# Association between B vitamin intake and plasma homocysteine concentration in the general Dutch population aged 20–65 y[1–3]

*Angelika de Bree, WM Monique Verschuren, Henk J Blom, and Daan Kromhout*

**ABSTRACT**

**Background:** An elevated plasma total homocysteine (tHcy) concentration is associated with an increased risk of cardiovascular diseases. Folate, riboflavin, vitamin B-6, and vitamin B-12 are essential in homocysteine metabolism.

**Objective:** The objective was to describe the association between dietary intakes of folate, riboflavin, vitamin B-6, and vitamin B-12 and the nonfasting plasma tHcy concentration.

**Design:** A random sample of 2435 men and women aged 20–65 y from a population-based Dutch cohort examined in 1993–1996 was analyzed cross-sectionally.

**Results:** Univariately, intakes of all B vitamins were inversely related to the plasma tHcy concentration. In multivariate models, only folate intake remained inversely associated with the plasma tHcy concentration. Mean plasma tHcy concentrations (adjusted for intakes of riboflavin, vitamin B-6, vitamin B-12, and methionine and for age, smoking, and alcohol consumption) in men with low (first quintile: 161 μg/d) and high (fifth quintile: 254 μg/d) folate intakes were 15.4 and 13.2 μmol/L, respectively; in women, plasma tHcy concentrations were 13.7 and 12.4 μmol/L at folate intakes of 160 and 262 μg/d, respectively. In men, the difference in the mean plasma tHcy concentration between men with low and high folate intakes was greater in smokers than in nonsmokers (2.8 compared with 1.6 μmol/L) and greater in nondrinkers than in drinkers of >2 alcoholic drinks/d (3.5 compared with 1.4 μmol/L). In women, the association between folate intake and plasma tHcy was not modified by smoking or alcohol consumption.

**Conclusions:** In this Dutch population, folate was the only B vitamin independently inversely associated with the plasma tHcy concentration. Changing dietary habits may substantially influence the plasma tHcy concentration in the general population. *Am J Clin Nutr* 2001;73:1027–33.

**KEY WORDS** Folate, riboflavin, vitamin B-6, vitamin B-12, plasma homocysteine, population-based study, Netherlands

## INTRODUCTION

The relation between elevated plasma homocysteine concentrations and cardiovascular diseases has been suspected since the 1960s. Extremely high plasma homocysteine concentrations in patients with homocystinuria, a rare genetic disorder of impaired homocysteine metabolism, are associated with the development of atherosclerosis (1, 2). More recently, numerous studies showed that a moderate elevation of the plasma total homocysteine (tHcy) concentration is also associated with an increased risk of vascular diseases (3, 4). Although the exact mechanism underlying this association is unidentified, it is suggested that homocysteine or one of its metabolites contributes to the development of vascular diseases (5–8).

Homocysteine is a sulfur-containing amino acid derived from the metabolism of the essential amino acid methionine, which is the only dietary precursor of homocysteine. Several B vitamins are involved in homocysteine metabolism. Vitamin B-6 is the cofactor of cystathionine β-synthase, the enzyme that irreversibly converts homocysteine to cystathionine. Vitamin B-12 is the cofactor of 5-methyltetrahydrofolate–homocysteine *S*-methyltransferase, which remethylates homocysteine to methionine. Folate in the 5-methyltetrahydrofolate form donates the methyl group in this reaction. For one of the recycling steps to reform 5-methyltetrahydrofolate, the enzyme methylenetetrahydrofolate reductase (NADPH) is necessary. This enzyme, in turn, uses riboflavin (vitamin B-2) as a cofactor (9, 10).

Several intervention studies have provided evidence for the importance of B vitamins in homocysteine metabolism. Supplements containing folic acid (synthetic form of folate) and also combinations of folic acid, riboflavin, vitamin B-6, and vitamin B-12 effectively reduced plasma tHcy concentrations in subjects with normal (11, 12) and elevated (13–16) baseline concentrations.

Although information on the relation between dietary intakes of folate, riboflavin, vitamin B-6, and vitamin B-12 and the plasma tHcy concentration is available for middle-aged (17) and elderly (18, 19) populations, it is scarce for the general adult

[1] From the National Institute of Public Health and the Environment, the Department of Chronic Disease Epidemiology, Bilthoven, Netherlands, and the University Hospital St Radboud, the Laboratory of Pediatrics and Neurology, the University Hospital Nijmegen, Netherlands.

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[3] Address reprint requests to A de Bree, Department of Chronic Disease Epidemiology (CZE, Pb 101), National Institute of Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, Netherlands. E-mail: angelika.de.bree@rivm.nl.

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population. This information is essential for an understanding of to what extent the plasma tHcy concentration can be lowered in the general population through dietary interventions. The only available large population-based study of adults (40–67 y), the Hordaland Homocysteine Study, unfortunately did not provide this information because it lacked detailed information on dietary intakes (20). The present study contains detailed information on intakes of folate, riboflavin, vitamin B-6, and vitamin B-12 and describes the association between these vitamins and plasma tHcy concentrations in a random sample ($n$ = 2435) of the general Dutch population aged 20–65 y.

## SUBJECTS AND METHODS

### Study population

Data were used from the MORGEN study (21), a cross-sectional investigation into the prevalence of risk factors for chronic diseases and certain chronic conditions. The population of this study consisted of a random sample of subjects aged 20–65 y from 3 cities in the Netherlands: Amsterdam, Doetinchem, and Maastricht. The external Medical-Ethical Committee of the TNO Toxicology and Nutrition Institute, which follows the guidelines of the Helsinki Declaration, approved the protocol of the MORGEN study.

Of the 19066 subjects who participated in the MORGEN study during 1993–1996, 14356 were considered eligible for the present study because they had completed questionnaires, had blood available for all aimed biochemical analyses, and had recorded time of drawing and centrifugation of blood. Of those subjects, 7992 were excluded because their whole blood samples had been stored at room temperature for >1 h, which may have artificially increased the plasma tHcy concentration (22, 23). A sample of 6364 subjects remained, from which an age- and sex-stratified random sample of 3025 subjects was drawn. Demographic variables and cardiovascular disease risk factors of this sample did not differ significantly from those of the MORGEN (1993–1996) study. Furthermore, the distribution of demographic characteristics (eg, sex, employment, and education) of our sample was comparable with nationwide data (23).

### Data collection

Respondents answered 2 self-administered questionnaires: a general and a semiquantitative food-frequency questionnaire. Subsequently, trained research assistants performed a physical examination of each subject at the Municipal Health Service.

From the general questionnaire we extracted information on sex, age, smoking, and alcohol consumption. The food-frequency questionnaire assessed the habitual consumption of 178 food items and vitamin supplements during the previous year. The frequency of consumption of main items could be indicated in *1*) times per day, *2*) times per week, *3*) times per month, *4*) times per year, or *5*) never. The amount eaten was estimated in commonly used units by using household measures or colored photographs of foods showing different portion sizes (24).

Average daily intakes of folate, methionine, total energy, riboflavin, vitamin B-6, and vitamin B-12 were estimated by multiplying the frequency of consumption of the food items by the portion size and the nutrient content per gram. Riboflavin, vitamin B-6, and energy intakes were calculated by using an extended version of the 1996 computerized Dutch food-composition table (25). This table does not contain data on folate, vitamin B-12, or methionine. Therefore, folate data were derived from a validated HPLC method with which Dutch foods were analyzed (26, 27). Vitamin B-12 and methionine data were compiled from food-composition tables from the United States, United Kingdom, Germany, and Sweden.

The food-frequency questionnaire did not collect information on the doses and contents of vitamin supplements. Hence, we excluded from all analyses subjects who used supplements that might have contained B vitamins (217 men and 371 women) to reduce misclassification of B vitamin intake. In addition, one man and one woman were excluded because of unreliable data in the food-frequency questionnaire.

### Blood sampling and biochemical determinations

During the physical examination, nonfasting venous blood samples were obtained from subjects in a sitting position. The samples used for the preparation of plasma were collected in evacuated tubes containing 7.5% tripotassium EDTA (Safety-Monovette tubes; Sarstedt, Tilburg, Netherlands) and were centrifuged at room temperature for 10 min at 3000 × $g$ within 1 h of collection. After centrifugation the plasma was separated from blood cells immediately and stored at −20°C or −80°C until homocysteine was measured.

The plasma tHcy concentration, which includes both the unbound and bound fractions of homocysteine, was measured by HPLC with fluorescence detection as described by Fiskerstrand et al (28), with some modifications (29). All samples were measured between July 1997 and January 1999; the within- and between-run CVs for reference plasma (≥2 samples in each run) were 3.2% and 8.6%, respectively.

### Statistical analysis

Because users of B vitamin supplements were excluded, all analyses were based on the data of 1275 men and 1160 women. Differences in characteristics between men and women were tested with Wilcoxon's two-sample test. The distributions of nutrient and energy intakes and plasma tHcy concentrations were positively skewed; therefore, we used natural logarithmic transformations to normalize their distributions. Thus, geometric means (antilogarithms of the transformed means) are presented unless stated otherwise.

To assess the association between intake of B vitamins and the plasma tHcy concentration, the nutrients (B vitamins and methionine) were energy-adjusted by regressing nutrient intake on total energy intake for each individual. The energy-independent residuals of this analysis were standardized to the predicted nutrient intake at the average energy intake (9391 kJ/d) in our population (30). Energy adjustments were applied to prevent multicollinearity in the multivariate models. The adjustments reduced the Spearman correlation between the nutrients from 0.46–0.81 to 0.12–0.69 and resulted in a maximum variance inflation factor of 2.7. Multicollinearity is a concern when the variance inflation factor is >10.0 (31).

Sex-specific quintiles of B vitamin intake were created after the logarithmic transformations and energy adjustments. Each relation between B vitamin and plasma tHcy was evaluated by calculating the mean plasma tHcy concentration per quintile of B vitamin intake. Adjusted mean plasma tHcy concentrations were estimated by analysis of covariance. Adjustments were made for age (y), methionine intake (mg/d), smoking (no or yes),

**TABLE 1**
Selected characteristics of the study population[1]

| Characteristics | Men ($n$ = 1275) | Women ($n$ = 1160) |
|---|---|---|
| Age (y) | 40.5 ± 12.1 | 40.8 ± 12.5 |
| Plasma tHcy (μmol/L)[2] | 14.8 ± 6.2 | 13.4 ± 4.8[3] |
| Folate intake (μg/d)[2] | 239 ± 73 | 192 ± 54[3] |
| Riboflavin (mg/d)[2] | 1.8 ± 0.7 | 1.5 ± 0.5[3] |
| Vitamin B-6 (mg/d)[2] | 2.1 ± 0.6 | 1.6 ± 0.4[3] |
| Vitamin B-12 (μg/d)[2] | 5.7 ± 2.8 | 4.3 ± 2.1[3] |
| Methionine (mg/d)[3] | 2.1 ± 0.6 | 1.7 ± 0.5 |
| Alcohol (drinks/d) | 1.7 ± 2.2 | 0.5 ± 1.0[3] |
| Current smokers (%) | 35.1 | 36.5 |

[1] $\bar{x}$ ± SD. tHcy, total homocysteine.
[2] Arithmetic values.
[3] Significantly different from men, $P$ = 0.0001 (Wilcoxon's two-sample test).

and alcohol consumption. Alcohol consumption was included as 2 indicator variables: ≤ or >2 drinks/d, with nondrinkers as a reference. Nondrinkers comprised abstainers (128 men and 273 women) and those drinking <1 drink/wk (209 men and 397 women). These variables were associated with B vitamin intake and with the plasma tHcy concentration. We also adjusted for the intake of the 3 other B vitamins under study (included continuously in the model when considered as confounders) to assess the independent effect of each B vitamin on the plasma tHcy concentration.

Trends across the quintiles were evaluated with linear regression, in which the quintiles were modeled as continuous variables. For the multivariate models, similar adjustments were made as in the analyses of covariance. The regression coefficients of these analyses express a proportional change because the plasma tHcy concentration was logarithmically transformed.

The relation between folate intake and the plasma tHcy concentration was visualized by plotting the adjusted mean plasma tHcy concentration against the mean folate intake in sex-specific deciles of folate intake. The adjusted means were calculated in a manner similar to that described for the quintiles.

Because both smoking and alcohol consumption are known to interfere with B vitamin metabolism (32–35), they were also evaluated as effect modifiers. For this evaluation the $P$ value of the interaction terms (eg, smoking × folate intake) was assessed for significance in the multivariate linear regression models.

Differences in the mean plasma tHcy concentration compared with a referent category were tested by using Bonferroni's adjustment for multiple comparisons. Findings were considered statistically significant if the two-sided $P$ value was <0.05. We used SAS statistical software (version 6.12) for all statistical analyses (SAS Institute Inc, Cary, NC).

## RESULTS

### Population characteristics

Selected characteristics of the study population are shown in **Table 1**. Because B vitamin supplements are used more frequently by women than by men, the exclusion of B vitamin supplement users resulted in a greater number of men in the study. However, the age range of the men and women was similar. The plasma tHcy concentration was significantly higher in men than in women, as were B vitamin and methionine intakes and alcohol consumption. The percentage of smokers was similar between the sexes.

### Association between B vitamin intake and plasma tHcy concentration

Mean plasma tHcy concentrations by quintiles of B vitamin intake are shown in **Table 2**. Univariately, the intake of all B vitamins was inversely associated with the plasma tHcy concentration in both men and women. An increase in intake of the different B vitamins from the lowest (first) to the highest (fifth) quintile was associated with decreases in the plasma tHcy concentration of 0.7–2.8 μmol/L. The largest decrease was observed across quintiles of folate intake; the plasma tHcy concentration decreased from 15.8 to 13.0 μmol/L in men and from 14.2 to 12.2 μmol/L in women.

Multivariate regression analyses showed that, after adjustment for age, intake of the other B vitamins and methionine, smoking, and alcohol consumption, only folate intake remained inversely associated with the plasma tHcy concentration (Table 2). The mean plasma tHcy concentration decreased from 15.4 μmol/L in the first quintile of folate intake to 13.2 μmol/L in the fifth quintile in men and from 13.7 to 12.4 μmol/L in women. Each quintile increase in folate intake was associated with a 3.2% decrease in plasma tHcy in men and a 2.5% decrease in women.

The association between folate intake and the plasma tHcy concentration in both sexes described a curve, especially in women, with a steeper slope in the low folate intake range (140–210 μg/d), which gradually became flatter at higher folate intakes (**Figure 1**). Across the range of folate intakes, the curve continued to describe an inverse association.

In men the relation between folate intake and the plasma tHcy concentration differed by smoking status (**Figure 2**). An increase in folate intake of one quintile was associated with a 2.6% decrease in the plasma tHcy in nonsmokers ($P$ for trend <0.01) and a 4.1% decrease in smokers ($P$ for trend <0.001). In nonsmokers the mean plasma tHcy concentration decreased 1.6 μmol/L from the first to the fifth quintile, whereas in smokers it decreased 2.8 μmol/L. In women we observed that the relation between folate intake and the plasma tHcy concentration was also somewhat stronger in smokers than in nonsmokers, although not significantly so.

In men we observed that alcohol consumption also modified the relation between folate intake and the plasma tHcy concentration (**Figure 3**). An increase of one quintile in folate intake was associated with a decrease in the plasma tHcy of 5.4% ($P$ for trend <0.001) in nondrinkers, 2.8% ($P$ for trend <0.01) in drinkers of ≤2 drinks alcohol/d, and 1.5% ($P$ for trend = 0.2) in drinkers of >2 drinks alcohol/d. The highest plasma tHcy concentration was observed in nondrinkers with a low folate intake (first quintile: 16.5 μmol/L). In women, alcohol did not modify the relation between folate intake and the plasma tHcy concentration.

## DISCUSSION

This was the first study in which the association between dietary intake of the B vitamins involved in homocysteine metabolism and the nonfasting plasma tHcy concentration was investigated for a large population-based sample aged 20–65 y. Our results indicate that after adjustment for confounders, folate was the only B vitamin independently inversely associated with the plasma tHcy concentration. Furthermore, we showed that in men this relation was stronger in smokers and weaker in alcohol drinkers.

In an earlier study we showed that our measurement of plasma tHcy concentrations is precise and valid, and thus suitable for

**TABLE 2**
Mean plasma total homocysteine (tHcy) concentrations by quintile (Q) of B vitamin intake[1]

| | Plasma tHcy[2] | Adjusted plasma tHcy[3] |
|---|---|---|
| Folate (μg/d) | | |
| Men | | |
| Q1: 161 | 15.8 | 15.4 |
| Q2: 187 | 14.4[4] | 14.3[5] |
| Q3: 204 | 13.5[4] | 13.6[4] |
| Q4: 223 | 13.8[4] | 13.9[4] |
| Q5: 254 | 13.0[4] | 13.2[4] |
| *P* for trend | <0.001 | <0.001 |
| Women | | |
| Q1: 160 | 14.2 | 13.7 |
| Q2: 188 | 13.0[4] | 13.1 |
| Q3: 206 | 12.6[4] | 12.7[5] |
| Q4: 224 | 12.3[4] | 12.5[4] |
| Q5: 262 | 12.2[4] | 12.4[4] |
| *P* for trend | <0.001 | <0.001 |
| Riboflavin (mg/d) | | |
| Men | | |
| Q1: 1.1 | 14.5 | 13.8 |
| Q2: 1.3 | 14.5 | 14.4 |
| Q3: 1.5 | 13.9 | 14.0 |
| Q4: 1.7 | 13.8 | 14.0 |
| Q5: 2.1 | 13.7 | 14.2 |
| *P* for trend | <0.01 | 0.6 |
| Women | | |
| Q1: 1.1 | 13.4 | 12.8 |
| Q2: 1.4 | 13.2 | 12.9 |
| Q3: 1.6 | 12.8 | 12.7 |
| Q4: 1.8 | 12.5[5] | 12.7 |
| Q5: 2.2 | 12.5[5] | 13.2 |
| *P* for trend | <0.001 | 0.6 |
| Vitamin B-6 (mg/d) | | |
| Men | | |
| Q1: 1.5 | 15.4 | 14.6 |
| Q2: 1.7 | 14.0[4] | 13.9 |
| Q3: 1.8 | 13.9[4] | 13.9 |
| Q4: 2.0 | 13.8[4] | 14.1 |
| Q5: 2.2 | 13.3[4] | 13.8 |
| *P* for trend | <0.001 | 0.2 |
| Women | | |
| Q1: 1.4 | 14.0 | 13.1 |
| Q2: 1.6 | 12.8[4] | 12.6 |
| Q3: 1.7 | 13.0[4] | 13.1 |
| Q4: 1.9 | 12.6[4] | 12.9 |
| Q5: 2.1 | 12.1[4] | 12.6 |
| *P* for trend | <0.001 | 0.6 |
| Vitamin B-12 (mg/d) | | |
| Men | | |
| Q1: 2.9 | 14.7 | 14.0 |
| Q2: 3.9 | 14.5 | 14.3 |
| Q3: 4.6 | 14.2 | 14.1 |
| Q4: 5.5 | 13.5[4] | 13.6 |
| Q5: 7.2 | 13.4[4] | 14.3 |
| *P* for trend | <0.001 | 0.8 |
| Women | | |
| Q1: 2.9 | 13.6 | 12.8 |
| Q2: 3.7 | 13.0 | 12.8 |
| Q3: 4.4 | 12.6[5] | 12.6 |
| Q4: 5.3 | 12.5[5] | 12.7 |
| Q5: 6.8 | 12.6[5] | 13.4 |
| *P* for trend | <0.001 | 0.3 |

[1] Geometric median B vitamin intakes standardized to the average energy intake (9391 kJ/d) of the population.
[2] $n$ = 1275 men and 1160 women.
[3] $n$ = 1254 men and 1148 women because of missing values. Values adjusted for intakes of other B vitamins and methionine, age, smoking (no or yes), and alcohol consumption [drinkers (≤2 and >2 drinks/d) and nondrinkers].
[4,5] Significantly different from Q1: [4] $P < 0.01$, [5] $P < 0.05$.

use when studying associations (23). Habitual dietary intake data were collected with a semiquantitative food-frequency questionnaire. A validation study indicated that the reproducibility and relative validity of food groups predominantly contributing to the intake of the B vitamins (eg, milk and milk products, meat, potatoes, bread, and vegetables) for this questionnaire were acceptable and comparable with those of other food-frequency questionnaires (24).

Our finding that folate is an important dietary correlate of the plasma tHcy concentration confirms the findings of observational studies in middle-aged (17) and elderly (18, 19) subjects. In contrast with those studies, however, we observed no association between plasma tHcy and riboflavin, vitamin B-6 (17–19), and vitamin B-12 (17), probably because of the more detailed statistical adjustment that we applied. For example, using the same model as used in the Framingham study (18), we found an association between vitamin B-6 and plasma tHcy in men only but not in our complete model. Alcohol consumption emerged as the strongest confounder in this association; methionine intake was the next strongest confounder. Moreover, the other 2 studies (17, 19) did not correct the intake of one B vitamin for the intake of the others. We suspect that if this had been done, there would have been no associations between the plasma tHcy concentration and riboflavin, vitamin B-6, and vitamin B-12. This suspicion is suggested by the weakening of these associations after correction for breakfast cereals, many of which are fortified with vitamins in the United States (17).

Because different B vitamins are frequently present in the same foods, the intake of one vitamin can be a marker for the intake of another vitamin. Our results showed that the univariate associations between the plasma tHcy concentration and riboflavin, vitamin B-6, and vitamin B-12 existed mainly because of confounding by folate intake. The importance of folate can be explained by its role in homocysteine metabolism. Folate, in the 5-methyltetrahydrofolate form, is the substrate donor in the remethylation of homocysteine to methionine (9). In contrast, the other B vitamins are cofactors of enzymes and are not used up during homocysteine degradation; thus, they are not often a limiting factor.

The cross-sectional design of our study limited our identification of causal relations; however, recent intervention trials showed that increases in the dietary folate intake resulted in decreases in plasma tHcy concentrations in apparently healthy subjects (36, 37). Thus, the relation between folate and plasma tHcy is likely to be causal. Assuming causality, 3 important conclusions can be drawn from the dose-response relation between folate intake and the plasma tHcy concentration. First, it describes a curve with a slope that is somewhat steeper at its beginning but that gradually becomes flatter at higher folate intakes, similar to what was shown in other observational studies (18, 20). This finding implies that an increase in folate intake [eg, achieved by consuming an additional bowl of salad and a glass of orange juice each day (providing >50 μg folate)] has the largest effect on the plasma tHcy concentration when folate intakes are low (<200 μg/d). Second, plasma tHcy concentrations decrease at folate intakes >200 μg/d, which is the recommended dietary intake in the Netherlands (38) and many other countries (39); thus, this intake is not sufficient to maintain an optimal low plasma tHcy concentration, as also suggested by other researchers (40–42). Finally, the curve did not reach its nadir within this range of folate intakes; thus, the recommended intake should be >300 μg/d.

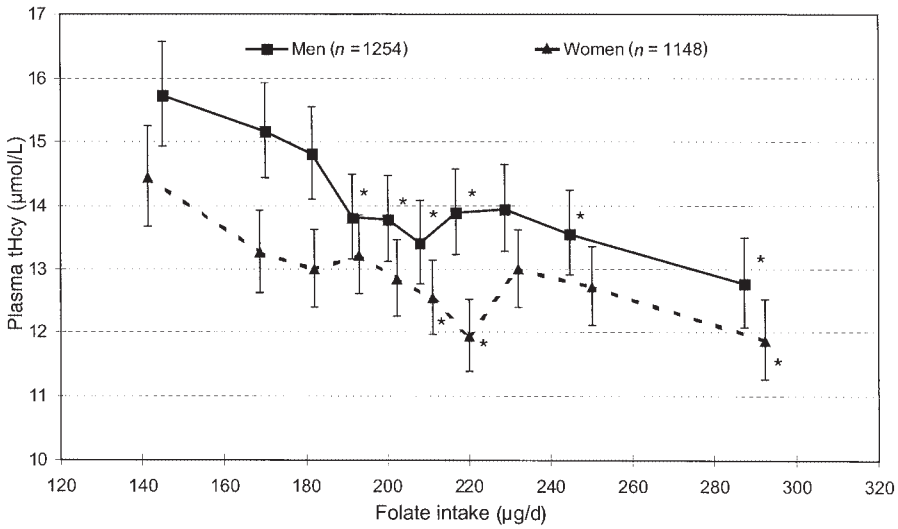


**FIGURE 1.** Mean plasma total homocysteine (tHcy) concentrations in men and women by deciles of folate intake. Means were adjusted for intakes of riboflavin, vitamin B-6, vitamin B-12, and methionine; age; smoking (no or yes); and alcohol consumption [drinkers (≤2 d and >2 drinks/d) and nondrinkers]. Folate intake was standardized to the average energy intake in our population (9391 kJ/d). *Significantly different from the lowest decile of folate intake, $P < 0.05$. Bars represent 95% CIs.

In men, we observed a stronger inverse relation between folate intake and plasma tHcy in smokers. Although it might appear that the interaction was drawn by the high mean plasma tHcy concentration at the lowest folate intake quintile in smokers, the interaction was also highly significant ($P = 0.002$) when folate intake was analyzed on a continuous scale. In women we observed a comparable pattern in the plasma tHcy concentration after stratification for smoking, but there was no statistical interaction. The interaction was not explained by a lower plasma folate concentration in smokers (32, 33, 43) because in our study population the plasma folate concentration in smokers was similar to that of nonsmokers after correction for folate intake. The much higher plasma tHcy concentration in smokers with a similar low folate intake to that of nonsmokers possibly points to the induction of a local folate deficiency in cells exposed to cigarette smoke (33). A high folate intake in smokers might secure enough folate for the remethylation of homocysteine within cells, preventing the export of an excess in homocysteine to plasma.

In men the relation between folate intake and the plasma tHcy concentration was also modified by alcohol consumption. This relation was strongest in nondrinkers and absent in drinkers of >2 drinks/d. We observed no interaction in women, likely because of the small range of alcohol intake: only 42% of the women regularly drank alcohol and only 7% drank >2 drinks/d.

A priori, we expected that, in subjects with a low folate intake, alcohol drinkers would have higher plasma tHcy concentrations

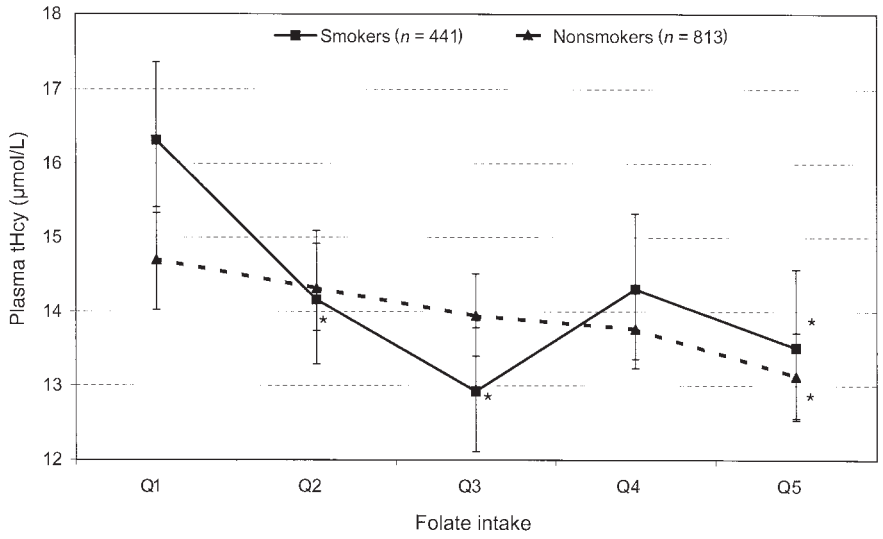


**FIGURE 2.** Mean plasma total homocysteine (tHcy) concentrations by quintile (Q) of folate intake. Means were adjusted for intakes of riboflavin, vitamin B-6, vitamin B-12, and methionine; age; and alcohol consumption [drinkers (≤2 and >2 drinks/d) and nondrinkers]. *Significantly different from the lowest quintile of folate intake, $P < 0.05$. Bars represent 95% CIs.

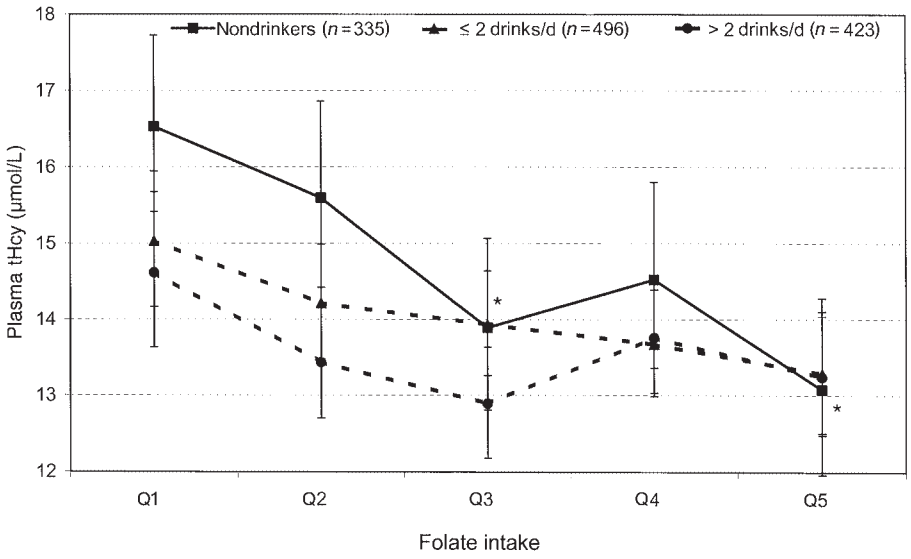


**FIGURE 3.** Mean plasma total homocysteine (tHcy) concentrations in male drinkers and nondrinkers by quintile (Q) of folate intake. Means were adjusted for intakes of riboflavin, vitamin B-6, vitamin B-12, and methionine; age; and smoking (no or yes). *Significantly different from the lowest quintile of folate intake, $P < 0.05$. Bars represent 95% CIs.

than would nondrinkers because of the adverse effect of alcohol on B vitamin status (34, 35). Quite the opposite was true (Figure 3). This contrast with the cited studies (in which a high amount of alcohol was studied) may have been due to the moderate alcohol consumption in our study (10% of the male drinkers in the present study consumed >5 alcoholic drinks/d). These findings add to the evidence for a potential beneficial health effect of moderate alcohol consumption on the cardiovascular system (44).

We evaluated whether the interaction was due to the type of alcohol consumed. For men in the highest alcohol category, beer was an important contributor to total folate intake. Men in the lowest folate intake quintile were more frequently wine drinkers: they drank twice as much wine (≈1 drink/d) as did men in the other quintiles. Wine contains betaine (45), which serves as a methyl donor for the less common remethylation of homocysteine by betaine-homocysteine methyltransferase (restricted to liver, kidney, and adrenal gland tissues) (9). This process might, at least partly, explain why these male drinkers had a low plasma tHcy concentration despite their low folate intake. We were not able to adjust for betaine intake because of the lack of dietary data for this nutrient.

We conclude that, of the B vitamins involved in homocysteine metabolism, folate is the most important dietary determinant of the plasma tHcy concentration. In the Dutch population studied, the Dutch folate recommendation was not met by 31% of the men and 61% of the women. It is important to note that even if all of the subjects had met the recommendation of 200 μg/d, this amount would not lead to an optimal low plasma tHcy concentration. Our study showed that a high dietary intake of folate can make a substantial contribution to a reduction in plasma tHcy concentrations in the general population, which is important because each 1-μmol/L decrease in tHcy may be associated with a 10% reduction in risk of cardiovascular diseases (3). These results provide additional scientific foundation for current public health educational programs targeted at increasing the consumption of plant foods, which are particularly rich in folate, to reduce chronic diseases.

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## REFERENCES

1. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56:111–28.
2. Wilcken DE, Wilcken B. The pathogenesis of coronary artery disease. A possible role for methionine metabolism. J Clin Invest 1976; 57:1079–82.
3. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 1995;274:1049–57.
4. Verhoef P, Kok FJ, Kruyssen DA, et al. Plasma total homocysteine, B vitamins, and risk of coronary atherosclerosis. Arterioscler Thromb Vasc Biol 1997;17:989–95.
5. Tsai JC, Perrella MA, Yoshizumi M, et al. Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. Proc Natl Acad Sci U S A 1994;91:6369–73.
6. Lentz SR. Homocysteine and vascular dysfunction. Life Sci 1997;61: 1205–15.
7. Harpel PC, Zhang X, Borth W. Homocysteine and hemostasis: pathogenic mechanisms predisposing to thrombosis. J Nutr 1996;126: 1285S–9S.
8. Duell PB, Malinow MR. Homocyst(e)ine: an important risk factor for atherosclerotic vascular disease. Curr Opin Lipidol 1997;8:28–34.
9. Finkelstein JD. Methionine metabolism in mammals. J Nutr Biochem 1990;1:228–37.
10. Guenther BD, Sheppard CA, Tran P, Rozen R, Matthews RG, Ludwig ML. The structure and properties of methylenetetrahydrofolate reductase from *Escherichia coli* suggest how folate ameliorates human hyperhomocysteinemia. Nat Struct Biol 1999;6: 359–65.
11. Brouwer IA, van Dusseldorp M, Thomas CMG, et al. Low-dose folic acid supplementation decreases plasma homocysteine concentrations: a randomized trial. Am J Clin Nutr 1999;69:99–104.

12. Ward M, McNulty H, McPartlin J, Strain JJ, Weir DG, Scott JM. Plasma homocysteine, a risk factor for cardiovascular disease, is lowered by physiological doses of folic acid. Q J Med 1997;90:519–24.
13. Olszewski AJ, Szostak WB, Bialkowska M, Rudnicki S, McCully KS. Reduction of plasma lipid and homocysteine levels by pyridoxine, folate, cobalamin, choline, riboflavin, and troxerutin in atherosclerosis. Atherosclerosis 1989;75:1–6.
14. Ubbink JB, van der Merwe A, Vermaak WJ, Delport R. Hyperhomocysteinemia and the response to vitamin supplementation. Clin Invest 1993;71:993–8.
15. Ubbink JB, Vermaak WJ, van der Merwe A, Becker PJ, Delport R, Potgieter HC. Vitamin requirements for the treatment of hyperhomocysteinemia in humans. J Nutr 1994;124:1927–33.
16. Brattstrom LE, Israelsson B, Jeppsson JO, Hultberg BL. Folic acid—an innocuous means to reduce plasma homocysteine. Scand J Clin Lab Invest 1988;48:215–21.
17. Shimakawa T, Nieto FJ, Malinow MR, Chambless LE, Schreiner PJ, Szklo M. Vitamin intake: a possible determinant of plasma homocyst(e)ine among middle aged adults. Ann Epidemiol 1997;7: 285–93.
18. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993;270:2693–8.
19. Bates CJ, Mansoor MA, van der Pols J, Prentice A, Cole TJ, Finch S. Plasma total homocysteine in a representative sample of 972 British men and women aged 65 and over. Eur J Clin Nutr 1997;51:691–7.
20. Nygard O, Refsum H, Ueland PM, Vollset SE. Major lifestyle determinants of plasma total homocysteine distribution: the Hordaland Homocysteine Study. Am J Clin Nutr 1998;67:263–70.
21. Smit HA, Verschuren WMM, Bueno de Mesquita HB, Seidell JC. Monitoring van risicofactoren en gezondheid in Nederland (MORGEN-project): doelstelling en werkwijze. [The monitoring project on risk factors for chronic diseases in the Netherlands (MORGEN-project): aim and method.] Bilthoven: Rijksinstituut voor Volksgezondheid en Milieu, 1994:1–93 (in Dutch). (Report No. 26320000).
22. Willems HPJ, Bos GMJ, Gerrits WBJ, den Heijer M, Vloet S, Blom HJ. Acidic citrate stabilized blood samples for assay of total homocysteine. Clin Chem 1998;44:342–5.
23. de Bree A, Verschuren WMM, Blom HJ, De Graaf-Hess A, Trijbels FJM, Kromhout D. The homocysteine distribution: (mis)judging the burden. J Clin Epidemiol 2001;54:462–9.
24. Ocke MC, Bueno de Mesquita HB, Goddijn HE, et al. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int J Epidemiol 1997;26(suppl):S37–48.
25. Stichting NEVO. NEVO tabel. Nederlands Voedingsstoffenbestand 1996. ( NEVO table. Dutch nutrient database 1996). Den Haag: Voorlichtingsbureau voor de Voeding, 1996 (in Dutch).
26. Konings EJM. A validated liquid chromatographic method for determining folates in vegetables, milk powder, liver, and flour. J AOAC Int 1999;82:119–27.
27. Konings EJM, Roomans HHS, Dorant E, Goldbohm RA, Saris WHM, van Den Brandt PA. Folate intake of the Dutch population according to newly established liquid chromatography data for foods. Am J Clin Nutr 2001;73:765–76.
28. Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM. Homocysteine and other thiols in plasma and urine: automated determination and sample stability. Clin Chem 1993;39:263–71.
29. te Poele-Pothoff MT, van den Berg M, Franken DG, et al. Three different methods for the determination of total homocysteine in plasma. Ann Clin Biochem 1995;32:218–20.
30. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 1997;65(suppl): 1220S–8.
31. Kleinbaum DG, Kupper LL, Muller KE, Nizam A. Regression diagnostics. In: Kleinbaum DG, Kupper LL, Muller KE, Nizam A, eds. Applied regression analysis and multivariable methods. Pacific Grove, CA: Duxbury Press, 1998:212–80.
32. Bergmark C, Mansoor MA, Swedenborg J, de Faire U, Svardal AM, Ueland PM. Hyperhomocysteinemia in patients operated for lower extremity ischaemia below the age of 50—effect of smoking and extent of disease. Eur J Vasc Surg 1993;7:391–6.
33. Piyathilake CJ, Hine RJ, Dasanayake AP, et al. Effect of smoking on folate levels in buccal mucosal cells. Int J Cancer 1992;52:566–9.
34. Shaw S, Jayatilleke E, Herbert V, Colman N. Cleavage of folates during ethanol metabolism. Role of acetaldehyde/xanthine oxidase-generated superoxide. Biochem J 1989;257:277–80.
35. Cravo ML, Gloria LM, Selhub J, et al. Hyperhomocysteinemia in chronic alcoholism: correlation with folate, vitamin B-12, and vitamin B-6 status. Am J Clin Nutr 1996;63:220–4.
36. Brouwer IA, Van Dusseldorp M, West CE, et al. Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled trial. J Nutr 1999;129: 1135–9.
37. Riddell LJ, Chisholm A, Williams S, Mann JI. Dietary strategies for lowering homocysteine concentrations. Am J Clin Nutr 2000;71: 1448–54.
38. Nederlandse Voedingsraad. Nederlandse voedingsnormen 1989. (Dutch dietary recommendations 1989.) Den Haag, Netherlands: Voorlichtingsbureau voor de Voeding, 1992 (in Dutch).
39. de Bree A, van Dusseldorp M, Brouwer IA, van het Hof KH, Steegers Theunissen RPM. Folate intake in Europe: recommended, actual and desired intake. Eur J Clin Nutr 1997;51:643–60.
40. Verhoef P, Stampfer MJ, Buring JE, et al. Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B6, B12, and folate. Am J Epidemiol 1996;143:845–59.
41. Jacob RA, Wu MM, Henning SM, Swendseid ME. Homocysteine increases as folate decreases in plasma of healthy men during short-term dietary folate and methyl group restriction. J Nutr 1994;124: 1072–80.
42. Nygard O, Refsum H, Ueland PM, et al. Coffee consumption and plasma total homocysteine: The Hordaland Homocysteine Study. Am J Clin Nutr 1997;65:136–43.
43. Chen AT, Reidy JA, Annest JL, Welty TK, Zhou HG. Increased chromosome fragility as a consequence of blood folate levels, smoking status, and coffee consumption. Environ Mol Mutagen 1989;13:319–24.
44. Thakker KD. An overview of health risks and benefits of alcohol consumption. Alcohol Clin Exp Res 1998;229(suppl):285S–98S.
45. Mar MH, Zeisel SH. Betaine in wine: answer to the French paradox? Med Hypotheses 1999;53:383–5.