Effect of Homocysteine-Lowering Treatment With Folic Acid and B Vitamins on Risk of Type 2 Diabetes in Women

A Randomized, Controlled Trial

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OBJECTIVE—Homocysteinemia may play an etiologic role in the pathogenesis of type 2 diabetes by promoting oxidative stress, systemic inflammation, and endothelial dysfunction. We investigated whether homocysteine-lowering treatment by B vitamin supplementation prevents the risk of type 2 diabetes.

RESEARCH DESIGN AND METHODS—The Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS), a randomized, double-blind, placebo-controlled trial of 5,442 female health professionals aged ≥40 years with a history of cardiovascular disease (CVD) or three or more CVD risk factors, included 4,252 women free of diabetes at baseline. Participants were randomly assigned to either an active treatment group (daily intake of a combination pill of 2.5 mg folic acid, 50 mg vitamin B6, and 1 mg vitamin B12) or to the placebo group.

RESULTS—During a median follow-up of 7.3 years, 504 women had an incident diagnosis of type 2 diabetes. Overall, there was no significant difference between the active treatment group and the placebo group in diabetes risk (relative risk 0.94 [95% CI 0.79–1.11]; $P = 0.46$), despite significant lowering of homocysteine levels. Also, there was no evidence for effect modifications by baseline intakes of dietary folate, vitamin B6, and vitamin B12. In a sensitivity analysis, the null result remained for women compliant with their study pills (0.92 [0.76–1.10]; $P = 0.36$).

CONCLUSIONS—Lowering homocysteine levels by daily supplementation with folic acid and vitamins B6 and B12 did not reduce the risk of developing type 2 diabetes among women at high risk for CVD. ***Diabetes*** **58:1921–1928, 2009**

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Received 20 January 2009 and accepted 7 May 2009.

Published ahead of print at http://diabetes.diabetesjournals.org on 2 June 2009. DOI: 10.2337/db09-0087. Clinical trial reg. no. NCT00000541, clinicaltrials.gov.

See accompanying commentary, p. 1730.

Homocysteinemia may promote insulin resistance and β-cell dysfunction through its adverse metabolic effects, ultimately contributing to the pathogenesis of type 2 diabetes and associated complications (1–3). Several lines of evidence from both in vitro and in vivo studies support this hypothesis. First, homocysteinemia directly elicits oxidative stress by increasing reactive oxygen species production and diminishing intracellular antioxidant defense (2). Experimental studies have suggested that oxidative stress interferes with insulin signaling and impairs pancreatic β-cell insulin secretion (4,5), thereby accelerating the progression from insulin resistance to overt type 2 diabetes. Second, elevated levels of homocysteine promote systemic inflammation via the activation of a cascade of inflammatory pathways including interleukin-6, tumor necrosis factor-α, and adhesion molecules (3). Low-grade chronic inflammation, as reflected by elevated circulating levels of inflammatory cytokines, may promote insulin resistance in liver, skeletal muscle, and vascular endothelium (6,7). Last, homocysteine can exert its damaging effects on the endothelium through mechanisms involving impaired nitric oxide (NO)-dependent vasodilation, endothelial toxicity and injury, oxidative stress, and systemic inflammation (2,8). The resultant endothelial dysfunction, especially in the capillary and arteriolar endothelium, can reduce insulin delivery to insulin-sensitive peripheral tissues, which in turn impairs insulin-mediated glucose metabolism (9–11). Collectively, we speculate that elevated homocysteine levels may play an etiologic role in the development of insulin resistance and type 2 diabetes primarily by promoting oxidative stress, systemic inflammation, and endothelial dysfunction.

Homocysteinemia has been recognized as a vascular risk factor for diabetic angiopathy (12), whereas few human data are currently available on the relation between homocysteine levels and risk of developing type 2 diabetes. In observational studies, homocysteine levels in nondiabetic individuals have been positively correlated with several biomarkers of insulin resistance and/or glucose intolerance in some (13–15) but not all (16–18) studies. In a 4-year prospective cohort study, elevated levels of homocysteine were independently associated with a 3.6-fold increased risk of type 2 diabetes among 170 women with a history of gestational diabetes mellitus (19). These observations not only provided suggestive evidence linking elevated levels of homocysteine to the develop-

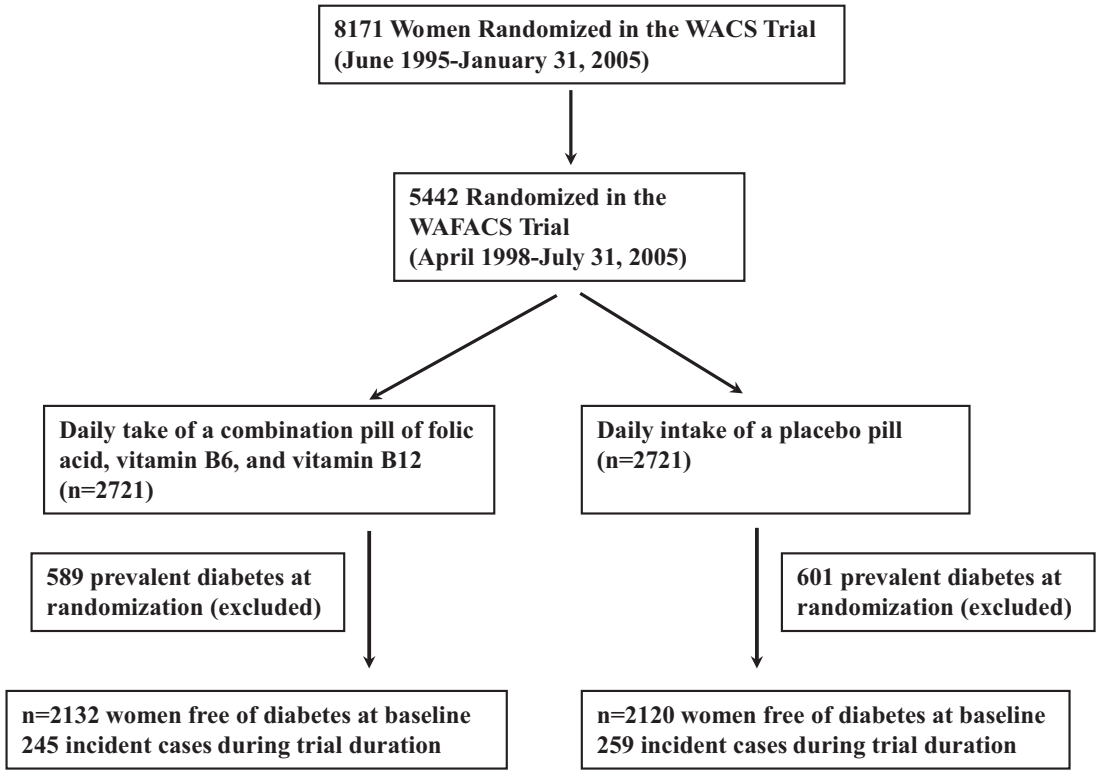


FIG. 1. Flow diagram illustrating diabetes outcomes in the randomly assigned treatment of folic acid and vitamins B6 and B12 of the WAFACS. A total of 1,190 participants who had a diagnosis of diabetes at baseline were excluded in the analysis.

ment of type 2 diabetes but also led to the suggestion that lowering homocysteine levels may prevent or reduce risk of type 2 diabetes.

Dietary folic acid and vitamins B6 and B12 are the most important modifiable determinants of homocysteine levels, and adequate intake of B vitamins may be potentially beneficial for prevention of type 2 diabetes in the general population. However, no previous prospective cohort studies have specifically examined intakes of individual B vitamins and diabetes risk. Some small and short-term randomized trials for secondary prevention of diabetes complications have been conducted but yielded inconsistent results; some reported that folic acid supplementation (5–10 mg/day) reduced oxidative stress and improved endothelial function in diabetic patients during a period of 2–12 weeks (20–23). To the best of our knowledge, there are no previous randomized clinical trials assessing the efficacy of B vitamin supplements for primary prevention of type 2 diabetes. In a large cardiovascular disease (CVD) prevention trial, the Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS), we specifically examined the homocysteine-lowering effect by daily supplementation with folic acid, vitamin B6, and vitamin B12 on the risk of type 2 diabetes in women at high risk for CVD.

RESEARCH DESIGN AND METHODS

The WAFACS is a randomized, double-blind, placebo-controlled trial evaluating the effects of a combination pill of folic acid (2.5 mg/day), vitamin B6 (50 mg/day), and vitamin B12 (1 mg/day) in the secondary prevention of important vascular events among high-risk women with either a history of CVD or at least three cardiovascular risk factors. Briefly, the WAFACS began in 1998, when the folic acid, vitamin B6, and vitamin B12 component was added to the Women's Antioxidant Cardiovascular Study (WACS), an ongoing 2×2×2 factorial trial of three antioxidant vitamins (vitamins C and E and β-carotene), which expanded it to a four-group factorial trial. Details of the overall trial design and the main results from the WAFACS and WACS have been reported previously (24–26). The study was sponsored by the National Heart, Lung, and Blood Institute of the National Institutes of Health. The study vitamins and matching placebo were provided by BASF Corporation (Mount Olive, NJ). The trial was approved by the institutional review board of Brigham and Women's Hospital (Boston, MA), and all patients provided written informed consent. An external independent data and safety-monitoring board monitored the safety of the participants and the overall quality and scientific integrity of the study.

In the WACS parent trial, 8,171 female health professionals were randomized into the trial from June 1995 through October 1996 to receive vitamin C (500 mg/day), vitamin E (600 IU every other day), and β-carotene (50 mg every other day) versus respective matching placebos. Women were eligible for WACS if they were at least 40 years old, postmenopausal or had no intention of becoming pregnant, and had a self-reported history of CVD (including myocardial infarction, stroke, coronary revascularization, or angina) or had at least three traditional cardiac risk factors (26).

In April 1998, 5,442 of these women provided consent and were additionally randomized in a factorial design to receive a combination pill containing 2.5 mg folic acid, 50 mg vitamin B6, and 1 mg vitamin B12 (active treatment) or a matching placebo daily. All study investigators, staff, and participants were unaware of the participants' treatment assignments. From the 5,442 women randomized in the WAFACS trial, we excluded those with diabetes at baseline (n = 1,190) for the present analyses, leaving 4,252 nondiabetic women at baseline (Fig. 1).

Follow-up procedures. Following randomization and annually thereafter, participants were mailed monthly calendar packs containing active agents or placebos, along with questionnaires on adherence, use of nonstudy supplements, and occurrence of major illnesses or adverse events. A semiquantitative food-frequency questionnaire at baseline was used to assess dietary nutrient intake (27). Written permission for medical records was sought from participants who reported cardiovascular end points or from the next of kin in case of death. Death certificates were also obtained. An end points committee of physicians who were blinded to randomized treatment assignment adjudicated all primary and secondary cardiovascular outcome events. Study medications and end point ascertainment were continued in a blinded fashion until the scheduled end of the trial, 31 July 2005, for a follow-up duration of 7.3 years. At the scheduled end of the trial, morbidity and mortality follow-up was 92.6% complete. If assessed in terms of person-time, mortality and morbidity information was complete for 98.9 and 98.0%, respectively, of person-years of follow-up.

Adherence was assessed through self-report on annual study questionnaires and was defined as taking at least two-thirds of the study pills. Average adherence over the course of follow-up was ~83% for active and placebo agents, with no significant difference between active and placebo groups. Use of open-label folic acid supplements, vitamin B6, or vitamin B12 supplements containing more than the recommended daily allowance for at least 4 days per month ranged from 2 to 11% in the active group to 2 to 13% in the placebo group over the course of the study. There were no serious adverse events reported that were conclusively related to study interventions.

Women in the WAFACS provided a baseline blood sample in 1996, prior to the initiation of background dietary folic acid fortification in the U.S. food supply in 1998. Randomly selected from participants who were adherent with study medications, 300 (150 in the active treatment and 150 in the placebo group) provided a blood sample at the end of randomized treatment.

As reported previously (25), median folate and homocysteine levels were similar between the active treatment group and the placebo group at baseline. At the end of study follow-up, the median folate level increased significantly in both groups; however, the relative increase in folate level was greater in the active treatment group. Despite significant increases in folate levels among the placebo group, there was no apparent reduction in homocysteine levels at the end of the study compared with participants measured at the beginning of the study in the placebo group ($P = 0.99$). In comparison, homocysteine levels were significantly reduced in the active treatment group; the geometric mean homocysteine level was decreased by 18.5% (95% CI 12.5–24.1; $P < 0.001$) in the active group over that observed in the placebo group for a difference of 2.27 μmol/l (1.54–2.96) from the placebo geometric mean homocysteine level of 12.28 μmol/l (25).

Ascertainment of incident type 2 diabetes. Diabetes status was evaluated at baseline, and all the participants were also asked annually whether and when they had been diagnosed with diabetes after randomization. Women who reported a diagnosis of diabetes during the follow-up were mailed supplementary questionnaires to confirm their self-reported diagnoses. The supplementary diabetes questionnaire was specifically designed to collect further detailed information on diabetes symptoms, screening test, and hypoglycemic medication. Based on the American Diabetes Association diagnostic criteria (28), actual glucose levels at fasting or oral glucose tolerance testing, diabetic symptoms, and/or hypoglycemic medication were combined together to confirm the self-reported incident cases of diabetes in a blinded fashion. The screening rate of having blood glucose testing among our study population was relatively high (85–90%). The observed high agreement between annual follow-up questionnaire and supplementary questionnaire (positive predictive value = 96%) suggests that self-reported diabetes possesses excellent predictive ability for true diabetes status in this cohort of U.S. female health professionals, who are likely to report accurate diagnostic information (29). Thus, we believe that self-reported type 2 diabetes is valid in our study population.

Statistical analysis. Primary analyses were performed on an intention-to-treat basis, including all randomized women after excluding those with self-reported diabetes at baseline. Baseline characteristics were compared by randomized groups using two-sample t tests for continuous variables and χ^2 statistics for categorical variables. Kaplan-Meier survival curves were used to estimate the overall cumulative incidence over time for the active vitamin group and the placebo group. The log-rank test was computed to compare the curves. We used Cox proportional hazards models to calculate the estimates of hazard ratio expressed as relative risks (RRs) and 95% CI for randomized treatment versus placebo, after adjustment for age and other randomized treatments (vitamin E, vitamin C, and β-carotene). To test the proportionality assumption, we included an interaction term for treatment with the logarithm of time in the Cox models. The tests showed that the proportional hazard assumption was not violated for any of the models. To examine the effect of actual as opposed to assigned folic acid/B vitamin treatment, we carried out a sensitivity analysis according to compliance. Women were censored if and when they stopped taking at least two-thirds of their study pills or were missing compliance information.

Subgroup analyses were conducted to examine the effect of active treatment on risk of type 2 diabetes according to prespecified risk factors for type 2 diabetes at baseline, including age-groups (45–54, 55–64, and ≥65 years), BMI (kg/m^2), smoking status (current, past, or never), alcohol use (never/rarely or one or more drinks per month), family history of diabetes (yes or no), physical activity (estimated energy expenditure from leisure activities of <1,000 or ≥1,000 kcal per week), menopausal status and hormone therapy (uncertain menopausal status, premenopausal, or postmenopausal including current, past, or never users of hormone therapy), history of hypertension (yes or no), history of hyperlipidemia (yes or no), and baseline dietary intakes of folate, vitamin B6, or vitamin B12 (tertiles for each). We assessed effect modification using interaction terms between subgroup indicators and randomized assignment, testing for trend when subgroup categories were ordinal. All analyses were conducted with SAS version 9 (SAS Institute, Cary, NC), and a two-sided test with a significance level of $\alpha = 0.05$ ($P < 0.05$) was used.

RESULTS

During a median follow-up period of 7.3 years, 504 women were diagnosed with type 2 diabetes among 4,252 nondiabetic participants who underwent randomization in the WAFACS. There were no statistically significant differences in baseline characteristics between the folic acid/B vitamin active treatment group and its corresponding placebo group (Table 1).

Overall, there was no significant effect of folic acid/B vitamins on the development of type 2 diabetes compared with the placebo group (Table 2 and Fig. 2). In total, there were 245 incident cases (11.5%) in the active treatment group and 259 (12.2%) in the placebo group (172.5/10,000 person-years vs. 184.8/10,000 person-years), corresponding to an overall RR of 0.94 (95% CI 0.79–1.11; $P = 0.46$) after controlling for age and antioxidant treatment assignments (Table 2). Figure 2 shows the cumulative incidence of type 2 diabetes events among women in the treatment and placebo groups by year of follow-up. There appeared to be a trend toward a modest reduction in risk of type 2 diabetes for the treatment group versus placebo group over the follow-up period, but the log-rank test for the overall difference was not statistically significant (P for log-rank test = 0.44) (Fig. 2).

When we subdivided the period of risk into years 1 and 2 combined and year 3 onward combined, we did not observe a statistically significant effect in any time period (Table 2). In sensitivity analyses to minimize potential bias due to the inclusion of undiagnosed diabetes at baseline, folic acid/B vitamin treatment was not significantly associated with risk of type 2 diabetes when excluding those cases that occurred in the first 2 years (RR 0.98 [95% CI 0.80–1.21]; $P = 0.87$). In a separate analysis where women were censored at the time they stopped taking at least two-thirds of their study pills or started to use outside folic acid/B vitamin supplements on ≥4 days per month, findings were similarly nonsignificant (0.92 [0.76–1.10]; $P = 0.36$). The results were unchanged after further adjustment for diabetes risk factors including BMI, physical activity, family history of diabetes, smoking, postmenopausal hormone use, multivitamin use, alcohol intake, and coffee consumption.

To determine whether certain subgroups of women were at particularly high or low risk for type 2 diabetes with folic acid/B vitamin treatment, we conducted multiple subgroup analyses stratified by several prespecified diabetes risk factors (Fig. 3). Overall, there was no evidence that any of these factors modified the treatment effect on the risk of type 2 diabetes. Similarly, neither baseline dietary folate intake nor intake of vitamin B6 or vitamin B12 modified the treatment effect. There was a significant reduction in risk of type 2 diabetes among women who had a family history of diabetes (RR 0.77 [95% CI 0.60–0.99]; $P = 0.04$) but not among those who had no such family history (1.09 [0.85–1.41]; $P = 0.49$), and the interaction was marginally significant ($P = 0.06$). However, these results from such subgroup analyses should be treated with caution, since they could be explained by chance alone due to multiple comparisons or imbalance of diabetes risk factors at baseline in small subgroups. Finally, we did a separate analysis to assess whether the randomly assigned treatment with vitamin E, vitamin C, or β-carotene may have modified the results (Table 3). We

TABLE 1
Baseline characteristics of nondiabetic women according to randomized groups in the WAFACS

Characteristics	*n*	Folic acid and B vitamins		*P* value
		Active (*n* = 2,132)	Placebo (*n* = 2,120)	
Age (years)	4,252	63.1 ± 8.9	62.9 ± 8.9	0.42
45–54	893	20.4	21.7	
55–64	1,526	37.1	34.7	
≥65	1,833	42.6	43.6	
BMI (kg/m^2)	4,252	29.7 ± 6.4	29.8 ± 6.3	0.56
<25	1,078	26.6	24.1	
25 to <30	1,276	29.2	30.8	
≥30	1,898	44.1	45.1	
Smoking status				0.64
Current	519	12.0	12.5	
Past	1,846	43.0	43.9	
Never	1,887	45.1	43.7	
Alcohol use				0.33
Never/rarely	2,102	48.6	50.3	
At least one drink/month	565	13.8	12.7	
1–6 drinks/week	1,156	28.0	26.4	
Daily	429	9.62	10.6	
Physical activity (kcal/week)	4,252	1,270 ± 1,768	1,209 ± 1,700	0.25
≤1,000	2,681	62.5	63.6	
>1,000	1,571	37.5	36.4	
Menopause and hormone therapy use				0.58
Premenopausal	269	5.86	6.79	
Uncertain	91	2.25	2.03	
Postmenopausal, current hormone therapy use	2,221	52.8	51.7	
Postmenopausal with no hormone therapy use	1,671	39.1	39.5	
Family history of diabetes				0.99
Yes	1,450	35.7	35.6	
No	2,618	64.4	64.4	
Baseline dietary intake				
Folic acid (mg)	4,053	492 ± 243	494 ± 240	0.60
Vitamin B6 (mg)	4,053	4.93 ± 15.3	5.19 ± 16.0	0.94
Vitamin B12 (mcg)	4,053	8.81 ± 8.75	8.83 ± 8.44	0.82

Data are means ± SD unless otherwise indicated.

found no significant differences in diabetes incidence in any of the subgroups and no evidence for interaction by the other interventions.

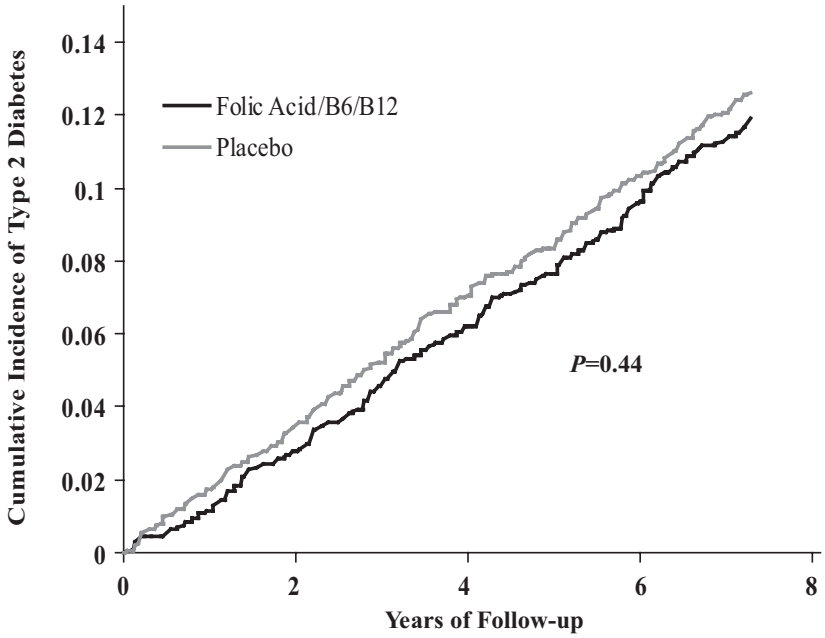
DISCUSSION

In this large, randomized, double-blind, placebo-controlled trial with 7.3 years of treatment among 4,252 women at

TABLE 2
RRs of self-reported type 2 diabetes by randomized folic acid and B vitamins intervention in the WAFACS

	Number of events/number of participants (%)		RR (95% CI)	
	Active (*n* = 2,132)	Placebo (*n* = 2,120)	Age-adjusted model*	Multivariate-adjusted model†
Follow-up interval (years)				
1–2	59 (2.77)	72 (3.40)	0.81 (0.58–1.14)	0.79 (0.55–1.12)
3–5	101 (4.74)	101 (4.76)	0.99 (0.75–1.31)	0.97 (0.73–1.28)
0–5	160 (7.50)	173 (8.16)	0.92 (0.74–1.14)	0.89 (0.72–1.11)
≥5	85 (3.99)	86 (4.06)	0.98 (0.72–1.32)	0.95 (0.70–1.30)
Total	245 (11.5)	259 (12.2)	0.94 (0.79–1.11)	0.91 (0.76–1.09)
Sensitivity analyses				
Excluding first 2 years	186 (8.72)	187 (8.82)	0.98 (0.80–1.21)	0.97 (0.78–1.19)
Compliance analyses				
Taking two-thirds or more of folic acid/B vitamins/placebo pills	223 (10.5)	238 (11.2)	0.92 (0.76–1.10)	0.87 (0.72–1.05)

*Adjusted for age and other randomized assignments (vitamins C and E and β-carotene). †Additionally adjusted for baseline variables, including BMI, smoking status, postmenopausal hormone use, multivitamin use, alcohol intake, coffee intake, physical activity, and family history of diabetes.



No. at Risk					
Folic Acid/B6/B12 vitamins Group	2132	2044	1935	1815	1738
Placebo Group	2120	2017	1901	1789	1720

FIG. 2. Cumulative incidence of self-reported type 2 diabetes by randomized treatment assignment (active treatment versus placebo) in the WAFACS.

high risk for CVD, we found no significant effect of homocysteine-lowering treatment by a combination pill of folic acid and vitamins B6 and B12 on risk of type 2 diabetes. There remained no evidence for a treatment effect in a sensitivity analysis restricted to women compliant with their study pills over the follow-up period. Our study provides the first randomized trial data regarding the long-term effect of folic acid/B vitamin supplementation on the risk of type 2 diabetes, although our findings remain to be corroborated by future research.

It has been hypothesized that B vitamins may help reduce the risk of type 2 diabetes by ameliorating metabolic abnormalities, such as oxidative damage, inflammation, and endothelial dysfunction, which characterize all phases of insulin resistance and pancreatic β-cell function and are implicated in the development and progression of type 2 diabetes. Due to their relative safety and low cost, B vitamin supplements have been targeted as potential therapeutic agents in previous randomized controlled trials for prevention of vascular diseases in high-risk populations. In contrast, direct evidence from randomized trials of B vitamin supplementation for type 2 diabetes has been very limited. Some (20–23) but not all (30) secondary prevention trials have suggested that folic acid supplementation may be effective in the improvement of oxidative stress (23) and endothelial dysfunction (20–22) in patients with type 2 diabetes. To our knowledge, no large clinical trials have specifically examined homocysteine-lowering interventions on the primary prevention of type 2 diabetes. In the present study, we provide evidence that folic acid and B vitamins have a neutral effect on the risk of type 2 diabetes among nondiabetic women; this is consistent with the absence of benefit from this intervention in lowering risk of cardiovascular events (31). Given the efficacy of the intervention in reducing homocysteine levels, our trial casts doubt on the etiologic role of hyperhomocysteinemia in the development of type 2 diabetes.

A major concern has been raised about the limited effect of folic acid treatment in a folate-fortified population. To assess the changes in homocysteine levels in response to background folate fortification in the U.S. population and to the randomized treatment with folic acid and B vitamins in our trial, Albert et al. (25) have conducted an analysis of baseline and follow-up folate and homocysteine levels in a randomly chosen subpopulation (150 in the active group and 150 in the placebo group) of WAFACS participants. The prefortification prevalence of hyperhomocysteinemia (≥15.0 μmol/l) in our population (27.7%) was larger than the average prevalence estimates of increased homocysteine levels (≥13.0 μmol/l) for men and women (25%) in the National Health and Nutrition Examination Survey (32) and in the Framingham population (19%) (33). Despite significant elevation in plasma folate levels due to mandatory folate fortification, homocysteine levels changed relatively little over a 7-year period. In contrast, our folic acid/B vitamin intervention lowered homocysteine levels by ~18.5% (2.27 μmol/l) (25). This reduction in homocysteine levels, however, was not associated with protection against the development of type 2 diabetes in this randomized trial. It is unknown whether a greater magnitude of homocysteine-lowering would have conferred protection against diabetes.

Genetic variations in enzymes involved in homocysteine metabolism may modulate the effect of treatment on risk of type 2 diabetes via their effects on circulating homocysteine levels (34). Interindividual genetic variability in our study population is an unlikely explanation for our null findings, however, because genetic factors should have been comparable in the active treatment and placebo groups by the randomization procedure. There may be

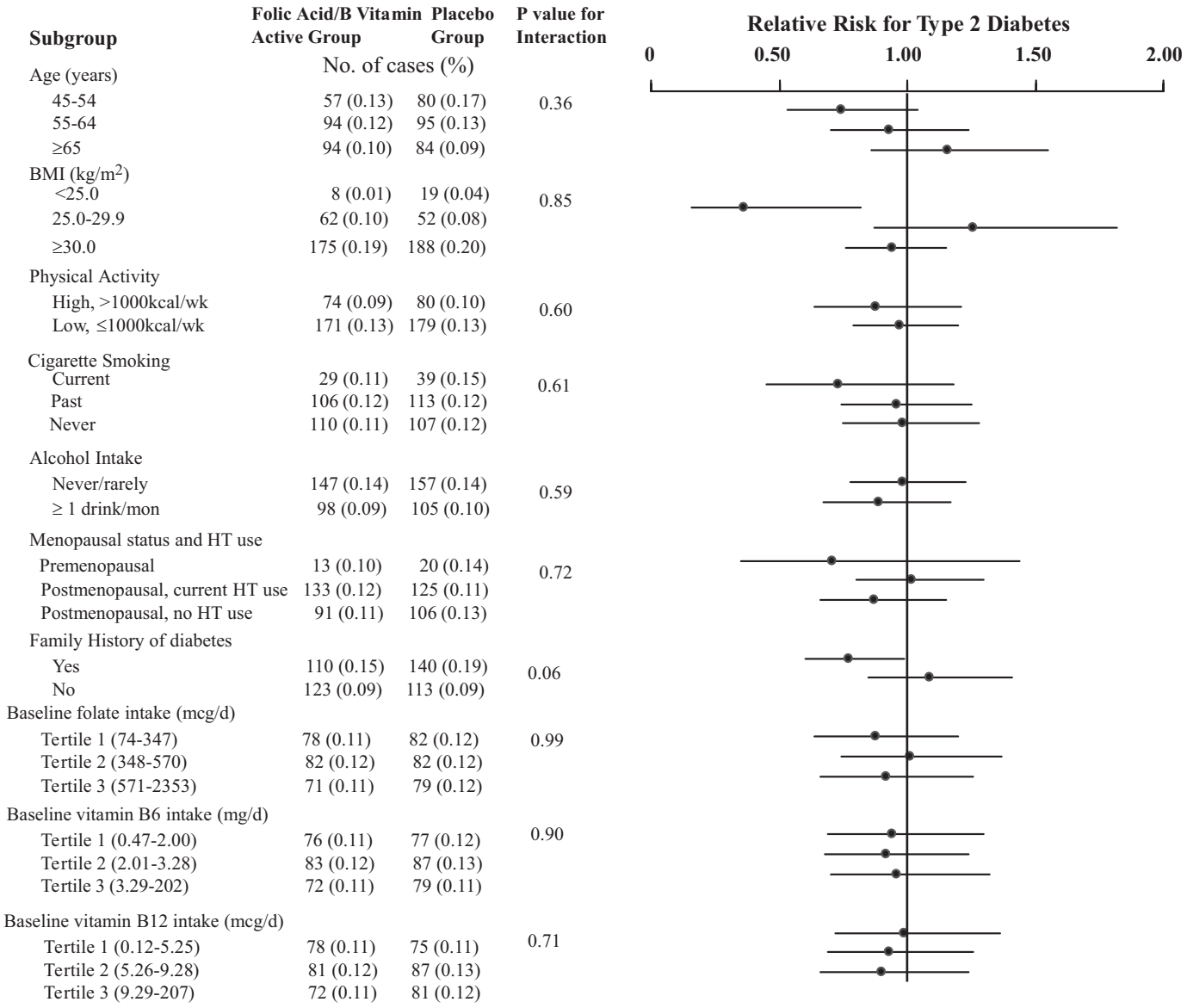


FIG. 3. RRs of self-reported type 2 diabetes by randomized intervention (active treatment versus placebo) within subgroups in the WAFACS. *HT*, hormone therapy.

TABLE 3
RRs of self-reported type 2 diabetes by homocysteine-lowering intervention according to other randomized treatment assignment groups in the WAFACS

	Number of events/number of participants (%)		RR (95% CI)	
Subgroups	Active group	Placebo group	Age-adjusted model*	*P* value for interaction
Vitamin C				
Active	114/1,059 (0.11)	119/1,059 (0.11)	0.95 (0.74–1.23)	0.86
Placebo	131/1,073 (0.12)	140/1,061 (0.13)	0.92 (0.73–1.17)	
Vitamin E				
Active	132/1,067 (0.12)	140/1,038 (0.13)	0.91 (0.72–1.16)	0.77
Placebo	113/1,065 (0.11)	119/1,082 (0.11)	0.96 (0.74–1.24)	
β-carotene				
Active	113/1,077 (0.10)	128/1,040 (0.12)	0.83 (0.65–1.07)	0.21
Placebo	132/1,055 (0.13)	131/1,080 (0.12)	1.04 (0.82–1.33)	

*Adjusted for age and two randomized assignments other than stratified groups.

subgroups of individuals with B vitamin deficiencies or genetic variants in the pathway of homocysteine metabolism that could particularly benefit from homocysteine-lowering therapy. Future well-designed and large-scale genetic studies within randomized trial settings are warranted to test the hypothesis. Of interest, our prespecified subgroup analyses showed a trend toward decreased risk associated with folic acid/vitamins B6 and B12 among women with a family history of diabetes, but these findings may have been due to chance and need to be confirmed in future investigations.

Some limitations of our trial also deserve consideration. First, declining compliance over time in the trial may have diluted the findings. In sensitivity analyses restricted to women compliant with their study pills, however, the overall null effects and trends were unchanged. Second, the use of a combination pill did not allow us to investigate the effects of individual components or potential interactions among them in relation to type 2 diabetes. Third, misclassification in the self-reported diagnosis of type 2 diabetes is another concern. Since the proportions of undiagnosed cases are likely to be similar in the treatment and placebo groups due to effective randomization and double-blinding strategies, such a misclassification is more likely to be nondifferential but could have attenuated the results. Fourth, we did not measure homocysteine and folate levels in all participants to assess a modifying effect of baseline levels; however, we found no evidence of benefits of B vitamin therapy among individuals with different levels of dietary folate, vitamin B6, and vitamin B12 intakes at baseline. Fifth, confounding by extraneous risk factors cannot be completely excluded, although the baseline characteristics were well balanced in the treatment and placebo group, as expected in a large-scale trial with effective randomization. Finally, our results based on women at high risk for CVD may not be generalizable to men or to the general population.

In conclusion, in this study within a large, randomized, placebo-controlled trial among over 5,400 women at high risk for CVD, we observed no apparent benefit or harm of folic acid and vitamins B6 and B12 supplementation on the risk of type 2 diabetes over 7 years of treatment. Our findings do not support recommending B vitamin supplements for diabetes prevention, although additional evidence from future large trials in populations with moderate-to-severe hyperhomocysteinemia or in regions without grain fortification will be needed.

ACKNOWLEDGMENTS

This study was supported by investigator-initiated grant HL46959 from the National Heart, Lung, and Blood Institute (NHLBI). Y.S. is supported by a grant (K01-DK078846) from the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

Vitamin E and its placebo were supplied by Cognis Corporation (LaGrange, IL). All other agents and their placebos were supplied by BASF Corporation (Mount Olive, NJ). Pill packaging was provided by Cognis and BASF. NHLBI, Cognis, and BASF did not provide any input into the design or conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript. No other potential conflicts of interest relevant to this article were reported.

An abstract of this article has been under consideration for publication in the pending supplemental issue of *Circulation* and was presented as an oral presentation during the American Heart Association Nutrition, Physical Activity, and Metabolism Conference 2009, Palm Harbor, Florida, 11 March 2009.

We acknowledge the contributions of the following members of the Data Safety and Monitoring Board: L. Cohen, R. Collins, T. Colton, D. DeMets, I.C. Henderson, A. La Croix, R. Prentice, N. Wenger (chair), M.F. Cotch, F. Ferris, L. Friedman, P. Greenwald, N. Kurinij, M. Perloff, E. Schron, A. Zonderman (ex officio members). We also acknowledge the invaluable contributions of the WACS staff, including Elaine Zaharris, Jean MacFadyen, Ellie Danielson, Marilyn Chown, Shamikhah Curry, Margarette Haubourg, Felicia Zangi, Tony Laurinaitis, Geneva McNair, Philomena Quinn, Harriet Samuelson, Ara Sarkissian, and Martin Van Denburgh. We also thank the end points reviewers, including Michelle Albert, Gavin Blake, Claudia Chae, Wendy Chen, Bill Christen, Carlos Kase, Tobias Kurth, I-Min Lee, Aruna Pradhan, Paul Ridker, Jackie Suk, James Taylor, and Simin Liu. Finally, we are indebted to the 8,171 dedicated WACS participants.

REFERENCES

1. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med 1998;338:1042–1050
2. Weiss N, Heydrick SJ, Postea O, Keller C, Keaney JF Jr, Loscalzo J. Influence of hyperhomocysteinemia on the cellular redox state: impact on homocysteine-induced endothelial dysfunction. Clin Chem Lab Med 2003;41:1455–1461
3. Hofmann MA, Lalla E, Lu Y, Gleason MR, Wolf BM, Tanji N, Ferran LJ Jr, Kohl B, Rao V, Kisiel W, Stern DM, Schmidt AM. Hyperhomocysteinemia enhances vascular inflammation and accelerates atherosclerosis in a murine model. J Clin Invest 2001;107:675–683
4. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and β-cell dysfunction? Diabetes 2003;52:1–8
5. Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol 2004;24:816–823
6. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 1997;389:610–614
7. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91
8. Stamler JS, Osborne JA, Jaraki O, Rabbani LE, Mullins M, Singel D, Loscalzo J. Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. J Clin Invest 1993;91:308–318
9. Pinkney JH, Stehouwer CD, Coppack SW, Yudkin JS. Endothelial dysfunction: cause of the insulin resistance syndrome. Diabetes 1997;46(Suppl. 2):S9–S13
10. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation 2006;113:1888–1904
11. Caballero AE. Endothelial dysfunction in obesity and insulin resistance: a road to diabetes and heart disease. Obes Res 2003;11:1278–1289
12. van Guldener C, Stehouwer CD. Diabetes mellitus and hyperhomocysteinemia. Semin Vasc Med 2002;2:87–95
13. Meigs JB, Jacques PF, Selhub J, Singer DE, Nathan DM, Rifai N, D'Agostino RB Sr, Wilson PW. Fasting plasma homocysteine levels in the insulin resistance syndrome: the Framingham Offspring Study. Diabetes Care 2001;24:1403–1410
14. Fonseca VA, Fink LM, Kern PA. Insulin sensitivity and plasma homocysteine concentrations in non-diabetic obese and normal weight subjects. Atherosclerosis 2003;167:105–109
15. Hajer GR, van der Graaf Y, Olijhoek JK, Verhaar MC, Visseren FL. Levels of homocysteine are increased in metabolic syndrome patients but are not

associated with an increased cardiovascular risk, in contrast to patients without the metabolic syndrome. Heart 2007;93:216–220
16. Abbasi F, Facchini F, Humphreys MH, Reaven GM. Plasma homocysteine concentrations in healthy volunteers are not related to differences in insulin-mediated glucose disposal. Atherosclerosis 1999;146:175–178
17. Sheu WH, Lee WJ, Chen YT. Plasma homocysteine concentrations and insulin sensitivity in hypertensive subjects. Am J Hypertens 2000;13:14–20
18. Godsland IF, Rosankiewicz JR, Proudler AJ, Johnston DG. Plasma total homocysteine concentrations are unrelated to insulin sensitivity and components of the metabolic syndrome in healthy men. J Clin Endocrinol Metab 2001;86:719–723
19. Cho NH, Lim S, Jang HC, Park HK, Metzger BE. Elevated homocysteine as a risk factor for the development of diabetes in women with a previous history of gestational diabetes mellitus: a 4-year prospective study. Diabetes Care 2005;28:2750–2755
20. Mangoni AA, Sherwood RA, Asonganyi B, Swift CG, Thomas S, Jackson SH. Short-term oral folic acid supplementation enhances endothelial function in patients with type 2 diabetes. Am J Hypertens 2005;18:220–226
21. van Etten RW, de Koning EJ, Verhaar MC, Gaillard CA, Rabelink TJ. Impaired NO-dependent vasodilation in patients with type II (non-insulin-dependent) diabetes mellitus is restored by acute administration of folate. Diabetologia 2002;45:1004–1010
22. Title LM, Ur E, Giddens K, McQueen MJ, Nassar BA. Folic acid improves endothelial dysfunction in type 2 diabetes: an effect independent of homocysteine-lowering. Vasc Med 2006;11:101–109
23. Hunter-Lavin C, Hudson PR, Mukherjee S, Davies GK, Williams CP, Harvey JN, Child DF, Williams JH. Folate supplementation reduces serum hsp70 levels in patients with type 2 diabetes. Cell Stress Chaperones 2004;9:344–349
24. Cook NR, Albert CM, Gaziano JM, Zaharris E, MacFadyen J, Danielson E, Buring JE, Manson JE. A randomized factorial trial of vitamins C and E and beta carotene in the secondary prevention of cardiovascular events in women: results from the Women's Antioxidant Cardiovascular Study. Arch Intern Med 2007;167:1610–1618
25. Albert CM, Cook NR, Gaziano JM, Zaharris E, MacFadyen J, Danielson E, Buring JE, Manson JE. Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: a randomized trial. JAMA 2008;299:2027–2036
26. Bassuk SS, Albert CM, Cook NR, Zaharris E, MacFadyen JG, Danielson E, Van Denburgh M, Buring JE, Manson JE. The Women's Antioxidant Cardiovascular Study: design and baseline characteristics of participants. J Womens Health (Larchmt) 2004;13:99–117
27. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65
28. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183–1197
29. Ding EL, Song Y, Manson JE, Pradhan AD, Buring JE, Liu S. Accuracy of administrative coding for type 2 diabetes in children, adolescents, and young adults. Diabetes Care 2007;30:141–143
30. Spoelstra-de MA, Brouwer CB, Terheggen F, Bollen JM, Stehouwer CD, Smulders YM. No effect of folic acid on markers of endothelial dysfunction or inflammation in patients with type 2 diabetes mellitus and mild hyperhomocysteinaemia. Neth J Med 2004;62:246–253
31. Bazzano LA, Reynolds K, Holder KN, He J. Effect of folic acid supplementation on risk of cardiovascular diseases: a meta-analysis of randomized controlled trials. JAMA 2006;296:2720–2726
32. Pfeiffer CM, Osterloh JD, Kennedy-Stephenson J, Picciano MF, Yetley EA, Rader JI, Johnson CL. Trends in circulating concentrations of total homocysteine among US adolescents and adults: findings from the 1991–1994 and 1999–2004 National Health and Nutrition Examination Surveys. Clin Chem 2008;54:801–813
33. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. N Engl J Med 1999;340:1449–1454
34. Lewis SJ, Ebrahim S, Davey Smith G. Meta-analysis of MTHFR 677C->T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate? BMJ 2005;331:1053