# Renal Insufficiency, Vitamin B12 Status, and Population Attributable Risk for Mild Hyperhomocysteinemia Among Coronary Artery Disease Patients in the Era of Folic Acid–Fortified Cereal Grain Flour

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# Renal Insufficiency, Vitamin $B_{12}$ Status, and Population Attributable Risk for Mild Hyperhomocysteinemia Among Coronary Artery Disease Patients in the Era of Folic Acid–Fortified Cereal Grain Flour

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***Abstract***—Fortification of enriched cereal grain flour products with folic acid has drastically reduced the prevalence of deficient plasma folate status, a major determinant of plasma total homocysteine (tHcy) levels. We hypothesized that even more liberally defined "suboptimal" plasma folate status might no longer contribute importantly to the population attributable risk (PAR) for mild hyperhomocysteinemia, a putative atherothrombotic risk factor. We determined fasting plasma tHcy, folate, vitamin $B_{12}$, and pyridoxal 5′-phosphate levels, along with serum creatinine and albumin levels, in 267 consecutive patients (aged 61±9 [mean±SD] years, 76.4% men and 26.6% women) with stable coronary artery disease (CAD) who were nonusers of vitamin supplements or had abstained from supplement use for at least 6 weeks before examination. Subjects were evaluated a minimum of 3 months after the implementation of flour fortification was largely completed. Relative risk estimates for the calculation of PAR were derived from a multivariable-adjusted logistic regression model with ≥12 μmol/L tHcy as the dependent variable and with age, sex, pyridoxal 5′-phosphate (continuous), albumin (continuous), <5 ng/mL folate, <250 pg/mL vitamin $B_{12}$, and ≥1.3 mg/dL creatinine as the independent variables. The prevalence of ≥12 μmol/L plasma tHcy was 11.2% (30 of 267 patients). PAR estimates (percentage) for ≥12 μmol/L tHcy were as follows: <5 ng/mL folate (<1%), <250 pg/mL vitamin $B_{12}$ (24.5%), and ≥1.3 mg/dL creatinine (37.5%). In the era of folic acid–fortified cereal grain flour, renal insufficiency and suboptimal vitamin $B_{12}$ status (but not folate status) contribute importantly to the PAR for mild hyperhomocysteinemia among patients with stable CAD. **(*Arterioscler Thromb Vasc Biol*. 2001;21:849-851.)**

**Key Words:** coronary arteriosclerosis ■ renal function ■ homocysteine ■ determinants

Ueland et al[1] recently reported a meta-analysis of 14 prospective studies of the relationship between baseline total homocysteine (tHcy) levels and (primarily) coronary artery disease (CAD) outcomes in population-based cohorts; this meta-analysis was reported through the end of 1999. The aggregate relative risk estimate (from a total of 2786 cases) per 5 μmol/L change in tHcy concentration was 1.20 (95% CI 1.14 to 1.25). Nephrosclerosis, specifically renal arteriolar hyalinization,[2–4] has been associated with systemic arteriosclerosis[2] and clinical[3] as well as subclinical CAD.[4] There is a strong independent (inverse) association between glomerular filtration rate directly determined by either iohexol clearance[5,6] or $^{51}$Cr-EDTA clearance[7] and fasting tHcy levels, which encompasses glomerular filtration rates throughout the normative range. A surrogate for glomerular filtration rate[8] and homocysteine generation,[9] creatinine is a significant determinant of tHcy levels in CAD patients[10,11] and general populations.[12] In a population-based sample with predominantly normal renal function, fortification of enriched cereal grain flour products with folic acid drastically reduced the prevalence of deficient plasma folate status and mild hyperhomocysteinemia.[13] These data contrast starkly with the very limited impact that this fortification policy has had on the prevalence of mild hyperhomocysteinemia among chronic renal transplant recipients,[14] who serve as a valid model for the hyperhomocysteinemia of chronic renal insufficiency, in general.[15] Moreover, renal function gauged as a simple creatinine determination is the major independent determinant of tHcy levels in each of these 2 patient groups with mild to moderate chronic renal insufficiency.[14,15] In light of such collective findings,[13–15] we hypothesized that even more liberally defined "suboptimal" plasma folate status might no longer contribute importantly to the population attributable risk (PAR) for mild hyperhomocysteinemia among stable

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CAD patients. Accordingly, we assessed fasting plasma tHcy and serum creatinine in conjunction with the other established determinants of tHcy levels (ie, age,[16] sex,[16] B-vitamin status,[16] and albumin[17]) among 267 consecutive patients with clinically stable CAD, all of whom were examined at least 3 to 4 months after fortification of enriched cereal grain flour with folic acid was largely completed.[13]

## Methods

The institutional review board at Memorial Hospital of Rhode Island (Pawtucket) approved the study protocol, and all participants provided written informed consent. Study participants were 267 stable CAD patients (ie, they were studied at least 3 months after myocardial infarction or coronary angioplasty and/or at least 6 months after coronary artery bypass graft surgery). CAD status was confirmed by established 12-lead ECG and cardiac isoenzyme (ie, creatine phosphokinase-MB) criteria for definite myocardial infarction and/or unstable angina with angiographically proven ≥50% stenosis of at least 1 major epicardial coronary artery. Participants lived in the Pawtucket and Providence, RI, metropolitan areas, and were examined between October 1997 and May 1999. Information regarding prior vitamin supplement use was obtained by standardized interview, and subjects were either nonusers of any supplements containing folic acid or had abstained from using such supplements for at least 6 weeks by the time of their examination. However, all participants were examined at least 3 to 4 months after the widespread availability in New England (John Watson, Watson Foods, New Haven, Conn, personal communication, 1998) of cereal grain flour products fortified with folic acid at 140 μg per 100 g flour.[18]

Overnight (10- to 14-hour) fasting blood samples were collected from each participant. Plasma tHcy levels were determined by high-performance liquid chromatography with fluorescence detection, and plasma pyridoxal 5′-phosphate (PLP) levels were measured by radioenzymatic (tyrosine decarboxylase) assay, as reported earlier.[16] Plasma folate and vitamin $B_{12}$ levels were measured by radioassay (Bio-Rad Quantaphase II). Serum creatinine levels (by Jaffe's test) and albumin levels (by bromcresol method) were determined by using standard techniques adapted for automated clinical chemistry laboratory analyzers.

Descriptive data included arithmetic means with standard deviations and complete ranges or geometric means with interquartile and complete ranges for continuous variables and prevalences (percentages) for discrete variables. The odds ratios (an estimate of relative risk) for the calculation of PAR percentage (PAR%)[19] were derived from a multivariable-adjusted logistic regression model with ≥12 μmol/L tHcy as the dependent variable and age, sex, PLP (continuous), albumin (continuous), <5 ng/mL folate, <250 pg/mL vitamin $B_{12}$, and ≥1.3 mg/dL creatinine as the independent variables. The dichotomous cut points chosen for mild hyperhomocysteinemia and mild renal insufficiency were consistent with earlier operational definitions.[7,20,21] The dichotomous cut points chosen for suboptimal folate and vitamin $B_{12}$ status were deliberately selected to be slightly above the most common clinically defined cut points for folate deficiency and vitamin $B_{12}$ deficiency of <3 ng/mL and <200 pg/mL, respectively. The dearth of US subjects with clinically defined folate deficiency in the current era of folic acid–fortified cereal grain flour figured prominently in our decision regarding vitamin status cut points.[13,14] PAR% estimates were calculated from the following formula: $PAR\% = \{[prevalence_{risk\ factor}(RR-1)]/[prevalence_{risk\ factor}(RR-1)+1]\} \times 100$.[19] Reported probability values were based on 2-tailed calculations, and all statistical analyses were performed by using SYSTAT (version 9.0) software.

## Results

Key subject characteristics, expressed as means, geometric means, percentages, and complete ranges, are depicted in Table 1. Geometric mean fasting tHcy levels were greater in the men (n=196) than in the women (n=71) at 8.5 versus 7.7 μmol/L, respectively ($P=0.007$). The prevalence of ≥12 μmol/L plasma tHcy was 11.2% (30 of the 267 patients).

**TABLE 1. Subject Characteristics**

| | |
|---|---|
| Subjects, n | 267 |
| Sex, n (% women) | 71 (26.6%) |
| Age, y | |
| Mean±SD | 61±9 |
| Range | 30–77 |
| tHcy, μmol/L | |
| Geometric mean | 8.3 |
| 25th to 75th percentile range (full range) | 6.8–9.9 (3.8–23.4) |
| Creatinine, mg/dL | |
| Geometric mean | 1.1 |
| 25th to 75th percentile range (full range) | 1.0–1.2 (0.5–2.6) |
| Folate, ng/mL | |
| Geometric mean | 8.6 |
| 25th to 75th percentile range (full range) | 6.8–11.0 (2.2–41.1) |
| Vitamin B12, pg/mL | |
| Geometric mean | 396 |
| 25th to 75th percentile | 317–500 (165–938) |
| PLP, nmol/mL | |
| Geometric mean | 49.7 |
| 25th to 75th percentile range (full range) | 34.4–68.8 (9.2–373.8) |
| Albumin (mg/dL) | |
| Geometric mean | 4.4 |
| 25th to 75th percentile range (full range) | 4.2–4.6 (2.9–5.6) |

Logistic regression modeling was performed with ≥12 μmol/L tHcy as the dependent variable and age, sex, PLP (continuous), albumin (continuous), <5 ng/mL folate, <250 pg/mL vitamin $B_{12}$, and ≥1.3 mg/dL creatinine as the potential explanatory variables. Table 2 indicates the prevalence (percentage) of ≥1.3 mg/dL creatinine , <250 pg/mL vitamin $B_{12}$, and <5 ng/mL folate, as well as the multivariable-adjusted relative risk estimates and PAR% for a ≥12 μmol/L fasting tHcy conferred by these 3 potential explanatory variables. Stepwise forward selection and backward elimination revealed that ≥1.3 mg/dL serum creatinine ($P=0.002$) and <250 pg/mL plasma vitamin $B_{12}$ ($P=0.008$), but not <5 ng/mL plasma folate ($P=0.351$), were independently predictive of a ≥12 μmol/L fasting tHcy. PAR estimates (percentage) for a ≥12 μmol/L tHcy, were as follows: <5 ng/mL folate (<1%), <250 pg/mL vitamin $B_{12}$ (24.5%), and ≥1.3 mg/dL creatinine (37.5%).

## Discussion

All patients were examined at least several months after the widespread availability in southeast New England of cereal

**TABLE 2. PAR Percentages for Mild Fasting Hyperhomocysteinemia (ie, tHcy Levels ≥12 μmol/L)**

| | Prevalence, % | Odds Ratio* (95% CI) | PAR, % |
|---|---|---|---|
| Creatinine ≥1.3 mg/dL | 16.7 | 4.6 (1.8–2.7) | 37.5 |
| Vitamin $B_{12}$ <250 pg/mL | 7.9 | 5.1 (1.5–17.1) | 24.5 |
| Folate <5 ng/mL | 9.0 | 0.3 (0.1–3.1) | <1.0 |

*Odds ratio for tHcy ≥12 μmol/L from a logistic regression model adjusted for age, sex, PLP (continuous), albumin (continuous), and each of the dichotomous variables in the table.

grain flour products (ie, all enriched wheat, corn, and rice flour products) fortified with folic acid at 140 μg/100 g of flour.[18] Within a population-based sample of New England residents (ie, the Framingham Offspring cohort) who were nonusers of vitamin supplements, this fortification policy has doubled the plasma folate levels while reducing the prevalence of low folate levels (ie, <3 ng/mL) by >90% and the prevalence of >13 μmol/L fasting tHcy levels by nearly 50%.[13] The very low point prevalence of <3 ng/mL plasma folate (ie, 4 [1.5%] of 267 patients) among the CAD patients examined in the present study is completely consistent with the prevalence of <3 ng/mL folate (1.0%, 95% CI 0.0% to 4.8%) among nonusers of supplements in the Framingham Offspring Study, who were similarly examined after the advent of fortification.[13] Moreover, these results with respect to the current dearth of plasma folate deficiency in US populations are consistent with findings from Canadian general populations[22] and CAD populations[23] examined after the initiation of the same flour fortification policy in Canada. Improved, relatively "homogeneous" folate status secondary to cereal grain fortification may have contributed to the lack of association between plasma folate and fasting tHcy levels observed in the present study. In contrast, the median age of the CAD patient population (61 years) could have accentuated the impact of age-related decrements in renal function[8] and vitamin $B_{12}$ status[24] on fasting plasma tHcy levels.

Persistent mild hyperhomocysteinemia is characteristic of patients with chronic renal insufficiency and end-stage renal disease.[25] The etiology of this hyperhomocysteinemia remains unknown, although it has been hypothesized to result from the loss of intrarenal homocysteine metabolism[26] and/or uremia-induced extrarenal defects in homocysteine metabolism.[27] In conjunction with earlier reports,[5–7] the present findings from clearly nonuremic subjects with, at most, only mild to moderate chronic renal insufficiency[28] might suggest that intrarenal homocysteine metabolism is a major determinant of homocysteine levels. However, these data cannot rule out the possibility that subtle extrarenal defects in homocysteine metabolism that may accompany even such mild reductions in renal function could account for the resulting increases in tHcy levels.

In summary, ever since the advent of folic acid fortification of cereal grain flour, renal insufficiency and suboptimal vitamin $B_{12}$ status, but not folate status, contribute importantly to the PAR for mild hyperhomocysteinemia among patients with stable CAD.

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