The Effects of Folic Acid Supplementation on Recurrence and Metabolic Status in Endometrial Hyperplasia: A Randomized, Double-Blind, Placebo-Controlled Trial

Fereshteh Bahmani, PhD; Fatemeh Rahimi Galougahi, MSc; Zahra Vahedpoor, MD; Mehr Jamiilian, MD; Samaneh Mahmoodi, MD; Rahele Baghban, MD; Tayebeh Bagherian, MSc; Maryam Zarezade Mehrizi, MD; Zatollah Asemi, PhD

1Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, I.R. Iran
2Department of Gynecology and Obstetrics, School of Medicine, Kashan University of Medical Sciences, Kashan, I.R. Iran
3Endocrinology and Metabolism Research Center, Department of Gynecology and Obstetrics, School of Medicine, Arak University of Medical Sciences, Arak, Iran

Abstract

Background: Data on the effects of folic acid supplementation on clinical symptoms and metabolic profiles of patients with endometrial hyperplasia (EH) are limited. This investigation was performed to evaluate the effects of folic acid supplementation on clinical symptoms and metabolic status of patients with EH.

Methods: This randomized, double-blind, placebo-controlled trial was conducted among 60 women diagnosed with EH. Diagnosis of EH was made based on biopsy results. Participants were randomly allocated to 2 groups to take 5 mg/d folic acid supplements (n = 30) or placebo (n = 30) for 12 weeks.

Results: After the 12-week intervention, folic acid supplementation significantly decreased fasting plasma glucose (β = -3.99 mg/dL; 95% CI, -7.39, -0.59; P = 0.02), serum insulin levels (β = -2.82 μIU/mL; 95% CI, -4.86, -0.77; P = 0.008), homeostasis model assessment for insulin resistance (β = -0.68; 95% CI, -1.20, -0.17; P = 0.009), triglycerides (β = -16.47 mg/dL; 95% CI, -28.72, -4.22; P = 0.009) and very-low-density lipoprotein (VLDL) cholesterol (β = -3.29 mg/dL; 95% CI, -5.74, -0.84; P = 0.009), and significantly increased the quantitative insulin sensitivity check index (β = 0.01; 95% CI, 0.004, 0.03; P = 0.01) compared with the placebo. Additionally, folic acid intake resulted in a significant reduction in serum high sensitivity C-reactive protein (hs-CRP) (β = -0.36 mg/L; 95% CI, -0.52, -0.21; P < 0.001) compared with the placebo. Folic acid supplementation did not affect other metabolic parameters.

Conclusion: In conclusion, we found that folic acid administration for 12 weeks to subjects with EH improved glycemic control, triglycerides, VLDL-cholesterol and hs-CRP levels, but did not influence recurrence and other metabolic profiles.

Keywords: Endometrial hyperplasia, Folic acid supplementation, Metabolic profiles


Received: April 16, 2018, Accepted: July 4, 2018, ePublished: October 1, 2018

Introduction

Endometrial hyperplasia (EH) is a precursor to the most common female gynecologic diseases with a high risk for malignant transformations and relapses.1 EH may result in endometrial cancer in up to 50% of cases.2 Most cases of EH are due to unopposed, prolonged exposure of the endometrium to oestrogen.1 In addition, some studies have reported the association between insulin resistance, inflammation and oxidative stress, and the progression of EH. In a study by Mitsuhashi et al.4 it was observed that abnormal glucose metabolism and insulin resistance were highly prevalent in patients with EH and endometrial cancer. Increased inflammatory cytokines also increase the incidence of complex and atypical EH.3

Previous studies have shown that folic acid deficiency is associated with several abnormalities including breaking DNA strand, enhanced mutation rates and impaired DNA repair mechanisms.6-7 Data on the association between folic acid deficiency and EH are sparse and inconsistent. Animal experimental and epidemiological studies have demonstrated an association between the increased risk of various cancers and folic acid deficiency.5-9 The results of another study showed a significant inverse association between dietary folic acid intake and endometrial cancer risk among all subjects and non-B vitamin supplement users.10 However, higher dietary folic acid intake was associated with a modestly decreased risk of ovarian cancer.11 In addition, improvement in insulin sensitivity was observed following supplementation with 2.5 mg/d folic acid for 12 weeks among overweight subjects.12 In addition, we have previously shown that folic acid supplementation (5 mg/d) in subjects with polycystic ovary syndrome (PCOS) had beneficial effects on biomarkers of inflammation and oxidative stress.13

Folic acid intake may reduce cancer risk through increased...
5-methyltetrahydrofolic acid availability, increased values of methionine, and global hypomethylation of DNA. This evidence might suggest the beneficial effects of folic acid administration in control of proliferation in EH. Although there is no specific data on the prevalence of folic acid deficiency in Iranian women with EH, we expect a high rate of deficiency in these patients due to the low consumption of fruits and vegetables in Iranian diet. Mean daily intake of folic acid among women of childbearing age in Iran has been reported to be 198.3 μg/d, which is much lower than recommendation. Therefore, based on existing evidence, we hypothesized that clinical signs, metabolic profiles and biomarkers of inflammation and oxidative stress might be improved by folic acid supplementation in patients with EH. The aim of the current study was to evaluate the effects of folic acid supplementation on recurrence and metabolic status of patients with EH.

Materials and Methods

Participants
This randomized, double-blind, placebo-controlled clinical trial, registered in the Iranian website for registration of clinical trials (identifier: IRCT2016060122562N2; http://www.irct.ir), was carried out among 60 subjects with EH who were diagnosed with endometrial biopsy in the past year, aged 35–55 years old and referred to the Naghavi Clinic in Kashan, Iran, from October 2016 to January 2017. Exclusion criteria were smoking, unwilling to cooperate, menopausal women, history of cardiovascular diseases, diabetes mellitus, hypertension and untreated thyroid diseases.

Study Design
At the beginning of the study, to decrease potential confounding effects, randomization was stratified according to age and body mass index (BMI). Then, participants in each block were randomly allocated into 2 treatment groups to take either 5 mg folic acid (Tehran Darou, Tehran, Iran) or placebo (Barij Essence, Kashan, Iran) (n = 30 each group) per day for 12 weeks. All study participants followed the standard treatment protocol, consuming 5 mg/d medroxyprogesterone (2 weeks/month) for 12 weeks. Duration of intervention was used based on observed beneficial effects of folic acid supplements on metabolic status in patients with PCOS. Both folic acid supplements and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tablets had similar packaging and placebo (starch) tables...
method of Beutler et al. All inter- and intra-assay CVs for FPG, lipid fractions, hs-CRP, NO, TAC, GSH and MDA concentrations were less than 6%.

Randomization
Randomization assignment was conducted using computer-generated random numbers. Randomization and allocation were concealed from both researchers and patients until the final analyses were completed. The randomized allocation sequence, enrolling patients and allocating them to interventions were performed by a trained member at the gynecology clinic.

Sample Size
Sample size was calculated using the formula suggested for randomized clinical trials. EH recurrence and hs-CRP were considered as the primary study variables; therefore, we used hs-CRP to calculate sample size. Type one (α) and type 2 (β) errors were defined as 0.05 and 0.20, with the study power of 80%. Based on a previous study, we used 0.29 mg/dL as SD. In addition, 0.23 mg/dL was considered as effect size (the mean difference) of the hs-CRP. Based on this, we needed 25 patients in each treatment group. Allowing 20% dropouts in each group, the final sample size was considered to be 30 patients in each group.

Statistical Methods
The Kolmogorov-Smirnov test was done to determine the normality of data. To detect differences in anthropometric measures and dietary intakes between treatment groups, we used the independent-samples t-test. Multiple linear regression model was used to assess the intention-to-treat effect of treatment on study outcomes after adjusting for random confounding by the baseline values of outcome, age, and BMI. The effect sizes were presented as mean differences with 95% confidence intervals. Outcome log-transformation was used if model residual had non-normal distribution (tHcy, hs-CRP and QUICKI). P-values <0.05 were considered statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

Results
At baseline, we recruited 75 subjects; however, 15 subjects were excluded from the study because of not meeting the inclusion criteria. Sixty participants (folic acid [n = 30] and placebo [n = 30]) completed the trial (Figure 1). On average, the rate of compliance in the present study was high, and more than 90% of supplements were taken throughout the study in both groups. No side effects were reported following supplementation with folic acid in subjects with EH.

Mean age, height, baseline weight and BMI as well as their means before and after the 12-week treatment were not statistically different between folic acid and placebo groups (Table 1). In addition, taking folic acid for 12 weeks did not affect EH recurrence.

Based on the 3-day dietary records obtained throughout the trial, we found no significant difference in mean macronutrient and micronutrient intakes between 2 groups (Table 2).

After the 12-week intervention, folic acid supplementation significantly decreased FPG (β -3.99 mg/dL; 95% CI, -7.39, -0.59; P = 0.02), serum insulin levels (β -2.82 µIU/mL; 95% CI, -4.86, -0.77; P = 0.008), HOMA-IR (β -0.68; 95% CI, -1.20, -0.17; P = 0.009), triglycerides (β -16.47 mg/dL; 95% CI, -28.72, -4.22; P = 0.009) and very-low-density lipoprotein (VLDL) cholesterol (β -3.29 mg/dL; 95% CI, -5.74, -0.84; P = 0.009), and significantly increased QUICKI (β 0.01; 95% CI, 0.004, 0.03; P = 0.01) compared with the placebo (Table 3). Additionally, folic acid intake resulted in a significant reduction in serum hs-CRP

---

**Figure 1. Summary of Patients’ Flow Diagram.**

---
Table 1. General Characteristics of Study Participants

<table>
<thead>
<tr>
<th></th>
<th>Placebo Group (n = 30)</th>
<th>Folic Acid Group (n = 30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>44.7 ± 3.1</td>
<td>44.4 ± 6.5</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157.9 ± 5.3</td>
<td>156.4 ± 7.6</td>
<td></td>
</tr>
<tr>
<td>Weight at study baseline (kg)</td>
<td>76.1 ± 9.2</td>
<td>75.2 ± 12.2</td>
<td></td>
</tr>
<tr>
<td>Weight at end-of-trial (kg)</td>
<td>76.3 ± 9.3</td>
<td>75.3 ± 12.1</td>
<td></td>
</tr>
<tr>
<td>Weight change (kg)</td>
<td>0.2 ± 0.7</td>
<td>0.1 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>BMI at study baseline (kg/m²)</td>
<td>30.6 ± 3.8</td>
<td>30.4 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>BMI at end-of-trial (kg/m²)</td>
<td>30.6 ± 3.8</td>
<td>30.4 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>BMI change (kg/m²)</td>
<td>0.1 ± 0.3</td>
<td>0.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>EH recurrence (%)</td>
<td>22 (73.3)</td>
<td>25 (83.3)</td>
<td></td>
</tr>
</tbody>
</table>

EH, endometrial hyperplasia; BMI, Body mass index.

* Data are means ± SDs.

Table 2. Mean Dietary Intakes of Study Participants at Weeks 1, 3, 6, 9 and 12 of the Study

<table>
<thead>
<tr>
<th></th>
<th>Placebo Group (n = 30)</th>
<th>Folic Acid Group (n = 30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>2286 ± 130</td>
<td>2242 ± 116</td>
<td>0.17</td>
</tr>
<tr>
<td>Carbohydrates (g/d)</td>
<td>312.0 ± 35.8</td>
<td>306.1 ± 42.4</td>
<td>0.56</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>82.3 ± 10.6</td>
<td>82.5 ± 15.1</td>
<td>0.99</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>81.9 ± 12.9</td>
<td>79.5 ± 14.4</td>
<td>0.50</td>
</tr>
<tr>
<td>SFA (g/d)</td>
<td>23.4 ± 5.4</td>
<td>22.5 ± 6.5</td>
<td>0.55</td>
</tr>
<tr>
<td>PUFA (g/d)</td>
<td>28.4 ± 5.9</td>
<td>28.2 ± 6.4</td>
<td>0.92</td>
</tr>
<tr>
<td>MUFA (g/d)</td>
<td>21.6 ± 5.1</td>
<td>20.1 ± 5.0</td>
<td>0.25</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>189.5 ± 117.9</td>
<td>210.7 ± 146.9</td>
<td>0.54</td>
</tr>
<tr>
<td>TDF (g/d)</td>
<td>16.0 ± 4.4</td>
<td>16.1 ± 4.0</td>
<td>0.97</td>
</tr>
<tr>
<td>Vitamin B2 (µg/d)</td>
<td>1.6 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>0.26</td>
</tr>
<tr>
<td>Vitamin B6 (µg/d)</td>
<td>1.2 ± 0.3</td>
<td>1.3 ± 0.4</td>
<td>0.30</td>
</tr>
<tr>
<td>Folic acid (µg/d)</td>
<td>228.6 ± 89.1</td>
<td>227.7 ± 73.1</td>
<td>0.96</td>
</tr>
<tr>
<td>Vitamin B12 (µg/d)</td>
<td>3.7 ± 1.3</td>
<td>3.5 ± 1.4</td>
<td>0.66</td>
</tr>
</tbody>
</table>

* Data are means ± SDs.

Table 2. Mean Dietary Intakes of Study Participants at Weeks 1, 3, 6, 9 and 12 of the Study

**Note:** The values are means ± SDs. Table 2 displays the mean dietary intakes of study participants at weeks 1, 3, 6, 9, and 12 of the study.

**Discussion**

To our knowledge, the beneficial effects of folic acid administration on recurrence and metabolic status of EH have not been evaluated previously. For the first time, we found that folic acid supplementation at a dosage of 5 mg/d for 8 weeks for subjects with type 2 diabetes mellitus (T2DM) could reduce tHcy levels. Longer durations of the treatment and higher dosage of folic acid may result in more EH recurrence.

**Effects on Insulin Metabolism and Lipid Profiles**

Our data supported that folic acid supplementation for subjects with EH for 12 weeks led to a significant reduction in FPG, serum insulin, HOMA-IR, triglycerides and VLDL-cholesterol levels, and a significant rise in QUICKI, but did not affect other lipid profiles. We have previously demonstrated that 5 mg/d folic acid supplementation for 12 weeks for subjects with PCOS had beneficial effects on insulin metabolism and total-/HDL-cholesterol ratio, but did not affect FPG and other lipid concentrations. Supporting our results, folic acid supplementation at a dosage of 5 mg/d for 8 weeks lowered tHcy, and improved glycemic control in subjects with T2DM. In addition, in a meta-analysis study, folic acid supplementation reduced insulin levels and HOMA-IR, but did not affect FPG and HDL cholesterol levels among people with metabolic diseases. The same findings were seen following supplementation with 2.5 mg/d folic acid for 3 months in overweight subjects, and 7.5 mg/d folic acid for 8 weeks in healthy postmenopausal women. Likewise, 5 mg/d folic acid supplementation for 12 weeks for subjects with metabolic syndrome improved glycemic control, and decreased triglycerides and VLDL-cholesterol levels. However, taking folic acid supplements at a dosage of 5 mg/d by patients with T2DM did not affect glycemic control. No significant effect on lipid profiles was also seen following administration of 5 mg/d folic acid for 4 weeks in cigarette smokers. There is increasing evidence that insulin resistance, diabetes and hypertension are implicated in the etiology and development of endometrial cancer. Insulin resistance may result in more EH recurrence.

**Conclusions**

In conclusion, dietary folic acid intake decreased the risk of endometrial cancer. Furthermore, we have previously shown that folic acid supplementation (5 mg/d) for 6 months for women with cervical intraepithelial neoplasia grade 1 (CIN1) resulted in its recurrence. Improvement in cervical dysplasia also associated with folic acid therapy in users of oral contraceptives. However, folic acid supplementation at a dosage of 10 mg/d for 6 months for subjects with CIN1 or CIN2 did not alter the course of established disease. In addition, in a meta-analysis study conducted by Qin et al., it was shown that folic acid supplementation had no significant impact on total cancer incidence, colorectal cancer, prostate cancer, breast cancer or hematological malignancies, but decreased the risk of melanoma. Existing evidence suggests that folic acid plays an important role in DNA methylation, synthesis and repair. Folic acid intake may reduce cancer risk through increased 5-methyltetrahydrofolic acid availability, increased values of methionine, and global hypomethylation of DNA. In addition, folic acid supplementation has been reported to lower tHcy levels and also may have some benefits in patients with EH. In a meta-analysis study, folic acid supplementation for patients with type 2 diabetes mellitus (T2DM) could reduce tHcy levels. Longer durations of the treatment and higher dosage of folic acid may result in more EH recurrence.
carcinoma may be correlated to hyperinsulinemia. Furthermore, prior studies have shown a decreasing risk of CVD following supplementation of folic acid, due to reducing tHcy concentrations. For instance, in a meta-analysis study, folic acid supplementation decreased the risk of cardiovascular and cerebrovascular events by 12.9% compared with control groups. Two meta-analyses have documented that folic acid supplementation was beneficial for CVD prevention in people with kidney diseases. Therefore, due to its beneficial effects on insulin metabolism and triglycerides and VLDL-cholesterol levels, folic acid may decrease metabolic events related to diabetes and CVD in patients with EH. Folic acid intake may improve glycemic control and lipid profiles through increasing AMP-activated protein kinase (AMPK) activation and Hcy-linked effects and also inhibiting insulin-stimulated tyrosine phosphorylation of insulin receptor β-subunit and its substrates. It was suggested that AMPK plays an important role in metabolic control, and pharmacologic enhancement of AMPK activity is used to improve insulin resistance. Furthermore, it has been documented that in follic acid deficiency, homocysteine thiocysteamine inhibits the tyrosine phosphorylation of insulin receptor β-subunit and might reduce the p85 regulatory subunit of phosphatidylinositol 3-kinase activity. Hyperhomocysteine has been found in people with insulin resistance.

**Effects on Inflammatory Markers and Oxidative Stress**

The current study indicated that taking folic acid by women with EH for 12 weeks resulted in a significant decrease in serum hs-CRP levels, but did not affect other biomarkers of inflammation and oxidative stress. We have previously shown that taking folic acid supplements (5 mg/d) for 12 weeks by subjects with PCOS could decrease serum hs-CRP and plasma MDA, and increase plasma TAC and GSH concentrations. In addition, the administration of B-group vitamins containing 5 mg folic acid per day for 14 days resulted in a significant decrease in CRP concentrations among subjects with acute ischemic stroke. However, in elderly subjects with hyperhomocysteineemia, folic acid (400 µg) and vitamin B12 (500 µg) supplementation did not influence either endothelial function or low-grade systemic inflammation. Furthermore, no significant effect on plasma CRP levels was seen after taking 400 µg/d folic acid for 12 weeks in subjects with atherosclerosis risk factors. Increased inflammatory cytokines can result in disorders of the regulation of cell division, which in turn results in excessive mitosis, decreased apoptosis, mutations, and therefore initiation and promotion of neoplastic transformations. Furthermore, increased inflammatory markers in EH may be considered as a factor in promotion and progression of pathology, as well as an attributed risk factor for malignancy in EH patients. Also, increased levels of free radicals and reactive oxygen species seem to be involved in the onset and progress of carcinogenesis. Therefore, due to its beneficial effects on biomarkers of inflammation and oxidative stress, folic acid may decrease metabolic complications in patients with EH. Less production of parathyroid hormone

### Table 3. Metabolic Profiles at Baseline and After the 12-Week Intervention in Women With Endometrial Hyperplasia That Received Either Folic Acid Supplements Or Placebo

<table>
<thead>
<tr>
<th>Variables</th>
<th>Placebo Group (n = 30)</th>
<th>Folic Acid Group (n = 30)</th>
<th>Difference in Outcome Measures Between Folic Acid and Placebo Treatment Groups</th>
<th>β (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy (µmol/L)</td>
<td>19.3±5.7</td>
<td>18.7±5.7</td>
<td></td>
<td>-0.08 (-0.13, -0.03)</td>
<td>0.001</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>98.4±8.8</td>
<td>99.6±8.0</td>
<td></td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>12.1±4.2</td>
<td>12.6±4.7</td>
<td></td>
<td>13.4±5.1</td>
<td>0.008</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.9±1.1</td>
<td>3.1±1.2</td>
<td></td>
<td>3.3±1.4</td>
<td>0.009</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.32±0.02</td>
<td>0.32±0.01</td>
<td></td>
<td>0.32±0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>134.5±45.4</td>
<td>139.7±45.0</td>
<td></td>
<td>136.0±47.5</td>
<td>0.009</td>
</tr>
<tr>
<td>VLDL-cholesterol (mg/dL)</td>
<td>26.9±9.1</td>
<td>27.9±9.0</td>
<td></td>
<td>27.2±9.5</td>
<td>0.009</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>190.0±33.1</td>
<td>194.2±33.5</td>
<td></td>
<td>185.1±32.6</td>
<td>0.66</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>118.7±33.9</td>
<td>121.2±32.4</td>
<td></td>
<td>113.7±28.9</td>
<td>0.78</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>44.4±7.9</td>
<td>45.0±9.3</td>
<td></td>
<td>44.1±7.6</td>
<td>0.26</td>
</tr>
<tr>
<td>hs-CRP (µg/L)</td>
<td>4.2±2.2</td>
<td>4.4±1.9</td>
<td></td>
<td>3.6±3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NO (µmol/L)</td>
<td>45.6±7.8</td>
<td>45.3±8.1</td>
<td></td>
<td>50.4±8.0</td>
<td>0.05</td>
</tr>
<tr>
<td>TAC (mmol/L)</td>
<td>782.7±56.4</td>
<td>786.8±71.5</td>
<td></td>
<td>745.8±42.8</td>
<td>0.56</td>
</tr>
<tr>
<td>GSH (µmol/L)</td>
<td>596.6±90.2</td>
<td>599.1±101.1</td>
<td></td>
<td>676.6±97.3</td>
<td>0.23</td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>2.5±0.4</td>
<td>2.5±0.4</td>
<td></td>
<td>2.7±0.3</td>
<td>0.39</td>
</tr>
</tbody>
</table>

FPG, fasting plasma glucose; GSH, total glutathione; HOMA-IR, homeostasis model assessment for insulin resistance; HDL-cholesterol, high density lipoprotein-cholesterol; Hs-CRP, high sensitivity C-reactive protein; LDL-cholesterol, low density lipoprotein-cholesterol; MDA, malondialdehyde; NO, nitric oxide; QUICKI, quantitative insulin sensitivity check index; VLDL-cholesterol, very low density lipoprotein-cholesterol; tHcy, total homocysteine; TAC, total antioxidant capacity.

Data are mean ±SDs.

Outcome measures refer to the change in values of measures of interest between baseline and week 12. β (difference in the mean outcomes measures between treatment groups [folic acid group = 1 and placebo group = 0]).

* Obtained from multiple regression model (adjusted for baseline values of each biochemical variables, age and baseline BMI).
(PTH) due to decreased insulin resistance following supplementation of folic acid may decrease production of inflammatory factors. Furthermore, decreased expression of inflammatory cytokines due to decreased levels of Hcy and decreased activity of nuclear factor kappa B by folic acid supplementation may decrease inflammatory markers and oxidative stress.

The current study had a number of strengths. Firstly, we focused on some interesting questions using a randomized, double-blind, placebo-controlled trial. The findings of improved glycemic control, triglycerides, VLDL-cholesterol and hs-CRP levels in the folic acid group are interesting, but need to be confirmed in a larger study. Another strength of the current study was the absence of dropout. One of the major limitations of this study was the absence of measurement of serum levels of folic acid, vitamin B12 and B6 due to funding limitations. In addition, sample size and duration of intervention were not enough in the current study. Further studies are needed to confirm our findings with a larger sample size and a longer duration of the treatment. Also, longer duration of the treatment may result in higher EH recurrence.

In conclusion, we found that folic acid administration for 12 weeks at a dosage of 5 mg/d for subjects with EH improved glycemic control, triglycerides, VLDL-cholesterol, hs-CRP and MDA levels, but did not influence recurrence and other metabolic profiles. This suggests that folic acid supplementation may confer advantageous therapeutic potential for women with EH. Further research is needed in other populations and for longer periods to determine the beneficial effects of folic acid supplementation. Moreover, further studies should evaluate gene expression levels related to insulin metabolism, inflammation and oxidative stress to explore the plausible mechanisms and confirm our findings.

Authors’ Contribution
ZA contributed to conception, design, statistical analysis and drafting of the manuscript. FB, FR-G, ZV, MJ, SM, RB and TB contributed to conception, data collection and manuscript drafting. All authors approved the final version for submission.

Conflict of Interest Disclosures
The authors have no conflicts of interest.

Ethical Statement
This study was performed in accordance with the Declaration of Helsinki, and informed consent was taken from all subjects.

Clinical Trial Registration Number
https://www.irct.ir; IRCT2016060122562N2.

Guarantor
ZA is the guarantor of this work.

Acknowledgement
The current research was supported by a grant from the Vice-chancellor for Research, KAUMS, Iran.

References


