Chapter 3.1

Folic acid and vitamin-B12 supplementation and the risk of cancer: long-term follow-up of the B-vitamins for the Prevention Of Osteoporotic Fractures (B-PROOF) trial

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ABSTRACT

Background

Folic acid and vitamin-B12 play key roles in one-carbon metabolism. Disruption of one-carbon metabolism may be involved in the risk of cancer. Our aim was to assess the long-term effect of supplementation with both folic acid and vitamin-B12 on the incidence of overall cancer and on colorectal cancer in the B-PROOF trial.

Methods

Long-term follow-up of B-PROOF trial participants (N=2,524), a multi-center, double-blind randomized placebo-controlled trial designed to assess the effect of 2-3 years daily supplementation with folic acid (400 µg) and vitamin-B12 (500 µg) versus placebo on fracture incidence. Information on cancer incidence was obtained from the Netherlands cancer registry (Integraal Kankercentrum Nederland), using the International Statistical Classification of Disease (ICD-10) codes C00-C97 for all cancers (except C44 for skin cancer), and C18-C20 for CRC.

Results

Allocation to B-vitamins was associated with a higher risk of overall cancer (171 [13.6%] vs. 143 [11.3%]), HR 1.25; 95%CI 1.00-1.53, p=0.05). B-vitamins were significantly associated with a higher risk of colorectal cancer (43[3.4%] vs. 25[2.0%]), HR 1.77; 95%CI 1.08-2.90, p=0.02).

Conclusion

Folic acid and vitamin-B12 supplementation was associated with an increased risk of colorectal cancer.

Impact

Our findings suggest that folic acid and vitamin-B12 supplementation may increase the risk of colorectal cancer. Further confirmation in larger studies and in meta-analyses combining both folic acid and vitamin-B12 are needed to evaluate whether folic acid and vitamin B12 supplementation should be limited to patients with a known indication such as a proven deficiency.

INTRODUCTION

A large proportion of the population globally, especially older people, use dietary supplements to promote good health (1). Studies of National and International Food Consumption Surveys, reported for example in the USA, UK and The Netherlands that 56%, 39% and 27% of older adults respectively use dietary supplements (2-5). However, supplements may not always be favorable, and in certain cases or doses they may even have adverse health effects (6). In addition, potential effects need to be put into perspective according to different fortification policies in countries (e.g. mandated vs. voluntary folic acid fortification). Together with vitamin-B12, folic acid plays a key role in one-carbon metabolism being involved in DNA methylation and DNA synthesis (7, 8). Several studies have suggested that altered DNA methylation is associated with a higher risk of certain cancers, including breast, prostate and colorectal cancer (CRC). Until recently, it was believed that folate and folic acid supplementation may have a protective effect on the risk of malignancies (9, 10). However, over the past decade, there have been some concerns that folic acid supplementation may actually increase the risk of cancer (11-15), possibly by promoting the progression of pre-neoplastic and undiagnosed neoplastic lesions (8, 16). Although some countries, including The United States, South Africa and Australia, have introduced population-wide folic acid fortification to prevent neural tube defects in the fetus (17-21), mandatory folic acid fortification has not been implemented in New Zealand or in several Western European countries partly because of these concerns about potential adverse effects on cancer incidence or progression (21, 22).

A recent meta-analysis of 10 studies (n=19,106; age range 26-69 years), reported no significant excess risk of folic acid (0.4 to 1 mg) supplementation on overall cancer incidence (23). However, previous results from the B-PROOF study, a randomized controlled trial on vitamin-B12 and folic acid supplementation on fracture risk in older persons, reported a higher incidence of self-reported cancer in the intervention group relative to the control group after a follow-up of 2-3 years (HR 1.56; 95% CI 1.04-2.31). Additional subgroup analysis revealed that the excess risk was predominantly explained by a higher CRC incidence, and that the effect appeared to be strongest in people aged older than 80 years (24).

Since the adverse effect of folic acid and vitamin-B12 supplementation on self-reported (colorectal) cancer was previously observed within 2-3 years in the B-PROOF study (24), the objective of this study was to validate these findings with data on confirmed cancer diagnosis and assess the long-term effects of folic acid and vitamin-B12 co-supplementation on the risk of overall cancer incidence and on CRC using prolonged

follow-up of trial participants. As such this secondary analysis of the B-proof study will contribute to current understanding of the biological plausibility of the effect of folic acid and vitamin- B12 co-supplementation on cancer (CRC) risk which will contribute to the ongoing fortification debate ongoing in several countries.

MATERIALS AND METHODS

The B-PROOF study (B-vitamins for the Prevention Of Osteoporotic Fractures) is a large multi-center (Erasmus MC Rotterdam, VU University Medical Center Amsterdam (VUmc) and Wageningen University (WUR), the Netherlands), randomized, placebo-controlled, double blind study, investigating the effect of daily oral vitamin-B12 and folic acid supplementation over a period of 2 to 3 years on fracture incidence.

Recruitment of participants took place between September 2008 and March 2011. A detailed description and study protocol of the trial has been reported elsewhere (25). Participants (n=2919) aged 65 years and over with an elevated homocysteine level (Hcy 12-50 µmol/l) were included. Participants were excluded if they had a renal insufficiency (creatinine level > 150 µmol/l) or history of a malignancy (excluding non-melanoma skin cancer) in the past 5 years or if they used high dosages of B-vitamins (intramuscular injections of vitamin-B12 and/or folic acid intake >300 µg/day, this was reported at the time of recruitment and was asked again by the questionnaire at the baseline).

Written informed consent was obtained before allocated treatment for all participants. For the present analysis, we used only the information of participants who gave permission to contact health institutes and medical doctors for their health details and medical history (n=2,524).

The B-PROOF study was registered in the Netherlands Trial Register (NTRNTR1333) and ClinicalTrials.gov (NCT00696514). The Ethics Committee approval for the study protocol was obtained from the Medical Ethics committees of Erasmus MC, VUmc and WU universities, according to declaration of Helsinki (25).

The intervention group received a daily tablet with 500 µg vitamin-B12 and 400 µg folic acid. In addition, both the control and intervention groups received 15 µg (600 IU) of vitamin D3 daily to ensure a normal vitamin D status. The intervention and placebo tablets, produced by Orthica, Almere, the Netherlands, are indistinguishable in taste, smell and appearance. The duration of intervention was 2 years, and to

increase power, individuals who finished their participation extended their participation for 1 more year (n=339 had 3 years intervention)(25).

The primary outcome of this study was the incidence of any cancer defined based on the International Statistical Classification of Disease (ICD-10) codes C00-C97. Individual data were obtained by linkage to the Netherlands cancer registry (Integraal Kankercentrum Nederland IKNL) from baseline until May, 2017. The Netherlands Cancer Registry is linked to the International Agency for Research on Cancer (IARC) and delivers pseudonymous data to the European database of the European Network of Cancer Registries (ENCR). Hence, employees of the cancer registry were unaware of treatment allocation of the participants. We used C00-C97 ICD codes for overall cancer (except C44 for skin cancer), and C18-C20 for CRC (26).

At baseline, height was measured using a stadiometer in duplicate to the nearest 0.1 cm and weight by using a calibrated weighing device (SECA 761) to the nearest 0.5 kg, both without wearing shoes (25). Body mass index (BMI) was calculated as weight in kg/height in m^2. A structured questionnaire was used to assess self-reported medical history (cardiovascular disease and diabetes mellitus), current use of medication and supplements, alcohol intake and smoking habits. Blood was collected, plasma homocysteine (Hcy), serum folate, vitamin-B12, holotranscobalamin (HoloTC), 25(OH) D and methylenetetrahydrofolate reductase (MTHFR)-genotype were determined; details of the methods used have been described previously (25).

Statistical analyses

We extended the follow-up of the original B-proof study to study the incidence of pathology-proven solid cancers. For all variables, mean with standard deviations (SD) or percentages were reported for each group. Differences between groups were tested with the t-test for continuous variables, Mann-Whitney U for normally skewed variables, and Chi-squared test for categorical variables. The cumulative event-free survival for cancer was analyzed by a Kaplan-Meier event curve. We calculated follow-up time as the number of months from the baseline measurement until the first diagnosis of incident cancer, death, loss-to-follow-up, or end of the study period, whichever occurred first. The incidence rate ratio was calculated on the incidence-rate of cancer for both treatment groups, which is defined as the number of cancer cases divided by the total sum of the follow-up in each group (cases/persons years). To avoid bias, primary analysis was based on the intention to treat (ITT) principle, where participants were analyzed based on the initial treatment allocation. Unadjusted Cox proportional hazard analyses were conducted with treatment (intervention vs.

control group) as the independent variable and the cancer diagnosis as the dependent outcome variable. Multivariable Cox proportional hazard regression analyses were applied adjusted for serum HoloTC, because this variable differed significantly between the intervention and the control group despite randomization. All other potential confounders were equally distributed between both groups. Additionally, subgroup analyses were performed to assess whether the treatment effect was different in strata of sex, age, plasma Hcy and MTHFR polymorphism, interaction with these variables were evaluated in the multivariable model for both overall cancer and CRC. When *p* for interaction was <0.1, subgroup analyses were performed. Second, per protocol (PP) analyses were performed that included data only from subjects who were compliant (>80% of pills consumed) to the study protocol, details have been described previously (25) and also a sensitivity analysis was performed in participants who were not using folic acid and vitamin- B12 supplements. Exploratory analysis has been done by duration of treatment (2 years vs. 3 years). Furthermore, for comparison purposes, we also calculated the risk ratio (RR) and log-rank observed – expected statistic. p-values <0.05 were considered to be statistically significant. Analyses were performed using IBM SPSS 21.

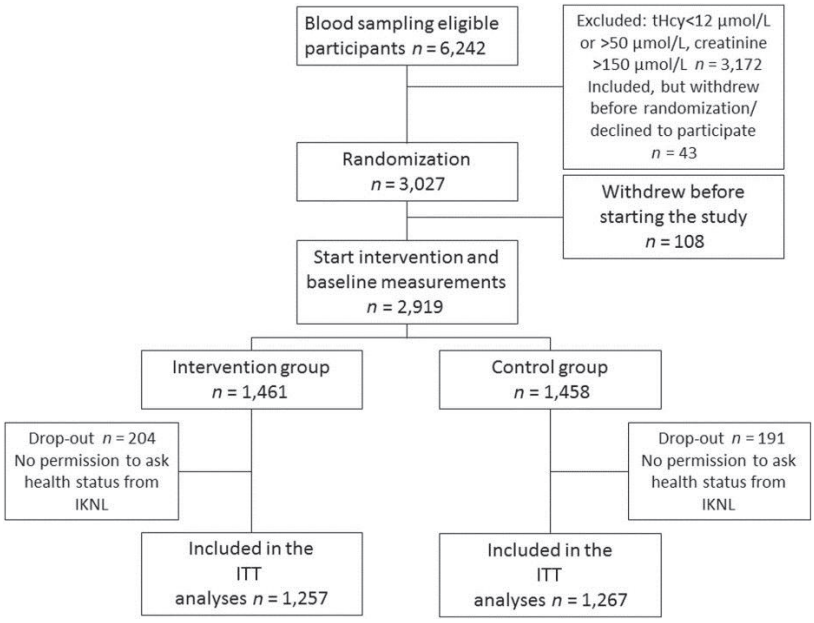

Figure 1 Flow-chart of the B-PROOF Trial based on the CONSORT 2010 Statement. A total of 2,524 participants were included in the intention to treat analyses.

Table 1. Selected characteristics of the trial population (n=2,524)

	Control: Vitamin D (n=1,267)	Missing (n)	Intervention: Folic acid, vitamin-B12 and vitamin D (n=1,257)	Missing (n)	p-value
Age(years)[a]	74.0 (6.2)	0	73.9 (6.6)	0	0.63
Sex (% Women)	48.6	0	50.4	0	0.36
Education years (%)		2		0	0.30
5-10	67.3		65.3		
11-18	32.7		32.7		
Alcohol consumption(%)		1		0	0.57
Light	66.7		66.9		
Moderate	28.8		29.6		
Excessive	4.0		3.0		
Very excessive	0.4		0.5		
Smoking status (%)		0		0	0.80
Never	33.9		33.5		
Current	9.4		10.2		
Former	56.7		56.3		
BMI[a]	27.2 (4.0)	8	27.1 (4.0)	10	0.67
Homocysteine (micromol/l)[b]	14.5 (13.0-16.7)	0	14.3 (13.0-16.5)	0	0.40
MTHFR (%)		161		145	0.27
CC	42.8		44.2		
CT	41.2		39.3		
TT	12.6		12.3		
Folic acid use supplements (%)		0		0	0.94
Yes	14.8		14.5		
No	83.3		83.8		
When necessary	1.9		1.8		
Vitamin-B12 use supplements (%)		0		0	0.96
Yes	15.1		14.5		
No	83.2		79.3		
When necessary	1.9		1.8		
Vitamin D use supplements (%)		0		0	0.85
Yes	19.7		18.8		
No	78.5		79.3		
When necessary	1.7		1.8		
Serum 25(OH)D (nmol/L)[a]	55.8 (23.9)	30	56.0 (25.9)	26	0.82
Serum Folate (nmol/L)[a]	20.1 (7.3)	44	20.3 (7.4)	45	0.47
Serum Vitamin-B12 (pmol/L)[a]	283.6 (115.0)	17	289.7 (116.2)	11	0.19
Serum Holotranscobalamin (pmol/L)[a]	70.9 (42.5)	11	74.3 (44.5)	7	0.05*

[a]mean (SD)[b]median (IQR) *p<0.05

RESULTS

A flow chart of 2,524 participants (86.5% of the initial 2,919 participants) is shown in **Figure 1**. Baseline characteristics were similar for the participants with and without informed consent for medical follow-up (n=2,524 vs. n=395).

Table 1 presents the selected baseline characteristics of the B-PROOF population, by allocated treatment. Mean (SD) age was 74 years (6.2) in both treatment and control groups and mean values for all other baseline characteristics were similar for treatment (n=1,257) and control groups (n=1,267), except for serum HoloTC concentration which was slightly higher in the treatment group (mean 74.3 (44.5 SD) vs. 70.9 (42.5 SD); p<0.05).

Intention To Treat (ITT) analyses showed that 314 persons were diagnosed with any cancer (171 cases [13.6%] in the intervention group vs 143 cases [11.3%] in the control group) and 68 persons were diagnosed with CRC (43 cases [3.4%] in the intervention group vs 25 cases [2.0%] in the control group) during a median follow up of 78 months; IQR: 74-83. Crude Cox proportional hazards models showed that persons in the intervention group did not have a significantly higher risk of any cancer than persons in the control group (HR 1.23; 95%CI 0.98-1.53; **Table 2**). However, the risk of CRC was significantly higher in the intervention group than persons in the control group (HR 1.76; 95% CI 1.07-2.88; **Table 2**) (**Figures 2 and 3**). After additional adjustment for baseline HoloTC, the risk of any cancer tended to be higher in the intervention group than in the control group (HR 1.25; 95%CI 1.00-1.57) and a significant increased risk remained for CRC (HR 1.77; 95%CI 1.08-2.90; **Table 2**).

Table 2. Effect of folic acid and vitamin-B12 on overall cancer, and on CRC incidence. Cox proportional hazard analysis of risk of cancers (ITT) in total group

Type of cancer (cases treatment vs. control group)	Cases/100 PY treatment vs. control group	HR [95%CI][a]	p-value	HR [95% CI][b]	p-value
Any cancers (171 vs. 143)	2.3 vs. 1.9	1.23 [0.98; 1.53]	0.07	1.25 [1.00; 1.57]	0.05
CRC (43 vs. 25)	0.6 vs. 0.3	**1.76 [1.07; 2.88]***	0.03	**1.77 [1.08; 2.90]***	0.02

[a] Crude model [b] adjusted for HoloTC (significant difference between intervention and control group) *p<0.05 CRC=colorectal cancer; HR: Hazard Ratio; ITT: Intention To Treat; PY= person years.

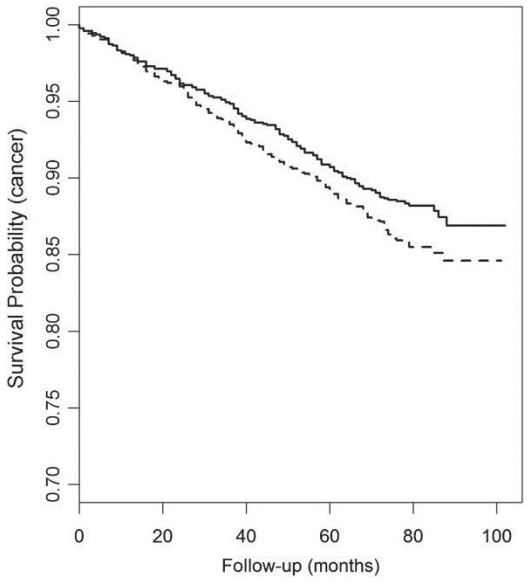

Figure 2. Kaplan-Meier curve of survival analysis of any cancers for the intervention (dashed line) and the control group (continuous line) and the follow-up time in months.

Interaction analyses revealed that the effect of the intervention did not significantly differ by age (<80 vs. >80 years), sex, plasma Hcy and MTHFR polymorphism (p-interaction > 0.10*)* for overall cancer and CRC.

Table 3. Effect of folic acid and vitamin-B12 on overall cancer, and on CRC incidence. Cox proportional hazard analysis of risk of cancers (PP) in compliance participants >80%

Type of cancer (cases treatment vs. control group)	Cases/PY treatment vs. control group	HR [95%CI][a]	p-value	HR [95% CI][b]	p-value
Any cancers (160 vs. 124)	2.3 vs. 1.7	**1.32 [1.05; 1.67]***	0.02	1.00 [0.99; 1.00]	0.10
CRC (40 vs. 19)	0.6 vs. 0.3	**2.15 [1.25; 3.72]***	0.01	**2.17 [1.26; 3.75]***	0.01

[a] Crude model [b] adjusted for HoloTC (significant difference between intervention and control group) *p<0.05 CRC=colorectal cancer; HR: Hazard Ratio; ITT: Intention To Treat; PY= person years.

Per Protocol (PP) analysis was conducted in compliant participants (n=2,330). After PP analyses, the HR on any cancer weakened but the HR on CRC became stronger relative to the ITT analyses (HR 1.00; 95%CI 0.99-1.00 and HR 2.17; 95%CI 1.26-3.75 respectively in the adjusted model; **Table 3**). Sensitivity analysis in participants who were not using folic acid and/or vitamin-B12 supplements, showed that the HR for

any cancer and CRC became stronger relative to the ITT analyses (HR 1.30; 95%CI 1.01-1.66 and HR 2.10; 95%CI 1.21-3.63 respectively in the adjusted model).

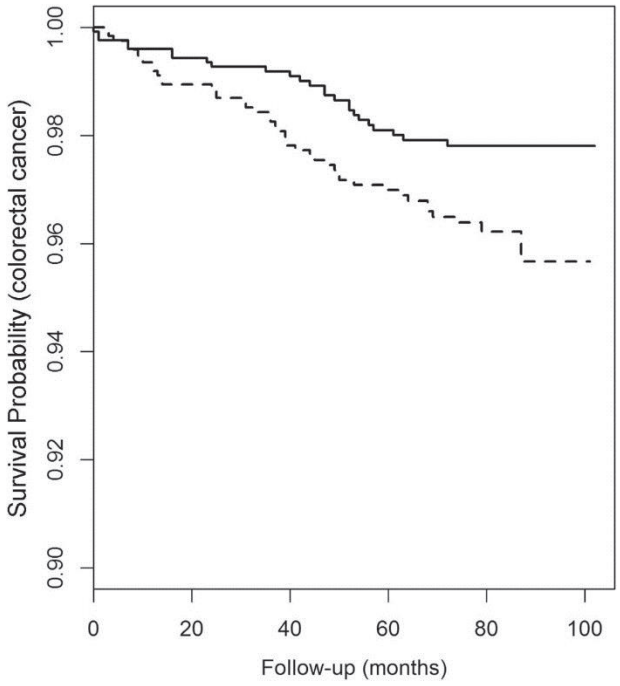

Figure 3. Kaplan-Meier curve of survival analysis of colorectal cancer (CRC) for the intervention (dashed line) and the control group (continuous line) and the follow-up time in months.

Exploratory analysis stratified by duration of the treatment (2 years vs. 3 years) showed that the HR on CRC were slightly weaker for participants with 2 years of intervention relative to the ITT analyses, but was still significant (HR= 1.72; 95%CI: 1.03-2.88 in the adjusted model).

We compared the results from the Cox proportional hazard model with the risk ratio (RR) and log-rank statistics and found similar results.

DISCUSSION

The findings of this study showed that allocating older persons with mildly elevated homocysteine levels to receive combined folic acid and vitamin-B12 supplementation was associated with a slight excess risk of overall cancer but a statistically significant increased risk for CRC when compared to placebo. The effect on CRC risk was even

more extreme in compliant participants (>80%). As difference in cancer risk was already apparent within the first years of follow-up, these findings are consistent with evidence that folic acid (combined with vitamin-B12) may promote the growth of early precursor mucosal lesions (8, 16). However, on the basis of the previous observations and the results of the B-PROOF study, we cannot yet ascertain whether this is due to an individual effect of folic acid or vitamin-B12, or an interactive effect of both folic acid and vitamin-B12 combined. The current findings confirm previous observations from the primary analyses of the B-PROOF trial (using self-reported cancer data), which showed an increased cancer risk in participants using folic acid and vitamin-B12 supplementation(24). A major strength of the current study is the extended follow-up of the B-PROOF study combined with the use of pathology-proven malignancies as an outcome measure. This is particularly important because of the long latency period between dietary risk-factors and cancer as well as the timeframe from premalignant lesions to cancer diagnosis in elderly. However, the findings differ from three recent meta-analyses, with mostly overlapping trials, which studied the effects of folic acid on cancer risk. Qin et al. found no significant overall effect of folic acid supplementation (mean dosage 1.64 mg) on cancer and CRC during a mean follow-up time of 5.3 years (mean age: 62.5 yrs.) (27). Vollset et al demonstrated no significant effect on cancer incidence including colon cancer in a time-frame of 1.8 to 7.4 years with a mean dosage of 4.7 mg (mean age: 64 yrs.) (28). In contrast, Baggott et al. (2012) reported a higher cancer risk in participants receiving folic acid supplementation during 3-8 years (mean dosage 1.3 mg), in a meta-analysis of a subset of these trials (mean age: 62.0)(29). The results of the present study differ from the three meta-analyses, probably because these studies addressed a younger population, as well as different dosages of supplementation and different outcome measures. It should also be noted that most studies included individual folic acid supplementation without vitamin-B12. Besides B-PROOF, only two other RCT's studied the effect of both folic acid and vitamin-B12. Although these trials included a selected population of people with ischemic heart disease, they also observed a significantly higher overall cancer risk (HR 1.21; 95% CI 1.03-1.41; p=0.02)(11), which is consistent with our findings. Most of the previous trials had a shorter follow-up, albeit they had a longer duration of treatment than B-PROOF. Whether folic acid, vitamin B-12, or both explain the results, cannot yet be confirmed. Especially because the studies of the effect of dietary, supplements, and plasma levels of folate and vitamin-B12 on cancer risk showed opposing results and none of these were randomized controlled trials (30-33). For example, Matejcic et al. (2017) found that overall, folate and vitamin-B12 status was not clearly associated with breast cancer risk in their prospective cohort study. They did, however, find potential interactions between vitamin-B12 and folate on the risk of breast cancer and suggested that low plasma folate concentrations

(mainly 5-methyl THF), as a consequence of high vitamin-B12 status, may impair DNA methylation (32). Price et al. (2016) reported a small increased risk of prostate cancer with higher folate and vitamin-B12 concentrations, in data from six cohorts (33). Another recent study, reported a 30-40% increase in lung cancer risk in men using vitamin-B12 supplements (not from multivitamins). They found no association of use of folic acid supplements in men and women in risk of lung cancer (31).

The previous analysis of the B-PROOF study showed that curves for cancer incidence separated shortly after the start of the intervention, which may imply that the effect of the treatment was on cancer progression rather that cancer induction. Since our previous analysis of the B-PROOF study showed a higher risk in persons aged >80y (24), and given that the results of the current study showed that 18 of the 28 excess cancers were CRC, it may be argued that the older age group has a higher prevalence of latent colorectal neoplastic cells (8) since the risk of CRC increases with advanced age (34) and older individuals may therefore be more prone to the effects of folic acid and vitamin-B12 supplementation. However, we did not have data on the presence of early neoplastic lesions in the colorectal mucosa to confirm this hypothesis.

The intervention dosage was 500µg vitamin-B12 and 400 µg folic acid per day. Although the dosage of folic acid was close to the recommended daily intake and well below the Tolerable Upper Intake Level for folic acid of 1 mg/d in Europe (35), the dosage of vitamin-B12 was almost 200 times higher than the recommended intake. For vitamin-B12, no systematic toxicological effects have been reported so far, (35), but we cannot rule out that the high dosage of vitamin-B12 supplementation influenced the risk of CRC in our study.

There may be several plausible mechanisms by which folic acid and vitamin-B12 supplementation increase the risk of CRC in particular. First, the epithelial cells of the colorectal mucosa have the most rapid turnover rate of any tissue in the body. Hence, it may be speculated that this tissue may be particularly sensitive to nutrients involved in cell growth such as B-vitamins. Folic acid and vitamin-B12 play a key role in one-carbon metabolism and cells require one-carbon units for DNA synthesis and methylation (36). Thus, these nutrients may influence pathways enhancing proliferation of cancer cells and modulate DNA and therefore the chance of developing a neoplastic cell (36). Folate has been demonstrated to affect neoplastic cells by enhancing growth in both animal and in-vitro models in DNA synthesis (36). Both folic acid and vitamin-B12 are essential for the synthesis of methionine and S-adenosyl methionine (SAM), which are required as the common methyl donor for the regulation of DNA methylation patterns in DNA influencing gene expression (36-38). DNA

methylation occurs mainly in CpG dinucleotides, concentrated in short CpG-rich DNA fragments so-called ÇpG islands' (39, 40). In normal cells, CpG island in active promoters can be methylated, which lead to long-term silencing of transcription. However, gene expression may be inactivated in genes that are hypermethylated at their CpG island-containing promotors, through which a neoplastic cell can develop (41). Currently little is known about the possible relation between vitamin-B12 and cancer risk. However, since vitamin-B12 has a key role in one-carbon metabolism and cells require one-carbon units for DNA synthesis, methylation as well as redox and reductive metabolism, vitamin-B12 may influence pathways enhancing the proliferation of cancer cells (42).

A second potentially relevant mechanism for CRC specifically may be via the gut microbiome. Several studies have shown that microbial imbalance of *Fusobacterium spp., Streptococcus gallolyticus susp. gallolyticus* may play a role in CRC etiology (43-45). Vitamin-B12 and folate can be synthesized by human gut microbes as a valuable resource in the gut (46). It has been suggested by others that competition and exchange of vitamin-B12 and cofactors from both dietary intake and gut microbes affect the gut microbial community (46). Thus, there may be an interaction between the gut microbiome and B-vitamins but further exploration of this hypothesis is needed.

The present study has several strengths as well as potential limitations. The main strengths of the present study were the randomized controlled study design, the pathology-proven cancer, the large sample size of elderly subjects, and the prolonged follow-up relative to other trials. A limitation of the present study is that it presents secondary analyses of a randomized controlled trial primarily designed to study the effect on fracture risk. As a result, a significant results of such analysis have to be interpreted in the context of other evidence in the literature. However, with a sample size of 2,524, an alpha of 5% and a power of 80% our study was able to detect a HR of 0.85/1.18 on overall cancer (47). In addition, the decision to study CRC in the B-PROOF study and other (non-site specific) was made on the basis of prior results on self-reported cancer as adverse event of the trial. We did not include other GI cancers, due to the limited power. It can be argued that this approach may increase the probability of type I errors because we did not adjust for multiple comparisons. For possible type I errors, stringent interpretation of p-values, especially for the results on all (non-site specific) cancers (p=0.05) should be made with caution. The initial B-PROOF study included 2,919 participants, but for the current extended follow-up analyses we collected data from a subgroup of 2,524 participants which could introduce a source of bias. However, there was no difference between the intervention and

control group in baseline characteristics between the participants with and without informed consent for medical follow-up.

Another source of potential bias was that the allocation to the intervention and control group was no longer blinded to the researchers. However, since the data collection of cancer was derived from the independent national cancer registry, and the physicians involved in the cancer diagnosis were blinded to the allocation of the intervention, observer bias is unlikely. Another possible limitation is that we only included Caucasian participants aged 65 years and over with elevated homocysteine levels in a country where no mandated folic acid fortification has been implemented. Therefore, the results may not be generalizable to other populations. Nonetheless, this trial is one of the few that were done in a population without mandated folic acid fortification and relatively low supplement use. As a result we were able to clearly discern the effect of supplementation in a population with limited intake of folic acid above the tolerable upper intake level.

CONCLUSION

The present study reported a higher risk of CRC among those allocated to folic acid and vitamin-B12 compared with placebo, which persisted over time (6-9 years). This was observed in older ambulant persons with mildly elevated homocysteine concentrations. The primary analyses of the B-PROOF trial did not show any protective effect of folic acid and vitamin-B12 supplementation on fracture, falls, and cardiovascular disease (with the exception of CVA). However, since secondary analyses of this trial, showed potential adverse effects on cancer, careful monitoring of long-term hazards of B-vitamins is required before making any recommendations for public health related to the implementation of fortification policies. To clarify the role of combined supplementation with B-vitamins on CRC, further confirmation for example by individual meta-analyses of existing, large RCT of folic acid and vitamin-B12, with additional information on the presence of early neoplastic lesions in the colorectal mucosa is needed.

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