CHAPTER 6

High circulating folate concentrations are associated with low-grade inflammation: an explanation for the homocysteine paradox?

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Abstract

Background

B-vitamins can decrease homocysteine concentrations, but are not effective in preventing cardiovascular events. Folate-induced enhancement of low-grade inflammation might account for these findings.

Objective

We aimed to study the association between concentrations of serum folate and serum C-reactive protein (CRP), a marker of inflammation, in an elderly population.

Design

Cross-sectional data were derived from the B-vitamins for the PRevention Of Osteoporotic Fractures (B-PROOF) study. Participants were aged ≥65 years (n=2,817, mean age 74.0 years). Non-linear cubic spline functions were used to examine the nature of the association for the total population, for persons having self-reported cardiovascular disease (CVD) (n=829), and for persons without CVD (n=1,346).

Results

A significant U-shaped association was observed between serum folate and CRP in the total study sample, with optimal serum folate concentrations around 20 nmol/L. In elderly with CVD, higher folate concentrations were significantly associated with higher CRP concentrations (regression coefficient B: 0.05; 95% confidence interval (CI): 0.01, 0.08). Below a threshold of approximately 17 nmol/L, a borderline significant inverse association between folate and CRP was observed in elderly without CVD (B: -0.12; 95% CI: -0.24, 0.00).

Conclusion

This study supports the existence of a U-shaped association between folate concentrations and inflammation in a population of elderly persons. High serum folate concentrations might be detrimental in elderly with CVD.

Introduction

Hyperhomocysteinemia is associated with increased cardiovascular risk. Several lines of evidence have demonstrated that homocysteine is an independent risk factor for atherosclerosis, arterial stiffness, and endothelial dysfunction.[1-3] However, recent meta-analyses failed to show a preventative effect of homocysteine-lowering by B-vitamin treatment on cardiovascular events in high risk patients.[4,5] These findings sparked a debate as to whether the homocysteine hypothesis in cardiovascular disease (CVD) is correct, or whether the association is in fact non-causal.

A potential explanation for the ineffectiveness of B-vitamins (particularly folic acid) that does not necessarily destabilize the homocysteine hypothesis, is that B-vitamin supplementation may have untoward effects that are independent of their homocysteine lowering effects. Such 'side effects' of B-vitamins may include stimulation of low-grade inflammation.[6] Because folate plays a crucial role in one-carbon metabolism, many critical cellular pathways depend on folate, including hundreds of methylation reactions, as well as DNA and RNA synthesis and maintenance.[7] This makes folate an essential molecule for metabolically active cells. Among cell types known to depend on folate are inflammatory cells, such as activated macrophages.[8,9] Activated macrophages are involved in many inflammatory processes, like for example advanced atherosclerosis.

Alternatively, hyperhomocysteinemia, which is associated with relatively low folate concentrations, has been shown to directly and indirectly enhance the production and release of several pro-inflammatory molecules, as reported by different in vitro studies.[10-13] In addition, an increase in expression of inflammatory mediators was observed in mouse monocyte cells under conditions of folate deficiency.[14]

Since both low and high folate concentrations might be associated with increased inflammation, we hypothesize a U-shaped association between folate and inflammation. This association might depend on health status:[6] folate might stimulate inflammation (e.g. macrophages) only to a detectable extent in patients with advanced atherosclerosis. Therefore, the current study aimed to examine the association between folate and the inflammation marker C-reactive protein (CRP) in an elderly population and in subgroups of elderly with and without prevalent CVD. Because aging in itself is associated with a propensity to develop low-grade inflammation,[15] this association is particularly relevant in an elderly population. We anticipate a U-shaped association in the total sample of elderly, and a positive association at higher folate concentrations in persons with CVD.

Subjects and methods

Study sample

Baseline data from the B-vitamins for the Prevention Of Osteoporotic Fractures (B-PROOF) study were used. The B-PROOF study is a double blind randomized placebo controlled intervention study that examines the efficacy of daily vitamin B12 and folic acid supplementation on fracture incidence in older individuals. The study is performed at three research institutions in the Netherlands: Wageningen University, Wageningen, VU University Medical Center, Amsterdam, and Erasmus MC, Rotterdam. Details on design, study sampling and data collection have been reported elsewhere.[16] Inclusion criteria were age ≥65 years, plasma homocysteine concentration ≥12 and ≤50 μmol/L, serum creatinine concentration ≤150 μmol/L, no diagnosis of cancer within the last 5 years, and no current or recent (<4 months) use of supplements with high doses of vitamin B12 (intramuscular injections) or folic acid (>300 μg). The number of included participants was 2,919. For the current study, baseline data were used from participants with complete data on serum folate and CRP concentrations (n=2,817). In the subgroup analyses, data were used from a subsample of participants with data on CVD (n=2,175). The Medical Ethics Committee of Wageningen University approved the study and local feasibility was provided by the Medical Ethics Committee of Erasmus MC and VU University Medical Center. All participants gave written informed consent.

Measurements

Participants were in fasted state, or had taken a light breakfast when morning venous blood samples were obtained. Serum concentrations of the inflammation marker CRP and serum folate were determined with electrochemiluminescence immunoassay on a Roche Modular E170 (Roche, Almere, The Netherlands). The inter-assay coefficient of variation was 5.3% at a level of 0.6 mg/L and 1.9% at a level of 42 mg/L.

Presence of CVD was self-reported via a structured questionnaire. CVD was defined as myocardial infarction, angina pectoris, cardiac arrythmia, congestive heart failure, peripheral arterial disease, thrombosis, cerebrovascular accident (including transient ischemic attack), or aneurysm. Pericarditis, atrial septal defects, and vascular spasms were excluded from the definition.

Potential confounders included age, sex, study location (Wageningen, Amsterdam or Rotterdam), smoking (former, current, never), physical activity, and serum creatinine. Physical activity (min/ day) was measured using the validated LASA Physical Activity Questionnaire (LAPAQ).[17] Serum creatinine was measured using the enzymatic colorimetric Roche CREA plus assay.

Plasma homocysteine was considered as covariate to check whether the association was independent from plasma homocysteine concentrations. Plasma homocysteine was

determined using HPLC, LC-MS/MS (Waters, Etten-Leur, the Netherlands), or the Architect i2000 RS analyzer (Abbott Laboratories, Abbott Park, IL, USA), depending on the study location.[16] Following a cross-calibration, the outcomes of the three study locations did not differ significantly.

Statistical analyses

Cubic spline regression analyses were performed to investigate the shape of the association between serum folate and serum CRP using R version 2.15.0, with folate as the independent variable and CRP as the dependent variable. Spline regression models are piecewise polynomial functions that join smoothly at points called knots. In contrast to categorical models that assume a constant association within categories, in spline models all data points are used, providing a better estimate of dose-response relationships.[18] Spline models were tested with 3 to 5 knots. The final models contained 3 or 4 knots. Non-linearity was tested by analysis of variance, which provides an overall p-value of non-linearity of the association between folate and CRP. Age and sex were added to the models. Other potential confounders were only included if they were associated with both the determinant (folate) and the outcome (CRP) ($p<0.10$). Analyses were performed in the total sample, and in a pre-specified subsample of persons with and without self-reported CVD. Post-hoc linear regression analyses were performed if no U-shape association was observed. IBM SPSS Statistics 20 was used. Significance was accepted at $p<0.05$.

Results

Characteristics of the study sample (n=2,817) are presented in Table 1. Participants were on average 74.0 ± 6.5 years of age, and 50% was female. The spline function of the association between folate and CRP in the total study sample is presented in Figure 1. A significant U-shaped association was observed after adjustment for age, sex, smoking, and study location. Analysis of variance within Spline regression analyses revealed that the overall p-value for non-linearity was 0.04. The optimal folate concentration was approximately 20 nmol/L.

Table 1 Characteristics of 2,817 men and women from the B-PROOF study.

	Total sample (n=2,817)	Persons with self-reported CVD (n=829)
Folate (nmol/L)[a]	20.1 ± 7.3	20.1 ± 7.4
Age (years)[a]	74.0 ± 6.5	74.2 ± 6.3
Sex (% women)	49.5	46.2
Study location		
- Wageningen (%)	29.6	23.9
- Amsterdam (%)	26.2	28.1
- Rotterdam (%)	44.2	48.0
CRP (mg/L)[b]	2 [1-3]	2 [1-3]
Homocysteine (µmol/L)[b]	14.4 [13.0-16.7]	14.6 [13.1-17.1]
Serum creatinine (µmol/L)[a]	84.0 ± 18.2	86.5 ± 19.2
Smoking (% current)	9.7	11.3
Physical activity (min/day)[b]	130 [84-192]	122 [71-184]

[a] Presented as mean ± standard deviation; [b] Presented as median [interquartile range]; CRP= C-reactive protein, CVD= cardiovascular disease.

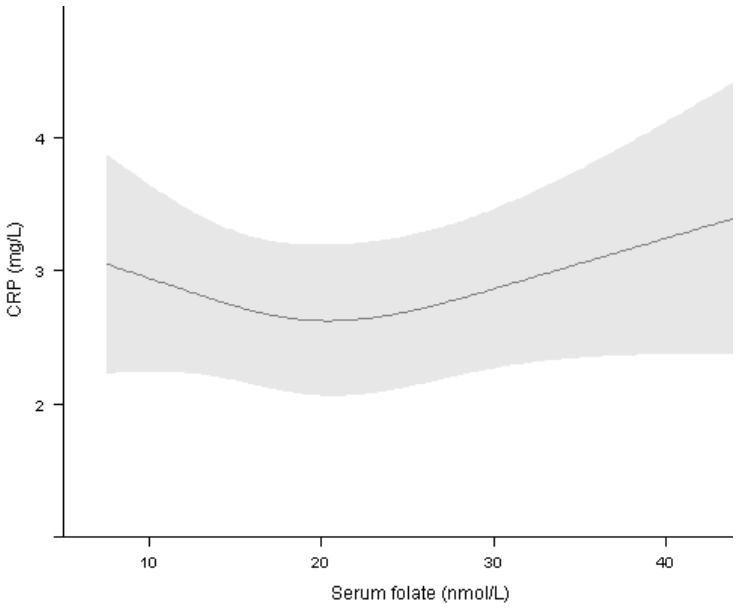


Figure 1 Spline functions for the association between folate and CRP in the total B-PROOF study sample (n=2,817), adjusted for age, sex, smoking, and study location. CRP= C-reactive protein.

Data on CVD were available in 2,175 participants. A total of 829 persons (38%) reported CVD. In this subgroup, higher folate concentrations were associated with higher CRP concentrations, above a folate threshold of approximately 20 nmol/L (Figure 2A), after adjustment for age, sex, smoking and physical activity. The overall p-value of non-linearity was not significant (p=0.08). Post-hoc linear regression analysis revealed a significant association between folate and CRP after adjustment for confounders (regression coefficient B: 0.05; 95% confidence interval (CI): 0.01, 0.08; p=0.02) among persons with CVD.

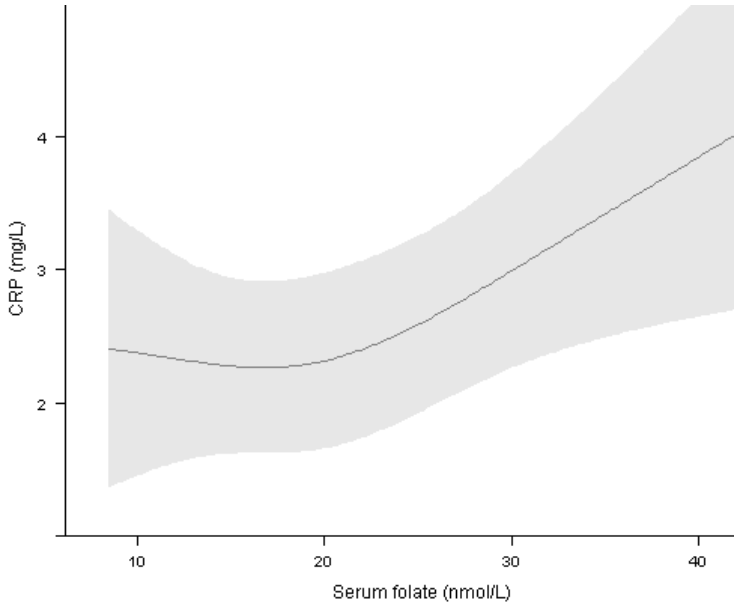
In persons without CVD (n=1,346), the spline function suggested that lower folate concentrations were associated with higher CRP concentrations, below a threshold of approximately 17 nmol/L (Figure 2B), after adjustment for age, sex, and study location. The overall p-value for non-linearity was not significant (p=0.54). Result from post-hoc linear regression analysis also indicated no association between folate and CRP (B: 0.01, 95% CI: -0.02, 0.04; p=0.64). However, a borderline significant inverse association between folate and CRP for folate concentrations <17 nmol/L was observed (B: -0.12; 95 %CI: -0.24, 0.00; p=0.06).

Discussion

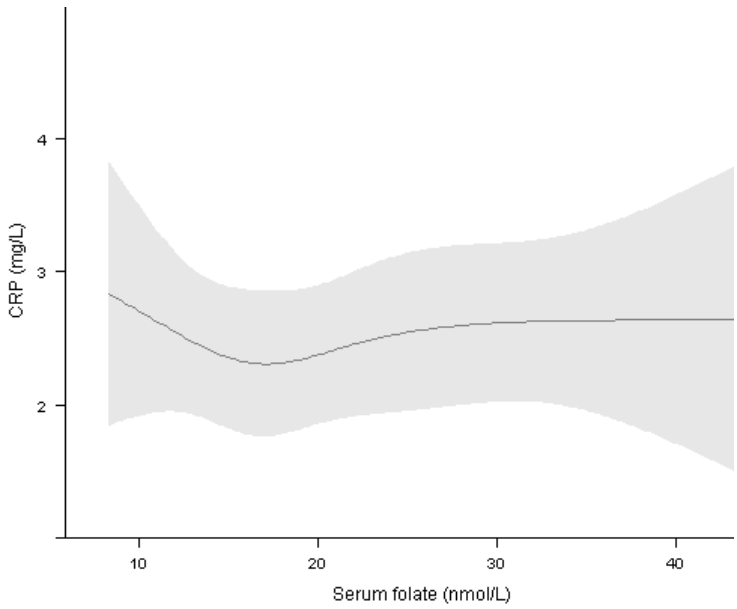
The findings of this study support our hypothesis that high folate concentrations are associated with higher levels of inflammation. This appears particularly true in a pro-inflammatory state, such as having CVD at a relatively high age. The optimal folate concentration in our total sample was 20 nmol/L. We further hypothesized that low folate concentrations would be associated with higher levels of inflammation as well. This was observed particularly in persons without CVD, although the results did not reach statistical significance.

The association between folate and inflammation was investigated earlier in the InCHIANTI Study, which suggested that folate was not associated with inflammatory markers among men and women (median age 69 years).[19] However, linear models were used in the analyses, in which a potential U-shaped association might be overlooked. It emphasizes the need for non-linear modeling.

To our knowledge, our hypothesis that high folate concentrations might accelerate inflammation, and thereby exert untoward effects on atherosclerosis, has not been studied previously, although previous empirical findings are supportive. First, a comparison of young and older individuals showed that high folate concentrations are associated with a decreased risk of coronary heart disease in young individuals, but with an increased risk in the elderly.[20] As the development of atherosclerosis progresses with aging, the lack of preventative effects in elderly might be explained by the presence of atherosclerosis and progression of atherosclerotic lesions by folate supplementation.



A



B

Figure 2 Spline functions for the association between folate and CRP in different subgroups from the B-PROOF study. A: Self-reported CVD, adjusted for age, sex, smoking and physical activity (n=829), and B: Persons with no self-reported CVD, adjusted for age, sex, and study location (n=1,346). Gray areas represent 95% confidence intervals. CRP= C-reactive protein; CVD= Cardiovascular disease.

Second, the currently reported association between folate and CRP might provide an explanation for the disappointing effects from previous B-vitamin supplementation trials on cardiovascular endpoints or inflammation. Effects on cardiovascular endpoints were mainly studied among high risk populations for cardiovascular events, for instance patients with non-disabling cerebral infarction,[21] patients with vascular disease or diabetes,[22] or patients with myocardial infarction.[23] With respect to inflammation, no favourable effects were observed among older adults,[24,25] or high risk patients for CVD.[26-28] The findings from these trials might suggest that indeed there is no effect. Alternatively, it might imply that the potentially favourable effect of homocysteine lowering could be offset by untoward effects of the intervention, such as acceleration of inflammation among such high risk populations for cardiovascular events. The net effect of the studies might depend on initial folate and homocysteine concentrations, age, and CVD risk.

How might folate accelerate inflammation? Folate accumulates at sites of inflammation. In atherosclerotic plaques, a higher number of folate dependent macrophages produce inflammatory mediators.[8,9] Folate has been shown to enter and stimulate activated macrophages via up-regulated folate receptor beta.[9] Furthermore, folate plays an important role in methylation reactions, since it is an important cofactor of the transmethylation in the 1-carbon metabolic cycle. Methylation of DNA and RNA have been shown to depend on folate availability.[29]

Some study limitations should be addressed. The cross-sectional study design limits conclusions regarding cause and effect. It remains therefore speculative whether folate affects CRP concentrations, or that the association is in fact non-causal. Moreover, CVD was self-reported, and might therefore be less accurate and might have led to misclassification. Further, although CRP is an independent risk factor for atherosclerosis and has been associated with future cardiovascular events,[30] other markers of inflammation, besides CRP, for instance IL-6, TNF-α, are of interest as well.[31] Unfortunately, other markers were not available.

More research is needed to confirm our findings and to address the potential implications for clinical practice and public health, for instance the need for alternative approaches to reduce homocysteine concentrations in the elderly. Our hypothesis needs to be further examined using longitudinal data from intervention trials in populations with different health states.

In conclusion, this study supports the theoretical possibility of a U-shaped association between folate concentrations and inflammation in the elderly. Furthermore, the results suggested that high serum folate concentrations might be detrimental in elderly with a high risk for CVD. Together with previous supportive empirical findings, the current data offer a new perspective.

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