Vitamin supplementation on the risk of venous thrombosis: results from the MEGA case-control study[1–4]

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ABSTRACT

Background: Whether vitamin supplements decrease venous thrombosis risk is controversial. Previous reports did not all take confounding fully into account, either by randomization or by extensive adjustment.

Objective: The aim of our study was to determine whether vitamin supplementation decreases the risk of venous thrombosis.

Design: A large case-control study included 2506 patients with venous thrombosis, 2506 partner controls, and 2684 random-digit dialing (RDD) controls. When patients were compared with RDD controls, unconditional logistic regression was used to calculate ORs with 95% CIs. When patients were compared with partner controls, conditional logistic regression was used, providing further adjustment for unmeasured confounding.

Results: Vitamin use yielded a 37% lower risk of venous thrombosis than no vitamin use (OR: 0.63; 95% CI: 0.57, 0.70) when comparing patients with RDD controls. Adjustment for several putative confounders did not change the estimate (OR: 0.68; 95% CI: 0.61, 0.77). The fully adjusted ORs for vitamin A, vitamin B-6, vitamin B-12, folic acid, vitamin C, vitamin D, vitamin E, and multivitamin use were in the same range. However, when patients were compared with partner controls, ORs attenuated to unity. Results were similar for provoked and unprovoked events, as well as for deep vein thrombosis and pulmonary embolism.

Conclusions: After extensive adjustments, vitamin supplementation was no longer associated with a decreased risk of venous thrombosis in this study. Previous positive results may have been spurious as a result of uncontrolled confounding. *Am J Clin Nutr* 2015;101:606–12.

Keywords cardiovascular diseases, epidemiology, public health, risk, venous thrombosis

INTRODUCTION

Venous thrombosis is one of the leading causes of morbidity and mortality worldwide, occurring each year in about one in 1000 people in industrialized countries (1). The condition can be prevented and treated with anticoagulants, but as a side effect, bleeding often occurs (2). Therefore, strategies for the prevention of venous thrombosis that are not based on oral anticoagulant treatment are needed. Both basic research and observational epidemiologic studies have supported the hypothesis that vitamins may inhibit venous thrombosis. For example, based on early findings that elevated homocysteine concentrations are associated with thrombotic disease (3, 4), as well as the knowledge that homocysteine concentrations depend on a series of intracellular metabolic reactions in which folate acts as a substrate and vitamin B-12 as a coenzyme, it was believed that adequate supplementation of B vitamins could lower homocysteine and thus decrease the risk of thrombotic events (5). However, initial therapeutic trials with vitamin B supplements that induce a decrease in homocysteine concentration have not resulted in an improvement of the thrombotic risk (6–8), probably because of the existence of a more complicated metabolic network than what was assumed at first or the absence of a causal relation between hyperhomocysteinemia and thrombotic risk (9). As a consequence, multivitamin supplementation became of interest irrespective of the homocysteine concentration, and several studies dealing with the possible connection of different vitamins and thrombotic risk have been designed. Most of these studies investigated the risk of arterial thrombosis and yielded inconsistent results (10–12). For venous thrombosis, studies are scarce. Some showed that vitamin D or E supplementation decreases the risk of venous thrombosis (13, 14); others showed no effect (15). As far as we know, no other observational studies or trials have analyzed whether other vitamins such as vitamin A and vitamin C are associated with a decreased risk of venous thrombosis.

One issue to keep in mind when studying the effect of vitamin use on venous thrombosis is that studies on vitamin therapy are generally not randomized and lack proper adjustment for the many lifestyle-related factors that could confound the relation.

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[3] Supplemental Tables 1–3 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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In this study, we used data from the Multiple Environmental and Genetic Assessment (MEGA) case-control study to analyze whether use of vitamin A, vitamin B-6, vitamin B-12, vitamin C, vitamin D, vitamin E, folic acid, or multivitamins decreased the risk of venous thrombosis. This study provided an excellent opportunity to study this because both measured and unmeasured confounding factors could be taken into account by comparing patients with population-derived random-digit dialing (RDD) controls and with patients' partners, which formed the 2 control groups of the study.

METHODS

Study design

The design of the MEGA case-control study is described elsewhere (16). In short, 4956 consecutive patients aged 18–70 y, with a first diagnosis of deep vein thrombosis or pulmonary embolism, were included from 6 anticoagulation clinics in The Netherlands (Amersfoort, Amsterdam, The Hague, Leiden, Rotterdam, and Utrecht) between March 1999 and September 2004. Diagnostic information was obtained from hospital discharge reports and general practitioners. The diagnosis of deep vein thrombosis was confirmed with Doppler ultrasonography, whereas the diagnosis of pulmonary embolism was confirmed with a ventilation perfusion lung scan, spiral computed tomography, or angiogram. Patients' partners were invited to participate as controls if they were aged 18–70 y and had no history of venous thrombosis. In total, 3297 partners participated, forming a first control group. Also, from January 2002 through September 2004, a total of 3000 additional controls, who were recruited by using an RDD method, formed a second control group. These participants were also aged 18–70 y with no previous history of venous thrombosis.

All participants gave written informed consent. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center, Leiden, The Netherlands.

Data collection and definitions

The index date for patients and partner controls was defined as the date of diagnosis of the thrombotic event. For RDD controls, the index date was the date of informed consent signing. Participants completed a standardized questionnaire, including items on demographic and lifestyle factors, as well as on potential risk factors for venous thrombosis (17). Also, self-reported information was obtained on weight, height, and smoking habits, according to which participants were classified as current smokers, previous smokers, or nonsmokers (18). BMI was calculated according to the following formula: weight (kg)/height squared (m^2), and participants were classified into 3 categories (in kg/m^2): normal weight (<25), overweight (25–30), and obese (>30). A structured questionnaire was taken from all participants regarding, among others, medication use. Participants were classified as vitamin users if they reported regular use of one or more of the following: folic acid, multivitamins, or vitamins A, B-6, B-12, C, D, or E in the 12 mo before the onset of venous thrombosis (for patients) or before enrollment in the MEGA study (controls). No information was obtained on the dosage of vitamin intake.

Provoked venous thrombosis was defined as venous thrombosis preceded by surgery, plaster cast immobilization, bed rest, leg injury, or hospitalization in the 3 mo before the index date or long-distance travel in the 2 mo before the index date.

Inclusion/exclusion criteria

Of the 4956 patients, we excluded 182 women who were pregnant at the index date or within the previous 3 mo. These women were excluded as guidelines recommend that women should take folic acid during pregnancy, and pregnancy itself affects risk (19). Next we excluded 517 patients from this study for whom information on vitamin consumption was missing, which left 4257 patients. Of these patients, 2506 had a partner who fulfilled the inclusion criteria and was willing to participate, so 2506 complete couples remained. After application of the same exclusion criteria on the RDD control group, 2684 RDD control participants could be included in our analysis.

Statistical analysis

Participants were analyzed as current vitamin users or nonusers but also as users and nonusers of different types of vitamins (folic acid, multivitamins, and vitamins A, B-6, B-12, C, D, and E). Because the control population consisted of either RDD controls or partners of patients, we could perform 2 analyses. In the first analysis, we compared patients with RDD controls and adjusted for all measured confounding factors. In the second analysis, we used the partners of the patients as control individuals.

When patients were compared with RDD controls, unconditional logistic regression was used to calculate ORs with 95% CIs as a measure of the relative risks for venous thrombosis in vitamin users compared with nonusers. This analysis is unconditional, because controls were not individually matched to the patients, apart for frequency matching for age and sex, for which we adjusted. In the unconditional logistic regression analysis, all patients (n = 4257) were compared with all RDD controls (n = 2684). In a separate analysis, we also compared patients who had a partner (n = 2506) with RDD controls to see whether this would affect our risk estimates. Analyses were adjusted for age, sex, BMI, smoking habits, and prevalent arterial cardiovascular diseases, which included prior myocardial infarction or ischemic stroke. To avoid that effects of vitamin use on the risk of venous thrombosis were attributable to residual lifestyle-related confounders that are associated with vitamin use (20–22), we also included statin use, hormonal drug use (defined as oral contraception or postmenopausal hormone therapy), and physical activity as potential sources of confounding. Hormonal drugs were added to the model as a dichotomous variable in which all men were classified as unexposed (23, 24). Hyperhomocysteinemia was not added as a confounding variable in the models because vitamin supplementation in hyperhomocysteinemia is not common in The Netherlands. Patients with provoked and unprovoked venous thrombosis, as well as patients with deep vein thrombosis and pulmonary embolism, were combined in most analyses but also analyzed separately.

Vitamin use may be related to a general health-conscious behavior, which may affect the risk of thrombosis and therefore

act as a confounder. Such behavior is not easily adjusted for and measured. Partners of patients are likely to resemble the patients in health consciousness more than RDD controls, and therefore we performed a 1:1 matched analysis by conditional logistic regression, which adjusts for associations within matched pairs. This method provides adjustment for all unmeasured factors for which couples tend to be similar (25). The analysis is conditional because many clinical characteristics of controls, who are individually matched to the patients, are likely to be similar to patient characteristics. One needs to take this into account in the analysis because otherwise, the frequency (of vitamin intake, for example) would become similar in cases and controls, leading to biased null findings. In this analysis, we also adjusted for all aforementioned potential confounding factors. Although using partners as controls results in most controls having the opposite sex as their matched case, one can adjust for sex in a partner-matched case-control study by allowing for sex with an indicator variable (24).

All statistical analyses were performed with SPSS for Windows, version 20.0 (SPSS Inc.). Conditional logistic regression was performed by using the COXREG procedure, as explained on the SPSS tutorial page at http://www-01.ibm.com/support/docview.wss?uid=swg21477360.

RESULTS

A total of 7696 participants (2506 patients, 2506 partner controls, and 2684 RDD controls) were included in this study (**Figure 1**). The main characteristics of participants are presented in **Table 1** and **Supplemental Table 1**. The mean age was 49 y (range, 18–70 y) in patients and partner controls and 46 y (range, 18–70 y) in RDD controls. Vitamin supplements were used by 796 (32%) patients, 818 (33%) partner controls, and 1181 (44%) RDD controls. Because patients were matched to their partners, only exposure-discordant couples (i.e., couples in whom vitamin consumption differs between patient and partner) were relevant to the univariable risk analyses (26). In total, there were 744 discordant couples (i.e., in whom only one of the two used vitamins). Participants who used vitamins and those who did not were of similar age. Female participants used vitamins more frequently than did men. Patients and RDD controls with malignancy used vitamins more frequently (40% compared with 54%) than did participants without malignancy (32% compared with 44%), respectively, while in partner controls, there was no difference regarding vitamin use between those with and without malignancy. Also, participants with classic venous thrombosis risk factors used vitamins more frequently than did those without these risk factors, but this was mainly associated with hormone use. In vitamin users, the prevalence of hormone use and sporting was higher, whereas the prevalence of statin use was lower than in nonusers. Also, participants who used vitamins were less likely to be overweight or obese than participants who did not use vitamins. No other characteristics were associated with vitamin use.

Overall, vitamin use was associated with a decreased risk of venous thrombosis when comparing all patients with RDD controls (OR: 0.63; 95% CI: 0.57, 0.70) (**Table 2**). Adjustment for age, sex, BMI, smoking, statin use, and hormone use yielded an OR of 0.68 (95% CI: 0.61, 0.77). The fully adjusted ORs for vitamin A, vitamin B-6, vitamin B-12, folic acid, vitamin C, vitamin D, vitamin E, and multivitamin use were in the same range. The results were the same when we compared patients with a partner with RDD controls (**Supplemental Table 2**).

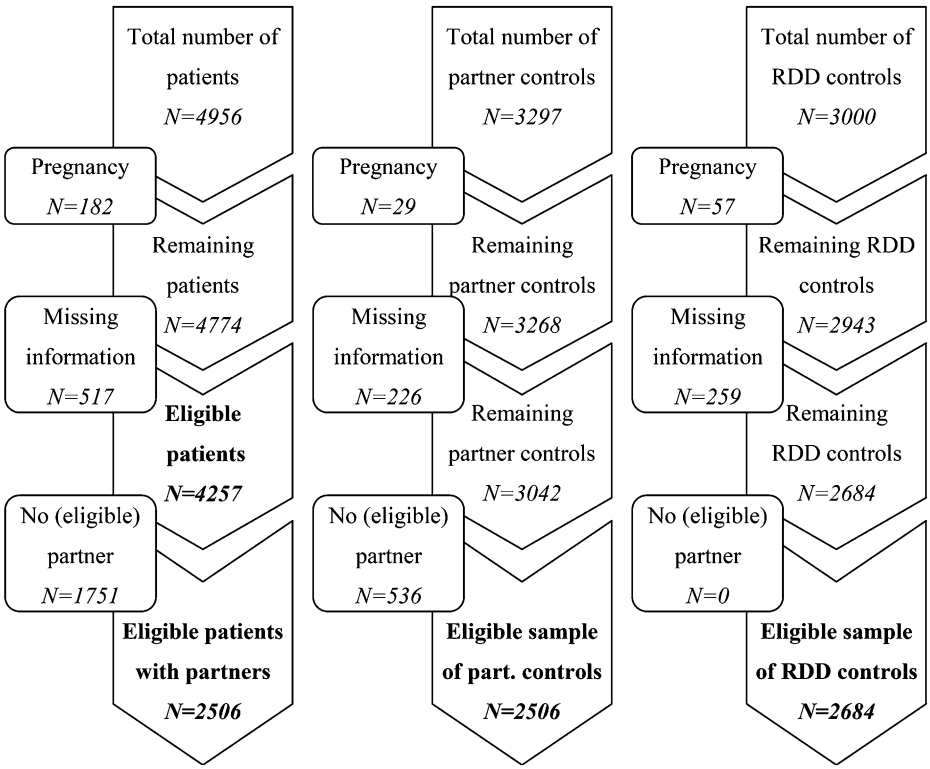

FIGURE 1 Selection of patients and controls in the Multiple Environmental and Genetic Assessment study. part., partner; RDD, random-digit dialing.

TABLE 1
Clinical characteristics of the MEGA case-control study[1]

Characteristic	Patients (n = 4257)		Partner controls (n = 2506)		RDD controls (n = 2684)	
Vitamin use[2]	No	Yes	No	Yes	No	Yes
General characteristics						
Total	2844 (67)	1413 (33)	1688 (67)	818 (33)	1503 (56)	1181 (44)
Men	1479 (52)	544 (38)	905 (54)	294 (36)	772 (51)	404 (34)
Age at enrollment,[3] y	49 (18–70)	49 (18–70)	49 (18–70)	49 (18–70)	46 (18–71)	45 (18–70)
BMI,[3] kg/m^2	27 (14–63)	26 (15–55)	26 (17–48)	25 (17–45)	25 (16–53)	25 (16–50)
Malignancy	232 (8)	153 (11)	28 (2)	13 (2)	25 (2)	29 (3)
Classic venous thrombosis risk factors						
Present[4]	1465 (52)	844 (61)	255 (16)	169 (23)	347 (23)	302 (26)
Without hormonal risk factors	655 (33)	346 (39)	70 (5)	45 (7)	75 (6)	54 (6)
With hormonal risk factors (in women)	798 (60)	483 (58)	179 (26)	119 (26)	271 (38)	243 (32)
Absent[4]	1359 (48)	549 (39)	1343 (84)	581 (77)	1142 (77)	858 (74)
Arterial cardiovascular disease risk factors						
Overweight	1240 (45)	516 (38)	707 (43)	282 (36)	514 (36)	357 (31)
Obesity	585 (21)	260 (19)	259 (16)	111 (14)	192 (13)	97 (9)
Previous smoking	854 (30)	421 (30)	502 (30)	260 (32)	410 (27)	317 (27)
Current smoking	1036 (37)	482 (34)	552 (33)	247 (31)	466 (31)	359 (31)
Self-reported prior CVD[5]	112 (4)	49 (4)	44 (3)	11 (1)	38 (2)	26 (2)
Statin use	120 (4)	30 (2)	97 (6)	37 (4)	98 (7)	61 (5)
Regular sports activity	845 (33)	470 (37)	539 (36)	319 (44)	595 (45)	550 (53)

[1]Values are n (%) unless otherwise indicated. CVD, cardiovascular disease; MEGA, Multiple Environmental and Genetic Assessment; RDD, random-digit dialing.
[2]Use of folic acid, multivitamins, or vitamin A, B-6, B-12, C, D, or E.
[3]Values are means; ranges in parentheses.
[4]Classic risk factors include surgery, malignancy, immobilization, trauma, plaster cast, oral contraceptive, hormonal replacement therapy, and recent travel.
[5]CVD denotes self-reported myocardial infarction or ischemic stroke.

When we compared patients with their partner controls, 361 couples were present in whom the patient had been taking vitamins but the partner had not, as well as 383 couples in whom it was the other way around, resulting in ORs close to unity; the fully adjusted ORs also were in the same range. An exception was vitamin A therapy, in which the OR was 0.47 (95% CI: 0.24, 0.91), and the fully adjusted OR was 0.46 (95% CI: 0.18, 1.20) (**Table 3**). In a further analysis, we restricted the outcome to deep vein thrombosis or pulmonary embolism and analyzed patients with and without classic provocative risk factors for venous thrombosis. These analyses showed similar results (**Tables 4** and **5** and **Supplemental Table 3**).

DISCUSSION

We analyzed data from a large case-control study with the aim to investigate whether vitamin supplements decrease venous thrombosis risk by using both population and partner controls and found that vitamin B, C, D, and E supplementation is not associated with a decreased risk.

In the initial analyses, in which patients were compared with RDD controls, we observed a 37% decrease in risk for venous thrombosis in vitamin users compared with no vitamin users. After adjustment for many lifestyle-related factors such as BMI, smoking, statin use, hormone use, and sports activity, this

TABLE 2
Risk of venous thrombosis by categories of vitamin supplementation for all patients and RDD controls[1]

Vitamin supplementation	All patients, n (%)	RDD controls, n (%)	OR[2] (95% CI)	OR[3] (95% CI)	OR[4] (95% CI)	OR[5] (95% CI)
No vitamin therapy	2844 (67)	1503 (56)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Vitamin therapy	1413 (33)	1181 (44)	0.63 (0.57, 0.70)	0.60 (0.53, 0.68)	0.68 (0.61, 0.76)	0.68 (0.61, 0.77)
Vitamin A	40 (1)	43 (2)	0.57 (0.37, 0.89)	0.61 (0.38, 0.97)	0.63 (0.38, 1.03)	0.65 (0.38, 1.12)
Vitamin B-6	112 (3)	109 (4)	0.63 (0.48, 0.83)	0.63 (0.48, 0.84)	0.64 (0.48, 0.85)	0.61 (0.44, 0.84)
Vitamin B-12	141 (3)	128 (5)	0.67 (0.52, 0.86)	0.69 (0.53, 0.89)	0.68 (0.52, 0.89)	0.65 (0.48, 0.87)
Folic acid	143 (5)	147 (3)	0.67 (0.53, 0.85)	0.65 (0.50, 0.83)	0.80 (0.62, 1.05)	0.85 (0.64, 1.13)
Vitamin C	364 (9)	396 (15)	0.55 (0.47, 0.64)	0.57 (0.49, 0.68)	0.57 (0.48, 0.67)	0.57 (0.48, 0.69)
Vitamin D	86 (2)	57 (2)	0.90 (0.64, 1.27)	0.98 (0.69, 1.40)	1.06 (0.72, 1.54)	1.16 (0.77, 1.75)
Vitamin E	96 (2)	79 (3)	0.71 (0.53, 0.97)	0.73 (0.53, 1.00)	0.81 (0.58, 1.13)	0.87 (0.60, 1.25)
Multivitamins	792 (19)	782 (29)	0.57 (0.51, 0.64)	0.60 (0.53, 0.68)	0.60 (0.53, 0.68)	0.63 (0.55, 0.72)

[1]ORs (95% CIs) were estimated by means of unconditional logistic regression. RDD, random-digit dialing.
[2]Adjusted for age, sex, and partnership where applicable.
[3]Adjusted for age, sex, BMI, smoking, and partnership where applicable.
[4]Adjusted for age, sex, BMI, smoking, statin use, hormone use, and partnership where applicable.
[5]Adjusted for age, sex, BMI, smoking, statin use, hormone use, cardiovascular disease, sports activity, and partnership where applicable.

TABLE 3
Risk of venous thrombosis by categories of vitamin supplementation for all patients with partner controls[1]

Vitamin supplementation	Patients with partners, *n* (%)	Partner controls, *n* (%)	OR[2] (95% CI)	OR[3] (95% CI)	OR[4] (95% CI)	OR[5] (95% CI)
No vitamin therapy	1710 (68)	1688 (67)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Vitamin therapy	796 (32)	818 (33)	0.98 (0.84, 1.14)	1.03 (0.88, 1.20)	1.13 (0.95, 1.35)	1.11 (0.91, 1.35)
Vitamin A	38 (1)	23 (1)	0.47 (0.24, 0.91)	0.47 (0.23, 0.95)	0.58 (0.26, 1.25)	0.46 (0.18, 1.20)
Vitamin B-6	56 (2)	59 (2)	0.97 (0.65, 1.44)	0.96 (0.63, 1.46)	0.97 (0.61, 1.54)	0.86 (0.49, 1.49)
Vitamin B-12	75 (3)	65 (3)	1.22 (0.85, 1.77)	1.24 (0.84, 1.84)	1.04 (0.67, 1.61)	0.94 (0.55, 1.61)
Folic acid	73 (3)	69 (3)	1.10 (0.78, 1.56)	1.08 (0.74, 1.56)	1.19 (0.79, 1.79)	1.04 (0.65, 1.67)
Vitamin C	193 (8)	218 (9)	0.82 (0.64, 1.06)	0.88 (0.68, 1.15)	0.95 (0.71, 1.28)	0.96 (0.68, 1.36)
Vitamin D	39 (2)	45 (2)	0.85 (0.53, 1.38)	1.01 (0.61, 1.70)	0.87 (0.48, 1.55)	1.23 (0.61, 2.45)
Vitamin E	53 (2)	46 (2)	1.21 (0.77, 1.91)	1.38 (0.85, 2.25)	1.36 (0.79, 2.33)	1.34 (0.73, 2.47)
Multivitamins	454 (18)	488 (19)	0.89 (0.74, 1.07)	0.94 (0.77, 1.14)	0.98 (0.79, 1.12)	0.99 (0.77, 1.26)

[1]ORs (95% CIs) were estimated by means of conditional logistic regression.
[2]Adjusted for age, sex, and partnership where applicable.
[3]Adjusted for age, sex, BMI, smoking, and partnership where applicable.
[4]Adjusted for age, sex, BMI, smoking, statin use, hormone use, and partnership where applicable.
[5]Adjusted for age, sex, BMI, smoking, statin use, hormone use, cardiovascular disease, sports activity, and partnership where applicable.

decrease was still 32%. For the individual vitamins, a protective effect was found for vitamin B-6, vitamin B-12, folic acid, and vitamin C, with a 39%, 35%, 35%, and 43% decrease in risk after adjustment, respectively. On the basis of these results, we could have concluded that one should prescribe vitamins to prevent venous thrombosis, especially considering the popular opinion that vitamins are otherwise harmless (27). However, comparison of patients with venous thrombosis and their partners showed that the above-mentioned risk estimates (with the possible exception of vitamin A) were likely to be confounded, because in this analysis, no decreased risk estimates were found for venous thrombosis in vitamin users compared with nonusers of vitamins (fully adjusted OR: 1.11; 95% CI: 0.91, 1.35). Similar results were obtained for all individual vitamins. This could be due to the influence of lifestyle-related confounders, which the comparison with partners adjusts for.

Most studies investigating the influence of vitamin intake on the risk of venous thrombosis focused on vitamin B-6, vitamin B-12, and folic acid. Because folic acid decreases homocysteine concentration, appears to interact with the metabolism of nitric oxide, and reduces superoxide anion generation (5, 28), it was assumed that supplementation of this vitamin would lead to a decreased risk of venous thrombosis. Our results showed no decrease in risk for venous thrombotic events in vitamin B users, which is consistent with the results of large clinical trials on first and recurrent venous thrombosis, such as the Heart Outcomes Prevention Evaluation and the Vitamins and Thrombosis trial (7, 29), which showed no benefit of homocysteine-lowering therapy. Regarding the other vitamins, some studies showed that combined antioxidant treatment with vitamins C and E for 4 wk improves endothelial function and decreases the plasminogen activator inhibitor 1/tissue plasminogen activator ratio (30). The Women's Health Study reported a 21% risk reduction of venous thrombosis in women taking vitamin E over a 10-y follow-up period (14). However, results of this study are difficult to interpret. The Women's Health Study used a 2×2 factorial randomized design (vitamin E, aspirin, placebo) but failed to show an effect of vitamin E alone compared with placebo on venous thrombosis risk. It is therefore possible that it was not vitamin E but aspirin, or a combination of both, that decreased the risk of venous thrombosis and not vitamin E itself. No other

TABLE 4
Risk of venous thrombosis by vitamin supplementation for all patients and RDD controls, subgroup analysis[1]

Characteristic	All patients, *n* (%)	RDD controls, *n* (%)	OR (95% CI)
Provoked venous thrombosis			
No vitamin therapy	1465 (63)	1503 (57)	1.0 (reference)
Vitamin therapy	844 (37)	1181 (44)	0.77 (0.66, 0.89)
Unprovoked venous thrombosis			
No vitamin therapy	1359 (71)	1503 (56)	1.0 (reference)
Vitamin therapy	549 (29)	1181 (44)	0.61 (0.52, 0.72)
Deep vein thrombosis only			
No vitamin therapy	1664 (68)	1503 (57)	1.0 (reference)
Vitamin therapy	795 (32)	1181 (44)	0.67 (0.59, 0.77)
Pulmonary embolism ± deep vein thrombosis			
No vitamin therapy	1180 (66)	1503 (56)	1.0 (reference)
Vitamin therapy	618 (34)	1181 (44)	0.71 (0.61, 0.82)

[1]Estimated by means of unconditional logistic regression and adjusted for age, sex, BMI, smoking, statin use, hormone use, cardiovascular disease, and sports activity. RDD, random-digit dialing.

TABLE 5
Risk of venous thrombosis by vitamin supplementation for patients with partner controls, subgroup analysis[1]

Characteristic	Patients with partners, *n* (%)	Partner controls, *n* (%)	OR (95% CI)
Provoked venous thrombosis			
No vitamin therapy	865 (64)	1688 (67)	1.0 (Reference)
Vitamin therapy	486 (36)	818 (33)	1.05 (0.93, 1.19)
Unprovoked venous thrombosis			
No vitamin therapy	837 (74)	1688 (67)	1.0 (Reference)
Vitamin therapy	302 (26)	818 (33)	0.94 (0.81, 1.09)
Deep vein thrombosis only			
No vitamin therapy	991 (69)	1688 (67)	1.0 (Reference)
Vitamin therapy	447 (31)	818 (33)	0.98 (0.86, 1.11)
Pulmonary embolism ± deep vein thrombosis			
No vitamin therapy	719 (67)	1688 (67)	1.0 (Reference)
Vitamin therapy	349 (33)	818 (33)	1.02 (0.88, 1.18)

[1]Estimated by means of conditional logistic regression and adjusted for partnership, age, sex, BMI, smoking, statin use, hormone use, cardiovascular disease, and sports activity.

evidence is present that supplementation with these antioxidant vitamins leads to a decreased risk for venous thrombosis. Our results showed no protective effect of vitamins C, E, or D on venous thrombotic risk, but vitamin A consumption showed a beneficial effect. This latter result should be interpreted with caution because numbers were small in this analysis. Also, in the argument to take vitamins or not, it should be considered that vitamin supplements may not be that harmless. For example, use of folic acid might promote progression of atherosclerosis (31) and increase the risk for carcinogenesis (32). Moreover, antioxidant vitamins interfere with essential defensive mechanisms such as apoptosis, phagocytosis, and detoxification and might lead to increased mortality (33), which makes prescription of these supplements less attractive.

Limitations of our study are that data on vitamin use were self-reported without information on duration of vitamin use or exact dose for every single vitamin. Also, because we could only investigate associations between vitamin supplements and venous thrombosis, our findings should not be applied directly to natural vitamins (as in food). Given that we did not have data about vitamin status before or after vitamin consumption, we were unable to evaluate whether some vitamins would be beneficial against venous thrombosis in people with vitamin deficiency at baseline. Furthermore, it would have been interesting to consider vitamin K status in MEGA because vitamin K–dependent coagulation factors can determine venous thrombosis risk. Unfortunately, this information was not available. A strength of our research lies in the large study size and in the study design, which included both RDD and partner controls, making it possible to show that for this research question, protective risk estimates can easily be found if not all lifestyle-dependent confounding is accounted for and measured. We consider it unlikely that the accuracy or completeness of the recollections retrieved by study participants regarding vitamin use in the past is different among RDD controls, partner controls, and the patients with venous thrombosis. In addition, if somehow the patients did erroneously recall their vitamin use after venous thrombosis diagnosis, this would not explain the difference in risk estimates when patients were compared with RDD controls or partner controls. It should be noted, however, that using partner controls in case-control studies has drawbacks, too. First, patients without a partner are not included, which may lead to selection of a certain "type" of patient. This, however, should not compromise internal validity but could at most hamper generalization to other patients. Second, it is possible that partner controls are more similar to the cases with respect to the frequency of exposure than a random sample from the population. This would lead to selection bias and to an underestimation of the effect. However, carrying out a matched analysis (such as we did) takes this into account and adjusts for such bias should it have occurred. Although the number of comparisons in this study was extensive, no adjustment for multiple testing was performed. We decided not to adjust for multiple testing because nearly all our analyses pointed toward a lower risk of venous thrombosis in vitamin users when patients were compared with RDD controls, whereas relative risks were all close to unity when patients were compared with partners. This consistent pattern agrees with our null hypothesis (i.e., no decreased risk of venous thrombosis in vitamin supplement users after extensive adjustments for confounding), and therefore there is no risk of falsely rejecting it (i.e., no risk of a type I error) and no need for adjustment for multiple testing. Of note, some of the estimates in this analysis had confidence intervals that were wide and sometimes included 1.0. However, our most robust estimates (in which we pooled all vitamin users together in one group) showed that the risk of venous thrombosis was lower (OR: 0.68; 95% CI: 0.61, 0.77) and still confounded when patients were compared with RDD controls as opposed to a more rigorous adjustment for confounding when patients were compared with partners (OR for venous thrombosis: 1.11; 95% CI: 0.91, 1.35). Results for the individual vitamin supplements pointed toward the same direction (i.e., lower risk of venous thrombosis compared with RDD controls as opposed to partners), indicating that relative risk estimates on any vitamin use were likely to be confounded by unmeasured confounding factors when patients were compared with population-derived RDD controls.

In conclusion, our findings confirm that vitamin B supplementation is not associated with a decreased risk of venous thrombosis and adds as a novelty that vitamins C, D, and E are not associated with this disease. Furthermore, our study demonstrated that initial protective risk estimates were found due to control selection, reinforcing that when studying health-conscious related exposures, the control group must be selected with care, and lifestyle-dependent confounding should be measured as much as possible.

The authors' responsibilities were as follows—SCC, FRR, and WML: designed the research; BAV, NvR, and WML: conducted the research and analyzed data; BAV, NvR, SCC, FRR, and WML: wrote the manuscript; and WML: had primary responsibility for the final content. All authors read and approved the final manuscript. The authors declared no conflicts of interest with respect to this study.

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