic pteridine cofactor "DL,2-amino4-hydroxy-6,7,dimethyltetrahydropteridine".[17]Moreover, folate deficiency was associated with decreased serotonin activity,[35] and supplementation with folic acid increased CSF levels of 5-HIAA in folate deficient patients with depression.[18] Interestingly, it was found that the regional distribution of

5-methyltetrahydrofolate in the brain was similar to that of serotonin.[36]

Four small studies suggested that folic acid supplementation significantly ameliorated hot flushes in postmenopausal women. The first study, including two groups (n =23 each) of postmenopausal women, reported significant improvement of hot flushes and lowering in plasma levels of 3-methoxy 4-hydroxy phenyl glycol (MHPG, the end metabolite of brain noradrenaline) with daily folic acid 5 mg supplementation for 4 weeks when compared with placebo. There was also significant negative correlation between improvement in hot flushes and the plasma level of MHPG.[19] The second study, including two groups (n =20 each) of postmenopausal women, demonstrated an average of 57% reduction in the frequency in hot flushes with daily folic acid 5 mg supplementation for 4 weeks when compared with no treatment.[20] The third study, including 2 groups (n =35 each) of postmenopausal women, revealed significant improvement in severity, duration, and frequency of hot flushes with daily folic acid 1 mg supplementation for 4 weeks as well as with placebo tablets. However; there was more improvement in the folic acid group.[21] The fourth study included 3 groups (n =40 each) of postmenopausal women taking daily supplementation of folic acid 1 mg, omega-3 1000 mg, or placebo tablets for 12 weeks. There was statistically significant improvement in severity, duration, and frequency of hot flushes in the folic acid group when compared with placebo.[22]Nonetheless, all these studies had serious methodologic flaws. First, they were underpowered with small number of participants. Second, folic acid supplementation was given for a short duration of 4 weeks raising the suspicion of placebo effect. Third, the bias in allocation and assessment cannot be excluded given the poor reporting of the methods. None of the studies specified the method of concealment of study-group assignments. In one study, placebo was not used for comparison. In all studies, women were allocated (by alternation) into the groups. Fourth, two studies used a small dose of 1 mg of folic acid and reported positive results. Last, no validated method to assess the frequency and intensity of the flushes was used, and the improvement was subjectively described by women based on their overall feelings.

Strengths and limitations:

This is the first well-designed trial robustly investigating the hypothesis that folic acid could ameliorate hot flushes in postmenopausal women. The standard therapeutic dose of 5mg was used. The study screening criteria were precise and ensured that a carefully characterised group of women with normal folate levels were included. Randomisation and study conduct were according to a protocol using double-blinding methods of concealment and computerised randomisation. Drop-out rate was lower than expected and the study included a modified-intention-to-treat sensitivity analysis.

The trial demonstrated that folic acid was safe and well-tolerated. In contrast to HRT, literatures reported no concerns about safety of folic acid and daily oral supplement of up to 10 mg folic acid rarely caused side effects in healthy individuals. A few cases of allergic reactions were.[37, 38] A meta-analysis, including 13 RCTs with 49621 participants that compared folic acid *versus* placebo, found no change in overall and site-specific cancer incidence when folic acid supplementation was used at doses higher than those from fortification for average duration of 5.2 years.[39]

We acknowledge that no formal measure was taken to address the issue of multiple testing in the secondary outcome measures. The sample size used in the study assumed a within-group standard deviation of 7.1 for the change from baseline, but we observed greater variability with a standard deviation greater than this in both treatment groups. This combined with observing a smaller difference than anticipated, has resulted in the study being underpowered to detect the clinically relevant difference specified in the design. The planned sensitivity analysis increased the patient population for the primary outcome by a further 15 women. As a result, the analysis showed a trend towards a statistically significant result for the unadjusted (p =0.099) and adjusted (p =0.071) analyses. The overall treatment effect over time from the multilevel mixed-effects model was not statistically significant (p= 0.614). We also considered the treatment effect in women with high frequency of hot flushes at baseline using a cut-off of 72 which is the median number of hot flushes. The treatment effect was -4.62 (-10.66, 1.42) and -0.50 (-3.20, 2.21) in the >72 group and [?]72 group, respectively.

Conclusion:

This rigorously conducted RCT showed that folic acid has greater benefit in reducing Hot Flush Score in postmenopausal women when compared with placebo. However; the difference between groups did not reach statistical significance. While the study was unable to demonstrate an unequivocal benefit, folic acid supplementation represents a cheap, safe and well-tolerated alternative to conventional HRT, and it remains a potential option for those who are not able to take HRT such as breast cancer survivors. Definitive evidence of benefit would however require a larger study.

Acknowledgment

We thank all the women who participated in this trial; Participating Centres: University Hospitals Birmingham NHS Foundation Trust, Sandwell and West Birmingham NHS Trust, London North West University Healthcare NHS Trust, University Hospitals of Leicester NHS Trust, University Hospitals of Derby and Burton NHS Foundation Trust, The Shrewsbury and Telford Hospital NHS Trust, The Royal Wolverhampton NHS Trust, East Cheshire NHS Trust and University Hospital Coventry and Warwickshire; the research nurses who assisted in data collection: Anne Rutland, Laura Butler, Harriet Goddard, Jeanette Brown, Sam Stafford, Rupa Modi, Alison Tilley, Katy English, Elena Michael, Sally Potts, Vanda carter, Emily Eaton, Sharon Rudge, Lisa Hardstaff, Lesley Wilkinson, Pauline Kelly and Kay Sanders; the Senior nurse specialist: Kathy Abernethy; trial pharmacists; Yin May Chin, Rahima Khatun, Matilda Lans, Alexis Spratt, Jill Lyons, Pattrick Lisbett, Ann Adams, Renee Poulsom, Gurminder Sahota, Julie Whitehead, Mojid Khan; CRCTU staff: Claire Gaunt (Team Leader), Cassandra Brookes (senior statistician), Yolande Jefferson-Hulme (senior trial coordinator), Rachel Blundred (senior trial coordinator), Julie Joseph (trial coordinator) and Dalbir Kaur (trial coordinator); Trial Steering Committee: David Sturdee (chair), Adele Francis, Kulsum Jaffer, Jocelyn Bell, Sean Jennings, Elizabeth Benns and Elizabeth Mortlock; Data Monitoring Committee: Saad Amer (chair), Kanna Jayaprakasan and Karen Thomas; and all those not otherwise mentioned above who have contributed to the trial.

Declaration of interest statement

No potential conflict of interest was reported by the authors

The funder and the manufacturers had no role in the design of the study; the collection, analysis, or interpretation of the data; or the writing of the report.

Permissions obtained

Greene Climacteric Scale: Permission has been granted by Menopause Matters

for the Scale to be used for this trial.

Sloan Diary: Permission has been granted by Dr Jeff Sloan at Mayo Clinic, Rochester for the Diary to be used for this trial.

Utian QoL Scale: Questionnaire published by North American Menopause Society to be used freely for clinical or research purposes.

Contribution to Authorship

AE : developed the hypothesis, chief investigator, contributed to the planning, recruitment, data collection and interpretation, and wrote the manuscript.

IA : produced the statistical analysis plan and the statistical analysis report, and critically reviewed the manuscript.

FAL : contributed to the conception, planning, carrying out and collection of data and interpretation, writing up and critical review of the manuscript.

JP : contributed to the carrying out and collection of data, writing up and critical review of the manuscript.

PG : contributed to the planning, carrying out and collection of data, writing up and critical review of the manuscript.

MP : contributed to the carrying out and collection of data, writing up and critical review of the manuscript.

BS : contributed to the carrying out and collection of data, writing up and critical review of the manuscript.

AEG : contributed to the carrying out and collection of data, writing up and critical review of the manuscript.

LB : contributed to the carrying out and collection of data, writing up and critical review of the manuscript.

JW : contributed to the carrying out and collection of data, writing up and critical review of the manuscript.

JB : contributed to the planning, carrying out, and critical review of the manuscript.

SB : contributed to the conception, planning, carrying out, writing up and critical review of the manuscript.

DS: produced the initial statistical analysis plan and power calculation, writing up and critical review of the manuscript.

LB : critically reviewed the statistical analysis, and contributed to writing up and critical review of the manuscript.

SS : contributed to the conception, planning, carrying out, writing up and critical review of the manuscript.

DR : contributed to the conception, planning, carrying out, data collection and interpretation, writing up and critical review of the manuscript.

Details of Ethics Approval

The trial was approved by the United Kingdom Medicines and Healthcare Products Regulatory Authority (MHRA), West Midlands-The black Country Research Ethics Committee (REC Ref No.: 14/WM/0093, Date: 06/05/2014), and the research and development departments at the 9 participating NHS hospitals.

Funding

The trial was funded by a grant from "Research for Patient Benefit", Ref: PB-PG-1111-26094. The awarded grant included external peer review for scientific quality and priority assessment by a public involvement panel.

References

1. Freedman RR. Hot flashes: behavioral treatments, mechanisms, and relation to sleep. The American journal of medicine. 2005 Dec 19;118 Suppl 12B:124-30.

2. Maclennan AH, Broadbent JL, Lester S, Moore V. Oral oestrogen and combined oestrogen/progestogen therapy versus placebo for hot flushes. Cochrane database of systematic reviews (Online). 2004 Oct 18(4):CD002978.

3. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. Jama. 2002 Jul 17;288(3):321-33.

4. Kargozar R, Azizi H, Salari R. A review of effective herbal medicines in controlling menopausal symptoms. Electron Physician. 2017 Nov;9(11):5826-33.

5. Albertazzi P. A review of non-hormonal options for the relief of menopausal symptoms. Treat Endocrinol. 2006;5(2):101-13.

6. Singh P, Oehler MK. Hormone replacement after gynaecological cancer. Maturitas. 2010 Mar;65(3):190-7.

7. Biglia N, Mariani L, Marenco D, Robba C, Peano E, Kubatzki F, et al. Hormonal replacement therapy after gynaecological cancer. Gynakologisch-geburtshilfliche Rundschau. 2006;46(4):191-6.

8. Morales L, Neven P, Timmerman D, Christiaens MR, Vergote I, Van Limbergen E, et al. Acute effects of tamoxifen and third-generation aromatase inhibitors on menopausal symptoms of breast cancer patients. Anticancer Drugs. 2004 Sep;15(8):753-60.

9. Nedrow A, Miller J, Walker M, Nygren P, Huffman LH, Nelson HD. Complementary and alternative therapies for the management of menopause-related symptoms: a systematic evidence review. Arch Intern Med. 2006 Jul 24;166(14):1453-65.

10. Freedman RR. Biochemical, metabolic, and vascular mechanisms in menopausal hot flashes. Fertility and sterility. 1998 Aug;70(2):332-7.

11. Freedman RR, Woodward S, Sabharwal SC. Alpha 2-adrenergic mechanism in menopausal hot flushes. Obstetrics and gynecology. 1990 Oct;76(4):573-8.

12. Berendsen HH. The role of serotonin in hot flushes. Maturitas. 2000 Oct 31;36(3):155-64.

13. Blum I, Vered Y, Lifshitz A, Harel D, Blum M, Nordenberg Y, et al. The effect of estrogen replacement therapy on plasma serotonin and catecholamines of postmenopausal women. Israel journal of medical sciences. 1996 Dec;32(12):1158-62.

14. Lippert TH, Filshie M, Muck AO, Seeger H, Zwirner M. Serotonin metabolite excretion after postmenopausal estradiol therapy. Maturitas. 1996 May;24(1-2):37-41.

15. Miller AL. The methylation, neurotransmitter, and antioxidant connections between folate and depression. Altern Med Rev. 2008 Sep;13(3):216-26.

16. Brocardo PS, Budni J, Kaster MP, Santos AR, Rodrigues AL. Folic acid administration produces an antidepressant-like effect in mice: evidence for the involvement of the serotonergic and noradrenergic systems. Neuropharmacology. 2008 Feb;54(2):464-73.

17. Lucock MD, Green M, Levene MI. Methylfolate modulates potassium evoked neuro-secretion: evidence for a role at the pteridine cofactor level of tyrosine 3-hydroxylase. Neurochemical research. 1995 Jun;20(6):727-36.

18. Botez MI, Young SN, Bachevalier J, Gauthier S. Effect of folic acid and vitamin B12 deficiencies on 5-hydroxyindoleacetic acid in human cerebrospinal fluid. Annals of neurology. 1982 Nov;12(5):479-84.

19. Gaweesh SS, Abdel-Gawad MM, Nagaty AM, Ewies AA. Folic acid supplementation may cure hot flushes in postmenopausal women: a prospective cohort study. Gynecol Endocrinol. 2010 Sep;26(9):658-62.

20. Grodnitskaya E, Kurtser MA. Effect of folic acid supplementation on hot flushes in healthy menopausal women. Climacteric. 2011 Jun 2011;14:87.

21. Bani S, Hasanpour S, Farzad Rik L, Hasankhani H, Sharami SH. The effect of folic Acid on menopausal hot flashes: a randomized clinical trial. J Caring Sci. 2013 Jun;2(2):131-40.

22. Jahdi F, Chamani M, Neisani SL, Haghani H, Mojab F. Comparing the effect of Omega3 and that of Folic Acid on menopause-related hot flashes. Complementary Medicine Journal. 2018;3(28):2353.

23. Gaweesh S, Ewies AA. Folic acid supplementation cures hot flushes in postmenopausal women. Med Hypotheses. 2010 Feb;74(2):286-8.

24. Sloan JA, Loprinzi CL, Novotny PJ, Barton DL, Lavasseur BI, Windschitl H. Methodologic lessons learned from hot flash studies. J Clin Oncol. 2001 Dec 1;19(23):4280-90.

25. (MHRA) MaHpRA. Find product information about medicines. Last accessed 28 December 2019 on: https://wwwgovuk/government/organisations/medicines-and-healthcare-products-regulatory-agency. 2019.

26. Greene JG. A factor analytic study of climacteric symptoms. J Psychosom Res. 1976;20(5):425-30.

27. Greene JG, Cooke DJ. Life stress and symptoms at the climacterium. Br J Psychiatry. 1980 May;136:486-91.

28. Greene JG. Constructing a standard climacteric scale. Maturitas. 1998 May 20;29(1):25-31.

29. Utian WH, Janata JW, Kingsberg SA, Schluchter M, Hamilton JC. The Utian Quality of Life (UQOL) Scale: development and validation of an instrument to quantify quality of life through and beyond menopause. Menopause (New York, NY. 2002 Nov-Dec;9(6):402-10.

30. Little R, Yau L. Intent-to-Treat Analysis for Longitudinal Studies with Drop-Outs. Biometrics. 1996;52(4):1324-33.

31. Bottiglieri T. Homocysteine and folate metabolism in depression. Progress in neuro-psychopharmacology & biological psychiatry. 2005 Sep;29(7):1103-12.

32. Bottiglieri T, Hyland K, Laundy M, Godfrey P, Carney MW, Toone BK, et al. Folate deficiency, biopterin and monoamine metabolism in depression. Psychological medicine. 1992 Nov;22(4):871-6.

33. Abou-Saleh MT, Anderson DN, Collins J, Hughes K, Cattell RJ, Hamon CG, et al. The role of pterins in depression and the effects of antidepressive therapy. Biological psychiatry. 1995 Oct 1;38(7):458-63.

34. Hyndman ME, Verma S, Rosenfeld RJ, Anderson TJ, Parsons HG. Interaction of 5-methyltetrahydrofolate and tetrahydrobiopterin on endothelial function. American journal of physiology. 2002 Jun;282(6):H2167-72.

35. Botez MI, Young SN, Bachevalier J, Gauthier S. Folate deficiency and decreased brain 5-hydroxytryptamine synthesis in man and rat. Nature. 1979 Mar 8;278(5700):182-3.

36. Korevaar WC, Geyer MA, Knapp S, Hsu LL, Mandell AJ. Regional distribution of 5-methyltetrahydrofolic acid in brain. Nature: New biology. 1973 Oct 24;245(147):244-5.

37. Butterworth CE, Jr., Tamura T. Folic acid safety and toxicity: a brief review. The American journal of clinical nutrition. 1989 Aug;50(2):353-8.

38. Hellstrom L. Lack of toxicity of folic acid given in pharmacological doses to healthy volunteers. Lancet. 1971 Jan 9;1(7689):59-61.

39. Vollset SE, Clarke R, Lewington S, Ebbing M, Halsey J, Lonn E, et al. Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: meta-analyses of data on 50,000 individuals. Lancet. Mar 23;381(9871):1029-36.

Table/Figure Caption List

Figure 1: Trial Profile

Figure 2: Comparison between the treatment groups in the mean change in hot flushes, menopausal symptoms and quality of life over time from randomisation to week 12

Table 1. Baseline characteristics of trial participants

Table 2: Rate of compliance with treatment

Supplementary

Figure S1: Figure S1: Comparison between the treatment groups in the mean change in the Hot Flush Scores over time from randomisation to week 12

Figure S2: Figure S2: Comparison between the treatment groups in the mean change in the domain scores of the Greene Climacteric Scale from randomisation to week 12

Figure S3: Figure S3: Comparison between the treatment groups in the mean change in the domain scores of the Utian Quality of Life Scale from randomisation to week 12

Table S1: Changes in Hot Flush Score B from randomisation - primary and secondary outcomes

Table S2: Changes in Greene Climacteric Scale and Utian Quality of Life Score from randomisation – secondary outcome measures

Table S3: Multilevel mixed-effects model fitted to weekly Hot Flush Score from randomisation to week 12 based on Sloan Diary Composite Score B

Table S4: Treatment effect from multilevel mixed-effects model fitted to all secondary outcomes

Table S5: Comparison of treatment groups as regards week 12 Folate levels

Table S6: Sensitivity analysis for primary outcome measure of change in daily Hot Flush Score at week 12 from randomisation from Sloan Diary composite score B

Table S7: Reported Adverse Events

	Folic Acid n=83	**Placebo** n=81	**Total** n=164
Age (years)			
Mean ± SD	55.4 ±5.1	56.2±5.9	55.8 ±5.5
Patient Subgroups n (%)			
Healthy Woman	67 (81)	66 (82)	133 (81)
Breast Cancer Survivor	14 (17)	14 (17)	28 (17)
Endometrial Cancer Survivor	2 (2)	1 (1)	3 (2)
Body Mass Index n (%)			?¿?
30	64 (77)	63 (78)	127 (77)
>30	19 (23)	18 (22)	37 (23)
Number of Hot Flushes at Screening			
Mean ± SD	85 ±37	85 ±51	85 ±44
Baseline Folate Level (µg/L)			
Mean ± SD	7.8 ±3.1	7.6 ±3.1	7.7 ±3.1

Table 1. Baseline characteristics of trial participants

SD = Standard Deviation

	Folic Acid n=83	**Placebo** n=81	**Total** n=164
Compliance n (%)			
0%	6 (7)	7 (8)	13 (8)
33%	11 (13)	12 (15)	23 (14)
67%	11 (13)	12 (15)	23 (14)
100%	55 (67)	50 (62)	105 (64)

Table 2: Rate of compliance with treatment

Note: Compliance data was collected at week 4, 8 and 12

100% - women were compliant throughout the trial

67% - women were compliant for two thirds of the treatment period

33% - women were compliant for one third of the treatment period

Hosted file

Figure 1-Final.docx available at https://authorea.com/users/345939/articles/472010-folic-acid-supplementation-in-postmenopausal-women-with-hot-flushes-phase-iii-randomised-double-blind-placebo-controlled-trial

Hosted file

Figure 2-Final.docx available at https://authorea.com/users/345939/articles/472010-folic-acid-supplementation-in-postmenopausal-women-with-hot-flushes-phase-iii-randomised-double-blind-placebo-controlled-trial