Folic acid and reduction of plasma homocysteine concentrations in older adults: a dose-response study[1–3]

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ABSTRACT

Background: Elevated homocysteine concentrations, a likely risk factor for cardiovascular disease, can be lowered effectively with folic acid. The minimum dose of folic acid required for maximal reduction of homocysteine concentrations is not yet known reliably.

Objective: We aimed to determine the lowest folic acid dose that decreases plasma homocysteine concentrations adequately in healthy older adults.

Design: A dose-response trial with a randomized, double-blind, parallel-group, placebo-controlled design was carried out among 316 Dutch men and women aged 50–75 y. Subjects received daily for 12 wk either a placebo or 1 of the 6 following folic acid doses: 50, 100, 200, 400, 600, or 800 μg. The relative changes in plasma homocysteine concentration in response to increasing doses of folic acid were used to calculate the dose-response curve. An adequate dose of folic acid was defined as the dose that induced ≥90% of the maximal reduction in homocysteine concentration.

Results: The relative decrease in plasma homocysteine concentration was associated exponentially with increasing doses of folic acid. From the dose-response curve, the adequate daily dose of folic acid was estimated to be 392 μg, which decreased plasma homocysteine concentrations 22%.

Conclusion: In older adults, daily supplementation with folic acid effectively lowers plasma homocysteine concentrations, and a daily dose of ≈400 μg is the minimum dose required for adequate homocysteine reduction. *Am J Clin Nutr* 2003;77:1318–23.

KEY WORDS Folic acid, homocysteine, dose response, food fortification, dose-response curve, adult population

INTRODUCTION

Folic acid supplementation can reduce the risk of neural tube defects (1) and effectively lowers elevated plasma total homocysteine concentrations (2), a likely risk factor for cardiovascular disease (3–7). In the United States, mandatory fortification of flour with folic acid at a level of 1.4 mg/kg was introduced in 1998 (8). This fortification nearly doubled the mean daily intake of folic acid (9, 10), substantially reduced mean blood total homocysteine concentrations (11–13), and reduced the incidence of neural tube defects by 19% (14). In some countries, even higher levels of fortification are being recommended to reduce homocysteine concentrations in the population. The current British recommendations, for instance, are to fortify flour with folic acid at 2.4 mg/kg (15, 16). In the Netherlands, the fortification of foods with folic acid is not permitted (17). There is concern that, especially in older populations, extra folic acid may mask the symptoms associated with vitamin B-12 deficiency, which may lead to delayed diagnosis and potential progression of the neurologic abnormalities that result from this deficiency (15–17).

The minimum dose of folic acid required for maximal lowering of homocysteine concentrations is not known reliably. The initial studies on the use of folic acid to lower homocysteine concentrations used daily doses of ≥5 mg (18). A meta-analysis of 12 randomized trials of folic acid–based multivitamin supplements to lower homocysteine concentrations showed that daily doses from 0.4 to 5 mg folic acid were equally effective (2). However, there is little or no available evidence from randomized trials for the homocysteine-lowering effects of daily doses of <0.4 mg folic acid in healthy adults. (2). Trials comparing the homocysteine-lowering effects of one dose of folic acid with another at daily doses <0.5 mg have either used a sequential design (19) or have been carried out in young populations (20, 21) or people with cardiovascular disease (16). A dose-response study in an older population—including a wide range of low doses—could inform the debate on the level of folic acid to use for food fortification in the light of possible adverse effects.

The aim of this dose-response trial was to estimate the adequate dose of folic acid, which was defined as the dose that induces 90% of the maximal decrease in plasma total homocysteine concentration. We compared the effects on plasma total homocysteine concentrations of daily supplementation with folic acid at doses of 50, 100, 200, 400, 600, or 800 μg with the effect of placebo in a group of healthy, older Dutch subjects.

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SUBJECTS AND METHODS

Subjects

We recruited healthy adults aged 50–75 y either from a random sample of people living in the community near Wageningen, Netherlands, or through a database of volunteers who had previously indicated interest in participating in such studies by Wageningen University. A summary of the recruitment procedures is shown in **Figure 1**. Persons with a history of cardiovascular diseases were excluded, as were persons with any chronic disease that might interfere with folate or homocysteine metabolism (eg, renal disease, thyroid disease, epilepsy) and persons who used medication known to interfere with folate or homocysteine metabolism (eg, methotrexate, malaria prophylactics, antiepileptic drugs). All of the women were postmenopausal, and only one received hormone replacement therapy, which was permitted if participants received it ≥3 mo before screening and intended to continue the therapy for the duration of the trial. Persons who took dietary supplements containing B vitamins or yeast extracts within 3 mo before the study were excluded. Persons with a plasma homocysteine concentration >26 μmol/L, a serum vitamin B-12 concentration <160 pmol/L, or a serum creatinine concentration >125 μmol/L were also excluded. Among the 316 persons who were randomly assigned, 311 subjects completed the trial. Reasons for dropout included medical complications, the use of medications that interfere with folate or homocysteine metabolism (n = 4), and personal reasons (n = 1). All participants gave written informed consent to a protocol that was approved by the Medical Ethical Committee of Wageningen University.

Study design

After the screening visit, subjects commenced a run-in period of 3–4 wk, during which they took placebo capsules to assess compliance with study procedures. The run-in period was followed immediately by an intervention period of 12 wk. A fasting sample of venous blood was collected at the screening visit, at the randomization visit (baseline), and at 4 and 12 wk after the start of the treatment. Subjects were randomly assigned to receive 1 of 7 treatments: placebo or 50, 100, 200, 400, 600, or 800 μg folic acid/d. In the randomization procedure, we took account of pretreatment plasma homocysteine concentrations by stratifying treatment allocation by quartile of homocysteine concentration at screening. At the baseline visit (week 0), the subjects received all of the supplements for the 12 wk of intervention. The capsules were made especially for this intervention by the pharmacy of the hospital of Ede in the Netherlands (Ziekenhuis de Gelderse Vallei, Ede, Netherlands). Roche Vitamins (Basel, Switzerland) supplied the folic acid for the capsules. We asked the subjects to maintain their regular diet but to avoid the intake of liver, yeast extracts, or supplements containing B vitamins during the trial and to avoid the consumption of liver products within 3 d before blood sampling. Fortification of foods with folic acid is not allowed in the Netherlands; therefore, the subjects' dietary intake of folate was restricted to the natural content of folate in foods.

Data collection

At the screening visit, height and weight were measured. All subjects kept a diary throughout the study in which they reported their daily intake of capsules, any illnesses they experienced, and their use of medication. Smoking habits were also recorded.

In the blood collected at the screening visit, we measured total plasma homocysteine, serum vitamin B-12, and serum creatinine concentrations. Total plasma homocysteine and serum folate concentrations were measured in the blood samples obtained at baseline and at 4 and 12 wk of intervention. We measured folate concentrations in red blood cells at baseline and at 12 wk of intervention.

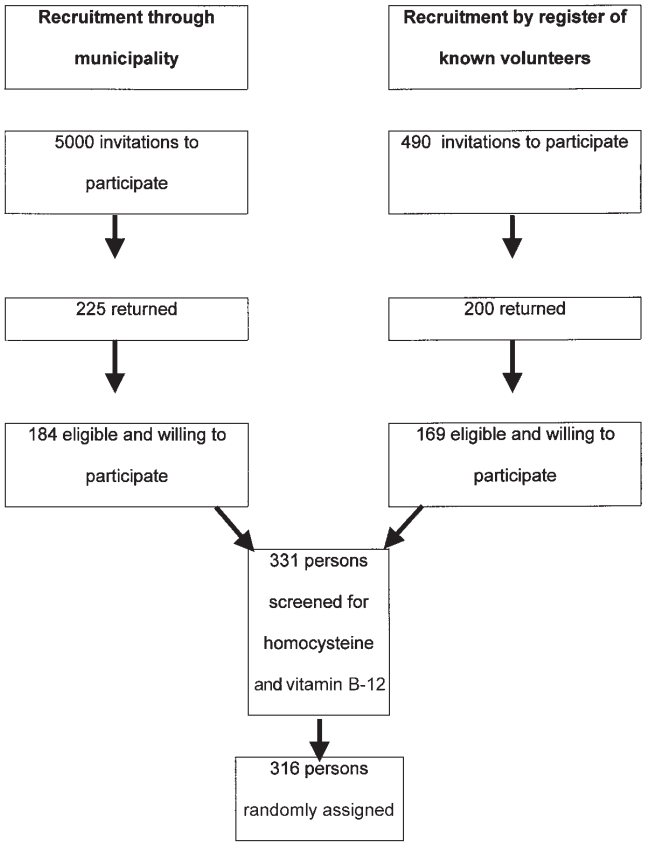

FIGURE 1. Flow schedule of the recruitment procedure.

Laboratory procedures

Blood samples were drawn into EDTA-containing evacuated tubes. Samples for the measurement of plasma homocysteine concentrations were immediately placed on ice, and the plasma was separated from blood cells within 30 min. Samples for serum vitamin B-12, creatinine, and folate measurements were placed in the dark and stored at room temperature for ≥30 min before centrifugation for 10 min at 2600 × g and 4 °C. We assessed hematocrit values immediately and diluted whole blood with 4 volumes sodium ascorbate (10 g/L) for the measurement of folate concentrations in red blood cells. All samples were stored at −80 °C.

Samples collected from each subject at baseline and at 4 and 12 wk of intervention were analyzed in the same batch to minimize variability. Total plasma homocysteine concentrations were measured by HPLC with fluorimetric detection at the Division of Human Nutrition and Epidemiology, Wageningen University, Netherlands (intraassay and interassay CVs were 2% and 7%, respectively) (22, 23). We measured serum and red blood cell folate concentrations and serum vitamin B-12 concentrations with the use of a commercial chemiluminescent immunoassay analyzer (Immulite 2000; Diagnostic Products Company, Los Angeles). The samples for red blood cell folate were further diluted with a concentrated human protein–based matrix (Immulite 2000 diluent;

Diagnostic Products Company) before measurement. Folate concentrations were measured at the clinical laboratory of the University Medical Centre St Radboud, Nijmegen, Netherlands. The intraassay CVs of the serum folate and red blood cell folate assays were < 10% and < 9%, respectively. Creatinine concentrations were measured by using a modification of the kinetic Jaffé reaction (DuPont Dimension, Boston; Dade BV, Leusden, Netherlands). All laboratory staff were blinded to the treatment allocation.

We used an HPLC method with fluorescence and diode array detection to measure the folic acid content of the capsules for treatment (24). The mean concentrations (with ranges in parentheses) of folic acid in the placebo and 50-, 100-, 200-, 400-, 600-, and 800-μg capsules were 0 (0–0), 49 (48–52), 99 (95–102), 198 (192–205), 408 (396–431), 633 (619–655), and 872 (839–898) μg, respectively. The folic acid content of the capsules did not vary by > 6%.

Statistical analysis

We calculated absolute changes in homocysteine concentration from concentrations at baseline to those at 12 wk of intervention. Furthermore, we calculated individual relative changes by dividing each subject's absolute change in plasma homocysteine concentration at 12 wk of intervention by his or her baseline plasma homocysteine concentration. Curve fitting by nonlinear regression was used to assess the adequate folic acid dose: mean relative changes in plasma homocysteine concentration were plotted by dose of folic acid, and nonlinear regression was used to find the best-fit curve through the relative decreases in homocysteine concentration. The adequate dose was arbitrarily defined as the dose that induces 90% of the maximal decrease in plasma homocysteine concentration as predicted by the asymptote of the best-fit curve (ie, the decrease at infinite folic acid intake). In addition, we calculated the adequate dose on the basis of 95% of the maximal decrease (a more strict definition). The independent variable was the dose of folic acid as measured in the capsules. We used SAS statistical software (version 6.12; SAS Institute Inc, Cary, NC) for calculating means and GRAPHPAD PRISM (version 3.00; Graph Pad Software Inc, San Diego) for curve fitting.

RESULTS

There were no differences between the intervention groups in baseline characteristics. The mean (± SD) age of the study participants was 60 ± 6 y, 59% (n = 182) were male, and 15% (n = 48) were smokers. The mean body mass index (in kg/m^2) was 27 ± 4, and the mean serum vitamin B-12 concentration was 315 ± 128 pmol/L. One subject took the capsules of his partner, who had been allocated to a different treatment, and thus the data from both of these subjects were excluded from the analyses. The data from another subject were also excluded because of the subject's poor compliance (42%). The remaining 308 subjects had a mean compliance of 99% (all subjects ≥ 80%) as estimated by pill counting.

As shown in **Table 1**, the baseline serum and red blood cell folate concentrations in all treatment groups were well matched. Serum folate concentrations increased rapidly and linearly with increasing doses of folic acid, as shown by the observed increases after 4 wk and the further increases in the following 8 wk. The Spearman correlation coefficient for the correlation between folic acid dose and change in serum folate concentration after 12 wk was 0.90 (P < 0.0001; n = 306). There was a slight increase in serum folate concentrations in the placebo group after 12 wk, which reflects random error. Folate concentrations in red blood cells increased in all the groups receiving folic acid but did not change in the placebo group. As with the change in serum folate concentration, the change in red blood cell folate concentration correlated strongly with folic acid dose (Spearman correlation coefficient = 0.89, P < 0.0001; n = 296).

TABLE 1
Serum and red blood cell folate concentrations before and after 4 and 12 wk of intervention and absolute changes after 12 wk of intervention by intervention group[1]

	Week			
	0	4	12	Absolute change[2]
	nmol/L			*nmol/L*
Serum folate				
Placebo (n = 51)	13.3 ± 3.6[3]	12.9 ± 3.8	14.2 ± 4.2	1.0 (0.1, 2.0)
50 μg/d (n = 42)	12.0 ± 3.1	14.7 ± 3.7	16.3 ± 4.4	4.3 (3.2, 5.3)
100 μg/d (n = 41)	12.7 ± 4.6	17.4 ± 6.2	19.9 ± 7.6	7.2 (5.7, 8.7)
200 μg/d (n = 43)	12.3 ± 4.2	19.8 ± 5.7	24.6 ± 7.5	12.3 (10.7, 13.8)
400 μg/d (n = 43)	13.8 ± 5.3	31.9 ± 15.3	43.2 ± 21.0	29.4 (23.4, 35.4)
600 μg/d (n = 43)	12.9 ± 4.9	41.4 ± 19.4	55.6 ± 24.5	42.7 (35.2, 50.3)
800 μg/d (n = 43)	12.9 ± 3.6	53.4 ± 28.3	74.8 ± 43.3	61.9 (48.5, 75.4)
Red blood cell folate				
Placebo (n = 50)	721 ± 257	—	733 ± 231	14 (−17, 46)
50 μg/d (n = 42)	701 ± 223	—	755 ± 184	53 (21, 86)
100 μg/d (n = 39)	722 ± 271	—	837 ± 242	120 (83, 157)
200 μg/d (n = 43)	695 ± 251	—	979 ± 283	284 (245, 322)
400 μg/d (n = 42)	836 ± 286	—	1381 ± 366	531 (466, 595)
600 μg/d (n = 38)	679 ± 231	—	1350 ± 293	674 (604, 745)
800 μg/d (n = 42)	761 ± 237	—	1593 ± 380	832 (750, 914)

[1] Spearman correlation coefficients for the correlation between folic acid dose and change in folate status after 12 wk were 0.90 (P < 0.0001; n = 306) for serum folate and 0.89 (P < 0.0001; n = 296) for red blood cell folate.
[2] 95% CI in parentheses.
[3] $\bar{x}$ ± SD.

TABLE 2
Plasma homocysteine concentrations before and after 4 and 12 wk of intervention and absolute and percentage changes after 12 wk of intervention by intervention group

	Week				
	0	4	12	Absolute change[1]	Percentage change[1]
		μmol/L		*μmol/L*	*%*
Placebo (n = 52)	10.9 ± 2.3[2]	11.3 ± 2.4	11.3 ± 2.7	0.4 (−0.05, 0.8)	4.0 (0.1, 7.9)
50 μg/d (n = 43)	11.5 ± 2.7	11.3 ± 2.6	11.1 ± 2.5	−0.4 (−0.9, 0.03)	−2.9 (−7.0, 1.2)
100 μg/d (n = 41)	11.7 ± 3.2	11.3 ± 3.1	10.8 ± 2.6	−0.9 (−1.5, −0.3)	−6.1 (−10.1, −2.0)
200 μg/d (n = 43)	11.8 ± 3.3	10.4 ± 2.5	10.0 ± 2.4	−1.8 (−2.4, −1.3)	−14.0 (−17.4, −10.7)
400 μg/d (n = 43)	12.0 ± 3.2	10.2 ± 2.7	9.6 ± 1.5	−2.4 (−3.2, −1.7)	−17.3 (−21.5, −13.2)
600 μg/d (n = 43)	11.8 ± 3.6	9.6 ± 2.3	8.8 ± 1.7	−3.0 (−3.7, −2.2)	−22.1 (−26.6, −17.6)
800 μg/d (n = 43)	11.5 ± 2.8	9.6 ± 2.2	9.1 ± 2.0	−2.4 (−2.9, −1.9)	−19.9 (−22.9, −16.8)

[1] 95% CI in parentheses.
[2] $\bar{x}$ ± SD.

As shown in **Table 2**, plasma homocysteine concentrations were well matched among the treatment groups at baseline (ie, randomization was successful). Plasma homocysteine concentrations decreased in all of the folic acid groups during the first 4 wk of intervention and decreased further between the 4th and 12th wk of intervention. Relative to baseline concentrations, the decrease in plasma homocysteine concentrations after 12 wk varied from 0.8 μmol/L (6.9%) to 3.4 μmol/L (26.9%) after correction for the 0.4-μmol/L (4%) increase in the placebo group.

The best-fitting dose-response curve plotted through the percentage changes in homocysteine concentration after 12 wk of intervention is shown in **Figure 2**, top panel. This dose-response curve was an exponential curve, and was described by the following equation:

$$\text{Change in homocysteine concentration after 12 wk (\%)} = 24.8 \times \exp[-0.0059 \times \text{folic acid intake (in μg/d)}] - 20.9 \quad (1)$$

The curve had an R^2 of 0.9997, which indicates a very good fit. The decrease at infinite folic acid intake (or the asymptote) was −20.9% because the exponential term then equals zero. When no folic acid is supplemented, the change in plasma homocysteine concentration equals 24.8 − 20.9 = 3.9%, which is the estimated placebo effect. The maximum decrease (or decrease at infinite folic acid intake) in plasma homocysteine concentration corrected for placebo was therefore 24.8%. As clearly shown in the top panel of Figure 2, the decrease achieved with the 600- and 800-μg doses was almost identical to that estimated at infinite folic acid intake. The lowest dose that achieved 90% of the maximal reduction was estimated to be 392 μg (95% CI: 274, 697 μg). At this dose, homocysteine concentrations decreased 22.3%. The same dose-response curve shown in the top panel of Figure 2 is also shown in the lower panel, but with dotted lines added to estimate the lowest dose required to achieve 90% and 95% of the maximal decrease in plasma homocysteine concentration. As shown in this panel, the adequate dose of folic acid varied from 392 to 511 μg for 90% and 95%, respectively, of the maximal decrease in plasma homocysteine concentration.

DISCUSSION

This trial showed that daily supplementation with folic acid at a dose of 400 μg, which decreased homocysteine concentrations ≈22%, is associated with 90% of the maximal decrease in plasma homocysteine concentration. Furthermore, supplementation with daily doses of folic acid as low as 50 or 100 μg decreased homocysteine concentrations ≈10%.

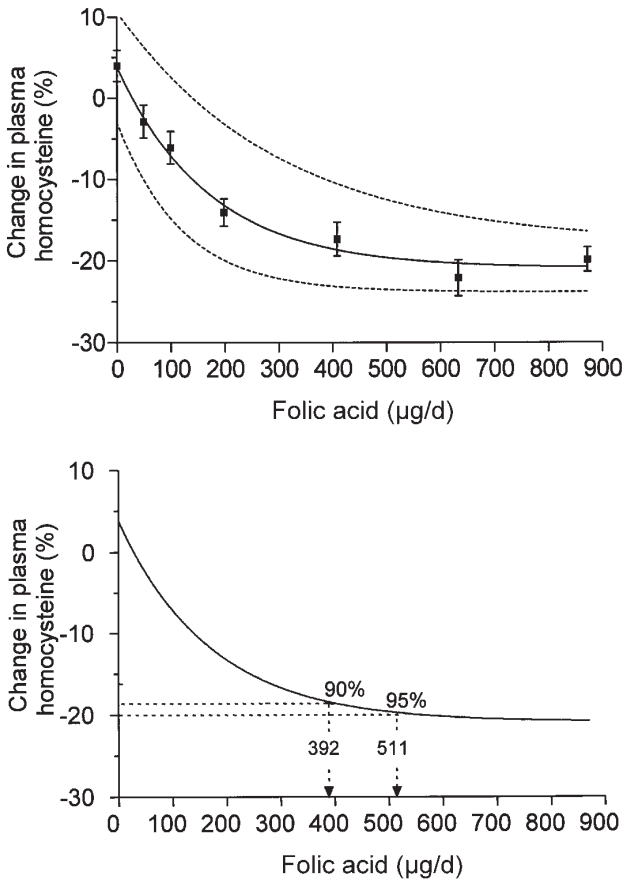

FIGURE 2. Percentage change in plasma homocysteine concentration after 12 wk of intervention plotted against daily folic acid dose. Top: The error bars are the 95% CIs. The solid line describes the best-fitting curve through the points, and the dotted lines describe the 95% CI for this curve. The curve is described by the following equation: Change (%) = 24.8 × exp(−0.0059 × dose) − 20.9. Goodness of fit is indicated by R^2 = 0.9997. Bottom: The solid line indicates the best-fitting curve. The dotted lines indicate the minimum dose of folic acid required to achieve 90% or 95% of the maximal reduction in plasma homocysteine concentration.

One of the strengths of this trial was the use of stratification of pretreatment plasma homocysteine concentrations into quartiles before randomization to avoid any imbalance in homocysteine concentrations in the treatment groups. This enabled the present trial to control for the confounding effect of initial homocysteine concentrations on the observed reductions in homocysteine concentration achieved by different doses of folic acid (19, 20). We fitted a dose-response curve on the basis of proportional changes from baseline values in homocysteine concentration after treatment to estimate the dose of folic acid that was adequate to lower homocysteine concentrations.

Different definitions of an adequate dose of folic acid are possible. The strengths of the definition adopted in the present trial were that it took account both of observed changes in the placebo group and of baseline concentrations. From Table 2 we can conclude that the adequate dose of folic acid would not have been substantially different whether it was based on absolute changes or final homocysteine concentrations at week 12. The choice of a cutoff of 90% of the maximal effect was arbitrary. When 95% of the maximal effect was used instead, the adequate dose was ≈100 μg higher.

In addition to the present study, one meta-analysis and 3 dose-response studies sought to determine the lowest effective dose of folic acid to lower homocysteine concentrations. The meta-analysis of 12 randomized trials of folic acid–based multivitamin supplements to lower homocysteine concentrations showed that a daily dose of 400 μg was as effective in lowering homocysteine as were daily doses up to 5 mg, but no studies with daily doses < 400 μg were available (2). In a parallel 3-mo study with 151 patients with ischemic heart disease (mean age: 65 y), Wald et al (16) compared the effects on homocysteine concentrations of daily doses of folic acid ranging from 200 μg to 1 mg. They concluded that the maximum reduction in homocysteine was achieved by a dose of 800 μg and that no further reduction was achieved by supplementation with 1 mg/d (16). However, the failure to take account of differences in pretreatment homocysteine concentration before randomization in that trial may have resulted in the imbalance observed in initial homocysteine concentrations. Furthermore, participants in that trial had ischemic heart disease (and higher baseline homocysteine concentrations) and may have required a higher dose of folic acid to reduce their homocysteine concentrations. In a sequential-design study of 30 healthy men aged 34–65 y, supplementation with 400 μg folic acid/d for 14 wk was as effective as supplementation with 200 μg/d for 6 wk, whereas supplementation with 100 μg/d for 6 wk was less effective than either of the other 2 dosages (19). A sequential design without a placebo group is constrained because each dose cannot be compared with a placebo and because the influence of any carryover effect of a previous dose cannot be excluded from the observed effects. In the present study, we stratified the subjects by initial homocysteine concentration before randomization to ensure that initial homocysteine concentrations were similar in all the treatment groups. Furthermore, the use of a parallel group design provided a more reliable comparison of the effects of the different doses of folic acid with the effect of placebo. Rydlewicz et al (25) recently published the results of a dose-response study in a group of healthy elderly subjects from which they conclude that daily supplementation with 600 μg folic acid is the most effective therapy for homocysteine lowering. However, whereas we aimed to find the lowest adequate dose of folic acid for reducing homocysteine concentrations, the aim of Rydlewicz et al was to find the adequate dose to bring the plasma homocysteine concentrations of 95% of the population below a cutoff of 10 μmol/L. There is no consensus yet on cutoffs for healthy homocysteine concentrations. Furthermore, variation between laboratory procedures in sample collection and analysis of homocysteine concentrations makes it difficult to generalize conclusions. Therefore, we chose to show the dose-response curve, allowing the reader to extrapolate the effect of any given dose on homocysteine reduction.

Relative to placebo, the daily doses of 50–100 μg folic acid lowered plasma homocysteine concentrations 7–10%. Very low doses of folic acid can thus lower plasma homocysteine concentrations by approximately one-half of the maximal decrease. On the basis of our data, we expect that improvement in the bioavailability of folate in foods or advice to increase daily dietary folate intake could substantially contribute to a reduction in homocysteine concentrations. However, an increase in folic acid intake of 400 μg/d can most likely only be achieved by food fortification.

The present trial shows that daily supplementation with folic acid at a dose of ≥ 400 μg leads to a maximum reduction of homocysteine. The current evidence that an elevated homocysteine concentration is a causal risk factor for cardiovascular disease is promising (5–7), and the ongoing trials of folic acid supplementation in patients with cardiovascular disease might further substantiate this claim.

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All authors contributed to the study design; FVAvO and AM-B performed data collection; FVAvO, AM-B, and PV contributed to data analysis; and FVAvO drafted the manuscript, and all other authors participated in critically revising the manuscript for important intellectual content. None of the authors had any financial or personal interest, including advisory board affiliations, in any company or organization sponsoring the research. The Wageningen Centre for Food Sciences is an alliance of major Dutch food industries, the TNO Nutrition and Food Research, and Wageningen University and Research Centre that receives financial support from the Dutch government.

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