Effect of folic acid supplementation on biochemical indices in overweight and obese men with type 2 diabetes

Bahram Pourghassem Gargari a,*, Vahide Aghamohammadi a, Akbar Aliasgharzadeh b

a Nutritional Research Center, Department of Biochemistry & Nutrition, Faculty of Health & Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran
b Faculty of Medicine, Endocrine and Metabolism Section, Imam Reza Teaching Hospital, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT
Aims: This study performed to determine the effects of folate supplementation on indices of glycemic control, insulin resistance and lipid profile in overweight and obese men with type 2 diabetes under metformin (at least 1500 mg daily) treatment.

Methods: The study was a double-blind randomized controlled clinical trial. Forty-eight overweight and obese men (aged 58.2 ± 8.9 years; BMI = 28.6 ± 2.9 kg/m²) with type 2 diabetes participated in the study. Patients were divided randomly into two groups of folic acid (5 mg/d) and placebo. All patients received the tablets for eight weeks.

Results: Supplementation with folic acid led to 8% decrease in HbA1C (p = 0.048), 7.5% in fasting blood glucose (p = 0.051), 16.2% in serum insulin (p = 0.021), 20.5% in insulin resistance (p = 0.041) and 21.2% in plasma homocysteine (p = 0.000). A significant increase in serum folate and B12 levels (19% and 17.3%, p = 0.000, respectively) were observed in the folic acid group, whereas no significant changes occurred in the placebo group. Also, in the folic acid and placebo groups, there were no significant changes in body weight.

Conclusions: Folic acid supplementation lowered plasma level of homocysteine, improved glycemic control and insulin resistance in patients with type 2 diabetes.

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1. Introduction

Type 2 diabetes is a chronic, progressive disorder that results from inadequate β-cell compensation for, or adaptation to, insulin resistance [1]. Metformin, a biguanide, acts by decreasing hepatic glucose production and increasing glucose clearance; it is the recommended first-line pharmacologic treatment for type 2 diabetes worldwide [2]. However, it reduces levels of folate and vitamin B12, thereby increasing the homocysteine (Hcy) level, probably due to malabsorption [3–6]. How metformin affects folate and B12 status and Hcy concentration is not fully understood, but these effects depend on the duration and amount of metformin received [7]. Hcy is a nonessential sulfur-cotaining amino acid formed from the demethylation of an essential amino acid, methionine [5].

In patients with diabetes, elevated Hcy levels have been reported to be associated with endothelial dysfunction, insulin resistance, prothrombotic state, macroangiopathy, nephropathy, dyslipidemia, oxidative stress and poor control of disease [8].

Controversial data on the association between insulin resistance and plasma Hcy levels have been reported. In two large epidemiological studies, hyperhomocysteinemia was modestly but significantly associated with insulin levels and insulin resistance [9,10].

Subjects with impaired fasting glucose had significantly higher fasting serum Hcy levels than those with normal fasting glucose [11].

* Corresponding author. Tel.: +98 4113376229; fax: +98 4113340634.
E-mail addresses: bahrampg@yahoo.com, pourghassem@tbzmed.ac.ir (B.P. Gargari).
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Due to positive relation between body weight and Hcy concentration [12,13] and a lack of knowledge on the effect of folate on indices of glycemic control, insulin resistance and lipid profile in patients with type 2 diabetes, this clinical trial was performed to evaluate the effects of oral folic acid supplementation for eight weeks on levels of fasting blood glucose, HbA1C, serum insulin, insulin resistance, total cholesterol, TG, LDL-C, HDL-C, serum folate and B12, plasma Hcy and body weight in overweight and obese men with type 2 diabetes under metformin (at least 1500 mg daily) treatment.

2. Materials and methods

2.1. Subjects and intervention

The study was approved by the Ethics Committee of Tabriz University of Medical Sciences and it was registered on the Iranian Registry of Clinical Trials website (IRCT138811033140N1). Forty-eight overweight men with type 2 diabetes who met inclusion criteria was included in the study. Inclusion criteria consisted of having the type 2 diabetes, Body Mass Index (BMI) ≥25 kg/m², voluntary consenting for participation in the study and under metformin treatment of at least 1500 mg daily for more than six months. Subjects were excluded if they had hepatic disorders, renal failure, cardiovascular disease, celiac or gastrointestinal disorders, rheumatic, endocrine and thyroid disorders, leukemia, inborn errors in the enzymes that metabolize homocysteine. In addition, subjects who had insulin therapy, nutrient supplementation, smoking, alcohol intake and using corticosteroids, anticonvulsants or isoniazid were excluded.

After informed consent, each participant was assigned randomly and double-blindly to one of two groups: folic acid group (n = 24) received folate supplementation for 8 weeks (5 mg daily), and placebo group (n = 24) received placebo. Both folic acid and placebo tablets were supplied by Rouzdarou Pharmacy, Tehran, Iran. The placebo was similar to the folic acid tablets in appearance and taste. Two groups were matched for age, BMI and medication. All patients were advised to avoid changes in their habitual diet or exercise levels during their participation in the study.

Each patient was interviewed with a questionnaire designed for the study to collect information on age, medication, diabetes duration (years) and physical activity. Weight and height were measured to the nearest 0.5 kg and 0.1 cm, respectively. BMI was calculated as weight (kg) divided by height squared (m²). They were also interviewed about their dietary intake using 24-h dietary recalls at baseline and at the end of the study. In order to analyze 24-h dietary recalls Nutritionist III software was used. All subjects were tested to exclude vitamin B12 deficiency before entry, which precludes folic acid treatment.

2.2. Blood sampling and biochemical assays

At baseline and the eighteen week of the trial, 10 ml venous blood samples were collected after overnight fasting, into tubes containing ethylene diamine tetra acetic acid and tubes without anticoagulant. The samples were centrifuged for 15–20 min at 1500 × g to obtain either plasma or serum. Plasma samples were stored at –70 °C and serum samples at –20 °C until analysis.

Lipid profile (total cholesterol (total-C), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglyceride (TG)) and glucose were analyzed on the day of sampling. Levels of fasting blood glucose (FBG), total-C, HDL-C and TG were determined by the enzymatic method using an Abbot Model Aclyon 300 autoanalyzer with kits from Pars-Azmoon (Tehran, Iran). LDL-C levels were calculated by the Friedwald equation [14]. HbA1C was measured using an automated high performance liquid chromatography analyzer with a kit Bio-Rad D-10 Laboratories, Schiltigheim, France.

Serum insulin concentration was measured by chemiluminescent immunoassay (CLIA) method (LIAISON kit, Bio-Rad, Italy).

Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated by the following formula: fasting glucose (mg/dl) × fasting insulin (μU/mL)/405.

Serum levels of folate and B12 were measured using a radioassay method with a Simul TRAC-SNB kit (USA). In this assay, serum folate and B12 compete with folate marked with 125I and B12 marked with CO15 to bind to a specific protein.

Plasma Hcy was determined using an enzyme immunoassay method and an Hcy kit (Axis-Shield Diagnostics, UK). Briefly, mixed disulfides and protein-bound forms of Hcy were reduced to free Hcy by dithiotheritol and enzymatically changed to S-adenosyl-L-homocysteine (SAH) by SAH hydrodase. Plasma Hcy concentration was measured using an enzyme-linked immnosorobent assay reader at 450 nm. In this study, plasma Hcy level of 15 μmol/L or more was considered to be hyperhomocysteinemia (HHC) [15].

2.3. Statistical analysis

The data were analyzed using SPSS version 11.5. A p value less than 0.05 was considered to be statistically significant. The normality of variables was tested using the Kolmogorov-Smirnov test. The chi-square and Student’s t-test were used for categorical and quantitative data comparison between groups, respectively. Mean values before and after the trial were compared within groups using the paired t-test. Bivariate associations were estimated using Pearsons correlation coefficient. Data are expressed as mean ± standard deviation.

3. Results

Baseline and anthropometric characteristics of the patients in the study groups are shown in Table 1. The initial characteristics were similar in the folic acid and placebo treated groups. Results of 24-h recall showed no significant differences in intake of energy, macronutrients, vitamins B2, B6, B12 and folate or caffeine between the two groups at baseline and at the end of the trial period.

At baseline entry, no patients had folate or B12 deficiency (serum folate >3 ng/mL and B12 >200 pg/mL) and 59.6% patients had HHC.
3.1. Glycemic control, HOMA-IR and body weight

As shown in Table 2, the serum level of insulin, HOMA-IR and HbA1C in the folic acid group decreased significantly (16.2%; \( p = 0.021 \), 20.5%; \( p = 0.041 \) and 7.5%; \( p = 0.048 \), respectively). After folate supplementation in patients in the folic acid group, decrease of FBG did not quite reach statistical significance (138.3 ± 35.6 to 127.9 ± 30.0, \( p = 0.051 \)). In the folic acid, there were no significant changes in body weight (\( p = 0.122 \)). Placebo treatment over the eight weeks did not produce any statistically significant changes in these variables.

3.2. Lipid profile

Supplementation with folic acid had no significant effect on total-C, TG, LDL-C and HDL-C levels. These results are presented in Table 3.
Table 4 – Levels of folate, B12 and plasma Hcy in folic acid and placebo groups at baseline and the 8th week of study.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Folic acid group (n = 24)</th>
<th>Placebo group (n = 24)</th>
<th>p1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum folate (ng/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.8 ± 0.6</td>
<td>6.2 ± 1.0</td>
<td>0.086</td>
</tr>
<tr>
<td>After 8 weeks</td>
<td>6.9 ± 0.9</td>
<td>6.3 ± 0.9</td>
<td>0.046</td>
</tr>
<tr>
<td>p1</td>
<td>0.000</td>
<td>0.108</td>
<td></td>
</tr>
<tr>
<td>Serum B12 (pg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>517.2 ± 102.5</td>
<td>512.2 ± 136.3</td>
<td>0.885</td>
</tr>
<tr>
<td>After 8 weeks</td>
<td>606.8 ± 138.5</td>
<td>522.7 ± 121.5</td>
<td>0.030</td>
</tr>
<tr>
<td>p1</td>
<td>0.000</td>
<td>0.200</td>
<td></td>
</tr>
<tr>
<td>Hcy (μmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>15.1 ± 3.3</td>
<td>16.2 ± 4.9</td>
<td>0.393</td>
</tr>
<tr>
<td>After 8 weeks</td>
<td>11.9 ± 3.0</td>
<td>16.4 ± 4.6</td>
<td>0.000</td>
</tr>
<tr>
<td>p1</td>
<td>0.000</td>
<td>0.372</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation.

1 p-value was calculated using Student’s t-test.

2 p-value was calculated using paired t-test.

3.3. Homocysteine, folate and vitamin B12

After folate supplementation in patients in the folic acid group, serum folate and B12 levels increased significantly (19% and 17.3%; p = 0.000, respectively). As shown in Table 4, the plasma level of Hcy in the folic acid group decreased significantly (21.2%; p = 0.000), Whereas no significant changes occurred in the placebo group.

There was a negative correlation between folate change and Hcy change(r = −0.360, p = 0.007). Also, there was a small but statistically significant correlation between Hcy change and HbA1C change(r = 0.274, p = 0.031).

Additionally, univariate general linear modeling was applied to control for time of metformin use. Significant difference by group in endpoint HbA1C, serum insulin, HOMA-IR, Hcy, serum folate and serum B12 persisted even after adjustment for time of metformin use.

4. Discussion

This study showed that folate supplementation with folic acid (5 mg/d) for eight weeks in overweight and obese men with type 2 diabetes receiving metformin treatment led to significant increases in serum folate and B12, and decreases in the plasma level of Hcy and FBG and serum level of insulin and HOMA-IR.

Our findings shows that folate supplementation decreased the level of Hcy, which is in agreement with results of earlier studies; however, the percentage decrease differs [16–19]. Differences in duration of therapy and dose of folic acid could account for these differences. Folic acid is far more effective as an Hcy-lowering agent than vitamins B6 and B12, which cause little, if any, reduction in Hcy [20].

Folate supplementation of as low as 0.5 mg/day has resulted in a reduction of plasma Hcy, although the dose varies considerably between studies (0.5–10 mg/day) [19]. Folate administration also reduces Hcy when both folate and Hcy levels are within the reference range [21].

In the present study, at baseline all patients had folate and B12 levels within the reference range. This indicates that vitamin B12 or folate deficiency is only a rare complication of long-term metformin use. This observation is accordance with the fact that only a few cases of megaloblastic anemia have been reported in metformin-treated patients [22].

There are growing data that folic acid supplementation may improve insulin resistance [23]. A study showed that 3 months of supplementation with folic acid (2.5 mg/d) significantly reduced plasma insulin concentrations and HOMA-IR in overweight subjects [24]. Piatti and colleagues showed that 6 months administration of folic acid not only decreased Hcy levels but also ameliorated insulin sensitivity and endothelial dysfunction [25]. Another study by Setola and co-workers showed that folic acid supplementation (5 mg/d) plus B12 (0.5 mg/d) for 8 weeks in adults with the metabolic syndrome resulted in an improvement in plasma insulin level [26]. In Mao and colleagues’ study, folic acid supplementation combined with enalapril showed a greater beneficial effect on reduction of fasting blood glucose in a dose related fashion than did enalapril alone among subjects with hyperglycemia [27]. Although the mechanisms by which folic acid decreases blood glucose concentration are not clearly understood, several hypotheses have been suggested. The possible mechanism of this relation may be that Hcy thiolactone, the active form of Hcy, may inhibit the insulin-stimulated tyrosine phosphorylation of insulin receptor β-subunit and its substrates and decrease the p85 regulatory subunit of phosphatidylinositol 3-kinase activity, including a reduction in insulin-stimulated glycogen synthesis [28]. This naturally leads to insulin resistance and blood glucose increase. Another possible mechanism is that folic acid will ameliorate endothelial dysfunction induced by elevated Hcy, convert L-arginine to nitric oxide and L-citrulline, scavenger reactive oxygen species such as O2− and peroxynitrite, maintain a coupled endothelial nitric oxide synthase reaction, and prevent nitric oxide synthase dysfunction. All of these may be beneficial to glycometabolism.

There are concerns that, in populations with vitamin B12 deficiencies (such as elderly and vegans), high dose folic acid supplementation may mask the hematologic manifestations of the B12 deficiency and propagate the progression of central and peripheral neurologic damage. In this study, all subjects were tested to exclude vitamin B12 deficiency before entry, and at baseline all patients had folate and B12 levels within the reference range. There is also a concern that high dose folic acid therapy might lead to an increase in incidence of colon or prostate carcinogenesis [29]. Two studies have reported an increased risk of prostate cancer in association with high folate intake, but these results were not statistically significant and were limited to early-stage cancers [30,31]. It has also been suggested that high intakes of folic acid may not lead to de
novo carcinogenesis, but may only increase the rate of proliferation of already established tumor cells [32]. Matching two groups for age distribution and metformin dose, evaluating dietary and caffeine intake and using a placebo are distinctions of our study.

5. Conclusions

Folic acid supplementation decreased plasma level of homocysteine and improved glycemic control, insulin resistance and folate and B12 levels in type 2 diabetic patients treated with high doses of metformin. This finding offers a safe and inexpensive therapy for lowering homocysteine and improving the overall management of diabetic patients. The beneficial effect of metformin may be even more pronounced by lowering of Hcy levels, but they need to be confirmed by additional long term follow-up studies.

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Conflict of interest

There are no conflict of interest.

REFERENCES


