
# Effect of folate supplementation on insulin sensitivity and type 2 diabetes: a meta-analysis of randomized controlled trials

# Effect of folate supplementation on insulin sensitivity and type 2 diabetes: a meta-analysis of randomized controlled trials

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**ABSTRACT**

**Background:** Various mechanisms link higher total homocysteine to higher insulin resistance (IR) and risk of type 2 diabetes (T2D). Folate supplementation is recognized as a way to lower homocysteine. However, randomized controlled trials (RCTs) show inconsistent results on IR and T2D outcomes.

**Objective:** The aim of this study was to examine the effect of folate supplementation on IR and T2D outcomes.

**Design:** We conducted a systematic literature search in PubMed, Web of Science, and EMBASE and prior systematic reviews and meta-analyses and identified 29 RCTs (22,250 participants) that assessed the effect of placebo-controlled folate supplementation alone or in combination with other B vitamins on fasting glucose, insulin, homeostasis model assessment for insulin resistance (HOMA-IR), glycated hemoglobin (HbA1c), or risk of T2D. The meta-analysis was conducted using both random- and fixed-effects models to calculate weighted mean differences (WMDs) or risk ratios with 95% CIs. Subgroup analyses were conducted based on intervention type (folate alone or in combination with other B vitamins), as well as analysis based on population characteristics, duration, dose, and change in homocysteine.

**Results:** When compared with placebo, folate supplementation lowered fasting insulin (WMD: −13.47 pmol/L; 95% CI: −21.41, −5.53 pmol/L; $P < 0.001$) and HOMA-IR (WMD: −0.57 units; 95% CI: −0.76, −0.37 units; $P < 0.0001$), but no overall effects were observed for fasting glucose or HbA1c. Heterogeneity was low in all meta-analyses, and subgroup analysis showed no signs of effect modification except for change in homocysteine, with the most pronounced effects in trials with a change of >2.5 μmol/L. Changes in homocysteine after folate supplementation correlated with changes in fasting glucose ($\beta = 0.07$; 95% CI: 0.01, 0.14; $P = 0.025$) and HbA1c ($\beta = 0.46$; 95% CI: 0.06, 0.85; $P = 0.02$). Only 2 studies examined folate supplementation on risk of T2D, and they found no change in RR (pooled RR: 0.91; 95% CI: 0.80, 1.04; $P = 0.16$).

**Conclusion:** Folate supplementation might be beneficial for glucose homeostasis and lowering IR, but at present there are insufficient data to conclusively determine the effect on development of T2D. This trial was registered on the Prospero database as CRD42016048254. *Am J Clin Nutr* 2019;109:29–42.

**Keywords:** metabolic syndrome, homocysteine, insulin resistance, methyl donor metabolism, folic acid, one-carbon metabolism

## Introduction

Type 2 diabetes (T2D) is an increasing problem worldwide and a major health care burden in developed and developing societies alike. The most cost-effective way to treat T2D is through early detection, prevention, and treatment in the early stages, before onset of major systemic damage such as microvascular complications and retino- and nephropathy (1).

High circulating concentrations of homocysteine have been established as a risk factor for stroke and other cardiovascular diseases (CVDs) (2). Furthermore, high homocysteine has been associated with insulin resistance (IR) (3) and increased risk of T2D in women with gestational diabetes (4), and a Mendelian randomization study suggested that high homocysteine might play a causal role in the development of T2D (5). In addition, high homocysteine has also been linked to vascular complications of diabetes (6, 7) as well as nephropathy (8) and has been shown in patients with T2D to predict risk of early mortality (9). Folate, alone or in combination with other B vitamins, has been proven to be an effective way of lowering homocysteine concentrations (10). A recent study showed that folate supplementation reduced stroke incidence more among participants with high fasting blood

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Supplemental Tables 1–10 and Supplemental Figures 1–3 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: CVD, cardiovascular disease; HbA1c, glycated hemoglobin; IR, insulin resistance, RCT, randomized controlled trial; T2D, type 2 diabetes; WMD, weighted mean difference.

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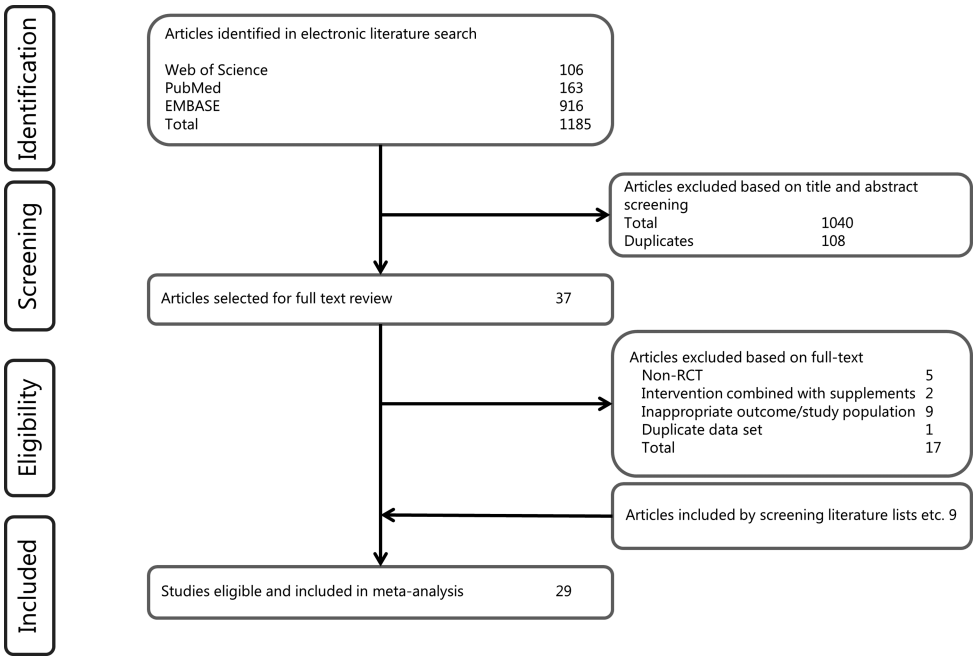


**FIGURE 1** Flow diagram showing the flow of articles through the search strategy and selection of studies. RCT, randomized controlled trial.

glucose or diabetes, suggesting that folate might especially benefit this subgroup (11). A complex link between homocysteine, IR, T2D, and CVD outcomes has been hypothesized (12), and although a great number of randomized controlled trials (RCTs) have investigated the effects of folate supplementation, only a limited number have reported on outcomes related to glucose homeostasis or T2D development. Therefore, further attention is needed on folate supplementation as a potential modifier of homocysteine status as a way to affect T2D risk and complications.

The results from RCTs examining folate supplementation and IR are inconclusive due to the high variability between the trials (13–21). The aim of this systematic review and meta-analysis was to examine if folate improves glycemic control and thereby prevents development of T2D. The present meta-analysis includes all RCTs examining the effect of folate supplementation alone or in combination with vitamin B-6 and/or vitamin B-12 on markers of glucose homeostasis and T2D development. Changes in fasting insulin, glucose, glycated hemoglobin (HbA1c), and HOMA-IR, as well as future development of T2D, were included as outcomes.

## Methods

The present meta-analysis was carried out using a predetermined protocol as published in the Prospero database (registration no. CRD42016048254) and in accordance with PRISMA guidelines (22). The flow diagram of the search, identification, screening, and inclusion of studies is outlined in **Figure 1**.

### Data sources and identification of potentially relevant studies

A systematic literature search followed by study selection according to the predefined eligibility criteria, data mining, and statistical analyses was performed by the researchers MVL and JNE as outlined in the protocol. Data sources included the following bibliographic databases: MEDLINE via PubMed from 1953, Web of Science from 1900, and EMBASE from 1974. The data search was conducted in the period from March to October 2016. An updated search was conducted in December 2017. No new studies were identified.

RCTs investigating the effects of folate supplementation on glucose, fasting insulin, HOMA-IR, HbA1c, and T2D were sought using the predefined search terms built into 3 main search blocks covering study intervention, outcomes, and study design (see Supplemental Methods 1). The search was limited to human studies that had been published in English. The outcomes addressed were seldom primary intervention outcomes in the included studies, and therefore reference lists as well as other systematic reviews were inspected to identify any additional published studies not identified by the database search. The knowledge of known experts in the field was also sought.

### Criteria for study consideration

#### *Study design and population.*

RCTs in adults (aged >18 y), both parallel and crossover interventions of >24-h duration (with no upper limit), were considered eligible (**Supplemental Table 1**). No restriction on medication use or baseline health condition, including status of insulin sensitivity, was set. In studies with multiple eligible intervention arms, results were obtained from all eligible arms (20, 23–25).

#### *Intervention type and outcome.*

Folate is usually supplemented as folic acid, a synthetic form of the vitamin that is converted to the biologically active form,

5-methyltetrahydrofolate. Articles were included if they examined any form of folate alone or in combination with vitamin B-12 or vitamin B-6. Most studies gave folate as folic acid, although 2 gave folate as 5-methyltetrahydrofolate. Because only 2 studies gave the latter (26, 27), we combined the results across studies and refer to this as folate supplementation. Studies including other vitamins (except for vitamin B-12 and vitamin B-6) or bioactive components as part of the intervention were excluded. There were no restrictions on diet or medication, although comparable background diets and/or medication protocols in the intervention and control groups were required. Studies that met the above criteria were included if ≥1 of the following outcomes were measured: change in glucose, insulin, HOMA-IR, or HbA1c. The primary outcome of the analysis was HOMA-IR. Studies reporting on the development of new diabetic cases in participants receiving the folate or placebo intervention were also included.

### Screening and eligibility

To assess eligibility, 2 authors (MVL and JNE) independently screened all titles and abstracts from each single retrieved publication found in the database search. Duplicate articles on the same study were removed. Eligible publications were screened at the full-text level by the same 2 authors. Differences of opinion were resolved by a third author (LL or ABR). For eligible studies based on defined inclusion criteria, 2 investigators (MVL and JNE) extracted relevant information on both population level and intervention characteristics using data-extraction templates, including *1*) general information, *2*) trial characteristics, *3*) risk of bias (28), *4*) intervention (e.g., type of supplement, dose), *5*) participants (i.e., sex, age), *6*) baseline and postintervention characteristics (i.e., plasma folate and homocysteine), and *7*) outcomes (**Supplemental Table 2**). Data extraction was conducted independently by the 2 authors from the original publications. When relevant data were missing from publications, authors were contacted to obtain the missing information.

### Assessment of reviewer agreement and risk of bias for included studies

Study quality and risk of bias were assessed independently by 2 of the authors (MVL and JNE) using the Cochrane Collaboration's Tool for Bias Assessment (28). Discrepancies in opinions between the 2 authors were discussed until agreement was reached. The following domains were considered: *1*) random-sequence generation, *2*) allocation concealment, *3*) blinding of participants and personnel, *4*) blinding of outcome assessors, *5*) selective reporting, and *6*) other bias. Each of these key components of methodologic quality was assessed according to the categorizations of low, unclear, or high.

### Data synthesis

The extracted data were transformed to harmonize the units reported before analysis. All units in the studies were converted into millimoles per liter for glucose and picomoles per liter for insulin. The conversion factors used in this analysis are as follows: 6.945 pmol/L per μIU insulin/mL and 0.0555 mmol/L per mg glucose/dL. HOMA-IR was calculated as fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5 (29, 30), and HbA1c is reported as percentages.

We calculated the difference in means for all the extracted continuous outcomes. In crossover trials in which the SDs for paired differences were lacking, the SDs were taken for the last measurements in the groups ending the intervention and control periods. Net changes in outcome measures were calculated as the difference (folate minus control) between changes (postintervention minus baseline) in these mean values. When variances for net changes were not reported directly, they were calculated from the SEs, SDs, and 95% CIs by using the largest value determined, which mostly included using variability data for end measurements. Where the data were not shown but were reported not to be different, we contacted the authors to try to obtain the true values. However, if this was not possible, we assumed that there was no difference. Pooled SDs from the other studies included were used in the reporting. For outcomes that only reported postintervention values, the mean differences were subtracted together with the SDs from the 2 groups. For one study (14), the outcome for HbA1c was found in another meta-analysis (21) and the reported mean difference was used. In addition, HOMA-IR was calculated for Villa et al. (31) based on reported values of fasting insulin and glucose. For Kurt et al. (32) and Mashavi et al. (17), insulin was calculated from HOMA-IR and fasting glucose values based on the equation for HOMA-IR. The SD for all of these was based on the pooled SDs from the rest of the included studies. We did the analysis with and without the calculated means and found that this did not affect the overall estimate or interpretation of the results. For 2 studies (33, 34), changes in homocysteine were reported elsewhere (35, 36). To avoid double-counting participants in studies where there were >2 intervention groups, the control group was halved.

### Statistical analyses

Statistical analyses were conducted with Review Manager software (version 5.3.5; Cochrane Collaboration) and R (version 3.3.2) using the Metafor package (37). The effects of folate on glucose metabolism outcomes were analyzed using a random-effects model for both main outcomes and subgroup analyses. For some studies, a control group was included in more than one meta-analytic estimate; thus, we inflated the SEs to avoid double-counting of participants (20, 23–25). The effect of folate supplementation was tested according to predefined and post hoc subgroups (Supplemental Table 2). Tests for subgroup differences were done according to Borenstein et al. (38). For subgroup analysis, both studies defining subjects as diabetics and studies reporting on intake of antidiabetic medication were included in the subgroup analysis for disease state. For the T2D development outcome, fixed-effects Mantel-Haenszel analysis of the risk ratio was used. Funnel plots were used to evaluate potential small-study effects, as well as Egger's regression and the Begg and Mazumdar test for heterogeneity. Heterogeneity between studies was tested using the Q-statistic and quantified using the $I^2$ metric. The $I^2$ metric ranged between 0% and 100%, with higher values indicating greater heterogeneity between

studies. Sensitivity analyses were performed in which each study (and study arm) was excluded from analysis in turn to assess the impact of the individual studies on the overall effect size. A restricted maximum likelihood–based (i.e., random-effects) meta-regression analysis was applied to assess whether dose, duration, or changes in homocysteine were associated with glycemic control outcomes.

## Results

### Study characteristics and designs

The initial literature search identified 1185 publications reporting potentially relevant studies. After screening based on title and abstracts, 37 articles were selected for full-text review. In total, 29 studies that met the inclusion criteria were identified and included for glucose metabolism measurements (Figure 1, **Table 1**). Most of the included studies ($n$ = 26) reported on fasting circulating glucose. For fasting insulin and HOMA-IR, 11 studies were included, and 8 studies were included for HbA1c. Only 2 studies were included for T2D development as an outcome, with follow-ups of 4.5 and 7.3 y, respectively (33, 34). Of the studies, 4 were crossover designs and 25 were parallel designs. The duration of the studies reporting on glucose metabolism outcomes ranged from 2 wk (13) to 2 y (39), with the majority of studies lasting between 4 and 8 wk. There were 2 studies that included a weight-loss regime (16, 19), and 21 studies included supplementation with folic acid alone, whereas 3 included vitamin B-12 (15, 32, 40), 1 included vitamin B-6 (41), and 4 included both vitamin B-12 and vitamin B-6 (17, 27, 34, 39). All trials showed a mean reduction in homocysteine ranging from 0.3 to 4.3 μmol/L compared with the placebo treatment (**Table 2**). The dosages used were between 0.4 and 15 mg folic acid/d, with most studies using dosages between 0.8 and 5 mg/d (Table 1).

### Participant characteristics

For glucose metabolism outcomes, 17,765 participants were included in the analysis (Table 1), 15,951 of whom were from the same study (33). Many studies examined effects on populations with varying health status, including 1 study in women with polycystic ovary syndrome (20); 8 studies in subjects with T2D (14, 17, 18, 25, 27, 42–44); 6 in subjects with metabolic syndrome traits—either overweight, obese, hypertensive, or having multiple variables of the metabolic syndrome (15, 16, 19, 23, 33, 45); 7 in people with CVD (13, 24, 39, 40, 46–48); 1 in people with HIV (49); 1 included women with cervical intraepithelial neoplasia (50); and 5 studies included apparently healthy participants (26, 31, 32, 41, 51). Most studies included both genders, but 6 were in women only (19, 20, 26, 31, 34, 50) and 5 were in men only (14, 18, 40, 41, 51). Most studies were performed in European populations (12), although 6 were in Arabic populations, 5 were in East-Asian populations, 3 were North American, 2 were Australian, 1 was Israeli, and 1 was South American. The 2 studies on T2D development outcome had a total of 24,954 participants, with 916 new diabetes cases identified during follow-up (33, 34).

### Fasting glucose

We found no effect of folate supplementation on fasting glucose concentrations [weighted mean difference (WMD): −0.02 mmol/L; 95% CI: −0.06, 0.02 mmol/L; $P = 0.43$] (**Figure 2**). In total, 30 study arms were included in this analysis, 23 of which were with folate supplementation alone, with the other 7 being in combination with supplementation of other B vitamins. The test for subgroup differences between folate alone and with other B vitamins was significant ($P = 0.01$); however, the overall test on heterogeneity resulted in an $I^2$ value of 0% ($P = 0.68$). Studies that combined folate and other B vitamins showed lower fasting glucose (WMD: −0.18 mmol/L; 95% CI: −0.31, −0.04 mmol/L; $P = 0.01$) compared with placebo, whereas folate supplementation alone showed no difference between treatment and placebo groups (WMD: −0.00 mmol/L; 95% CI: −0.04, 0.04 mmol/L; $P = 0.97$). There was no indication of heterogeneity within the 2 subgroups ($I^2 = 0\%$ for both) (Figure 2). No indication of funnel plot asymmetry was found using Egger's test ($P = 0.12$) or the Begg and Mazumdar test ($P = 0.17$) (**Supplemental Table 3**).

### Fasting insulin

Folate supplementation resulted in a decrease in fasting insulin (WMD: −13.47 pmol/L; 95% CI: −21.41, −5.53 pmol/L; $P < 0.001$) (**Supplemental Figure 1**). This analysis included 12 study group arms, of which 9 were with folate supplementation only and 3 gave folate together with other B vitamins. Folate supplementation alone showed a significant decrease in the intervention group (WMD: −12.19 pmol/L; 95% CI: −20.49, −3.88 pmol/L; $P = 0.004$), whereas no difference was found in the combination group (WMD: −15.63 pmol/L; 95% CI: −38.58, 7.32 pmol/L; $P = 0.18$) compared with placebo (Supplemental Figure 1). There was no indication of any subgroup differences ($P = 0.78$), but an overall significant heterogeneity between studies ($I^2 = 55\%$; $P = 0.01$) and funnel plot asymmetry using Egger's test ($P = 0.01$) or the Begg and Mazumdar test ($P = 0.03$) were found (Supplemental Table 3).

### HOMA-IR

Folate supplementation resulted in an overall decrease in HOMA-IR (WMD: −0.57 units; 95% CI: −0.76, −0.37 units; $P < 0.001$), with no subgroup differences between folate alone or in combination with other B vitamins ($P = 0.30$) (**Figure 3**). The analysis included results from 12 study arms, of which 9 were with folate supplementation alone and 3 were with folate combined with other B vitamins. No heterogeneity between studies ($I^2 = 0\%$; $P = 0.32$) were observed and funnel plot asymmetry was not indicated by Egger's test ($P = 0.26$) or the Begg and Mazumdar test ($P = 0.95$) (Supplemental Table 3).

### HbA1c

No overall effect was observed after folate supplementation on HbA1c (WMD: −0.06%; 95% CI: −0.24%, 0.12%; $P = 0.34$), and there were no differences between subgroups of folate alone or combined with other B vitamins ($P = 0.53$) (**Supplemental Figure 2**). In total, 8 studies were included in the HbA1c analyses

**TABLE 1** Characteristics of included studies, participants, and intervention[1]

| Study: first author, year of publication (ref) | Country | Design | Duration, wk | No. of subjects | Sex | Age, y | Intervention and dose | Outcomes reported[2] | Subject health state | Other study interventions | T2D or glucose metabolism medication |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Aarsand, 1998 (39) | Norway | P | 12 | 28 | M = 21, F = 7 | 59 ± 3 | 0.25 mg folate | FBG, HbA1c | T2D | Fe supplement | Metformin >1000 mg/d |
| Aghamohammadi, 2011 (14) | Iran | P | 8 | 68 | M | 57 ± 8 | 5 mg FA | HbA1c | T2D | None | Yes, metformin ≥1500 mg |
| Araki, 2006 (40) | Japan | P | 2 | 17 | M | 22 ± 1 | 0.8 mg FA | FBG | Healthy | None | None |
| Asemi, 2014 (20) | Iran | P | 8 | 81 | F | 25 ± 5 | Group 1: 1 mg FA; group 2: 5 mg FA | FPG, insulin, HOMA-IR | PCOS, overweight or obese | None | None |
| Asemi, 2016 (41) | Iran | P | 26.1 | 58 | F | 38 ± 9 | 5 mg FA | FBG, insulin, HOMA-IR | Cervical intraepithelial neoplasia patients | None | Not known |
| Cagnacci, 2009 (26) | Italy | P | 3 | 30 | F | 55 ± 1 | 15 mg 5-MeTHF | FBG, insulin HOMA-IR | Healthy postmenopausal | None | None |
| Chambers, 2000 (42) | UK | P | 8 | 89 | M | 56 ± 7 | 5 mg FA + 1 mg vitamin B-12 | FBG | CHD | CHD medication | Not known |
| Doshi, 2001 (43) | UK | CO | 6 | 50 | M = 44, F = 6 | 57 ± 8 | 5 mg FA | FBG | CAD (or CHD) patients | CAD medication | Not known |
| Doshi, 2002 (44) | UK | P | 6 | 33 | M = 30, F = 3 | 56 ± 7 | 5 mg FA | FBG | CAD patients | CAD medication | Not known |
| Fonseca, 2013 (27) | USA | P | 24 | 214 | M = 148, F = 66 | 63 ± 9 | 3 mg 5-MeTHF + 2 mg vitamin B-12 + 35 mg vitamin B-6 | HbA1c | T2D + neuropathy | None | Antidiabetic medication + insulin |
| Gargari, 2011 (18) | Iran | P | 8 | 48 | M | 58 ± 9 | 5 mg FA | FBG, insulin, HOMA-IR, HbA1c | T2D | None | Yes, metformin ≥1500 mg |
| Grigoletti, 2013 (45) | Brazil | P | 4 | 30 | M = 14, F = 16 | 45 ± 2 | 5 mg FA | FBG | HIV | ART treatment | None |
| Haglund, 1993 (46) | Sweden | CO | 4 | 12 | M | 48 ± 12 | 10 mg FA + 80 mg vitamin B-6 | FBG | Healthy, with slightly increased blood lipids | Fish oil (30 mL) | None |
| Kurt, 2010 (32) | Turkey | P | 8 | 44 | M = 20, F = 24 | 65 ± 3 | 5 mg folate + 0.5 vitamin B-12 | FBG (insulin), HOMA-IR | Older males and females with low vitamin B-12 (<180 mg/dL) | None | Excluded people with T2D |
| Mangoni, 2005 (47) | Australia | P | 4 | 26 | M = 14, F = 12 | 57 ± 1 | 5 mg FA | FBP, HbA1c | T2D | None | Antidiabetic medication + insulin |
| Mao, 2008 (23) | China | P | 8 | 443 | M = 189, F = 254 | 57 ± 10 | Group 1: 0.4 mg FA; group 2: 0.8 mg FA | FBP | Hypertensive subjects; 1.8% had diabetes | None | |
| Mashavi, 2008 (17) | Israel | P | 17.4 | 57 | M = 28, F = 29 | 61 ± 7 | 1 mg folate + 0.4 vitamin B-12 + 10 mg vitamin B-6 | FBP (insulin), HOMA-IR, HbA1c | T2D | None | Antidiabetic medication + insulin |
| Moat, 2006 (24) | UK | P | 6 | 84 | M = 73, F = 11 | 60 ± 7 | Group 1: 0.4 mg FA; group 2: 5 mg FA | FBP | CAD patients | None | Subjects with T2D were excluded |
| Moens, 2007 (48) | Netherlands | CO | 6 | 40 | M = 35, F = 5 | 56 ± 13 | 10 mg FA | FBP | AMI/CVD participants 10% had T2D | None | — |

(*Continued*)

**TABLE 1** (*Continued*)

| Study: first author, year of publication (ref) | Country | Design | Duration, wk | No. of subjects | Sex | Age, y | Intervention and dose | Outcomes reported[2] | Subject health state | Other study interventions | T2D or glucose metabolism medication |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Potter, 2008 (49) | Australia | P | 104 | 162 | M = 117, F = 45 | 65 ± 14 | 2 mg FA + 0.5 mg vitamin B-12 + 25 mg vitamin B-6 | FBG, HbA1c | Patients with a history of stroke; 17% had T2D | None | — |
| Qin, 2016 (33) | China | P | 4.5-y follow-up | 20,030 (15,951 for FBG) | M = 8295, F = 11,735 | 60 ± 8 | 0.8 mg FA | FBG, risk of diabetes | Hypertensive patients | None | No diabetes or glucose-lowering drugs |
| Setola, 2004 (15) | Italy | P | 4 | 50 | M = 41, F = 9 | 67 ± 1 | 5 mg FA + 0.5 mg vitamin B-12 | FBG, insulin, HOMA-IR | Metabolic syndrome + hyperinsulinemia | Both groups treated with diet for 8 wk | NA |
| Sheu, 2005 (19) | Taiwan | P | 12 | 74 | F | 42 ± 2 | 5 mg FA | Insulin, FBG, HOMA-IR | Obese | Weight-loss study | NA |
| Solini, 2006 (16) | Italy | P | 12 | 60 | M = 19, F = 41 | 49 ± 8 | 2.5 mg FA | FBG, insulin, HOMA-IR | Overweight | Weight-loss study | No T2D |
| Song, 2009 (34) | USA | P | 7.3-y follow-up | 4252 | F | 63 ± 9 | 2.5 mg FA + 1 mg vitamin B-12 + 50 mg vitamin B-6 | Risk of diabetes | History of CVD or ≥3 CVD factors. | Added to a 2 × 2 × 2 factorial trial of 3 antioxidant vitamins (vitamins C and E and $\beta$-carotene) | No T2D |
| Spoelstra-de Man, 2004 (50) | Netherlands | P | 26.1 | 41 | M = 24, F = 17 | 65 ± 9 | 5 mg FA | HbA1c | T2D | None | 50% Oral hypoglycemia agents |
| Talari, 2016 (51) | Iran | P | 12 | 60 | M = 26, F = 37 | 64 ± 10 | 5 mg FA | FBG, insulin, HOMA-IR | Metabolic syndrome/ CHD/T2D | None | Not reported |
| Title, 2006 (13) | Canada | CO | 2 | 19 | M = 9, F = 10 | 55 ± 6 | 10 mg FA | FBG | CAD | None | Excluded T2D patients and no diabetes medication |
| Villa, 2005 (31) | Italy | P | 8 | 20 | F | 54 ± 2 | 7.5 mg FA | FBG, insulin (HOMA-IR) | Healthy postmenopausal | None | None |
| Weijun, 2008 (25) | China | P | 2 | 90 | M = 33, F = 27 | 58 ± 10 | 5 mg FA | FBG | T2D | None | Not reported |

[1]AMI, acute myocardial infarction; ART, antiretroviral therapy; CAD, coronary artery disease; CHD, coronary heart disease; CO, crossover; CVD, cardiovascular disease; FA, folic acid; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; MeTHF, methyl-tetrahydrofolate; NA, not available; P, parallel; PCOS, polycystic ovary syndrome; ref, reference; T2D, type 2 diabetes.

[2]Outcomes in parentheses were calculated based on FBG, insulin, or HOMA-IR.

**TABLE 2** Characteristics of participant baseline vitamin and homocysteine concentrations and BMI and glucose metabolism measurements in included studies[1]

| | Baseline | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Study: first author, year of publication (ref) | Folate, nmol/L | Vitamin B-12, pmol/L | Homocysteine, μmol/L | BMI, kg/m$^2$ | Glucose, mmol/L | Insulin, pmol/L | HOMA-IR, units | HbA1c, % | Mean change in homocysteine,[2] μmol/L |
| Aarsand, 1998 (39) | 9 ± 5 | 348 ± 40 | 10.5 ± 1.2 | 29 ± 1.8 | 9.20 ± 1.2 | — | — | 8.1 ± 0.6 | 2.4 ± 0.9 |
| Aghamohammadi, 2011 (14) | — | — | 15.5 ± 6.0 | 28 ± 5 | 7.53 ± 2.75 | — | — | 7.6 ± 2.1 | 3.5 ± 5.5 |
| Araki, 2006 (40) | 12 ± 6.2 | 688 ± 550 | 6.5 ± 4.24 | 21 ± 1 | — | — | — | — | 1.1 ± 0.8 |
| Asemi, 2014[3] (20) | — | — | 17.0 ± 7.6 | 28 ± 8 | 4.28 ± 1.05 | 78.9 ± 63.6 | 2.17 ± 1.97 | — | 2.7 ± 7.9/3.2 ± 8.3 |
| Asemi, 2016 (41) | — | — | 12.9 ± 4.7 | 29 ± 7 | 4.31 ± 1.27 | 112.5 ± 78.3 | 3.15 ± 2.34 | — | 2.1 ± 5.5 |
| Cagnacci, 2009 (26) | — | — | 11.8 ± 5.3 | 27 ± 7 | 5.12 ± 1.58 | 75.6 ± 54.1 | 2.50 ± 0.19 | — | 3.0 ± 5.4 |
| Chambers, 2000 (42) | 24 ± 15 | 223 ± 95 | 13.8 ± 6.4 | 27 ± 5 | 5.85 ± 2.83 | — | — | — | 4.1 ± 7.2 |
| Doshi, 2001 (43) | 20 ± 8 | 319 ± 86 | 11.2 ± 2.7 | 29 ± 4 | 5.30 ± 0.60 | — | — | — | 2.1 ± 3.2 |
| Doshi, 2002 (44) | 24 ± 11 | 309 ± 128 | 10.7 ± 3.4 | 29[4] | 5.55 ± 1.07 | — | — | — | 2.3 ± 2.9 |
| Fonseca, 2013 (27) | 43 ± 14 | 426 ± 433 | 9.6 ± 5.8 | — | — | — | — | 7.1 ± 1.2 | 3.2 ± 2.0 |
| Gargari, 2011 (18) | 14 ± 3 | 380 ± 126 | 15.7 ± 5.9 | 29 ± 4 | 7.54 ± 2.76 | 92.7 ± 66.8 | 4.40 ± 3.48 | 7.6 ± 2.2 | 3.4 ± 5.1 |
| Grigoletti, 2013 (45) | 23 ± 11 | 268[4] | 8.9[4] | 24[4] | 5.55 ± 1.07 | — | — | — | 1.1[4] |
| Haglund, 1993 (46) | 17 ± 10 | — | 4.3 ± 1.6 | 26 ± 5 | 5.10 ± 0.78 | — | — | — | 1.7 ± 1.6 |
| Kurt et al., 2010 (32) | 13 ± 7 | 95 ± 32 | 10.4 ± 4.3 | 28 ± 4 | 5.13 ± 0.79 | 57.8[4] | 1.90 ± 1.12 | — | 4.3 ± 3.9 |
| Mangoni, 2005 (47) | 18 ± 7 | 307 ± 124 | 11.7 ± 2.0 | 31 ± 6 | 11.40 ± 4.08 | — | — | 8.3 ± 1.5 | 2.3 ± 3.7 |
| Mao, 2008[3] (23) | 13 ± 3 | — | 13.2 ± 2.8 | 26 ± 6 | 5.47 ± 2.08 | — | — | — | 1.7 ± 1.9/1.9 ± 1.9 |
| Mashavi, 2008 (17) | 19 ± 9 | 460 ± 248 | 9.0 ± 5.1 | 31 ± 7 | 8.84 ± 3.13 | 185.5[4] | 10.50 ± 30.04 | 8.4 ± 2.1 | 2.4 ± 4.8 |
| Moat, 2006[3] (24) | 22 ± 16 | 342 ± 212 | 12.2 ± 6.2 | 29 ± 7 | 5.43 ± 1.11 | — | — | — | 1.9 ± 5.2/3.5 ± 4.7 |
| Moens, 2007 (48) | 14 ± 9 | 330 ± 297 | 14.6 ± 7.8 | — | 6.19 ± 4.23 | — | — | — | 4.0[4] |
| Potter, 2008 (49) | — | 307[4] | 11.0[4] | 29 ± 7 | 5.45[4] | — | — | — | 2.5[4] |
| Qin, 2016 (33) | 18[4] | 279[4] | 12.6[4] | 25 ± 11 | 5.70 ± 1.98 | — | — | — | 1.7[4] |
| Setola, 2004 (15) | 29 ± 14 | 219 ± 273 | 11.6 ± 6.9 | 29 ± 4 | 6.30 ± 7 | 136.1 ± 49.1 | 5.43 ± 2.9 | 5.8 ± 0.7 | 3.2 ± 6.4 |
| Sheu, 2005 (19) | 22 ± 11 | 510 ± 254 | 7.8 ± 2.7 | 30 ± 4 | 5.36 ± 0.95 | 96.2 ± 84.0 | 3.40 ± 3.44 | — | 0.3 ± 2.2 |
| Solini, 2006 (16) | 10 ± 6 | 598 ± 237 | 11.2 ± 3.9 | 27 ± 1 | 4.50 ± 0.71 | 88.0 ± 37.8 | 2.95 ± 1.38 | — | 1.3 ± 3.4 |
| Song, 2009 (34) | — | — | — | — | — | — | — | — | 2.3[4] |
| Spoelstra-de Mar, 20045 (50) | 13 ± 6 | 250 ± 106 | 17.5 ± 21.5 | 29 ± 5 | — | — | — | 7.5 ± 1.8 | 19%[b,4] |
| Talari, 2016 (51) | — | — | 17.3 ± 9.1 | 30 ± 6 | 6.18 ± 3.49 | 128.1[4] | 5.00 ± 4.39 | — | 3.9 ± 9.9 |
| Title, 2006 (13) | 37[4] | — | 9.4[4] | — | 7.10 ± 1.30 | — | — | 6.5 ± 1.1 | 0.3[4] |
| Villa, 2005 (31) | 16 ± 32 | 232 ± 79 | 8.1 ± 3.5 | 28 ± 8 | 4.57 ± 0.51 | 61.7 ± 28.3 | 1.81[4] | — | 1.2 ± 1.0 |
| Weijun, 2008[3] (25) | 17[4] | 347[4] | 9.4[4] | 25 ± 6 | — | — | — | 7.9 ± 3.3 | 0.3/3.5[4] |

[1] Values are means ± SDs unless otherwise indicated. HbA1c, glycated hemoglobin; ref, reference.
[2] Where there are 2 numbers, there are changes in the 2 substudies.
[3] The 2 numbers in the mean change in homocysteine indicate the changes in the 2 study arms.
[4] SD not reported.
[5] One study only reported the percentage change.

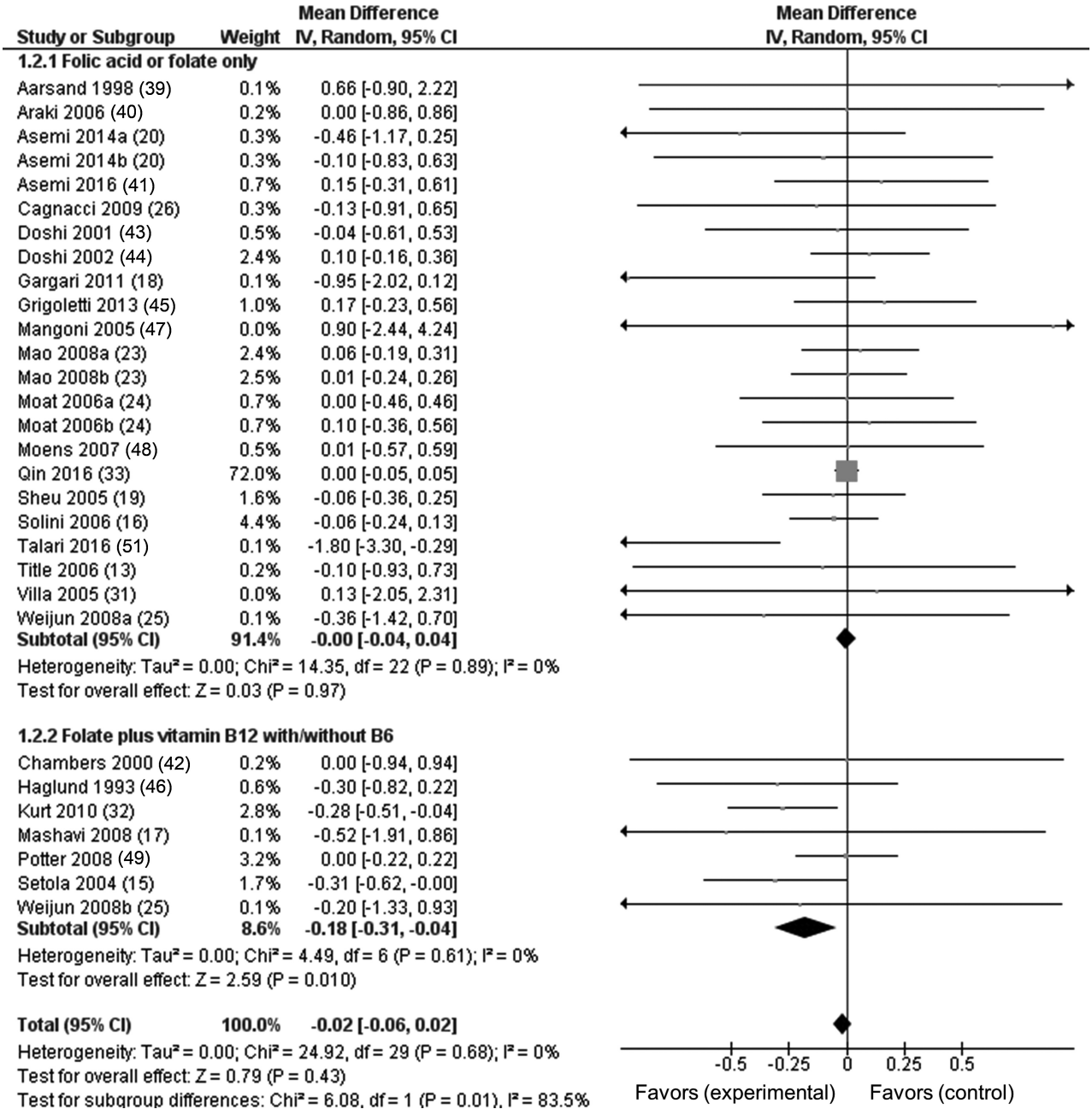


**FIGURE 2** Forest plot of fasting glucose (mmol/L) (folate alone or in combination with other B vitamins compared with control) by individual trials and pooled results using a random-effects model. For each study, the square represents the point estimate of the intervention effect. The horizontal lines show the lower and upper 95% CI limits of this effect. The area of the squares reflects the relative weight of each study in the meta-analysis. The diamonds represent the subgroup weighted mean differences and the combined mean difference. IV, inverse variance.

and, of these, 5 were conducted with folate alone and 3 included folate in combination with other B vitamins. Low heterogeneity was found between studies ($I^2 = 18\%$; $P = 0.29$) and funnel plot asymmetry was not indicated by Egger's test ($P = 0.09$) or the Begg and Mazumdar test ($P = 0.06$) (Supplemental Table 3).

### T2D development

Only 2 studies reported on development of new T2D cases in the follow-up period. Of these, one used folic acid only (33) and the other used folic acid, vitamin B-12, and vitamin B-6 (34). No differences were found for folate supplementation on overall T2D risk (0.91; 95% CI: 0.80, 1.04; $P = 0.16$) (**Supplemental Figure 3**), and no heterogeneity was found in the results of the 2 studies ($I^2 = 0\%$; $P = 1.00$).

### Subgroup and meta-regression analyses of effects of folate supplementation

No subgroup effects were observed on any of the glucose metabolism outcomes after stratifying on age (<58 y, ≥58 y),

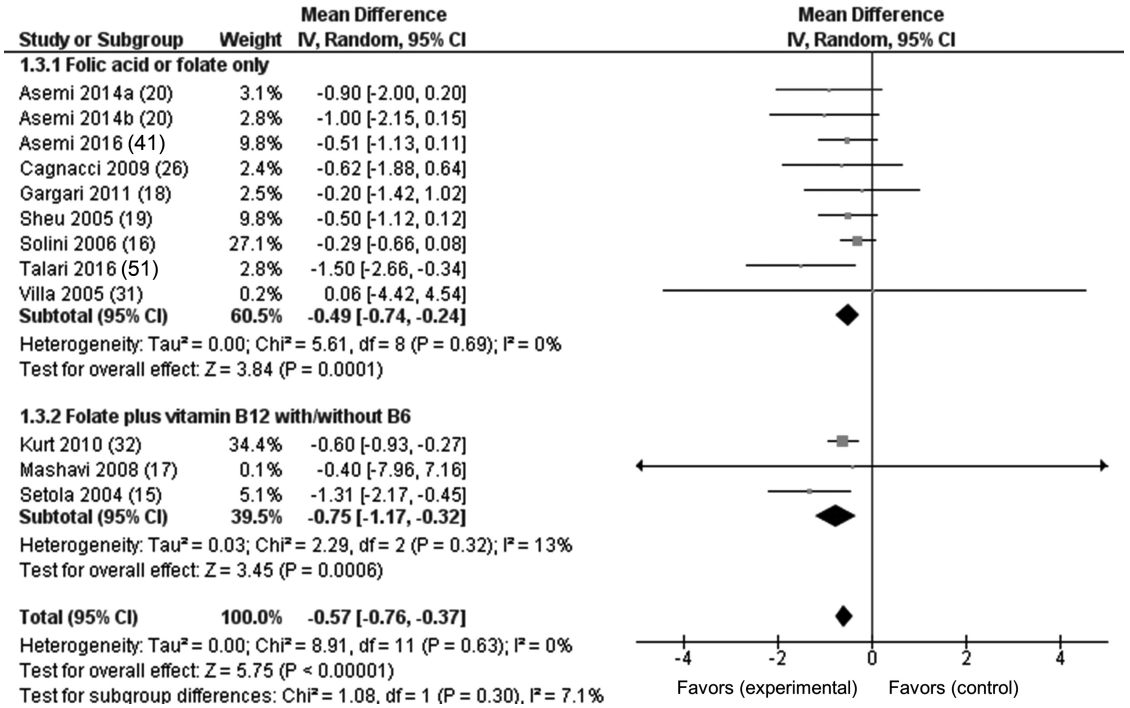


**FIGURE 3** Forest plot of HOMA-IR (units) (folate alone or in combination with other B vitamins compared with control) by individual trials and pooled results using a random-effects model. For each study, the square represents the point estimate of the intervention effect. The horizontal lines show the lower and upper 95% CI limits of this effect. The area of the squares reflects the relative weight of each study in the meta-analysis. The diamonds represent the subgroup weighted mean differences and the combined mean difference. IV, inverse variance.

sex (<33% men, 33–66% men, >66% men), baseline folate concentrations (≤18 pmol/L, >18 pmol/L), baseline vitamin B-12 concentrations (≤325 pmol/L, >325 pmol/L), baseline homocysteine concentrations (≤12 μmol/L, >12 μmol/L), baseline fasting glucose concentration (<5.6 mmol/L, ≥5.6 mmol/L), baseline BMI (kg/m$^2$; <28, ≥28), percentage homocysteine changes (≤20%, >20%), health status of participants (T2D, CVD, no T2D) dosage of folate used (<5 mg/d, ≥5 mg/d), or duration of study (<8 wk, ≥8 wk) (**Table 3**, **Supplemental Tables 4–7**). No significant associations were found for any outcomes in meta-regression for folate dosage or trial duration (**Supplemental Table 8**). We also tested changes in homocysteine as a mediator of changes in glucose metabolism and found that consistently larger reductions in homocysteine (>2.5 μmol/L) were associated with improved outcomes of glycemic control ($P$ values of 0.004–0.07). Further, post hoc meta-regression analyses showed positive associations between absolute decreases in homocysteine and absolute decreases in glucose measurement outcomes, which reached statistical significance for fasting glucose and HbA1c [$\beta$ (95% CI) = 0.07 (0.01, 0.14) mmol/L per μmol/L change in homocysteine, $P$ = 0.025, and $\beta$ = 0.46% (0.06%, 0.85%) per μmol/L change in homocysteine, $P$ = 0.02, respectively] (**Figure 4**, Supplemental Table 8).

### Assessment of study bias and sensitivity analyses

Overall, most studies had some risk of bias, including unclear reporting of random-sequence generation and blinding of outcome assessment (**Supplemental Table 9**). Sensitivity analyses were performed by leaving any single study or study arm out of the analyses, but this did not greatly affect the effect sizes (**Supplemental Table 10**). Sensitivity analysis stratified on bias assessment was not possible due to an insufficient number of studies.

## Discussion

In this meta-analysis, folate supplementation reduced fasting insulin concentrations as well as HOMA-IR, whereas no overall effect was observed for HbA1c or fasting glucose. Better glycemic control and lower IR are protective against development of T2D; however, only 2 studies examined folate supplementation on T2D risk and found no effect. The discrepancy between the findings on markers of IR and T2D development could be because of the low number of studies ($n$ = 2) on T2D development. The adjusted risk ratios from both studies on T2D development found a nonsignificant 9% decrease in relative risk, which is very close to the overall effect estimate of folate supplementation on stroke reduction (10%) (2). However, in order to detect such potential reductions in relative risk, large sample sizes are needed, and for the latest meta-analysis on folate and stroke 82,334 participants were included (2), in contrast to the ∼25,000 participants included in the present meta-analysis. Two other systematic reviews have recently addressed the role of folate supplementation on glucose metabolism and risk of T2D (52, 53). One was among patients with metabolic disturbances and included children as well (52), whereas the other had similar inclusion criteria as the present analysis but included fewer studies, showing that the present meta-analysis is more comprehensive in covering relevant studies (53). The overall

**TABLE 3** Subgroup analyses for glucose metabolism outcomes stratified by study design variables and participant characteristics[1]

| | No. of RCTs | No. of subjects | $P$[2] | Effect size (95% CI) | $P$[3] | $I^2$, % | $P$[4] |
|---|---|---|---|---|---|---|---|
| Fasting plasma glucose, mmol/L | | | | | | | |
| Dosage | | | | | | | |
| <5 mg/d | 9 | 16,804 | 0.14 | −0.00 (−0.05, 0.04) | 0.89 | 0 | 0.90 |
| ≥5 mg/d | 19 | 871 | | −0.10 (−0.21, 0.01) | 0.06 | 0 | 0.47 |
| Duration | | | | | | | |
| <8 wk | 12 | 500 | 0.90 | −0.04 (−0.17, 0.09) | 0.58 | 0 | 0.89 |
| ≥8 wk | 14 | 17,175 | | −0.04 (−0.12, 0.04) | 0.29 | 15 | 0.29 |
| Homocysteine reductions | | | | | | | |
| ≤2.5 μmol/L | 18 | 17,149 | <0.01 | 0.00 (−0.04, 0.04) | 0.97 | 0 | 1.00 |
| >2.5 μmol/L | 10 | 526 | | −0.24 (−0.39, −0.09) | <0.01 | 0 | 0.46 |
| Health status | | | | | | | |
| T2D | 6 | 309 | | −0.50 (−1.03, 0.03) | 0.07 | 10 | 0.35 |
| CVD | 7 | 496 | | 0.03 (−0.10, 0.17) | 0.65 | 0 | 1.00 |
| Without T2D | 20 | 17,366 | | −0.01 (−0.06, 0.03) | 0.49 | 0 | 0.88 |
| Fasting insulin, pmol/L | | | | | | | |
| Dosage | | | | | | | |
| <5 mg/d | 3 | 158 | 0.31 | −6.34 (−17.27, 4.59) | 0.26 | 27 | 0.26 |
| ≥5 mg/d | 9 | 424 | | −16.82 (−27.47, −6.18) | <0.01 | 62 | 0.01 |
| Duration | | | | | | | |
| <8 wk | 2 | 80 | 0.08 | −28.67 (−47.85, −9.49) | <0.01 | 29 | 0.23 |
| ≥8 wk | 9 | 502 | | −10.26 (−17.81, −2.72) | 0.01 | 44 | 0.06 |
| Homocysteine reductions | | | | | | | |
| ≤2.5 μmol/L | 5 | 269 | 0.03 | −6.26 (−14.73, 2.20) | 0.15 | 46 | 0.12 |
| >2.5 μmol/L | 6 | 313 | | −20.70 (−31.21, −10.19) | <0.01 | 26 | 0.23 |
| Health status | | | | | | | |
| T2D | 3 | 165 | | −4.88 (−24.25, 14.49) | 0.62 | 52 | 0.12 |
| CVD | — | — | | — | | — | |
| Without T2D | 8 | 417 | | −15.99 (−24.91, −7.06) | <0.01 | 57 | 0.02 |
| HOMA-IR, units | | | | | | | |
| Dosage | | | | | | | |
| <5 mg/d | 3 | 158 | 0.15 | −0.35 (−0.70, 0.00) | 0.05 | 0 | 0.59 |
| ≥5 mg/d | 9 | 424 | | −0.66 (−0.89, −0.43) | <0.01 | 0 | 0.67 |
| Duration | | | | | | | |
| <8 wk | 2 | 80 | 0.13 | −1.09 (−1.8, −0.38) | <0.01 | 0 | 0.38 |
| ≥8 wk | 9 | 502 | | −0.53 (−0.73, −0.32) | <0.01 | 0 | 0.75 |
| Homocysteine reductions | | | | | | | |
| ≤2.5 μmol/L | 5 | 269 | 0.07 | −0.38 (−0.66, −0.10) | 0.01 | 0 | 0.97 |
| >2.5 μmol/L | 6 | 313 | | −0.74 (−1, −0.47) | <0.01 | 0 | 0.53 |
| Health status | | | | | | | |
| T2D | 3 | 165 | | −0.87 (−1.82, 0.08) | 0.07 | 13 | 0.32 |
| CVD | — | — | | — | | — | |
| Without T2D | 8 | 417 | | −0.55 (−0.75, −0.35) | <0.01 | 0 | 0.64 |
| HbA1c, % | | | | | | | |
| Dose | | | | | | | |
| <5 mg/d | 4 | 461 | 0.32 | 0.01 (−0.17, 0.19) | 0.92 | 0 | 0.65 |
| ≥5 mg/d | 4 | 183 | | −0.23 (−0.67, 0.20) | 0.29 | 49 | 0.11 |
| Duration | | | | | | | |
| <8 wk | 1 | 26 | 0.38 | 0.15 (−0.3, 0.60) | 0.51 | 0 | 1.00 |
| ≥8 wk | 7 | 618 | | −0.10 (−0.3, 0.11) | 0.35 | 23 | 0.25 |
| Homocysteine reductions | | | | | | | |
| ≤2.5 μmol/L | 4 | 273 | 0.05 | 0.08 (−0.12, 0.28) | 0.44 | 0 | 0.78 |
| >2.5 μmol/L | 3 | 330 | | −0.36 (−0.77, 0.05) | 0.09 | 43 | 0.17 |
| Health status | | | | | | | |
| T2D | 7 | 482 | | −0.12 (−0.33, 0.10) | 0.29 | 16 | 0.31 |
| CVD | 1 | 162 | | 0.10 (−0.18, 0.38) | 0.48 | 0 | 1.00 |
| Without T2D | 1 | 162 | | 0.10 (−0.18, 0.38) | 0.48 | 0 | 1.00 |

[1] CVD, cardiovasular disease; HbA1c, glycated hemoglobin; RCT, randomized controlled trial; T2D, type 2 diabetes.
[2] $P$ values for between-group test for subgroup differences performed as described by Borenstein et al. (38).
[3] $P$ values for within-subgroup analyses using a random-effects model.
[4] $P$ values for test for heterogeneity within subgroups.

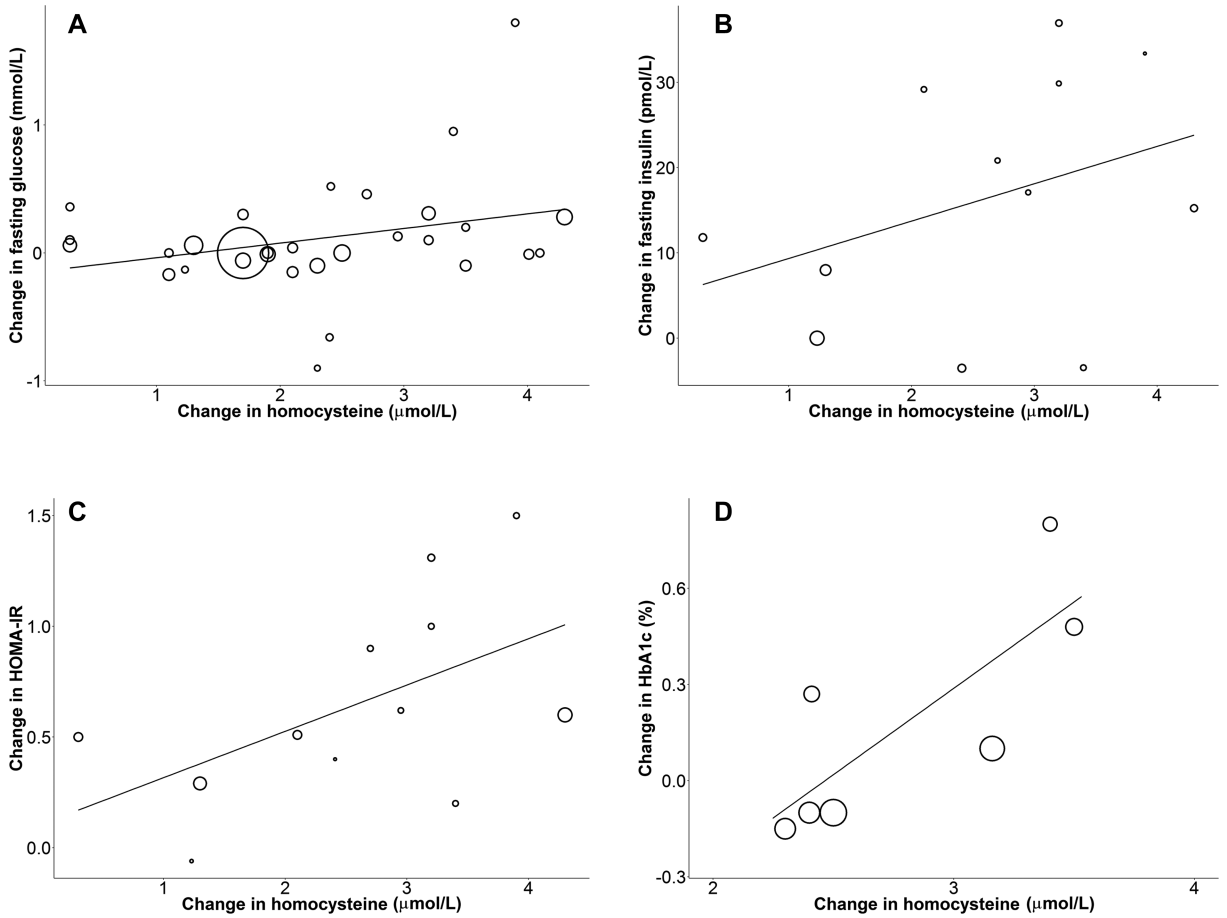


**FIGURE 4** Meta-regression plots of outcomes as a function of absolute change in homocysteine. The size of the circles is proportional to the precision of the estimate used in the meta-regression. Regression lines: (A) glucose [$\beta$ (95% CI)= 0.07 (0.01, 0.14) mmol/L per μmol/L change in homocysteine, $P = 0.025$] ($n_{\text{studies}} = 28$); (B) insulin [$\beta = 4.40$ (−2.14, 10.93) pmol/L per μmol/L change in homocysteine, $P = 0.19$] ($n_{\text{studies}} = 11$); (C) HOMA-IR [$\beta = 0.10$ (−0.05, 0.24) units per μmol/L change in homocysteine, $P = 0.19$] ($n_{\text{studies}} = 11$); and (D) HbA1c [$\beta = 0.46$ (0.06, 0.85) % per μmol/L change in homocysteine, $P = 0.02$] ($n_{\text{studies}} = 7$). HbA1c, glycated hemoglobin.

results obtained in these 2 meta-analyses were comparable to our results, which shows that the findings of the present analyses are robust and that the effects of folate supplementation on glycemic markers and risk of T2D require further research.

Circulating concentrations of homocysteine has been suggested as a potential mediating factor for IR and T2D development, and we therefore examined whether changes in homocysteine were associated with changes in related glycemic control outcomes. Our meta-regression analyses showed an association between a reduction in homocysteine concentrations and a lowering of fasting glucose and HbA1c concentrations, highlighting the importance of homocysteine as a mediator. Results could suggest that a substantial decrease in homocysteine is needed for better glycemic control, which could optimally be achieved through greater compliance and/or a higher dose of the folate supplement. The doses used by Qin et al. (33) (0.8 mg/d) and Song et al. (34) (2.5 mg/d) for T2D risk outcomes were in the lower end compared with what was used in the studies of glucose metabolism outcomes (median intake of 5 mg/d), and this too could contribute to the discrepancy between the results for the glucose homeostasis markers and the actual development of T2D.

The health status of the population has been argued to be a highly relevant effect modifier for the role of homocysteine in CVD and stroke prevention (54). Subgroups such as elderly people, as well as people with cardiovascular or kidney diseases, have previously shown a high prevalence of hyperhomocystinemia, indicating that these populations could be relevant targets for folate intervention. In the present meta-analysis, we found no indication of subgroup differences between T2D patients, CVD patients, and other groups. However, our analysis might be limited by the small number of studies included within each subgroup. Furthermore, it could be relevant to evaluate other specific groups, such as pregnant women, to assess how folate supplementation during pregnancy might influence gestational diabetes.

Multiple mechanisms have been proposed for the role of folate supplementation and homocysteine lowering in the development of IR and T2D. It has been speculated that a sufficient pool of methyl donors may lower homocysteine and thus reduce oxidative stress and systemic inflammation, both of which can interfere with insulin signaling and impair pancreatic $\beta$ cell insulin secretion (34). Furthermore, it has been suggested that increased homocysteine and impaired endothelial dysfunction

could reduce insulin delivery to peripheral tissue, thus impairing glucose uptake (34). This is in agreement with cell studies that have shown direct effects of both acute and long-term increased homocysteine concentration on $\beta$ cell glucose metabolism (55, 56), and further, that higher concentrations of homocysteine are associated with impaired insulin signaling in peripheral tissue (57, 58). However, because homocysteine is a central molecule for multiple metabolic pathways, including one-carbon metabolism, high concentrations might affect multiple pathways, including lipid metabolism and epigenetic regulation such as DNA methylation (59). DNA methylation has been shown to play a role in T2D development (60), although a clear link between folate supplementation and T2D via epigenetics in humans is yet to be demonstrated.

The present meta-analysis has several methodological strength and limitations. Because only RCTs were included, the meta-analysis findings are unlikely to be affected by confounding factors. Furthermore, sensitivity analyses showed that no one study substantially influenced the combined results. Considerable heterogeneity and indication of small-study effects were observed for insulin and should be taken into account when interpreting these results. Changes in medication and other treatment regimens potentially interfering with glycemic control during the study period were difficult to assess and could potentially influence the present results. Our subgroup and meta-regression analysis did not indicate any dependence on trial duration or folate dose for any of the outcomes. However, evidence of a dose-dependent association was observed between reduction in homocysteine and reduction in IR markers, which could support the hypothesis about a causal link between homocysteine reduction and T2D prevention. Changes in plasma homocysteine concentration could be an indicator that compliance is an important determinant of treatment effect, thus highlighting the importance of including changes in homocysteine when examining folate supplementation in T2D prevention in future studies. Only changes at a study level were included in the present analysis; including individual-level data would have been helpful for examining treatment compliance and the effect of homocysteine lowering.

Cost-effective prevention of T2D is urgently needed, given its increasing prevalence (1). Intensive lifestyle intervention and metformin treatment have both been shown to be effective in lowering HOMA-IR and preventing T2D in individuals with impaired glucose tolerance (61, 62). In the 1-y follow-up of the Diabetes Prevention Program, HOMA-IR was lowered by 1.46 units in the metformin group and by 1.95 units in the lifestyle group compared with placebo (62). The effect of folate supplementation on HOMA-IR in the present analysis was approximately one-third of the effect of metformin treatment (62). A similar effect size difference was found for T2D prevention, where folate supplementation showed approximately one-third of the effect of metformin (61), suggesting that folate is less effective than metformin. However, the interventions included in this meta-analysis were not just in subjects with impaired fasting glucose, as in the Diabetes Prevention Program, and effect sizes could potentially be higher in populations with impaired fasting glucose or in populations with both impaired fasting glucose and elevated homocysteine. In parallel with results showing that folate might be especially effective in prevention of stroke in adults with high fasting plasma glucose (11), a potential lowering effect on HOMA-IR and T2D risk would be an added benefit. This suggests that some subgroups might benefit substantially from folate, which may improve the cost-effectiveness of supplementation, although this was not shown in the subgroup analysis of the present study. Some of the included studies also gave folate in combination with metformin (14, 18, 44); yet, at this point, it is unclear whether there could be an additional effect of supplementing with folate in addition to metformin or other antidiabetic medication. Moreover, the benefits of folate supplementation are not universal; for example, in T2D patients with diabetic nephropathy, folate supplementation leads to a greater decrease in glomerular filtration rate and an increase in vascular events (63). Other groups, such as the Health Assessment Workspace Collaborative, have addressed the potential adverse effects of folic acid supplementation in more detail (https://hawcproject.org/assessment/public/). Thus, uncritically supplementing with high doses of folate is not recommended, and future studies could benefit from focusing on identifying subjects and populations in which targeted supplementation could have maximum clinical benefits.

The results from this meta-analysis indicate that folate supplementation alone or in combination with other B vitamins might be beneficial for glucose homeostasis and lowering of IR; however, at present, there are insufficient data to conclusively determine if folate supplementation can affect development of T2D. A way to further increase our knowledge within this area would be to encourage reporting of glucose homeostasis and T2D outcomes from previously conducted RCT studies on folate supplementation that did not include these in the previously reported outcomes. Our findings strongly suggest that folate supplementation could improve some markers of T2D risk and warrant additional studies that investigate folate supplementation and T2D risk as a primary outcome in high-risk population groups.

The authors' responsibilities were as follows—MVL: designed the research and performed the statistical analysis; MVL and JNE: conducted the research, drafted the manuscript, and had primary responsibility for final content; ABR, JNE, LL, MK, MVL: provided input, assisted in interpretation of results, assisted in the revision of the manuscript, and read and approved the final version; and ABR, LL and MK: Aided in search optimization, and discussion of eligability of studies. None of the authors had a conflict of interest to declare.

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