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Effect of Active Folate Compared With Folic Acid on Homocysteine Levels in Postmenopausal Women With Metabolic Syndrome: A Triple-Blind Randomized Clinical Trial

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Abstract

Background: Hyperhomocysteinemia is associated with cardiovascular risk and may be particularly relevant in postmenopausal women with metabolic syndrome. Folic acid lowers homocysteine, but its conversion to biologically active folate depends on enzymatic pathways that may be inefficient in some individuals. This trial compared the effects of folic acid and 5-methyltetrahydrofolate on plasma homocysteine and cardiometabolic outcomes in postmenopausal women with metabolic syndrome.

Methods: This randomized, triple-blind clinical trial was conducted among postmenopausal women aged 50 to 70 years with metabolic syndrome who were referred to the gynecology clinic of Baharloo Hospital, Tehran, Iran, during 2024 to 2025. Potential participants were consecutively screened for eligibility. Fifty eligible women were randomly assigned in a 1:1 ratio to receive either folic acid 1 mg/day or 5-methyltetrahydrofolate 0.6 mg/day for 3 months. The primary outcomes were changes in plasma homocysteine and serum folate. Secondary outcomes included changes in body mass index, waist circumference, blood pressure, fasting blood glucose, lipid profile, and liver enzymes.

Results: Fifty women were randomized, with 25 participants assigned to each group. The mean age of the study population was 58.06 ± 3.64 years. Serum folate increased significantly in both groups, with a greater increase in the 5-methyltetrahydrofolate group than in the folic acid group. Plasma homocysteine decreased significantly within both groups after 3 months; however, the between-group difference in homocysteine reduction was not statistically significant. In the ANCOVA model adjusted for baseline homocysteine, the adjusted mean difference was −0.36 μmol/L (95% CI, −0.90 to +0.18; p = 0.190). In the responder analysis, at least a 20% reduction in homocysteine was achieved by 58.3% of participants in the folic acid group and 66.7% in the 5-methyltetrahydrofolate group, with no significant between-group difference (p = 0.766). Secondary cardiometabolic outcomes showed no meaningful within-group changes or between-group differences.

Conclusions: In postmenopausal women with metabolic syndrome, both folic acid and 5-methyltetrahydrofolate lowered plasma homocysteine over 3 months. Although 5-methyltetrahydrofolate produced a greater increase in serum folate, it offered no significant advantage over folic acid in reducing plasma homocysteine or improving short-term cardiometabolic outcomes

Introduction

Hyperhomocysteinemia is an independent risk factor for atherosclerotic disease, including ischemic heart disease, stroke, and peripheral vascular disease [1–3]. Homocysteine is produced during methionine catabolism [2], and higher levels are associated with endothelial dysfunction, impaired vascular tone, inflammatory-cell adhesion, and reduced endothelial antithrombotic activity [4–6]. Homocysteine also predicts cardiovascular complications in diabetes, and lowering elevated plasma homocysteine may reduce cardiovascular risk [7, 8]. Hyperhomocysteinemia may be a stronger coronary heart disease risk factor in women than in men, probably because of hormonal changes and age-related low folate intake [4, 9].

Homocysteine is not usually dietary and is synthesized from methionine, then remethylated to methionine or converted to cysteine through B-vitamin-dependent pathways. Deficiency of folic acid, vitamin B6, or vitamin B12 may increase homocysteine levels [10]. Age, sex, circulating folate and vitamin B12, and probably estrogen status influence total plasma homocysteine [2]. Without sufficient folic acid, cobalamin, and 5-methyltetrahydrofolate, homocysteine may accumulate [11].

Women spend more than one-third of life after menopause, a period associated with increased risk of several disorders, particularly cardiovascular disease [12–15]. Homocysteine concentrations rise with age in both sexes, probably because of clinical or subclinical folate and vitamin B12 deficiency [16]. Plasma homocysteine increases significantly after menopause, probably because of hormonal changes [17, 18]. Before menopause, plasma homocysteine is lower in women than in young men, but after menopause it approaches male levels and may increase significantly [19, 20].

Metabolic syndrome predisposes individuals to cardiovascular disease [21]. Greater obesity and metabolic syndrome are associated with advanced atherosclerosis and higher coronary heart disease incidence [22, 23]. Insulin resistance is central to metabolic syndrome and its cardiovascular effects [21]. Because each component is a cardiovascular risk factor, their coexistence accelerates cardiovascular disease, with greater excess risk in women than in men [24, 25]. In postmenopausal women, reduced estrogen protection, lipid changes, and increased coagulation factors make cardiovascular disease a major cause of mortality. Hormone therapy has not produced the expected cardiovascular benefit and increases thromboembolic risk [26]. In Iran, metabolic syndrome prevalence in postmenopausal women exceeds 50%, and menopause itself is a risk factor for metabolic syndrome [27, 28].

Thus, postmenopausal women with metabolic syndrome are a high-risk cardiovascular group. Folic acid is a simple, relatively safe, low-cost intervention that may reduce homocysteine [7, 9, 29]. However, folic acid requires conversion by dihydrofolate reductase and methylenetetrahydrofolate reductase to 5-methyltetrahydrofolate, the active folate in the methionine-homocysteine cycle [30, 31]. Weak or variable enzyme activity and MTHFR polymorphisms may reduce active folate production and increase unmetabolized folic acid [32, 33]. Unmetabolized folic acid may mask vitamin B12 deficiency [10, 11], reduce natural killer-cell activity in postmenopausal women, and has been linked to breast cancer and possible organ fibrosis [34–36]. In contrast, 5-methyltetrahydrofolate can bypass these enzymatic steps and is less likely to mask hematologic signs of vitamin B12 deficiency [37, 38]. Previous studies show that folic acid reduces homocysteine in postmenopausal women, while active folate may improve folate status or reduce homocysteine in other populations [39–41]. However, based on the literature reviewed, no independent study has directly compared these supplements for reducing homocysteine in postmenopausal women with metabolic syndrome. Therefore, this triple-blind randomized clinical trial compared folic acid and active folate effects on plasma homocysteine and other cardiovascular risk-related factors in postmenopausal women with metabolic syndrome.

Materials and Methods

Study Design and Setting

This randomized, triple-blind clinical trial was conducted among postmenopausal women referred to the gynecology clinic of Baharloo Hospital, Tehran, Iran, during 2024 to 2025. The study was performed after approval by the Ethics Committee of Tehran University of Medical Sciences (ethics code: IR.MEDICINE.REC.1403.166) and registered in the Iranian Registry of Clinical Trials (IRCT registration number: IRCT20240722062513N1, 2024-08-16). Participants were recruited by convenience sampling from eligible clinic attendees. Potential participants were consecutively screened for eligibility at the gynecology clinic. Women who met the predefined inclusion criteria and had no exclusion criteria were invited to participate. Those who agreed to participate provided written informed consent before randomization. The sample size was calculated using a two-sided significance level of 0.05 and 80% power, based on a similar study by Almassinokiani et al. [40]. The required sample size was 20 participants per group; after allowing for 25% attrition, 25 women were enrolled in each group, for a total sample of 50 participants.(Fig. 1)

Participants and Eligibility Criteria

Eligible participants were women aged 50 to 70 years who had been postmenopausal for at least 2 years, met criteria for metabolic syndrome, and provided written and verbal informed consent. Metabolic syndrome in women was defined as the presence of at least three of the following five criteria: fasting blood glucose greater than 100 mg/dL, blood pressure of at least 130/85 mmHg, triglycerides of at least 150 mg/dL, HDL cholesterol below 50 mg/dL, and waist circumference of at least 88 cm.

Participants were excluded if they withdrew consent, changed their medication regimen, used weight-loss or appetite-suppressant drugs, used vitamin or mineral supplements including vitamins B6 or B12, or used medications interfering with folate metabolism. These included antifolates, folate-lowering antiepileptic drugs, metformin with suspected vitamin B12 deficiency, recent nitrous oxide exposure, cholestyramine, high-dose niacin, levodopa, fibrates, theophylline, and cyclosporine. Additional exclusion criteria were advanced chronic kidney disease, untreated hypothyroidism, pernicious anemia, malabsorption disorders such as active celiac disease or active inflammatory bowel disease, chemotherapy, and active smoking or use of other nicotine products, because smoking is associated with higher homocysteine levels [42].

Randomization, Blinding, and Interventions

Participants were randomly assigned in a 1:1 ratio to receive either folic acid 1 mg daily or 5-methyltetrahydrofolate 0.6 mg daily for 3 months. The randomization sequence was generated using a computer-generated random-number list with a block size of four by an independent researcher who was not involved in recruitment, intervention delivery, or outcome assessment. Allocation concealment was achieved by repackaging both supplements into identical opaque containers labeled only as A or B

according to the randomization list by an independent researcher who was not involved in participant recruitment, outcome assessment, data analysis, or interpretation of the results .To maintain triple blinding, supplement containers were coded as A and B by a person not involved in assessment or analysis. Participants, the investigator, the sample collector, and the statistician were unaware of treatment allocation. The tablets were provided by Abidi Pharmaceutical Company and were identical in shape and size. Because the original packaging differed, all tablets were repackaged by the research team in identical opaque containers labeled only as A or B. Abidi Pharmaceutical Company provided only the study supplements and had no role in participant recruitment, randomization, data collection, outcome assessment, statistical analysis, interpretation of the results, or manuscript preparation. No participant received other vitamin or mineral supplements during the trial. Adherence was assessed by monthly telephone follow-up and tablet counting based on the number of unused tablets returned at the final visit.

. The folic acid dose was considered low and safe, and previous human studies have reported safety across doses of 0.5 to 10 mg/day [43].

Data Collection and Laboratory Measurements

Baseline data, including age, medical history, medication use, smoking status, physical activity, and supplement use, were obtained by interview and questionnaire. Anthropometric indices, including weight, height, body mass index, waist circumference, and hip circumference, and blood pressure were assessed at baseline and after 3 months by the same trained operator using standard calibrated equipment.

After 12 to 14 hours of fasting, venous blood samples were obtained at baseline and after the intervention. Plasma homocysteine and serum folate were measured by enzyme-linked immunosorbent assay in the Baharloo Hospital laboratory under Tehran University of Medical Sciences supervision, with sample handling and quality control performed according to standard laboratory procedures. Fasting blood glucose, triglycerides, HDL cholesterol, LDL cholesterol, total cholesterol, AST, and ALT were also assessed.

Outcomes and Statistical Analysis

Primary outcomes were changes in plasma homocysteine and serum folate. Secondary outcomes included changes in body mass index, waist circumference, blood pressure, fasting blood glucose, lipid profile, and liver enzymes. Continuous variables were summarized as mean ± standard deviation, and categorical variables as frequency and percentage. Baseline continuous characteristics were compared between groups using two-sided Welch independent-samples t tests. Analyses were conducted using available data for each outcome, and no missing-data imputation was performed. Normality of change values was assessed using the Shapiro-Wilk test. Within-group changes from baseline were analyzed using paired t tests or Wilcoxon signed-rank tests, as appropriate. Between-group differences in change

scores, calculated as post-intervention minus baseline, were analyzed using Welch independent-samples t tests or Mann-Whitney U tests, according to distribution. ANCOVA models adjusted for baseline values were used for serum folate, plasma homocysteine, and systolic blood pressure. A responder analysis defined clinically meaningful homocysteine reduction as at least 20% from baseline, and proportions were compared using Fisher's exact test. All tests were two-sided, with $p < 0.05$ considered statistically significant. Statistical analyses were performed using SPSS version 27.

Results

Between 2024 and 2025, a total of 63 women were assessed for eligibility. Thirteen women were excluded before randomization, including 10 who did not meet the inclusion criteria and 3 who declined to participate. The remaining 50 eligible participants were randomized in a 1:1 ratio to receive either folic acid or 5-methyltetrahydrofolate. During follow-up, one participant in each group was lost to follow-up, leaving 24 participants per group available for the final analysis (Fig. 1)A total of 50 postmenopausal women with metabolic syndrome were randomized in a 1:1 ratio to receive either folic acid 1 mg/day or 5-methyltetrahydrofolate 0.6 mg/day for 3 months. Twenty-five participants were assigned to each treatment group. The mean age of the study population was 58.06 ± 3.64 years. Mean age was 57.68 ± 3.64 years in the folic acid group and 58.44 ± 3.51 years in the 5-methyltetrahydrofolate group, with no significant between-group difference. Baseline clinical, anthropometric, and biochemical characteristics were generally comparable between groups. The only statistically significant baseline imbalance was BMI, which was higher in the folic acid group than in the 5-methyltetrahydrofolate group. Full baseline characteristics are presented in Table 1.

Table 1
Baseline characteristics

Variables	Folic acid 1 mg	5-MTHF 0.6 mg
Age	57.68 ± 3.64	58.44 ± 3.51
BMI (kg/m²)	30.73 ± 1.01	30.10 ± 0.93
Waist (cm)	99.92 ± 3.01	99.72 ± 2.63
Systolic BP (mmHg)	136.96 ± 5.84	137.25 ± 5.18
Diastolic BP (mmHg)	84.36 ± 3.97	83.16 ± 4.4
Serum Folate (ng/mL)	12.59 ± 1.65	13.25 ± 1.86
Homocysteine (μmol/L)	15.25 ± 0.97	14.96 ± 0.93
Vitamin B12 (pg/mL)	511.12 ± 52.03	484.92 ± 60.61
LDL-C (mg/dL)	145.55 ± 8.58	146.18 ± 10.40
HDL-C (mg/dL)	51.04 ± 3.64	49.16 ± 3.00
Total Cholesterol (mg/dL)	232.64 ± 9.59	231.76 ± 11.53
Triglycerides (mg/dL)	172.55 ± 21.14	177.82 ± 21.55
Fasting Glucose (mg/dL)	111.76 ± 3.00	111.00 ± 3.59
AST (U/L)	25.36 ± 3.92	24.09 ± 3.88
ALT (U/L)	26.08 ± 6.22	24.50 ± 4.81

Primary Outcomes

Serum folate increased significantly in both treatment groups. In the folic acid group, serum folate increased from 12.60 ± 1.65 ng/mL to 21.49 ± 4.24 ng/mL, corresponding to a mean increase of 8.90 ± 3.54 ng/mL and a 70.63% increase from baseline. In the 5-methyltetrahydrofolate group, serum folate increased from 13.26 ± 1.87 ng/mL to 27.56 ± 5.85 ng/mL, corresponding to a mean increase of 14.30 ± 4.93 ng/mL and a 107.87% increase from baseline. Within-group changes were significant in both groups. The between-group comparison of change in serum folate was statistically significant and favored 5-methyltetrahydrofolate. Detailed within-group and between-group comparisons are shown in Table 2.

Table 2
Primary outcomes and responder analysis

Outcome	Group	n	Baseline	3 months	Change	Within-group p-value	Between-group p-value
Serum folate, ng/mL	Folic acid 1 mg/day	25	12.60 ± 1.65	21.49 ± 4.24	+ 8.90 ± 3.54	< 0.001	< 0.001
	5-MTHF 0.6 mg/day	23	13.26 ± 1.87	27.56 ± 5.85	+ 14.30 ± 4.93	< 0.001	
Plasma homocysteine, μmol/L	Folic acid 1 mg/day	24	15.25 ± 0.97	12.02 ± 1.15	−3.23 ± 0.78	< 0.001	0.375
	5-MTHF 0.6 mg/day	24	14.97 ± 0.94	11.48 ± 1.03	−3.49 ± 1.15	< 0.001	
Homocysteine responder, ≥ 20% reduction	Folic acid 1 mg/day	24		14/24 (58.3%)			0.766
	5-MTHF 0.6 mg/day	24		16/24 (66.7%)			

Plasma homocysteine decreased significantly within both groups after 3 months of supplementation. However, the between-group difference in homocysteine change was not statistically significant. In the ANCOVA model adjusted for baseline homocysteine, the adjusted mean difference was − 0.36 μmol/L, with a 95% confidence interval from − 0.90 to + 0.18 and p = 0.190, indicating no superiority of 5-methyltetrahydrofolate over folic acid for homocysteine reduction. Primary outcome results are summarized in Table 2.

Responder Analysis and Secondary Outcomes

In the responder analysis, defined as at least a 20% reduction in plasma homocysteine from baseline, 14 of 24 participants in the folic acid group (58.3%) and 16 of 24 participants in the 5-methyltetrahydrofolate group (66.7%) met the response criterion. The absolute risk difference was + 8.3 percentage points in favor of 5-methyltetrahydrofolate, but this difference was not statistically significant (95% CI, − 28.8 to + 43.2; Fisher's exact p = 0.766).

Secondary Outcomes

Anthropometric and hemodynamic outcomes changed only slightly from baseline to 3 months within each treatment group, and these changes were not significantly different between the folic acid and 5-

methyltetrahydrofolate groups. Within each group, changes in body mass index, waist circumference, and systolic and diastolic blood pressure were small and generally not statistically significant; between-group comparisons of these changes were also not significant (Table 3). Secondary biochemical outcomes followed the same pattern. Vitamin B12 concentrations, fasting blood glucose, lipid parameters, including LDL cholesterol, HDL cholesterol, total cholesterol, and triglycerides, and liver enzymes, including AST and ALT, remained stable in both groups, with no significant within-group changes or between-group differences over the 3-month intervention period. Detailed baseline values, 3-month values, within-group p-values, and between-group p-values for all secondary outcomes are presented in Table 3. No adverse events, serious adverse events, or unintended effects related to either intervention were reported during the study period.

Table 3
Secondary outcomes

Outcome	Group	Baseline	3 months	Change	Within-group p-value	Between-group p-value
BMI	Folic acid	30.73 ± 1.01	30.79 ± 1.02	+ 0.06 ± 0.16	0.06	0.12
	5-MTHF	30.10 ± 0.93	30.09 ± 0.91	−0.01 ± 0.18	0.74	
Waist circumference	Folic acid	99.92 ± 3.01	99.56 ± 2.86	−0.36 ± 1.50	0.24	0.49
	5-MTHF	99.72 ± 2.63	99.68 ± 3.41	−0.04 ± 1.79	0.91	
Systolic BP	Folic acid	136.96 ± 5.84	137.64 ± 6.13	+ 0.68 ± 2.39	0.16	0.72
	5-MTHF	137.25 ± 5.18	137.67 ± 6.70	+ 0.42 ± 2.80	0.47	
Diastolic BP	Folic acid	84.36 ± 3.97	83.76 ± 5.44	−0.60 ± 3.33	0.37	0.32
	5-MTHF	83.16 ± 4.40	83.46 ± 4.74	+ 0.29 ± 2.91	0.62	
Vitamin B12, pg/mL	Folic acid	511.12 ± 52.03	511.00 ± 57.74	−0.12 ± 20.50	0.97	0.85
	5-MTHF	484.92 ± 60.61	486.04 ± 70.39	+ 1.12 ± 27.43	0.84	
LDL-C, mg/dL	Folic acid	145.55 ± 8.58	144.75 ± 12.10	−0.80 ± 14.22	0.80	0.77
	5-MTHF	146.18 ± 10.40	146.50 ± 11.22	+ 0.32 ± 10.01	0.88	
HDL-C, mg/dL	Folic acid	51.04 ± 3.64	50.42 ± 4.38	−0.63 ± 3.05	0.32	0.10
	5-MTHF	49.16 ± 3.00	50.13 ± 4.83	+ 0.96 ± 3.56	0.20	
Total Cholesterol, mg/dL	Folic acid	232.64 ± 9.59	232.80 ± 11.08	+ 0.16 ± 8.10	0.92	0.71
	5-MTHF	231.76 ± 11.53	232.68 ± 11.24	+ 0.92 ± 6.40	0.47	
Triglycerides, mg/dL	Folic acid	172.55 ± 21.14	174.45 ± 24.40	+ 1.90 ± 19.05	0.66	0.70

Outcome	Group	Baseline	3 months	Change	Within-group p-value	Between-group p-value
	5-MTHF	177.82 ± 21.55	177.65 ± 27.87	−0.17 ± 15.61	0.95	
FBS, mg/dL	Folic acid	111.76 ± 3.00	110.40 ± 4.19	−1.36 ± 3.82	0.08	0.71
	5-MTHF	111.00 ± 3.59	110.04 ± 5.60	−0.96 ± 3.91	0.23	
AST, U/L	Folic acid	25.36 ± 3.92	25.68 ± 4.61	+ 0.32 ± 3.12	0.61	0.90
	5-MTHF	24.09 ± 3.88	24.52 ± 5.91	+ 0.43 ± 3.01	0.52	
ALT, U/L	Folic acid	26.08 ± 6.22	26.71 ± 8.05	+ 0.63 ± 3.93	0.44	0.56
	5-MTHF	24.50 ± 4.81	25.82 ± 5.38	+ 1.32 ± 4.09	0.14	

Discussion

This randomized triple-blind clinical trial compared the effects of folic acid and 5-methyltetrahydrofolate on plasma homocysteine and cardiometabolic outcomes in postmenopausal women with metabolic syndrome. The principal finding is that both supplements significantly lower plasma homocysteine after 3 months, but 5-methyltetrahydrofolate does not show superiority over folic acid for homocysteine reduction. Serum folate increases in both groups, with a significantly greater increase after 5-methyltetrahydrofolate. The responder analysis is consistent with the continuous outcome analysis and showed the same pattern.

Secondary cardiometabolic outcomes, including body mass index, waist circumference, blood pressure, fasting blood glucose, lipid parameters, vitamin B12, and liver enzymes, showed no meaningful within-group changes or between-group differences over the intervention period.

The observed reduction in homocysteine after folic acid supplementation agrees with previous studies in postmenopausal women. De Leo et al. report that low-dose folic acid reduces plasma homocysteine in postmenopausal women, supporting the biological responsiveness of this population to folate supplementation [44]. Villa et al. also describe homocysteine lowering after L-folic acid supplementation in healthy postmenopausal women, with evaluation of glycolipid metabolism as a related cardiometabolic domain [45]. Together, these studies support the responsiveness of homocysteine to folate supplementation in postmenopausal populations.

The comparison between folic acid and active folate is more nuanced. Lamers et al. report that supplementation with 5-methyltetrahydrofolate or folic acid similarly reduces plasma total homocysteine in healthy women, which is closely aligned with our finding of comparable homocysteine reduction between formulations [43]. Henderson et al. report that L-5-methyltetrahydrofolate increases blood folate concentrations more than folic acid in Malaysian women, while not producing a greater homocysteine-lowering effect, which parallels the present pattern of greater folate rise without superior homocysteine reduction [46].

Some findings in the literature contrast with our results. Venn et al. report greater homocysteine reduction with L-5-methyltetrahydrofolate than with folic acid in a randomized trial, suggesting that active folate may be more effective under some conditions [37]. Differences in population characteristics, baseline folate status, dose, treatment duration, genetic background, and baseline homocysteine may explain the inconsistency. In addition, Cagnacci et al. report blood pressure improvement after high-dose folate administration in postmenopausal women, whereas our study shows no significant blood pressure advantage for either supplement [47]. This difference may reflect the lower dose used in our trial, the short follow-up, and the distinction between vascular functional effects and measurable blood pressure change.

The biochemical pattern observed in this trial is plausible. Unlike folic acid, 5-methyltetrahydrofolate enters one-carbon metabolism downstream of key enzymatic conversion steps. Therefore, it may increase circulating folate more efficiently, particularly in individuals with reduced activity of enzymes involved in folate metabolism. Some studies have also suggested that genetic variation in methylenetetrahydrofolate reductase can influence the homocysteine-lowering response to folic acid or 5-methyltetrahydrofolate [48]. Homocysteine metabolism also depends on vitamin B12, vitamin B6, renal function, baseline homocysteine, and other metabolic factors. In a population without severe baseline hyperhomocysteinemia, both interventions may provide enough folate activity to support remethylation, leading to a similar reduction in homocysteine despite different folate concentrations.

The lack of significant effects on blood pressure, lipids, fasting glucose, and liver enzymes is also interpretable. Folate-based interventions may improve proximal biochemical or endothelial markers before measurable changes occur in broader cardiometabolic outcomes. Meta-analytic and mechanistic evidence suggests that folic acid can affect endothelial function, but such effects may not consistently appear as short-term changes in blood pressure or lipid profile [49, 50]. The 3-month duration of this study may be adequate for folate and homocysteine changes but insufficient for modest changes in body composition, lipid metabolism, or hemodynamic outcomes.

Clinically, these findings support a cautious and targeted interpretation of folate supplementation. Both folic acid and 5-methyltetrahydrofolate appear to be reasonable short-term options for lowering homocysteine. The greater folate increase with 5-methyltetrahydrofolate may be pharmacologically attractive, especially when impaired folate activation is suspected. However, routine preference for 5-

methyltetrahydrofolate over folic acid cannot be justified solely on the expectation of greater homocysteine lowering.

Homocysteine reduction may improve a proximal risk marker, but this trial does not demonstrate improvement in blood pressure, lipid profile, glucose, liver enzymes, or anthropometric indices. Larger cardiovascular outcome trials of homocysteine-lowering B-vitamin therapy have produced mixed results, with some showing no major reduction in composite cardiovascular events despite homocysteine lowering [51, 52]. Therefore, folate supplementation should be viewed as a targeted adjunctive biochemical intervention, not as a substitute for established cardiometabolic risk-reduction strategies.

Strengths and Limitations

This study has several strengths. Its randomized triple-blind design reduces selection, performance, and assessment bias. The use of two active comparators addresses a clinically relevant question rather than comparing supplementation with no treatment. Baseline assessment of major cardiometabolic variables, monitoring of adherence, and analysis of both continuous outcomes and responder status improve interpretability. The statistical strategy, including baseline-adjusted ANCOVA for key outcomes, further supports the interpretability of the main conclusion.

Several limitations should also be considered. The sample size is modest, which limits power to detect small between-group differences, particularly for secondary outcomes. The follow-up period is short and may not capture delayed changes in blood pressure, lipids, glycemic markers, or anthropometric measures. Participants are not stratified by MTHFR genotype, although genotype may modify response to folate formulation. Adherence is monitored, but imperfect reporting of supplement intake cannot be fully excluded. Single-center recruitment and the focus on postmenopausal women with metabolic syndrome also limit generalizability to men, younger adults, and individuals with substantially higher baseline homocysteine.

Future research directions

Future trials should enroll larger samples and extend follow-up to evaluate whether sustained homocysteine reduction translates into changes in vascular function, blood pressure, lipid profile, glycemic indices, inflammatory markers, or clinical cardiovascular outcomes. Stratification by MTHFR genotype, baseline folate status, vitamin B12 status, and baseline homocysteine may help identify subgroups more likely to benefit from 5-methyltetrahydrofolate. Studies in populations with higher baseline homocysteine may also clarify whether active folate has an advantage when the remethylation pathway is under greater metabolic demand. Finally, future work should include complete inflammatory and glycemic endpoints, including CRP and HbA1c, and should prespecify clinically meaningful responder thresholds.

Conclusion

In postmenopausal women with metabolic syndrome, 3 months of supplementation with either folic acid or 5-methyltetrahydrofolate significantly lowers plasma homocysteine. Although 5-methyltetrahydrofolate produces a greater increase in serum folate, it does not provide superior homocysteine reduction or measurable short-term benefit for anthropometric, hemodynamic, lipid, glycemic, vitamin B12, or liver-enzyme outcomes compared with folic acid. These findings suggest that both formulations may be considered for short-term homocysteine reduction in this high-risk population, while the choice of active folate over folic acid should not be based solely on expectation of greater homocysteine lowering.

Declarations

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The authors declare no personal financial conflicts of interest. Abidi Pharmaceutical Company provided only the study supplements and had no role in the design, conduct, analysis, interpretation, or reporting of the study.

All participants provided written and verbal informed consent and were informed that they could withdraw at any time without loss of routine care. Patient information was stored confidentially, identifying information was removed before analysis, and all procedures followed the Declaration of Helsinki.

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Figures

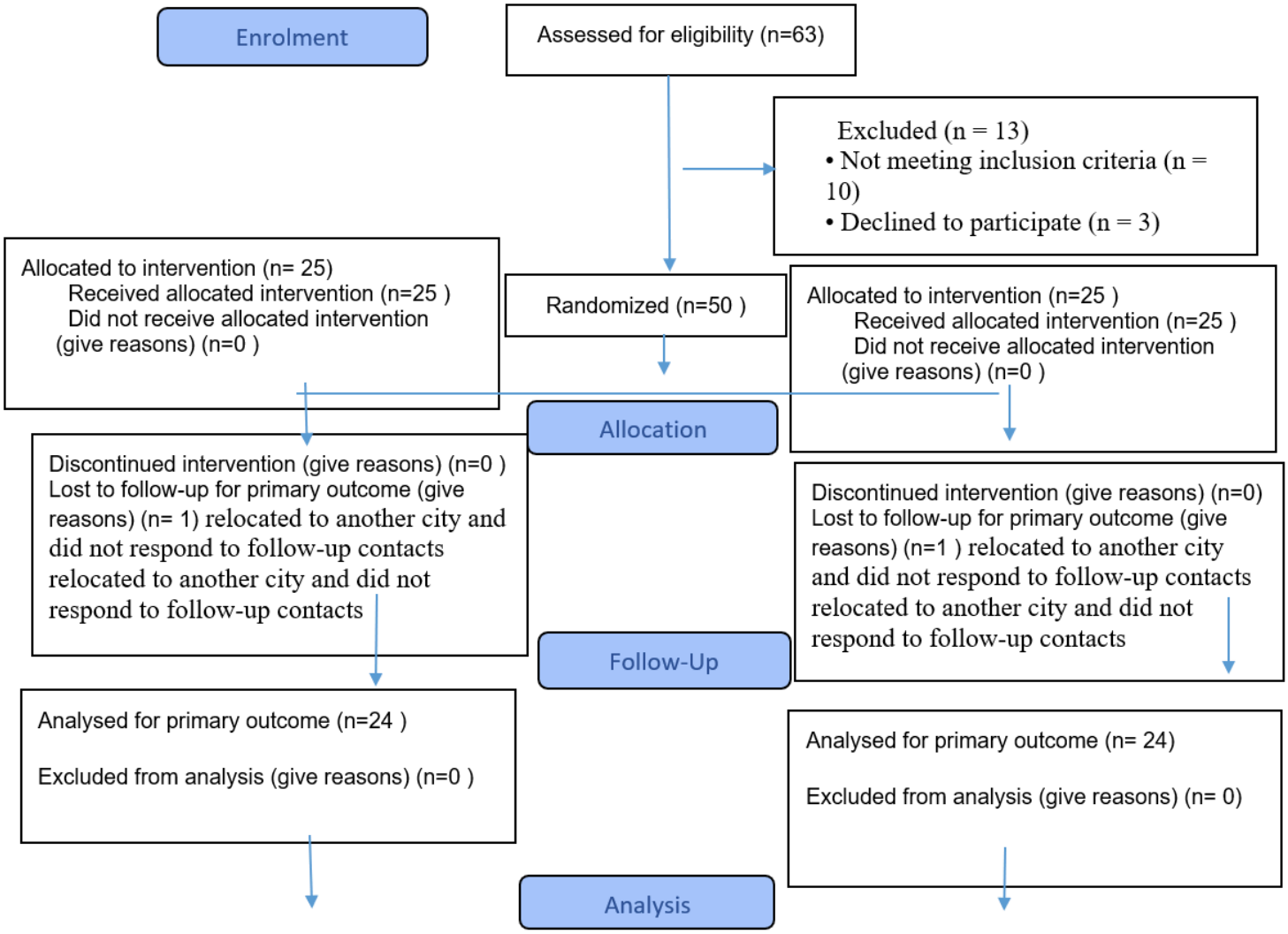


Figure 1

CONSORT flow diagram of participant enrollment and follow-up.