Folic acid supplementation in postmenopausal women with hot flushes: phase III randomised double-blind placebo-controlled trial

AAA Ewies, a,b I Ahmed, c F Al-Azzawi, d J Pitkin, e,f P Gupta, g M Persic, h B Sahu, i A Elgobashy, j L Barracough, k J Woodman, l J Babrah, c S Bowden, c D Stocken, m L Billingham, c S Sundar, a,b D Rea b,n

a Sandwell and West Birmingham Hospitals NHS Trust, Birmingham, UK b Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, UK c Cancer Research UK Clinical Trials Unit (CRCTU), University of Birmingham, Birmingham, UK d University Hospitals of Leicester NHS Trust, Leicester, UK e London Northwest University Healthcare NHS Trust, Harrow, UK f Imperial College London, London, UK g University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK h University Hospital of Derby and Burton NHS Foundation Trust, Derby, UK i Princess Royal Hospital, Shrewsbury and Telford NHS Trust, Shrewsbury, UK j The Royal Wolverhampton NHS Trust, Wolverhampton, UK k The Christie NHS Foundation Trust, Manchester, UK l University Hospital Coventry and Warwickshire, Coventry, UK m Leeds Institute of Clinical Trials Research, University of Leeds, Leeds, UK n University Hospitals of Birmingham, Birmingham, UK

Correspondence: AAA Ewies, Pan Birmingham Gynaecological Cancer Centre, Birmingham City Hospital, Dudley Road, Birmingham B18 7QH, UK. Emails: e.ayman@bham.ac.uk; ayman.ewies@nhs.net

Accepted 9 February 2021.

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 5 mg tablet or placebo daily for 12 weeks. Participants recorded frequency and severity of hot flushes in a Sloan Diary daily and completed Greene Climacteric and Utian Quality of Life (UQoL) Scales at 4-week 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 were 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 to 0.87) (P = 0.149) and in the adjusted mean change −2.61 (95% CI −5.72 to 0.49) (P = 0.098). Analysis of secondary outcomes indicated 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 The study was not able to demonstrate that folic acid had a statistically significant greater benefit in reducing Hot Flush Score over 12 weeks in postmenopausal women when compared with placebo.

Keywords Folic acid, hot flushes.

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

Please cite this paper as: Ewies AAA, Ahmed I, Al-Azzawi F, Pitkin J, Gupta P, Persic M, Sahu B, Elgobashy A, Barracough L, Woodman J, Babrah J, Bowden S, Stocken D, Billingham L, Sundar S, Rea D. Folic acid supplementation in postmenopausal women with hot flushes: phase III randomised double-blind placebo-controlled trial. BJOG 2021; https://doi.org/10.1111/1471-0528.16739.

Registration: The trial was registered with International Standard Randomised Controlled Trials Number (ISRCTN): 98158824. https://www.isrctn.com/ISRCTN98158824?q=FOLIC%20ACID&filters=sponsorOrganisation:University%20of%20Birmingham&sort=&offset=2&totalResults=2&page=1&pageSize=10&searchType=advanced-search. European Union Drug Regulating Authorities Clinical Trials Database (Eudract) number: 2013-004246-41.

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Introduction

Hot flushes are experienced by 75% of menopausal women and half of them seek medical advice for severe symptoms. Since oestrogen deficiency is the cause, hormone replacement therapy (HRT) is the first line therapy. However, the publication of the Women Health Initiative trial in 2002 has dissuaded women from taking HRT due to concerns over potential adverse effects. Furthermore, the use of HRT in breast cancer survivors is not recommended for fear of recurrence, and the management of vasomotor symptoms in these women has become a pressing clinical challenge due to improved survival rates such that good quality of life has become a benchmark for successful therapy. The increased use of aromatase inhibitors leading to profound oestrogen deprivation and the limited relief achieved by the currently available non-hormonal remedies have made it necessary to search for a new effective and safe therapy.

Hot flushes are triggered by instability in the thermoregulatory centre within the hypotalamus or the vasomotor centres in the medulla due to increased central noradrenergic activity. In addition, the reduction in oestrogen activity increases the expression of 5-HT2A receptors, leading to lowered threshold to internal or external stimuli and resulting in peripheral vasodilation. Oestrogen therapy regulates monoamine neurotransmitters in the brain, noradrenaline and serotonin, which are the basis of its ability to prevent hot flushes. It was found significantly to decrease plasma noradrenaline, increase plasma serotonin and augment serotonergic activity in postmenopausal women.

Folic acid is involved, via donation of a methyl group, in the synthesis of monoamine neurotransmitters. Studies have reported that it reduced noradrenaline secretion and increased serotonin activity. In rodents treated with folic acid, an antidepressant effect was observed through the regulation of the noradrenergic receptors (α1 and α2) and serotonergic receptors (5-HT1A and 5-HT2A). Four small studies have reported that folic acid ameliorates hot flushes in postmenopausal women; however, these studies had substantial methodological 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, lowering noradrenaline and increases serotonin activities. The present 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 the Phase III randomised study of FOlic Acid supplementation in the management of Menopausal 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). The trial was funded by a grant from ‘Research for Patient Benefit’, Ref: PB-PG-1111-26094. 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 available on request.

Study participants

The participants were recruited from nine hospitals across the UK via menopause, oncology or research clinics. Women were eligible for enrolment in the study if they were 40–70 years of age, with normal baseline serum folate level (3.1–20.0 µg/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 recordings for 7 days prior to randomisation. 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. coeliac 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) phaeochromocytoma or carcinoid syndrome; (8) allergy to folic acid; taking prohibited medications unless the participant was willing and it was safe to discontinue doing so. In such cases, wash out periods were allowed before randomisation and were estimated based on the drug specifications published on MHRA website.

Study design and drug regimen

Participants were randomly assigned in a 1:1 ratio to receive tablets containing either folic acid 5 mg or matched
placebo to be taken orally once a day 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) \( \leq 30 \) versus \( >30 \). Participants were required to record the frequency and severity of hot flushes on a daily basis in a Sloan Diary over 12 weeks while taking the study medications. Participants were also requested to complete the Greene Climacteric Scale and Utian QoL Scale 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. 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 \( \geq 50\% \)) 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 \( \leq 30 \) versus \( >30 \). Data on planned exploratory translational outcomes were not generated for logistical reasons in relation to the Covid-19 pandemic.

### 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 between 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 reduction (\(-25\%\)) 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 \( \geq 20\% \) reduction with folic acid over and above the placebo effect, which translates to \( \geq 7 \)-point reduction (~45%) 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 with placebo using a 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.

### Analysis

All outcome measures were recorded longitudinally at screening, 4, 8 and 12 weeks. The analysis used an intention-to-treat type approach in which all women were included regardless of their compliance with treatment. 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 with a significance level of \( P < 0.05 \). 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 into the model as fixed effects. Where the shape of the data appeared to be quadratic over time (week), time was 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. \( P \)-values for all secondary outcomes were included as indicators of the strength of evidence, not for decision-making.

A planned sensitivity analysis was performed which accounted for missing data via multiple imputation for the primary outcome analysis. For week 1 to be included, women were required to have data available. 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 into one inference. A sensitivity analysis using the Last Observation Carried Forward (LOCF) imputation procedure was also performed, which used the last observed value for a
participant to fill in missing values. The sensitivity analyses were unadjusted and adjusted as described above. Stata v16.0 (StataCorp LLC, College Station, TX, USA) was used for the analysis.

**Patient involvement**
Patient and public involvement in the study was minimal. It was limited to input into the funding application and occasional Steering Committee Meeting attendance. This was a reflection of the time when the study was designed and planned in year 2012.

**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 5 mg tablets (n = 83) or placebo (n = 81). As 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 with the 12 weeks of allocated treatment was recorded, with only 13 (8%) women receiving no treatment; compliance was balanced across treatment arms (Figure 1). The percentage of women with available data for the primary outcome was 87% (143; 74 in the folic acid group and 69 in the placebo group). The characteristics of the participants at baseline were similar in the two groups (Table 1). The compliance data were collected at weeks 4, 8 and 12 and are presented in Table 2.

**Primary outcome**
The mean Composite Hot Flush Score B decreased over time in both groups and the mean change (SD) at week 12 was −6.98 (10.30) and −4.57 (9.46) for the folic acid and placebo group, respectively. The difference in the mean change between groups was −2.41 (95% CI −5.68 to 0.87) with t-test giving P = 0.149. From the adjusted linear regression model, the difference in the mean change was 10.39 (95% CI 9.18–11.61) with P < 0.001 (Table S5).

As there were 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 the folic acid and the placebo group, respectively. The difference in the unadjusted mean change was −2.69 (95% CI −5.88 to 0.50) with P = 0.099. The difference in the adjusted mean change was −2.82 (95% CI −5.87 to 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 20 women: 22 in 12 women on folic acid and 21 in eight women on placebo. All events resolved spontaneously. The causality of the treatment with these adverse events is hard to ascertain but was considered unlikely to be related. Details of events and grades are provided in Table S7.

**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 the 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 the 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 with the exception of 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 the folic acid group than for the placebo group. A total of 151 women had data available for the total score and emotional score at trial entry and week 8: 77 in the folic acid group and 74 in the placebo group. The mean change (SD) from baseline in total score was 0.88 (12.54) and −4.34 (12.69) for the folic acid group and the 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. 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 (Tables 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) than in the placebo group (0.66 ± 3.15); the difference in the mean change was 10.39 (95% CI 9.18–11.61) with P < 0.001 (Table S5).

As there were 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 the folic acid and the placebo group, respectively. The difference in the unadjusted mean change was −2.69 (95% CI −5.88 to 0.50) with P = 0.099. The difference in the adjusted mean change was −2.82 (95% CI −5.87 to 0.24) with P = 0.071. The sensitivity analysis was repeated using the LOCF procedure, which displayed similar results (Table S6).

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**Discussion**

**Main findings**
This RCT was not able to demonstrate that folic acid had a statistically significant greater benefit in reducing Hot Flush
Score over 12 weeks in postmenopausal women when compared with placebo. This may be due to a higher than expected response to placebo. 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 treatment group. 

**Figure 1.** Trial profile. *Concomitant medications breakdown (n = 426): HRT: 238 (56%), Serotonin reuptake inhibitors 48 (11%), LNG-IUS or oestrogen implant: 32 (8%), Tamoxifen: 27 (6%), None steroidal anti-inflammatory: 15 (34%), Herbal remedies: 9 (2%), Folic acid: 6 (1%), Others 50 (12%).
double-blinding methods of concealment and computerised and study conduct were according to a protocol using and ensured that a carefully characterised group of women 5 mg was used. The study screening criteria were precise postmenopausal women. The standard therapeutic dose of the hypothesis that folic acid can ameliorate hot flushes in This is the first well-designed trial robustly investigating Strengths and limitations
This is the first well-designed trial robustly investigating acid group. However; this difference disappeared at week 12. This finding was not replicated in a multilevel mixed-effects model analysis.

Table 1. Baseline characteristics of trial participants

|                        | Folic acid | Placebo | Total |
|------------------------|------------|---------|-------|
| n − 83                 |            |         |       |
| Age (y)                | 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 |

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.

Table 2. Rate of compliance with treatment

| s          | 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)      |

SD, standard deviation.

acidity. The 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, which is consistent with previous reports showing that a daily supplement of up to 10 mg folic acid rarely caused side effects in healthy individuals. A meta-analysis, including 13 RCTs with 49 621 participants that compared folic acid with placebo, found no change in overall or site-specific cancer incidence when folic acid supplementation was used at doses higher than those for fortification for an average duration of 5.2 years.

We acknowledge that no formal measures were taken to address the issue of multiple testing in the secondary outcome measures but intend these to be used only as indicators of the strength of evidence. A limitation of this study is that due to slow recruitment, the power was reduced to reduce the required sample size. 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 a 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 (95% CI, −10.66 to 1.42) and −0.50 (95% CI, −3.20 to 2.21) in the >72 group and ≤72 group, respectively.

The major limitation in this study was the higher than expected placebo response, which surpassed previously recorded responses in this field. The question that remained unanswered was whether a larger population size might have shown a statistically significant difference or that folic acid supplementation might not be superior to placebo.

Interpretation
It was plausible to hypothesise that folic acid ameliorates hot flushes in postmenopausal women. Tetrahydrofolates, the metabolically active forms of folic acid, are essential for the biosynthesis of serotonin and noradrenaline. 5-Methyltetrahydrofolate participates in re-methylation of the amino acid metabolite homocysteine, creating...
methionine. S-adenosylmethionine, the methionine metabolite, acts as a methyl donor allowing both the serotonin and catecholamine pathways to function properly. 5-Methyltetrahydrofolate has also been shown to augment production of tetrahydrobiopterin, which is an essential nutrient cofactor in the biosynthesis of serotonin and noradrenaline. Moreover, folate deficiency has been associated with reduced serotonin activity, and folic acid supplementation increased serotonin metabolite level in the cerebrospinal fluid of patients suffering from depression.

Four small studies suggested that folic acid supplementation significantly ameliorated hot flushes in postmenopausal women. The first study, which included two groups (n = 23 each), reported significant improvement of hot flushes and lowering in the brain noradrenaline end metabolite with daily folic acid 5 mg supplementation for 4 weeks when compared with placebo. The second study, which included two groups (n = 20 each), demonstrated an average 57% reduction in the frequency in hot flushes with daily folic acid 5 mg supplementation for 4 weeks when compared with no treatment. The third study, which included two groups (n = 35 each), 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, with more improvement in the folic acid group. The fourth study included three groups (n = 40 each) respectively taking a daily supplement of folic acid 1 mg, Omega-3 1000 mg or placebo tablets for 12 weeks. There was a statistically significant improvement in severity, duration and frequency of hot flushes in the folic acid group when compared with placebo. However, all these studies had serious methodological flaws. First, they were underpowered with small sample size. Secondly, folic acid supplementation was given for a short duration of 4 weeks, raising the suspicion of a placebo effect. Thirdly, bias in allocation and assessment cannot be excluded given the poor reporting of the methods. In one study, placebo was not used for comparison. In all studies, women were allocated by alternation into the

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. A – Composite Score B; B – Total Score of Greene Climacteric Scale; C – Total Score of Utian Quality of Life Scale.
groups. Fourthly, 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 overall feelings.

Conclusion
This RCT was not able to demonstrate that folic acid had a statistically significant greater benefit in reducing Hot Flush Score over 12 weeks in postmenopausal women when compared with placebo. This may be due to a higher than expected response to placebo. Definitive evidence of benefit would require a larger study.

Disclosure of interests
SB has reported grants from National Institute of Health Research (NIHR) Research for Patient Benefit during the conduct of the study. DR has reported personal fees from Pfizer, personal fees from Novartis, personal fees from Roche, personal fees from Daiichi-Sankyo, personal fees from Lily, grants from Roche, grants from Biotheranostics and grants from RNA diagnostics. All of these are outside the submitted work. No potential conflicts of interest were reported by the other authors. The trial was funded by a grant from ‘Research for Patient Benefit’, Ref: PB-PG-1111-26094. The funder and the manufacturers had no role in the design of the study, in the collection, analysis or interpretation of the data, or in the writing of the report.

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 the study and collection of data, writing up and critical review of the manuscript. PG: contributed to the planning, carrying out the study, and critical review of the manuscript. SS: contributed to the conception, planning and carrying out the study, and critical review of the manuscript. DR: contributed to the conception, planning and carrying out the study, 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. DS: produced the initial statistical analysis plan and power calculation, writing up and critical review of the manuscript. LB: critically reviewed the conception, planning and carrying out the study, writing up and critical review of the manuscript. DR: contributed to the conception, planning and carrying out the study, 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: 6 May 2014), and the research and development departments at the nine 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.

Acknowledgements
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 Khatoon, Matilda Lans, Alexis Spratt, Jill Lyons, Pattrick Lisbett, Ann Adams, Renee Poulson, 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 Dallbir 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.

**Data availability statement**
The data that support the findings of this study are available from the corresponding author upon reasonable request.

**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, MN, USA, for the Diary to be used in this trial.
*Uiitan QoL scale:* Questionnaire published by North American Menopause Society to be used freely for clinical or research purposes.

**Supporting Information**
Additional supporting information may be found online in the Supporting Information section at the end of the article.

- **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.** 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.** 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.
- **Figure S4.** Subgroup analysis of primary outcome measure.

- **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.

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