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B Vitamins and Hip Fracture: Secondary Analyses and Extended Follow-Up of Two Large Randomized Controlled Trials

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ABSTRACT

Elevated plasma homocysteine levels are associated with increased risk of fractures in observational studies. However, it is unsettled whether homocysteine-lowering treatment affects fracture risk. The aim of this study was to investigate the effect of an intervention with B vitamins on the risk of hip fracture in a secondary analysis of combined data from two large randomized controlled trials originally designed to study cardiovascular diseases. Both trials had identical design, intervention, and primary objective. Based on a two-by-two factorial design, the intervention consisted of a daily capsule with either (1) folic acid (0.8 mg) plus vitamin B_{12} (0.4 mg) and vitamin B_6 (40 mg); (2) folic acid (0.8 mg) plus vitamin B_{12} (0.4 mg); (3) vitamin B_6 alone (40 mg); or (4) placebo. The participants were followed with respect to hip fracture during the trial or during an extended follow-up (from the trial start for each patient until the end of 2012). No statistically significant association was found between folic acid plus vitamin B_{12} treatment and the risk of hip fracture, neither during the trial (median 3.3 years; hazard ratio [HR] 0.87; 95% confidence interval [CI], 0.48 to 1.59) nor during the extended follow-up (median 11.1 years; HR 1.08; 95% CI, 0.84 to 1.40). Nor were there significant differences in the risk of hip fracture between groups receiving versus not receiving vitamin B_6 during the trial (HR 1.42; 95% CI, 0.78 to 2.61). However, during the extended follow-up, those receiving vitamin B_6 showed a significant 42% higher risk of hip fracture (HR 1.42; 95% CI, 1.09 to 1.83) compared to those not receiving vitamin B_6. In conclusion, treatment with folic acid plus vitamin B_{12} was not associated with the risk of hip fracture. Treatment with a high dose of vitamin B_6 was associated with a slightly increased risk of hip fracture during the extended follow-up (in-trial plus post-trial follow-up). © 2017 American Society for Bone and Mineral Research.

KEY WORDS: HIP FRACTURE; VITAMIN B_6; FOLIC ACID; VITAMIN B_{12}; RANDOMIZED CONTROLLED TRIAL

Introduction

The use of vitamin supplements is common. According to the National Health and Nutrition Examination Survey (NHANES) (2003–2006), one-half of the US population used at least one dietary supplement, 10% reported taking more than five dietary supplements, and approximately 30% took supplements containing vitamin B_{12} or B_6.(1) For many nutrients, both too low and too high intakes may have adverse health consequences. According to randomized controlled trials (RCTs), high-dose vitamin supplementation may lead to unexpected side effects. For example, increased risk of lung cancer has been reported in

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men supplemented with beta carotene,[2] supplementation with high doses of vitamin E may increase all-cause mortality,[3] and contrary to expectation, increased risk of fracture in women has been reported in two randomized controlled trials after treatment with annual mega doses of vitamin D.[4,5] Large homocysteine-lowering B-vitamin trials have failed to demonstrate prevention of cardiovascular diseases[6,7] and cancer,[8,9] and possible side effects have been reported.[10,11]

Hip fractures constitute a major health problem in the elderly, are strongly associated with increased mortality, and are a burden for patients, the healthcare system and society.[12] The expected rise in life expectancy in the global population will most probably lead to a substantial increase in the number of fractures and its consequences.[13] The study and characterization of modifiable risk factors is essential for osteoporosis and fracture prevention. Several observational studies have showed an association between high levels of circulating total homocysteine (tHcy) and increased risk of osteoporosis and hip fracture.[14,15] In vitro studies have revealed possible mechanisms on how homocysteine may affect bone health by interfering with collagen cross-linkage formation (reducing bone quality) and by stimulating osteoclast activity (leading to bone loss).[16,17]

Vitamin B_{12} and folate play important roles in the metabolism of homocysteine, being cofactors for the methionine synthase enzyme. An increase in the concentration of these vitamins leads to a reduction in plasma tHcy levels.[18] Observational studies have reported a small but significant inverse association between folate and vitamin B_{12} and fracture risk.[15,19,20] A possible direct effect of B vitamins on bone physiology has also been investigated. Vitamin B_6 is an essential cofactor for the enzyme lysyl oxidase, which is important for collagen cross-linking formation, and deficiency of vitamin B_6 may lead to impaired cross-linking formation.[21] In vitro studies on a possible independent effect of folate and vitamin B_{12} on bone structure are limited and their results are inconsistent.[22]

Based on these premises, one RCT has been performed to evaluate a possible preventive effect of homocysteine-lowering treatment with B vitamins on fracture incidence,[23] and three have assessed fractures as secondary outcomes.[24–26] However, results of these studies are inconclusive, and it is unlikely that new, large RCTs will be performed in order to establish the scientific basis for clinical advice on the use of B vitamin supplements to prevent fractures. Hence, we utilized data from two Norwegian RCTs that were performed to assess the effect of intervention with high-dose oral B vitamin treatment on cardiovascular disease,[27,28] to study whether there were any associations between the intervention with B vitamins and the risk of hip fracture during the in-trial period and extended follow-up.

Subjects and Methods

Participants and study intervention

We combined data from two randomized, placebo-controlled, double-blind clinical trials performed in Norway: the Norwegian Vitamin Trial (NORVIT) (lasting from December 1998 to March 2004) and the Western Norway B Vitamin Intervention Trial (WENBIT) (lasting from January 2000 to October 2005). Median in-trial follow-up was 3.4 years for NORVIT and 3.2 years for WENBIT. These trials had identical design, intervention, central laboratory, and primary objective to determine if an intervention with folic acid, vitamin B_{12}, and vitamin B_6 could reduce cardiovascular morbidity and mortality in patients with ischemic heart disease. The objectives, design, and methods have been reported in more detail elsewhere,[27,28] and results from combined analyses of both studies have previously been reported concerning risk of cancer, cardiovascular diseases, and total mortality.[29,30] No benefit was found of folic acid plus vitamin B_{12} or vitamin B_6 treatment on cardiovascular outcomes.

In short, the studies had a two-by-two factorial design and patients were randomly assigned to one of four different groups receiving one capsule per day containing: (1) folic acid (0.8 mg) plus vitamin B_{12} (cyanocobalamin, 0.4 mg) and vitamin B_6 (pyridoxine hydrochloride, 40 mg); (2) folic acid (0.8 mg) plus vitamin B_{12} (0.4 mg); (3) vitamin B_6 alone (40 mg); or (4) placebo. Blood samples were collected at baseline, at 1 to 2 months, and at the end of the intervention. They were analyzed for serum creatinine, plasma homocysteine, serum cobalamin, serum folate, and plasma pyridoxal 5′phospate (PLP) as described.[29]

Among baseline characteristics registered in the NORVIT and WENBIT trials, the following variables were considered of special relevance to the current study: age, gender, body mass index (BMI), smoking status, hypertension (defined as medically treated), diabetes mellitus (previously diagnosed based on glucose levels), and presence of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene (NCBI Entrez Gene 4524) 677C>T polymorphism (in which the TT genotype encodes for an enzyme with less activity, leading to increased tHcy).[31]

Hip fracture ascertainment

The combined dataset from the NORVIT and WENBIT trials was linked to new hip fractures in the NORHip database using the unique Norwegian 11-digit personal identification number in order to identify incident hip fractures among trial participants during follow-up. NORHip is a database including data on all patients with hip fractures treated at Norwegian hospitals from 1994 to 2013 extracted from the hospitals' patient administrative systems. As described,[32] a new hip fracture in NORHip was identified in patients with a diagnosis code for cervical, trochanteric, or subtrochanteric hip fracture (International Classification of Diseases, Ninth Revision [ICD-9]: 820 with all subgroups; ICD-10: S72.0–S72.2) and a surgical procedure code indicating hip fracture surgery (98% of the fractures). A comprehensive algorithm was used to identify new hip fractures in cases with ambiguous information (classified as possible hip fractures) by taking surgical procedure codes, diagnosis codes, and time between hospitalizations into account (around 2% of the fractures). The study outcome was defined as the first hip fracture sustained by a trial participant from inclusion throughout follow-up. If a person sustained more than one fracture during follow-up, only the first was included.

Follow-up and statistical analyses

Analyses were prepared and described on a blinded dataset (the variable giving information on which group each individual was randomized to was not included) and an analysis plan was agreed upon with a statistician not otherwise involved in our study (see Supporting Information: Analysis Strategy). The in-trial follow-up for each participant included the time from inclusion until the end of the intervention. To assess any long-term association, the extended follow-up (in-trial plus post-trial) lasted from the start of the trial until December 31, 2012. For both the in-trial and the extended follow-up, observation time was calculated from the date of inclusion until date of first hip fracture, date of death, or end of follow-up. Date of death was obtained by linkage to the National Population Register.

There were 43 hip fractures in the study population during the in-trial follow-up and 236 during the extended follow-up. Based on these numbers a post hoc calculation was performed: given that 50% of the participants were exposed to folic acid plus vitamin B_{12}, the study had an 80% statistical power to find a relative risk of hip fracture of 0.39 at the 5% significance level during the in-trial follow-up, and a relative risk of 0.69 in the extended follow-up.

Changes in serum or plasma markers from baseline until the end of intervention were tested with the paired sample *t* test (only patients with serum or plasma measurements at both occasions were included in these analyses).

Cumulative hazard curves were constructed by Cox proportional hazard regression, and differences between the groups were tested by the score test. Unadjusted hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated by Cox proportional hazards regression stratified by trial. Adjusted HRs were also calculated, adjusting for the following baseline characteristics: age (as a continuous variable), gender, BMI (as a continuous variable), smoking status, hypertension, diabetes mellitus, and the MTHFR 677C>T polymorphism. We entered interaction terms to test if these variables interacted with the treatment modalities on fracture risk. Proportional hazard assumption was tested using Schoenfeld residuals, and no violation of this assumption was detected.

Subgroup analyses

The following six variables were dichotomized and displayed in forest-plots: gender; age, which was dichotomized based on the median age of participants (62.5 years); trial (NORVIT or WENBIT); smoking status (never smokers or ex-smokers >1 month versus current smokers); MTHFR 677C>T polymorphism (TT versus CC or CT); and plasma tHcy levels (based on a previous publication from the NORVIT study,[27] high baseline tHcy was defined as >13 μmol/L). STATA statistical software, version 14 (StataCorp, College Station, TX, USA) was used in the analysis.

Ethics

No further patient contribution was required for the hip fracture follow-up. The study was approved by the Regional Committee for Medical and Health Research Ethics (2014/602/REK vest). ClinicalTrials.gov identifier: NCT00671346.

Results

Patients

A total of 6837 participants were included in the analysis (see Supporting Fig. 1), 3749 (54.8%) from NORVIT and 3090 (45.2%) from WENBIT. There were no substantial differences between the intervention groups in baseline characteristics (Table 1). Mean age of participants was 62.3 ± 11.0 years, mean BMI was 26.6 ± 3.8 kg/m^2, and 23.5% were women. Thirty-nine percent of the participants were current smokers, 10.6% had diabetes mellitus, and 8.2% presented the MTHFR 677C>T polymorphism. Only 22 (1.4%) women and 7 (0.1%) men reported intake of osteoporosis medication at baseline. Median in-trial follow-up was 3.3 years (interquartile range [IQR], 2.6 to 3.5 years), and 11.1 years (IQR, 9.1 to 12.2 years) for the extended follow-up.

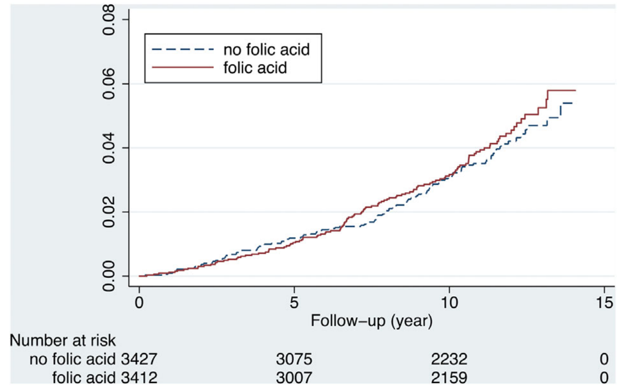

Fig. 1. Cumulative hazard curves for hip fracture. Comparisons were performed between folic acid versus no folic acid treatment groups.

Vitamin and homocysteine levels during study treatment

As previous published,[29] mean concentration of plasma tHcy at baseline was 12.2 ± 5.1 μmol/L. The reference range of homocysteine increases with age; for individuals older than 59 years it should be between 5.8 and 11.9 μmol/L. Hyperhomocysteinemia is considered with values of tHcy >15 μmol/L. Deficit in vitamin B_6 (PLP <20 nmol/L)[33] was found in 16.4% of the participants. Changes in circulating levels of the B vitamins from baseline to the end of the intervention are shown in Table 2. Although participants receiving folic acid plus vitamin B_{12} had a substantial decrease in plasma tHcy and a substantial increase in serum folate and serum cobalamin, only minor changes in these parameters were found in those not receiving folic acid plus vitamin B_{12}. Plasma PLP levels increased dramatically in both groups receiving intervention with vitamin B_6, whereas minor changes were seen for those not receiving vitamin B_6.

Fractures

A total of 43 patients (28 women) suffered a hip fracture during the in-trial follow-up, and 236 (118 women) during the extended follow-up. For baseline characteristics of those suffering a hip fracture, see Table 3.

As shown in Table 4 and Fig. 1, there were no significant differences in the risk of hip fracture between the intervention groups receiving folic acid plus vitamin B_{12} versus the groups not receiving it, with a HR of 0.87 (95% CI, 0.48 to 1.59) during the in-trial follow-up, and a HR of 1.08 (95% CI, 0.84 to 1.40) during the extended follow-up. No material differences were found in the results after adjusting for baseline characteristics (data not shown).

On the other hand, there was a statistically significant 42% (95% CI, 9% to 83%) increased risk of hip fracture in the groups receiving vitamin B_6 compared with groups not receiving vitamin B_6 during the extended follow-up. The corresponding *p* value for the score test was 0.008 (Fig. 2). For the in-trial follow-up, there was a nonsignificant association with a HR of 1.42 (95% CI, 0.78 to 2.61). Adjusting for baseline characteristics did not change the results substantially (data not shown).

Considering the four treatment groups separately, the incidence rate of hip fracture throughout the extended follow-up was highest in the group receiving both folic acid + vitamin B_{12} and vitamin B_6, followed by the group

Table 1. Baseline Characteristics of the NORVIT/WENBIT Population by Intervention Group

Characteristics	Folic acid + vitamin B_{12} and vitamin B_6 ($n = 1708$)	Folic acid + vitamin B_{12} ($n = 1703$)	Vitamin B_6 ($n = 1705$)	Placebo ($n = 1721$)
NORVIT study, *n* (%)	937 (54.9)	935 (54.9)	934 (54.8)	943 (54.8)
WENBIT study, *n* (%)	771 (45.1)	768 (45.1)	771 (45.2)	778 (45.2)
Age (years), mean ± SD	62.7 ± 11.2	62.3 ± 10.9	62.0 ± 10.9	62.3 ± 10.7
Gender male, *n* (%)	1310 (76.7)	1313 (77.1)	1304 (76.5)	1300 (75.5)
BMI, mean ± SD[a]	26.6 ± 3.9	26.5 ± 3.8	26.5 ± 3.7	26.7 ± 3.8
Smoking status, *n* (%)				
Never smoker (yes)	488 (28.6)	514 (30.2)	449 (26.4)	487 (28.4)
Ex-smoker <1 month (yes)[b]	553 (32.4)	565 (33.2)	538 (31.6)	552 (32.2)
Current smoker (yes)	665 (39.0)	621 (36.5)	715 (42.0)	676 (39.4)
Hypertension, yes (%)	627 (36.9)	605 (35.9)	615 (36.3)	643 (37.4)
Diabetes mellitus, yes (%)	187 (11.0)	175 (10.3)	163 (9.6)	199 (11.6)
Vitamin supplements, yes (%)[c]	401 (23.5)	398 (23.4)	390 (22.9)	392 (22.8)
MTHFR 677 genotype (yes), *n*/total (%)				
CC	806 (49.5)	862 (53.0)	810 (49.5)	816 (49.7)
CT	677 (41.6)	636 (39.1)	699 (42.7)	692 (42.1)
TT	144(8.9)	129 (7.9)	127 (7.8)	135 (8.2)
Serum or plasma biochemical values, mean ± SD				
Creatinine (μmol/L)	91.6 ± 23.0	90.8 ± 21.5	90.5 ± 21.4	91.6 ± 21.8
Plasma total homocysteine (μmol/L)	12.17 ±	12.06 ± 4.69	12.30 ± 5.60	12.29 ± 5.06
Serum cobalamin (vitamin B12) (pmol/L)	384.31 ± 305.86	387.20 ± 273.00	388.22 ± 467.32	378.67 ± 220.30
Serum folate (nmol/L)	12.63 ± 21.04	12.18 ± 23.54	10.85 ± 7.87	10.85 ± 7.23
Plasma pyridoxal 5′phosphate (vitamin B6) (nmol/L)	43.2 ± 44.0	38.5 ± 29.3	43.5 ± 44.3	39.3 ± 35.5

Information available for 6827 participants with BMI, 6823 with smoking status, 6796 with hypertension status, 6808 with Diabetes mellitus status, 6533 with MTHFR 677 genotype (out of a total of 6837 participants).

NORVIT = Norwegian Vitamin Trial; WENBIT = Western Norway B Vitamin Intervention Trial; BMI = body mass index; *MTHFR* = 5,10-methylenetetrahydrofolate reductase.

[a]BMI was calculated as weight in kilograms divided by height in meters squared.

[b]Defined as: participant quit smoking more than 1 month before trial entry.

[c]Daily or often use of vitamin supplements.

receiving vitamin B_6 alone (Table 4). Additional analyses showed that the group receiving treatment with folic acid plus vitamin B_{12} and vitamin B_6 had a statistically significant higher fracture risk compared to the placebo group (HR 1.49; 95% CI, 1.05 to 2.11) (results not included in Table 4).

There were no statistically significant differences between each of the other two groups compared with placebo for the risk of hip fracture, with an HR of 0.89 (95% CI, 0.60 to 1.32) for the group receiving folic acid plus vitamin B_{12} and a HR of 1.19 (95% CI, 0.83 to 1.72) for the group receiving vitamin B_6. However, the interaction term between the two treatment regimes and risk of hip fracture was not statistically significant ($p = 0.20$).

Stratified analyses

Overall, the interaction terms between the treatment modalities and relevant baseline characteristics were not statistically significant, indicating no effect modification (Figs. 3 and 4). The only exception was a statistically significant interaction between gender and the folic acid plus vitamin B_{12} treatment (Fig. 3; p value for interaction $= 0.027$). Women, but not men, receiving folic acid plus B_{12} had a statistically significant increased risk of fracture. To explore this further, we analyzed the four intervention groups separately. Compared to placebo (reference group), women receiving both B vitamin treatments (folic acid plus vitamin B_{12} and vitamin B_6) had an HR of 1.87 (95% CI, 1.14 to 3.07), whereas women receiving folic acid plus vitamin B_{12} had an HR of 1.13 (95% CI, 0.65 to 1.95) and women receiving vitamin B_6 alone had an HR of 1.03 (95% CI, 0.59 to 1.80) of sustaining a hip fracture. A similar analysis in men showed no significant associations (data not shown).

Sensitivity analyses

Six of the 236 participants who suffered a hip fracture during follow-up had a previous hip fracture before the trials started. Excluding these participants from the analyses did not change the results substantially (data not shown). In addition, five of the included hip fractures were classified as possible hip fractures in NORHip database. Sensitivity analyses excluding these fractures were performed, obtaining similar results (data not shown).

Discussion

This study showed that treatment with folic acid plus vitamin B_{12} in a population with ischemic heart disease during a mean of 3.0 years was not associated with the risk of hip fracture neither

Table 2. Circulating Homocysteine and B Vitamins During the Trials

	Baseline	End of study	Change	p[a]
Plasma total homocysteine (μmol/L)				
Folic acid + vitamin B_{12} + vitamin B_6 ($n = 1385$)	11.82 ± 4.27	8.84 ± 3.22	−2.97 ± 3.76	<0.001
Folic acid + vitamin B_{12} ($n = 1417$)	11.95 ± 4.72	9.13 ± 3.71	−2.82 ± 4.57	<0.001
Vitamin B_6 ($n = 1373$)	12.05 ± 5.19	12.25 ± 5.04	0.20 ± 4.65	0.127
Placebo ($n = 1400$)	11.97 ± 4.60	12.42 ± 5.26	0.46 ± 4.61	<0.001
Serum cobalamin (vitamin B_{12}) (pmol/L)				
Folic acid + vitamin B_{12} + vitamin B_6 ($n = 1376$)	380.54 ± 167.48	610.66 ± 324.54	230.12 ± 299.13	<0.001
Folic acid + vitamin B_{12} ($n = 1405$)	386.02 ± 268.32	626.76 ± 488.01	240.75 ± 430.86	<0.001
Vitamin B_6 ($n = 1364$)	388.69 ± 509.68	381.56 ± 274.39	−7.13 ± 502.70	0.600
Placebo ($n = 1385$)	376.14 ± 216.21	379.21 ± 223.51	3.07 ± 178.43	0.522
Serum folate (nmol/L)				
Folic acid + vitamin B_{12} + vitamin B_6 ($n = 1378$)	12.13 ± 17.88	59.35 ± 31.84	47.22 ± 34.81	<0.001
Folic acid + vitamin B_{12} ($n = 1411$)	12.34 ± 25.29	66.21 ± 36.89	53.87 ± 44.11	<0.001
Vitamin B_6 ($n = 1362$)	10.94 ± 7.77	11.40 ± 11.10	0.45 ± 11.99	0.162
Placebo ($n = 1388$)	10.84 ± 7.10	13.81 ± 14.08	2.97 ± 13.86	<0.001
Plasma pyridoxal 5′phosphate (vitamin B_6) (nmol/L)				
Folic acid + vitamin B_{12} + vitamin B_6 ($n = 1366$)	42.54 ± 39.37	292.35 ± 175.54	249.81 ± 176.17	<0.001
Folic acid + vitamin B_{12} ($n = 1397$)	38.66 ± 28.47	47.01 ± 42.36	8.34 ± 45.78	<0.001
Vitamin B_6 ($n = 1366$)	44.13 ± 44.05	289.90 ± 176.67	245.77 ± 177.40	<0.001
Placebo ($n = 1378$)	40.07 ± 36.88	48.11 ± 41.63	8.04 ± 49.23	<0.001

Patients with data both at the beginning and at the end of the intervention. Values are as mean ± SD.
[a]Paired t test for difference between values at the start and at the end of the intervention of the four intervention groups.

during in trial nor during an extended follow-up period (mean, 10.0 years). Treatment with vitamin B_6 was associated with an increased risk of hip fracture during the extended follow-up. However no statistical significant association was found for this treatment during the in-trial follow-up.

Strengths and limitations

A main strength of our study was the use of two large and well-designed RCTs with nearly 7000 participants. We were able to follow the participants with respect to hip fracture by linkage to the national NORHip database covering the complete

Table 3. Baseline Characteristics of the NORVIT/WENBIT Population Based on Sustaining Versus Not Sustaining a Hip Fracture During Extended Follow-Up

Characteristics	Hip fracture ($n = 236$)	No hip fracture ($n = 6603$)	p[a]
Study			<0.01
NORVIT, n (%)	152 (64.4)	3597 (54.4)	
WENBIT, n (%)	84 (35.6)	3006 (45.5)	
Age (years), mean ± SD	71.4 ± 8.4	62.0 ± 10.9	<0.01
Gender male, n (%)	118 (50)	5111 (77.4)	<0.01
BMI, mean ± SD[b]	24.7 ± 3.6	26.2 ± 3.8	<0.01
Smoking status, n (%)			0.05
Never smoker (yes)	93 (39.4)	1845 (28.0)	
Ex-smoker >1 month (yes)[c]	65 (27.5)	2144 (32.6)	
Current smoker (yes)	78 (33.1)	2592 (39.4)	
Hypertension (yes), n (%)	107 (45.7)	2385 (36.3)	<0.01
Diabetes mellitus (yes), n (%)	40 (17.2)	684 (10.4)	<0.01
MTHFR 677C>T polymorphism (yes), n/total (%)	16/224 (7.1)	519/6311 (8.2)	0.5
Serum or plasma biochemical values, (n) mean ± SD			
Creatinine (μmol/L)	(236) 93.2 ± 29.1	(6603) 91.0 ± 21.6	0.13
Homocysteine (μmol/L)	(235) 13.6 ± 5.2	(6581) 12.2 ± 5.0	<0.01
Cobalamin (pmol/L)	(234) 431.5 ± 517.6	(6517) 382.9 ± 320.8	0.02
Folate (nmol/L)	(233) 11.3 ± 8.3	(6542) 11.6 ± 16.9	0.70
Pyridoxal 5′phosphate (nmol/L)	(233) 35.1 ± 30.0	(6492) 41.4 ± 39.1	0.02

Percentages may not total 100 because of rounding.
NORVIT = Norwegian Vitamin Trial; WENBIT = Western Norway B Vitamin Intervention Trial; BMI = body mass index; *MTHFR* = 5,10-methylenetetrahydrofolate reductase.
[a]For interaction between variables (differences were tested by using a chi square test for categorical variables, and t test for continuous variables).
[b]BMI was calculated as weight in kilograms divided by height in meters squared.
[c]Defined as: participant quit smoking more than 1 month before trial entry.

Table 4. Hip Fracture Rates According to Randomization Groups and HRs of Hip Fracture With 95% CIs According to Folic Acid Versus No Folic Acid Treatment Groups and Vitamin B_6 Versus No Vitamin B_6 Treatment Groups

		Fractures *n* (rates per 1000 observation-years)				HR (95% CI), p^a	
	Total fractures (n)	Folic acid + vitamin B_{12} and vitamin B_6	Folic acid + vitamin B_{12}	Vitamin B_6	Placebo	Folic acid + vitamin B_{12} versus non–folic acid + vitamin B_{12} groups	Vitamin B_6 versus non–vitamin B_6 groups
In-trial	43	11 (2.1)	9 (1.7)	14 (2.7)	9 (1.7)	0.87 (0.48–1.59), 0.66	1.42(0.78–2.61), 0.25
Extended follow-up	236	75 (4.5)	46 (2.7)	62 (3.6)	53 (3.0)	1.08 (0.84–1.40), 0.54	1.42 (1.09–1.83), <0.01

All models are stratified by trial (NORVIT or WENBIT).
HR = hazard ratio; CI = confidence interval; NORVIT = Norwegian Vitamin Trial; WENBIT = Western Norway B Vitamin Intervention Trial.
[a]Bold values are significantc.

Norwegian population. These RCTs were performed in Norway, where there is no mandatory folic acid fortification of foods.[34] The decrease in tHcy plasma levels due to the folic acid plus vitamin B_{12} intervention in NORVIT/WENBIT trials was thus more pronounced than in trials performed in countries where such fortification is recommended or mandatory. As reported,[29] participants showed high adherence to the treatment, assessed by interview, capsule count, and corroborated by substantial changes in circulating concentrations of B vitamins and tHcy during in-trial follow-up. We do not know whether patients were taking vitamin supplements during the post-trial follow-up period. However, it is unlikely that they consumed the high doses used in the trials, because patients were discouraged from such supplement use when trial results from NORVIT and WENBIT were available. Further, we assume that post-trial B vitamin doses were moderate, since the doses of B vitamins in over-the-counter supplements in Norway are far lower than the doses given in the two trials. Finally, we believe that there were similar proportions of B vitamin supplement users across initial treatment groups.

It is a limitation that neither NORVIT nor WENBIT were originally designed or powered to study hip fractures. Hence, the registered baseline data did not include bone related variables such as bone mineral density, bone markers, or data on falls, which could have helped assessing possible mechanisms for the increased fracture risk. Nevertheless, a similar distribution of these parameters among the treatment groups is expended because of the randomized allocation of participants to the intervention groups. Information about hip fractures was registry-based, and the individual fracture was not verified by medical record review. However, a previous validation of the NORHip database showed good agreement with medical chart–verified and X-ray–verified hip fractures (Cohen's kappa = 0.95).[35] In other words, the possibility of a misclassification of a hip fracture diagnosis is low, and because of the design of the study they would be randomly distributed. We had no information of emigration in the current dataset. Nevertheless, based on a previous publication[29] it was less than 0.8% during a median of 6.5 years, so it is quite unlikely that it would have an impact in our results.

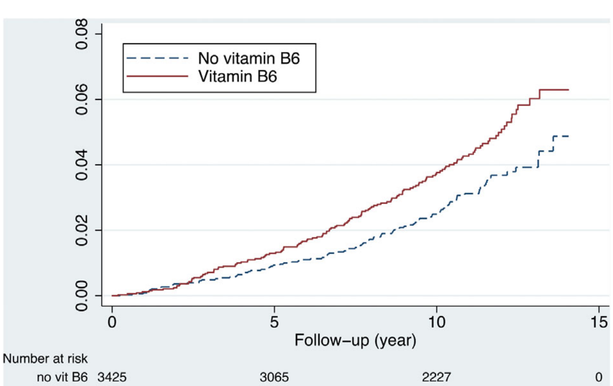

Fig. 2. Cumulative hazard curves for hip fracture. Comparisons were performed between vitamin B_6 and no vitamin B_6 treatment groups.

It is a limitation that the trials were performed among patients with ischemic heart disease. Thus, the generalizability of our findings must be interpreted with caution. On the other hand, ischemic heart disease and its treatments are common in the elderly. In addition, the study population was rather young with respect to the hip fracture outcome, and only 23% of the participants were women. It would be worth confirming our results in an older population with a higher female ratio and also to study other types of fracture.

The increased risk of hip fracture in those receiving vitamin B_6 was an unexpected finding, and we cannot exclude a spurious association. It is also puzzling that the association was evident years after the intervention had stopped. On the other hand, our analyses were based on two well-designed RCTs where recall bias and confounding were controlled for. We also performed additional analyses adjusting for possible confounders, outcome data was obtained from a validated database (NORHip), and the research questions (see Supporting Information: Analyses Strategy) were described before the analyses started.

Comparison with other studies

Regarding treatment with folic acid and vitamin B_{12}, the results of our study are in accordance with previous large RCTs that have failed to identify any significant association between folic acid plus vitamin B_{12} treatment and the incidence of osteoporotic fractures.[24–26] These RCTs, as is the case for the current study, were not designed to study fractures as the main outcome except for the Dutch B-PROOF (B Vitamin for the Prevention of Osteoporotic Fractures)[23] study. The B-PROOF study showed no effect of homocysteine lowering treatment on

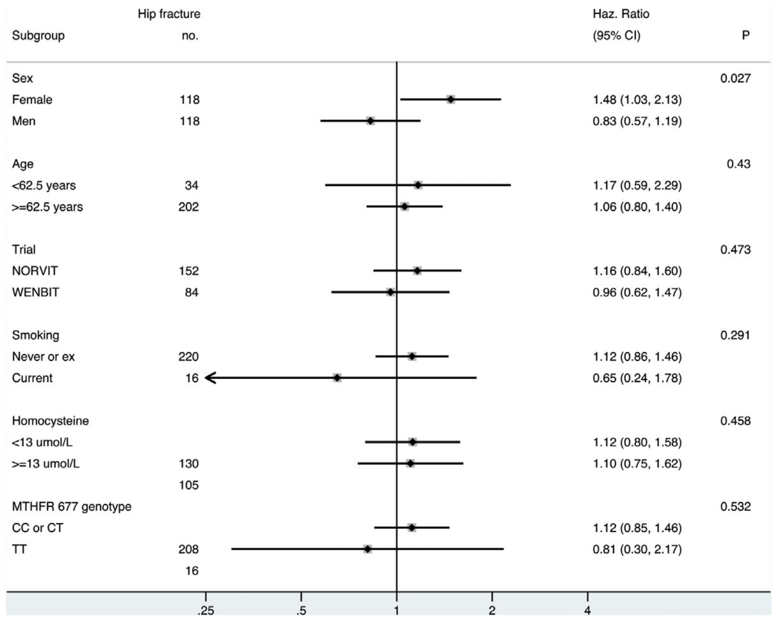

Fig. 3. Hazard ratios for sustaining a hip fracture in subgroups, and p values for interaction (*P*). The comparisons were performed for the extended follow-up (from randomization to December 31, 2013) between folic acid and no folic acid treatment groups.

fracture incidence except for a prespecified subgroup analysis among those older than 80 years, in which the intervention group had lower risk of osteoporotic fractures.

Although all of these RCTs presented differences in design and study population, they reached similar overall conclusions of no association between the intervention with folic acid and vitamin B_{12} and fractures. It should be added that a publication reporting that an intervention with folic acid plus vitamin B_{12} reduced the risk of hip fracture in patients with stroke has recently been retracted by the editors of *JAMA*.[36]

To the best of our knowledge, no RCT has been performed to assess the effect of vitamin B_6 alone on hip fracture risk. The HOPE-2[26] and VITATOPS[25] trials included vitamin B_6 as part of their intervention. However, because their design was based on

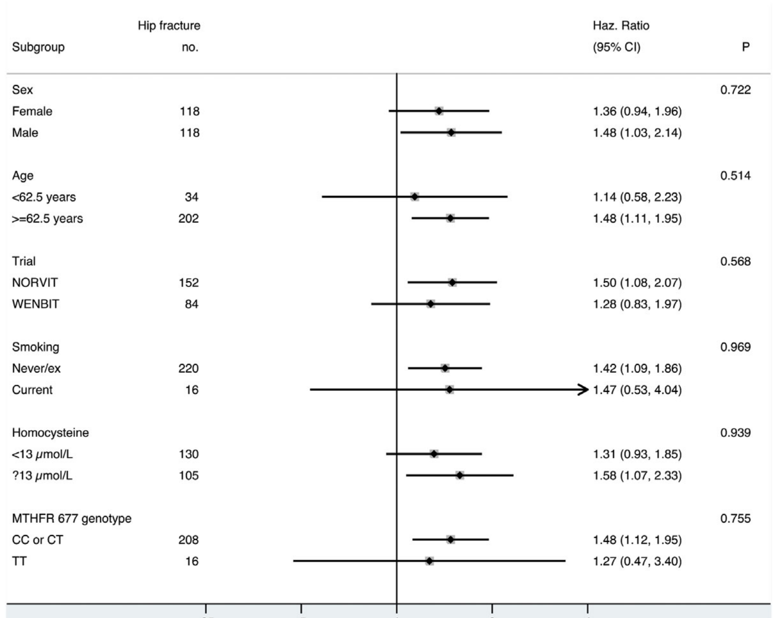

Fig. 4. Hazard ratios for sustaining a hip fracture in subgroups, and p values for interaction (*P*). The comparisons were performed for the extended follow-up (from randomization to December 31, 2013) and between vitamin B_6 and no vitamin B_6 treatment groups.

a combined intervention, assessing the association between vitamin B_6 alone and fracture incidence was not possible. In addition, the number of hip fractures in VITATOPS was limited to 70, and the number of hip fractures in HOPE-2 has not been reported. Observational studies on vitamin B_6 and fracture risk are limited. Although results so far may relate low vitamin B_6 intake to higher risk of hip fracture,[37,38] the possible role of vitamin B_6 on osteoporotic fractures is unclear.

Possible explanations

We can only speculate on possible mechanistic explanations for the increased fracture risk association with vitamin B_6 treatment. Of potential relevance is the fact that the vitamin B_6 dose of 40 mg/day used in our trials was substantially higher than the Recommended Dietary Allowances (1.3 to 1.7 mg, depending on adult age) and could be considered as a pharmacologic dose much higher than the average dietary intake (1.5 mg/day in women and 2 mg/day in men).[39,40] Tolerable upper daily intake has been set to 25 mg by the European Food Safety Authority (year 2000)[41] and to 100 mg by the US Food and Nutrition Board.[40] Ataxia, neuropathy, and decreased muscle tone have been described as side effects of high daily doses of vitamin B_6 (500 mg or more),[42] although minor neurological symptoms have been described with a dose of 50 mg daily.[43] The neurological damage may cause numbness, instability, and troubled walking, leading to higher possibilities of falling. The neurological effect appears to be dose- and time-dependent, but there is not adequate evidence concerning a total improvement of functions after discontinuing treatment.[44]

As a second possibility, vitamin B_6 has been suggested as a modulator of steroid receptors, reducing receptors response in the presence of high vitamin B_6 concentrations.[45,46] However, molecular basis of this effect are still unclear. However, in case high doses have such an effect, it is unlikely that participants would regain the bone mass they had lost after the treatment has ended, potentially leading to a long-lasting effect.

Common etiological factors have been suggested for cardiovascular diseases and osteoporosis. However, B vitamin treatment (either with the folic acid plus vitamin B_{12} intervention or vitamin B_6 intervention) had no effect on cardiovascular events in the original analysis of NORVIT and WENBIT, in contrast to the long-term association between vitamin B_6 treatment and hip fracture in the current analysis.

It should be noted that in the additional analysis of the four intervention groups separately, the group receiving both treatments (folic acid plus vitamin B_{12} and vitamin B_6) had the highest risk of hip fracture. These results are somewhat in line with the results from NORVIT, where the participants who received B vitamin combination therapy had an increased risk of cardiovascular events.[27] As for vitamin B_6, the dose of vitamin B_{12} was also much higher than the Recommended Dietary Allowances (400 μg versus 2.4 μg). However, we can not conclude that the association with hip fracture was different for the intervention with folic acid plus vitamin B_{12} and vitamin B_6 compared to the intervention with vitamin B_6 alone, because the interaction term between the two treatments and hip fractures was not statistically significant. On the other hand, the statistical power to detect such a difference was low.

Possible implications

Dietary supplements of vitamins and minerals are extensively available in a wide range of doses and combinations. In segments of the population, high-dose vitamin supplementation far exceeding the recommended doses is popular, with promise of health benefits and very little focus on potential side effects. Our findings have potential public health implications, mainly related to the need to respect the daily dosage recommendations when there is no deficiency of these vitamins. Use of supplements with high doses of vitamins could result in unexpected deleterious side effects for the individual.

Conclusion

These secondary analyses and extended follow-up of two large RCTs performed in Norway showed that treatment with folic acid plus vitamin B_{12} was not associated with the risk of hip fracture. However, treatment with high doses of vitamin B_6 was associated with a slightly increased risk of experiencing a hip fracture during the extended follow-up (3.3 years in-trial plus 7.8 years post-trial follow-up).

Disclosures

All authors state that they have no conflicts of interest.

Acknowledgments

Authors' roles: KHB, ME, ON, and PMU participated in the planning and completion of the NORVIT and WENBIT trials. GST, CGG, and HEM participated in the planning of the NORHip database (NOREPOS collaboration). HEM, ME, ON, KHB, GST, and CGG conceived and design the current analyses, whereas HEM and EFE obtained funding. MGL and HEM wrote the analysis plan and performed the statistical analyses. MGL and HEM wrote the first draft of the manuscript and subsequent revisions, and interpreted it in collaboration with CGG, EFE, GST, KHB, ME, ON, and PMU. MGL and HM had full access to the final data and had final responsibility for the content of the report and decision to submit for publication. All authors critically revised the paper for important intellectual content and approved the final version. MGL and HEM are the guarantors.

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