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Effect of Combination Folic Acid, Vitamin B_6, and Vitamin B_{12} Supplementation on Fracture Risk in Women: A Randomized, Controlled Trial

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ABSTRACT

Epidemiologic studies have demonstrated an association of elevated plasma homocysteine levels with greater bone resorption and fracture risk. Vitamins B_{12}, B_6, and folic acid are cofactors in homocysteine metabolism, and supplementation with B vitamins is effective in lowering homocysteine levels in humans. However, randomized trials of supplemental B vitamins for reduction of fracture risk have been limited. Therefore, we performed an ancillary study to the Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS), a large randomized trial of women with preexisting cardiovascular disease or three or more coronary risk factors, to test whether a daily B vitamin intervention including folic acid (2.5 mg/day), vitamin B_6 (50 mg/day), and vitamin B_{12} (1 mg/day) reduces nonspine fracture risk over 7.3 years of treatment and follow-up. Among 4810 women, we confirmed 349 nonspine fracture cases by centralized review of medical records. In a substudy of 300 women (150 in treatment group and 150 controls) with paired plasma samples at randomization and follow-up (7.3 years later), we measured two bone turnover markers, including C-terminal cross-linking telopeptide of type I collagen (CTX) and intact type I procollagen N-propeptide (P1NP). In Cox proportional hazards models based on intention-to-treat, we found no significant effects of B vitamin supplementation on nonspine fracture risk (relative hazard = 1.08; 95% confidence interval, 0.88 to 1.34). In a nested case-cohort analysis, there were no significant effects of B vitamins on fracture risk among women with elevated plasma homocysteine levels, or low levels of vitamins B_{12} or B_6, or folate at baseline. Furthermore, treatment with B vitamins had no effect on change in markers of bone turnover. We found no evidence that daily supplementation with B vitamins reduces fracture risk or rates of bone metabolism in middle-aged and older women at high risk of cardiovascular disease. © 2017 American Society for Bone and Mineral Research.

KEY WORDS: OSTEOPOROSIS; FRACTURE PREVENTION; B VITAMINS,; BIOCHEMICAL MARKERS OF BONE TURNOVER; THERAPEUTICS; NUTRITION

Introduction

Osteoporotic fractures are a leading cause of disability in older adults, and a major contributor to medical care costs around the world.[1] Therefore, the identification of modifiable risk factors for osteoporosis is of high importance to improve public health. It is especially important to identify inexpensive and effective treatments for osteoporosis that are available even to those with limited resources.

Dietary supplementation with the nutrients folic acid, vitamin B_{12}, and vitamin B_6 has been proposed as one such candidate treatment. These three vitamins are cofactors in homocysteine metabolism, and studies have shown that as many as two-thirds of cases of elevated homocysteine are associated with low plasma folate or vitamin B_{12}.[2] Randomized trials in adults have demonstrated that treatment with folic acid and B vitamins can significantly lower plasma homocysteine levels,[3,4] although the extent of

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homocysteine-lowering may depend on the prevalence of poor vitamin intake.[5]

Results from several epidemiologic studies have demonstrated an association between elevated plasma homocysteine levels and increased risk of osteoporotic fractures,[6–8] including hip fractures.[9] Furthermore, high homocysteine levels have been associated with increased bone resorption.[10–12] Additionally, a recent study based on data and samples from the National Health and Nutrition Examination Survey (NHANES) study demonstrated that elevated serum homocysteine and lower folate levels (but not B_{12} levels) correlated cross-sectionally with lower lumbar and total body bone mineral density (BMD).[13]

Despite relatively strong epidemiologic evidence, results of prior clinical trials to determine if administration of B vitamins can reduce fracture risk have been inconclusive.[14–17] For example, in the B-Vitamins for the Prevention of Osteoporotic Fractures (B-PROOF) study, over 2900 older adults with elevated homocysteine levels were randomized to receive daily B_{12} and folic acid supplementation or placebo for a period of 2 years. No significant differences in fracture incidence were observed overall, though there was a suggestion of protective effect among compliant individuals over 80 years old.[14] Two other studies, conducted among individuals at high risk of cardiovascular disease[15] or recent stroke,[17] found no significant association of homocysteine-lowering therapy and fracture risk. Only one study among older Japanese persons with ischemic stroke reported a significant reduction of fracture risk in those taking daily folic acid and vitamin B_{12} compared to placebo.[16] However, the study, by Sato and colleagues,[16] was recently retracted, invalidating those positive findings.[18] Similarly, results of most previous trials testing the effects of B vitamins on markers of bone turnover[19–22] have also failed to demonstrate significant effects. However, these studies have also suffered from small sample size and most were relatively short-term trials of 3 to 6 months.[20–22]

To test the hypothesis that treatment with a regimen of folic acid, vitamin B_6, and vitamin B_{12} would reduce the risk of incident fractures and influence levels of bone turnover markers pretreatment and posttreatment in women, we performed an ancillary study to the Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS), a large, double-blind, randomized placebo-controlled trial originally designed to test the effects of antioxidants and B vitamins on cardiovascular disease outcomes in women at high cardiovascular disease (CVD) risk. We further hypothesized that women with elevated homocysteine levels, or low serum levels of folic acid and vitamins B_6 or B_{12} would experience the greatest benefits of treatment.

Subjects and Methods

Study population

The WAFACS was a randomized, double-blind, placebo-controlled trial, originally designed to test the effects of antioxidant vitamins (vitamin C, vitamin E, and β-carotene) for the prevention of cardiovascular events among female health professionals aged ≥40 years at enrollment (mean age 60.6 years; range, 43 to 90 years), who had either preexisting CVD or three or more coronary risk factors.[3,23,24] The sample was approximately 94% white.[23]

Initially, the WAFACS cohort included 8171 participants who were randomly assigned in a $2 \times 2 \times 2$ factorial design to vitamin C, vitamin E, β-carotene, or placebo in 1995.[23,24] Additional funding was received to add the folic acid (2.5 mg/day), vitamin B_6, (50 mg/day), and vitamin B_{12} (1 mg/day) intervention to the study and 5442 willing participants were further randomized to either treatment with combination B vitamins or placebo in April 1998.[3] The design of the study is illustrated in Figure 1. The B vitamin intervention was designed with the objective of lowering homocysteine levels, thereby reducing the risk for CVD in this population of high risk women. A blood substudy of a random sample of 300 women (150 in each treatment group) confirmed that those receiving the B vitamin supplementation had higher folate levels, and lower homocysteine levels relative to baseline levels, and significantly more so than the placebo group.[3] To participate in the B vitamin intervention, women had to forego the use of B vitamin supplements at levels greater than the recommended dietary allowance (RDA) for the duration of the intervention.[3]

Treatment and follow-up of the B vitamin intervention were concluded in July 2005, with mean follow-up at study closeout of approximately 7.3 years. As previously published, all CVD risk factors, medication use, multivitamin use, and median intake of the relevant nutrients (folate, B_6, and B12) were very similar between randomization arms, with no significant differences.[3]

Fracture assessment

Throughout the treatment and follow-up period, annual follow-up questionnaires were mailed to participants to collect self-reported information on study outcomes including fractures that occurred during the preceding 12-month period. Women who reported a fracture were contacted by WAFACS staff, who requested detailed information on each fracture reported during the B vitamin intervention/follow-up period. Clinical documentation to confirm fractures, including medical and radiographic reports, were requested.

The primary endpoint was confirmed incident nonspine fracture during the B vitamin intervention trial. Spine fractures and pathological fractures were excluded. Radiographic reports or other clinical documentation for each reported fracture were reviewed and adjudicated centrally at the San Francisco Coordinating Center, without knowledge of treatment assignment. Women with inadequate fracture documentation were excluded from the main analysis ($n = 632$).

Case-cohort fracture study by baseline plasma levels of homocysteine and B vitamins

A case-cohort study design was used to test whether the effects of treatment on fracture risk differed among those with elevated baseline homocysteine levels, or low levels of vitamin B_6, vitamin B_{12}, or folate.[25] Approximately 75% of participants in the B vitamin intervention had provided EDTA plasma samples at baseline. Following completion of the fracture adjudication, among those with baseline plasma samples available, we selected a case-cohort sample of 833 participants independent of active versus placebo treatment status, which included all 274 participants with confirmed incident nonspine fractures, and a random cohort sample of 675 participants selected regardless of fracture status. Among the 675 women in the random cohort sample, 116 were fracture cases, and 559 did not have incident adjudicated nonspine fracture and served as the "control" group for analyses, resulting in an approximate control to case ratio of 2:1.

Archived blood specimens were assayed for biochemical parameters by the Nutrition Evaluation Laboratory at the Jean

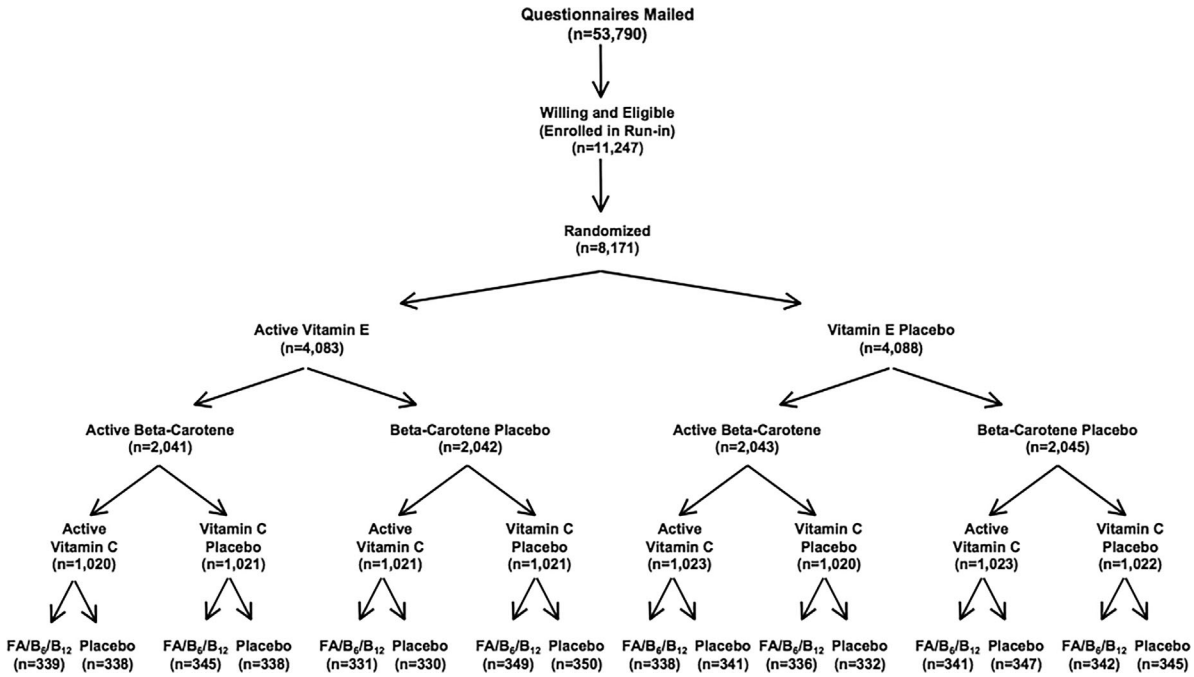

Fig. 1. Randomization scheme for the Women's Antioxidant and Folic Acid Cardiovascular Study.

Mayer USDA Human Nutrition Research Center on Aging at Tufts University. Plasma total homocysteine (tHcy) was measured by high-performance liquid chromatography using fluorescence detection (Empower HPLC System; Waters Corporation, Milford, MA, USA).[26] The detection limit of the tHcy assay is 1 nmol/mL and the intraassay and interassay coefficients of variation (CVs) are 4% and 7%, respectively. The reference range is 4.6 to 11.0 nmol/mL. Folate and vitamin B_{12} were measured by a competitive binding protein radioimmunoassay (Quantaphase II RIA kit; Bio-Rad Laboratories, Hercules, CA, USA). The detection limit for the folate assay is <0.1 ng/mL and the intraassay and interassay CVs are 3.8% to 5.2% and 4.1% to 8.7%, respectively. The reference range is 1.5 to 20.6 ng/mL. The detection limit of the vitamin B_{12} assay is 20 pg/mL and the intraassay and interassay CVs are 4.0% to 5.9% and 4.5% to 6.8%, respectively. The reference range is 200 to 1200 pg/mL. Pyridoxal 5′-phosphate (PLP: vitamin B_6) was determined by enzymatic radioimmunoassay using tyrosine decarboxylase apoenzyme.[27] The detection limit of the vitamin B_6 assay is 10 nmol/L and the intraassay and interassay CVs are 5% and 9%, respectively. The reference range is 20 to 100 nmol/L.

Markers of bone turnover

In a separate previous substudy,[3] a total of 300 women (150 in the active treatment and 150 in the placebo group) provided a fasting plasma sample at randomization for the B vitamin intervention, and at the end of randomized treatment and follow-up 7.3 years later. C-terminal cross-linking telopeptide of type I collagen (CTX; Roche Diagnostics, Mannheim, Germany), and marker of bone resorption, and intact type I procollagen N-propeptide (P1NP; Roche Diagnostics, Mannheim, Germany), a marker of bone formation, were measured using automated platforms at a specialized laboratory (CCBR-Synarc, Lyon, France). Intraassay and interassay CVs are $<4.4\%$ for P1NP and $<5.7\%$ for CTX.

In this blood subgroup, the concentration of baseline total homocysteine was previously determined by enzymatic assay using the Hitachi 917 analyzer (Roche Diagnostics, Basel, Switzerland). Baseline and follow-up samples were assayed in blinded fashion in the same analytical run.[3]

Other variables

Information regarding body mass index (BMI), menopausal status, menopausal hormone therapy (HT) use, multivitamin use, personal use of folic acid and other B vitamin supplements, smoking status (current, past, and never), physical activity (kcal/day) and history of prior fracture were collected by self-reported questionnaire at the time of the randomization in the B vitamin intervention.[3,23]

Statistical analysis

Characteristics of the B vitamin treatment and placebo groups were compared using *t* tests for continuous variables and chi-square statistics for categorical variables.

Following intention-to-treat analysis, the effect of B vitamin therapy versus placebo on fracture risk was assessed using Cox proportional hazards models. The primary outcome was adjudicated nonspine fracture. However, given that a relatively large number ($n = 632$) had inadequate fracture documentation, self-reported nonspine fracture was analyzed as a sensitivity analysis. Prespecified subgroup analyses were performed stratifying on adherence (defined as those who took at least two-thirds of their assigned pills at all follow-up time points) (Y/N), age ($\leq$65, $>$65 years), baseline use of B vitamin supplements (Y/N), current smoking (Y/N), and current HT use (Y/N). Because multivitamins often contain up to 100% of the recommended dietary allowance for some B vitamins, we performed a sensitivity analysis excluding those reporting use of B vitamins or multivitamins at baseline.

The case-cohort adaptation to the Cox proportional hazards model was used for analysis of the effects of treatment on risk of nonspine fracture among those with elevated baseline levels of homocysteine (defined as highest quartile), or low B_6, B_{12}, or folate levels (defined as lowest quartile). Because power for this analysis was limited, in sensitivity analyses we also explored the median as the cut point for these analyses.

For change in bone marker analysis, linear regression models were used to assess effects of the treatment of interest on percent change in untransformed P1NP and CTX, which was calculated by 100 × [(follow-up – baseline)/baseline]. Subgroup analyses were done by HT use status, menopause status, and baseline homocysteine level.

All analyses were conducted using SAS, version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

Among 5442 participants who were enrolled in the vitamin B intervention component of WAFACS, 1229 reported incident nonspine fractures on one or more annual questionnaires. Of these, 632 had inadequate fracture documentation (319 in the B vitamin group and 313 in the placebo group) and were excluded from analyses. An additional 248 reported fractures were adjudicated as nonfractures. The analytic cohort was therefore comprised of a total of 4810 women with 349 adjudicated nonspine fractures (including 22 hip fractures, and 67 wrist fractures). The vitamin and placebo groups were well matched at baseline with regard to age, postmenopausal status, previous fracture, and HT use (Table 1Table 1). Approximately two-thirds of women reported >66% compliance with their assigned intervention at all follow-up time points during the trial.

Fracture outcome

The risk of any nonspine fracture was similar among those randomized to vitamin or placebo (hazard ratio [HR] = 1.08; 95% confidence interval [CI], 0.88 to 1.34), as were risks of hip (HR = 0.99; 95% CI, 0.43 to 2.29), wrist (HR = 1.30; 95% CI, 0.80 to 2.11), and other fracture (HR = 1.03; 95% CI, 0.82 to 1.30) (Table 2). In a sensitivity analysis, results were also similar for the outcome of self-reported (unadjudicated) nonspine fracture (HR = 1.06; 95% CI, 0.93 to 1.20) (Table 2). Results were also unchanged by further adjustment for treatment assignment to vitamin C, E, or beta-carotene (results not shown).

Nonspine fracture results were similar among those who were considered adherent to study medications during the follow-up period, and among baseline subgroups defined by age, use of B vitamin supplements, HT use, and smoking status (all interaction p values >0.05) (Table 3). There were similarly no significant effects of treatment observed after excluding those who reported baseline use of either B vitamin supplements or multivitamins (HR = 1.07; 95% CI, 0.83 to 1.36).

In the case-cohort fracture substudy, there was no evidence of an interaction of treatment assignment with baseline plasma levels of homocysteine, B_{12}, or folate (Table 4). However, there was a significant interaction observed between baseline B_6 levels and treatment (interaction p value = 0.04). Among those in the lowest quartile of plasma B_6 levels, there was a nonsignificant protective effect of treatment (relative hazard [RH] = 0.69; 95% CI, 0.39 to 1.22), whereas there was a significant increased risk observed among those with normal B_6 levels (RH = 1.39; 95% CI, 1.01 to 1.91). In separate sensitivity analyses, we found no significant interactions of treatment assignment and baseline plasma levels of homocysteine, B_{12}, folate, or B_6 (nor did we observe any specific effects of treatment within strata) when we stratified based on median concentrations instead of high-risk quartile versus others.

Bone turnover markers outcome

There were no differences between treatment and placebo groups regarding baseline and follow-up bone turnover marker measurements and change in bone markers (Table 5). Change in

Table 1. Baseline Characteristics by Treatment Group Among WAFACS Women With Valid Fracture Information (n = 4810)

Variable	Folate/vitamin B group (n = 2402)	Placebo group (n = 2408)	p
Age (years), mean ± SD	62.6 ± 8.7	62.5 ± 8.7	0.65
BMI, mean ± SD	30.5 ± 6.6	30.6 ± 6.7	0.55
Postmenopause status, n (%)			0.47
Yes	2193 (91.3)	2204 (91.5)	
No	149 (6.2)	156 (6.5)	
Unsure	60 (2.5)	48 (2.0)	
Smoking status, n (%)			0.03
Never	1094 (45.6)	1035 (43.0)	
Past	1046 (43.6)	1071 (44.5)	
Current	262 (10.9)	302 (12.5)	
Current HT use, n (%)	1245 (51.8)	1248 (51.8)	0.99
Taking multivitamin, n (%)	555 (23.1)	546 (22.7)	0.73
Taking folate/B_6/B_{12} supplement, n (%)	184 (7.7)	166 (6.9)	0.31
Taking folate supplement	64 (2.7)	60 (2.5)	0.71
Taking B_6 supplement	81 (3.4)	80 (3.3)	0.92
Taking B_{12} supplement	90 (3.8)	75 (3.1)	0.23
Taking other supplement with high folate/B_6/B_{12}	51 (2.1)	57 (2.4)	0.57
Prior fracture, n (%)	891 (37.1)	903 (37.5)	0.77
Physical activity (kcal per week from exercise), mean ± SD	1259 ± 1779	1168 ± 1670	0.07
Adherent to treatment, n (%)[a]	1600 (67.2)	1636 (68.6)	0.30

[a]Adherence is defined as having 100% of annual follow-up questionnaires in which participant reports 67% or more study medications were taken.

Table 2. Incident Fractures by Treatment Group During 7.3 Years of Treatment/Follow-up

Variable	Folate/vitamin B group ($n = 2402$) n (%)	Placebo group ($n = 2408$) n (%)	Relative hazard (95% CI)	p[a]
Nonspine fracture	182 (7.6)	167 (6.9)	1.08 (0.88–1.34)	0.39
Hip fracture	11 (0.5)	11 (0.5)	0.99 (0.43–2.29)	0.99
Wrist fracture	38 (1.6)	29 (1.2)	1.30 (0.80–2.11)	0.26
Other nonspine fracture	146 (6.1)	142 (5.9)	1.03 (0.82–1.30)	0.79
Self-reported (unadjudicated) nonspine fracture	628 (23.1)	601 (22.1)	1.06 (0.93–1.20)	0.38

[a]Value of p from chi-square test.

bone markers were also similar with active and placebo treatment after stratifying for baseline HT use status (all $p > 0.3$). There were similarly no significant interactions between treatment and menopause status or baseline homocysteine levels.

Discussion

In this large randomized, placebo-controlled trial of women at high risk for CVD, we found no significant beneficial or harmful effects of daily B vitamin supplementation on nonspine fracture risk over 7.3.years of treatment and follow-up. In a case-cohort subsample, we similarly found no significant effects of B vitamins on nonspine fracture risk among those with elevated baseline plasma homocysteine levels. Results were similarly null in other prespecified subgroup analyses based on adherence, age, HT use, baseline use of B vitamin supplements, and smoking status.

Overall, our results add to the growing body of evidence from randomized trials, demonstrating minimal effect of B vitamin supplementation on risk of fracture.[14,15,17,28] For example, our findings are consistent with the recently published negative results in the B-PROOF study, in which over 2900 older adults with elevated homocysteine levels were randomized to receive daily B_{12} and folic acid supplementation or placebo for a period of 2 years.[14] However, whereas the B-PROOF study reported a possible protective effect among compliant individuals older than 80 years, our prespecified subgroup analyses revealed no significant interaction of treatment with age or compliance to assigned study pills. In contrast with the B-PROOF study, our intervention was longer (7.3 years versus 2 years in B-PROOF), and our study population comprised women at high risk of CVD, but were not otherwise selected for high baseline homocysteine levels. Nonetheless, our subgroup findings are consistent with those of B-PROOF, showing no significant effect of B vitamin supplementation in those with elevated baseline homocysteine levels.[14] Of the four baseline plasma biomarkers (homocysteine, folic acid, B_{12}, and B_6), we observed a significant interaction with treatment for only one (B_6), and therefore cannot rule out chance as an explanation for this finding.

Previous results from a Japanese study by Sato and colleagues[16] had suggested a significant protective benefit of B vitamin supplementation on fracture risk in patients with prior ischemic stroke; however, this study has since been

Table 3. Effect of Randomized Treatment Assignment on the Primary Outcome in Prespecified Subgroups

Characteristic	Patients (n)		Events (n)		RR (95% CI)	p[a]
	Active	Placebo	Active	Placebo		
Overall	2402	2408	182	167	1.08 (0.88–1.34)	
Age						0.14
≤65 years	1417	1415	104	107	0.95 (0.73–1.25)	
>65 years	985	993	78	60	1.32 (0.94–1.85)	
Taking supplements[b]						0.15
Yes	184	166	12	16	0.64 (0.30–1.35)	
No	2218	2242	170	151	1.13 (0.91–1.41)	
Current HRT use						0.052
Yes	1245	1248	78	88	0.87 (0.64–1.18)	
No	1157	1160	104	79	1.33 (0.99–1.78)	
Current smoker						0.94
Yes	262	302	13	14	1.06 (0.50–2.25)	
No	2140	2106	169	153	1.08 (0.87–1.35)	
Adherent[c]						0.62
Yes	1600	1636	129	124	1.05 (0.82–1.35)	
No	782	750	53	43	1.19 (0.79–1.77)	

[a]Value of p for interaction.

[b]Taking folic acid, B_6, B_{12}, and any other supplements which were above the US recommended daily allowance for folic acid, B_6, and B_{12} at baseline (400 μg folic acid, 2 mg B_6, and 6 μg B_{12}).

[c]Adherence is defined as having 100% of annual follow-up questionnaires in which participant reports 67% or more study medications were taken.

Table 4. Case-Cohort Analysis: Effect of Randomized Treatment Assignment on the Nonspine Fracture Risk by Baseline Plasma Levels of Homocysteine, B_6, and Folate

Characteristic	Patients (*n*)		Events (*n*)		RR (95% CI)	*p*[a]
	Active	Placebo	Active	Placebo		
Homocysteine						0.82
High (Q4)	94	109	34	36	1.11 (0.64–1.94)	
Normal (Q1–Q3)	309	321	107	97	1.20 (0.87–1.65)	
Vitamin B_{12}						0.31
Low (Q1)	109	101	37	37	0.91 (0.53–1.56)	
Normal (Q2–Q4)	294	329	104	96	1.27 (0.92–1.76)	
Folate						0.73
Low (Q1)	87	101	33	37	1.28 (0.74–2.22)	
Normal (Q2–Q4)	316	312	108	96	1.15 (0.83–1.58)	
Vitamin B_6						
Low (Q1)	92	110	25	38	0.69 (0.39–1.22)	0.04
Normal (Q2–Q4)	311	321	116	95	1.39 (1.01–1.91)	

High homocysteine is >15.1 nmol/mL; low B_{12} is ≤334 pg/mL; low folate is ≤6.4 ng/mL; low B_6 is ≤34.1 nmol/L.
[a]Value of *p* for interaction.

retracted.[18] To our knowledge, there are no randomized trials that have demonstrated significant effects of B vitamin supplementation for the prevention of fracture risk.

Finally, we found that a B vitamin intervention designed to lower homocysteine levels had no differential effects on changes in two contemporary markers of bone turnover over 7.3 years of treatment and follow-up. Results of previous trials, typically conducted over 1-year to 2-year periods, have been mixed,[19,20,22,29–31] with most studies confirming our findings of no significant effects of B vitamin supplementation on changes in bone turnover markers, despite successful lowering of plasma homocysteine or B vitamin levels.[19,20,30] To our knowledge, the only study to report significant changes in bone turnover markers was an intervention by Herrmann and colleagues,[31] in which B vitamin supplementation was conducted in men and women aged >54 years for a period of 1 year. In this study, both treatment and placebo group received supplemental calcium and vitamin D. Those in the treatment group demonstrated significantly decreased levels of bone alkaline phosphatase, tartrate resistant acid phosphatase, and osteocalcin compared to those in the placebo group. We did not examine these bone biomarkers in our study, nor did we administer supplemental calcium or vitamin D.

Because we did not measure BMD in our study, we were unable to examine the effects of B vitamin supplementation on changes in BMD, although most previous randomized trials that have investigated this endpoint have been null.[30,32] Herrmann and colleagues[30] reported a significant improvement in lumbar spine BMD after a year of supplementation with a high-dose combination of folic acid, vitamin B_6, and vitamin B_{12} among the small subset ($n = 8$) with low total homocysteine concentrations at baseline.

There is rationale for an effect of supplemental B vitamins in fracture prevention and bone health. Prior studies demonstrated that elevated homocysteine may interfere with collagen cross-linking.[33,34] Collagen cross-links play an important role in determining the stability and strength of the collagen network so that if deficient, the bone matrix could be compromised, leading to an increased risk of fracture. Alternatively, homocysteine may promote oxidative damage leading to increased fractures. For example, when homocysteine undergoes oxidative metabolism to homocystine and homocysteine thiolactone, cytotoxic reactive oxygen species, including the superoxide anion radical and hydroxyl radical are produced, which in turn have been shown to initiate lipid peroxidation.[35] Products of lipid and lipoprotein oxidation have been hypothesized to be risk factors for osteoporosis.[36] Other plausible mechanisms may involve adverse effects of homocysteine on endothelial-derived nitric oxide (eNOS), which has been shown to negatively impact osteoblast function in mice.[37,38] There may also be direct effects of B vitamin repletion on bone metabolism in vitamin-insufficient individuals, independently of homocysteine. With respect to the latter possibility, it is noteworthy that approximately 30% of participants were folate deficient at baseline (ie, plasma folate <7 ng/mL), but no deficiency was seen at the end of the trial in either the control or the placebo group.[3] The

Table 5. Change in Bone Turnover Markers by Treatment Group Over 7.3 Years (Pretreatment to Posttreatment)

Variable	Active group	Placebo group	*p*
Baseline CTX (ng/mL)	0.21 ± 0.14	0.23 ± 0.15	0.17
Baseline P1NP (ng/mL)	32.3 ± 18.2	32.7 ± 16.3	0.85
Follow-up CTX (ng/mL)	0.28 ± 0.20	0.28 ± 0.19	0.93
Follow-up P1NP (ng/mL)	42.5 ± 31.7	39.9 ± 23.6	0.43
% Change in CTX	113.2% ± 251.7%	105.4% ± 230.9%	0.78
% Change in P1NP	67.7% ± 162.1%	55.7% ± 114.7%	0.46

Values are mean ± SD. Total $n = 300$, including 150 in each treatment group.

improvement in the placebo group could have diminished our ability to detect a treatment effect. Nonetheless, results from randomized trials have largely conflicted with some animal studies as well as observational studies in humans showing an association between serum or plasma levels of homocysteine or B vitamins and bone health.

Our study has several strengths, given that it is a large, randomized placebo-controlled trial of B vitamin supplementation with centrally adjudicated nonspine fracture outcomes in middle-aged to older women. The availability of pretreatment and posttreatment markers of bone turnover is also a strength. Compared to prior studies, the treatment and follow-up period is relatively long at 7.3 years. Despite these major strengths, there are also some limitations. Although power for overall fracture effects was adequate, we had less power to examine effects of treatment on fracture subtypes, or effects of treatment in certain subgroups such as older women, or those with high baseline homocysteine levels. Furthermore, we had limited power to examine the effects of B vitamin supplementation among women who may meet criteria for deficiency (for example, only 1.9% of women, 2.0% in the treatment group, and 1.9% in the placebo group had vitamin B_{12} levels <200 pg/mL). Although our results are consistent with all other studies finding of no association of B vitamin intervention with fracture risk, we cannot dismiss the possibility that a different vitamin regimen or different dosages may yield different results. Some studies have suggested that relatively high doses of folic acid such as those used in our study may increase risk of cancer. However, previous analyses in WAFACS have found no significant effects of treatment assignment on risk of total invasive cancer or breast cancer.[39] Finally, our study was restricted to middle-aged to older women at high risk of CVD and may not be generalizable to younger women, men, or adults at high risk of fracture or usual risk for CVD. Because the primary outcome of the trial was CVD, we did not collect information on family history of fracture, nor is there information on sunlight exposure or calcium intake. Therefore, we were unable to determine if the treatment groups were balanced with respect to these fracture risk factors, and it is not possible to rule out randomization bias with respect to such factors. Previous intervention studies to date have been very heterogeneous with respect to study duration, study population, fortification status, and dosages.[40] The possibility of a benefit in terms of enhanced bone health for certain subgroups cannot be ruled out.

In a large randomized, placebo-controlled trial, we found no effects of long-term (7.3 year) B vitamin supplementation on risk of nonspine fracture or markers of bone resorption in women at high risk of CVD. Results were also null among women with elevated baseline plasma homocysteine levels. Overall, results from randomized trials thus far do not support a preventive benefit of supplemental B vitamins on fracture risk.

Disclosures

DK is consultant to Amgen, Inc. and Scientific Advisory Board Member for Kalytera, Inc.

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