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Original Article

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

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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**Cite this article as:** Bahmani F, Rahimi Galougahi F, Vahedpoor Z, Jamilian M, Mahmoodi S, Baghban R, et al. The effects of folic acid supplementation on recurrence and metabolic status in endometrial hyperplasia: a randomized, double-blind, placebo-controlled trial. Arch Iran Med. 2018;21(10):452–459.

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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.[3] 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.[5]

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.[8,9] 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

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5-methyltetrahydrofolic acid availability, increased values of methionine, and global hypomethylation of DNA.[14,15] 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,[16] 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.[17] Duration of intervention was used based on observed beneficial effects of folic acid supplements on metabolic status in patients with PCOS.[13] Both folic acid supplements and placebo (starch) tablets had similar packaging and patients and researchers were unaware of the content of the package until the end of study. Compliance was evaluated by counting the remaining supplements and placebos, and subtracting them from the number of supplements provided to the participants. To increase compliance rate, all subjects received reminder messages on their cell phones every day to remind them to take their capsules. In addition, compliance to the folic acid supplementation was assessed through quantification of plasma total homocysteine (tHcy) levels. All patients completed 3-day food records and three physical activity records as metabolic equivalents (METs) in hours per day[18] at baseline, weeks 3, 6, 9 and 12 of the intervention. Daily macro- and micro-nutrient intakes were analyzed by nutritionist IV software (First Databank, San Bruno, CA).

Assessment of Anthropometric Measures

A trained midwife at the gynecology clinic took anthropometric measurements at baseline and 12 weeks following the intervention. Weight (Seca, Hamburg, Germany) was measured in an overnight fasting status without shoes in a minimal clothing state. BMI was calculated as weight in kg divided by height in meters squared.

Outcomes

EH recurrence and high sensitivity C-reactive protein (hs-CRP) were considered as the primary study variables, and markers of insulin metabolism, lipid profiles, biomarkers of inflammation and oxidative stress were considered as secondary outcomes.

Clinical Assessment

Diagnosis of EH was made based on biopsy results at baseline and after the 12-week intervention. Endometrial biopsies were done using suction pipelles, fixed in 10% neutral buffered formalin, routinely processed, and paraffin embedded. Serial 5 micron sections were stained with hematoxylin and eosin and evaluated under the light microscope by a pathologist. Assessment of the pathological diagnosis was performed as concealment by a single experienced pathologist at baseline and at the end of the trial. Informed consent was taken from all participants for biopsy both at baseline and after the 12-week intervention.

Biochemical Assessment

At baseline and 12 weeks after the intervention, 10 mL fasting blood samples were taken from each patient at the Kashan reference laboratory, Kashan, Iran. Blood samples were immediately centrifuged (Hettich D-78532, Tuttlingen, Germany) at 3500 rpm for 10 minutes to separate the serum. The samples were then stored at -80°C until being analyzed at the Kashan reference laboratory. Plasma tHcy was assessed using an enzyme immunoassay method by tHcy kit (Axis-Shield Diagnostics, UK). Serum insulin concentrations were measured using an ELISA kit (DiaMetra, Milano, Italy) with intra- and inter-assay coefficient variances (CVs) of 2.5 and 4.3%, respectively. The homeostasis model assessment for insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) were determined according to the suggested formulas.[19] Enzymatic kits (Pars Azmun, Tehran, Iran) were used to quantify fasting plasma glucose (FPG) and lipid profiles. Serum hs-CRP concentrations were quantified by an ELISA kit (LDN, Nordhorn, Germany). The plasma nitric oxide (NO) concentrations were assessed using Griess method.[20] Plasma total antioxidant capacity (TAC) concentrations were evaluated by the method of ferric reducing antioxidant power developed by Benzie and Strain[21]; total glutathione (GSH) was evaluated using the

method of Beutler et al[22] and malondialdehyde (MDA) concentrations were assessed by the thiobarbituric acid reactive substances spectrophotometric test.[23] 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,[13] 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 the 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.[24] 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 macro- 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

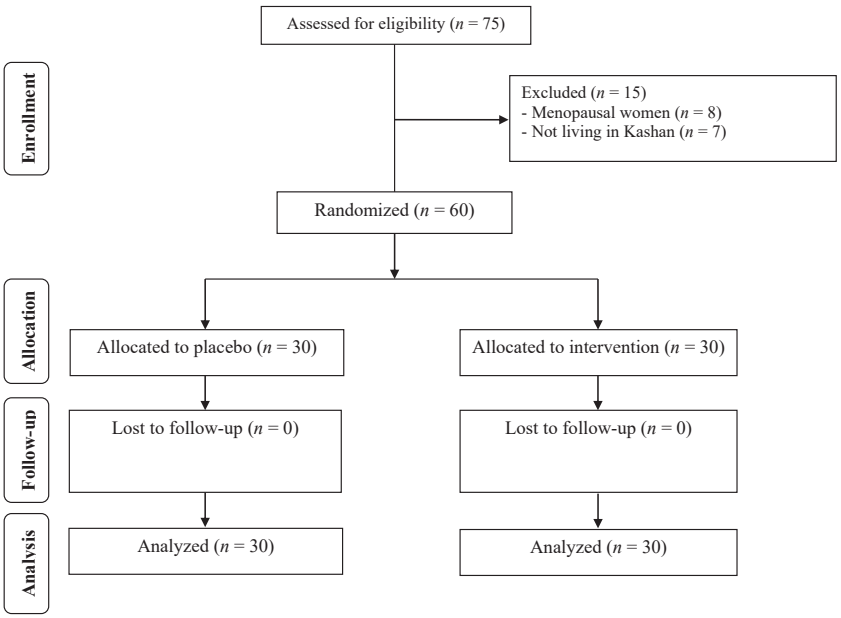

Figure 1. Summary of Patients' Flow Diagram.

Table 1. General Characteristics of Study Participants[a]

	Placebo Group (n = 30)	Folic Acid Group (n = 30)
Age (y)	44.7±3.1	44.4±6.5
Height (cm)	157.9±5.3	156.4±7.6
Weight at study baseline (kg)	76.1±9.2	75.2±12.2
Weight at end-of-trial (kg)	76.3±9.3	75.3±12.1
Weight change (kg)	0.2±0.7	0.1±0.6
BMI at study baseline (kg/m^2)	30.5±3.8	30.7±4.6
BMI at end-of-trial (kg/m^2)	30.6±3.8	30.8±4.6
BMI change (kg/m^2)	0.1±0.3	0.1±0.2
EH recurrence (%)	22 (73.3)	25 (83.3)

EH, endometrial hyperplasia; BMI, Body mass index.
[a] Data are means± SDs.

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

	Placebo Group (n = 30)	Folic Acid Group (n = 30)	*P*[b]
Energy (kcal/d)	2286±130	2242±116	0.17
Carbohydrates (g/d)	312.0±35.8	306.1±42.4	0.56
Protein (g/d)	82.3±10.6	82.5±15.1	0.96
Fat (g/d)	81.9±12.5	79.5±14.4	0.50
SFA (g/d)	23.4±5.4	22.5±6.5	0.55
PUFA (g/d)	28.4±5.9	28.2±6.4	0.92
MUFA (g/d)	21.6±5.1	20.1±5.0	0.25
Cholesterol (mg/d)	189.5±117.9	210.7±146.9	0.54
TDF (g/d)	16.0±4.4	16.1±4.0	0.97
Vitamin B2 (mg/d)	1.6±0.2	1.5±0.2	0.26
Vitamin B6 (mg/d)	1.2±0.3	1.3±0.4	0.30
Folic acid (µg/d)	228.6±89.1	227.7±73.1	0.96
Vitamin B12 (µg/d)	3.7±1.3	3.5±1.4	0.66

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TDF, total dietary fiber.
[a] Data are means± SDs.
[b] Obtained from independent *t* test.

(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.

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 patients with EH improved glycemic control, triglycerides, VLDL-cholesterol and hs-CRP levels, but did not influence recurrence and other metabolic profiles.

Effects on EH Recurrence

Subjects with EH are susceptible to endometrial cancer.[2] We found that folic acid supplementation for patients with EH did not influence EH recurrence. Earlier, it was shown that dietary folic acid intake decreased the risk of endometrial cancer.[10] Similar findings were reported by some others[25,26] in which higher folic acid intake accounted for a 20% to 40% reduced risk for endometrial cancer, but not other cancers.[27,28] 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.[29] Improvement in cervical dysplasia also associated with folic acid therapy in users of oral contraceptives.[30] 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.[31] In addition, in a meta-analysis study conducted by Qin et al,[32] 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 suggest 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.[14,15] 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.[33] 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.[34] 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.[35] In addition, in a meta-analysis study, folic acid supplementation reduced insulin levels and HOMA-IR, but did not affect FPG and HbA1c levels among people with metabolic diseases.[36] The same findings were seen following supplementation with 2.5 mg/d folic acid for 3 months in overweight subjects[12], and 7.5 mg/d folic acid for 8 weeks in healthy postmenopausal women.[37] 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.[38] However, taking folic acid supplements at a dosage of 5 mg/d by patients with T2DM did not affect glycemic control.[39] No significant effect on lipid profiles was also seen following administration of 5 mg/d folic acid for 4 weeks in cigarette smokers.[40] There is increasing evidence that insulin resistance,[41] diabetes and hypertension[42] are implicated in the etiology and development of endometrial cancer. Insulin resistance may act directly on endometrial tissue as a mitogenic and anti-apoptotic growth factor.[43] Also, higher prevalence of hypertension in people with endometrial

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[a]

Variables	Placebo Group (*n* = 30)		Folic Acid Group (*n* = 30)		Difference in Outcome Measures Between Folic Acid and Placebo Treatment Groups[b]	
	Baseline	Week 12	Baseline	Week 12	β (95% CI)	*P* [c]
tHcy (μmol/L)	19.3±5.7	18.7±5.7	20.5±7.1	16.4±4.4	-0.08 (-0.13, -0.03)	0.001
FPG (mg/dL)	98.4±8.8	99.6±8.0	100.6±12.5	97.2±12.3	-3.99 (-7.39, -0.59)	0.02
Insulin (μIU/mL)	12.1±4.2	12.6±4.7	13.4±5.1	10.7±5.0	-2.82 (-4.86, -0.77)	0.008
HOMA-IR	2.9±1.1	3.1±1.2	3.3±1.4	2.7±1.3	-0.68 (-1.20, -0.17)	0.009
QUICKI	0.32±0.02	0.32±0.01	0.32±0.02	0.33±0.03	0.01 (0.004, 0.03)	0.01
Triglycerides (mg/dL)	134.5±45.4	139.7±45.0	136.0±47.5	124.2±37.0	-16.47 (-28.72, -4.22)	0.009
VLDL-cholesterol (mg/dL)	26.9±9.1	27.9±9.0	27.2±9.5	24.8±7.4	-3.29 (-5.74, -0.84)	0.009
Total cholesterol (mg/dL)	190.0±33.1	194.2±33.5	185.1±32.6	186.9±39.5	-2.77 (-15.42, 9.87)	0.66
LDL-cholesterol (mg/dL)	118.7±33.9	121.2±32.4	113.7±28.9	118.5±39.7	1.74 (-11.24, 14.73)	0.78
HDL-cholesterol (mg/dL)	44.4±7.9	45.0±9.3	44.1±7.6	43.5±7.8	-1.20 (-3.35, 0.94)	0.26
hs-CRP (mg/L)	4.2±2.2	4.4±1.9	3.6±3.6	2.5±4.5	-0.36 (-0.52, -0.21)	<0.001
NO (μmol/L)	45.6±7.8	45.3±8.1	50.4±8.0	50.8±5.4	3.04 (0.00, 6.08)	0.05
TAC (mmol/L)	782.7±56.4	786.8±71.5	745.8±42.8	735.9±99.1	-10.86 (-48.66, 26.94)	0.56
GSH (μmol/L)	596.6±90.2	599.1±101.1	676.6±97.3	688.3±107.5	26.55 (-17.71, 70.82)	0.23
MDA (μmol/L)	2.5±0.4	2.5±0.4	2.7±0.3	2.6±0.2	-0.06 (-0.20, 0.08)	0.39

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.

[a]Data are mean ±SDs.

[b]"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]).

[c]Obtained from multiple regression model (adjusted for baseline values of each biochemical variables, age and baseline BMI).

carcinoma may be correlated to hyperinsulinemia.[44] 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.[45] Two meta-analyses have documented that folic acid supplementation was beneficial for CVD prevention in people with kidney diseases.[46,47] 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[48] Hcy-linked effects and also inhibiting insulin-stimulated tyrosine phosphorylation of insulin receptor β-subunit and its substrates.[49] It was suggested that AMPK plays an important role in metabolic control, and pharmacologic enhancement of AMPK activity is used to improve insulin resistance.[48] Furthermore, it has been documented that in folic acid deficiency, homocysteine thiolactone inhibits the tyrosine phosphorylation of insulin receptor b-subunit and might reduce the p85 regulatory subunit of phosphatidylinositol 3-kinase activity.[50] Hyperhomocysteinemia has been found in people with insulin resistance.[51,52]

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.[13] 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.[53] However, in elderly subjects with hyperhomocysteinemia, folic acid (400 μg) and vitamin B12 (500 μg) supplementation did not influence either endothelial function or low-grade systemic inflammation.[54] 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.[55] 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.[56] 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.[5] Also, increased levels of free radicals and reactive oxygen species seem to be involved in the onset and progress of carcinogenesis.[47] 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

(PTH) due to decreased insulin resistance[57] 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[58] 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.

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References

1. Montgomery BE, Daum GS, Dunton CJ. Endometrial hyperplasia: a review. Obstet Gynecol Surv. 2004;59(5):368-78.
2. Mittal K, Sebenik M, Irwin C, Yan Z, Popiolek D, Curtin J, et al. Presence of endometrial adenocarcinoma in situ in complex atypical endometrial hyperplasia is associated with increased incidence of endometrial carcinoma in subsequent hysterectomy. Mod Pathol. 2009;22(1):37-42. doi: 10.1038/modpathol.2008.138.
3. Clement NS, Oliver TR, Shiwani H, Saner JR, Mulvaney CA, Atiomo W. Metformin for endometrial hyperplasia: a Cochrane protocol. BMJ Open. 2016;6(8):e013385. doi: 10.1136/bmjopen-2016-013385.
4. Mitsuhashi A, Uehara T, Hanawa S, Shozu M. Prospective evaluation of abnormal glucose metabolism and insulin resistance in patients with atypical endometrial hyperplasia and endometrial cancer. Support Care Cancer. 2017;25(5):1495-501. doi: 10.1007/s00520-016-3554-y.
5. Kubyshkin AV, Aliev LL, Fomochkina, II, Kovalenko YP, Litvinova SV, Filonenko TG, et al. Endometrial hyperplasia-related inflammation: its role in the development and progression of endometrial hyperplasia. Inflamm Res. 2016;65(10):785-94. doi: 10.1007/s00011-016-0960-z.
6. Smith AD, Kim YI, Refsum H. Is folic acid good for everyone? Am J Clin Nutr. 2008;87(3):517-33. doi: 10.1093/ajcn/87.3.517.
7. Kruman, II, Kumaravel TS, Lohani A, Pedersen WA, Cutler RG, Kruman Y, et al. Folic acid deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer's disease. J Neurosci. 2002;22(5):1752-62.
8. Giovannucci E. Epidemiologic studies of folate and colorectal neoplasia: a review. J Nutr. 2002;132(8 Suppl):2350s-5s. doi: 10.1093/jn/132.8.2350S.
9. Mason JB. Folate consumption and cancer risk: a confirmation and some reassurance, but we're not out of the woods quite yet. Am J Clin Nutr. 2011;94(4):965-6. doi: 10.3945/ajcn.111.023796.
10. Xu WH, Shrubsole MJ, Xiang YB, Cai Q, Zhao GM, Ruan ZX, et al. Dietary folate intake, MTHFR genetic polymorphisms, and the risk of endometrial cancer among Chinese women. Cancer Epidemiol Biomarkers Prev. 2007;16(2):281-7. doi: 10.1158/1055-9965.epi-06-0798.
11. Tworoger SS, Hecht JL, Giovannucci E, Hankinson SE. Intake of folate and related nutrients in relation to risk of epithelial ovarian cancer. Am J Epidemiol. 2006;163(12):1101-11. doi: 10.1093/aje/kwj128.
12. Solini A, Santini E, Ferrannini E. Effect of short-term folic acid supplementation on insulin sensitivity and inflammatory markers in overweight subjects. Int J Obes (Lond). 2006;30(8):1197-202. doi: 10.1038/sj.ijo.0803265.
13. Bahmani F, Karamali M, Shakeri H, Asemi Z. The effects of folate supplementation on inflammatory factors and biomarkers of oxidative stress in overweight and obese women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled clinical trial. Clin Endocrinol (Oxf). 2014;81(4):582-7. doi: 10.1111/cen.12451.
14. Kim YI. Folate and carcinogenesis: evidence, mechanisms, and implications. J Nutr Biochem. 1999;10(2):66-88.
15. Rampersaud GC, Kauwell GP, Hutson AD, Cerda JJ, Bailey LB. Genomic DNA methylation decreases in response to moderate folate depletion in elderly women. Am J Clin Nutr. 2000;72(4):998-1003. doi: 10.1093/ajcn/72.4.998.
16. Abdollahi Z, Elmadfa I, Djazayery A, Golalipour MJ, Sadighi J, Salehi F, et al. Efficacy of flour fortification with folic acid in women of childbearing age in Iran. Ann Nutr Metab. 2011;58(3):188-96. doi: 10.1159/000329726.
17. Armstrong AJ, Hurd WW, Elguero S, Barker NM, Zanotti KM. Diagnosis and management of endometrial hyperplasia. J Minim Invasive Gynecol. 2012;19(5):562-71. doi: 10.1016/j.jmig.2012.05.009.

18. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc. 2000;32(9 Suppl):S498-504.
19. Pisprasert V, Ingram KH, Lopez-Davila MF, Munoz AJ, Garvey WT. Limitations in the use of indices using glucose and insulin levels to predict insulin sensitivity: impact of race and gender and superiority of the indices derived from oral glucose tolerance test in African Americans. Diabetes Care. 2013;36(4):845-53. doi: 10.2337/dc12-0840.
20. Tatsch E, Bochi GV, Pereira Rda S, Kober H, Agertt VA, de Campos MM, et al. A simple and inexpensive automated technique for measurement of serum nitrite/nitrate. Clin Biochem. 2011;44(4):348-50. doi: 10.1016/j.clinbiochem.2010.12.011.
21. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70-6. doi: 10.1006/abio.1996.0292.
22. Beutler E, Gelbart T. Plasma glutathione in health and in patients with malignant disease. J Lab Clin Med. 1985;105(5):581-4.
23. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med. 1990;9(6):515-40.
24. Mansournia MA, Altman DG. Invited commentary: methodological issues in the design and analysis of randomised trials. Br J Sports Med. 2018;52(9):553-5. doi: 10.1136/bjsports-2017-098245.
25. McCann SE, Freudenheim JL, Marshall JR, Brasure JR, Swanson MK, Graham S. Diet in the epidemiology of endometrial cancer in western New York (United States). Cancer Causes Control. 2000;11(10):965-74.
26. McCann SE, Marshall JR, Brasure JR, Graham S, Freudenheim JL. Analysis of patterns of food intake in nutritional epidemiology: food classification in principal components analysis and the subsequent impact on estimates for endometrial cancer. Public Health Nutr. 2001;4(5):989-97.
27. Potischman N, Swanson CA, Brinton LA, McAdams M, Barrett RJ, Berman ML, et al. Dietary associations in a case-control study of endometrial cancer. Cancer Causes Control. 1993;4(3):239-50.
28. Jain MG, Rohan TE, Howe GR, Miller AB. A cohort study of nutritional factors and endometrial cancer. Eur J Epidemiol. 2000;16(10):899-905.
29. Zima T, Fialova L, Mestek O, Janebova M, Crkovska J, Malbohan I, et al. Oxidative stress, metabolism of ethanol and alcohol-related diseases. J Biomed Sci. 2001;8(1):59-70. doi: 10.1159/000054014.
30. D'Adamo E, Marcovecchio ML, Giannini C, de Giorgis T, Chiavaroli V, Chiarelli F, et al. Improved oxidative stress and cardio-metabolic status in obese prepubertal children with liver steatosis treated with lifestyle combined with Vitamin E. Free Radic Res. 2013;47(3):146-53. doi: 10.3109/10715762.2012.755262.
31. Islam MR, Arslan I, Attia J, McEvoy M, McElduff P, Basher A, et al. Is serum zinc level associated with prediabetes and diabetes?: a cross-sectional study from Bangladesh. PLoS One. 2013;8(4):e61776. doi: 10.1371/journal.pone.0061776.
32. Mispireta ML, Caulfield LE, Zavaleta N, Merialdi M, Putnick DL, Bornstein MH, et al. Effect of maternal zinc supplementation on the cardiometabolic profile of Peruvian children: results from a randomized clinical trial. J Dev Orig Health Dis. 2017;8(1):56-64. doi: 10.1017/s2040174416000568.
33. Wang Q, Sun Y, Ma A, Li Y, Han X, Liang H. Effects of vitamin E on plasma lipid status and oxidative stress in Chinese women with metabolic syndrome. Int J Vitam Nutr Res. 2010;80(3):178-87. doi: 10.1024/0300-9831/a000015.
34. Asemi Z, Karamali M, Esmaillzadeh A. Metabolic response to folate supplementation in overweight women with polycystic ovary syndrome: a randomized double-blind placebo-controlled clinical trial. Mol Nutr Food Res. 2014;58(7):1465-73. doi: 10.1002/mnfr.201400033.
35. Gargari BP, Aghamohammadi V, Aliasgharzadeh A. Effect of folic acid supplementation on biochemical indices in overweight and obese men with type 2 diabetes. Diabetes Res Clin Pract. 2011;94(1):33-8. doi: 10.1016/j.diabres.2011.07.003.
36. Akbari M, Tabrizi R, Lankarani KB, Heydari ST, Karamali M, Kashanian M, et al. The Effects of Folate Supplementation on Diabetes Biomarkers Among Patients with Metabolic Diseases: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Horm Metab Res. 2018;50(2):93-105. doi: 10.1055/s-0043-125148.
37. Villa P, Perri C, Suriano R, Cucinelli F, Panunzi S, Ranieri M, et al. L-folic acid supplementation in healthy postmenopausal women: effect on homocysteine and glycolipid metabolism. J Clin Endocrinol Metab. 2005;90(8):4622-9. doi: 10.1210/jc.2004-1954.
38. Talari HR, Rafiee M, Farrokhian A, Raygan F, Bahmani F, Darooghegi Mofrad M, et al. The Effects of Folate Supplementation on Carotid Intima-Media Thickness and Metabolic Status in Patients with Metabolic Syndrome. Ann Nutr Metab. 2016;69(1):41-50. doi: 10.1159/000448295.
39. Marreiro DN, Geloneze B, Tambascia MA, Lerario AC, Halpern A, Cozzolino SM. Effect of zinc supplementation on serum leptin levels and insulin resistance of obese women. Biol Trace Elem Res. 2006;112(2):109-18.
40. O'Grady HL, Leahy A, McCormick PH, Fitzgerald P, Kelly CK, Bouchier-Hayes DJ. Oral folic acid improves endothelial dysfunction in cigarette smokers. J Surg Res. 2002;106(2):342-5.
41. Cust AE, Kaaks R, Friedenreich C, Bonnet F, Laville M, Lukanova A, et al. Plasma adiponectin levels and endometrial cancer risk in pre- and postmenopausal women. J Clin Endocrinol Metab. 2007;92(1):255-63. doi: 10.1210/jc.2006-1371.
42. Weiderpass E, Persson I, Adami HO, Magnusson C, Lindgren A, Baron JA. Body size in different periods of life, diabetes mellitus, hypertension, and risk of postmenopausal endometrial cancer (Sweden). Cancer Causes Control. 2000;11(2):185-92.
43. Cust AE, Kaaks R, Friedenreich C, Bonnet F, Laville M, Tjonneland A, et al. Metabolic syndrome, plasma lipid, lipoprotein and glucose levels, and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). Endocr Relat Cancer. 2007;14(3):755-67. doi: 10.1677/erc-07-0132.
44. Rosato V, Zucchetto A, Bosetti C, Dal Maso L, Montella M, Pelucchi C, et al. Metabolic syndrome and endometrial cancer risk. Ann Oncol. 2011;22(4):884-9. doi: 10.1093/annonc/mdq464.
45. Wang WW, Wang XS, Zhang ZR, He JC, Xie CL. A Meta-Analysis of Folic Acid in Combination with Anti-Hypertension Drugs in Patients with Hypertension and Hyperhomocysteinemia. Front Pharmacol. 2017;8:585. doi: 10.3389/fphar.2017.00585.
46. Campbell SE, Rudder B, Phillips RB, Whaley SG, Stimmel JB, Leesnitzer LM, et al. gamma-Tocotrienol induces growth arrest through a novel pathway with TGFbeta2 in prostate cancer. Free Radic Biol Med. 2011;50(10):1344-54. doi: 10.1016/j.freeradbiomed.2011.02.007.
47. Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Ann Intern Med. 2007;147(8):W163-94. doi: 10.7326/0003-4819-147-8-200710160-00010-w1.
48. Buettner R, Bettermann I, Hechtl C, Gabele E, Hellerbrand C, Scholmerich J, et al. Dietary folic acid activates AMPK and improves insulin resistance and hepatic inflammation

in dietary rodent models of the metabolic syndrome. Horm Metab Res. 2010;42(11):769-74. doi: 10.1055/s-0030-1263122.
49. Marcucci R, Zanazzi M, Bertoni E, Rosati A, Fedi S, Lenti M, et al. Vitamin supplementation reduces the progression of atherosclerosis in hyperhomocysteinemic renal-transplant recipients. Transplantation. 2003;75(9):1551-5. doi: 10.1097/01.tp.0000058810.59825.58.
50. Najib S, Sanchez-Margalet V. Homocysteine thiolactone inhibits insulin signaling, and glutathione has a protective effect. J Mol Endocrinol. 2001;27(1):85-91.
51. Shorakae S, Teede H, de Courten B, Lambert G, Boyle J, Moran LJ. The Emerging Role of Chronic Low-Grade Inflammation in the Pathophysiology of Polycystic Ovary Syndrome. Semin Reprod Med. 2015;33(4):257-69. doi: 10.1055/s-0035-1556568.
52. Melo AS, Kliemchen J, Junior AA, Ferriani RA, Navarro PA. Oxidative stress and polycystic ovary syndrome: evaluation during ovarian stimulation for ICSI. Reproduction. 2016. doi: 10.1530/rep-16-0084.
53. Ullegaddi R, Powers HJ, Gariballa SE. Antioxidant supplementation with or without B-group vitamins after acute ischemic stroke: a randomized controlled trial. JPEN J Parenter Enteral Nutr. 2006;30(2):108-14. doi: 10.1177/0148607106030002108.
54. van Dijk SC, Enneman AW, Swart KM, van Wijngaarden JP, Ham AC, de Jonge R, et al. Effect of vitamin B12 and folic acid supplementation on biomarkers of endothelial function and inflammation among elderly individuals with hyperhomocysteinemia. Vasc Med. 2016;21(2):91-8. doi: 10.1177/1358863x15622281.
55. Mierzecki A, Kloda K, Jastrzebska M, Chelstowski K, Honczarenko K, Kozlowska-Wojciechowska M, et al. Is there an effect of folic acid supplementation on the coagulation factors and C-reactive protein concentrations in subjects with atherosclerosis risk factors? Postepy Hig Med Dosw (Online). 2012;66:696-701. doi: 10.5604/17322693.1014655.
56. Gao Y, Li S, Li Q. Uterine epithelial cell proliferation and endometrial hyperplasia: evidence from a mouse model. Mol Hum Reprod. 2014;20(8):776-86. doi: 10.1093/molehr/gau033.
57. Zhao G, Ford ES, Li C. Associations of serum concentrations of 25-hydroxyvitamin D and parathyroid hormone with surrogate markers of insulin resistance among U.S. adults without physician-diagnosed diabetes: NHANES, 2003-2006. Diabetes Care. 2010;33(2):344-7. doi: 10.2337/dc09-0924.
58. Papatheodorou L, Weiss N. Vascular oxidant stress and inflammation in hyperhomocysteinemia. Antioxid Redox Signal. 2007;9(11):1941-58. doi: 10.1089/ars.2007.1750.